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Provisional Peer-Reviewed Toxicity Values for

3,5-Dinitroaniline
(CASRN 618-87-1)

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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U.S. Environmental Protection Agency
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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 (ORD) Center for Public Health and Environmental Assessment (CPHEA) website at https://ecomments.epa.gov/pprtv.

TABLE OF CONTENTS

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
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
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.

DRAFT PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 3,5-DINITROANILINE (CASRN 618-87-1)

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 U.S. Environmental Protection Agency (U.S. EPA) 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. 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 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 ORD CPHEA website at https://ecomments.epa.gov/pprtv.

1. INTRODUCTION

3,5-Dinitroaniline, CASRN 618-87-1, belongs to the class of compounds known as nitroaromatics, which are often used as intermediates in the preparation of dyes and pesticides (e.g., herbicides). 3,5-Dinitroaniline is a weak explosive but may be nitrated to yield the powerful explosive 2,3,4,5,6-pentanitroaniline (Talmage et al., 1999). It is not 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, 2015b).

Nitroanilines are normally formed by ammonolysis of the corresponding chloronitrobenzene (Amini and Lowenkron, 2003). 3,5-Dinitroaniline is also produced from the reduction of 1,3,5-trinitrobenzene with sodium sulfide (Booth, 2012). During the production of 2,4,6-trinitrotoluene (TNT), 3,5-dinitroaniline is formed as a byproduct. Thus, 3,5-dinitroaniline has been found in the environment near munitions production and processing facilities (Talmage et al., 1999).

The empirical formula of 3,5-dinitroaniline is $C_6H_5N_3O_4$ (see Figure 1). Table 1 summarizes its physicochemical properties. 3,5-Dinitroaniline is a solid in the form of yellow needles at room temperature (Talmage et al., 1999). Its low estimated vapor pressure and low Henry's law constant indicate that the solid compound is unlikely to volatilize from either dry or moist surfaces. The moderate estimated water solubility and moderate soil adsorption coefficient of 3,5-dinitroaniline indicate that it will have moderate potential to leach to groundwater or undergo runoff after a rain event.

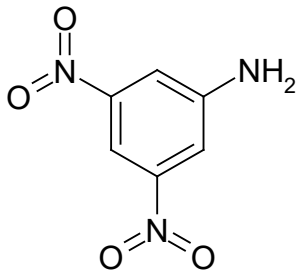


Figure 1. 3,5-Dinitroaniline (CASRN 618-87-1) Structure

Table 1. Physicochemical Properties of 3,5-Dinitroaniline (CASRN 618-87-1)[a]

Property (unit)	Value
Physical state	Solid
Boiling point (°C)	398 (experimental average)
Melting point (°C)	162 (experimental average)
Density (g/mL)	1.59 (predicted average)
Vapor pressure (mm Hg at 25°C)	1.39×10^{-5} (predicted average)
pH (unitless)	NA[b]
Acid dissociation constant (pKa) (unitless) (for protonated compound)	0.3[b]
Solubility in water (mol/L)	7.08×10^{-3} (experimental average)
Octanol-water partition coefficient (log K_{ow})	1.89
Henry's law constant (atm-m^3/mol at 25°C)	4.34×10^{-8} (predicted average)
Soil adsorption coefficient (K_{oc}) (L/kg)	253−507[c]
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	1.12×10^{-12} (predicted average)
Atmospheric half-life (d)	13 (estimated)[b]
Relative vapor density (air = 1)	NV[b]
Molecular weight (g/mol)	183.123
Flash point (closed cup in °C)	186[a, b]

[a]Unless otherwise noted, data were extracted from the U.S. EPA CompTox Chemicals Dashboard (CASRN 618-87-1; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=618-87-1#properties. Accessed July 15, 2020).
[b]U.S. EPA (2012).
[c]Talmage et al. (1999).

NA = not applicable; NV = not available.

No toxicity values for 3,5-dinitroaniline were identified from U.S. EPA or other agencies/organizations, as shown in Table 2.

Table 2. Summary of Available Toxicity Values for 3,5-Dinitroaniline (CASRN 618-87-1)

Source[a]	Value (applicability)	Reference[b]
Noncancer		
IRIS	NV	U.S. EPA (2020a)
HEAST	NV	U.S. EPA (2011b)
DWSHA	NV	U.S. EPA (2018)
ATSDR	NV	ATSDR (2018)
IPCS	NV	IPCS (2020)
CalEPA	NV	CalEPA (2019)
OSHA	NV	OSHA (2020a); OSHA (2020b)
NIOSH	NV	NIOSH (2018)
ACGIH	NV	ACGIH (2020)
Cancer		
IRIS	NV	U.S. EPA (2020a)
HEAST	NV	U.S. EPA (2011b)
DWSHA	NV	U.S. EPA (2018)
NTP	NV	NTP (2016)
IARC	NV	IARC (2018)
CalEPA	NV	CalEPA (2019)
ACGIH	NV	ACGIH (2020)

[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.

NV = not available.

Non-date-limited literature searches were conducted in May 2020 and updated in June 2021 for studies relevant to the derivation of provisional toxicity values for 3,5-dinitroaniline. Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. The HERO webpage capturing the search results can be found at https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2009. HERO searches the following databases: PubMed, TOXLINE[1] (including TSCATS1), and Web of Science. The following databases 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

[1]Note that this version of TOXLINE (https://www.nlm.nih.gov/databases/download/toxlinesubset.html) is no longer updated; therefore, it was not included in the literature search update from June 2021.

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 Integrated Risk Information System (IRIS), U.S. EPA Health Effects Assessment Summary Tables (HEAST), U.S. EPA Office of Water (OW), International Agency for Research on Cancer (IARC), U.S. EPA TSCATS2/TSCATS8e, U.S. EPA High Production Volume (HPV), Chemicals via IPCS INCHEM, Japan Existing Chemical Data Base (JECDB), Organisation for Economic Cooperation and Development (OECD) Screening Information Data Sets (SIDS), OECD International Uniform Chemical Information Database (IUCLID), OECD HPV, National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Occupational Safety and Health Administration (OSHA), and World Health Organization (WHO).

2. REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

As shown in Tables 3A and 3B, there are no potentially relevant short-term, subchronic, chronic, developmental, or reproductive toxicity studies of 3,5-dinitroaniline in humans or animals exposed by oral or inhalation routes. The phrase "statistical significance" and the term "significant," used throughout the document, indicate a *p*-value of < 0.05 unless otherwise specified.

Table 3A. Summary of Potentially Relevant Noncancer Data for 3,5-Dinitroaniline (CASRN 618-87-1)					
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**Reference (comments)**	**Notes**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					

ND = no data.

Table 3B. Summary of Potentially Relevant Cancer Data for 3,5-Dinitroaniline (CASRN 618-87-1)					
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**Reference (comments)**	**Notes**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					

ND = no data.

2.1. HUMAN STUDIES

2.1.1. Oral Exposures

No studies have been identified.

2.1.2. Inhalation Exposures

No studies have been identified.

2.2. ANIMAL STUDIES

2.2.1. Oral Exposures

No studies have been identified.

2.2.2. Inhalation Exposures

No studies have been identified.

2.3. OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Data pertaining to the toxicity of 3,5-dinitroaniline are limited to in vitro genotoxicity studies, as described below.

2.3.1. Genotoxicity

Genotoxicity studies of 3,5-dinitroaniline are summarized in Table 4. 3,5-Dinitroaniline was mutagenic when tested in *Salmonella typhimurium* strains TA98 and TA100 with or without metabolic activation (Assmann et al., 1997). Positive findings for 3,5-dinitroaniline were reported in *S. typhimurium* strains TA98, TA100, TA1537, and TA1538 without metabolic activation, and in TA1535 with or without metabolic activation, when tested at concentrations between 0.5 and 40 μg/plate (Spanggord et al., 1982). In the same study, 3,5-dinitroaniline was not mutagenic in strain TA100NR3 (mutant lacking nitroreductase activity) with or without activation.

Table 4. Summary of 3,5-Dinitroaniline (CASRN 618-87-1) Genotoxicity

Endpoint	Test System	Doses/Concentrations Tested (µg/plate)	Results without Activation	Results with Activation	Comments	References
Genotoxicity studies in prokaryotic organisms						
Mutation	*Salmonella typhimurium* strains TA98, TA100	0, 17, 34, 68, 135, 270	+	+	Plate incorporation assay. 3,5-Dinitroaniline induced a doubling of the spontaneous mutation rate at ≥34 µg/plate in TA98 and ≥17 µg/plate in TA100.	Assmann et al. (1997)
Mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538, TA100NR3 (nitroreductase-deficient strain)	0, 0.5−40	+ TA98, TA100, TA1535, TA1537, TA1538 − TA100NR3	+ TA1535 − TA98, TA100, TA1537, TA1538, TA100NR3	Plate incorporation assay. Effective dose(s) were not reported.	Spanggord et al. (1982)

3. DERIVATION OF PROVISIONAL VALUES

The lack of toxicity data precludes direct development of cancer or noncancer provisional reference values for 3,5-dinitroaniline. However, screening provisional reference dose (p-RfD) values and screening provisional reference concentration (p-RfC) values are derived based on available data for structurally similar compounds (see Appendix A).

3.1. DERIVATION OF ORAL REFERENCE DOSES

There are no data on the effects of 3,5-dinitroaniline in humans or animals exposed orally. Because of the lack of any available data for 3,5-dinitroaniline, subchronic and chronic p-RfDs cannot be 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, 4-nitroaniline is selected as the most appropriate analogue for 3,5-dinitroaniline for deriving a screening subchronic and chronic p-RfD.

3.2. DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

There are no data on the effects of 3,5-dinitroaniline in humans or animals exposed by inhalation. The absence of relevant inhalation data precludes deriving p-RfCs for 3,5-dinitroaniline directly. Instead, screening p-RfCs are derived in Appendix A using an alternative analogue approach. Based on the overall analogue approach presented in Appendix A, 4-nitroaniline is selected as the most appropriate analogue for 3,5-dinitroaniline for deriving a screening subchronic and chronic p-RfC.

3.3. SUMMARY OF PROVISIONAL REFERENCE VALUES

The noncancer provisional reference values for 3,5-dinitroaniline are summarized in Table 5.

Table 5. Summary of Noncancer Reference Values for 3,5-Dinitroaniline (CASRN 618-87-1)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD[a] (HED/HEC)	UF_C	Principal Study
Screening subchronic p-RfD (mg/kg-d)	Rat/F	Methemoglobinemia	7×10^{-4}	$BMDL_{1SD}$	0.22 (based on analogue POD)	300	Monsanto (1981) as cited in U.S. EPA (2009b)
Screening chronic p-RfD (mg/kg-d)	Rat/M	Methemoglobinemia	4×10^{-4}	$BMDL_{1SD}$	0.11 (based on analogue POD)	300	Nair et al. (1990) as cited in U.S. EPA (2009b)
Screening subchronic p-RfC (mg/m^3)	Rat/M	Methemoglobinemia	6×10^{-3}	$BMCL_{1SD}$	1.7 (based on analogue POD)	300	Nair et al. (1986) as cited in U.S. EPA (2009b)
Screening chronic p-RfC (mg/m^3)	Rat/M	Methemoglobinemia	2×10^{-3}	$BMCL_{1SD}$	1.7 (based on analogue POD)	1,000	Nair et al. (1986) as cited in U.S. EPA (2009b)

[a]As stated in the text, 4-nitroaniline is selected as the analogue for the screening subchronic and chronic noncancer oral and inhalation toxicity reference values.

BMCL = benchmark concentration lower confidence limit; BMDL = benchmark dose lower confidence limit, one standard deviation; F = female; HEC = human equivalent concentration; HED = human equivalent dose; M = male; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; SD = standard deviation; UF_C = composite uncertainty factor.

3.4. CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Under the U.S. EPA Cancer Guidelines (U.S. EPA, 2005a), there is *"Inadequate Information to Assess the Carcinogenic Potential"* of 3,5-dinitroaniline (see Table 6). No relevant studies are available in humans or animals. Within the current U.S. EPA Cancer Guidelines (U.S. EPA, 2005a), 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 is a qualitative level of *concern for potential carcinogenicity* of 3,5-dinitroaniline (see Appendix C).

Table 6. Cancer WOE Descriptor for 3,5-Dinitroaniline (CASRN 618-87-1)

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 due to the lack of adequate data in humans or animals to evaluate the carcinogenic potential of 3,5-dinitroaniline; however, a screening evaluation described in Appendix B indicates a level of *concern for potential carcinogenicity* of 3,5-dinitroaniline.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	No evidence of noncarcinogenicity is available.

NA = not applicable; NS = not selected; WOE = weight of evidence.

3.5. DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

The absence of suitable data precludes developing cancer risk estimates for 3,5-dinitroaniline (see Table 7).

Table 7. Summary of Cancer Risk Estimates for 3,5-Dinitroaniline (CASRN 618-87-1)

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

NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

APPENDIX A. SCREENING NONCANCER PROVISIONAL REFERENCE VALUES

Due to the lack of evidence described in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, it is inappropriate to derive provisional toxicity values for 3,5-dinitroaniline. However, some 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

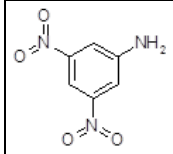
The analogue approach allows for the use of data from related compounds to calculate screening values when data for the compound of interest are limited or unavailable. Details regarding searches and specific methods for the tiered analogue analysis applied herein are presented in Wang et al. (2012). 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. All the available information is considered together as part of the final weight-of-evidence (WOE) approach to select the most suitable analogue both toxicologically and chemically.

An initial analogue search focused on identifying structurally related chemicals with toxicity values available in the Integrated Risk Information System (IRIS), PPRTV, 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. A total of 14 structurally related chemicals with oral and/or inhalation toxicity values are available as potential analogues for 3,5-dinitroaniline; structures of these chemicals are shown in Table A-1.

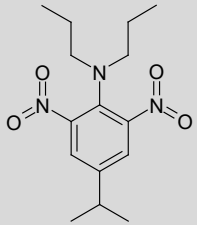
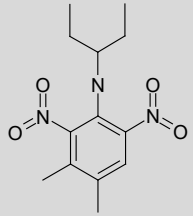
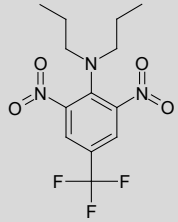
In selecting potential candidate analogues for this compound, chemicals of the following classes were initially considered: nitroanilines, dinitroaniline herbicides, trinitrobenzenes, dinitrobenzenes, trinitrotoluenes, dinitrotoluenes, and benzenediamines. Chemicals in these particular classes have two or three nitro or amino substituents on a benzene ring, and no other substituents apart from a methyl group. The nitro and amino substituents are likely important to the putative toxicological mode of action (MOA) for 3,5-dinitroaniline because many aromatic compounds with these substituents primarily induce methemoglobinemia and its sequelae of hematologic and splenic effects (Bingham and McGowan, 2012). Structure, reactivity, metabolism, and toxicity data for chemicals in these classes were examined to determine whether the list of candidate analogues could be further narrowed. Based on the available data and expert judgement, benzenediamines, dinitrotoluenes, and compounds with nitrogen-containing substituents in the *ortho* position to each other were omitted from the list of candidate analogues (see shaded structures in Table A-1), as described below.

Table A-1. Structures of Chemicals with Toxicity Values Initially Considered for Candidate Analogues[a]

3,5-Dinitroaniline (target compound; 618-87-1)



Dinitroaniline herbicides (nitro and alkylated amino groups)

2,6-Dinitro-*N*,*N*-dipropyl-4-isopropyl aniline (isopropalin; 33820-53-0)	3,4-Dimethyl-2,6-dinitro-*N*-(1-ethylpropyl)aniline (pendimethalin; 40487-42-1)	2,6-Dinitro-*N*,*N*-dipropyl-4-trifluoroaniline (trifluralin; 1582-09-8)
		
• Alkyl substituent on amine affects uptake and distribution relative to the target compound, which is an unsubstituted amine	• Alkyl substituent on amine affects uptake and distribution relative to the target compound, which is an unsubstituted amine • Metabolism data indicate cyclization reaction (to form benzimidazoles groups) which is not possible for target compound	• Alkyl substituent on amine affects uptake and distribution relative to the target compound, which is an unsubstituted amine • Metabolism data indicate cyclization reaction (to form benzimidazoles groups), which is not possible for target compound

Nitroanilines (nitro and amino groups)

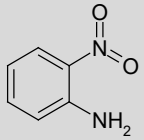
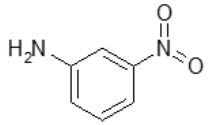
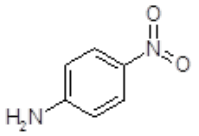
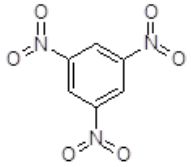
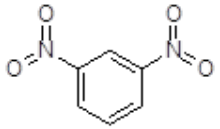
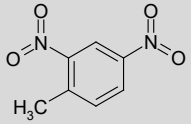
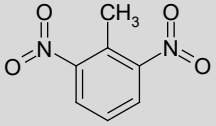
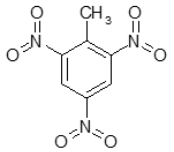
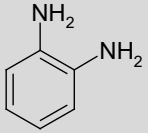
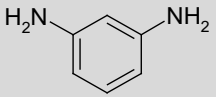
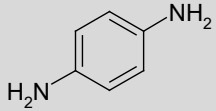
2-Nitroaniline (88-74-4)	3-Nitroaniline (99-09-2)	4-Nitroaniline (100-01-6)
		
• *Ortho* amino substituent causes steric metabolic hindrance		

Table A-1. Structures of Chemicals with Toxicity Values Initially Considered for Candidate Analogues[a]

Nitrobenzenes (nitro groups only)		
1,3,5-Trinitrobenzene (99-35-4)	1,3-Dinitrobenzene (99-65-0)	
		
Nitrotoluenes (nitro and methyl groups)		
2,4-Dinitrotoluene (121-14-2)	2,6-Dinitrotoluene (606-20-2)	2,4,6-Trinitrotoluene (118-96-7)
 • Primary metabolic pathway forms carboxylic acid (methyl group oxidation) • Not possible for target compound	 • Primary metabolic pathway forms carboxylic acid (methyl group oxidation) • Not possible for target compound	 • Retained because methyl group oxidation is NOT a primary metabolic pathway
Benzenediamines (amino groups only)		
1,2-Phenylenediamine (95-54-5)	1,3-Benzenediamine (108-45-2)	1,4-Benzenediamine (106-50-3)
 • Lacks nitro substituent (lower electron withdrawing potential) • Available metabolism data indicate formation of *N*-acetylated derivatives in liver/urine, which may contribute to different critical effects than those predicted for target compound	 • Lacks nitro substituent (lower electron withdrawing potential) • Available metabolism data indicate formation of *N*-acetylated derivatives in liver/urine, which may contribute to different critical effects (e.g. liver) than those predicted for target compound	 • Lacks nitro substituent (lower electron withdrawing potential) • Available metabolism data indicate formation of *N*-acetylated derivatives in liver/urine, which may contribute to different critical effects than those predicted for target compound

[a]Shading shows chemical classes omitted from consideration (see text for discussion).

Among dinitroanilines, toxicity values are available for the dinitroaniline herbicides isopropalin, pendimethalin, and trifluralin. However, these compounds include alkyl substituents on the amine that are expected to affect uptake and distribution (e.g., increased hydrophobicity) in the body relative to the unsubstituted amine of 3,5-dinitroaniline. In addition, metabolism data available for two of these compounds (pendimethalin and trifluralin) show cyclization reactions involving the alkyl groups that form benzimidazole groups (IARC, 1991; Zulalian, 1990); corresponding chemical reactions are not possible for 3,5-dinitroaniline, so the dinitroaniline herbicides were not considered further as candidate analogues.

Benzenediamines are the only chemical class among the candidate analogue classes that lack a nitro substituent. Because this class of chemicals lacks a nitro group, the benzenediamines exhibit lower electron-withdrawing potential than chemicals with nitro groups. Sabbioni and Jones (2002) noted that compounds with strong electron-withdrawing groups (including dinitrobenzenes, trinitrobenzenes, and trinitrotoluenes) can be reduced in erythrocytes, providing another site of bioactivation to the hydroxylamino intermediate that interacts with hemoglobin to produce methemoglobin. Available metabolism data on benzenediamines indicate that the primary metabolites in the liver and urine are *N*-acetylated derivatives [Nakao et al. (1980) as cited in ECHA (2015a); Lam and Bisgaard (1989) as cited in HSDB (2009)], and *N*-acetylation is a detoxification pathway for methemoglobin induction (Sabbioni and Jones, 2002). Consistent with this observation, liver effects represent the critical/most sensitive effect(s) used by IRIS to derive the chronic oral reference dose (RfD) for 1,3-benzenediamine (U.S. EPA, 2002b) rather than blood or splenic effects. For these reasons, benzenediamines were not considered further.

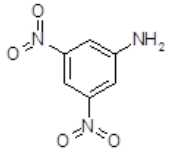
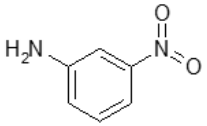
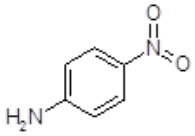
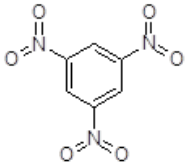
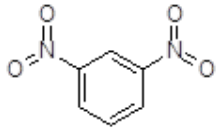
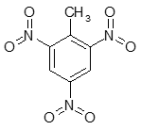
Dinitrotoluenes were omitted from consideration because the primary metabolic pathway for these compounds is methyl group oxidation (ATSDR, 2016) leading to the formation of a carboxylic acid, and this pathway is not possible for 3,5-dinitroaniline. Trinitrotoluenes were retained for consideration, however, as methyl group oxidation is not a primary metabolic pathway for these compounds (ATSDR, 1995b). Finally, data on the metabolism of aromatic nitro and amino compounds indicate that the position of the substituents on the ring affects metabolism to the hydroxylamino intermediates, with substituents in the *ortho* position to the nitro or amino group inhibiting bioactivation (Sabbioni and Jones, 2002). This is not likely due to electronic effects, but rather steric effects that decrease the rate of reaction of *ortho* substituents. The inhibition of bioactivation is borne out by available data on methemoglobinemia in rats treated orally with single doses of nitroanilines; 2-nitroaniline was inactive (as was the *ortho*-positioned 2,4-dinitroaniline, the only dinitroaniline tested), whereas 3- and 4-nitroaniline (non-*ortho* positioned) induced statistically significant increases in methemoglobin within 1 hour of dosing (SOCMA, 2000). Thus, candidate analogues with amino or nitro groups in the *ortho* position to another nitrogen-containing substituent (i.e., 2-nitroaniline) were not considered further, while those with nitrogen-containing substituents in the *meta* or *para* positions were included as candidate analogues.

Structural Analogues

Following the initial selection process outlined above, five structural analogues to 3,5-dinitroaniline with oral and/or inhalation noncancer reference values remained: 3-nitroaniline, 4-nitroaniline, 1,3,5-trinitrobenzene, 1,3-dinitrobenzene, and 2,4,6-trinitrotoluene (TNT). As described in Wang et al. (2012), structural similarity for analogues was evaluated using the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox (OECD, 2018) and the National Library of Medicine's (NLM's) ChemIDplus database (ChemIDplus, 2018). Table A-2 summarizes the

analogues' physicochemical properties and structural similarity scores. Physicochemical properties indicate that 3,5-dinitroaniline and the remaining candidate analogues are all water soluble and appear likely to be bioavailable via the oral route. Although the low vapor pressures and Henry's law constants of 3,5-dinitroaniline and the candidate analogues suggest limited potential for exposure via inhalation, the compounds can be inhaled if they are aerosolized or particle-bound, and once inhaled, absorption via the respiratory tract is likely. The ChemIDplus similarity scores for the remaining candidates exhibited a range between 56% for 3-nitroaniline and 86% for 1,3,5-trinitrobenzene. A ChemIDplus similarity score was not available for 4-nitroaniline. The OECD similarity scores ranged between 52.2% (4-nitroaniline) and 78.6% (1,3,5-trinitrobenzene). The similarity score predictions were similar across both tools. The identified candidate structural analogues all share similarities in functional groups (nitro and amino substituents), as well as similar physicochemical properties (detailed in Table A-2). In summary, any of these analogues may be considered an appropriate structural analogue for 3,5-dinitroaniline under the Wang et al. (2012) methodology.

Table A-2. Physicochemical Properties of 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues[a]

Type of Data	3,5-Dinitroaniline (target)	3-Nitroaniline	4-Nitroaniline	1,3,5-Trinitro-benzene	1,3-Dinitrobenzene	2,4,6-Trinitro-toluene
Structure						
CASRN	618-87-1	99-09-2	100-01-6	99-35-4	99-65-0	118-96-7
Molecular weight (g/mol)	183	138	138	213	168	227
ChemIDplus similarity score (%)[b]	100	56	NV	86	82	72
OECD similarity score (%)[c]	100	60.9	52.2	78.6	72	62.1
Melting point (°C)	162	113	148	122	90	80
Boiling point (°C)	398	306	297	315	296	240
Vapor pressure (mm Hg at 25°C)	1.39×10^{-5} (predicted average)	9.56×10^{-5}	3.2×10^{-6}	6.44×10^{-6}	9×10^{-4}	8.02×10^{-6}
Henry's law constant (atm-m^3/mole at 25°C)	4.34×10^{-8} (predicted average)	7.91×10^{-9}	1.26×10^{-9}	3.96×10^{-7} (estimated)	4.9×10^{-8}	3.92×10^{-7}
Water solubility (mg/L)	7.08×10^{-3}	7.95×10^{-3}	4.76×10^{-3}	1.29×10^{-3}	3.16×10^{-3}	5.67×10^{-4}
Octanol water partition coefficient (log K_{ow})	1.89	1.37	1.39	1.18	1.49	1.60
Acid dissociation constant (pKa) (for protonated compound)	0.3[d]	2.60	1.03	NV	NV	NV

[a]Data represent experimental average values as reported on the U.S. EPA's CompTox Chemicals Dashboard unless otherwise specified (CASRN 618-87-1; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=618-87-1#properties. Accessed July 15, 2020).
[b]ChemIDplus advanced similarity scores (ChemIDplus, 2018).
[c]OECD QSAR Toolbox (Version 4.1) Dice.
[d]U.S. EPA (2012).

NV = not available; OECD = Organisation for Economic Co-operation and Development.

Metabolic Analogues

No toxicokinetic information was located for 3,5-dinitroaniline. Although there were no quantitative data on the toxicokinetics of the candidate analogue compounds after inhalation exposure, there is qualitative evidence for uptake of 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, and TNT in humans believed to be occupationally exposed by inhalation (ATSDR, 1995a, b). Oral absorption, distribution, metabolism, and excretion information are available for all candidate analogues (see Table A-3) except 3-nitroaniline. As Table A-3 shows, 4-nitroaniline, 1,3-dinitrobenzene, and TNT are well absorbed after oral exposure (59 to >80% based on urinary excretion). For 1,3,5-trinitrobenzene, at least 24% was excreted in the urine after oral exposure; however, total recovery was not reported so it is uncertain whether the balance was retained or if overall recovery of radioactivity was low. Studies examining tissue distribution of the candidate analogues indicate little tissue accumulation and no preferential deposition in any particular tissue (U.S. EPA, 2009a, b, 1997; ATSDR, 1995a, b).

The aromatic nitro and amino compounds are well-studied chemical classes with a relatively well-defined MOA for noncancer toxicity [reviewed by Bingham and McGowan (2012); Sabbioni and Jones (2002)]. In general, these compounds induce methemoglobinemia via hydroxylamino intermediates formed during reduction of a nitro group and/or *N*-hydroxylation of an amino group (Bingham and McGowan, 2012). No studies identifying the primary metabolites of 3- or 4-nitroaniline were identified in the available literature. Available in vivo and in vitro data on metabolism of the other candidate analogues confirmed that the major pathways are nitroreduction, *N*-hydroxylation, ring-hydroxylation, *N*-acetylation, and sulfate or glucuronic acid conjugation of phenolic or *N*-hydroxylamine intermediates (U.S. EPA, 1997; ATSDR, 1995a, b). Nitroreduction may occur in the gut (via resident microbiota), liver, or erythrocytes (Sabbioni and Jones, 2002). *N*-Acetylation may occur in the liver or in the bladder, where the acidic pH subsequently promotes formation of nitrenium ions that form deoxyribonucleic acid (DNA) adducts (Sabbioni and Jones, 2002). Metabolism by other pathways occurs primarily in the liver [reviewed by Bingham and McGowan (2012); Sabbioni and Jones (2002)].

Identification of the hepatic and urinary metabolites produced by the candidate analogues with suitable data (see Table A-3) indicates that these compounds are generally metabolized as follows. 1,3,5-Trinitrobenzene undergoes sequential nitroreduction, yielding dinitroanilines (including 3,5-dinitroaniline, the target compound), diaminonitrobenzene, and triaminobenzene derivatives (U.S. EPA, 1997; ATSDR, 1995a). Similarly, 1,3-dinitrobenzene undergoes sequential nitroreduction followed by *N*-acetylation or ring hydroxylation; some of the resulting metabolites are subsequently conjugated with sulfate or glucuronic acid (HSDB, 2012; ATSDR, 1995a; Cossum and Rickert, 1985). Finally, TNT undergoes sequential nitroreduction (yielding amino dinitrotoluene or diamino nitrotoluene derivatives) as well as *N*- and ring-hydroxylation reactions (yielding hydroxylamino, dinitrotoluene, or aminodinitro cresol derivatives) (ATSDR, 1995b). Again, there were no experimental toxicokinetic data for 3-nitroaniline, and the metabolites for 4-nitroaniline were not fully identified. Taken together, the available metabolism data suggest the involvement of comparable pathways based on structural inference for the candidate analogues presented in Table A-3, making them plausible metabolic analogues for the target.

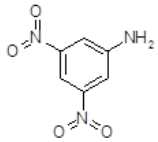
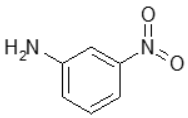
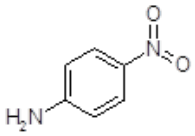
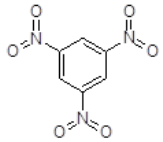
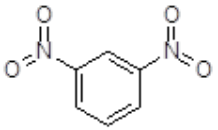
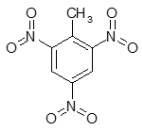
Table A-3. Comparison of Available ADME Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues					
3,5-Dinitroaniline (target)	**3-Nitroaniline**	**4-Nitroaniline**	**1,3,5-Trinitrobenzene**	**1,3-Dinitrobenzene**	**2,4,6-Trinitrotoluene**
					
Absorption after oral exposure					
ND	ND	Oral absorption in rats was ≥79% based on elimination via urine and expired air (see below)	Oral absorption in rats was ≥24−39% based on urine and expired air (see below); fecal elimination was low, and it is uncertain whether the balance of the dose was retained in the body or if overall recovery was low	Oral absorption in rabbits was ≥80% based on elimination via urine (see below)	Oral absorption was ≥59% in rats, mice, and dogs, based on radioactivity in urine (see below)
Distribution					
ND	ND	Rats exposed orally: Tissue radioactivity ranged from 0.1−0.36% of dose 72 h postdosing Rats exposed intravenously: Highest peak concentration in individual tissues (% dose/g tissue) as follows: Blood: 0.50 Urinary bladder: 3.33 Kidney: 1.79 Liver: 0.84 Lung: 0.73 Heart: 0.66 All tissue concentrations peaked 15 min postdosing	Rats exposed orally: Highest radioactivity in liver, kidney, skin, and lungs (0.02−0.03% of dose/g tissue 96 h postdosing)	ND	Rats exposed orally: Highest radioactivity in liver, skeletal muscle, blood, and fat (<0.1−5.4% of dose 24 h postdosing)

Table A-3. Comparison of Available ADME Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues					
3,5-Dinitroaniline (target)	**3-Nitroaniline**	**4-Nitroaniline**	**1,3,5-Trinitrobenzene**	**1,3-Dinitrobenzene**	**2,4,6-Trinitrotoluene**
Metabolites					
ND	ND	Rats exposed intravenously: Urinary: Nine unidentified metabolites; 56% of urinary radioactivity consisted of 2 sulfate conjugates and 3.5% was unmetabolized parent compound 7 of the 9 metabolites were detected in bile	Rats exposed orally: Urinary: 3,5-Dinitroaniline 1,3-Diamino-5-nitrobenzene 1,3,5-Triaminobenzene Fecal: 1,3-Diamino-5-nitrobenzene 1,3,5-Triaminobenzene Liver microsomes in vitro: 1,3-Diamino-5-nitrobenzene 3,5-Dinitroaniline	Rats exposed orally: Urinary: 3-Aminoacetanilide (22%) 4-Acetamido phenyl sulfate (6%) 1,4-Diacetamido benzene (7%) 3-Nitroaniline-*N*-glucuronide (4%) Hepatocytes and microsomes in vitro: 3-Nitroaniline Microsomal metabolism mediated by NADPH-CYP450 reductase. Rabbits exposed orally: Urinary: 3-Nitroaniline and 1,3-Benzenediamine (35%) 2,4-Diaminophenol (31%) 2-Amino-4-nitrophenol (14%) 4-Amino-2-nitrophenol (2%) 30% of the metabolites were conjugated with glucuronic acid and 6% with sulfate	Human: Urinary: 2-Amino-4,6-dinitro-toluene 4-Amino-2,6-dinitro-toluene 2,4-Diamino-6-nitro-toluene 4-Hydroxyl amino-2,6-dinitrotoluene 4-Amino-2,6-dinitro-*m*-cresol Similar metabolites identified in rat, mouse, rabbit, and dog urine

Table A-3. Comparison of Available ADME Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

3,5-Dinitroaniline (target)	3-Nitroaniline	4-Nitroaniline	1,3,5-Trinitrobenzene	1,3-Dinitrobenzene	2,4,6-Trinitrotoluene
Excretory pattern					
ND	ND	Rats exposed orally (% dose in 3 d): Urine: 75−96 Feces: 4−14 Expired air: 0.01−0.07 Biliary excretion in 4 h after intravenous dose: 19%	Rats exposed orally (% dose): Urine: 21−36 (in 4 d) Feces: 4 (in 4 d) Expired air: 3−5 (in 2 d) It is uncertain whether the balance of the dose was retained in the body or if overall recovery was low because the study was reported only in abstract form [Reddy and Gunnarson (1993) as cited in U.S. EPA (1997)]	Rabbits exposed orally (% dose in 2 d): Urine: 81 Feces: 0.3−5.2 Expired air: ND	Rats, mice, dogs, and rabbits exposed orally (% dose): Urine: 59−74 (in 24 h)
References					
NA	NA	Chopade and Matthews (1984) as cited in U.S. EPA (2009b)	ATSDR (1995a); U.S. EPA (1997); U.S. EPA (2009a)	ATSDR (1995a)	ATSDR (1995b)

ADME = absorption, distribution, metabolism, excretion; CYP450 = cytochrome P450; NA = not applicable; NADPH = reduced form of nicotinamide adenine dinucleotide phosphate; ND = no data.

Excretion of the candidate analogues following oral exposure (apart from 3-nitroaniline, for which there are no data) is primarily via the urine as tested in rats, mice, dogs, or rabbits in studies employing radioactive compounds; between 59 and 96% of an orally administered dose of 4-nitroaniline, 1,3-dinitrobenzene, or TNT is excreted in urine, and 21−36% of an oral dose of 1,3,5-trinitrobenzene is excreted in the urine (U.S. EPA, 2009b, 1997; ATSDR, 1995a, b). Small amounts of radioactivity are excreted in feces after exposure to 1,3,5-trinitrobenzene and 1,3-dinitrobenzene. Between 4 and 14% of an oral dose of 4-nitroaniline was excreted in feces of rats, and biliary excretion of this compound has been demonstrated (U.S. EPA, 2009b). There are no data on fecal or biliary excretion of TNT (ATSDR, 1995b).

In summary, although the most proximate metabolic analogue is 1,3,5-trinitrobenzene [because 3,5-dinitroaniline is a metabolite of 1,3,5-trinitrobenzene in rats exposed orally (U.S. EPA, 1997; ATSDR, 1995a)], metabolism of the three remaining candidate analogues occurs via pathways (including bioactivation to hydroxylamine intermediates) that are likely to be relevant to 3,5-dinitroaniline. There are no experimental toxicokinetic data for 3-nitroaniline. Thus, 4-nitroaniline, 1,3,5-trinitrobenzene, 1,3-dinitrobenzene, and 2,4,5-trinitrotoluene are plausible metabolic analogues.

Toxicity-Like Analogues

No toxicity data are available for 3,5-dinitroaniline apart from in vitro genotoxicity studies. Tables A-4, A-5, and A-6 summarize available oral subchronic toxicity values, oral chronic toxicity values, and inhalation subchronic and chronic toxicity values (respectively) for the compounds identified as candidate analogues.

As discussed earlier, methemoglobin induction is a consistently observed effect of aromatic nitro and amino compounds in laboratory animal studies (Bingham and McGowan, 2012). This effect occurs when nitro compounds undergo nitroreduction and/or when *N*-hydroxylamines are oxidized to nitroarenes in the blood, leading to oxidation of the ferrous ion in hemoglobin which prevents the hemoglobin from combining reversibly with oxygen (Bingham and McGowan, 2012; Sabbioni and Jones, 2002). Adverse sequelae of methemoglobinemia include hematologic effects such as decreased red blood cell (RBC) count and hemoglobin, leading to compensatory hematopoiesis and, as hemoglobin is degraded, hemosiderin deposition in the liver and/or spleen. Common comorbidities include increased splenic weight and extramedullary hematopoiesis.

Animals exposed to each of the candidate analogues exhibited signs of methemoglobin induction following both oral and inhalation exposure. Of note, only a single analogue (4-nitroaniline) has a published inhalation toxicity value (see Table A-6). Additionally, as shown in Tables A-4 through A-6, either methemoglobinemia or its related effects were the critical endpoints in the rat studies used to derive subchronic and chronic oral and inhalation toxicity values for all of the candidate analogues other than TNT. For TNT, hepatic effects (pathology) in dogs were the critical endpoint for deriving the chronic RfD (0.5 mg/kg-day); however, at higher doses, increased methemoglobin (8 or 32 mg/kg-day) and hemosiderin deposition in the liver (2 mg/kg-day) were observed. Related hematologic effects were observed in other species, including rats and mice following TNT exposure (U.S. EPA, 2002a). The differences in critical effect may be related to differences in species or metabolism of TNT. While the critical effects observed with 4-nitroaniline inhalation exposure were consistent with those observed orally and the putative mode of action for this class of chemicals, it does provide greater uncertainty because of the lack of inhalation data for other analogues. Thus, the available data show clear

commonalities in the toxic effects for all five analogues, providing support for the inference that 3,5-dinitroaniline would behave in a similar manner.

Table A-4. Comparison of Available Subchronic Oral Toxicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

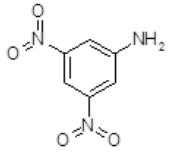
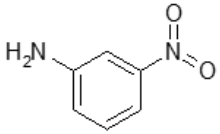
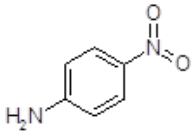
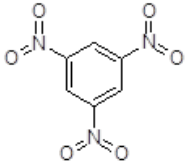
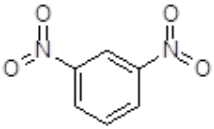
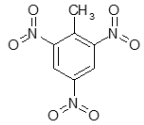
Type of Data	3,5-Dinitroaniline CASRN 618-87-1 (target)	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	1,3,5-Trinitrobenzene CASRN 99-35-4	1,3-Dinitrobenzene CASRN 99-65-0	2,4,6-Trinitrotoluene CASRN 118-96-7
Structure						
POD (mg/kg-d)	NV	15	0.95	NV	NV	NV
POD type	NV	LOAEL	$BMDL_{1SD}$	NV	NV	NV
UF_C	NV	10,000 (UF_A, UF_D, UF_H, UF_L, UF_S)	100 (UF_A, UF_H)	NV	NV	NV
RfD (mg/kg-d)	NV	1×10^{-3} (screening due to UF_C >3,000)	1×10^{-2}	NV	NV	NV
Critical effects	NV	Decreased RBCs and hemoglobin; histopathology of spleen (hemosiderin deposition, extramedullary hematopoiesis, congestion) and bone marrow (erythroid hyperplasia)	Increased methemoglobin	NV	NV	NV

Table A-4. Comparison of Available Subchronic Oral Toxicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

Type of Data	3,5-Dinitroaniline CASRN 618-87-1 (target)	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	1,3,5-Trinitrobenzene CASRN 99-35-4	1,3-Dinitrobenzene CASRN 99-65-0	2,4,6-Trinitrotoluene CASRN 118-96-7
Other effects	NV	Methemoglobinemia; increased absolute and relative spleen, liver, and kidney weights; decreased absolute and relative testes weight; hepatocyte swelling and hepatic hemosiderin deposition and extramedullary hematopoiesis; renal lipofuscin deposition; reduced spermatogenesis, multinucleated giant cell formation in the testes, and absence of spermatozoa in the epididymis	Decreased RBCs, hematocrit, hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin; splenic congestion, hemosiderosis, and extramedullary hematopoiesis	NV	NV	NV
Species	NV	Rat	Rat	NV	NV	NV
Duration	NV	28 d	90 d	NV	NV	NV
Route (method)	NV	Oral (gavage)	Oral (gavage)	NV	NV	NV
Dosing levels (critical study)	NV	0, 15, 50, 170 mg/kg-d	0, 3, 10, 30 mg/kg-d	NV	NV	NV

Table A-4. Comparison of Available Subchronic Oral Toxicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

Type of Data	3,5-Dinitroaniline CASRN 618-87-1 (target)	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	1,3,5-Trinitrobenzene CASRN 99-35-4	1,3-Dinitrobenzene CASRN 99-65-0	2,4,6-Trinitrotoluene CASRN 118-96-7
Additional toxicity data from other studies	NV	Hepatomegaly, enlarged and dark spleens, and difficult labor with litter losses in reproductive/ developmental toxicity study	Increased absolute and relative spleen weights and hemosiderosis of hepatic Kupffer cells in mice exposed subchronically In developmental studies: mortality and body-weight loss in rabbit does; decreased rat fetal weight; malformations of the tail, digits, and kidneys of rat pups	Decreased body-weight gain; increased methemoglobin and reticulocytes, decreased RBCs and hemoglobin; increased relative liver, spleen, and brain weights; decreased testes weight; spleen and bone marrow erythroid cell hyperplasia; seminiferous tubule degeneration; renal hyaline droplets, tubular degeneration, and mineralized foci (mice exposed subchronically) (U.S. EPA, 1997)	NV	NV
Source	NV	Onodera (date unknown) as cited in U.S. EPA (2009a)	Monsanto Co. (1981) as cited in U.S. EPA (2009b)	U.S. EPA (1997)	NV	NV

BMDL = benchmark dose lower confidence limit; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NV = not available; POD = point of departure; RBC = red blood cell; RfD = oral reference dose; SD = standard deviation; 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.

Table A-5. Comparison of Available Chronic Oral Toxicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

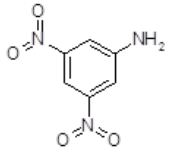
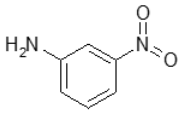
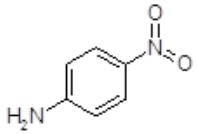
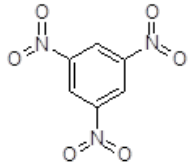
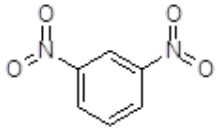
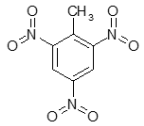
Type of Data	3,5-Dinitroaniline CASRN 618-87-1	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	1,3,5-Trinitrobenzene CASRN 99-35-4	1,3-Dinitrobenzene CASRN 99-65-0	2,4,6-Trinitrotoluene CASRN 118-96-7
Structure						
POD (mg/kg-d)	NV	NV	0.37	2.68	0.40	0.5
POD type	NV	NV	$BMDL_{1SD}$	NOAEL	NOAEL	LOAEL
UF_C	NV	NV	100 (UF_A, UF_H)	100 (UF_A, UF_H)	3,000 (UF_A, UF_D, UF_H, UF_S)	1,000 (UF_A, UF_H, UF_L, UF_S)
RfD (mg/kg-d)	NV	NV	4×10^{-3}	3×10^{-2}	1×10^{-4}	5×10^{-4}
Critical effects	NV	NV	Increased methemoglobin	Methemoglobinemia and spleen erythroid cell hyperplasia	Increased spleen weight (absolute or relative not reported)	Hepatocyte swelling (trace to mild severity)
Other effects	NV	NV	Increased absolute and relative spleen weight; hemosiderosis in liver and spleen	Decreased body weight; decreased hemoglobin and RBCs; seminiferous tubule degeneration; increased relative brain, spleen, liver, and/or kidney weights	Decreased body-weight gain in females, decreased hemoglobin and testicular atrophy in males, and hemosiderin deposits in spleen of both sexes	Increased methemoglobin; increased absolute and relative liver weight, cirrhosis, and hemosiderosis of the liver at higher doses
Species	NV	NV	Rat	Rat	Rat	Dog
Duration	NV	NV	2 yr	2 yr	16 wk	25 wk
Route (method)	NV	NV	Oral (gavage)	Oral (diet)	Oral (drinking water)	Oral (gelatin capsule)
Dosing levels (critical study)	NV	NV	0, 0.25, 1.5, 9.0 mg/kg-d	M: 0, 0.23, 2.68, 13.31 mg/kg-d[a] F: 0, 0.22, 2.64, 13.44 mg/kg-d)[b]	0, 0.4, 1.1, 2.7 mg/kg-d[b]	0, 0.5, 2, 8, 32 mg/kg-d

Table A-5. Comparison of Available Chronic Oral Toxicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

Type of Data	3,5-Dinitroaniline CASRN 618-87-1	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	1,3,5-Trinitrobenzene CASRN 99-35-4	1,3-Dinitrobenzene CASRN 99-65-0	2,4,6-Trinitrotoluene CASRN 118-96-7
Additional toxicity data from other studies	NV	NV	Increased sulfhemoglobin, reduced hematocrit and RBCs, increased reticulocytes, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration; increased absolute and relative spleen and liver weights; bone marrow hypercellularity, hemosiderosis of hepatic Kupffer cells, splenic congestion, and splenic extramedullary hematopoiesis; hemangiomas, and hemangiosarcomas in mice exposed chronically In developmental studies: mortality and body-weight loss in rabbit does; decreased rat fetal weight; malformations of the tail, digits, and kidneys of rat pups	Reduced numbers of motile sperm and splenic hemosiderosis in rat reproductive toxicity study; decreased maternal body weight, reduced fetal weight and crown-rump length, and increased incidence of skeletal variation in rat developmental toxicity study	Mortality, decreased spermatogenesis, reduced testicular weight in 8-wk rat study; ataxia, paresis equilibrium loss, muscle rigidity, absence of sperm in testis and epididymis cauda; decreased epididymis weight; and infertility in 12-wk rat study (U.S. EPA, 2005b; ATSDR, 1995a)	Anemia and hepatomegaly in mice; testicular degeneration and splenic effects in rats; urinary bladder papilloma and carcinoma in female rats; reported toxic effects in humans include cataracts, aplastic anemia, hepatitis, hepatomegaly, and liver cancer (U.S. EPA, 2002a; ATSDR, 1995b)

Table A-5. Comparison of Available Chronic Oral Toxicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

Type of Data	3,5-Dinitroaniline CASRN 618-87-1	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	1,3,5-Trinitrobenzene CASRN 99-35-4	1,3-Dinitrobenzene CASRN 99-65-0	2,4,6-Trinitrotoluene CASRN 118-96-7
Source	NV	NV	Nair et al. (1990) as cited in U.S. EPA (2009b)	Reddy et al. (1996) as cited in U.S. EPA (1997)	Cody et al. (1981) as cited in U.S. EPA (2005b)	U.S. DOD (1983) as cited in U.S. EPA (2002a); Levine et al. (1990) as cited in ATSDR (1995b)

[a]Study authors report doses as 0, 5, 60, and 300 ppm diet; these were converted to dosages as reported by Reddy et al. (1996) as cited in U.S. EPA (1997).
[b]Study authors report doses as 0, 3, 8, and 20 ppm drinking water. Drinking water concentrations were converted to dosages by investigators in Cody et al. (1981) as cited in U.S. EPA (2005b).

$BMDL_{1SD}$ = benchmark dose lower confidence limit, one standard deviation; F = female(s); LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NV = not available; POD = point of departure; RBC = red blood cell; RfD = reference dose; 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.

Table A-6. Comparison of Available Subchronic and Chronic Inhalation Toxicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

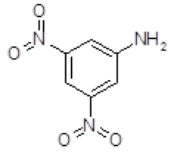
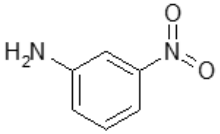
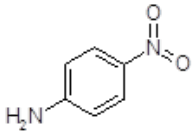
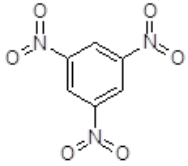
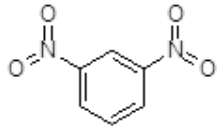
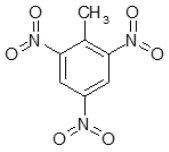
Type of Data	3,5-Dinitroaniline CASRN 618-87-1	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	1,3,5-Trinitro-benzene CASRN 99-35-4	1,3-Dinitrobenzene CASRN 99-65-0	2,4,6-Trinitro-toluene CASRN 118-96-7
Structure						
POD (mg/m^3)	NV	NV	1.7	NV	NV	NV
POD type	NV	NV	$BMCL_{1SD}$ (HEC)	NV	NV	NV
Subchronic UF_C	NV	NV	100 (UF_A, UF_D, UF_H)	NV	NV	NV
Subchronic RfC (mg/m^3)	NV	NV	2×10^{-2}	NV	NV	NV
Chronic UF_C	NV	NV	300 (UF_A, UF_D, UF_H, UF_S)	NV	NV	NV
Chronic RfC (mg/m^3)	NV	NV	6×10^{-3}	NV	NV	NV
Critical effects	NV	NV	Increased methemoglobin	NV	NV	NV
Other effects	NV	NV	Polychromasia and anisocytosis of RBCs; increased WBCs; increased absolute and relative spleen weights; hemosiderosis and extramedullary hematopoiesis of the spleen	NV	NV	NV
Species	NV	NV	Rat	NV	NV	NV
Duration	NV	NV	4 wk	NV	NV	NV
Route (method)	NV	NV	Inhalation (aerosol)	NV	NV	NV
Dosing levels (critical study)	NV	NV	0, 10, 32, 80 mg/m^3	NV	NV	NV

Table A-6. Comparison of Available Subchronic and Chronic Inhalation Toxicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

Type of Data	3,5-Dinitroaniline CASRN 618-87-1	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	1,3,5-Trinitro-benzene CASRN 99-35-4	1,3-Dinitrobenzene CASRN 99-65-0	2,4,6-Trinitro-toluene CASRN 118-96-7
Additional toxicity data from other studies	NV	NV	Decreased body weight; hematologic effects consistent with hemolytic anemia, methemoglobinemia, and compensatory hematopoiesis; splenic congestion; lymphoid cell atrophy of the spleen and thymus (2-wk study in rats)	NV	NV	NV
Source	NV	NV	Nair et al. (1986) as cited in U.S. EPA (2009b)	NV	NV	NV

BMCL = benchmark concentration lower confidence limit; HEC = human equivalent concentration; NV = not available; POD = point of departure; RBC = red blood cell; RfC = inhalation reference concentration; SD = standard deviation; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor; WBC = white blood cell.

Weight-of-Evidence Approach

A WOE approach is used to evaluate information from candidate analogues as described by Wang et al. (2012). Commonalities in structural/physicochemical properties, toxicokinetics, metabolism, toxicity, or MOA between candidate analogues and chemical(s) of concern are identified. Emphasis is given to toxicological and/or toxicokinetic similarity over structural similarity. Analogues are excluded if they do not have commonality or demonstrate significantly different physicochemical properties and toxicokinetic profiles that set them apart from the pool of analogues and/or chemical(s) of concern. From the remaining analogues, the most appropriate analogue (most biologically or toxicologically relevant analogue chemical) with the highest structural similarity and/or most conservative toxicity value is selected.

The available data provide concordance across structural, metabolism, and toxicity lines of evidence. The functional groups that are shared by 3,5-dinitroaniline and the analogues (nitro and amino substituents) have been associated with primary target organ toxicities, including methemoglobinemia and related toxicities for this group of chemicals via metabolic bioactivation. The available data suggest commonalities in the toxicokinetics and toxicity of the five candidate analogues. Among those with toxicokinetic data, all are absorbed after oral exposure and primarily excreted in the urine. The aromatic nitro compounds are a well-studied chemical class for which the proximal toxicant for methemoglobinemia is believed to be the hydroxylamine intermediates. Available in vivo and in vitro data on metabolism of the candidate analogues confirms that the major pathways are nitroreduction, *N*-hydroxylation and ring-hydroxylation, *N*-acetylation, and sulfate or glucuronic acid conjugation of phenolic or *N*-hydroxylamine intermediates. Although 1,3,5-trinitrobenzene undergoes sequential nitroreduction to yield 3,5-dinitroaniline, the efficiency of this pathway and the potential involvement of other pathways is unknown. In addition, toxicity data on the candidate analogues confirm methemoglobinemia as the most sensitive critical effect for 4-nitroaniline and 1,3,5-trinitrobenzene. Increased spleen weight, a comorbidity of methemoglobinemia, was the critical effect for 1,3-dinitrobenzene. 3-Nitroaniline had a critical effect of decreased red blood cells and hemoglobin, as well as spleen histopathology changes (hemosiderin deposition) and bone marrow pathology changes (erythroid hyperplasia), and, at higher doses, methemoglobinemia, increased spleen weight, liver pathology (hepatocyte swelling, hepatic hemosiderin disposition, and extramedullary hematopoiesis), and other effects. TNT exhibited hematologic effects (e.g., increased methemoglobin) at higher doses than the liver effects used as the basis for the chronic RfD. Although the specific critical effect was distinct among analogues, the constellation of toxic effects, including splenic and hepatic effects as well as effects on hematology (methemoglobinemia, red blood cells), is consistent, and there are conserved pathways that contribute to these effects. The observed differences are likely due to differences in exposure routes, species, and experimental designs that were used across principal studies. Thus, the available data show clear commonalities in the structure, metabolic pathways, and the toxicological effects for the candidate analogues, providing support for the inference that 3,5-dinitroaniline could be metabolized by the same pathways and exhibit similar toxicity.

For 3,5-dinitroaniline, no metabolism or toxicity data are available, precluding the use of biological and toxicokinetic data comparing this chemical with the candidate analogues as a means of choosing the most appropriate analogue. Structural similarity scores do not provide a meaningful or objective way to differentiate between analogues and thus were not used to select analogues. Thus, in addition to the overall WOE, availability of toxicity values, duration of key studies, and sensitivity of critical effects was also taken into consideration when choosing the most appropriate analogue, as described below.

Subchronic oral provisional reference doses (p-RfDs) derived by U.S. EPA are available for 3-nitroaniline and 4-nitroaniline (U.S. EPA, 2009a, b); formal subchronic oral toxicity values were not available for any of the other candidate analogues. However, the PODs from the chronic oral toxicity values developed for 1,3-dinitrobenzene [Reddy et al. (1996) as cited in U.S. EPA (1997)] and 2,4,6-trinitrotoluene [U.S. DOD (1983) as cited in U.S. EPA (2002a); Levine et al. (1990) as cited in ATSDR (1995b)] were also considered for development of a screening subchronic p-RfD because they were based on subchronic study designs for the principal study. 4-Nitroaniline is selected as the appropriate analogue for deriving a screening subchronic p-RfD for 3,5-dinitroaniline based on the following factors:

1) Although the POD (0.40 mg/kg-day, based on increased spleen weight) used by IRIS to derive a chronic RfD for 1,3-dinitrobenzene comes from a subchronic study [Cody et al. (1981) as cited in U.S. EPA (2005b)], methemoglobin changes were not evaluated in the Cody et al. (1981) [as cited in U.S. EPA (2005b)] study. As discussed above, increased methemoglobin was chosen as the critical effect for 4-nitroanline and 1,3,5-trinitrobenzene, and 2,4,6-trinitrotoluene and 3-nitroaniline were both observed to increase methemoglobin in dogs and rats, respectively.
2) On initial review, 1,3-dinitrobenzene has the lowest POD (0.4 mg/kg-day, used by IRIS to derive a chronic RfD) based on increased spleen weight from a NOAEL in a 16-week rat study that exposed animals via drinking water [Cody et al. (1981) as cited in U.S. EPA (2005b)]. Similar in magnitude is the POD used by IRIS to derive a chronic RfD for 2,4,6-trinitrotoluene (0.5 mg/kg-day) based on hepatocyte swelling from a LOAEL in a 25-week dog study that exposed animals via gelatin capsules [U.S. DOD (1983) as cited in U.S. EPA (2002a); Levine et al. (1990) as cited in ATSDR (1995b)]. The differences in species and routes of exposure make it challenging to do a direct comparison with other analogues and may contribute to the differences in observed critical effects. Methemoglobin changes were not evaluated for 1,3-dinitrobenzene in Cody et al. (1981) [as cited in U.S. EPA (2005b)], but were evaluated and observed at higher doses for TNT [U.S. DOD (1983) as cited in U.S. EPA (2002a); Levine et al. (1990) as cited in ATSDR (1995b)].
3) The POD used to derive the subchronic p-RfD for 4-nitroaniline (0.95 mg/kg-day, based on increased methemoglobin) was lower than the POD used to derive the 3-nitroaniline subchronic p-RfD (15 mg/kg-day, based on decreased RBC counts and hemoglobin).
4) The principal study upon which the subchronic p-RfD for 4-nitroaniline is based was of a longer duration (90 days) than the study upon which the subchronic p-RfD for 3-nitroaniline was based (28 days). Although the duration of the 1,3-dinitrobenzene study was 16 weeks and for 2,4,5-trinitrotoluene 25 weeks, there are also chronic toxicity data on methemoglobin effects (from a 2-year oral study) available for multiple time points following 4-nitroaniline exposure. The larger evidence base for a longer time frame, in addition to having inhalation toxicology data for the same analogue, strengthens the confidence in the body of available toxicology effects for 4-nitroaniline. The overall database for methemoglobinemia following 4-nitroaniline exposure is more robust, containing both subchronic and chronic (2-year bioassay) oral exposure data as well as inhalation data.

Chronic p-RfDs derived by U.S. EPA are available for 4-nitroaniline (U.S. EPA, 2009b), 1,3,5-trinitrobenzene (U.S. EPA, 1997), 1,3-dinitrobenzene (U.S. EPA, 2005b), and TNT (U.S. EPA, 2002a). Similar to the screening subchronic p-RfD, 4-nitroaniline is selected as the

appropriate analogue for deriving a screening chronic p-RfD for 3,5-dinitroaniline based on the following factors:

1) The POD for the 4-nitroaniline chronic p-RfD (0.37 mg/kg-day) was significantly lower (>sevenfold) than the POD for 1,3,5-trinitrobenzene (2.68 mg/kg-day), and also slightly lower than the PODs used to derive the other screening chronic p-RfDs (0.40 and 0.5 mg/kg-day for 1,3-dinitrobenzene and TNT, respectively), making the 4-nitroaniline value the most health conservative value.
2) Unlike the other candidate analogues, hepatic effects (hepatocyte swelling) in dogs were the most sensitive endpoints following oral TNT exposure, and increased methemoglobin levels and hemosiderin deposition in the liver were observed at higher doses (8 and 32 mg/kg-day), which adds uncertainty given that increased methemoglobin was chosen as the critical effect for 4-nitroanline and 1,3,5-trinitrobenzene. Because the TNT principal study used a different species (dogs) and exposure route (gelatin capsules), this may partially explain the differences in sensitive endpoints.
3) The principal study upon which the chronic p-RfD for 4-nitroaniline is based was a chronic 2-year rat study, while studies used as the basis for the chronic p-RfDs for 1,3-dinitrobenzene and TNT were subchronic in duration (16-week rat study and 25-week dog study, respectively).

Subchronic and chronic p-RfCs derived by U.S. EPA are available only for 4-nitroaniline; inhalation toxicity values were not available for any of the other candidate analogues. As stated above, 4-nitroaniline is an appropriate structural and metabolic analogue for 3,5-dinitroaniline. As with the toxic effects observed following oral exposure, inhalation exposure to 4-nitroaniline also results in increased methemoglobin levels in rats. Thus, based on the WOE approach and availability of p-RfCs, 4-nitroaniline is selected as the model analogue for deriving screening subchronic and chronic p-RfCs for 3,5-dinitroaniline.

ORAL NONCANCER REFERENCE VALUES

Derivation of a Screening Subchronic Provisional Reference Dose

Based on the overall WOE approach presented in this PPRTV assessment, 4-nitroaniline is selected as the analogue for 3,5-dinitroaniline for deriving a screening subchronic p-RfD. The principal study used to derive the subchronic p-RfD for 4-nitroaniline was a 90-day rat study [Monsanto (1981) and Houser et al. (1983) as cited in U.S. EPA (2009b)]. The PPRTV assessment (U.S. EPA, 2009b) described the study as follows:

> *Monsanto Co. (1981b) and Houser et al. (1983) reported a 90-day gavage study in which groups of 20 male and 20 female Sprague-Dawley rats were administered daily doses of 0, 3, 10, or 30 mg/kg-day of 4-nitroaniline (purity 99.85%) in corn oil. Animals were observed daily for mortality and clinical signs of toxicity. Body weights and food consumption were determined weekly. After 45 and again after 90 days of treatment, blood and urine samples were collected from 10 rats/sex/group for hematology, clinical chemistry, and urinalysis. At the end of the treatment period, all surviving animals were sacrificed and necropsied; selected organs were weighed, and histopathological examination was performed on comprehensive tissues.*

No treatment-related mortalities occurred, with only one mortality in the control group (female) during the course of the study (Monsanto Co., 1981b; Houser et al., 1983). Body weight and food consumption were comparable to controls in all 4-nitroaniline treatment groups. Ear paleness (indicative of anemia) was observed in males treated with 30 mg/kg-day during treatment Week 2 (2/20 rats) and Week 4 (20/20) and in females treated with 30 mg/kg-day during treatment Weeks 2 (2/20 rats), Week 4 (20/20), and Week 6 (20/20). Ear paleness was not observed in any rats on other weeks during the treatment period. No other significant clinical signs of toxicity were observed. Clinical chemistry parameters in treatment groups were comparable to controls. Treatment-related effects on hematology parameters and histopathological findings were consistent with the effects of increased blood concentrations of methemoglobin; specifically, accelerated red blood cell (RBC) destruction (hemolytic anemia), and compensatory erythropoiesis to maintain erythrocyte mass. Methemoglobin concentration and reticulocyte count were significantly increased in all 4-nitroaniline treatment groups after 90 days of treatment (see Table 2). Other significant hematology findings in both sexes included decreased erythrocyte count, Hct, and blood hemoglobin concentration in males and females treated with ≥10 mg/kg-day, and decreased mean cell hemoglobin (MCH) and mean cell volume (MCV) in the 30 mg/kg-day group. Comprehensive histopathologic examination of the controls and 30 mg/kg-day rats identified the spleen as the only organ with treatment-related lesions; therefore, the spleens of all rats were examined microscopically. Dose-related increases in splenic congestion, hemosiderosis, and extramedullary hematopoiesis were observed in all treated groups (see Table 3). The LOAEL for 90-day oral exposure has been identified as a daily average dose of 3 mg/kg-day for the development of methemoglobinemia and associated hematological and splenic changes; a NOAEL is not established.

The critical effect in this study was increased methemoglobin in female rats. A $BMDL_{1SD}$ of 0.95 mg/kg-day was derived from benchmark dose (BMD) modeling of the methemoglobin data in female rats and used as the POD for 4-nitroaniline (U.S. EPA, 2009b). This value is selected as the POD to derive the screening subchronic p-RfD for 3,5-dinitroaniline. The POD was not adjusted for molecular weight differences in the derivation of the 3,5-dinitroaniline provisional toxicity value because the molecular weight difference between the two compounds is less than twofold (Wang et al., 2012).

The $BMDL_{1SD}$ of 0.95 mg/kg-day is converted to a human equivalent dose (HED) according to current U.S. EPA (2011c) guidance. In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c), the Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches may include using some chemical-specific information, without a complete physiologically based toxicokinetic model. In the absence of chemical-specific models or data to inform the derivation of human equivalent oral exposures, U.S. EPA endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving an RfD is recommended when the observed effects are associated

with the parent compound or a stable metabolite but not for portal-of-entry effects or developmental endpoints.

A validated human physiologically based pharmacokinetic model for 4-nitroaniline is not available for use in extrapolating doses from animals to humans (U.S. EPA, 2009b). The selected POD is based on increased methemoglobin, which is not a portal-of-entry or developmental effect. Therefore, scaling by $BW^{3/4}$ is relevant for deriving HEDs for this effect.

Following U.S. EPA (2011c) guidance, the POD for increased methemoglobin in female rats 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 0.204 kg for female Sprague Dawley rats and a reference BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.23. Applying this DAF to the $BMDL_{1SD}$ of 0.95 mg/kg-day yields a POD (HED) as follows:

$$\begin{aligned} \text{POD (HED)} &= BMDL_{1SD} \text{ (mg/kg-day)} \times DAF \\ &= 0.95 \text{ mg/kg-day} \times 0.23 \\ &= 0.22 \text{ mg/kg-day} \end{aligned}$$

The U.S. EPA (2009b) subchronic p-RfD for 4-nitroaniline was derived using a composite uncertainty factor (UF_C) of 100, reflecting 10-fold uncertainty factors for both interspecies extrapolation and intraspecies variability (interspecies uncertainty factor [UF_A] and intraspecies uncertainty factor [UF_H]). An uncertainty factor for database uncertainties (UF_D) was not applied due to the availability of well-designed subchronic and chronic studies in two species, as well as developmental toxicity studies in two species and a multigeneration reproductive toxicity study (U.S. EPA, 2009b). Wang et al. (2012) indicated that the uncertainty factors typically applied in deriving a toxicity value for the chemical of concern are the same as those applied to the analogue unless additional information is available. In deriving the screening subchronic p-RfD for 3,5-dinitroaniline, a UF_A of 3 is applied to account for uncertainty associated with extrapolating from animals to humans when cross-species dosimetric adjustment (HED calculation) is performed. In addition, a UF_D of 10 is used for database uncertainties to account for the absence of any toxicity information for 3,5-dinitroaniline. A UF_H of 10 is applied to account for human-to-human variability. Thus, the screening subchronic p-RfD for 3,5-dinitroaniline was derived using a UF_C of 300 reflecting a UF_A of 3, UF_H of 10, and UF_D of 10.

$$\begin{aligned} \textbf{Screening Subchronic p-RfD} &= \text{Analogue POD (HED)} \div UF_C \\ &= 0.22 \text{ mg/kg-day} \div 300 \\ &= \mathbf{7 \times 10^{-4} \text{ mg/kg-day}} \end{aligned}$$

Table A-7 summarizes the uncertainty factors for the screening subchronic p-RfD for 3,5-dinitroaniline.

Table A-7. Uncertainty Factors for the Screening Subchronic p-RfD for 3,5-Dinitroaniline (CASRN 618-87-1)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following 3,5-dinitroanline exposure. The toxicokinetic uncertainty has been accounted for by calculating an HED through application of a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c). Dosimetric adjustment calculations were performed on the POD for the selected analogue, 4-nitroaniline.
UF_D	10	A UF_D of 10 is applied to account for the absence of toxicity data for 3,5-dinitroaniline.
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 3,5-dinitroaniline in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the analogue POD is a $BMDL_{1SD}$.
UF_S	1	A UF_S of 1 is applied because a subchronic study was selected as the principal study for the subchronic assessment.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

$BMDL_{1SD}$ = benchmark dose lower confidence limit, one standard deviation; DAF = dosimetric adjustment factor; 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; UF = uncertainty factor(s); 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 WOE approach presented in this PPRTV assessment, 4-nitroaniline is selected as the analogue for 3,5-dinitroaniline for deriving a screening chronic p-RfD. The principal study used to derive the chronic p-RfD for 4-nitroaniline was a 2-year rat study [Nair et al. (1990) as cited in U.S. EPA (2009b)]. U.S. EPA (2009b) described the study as follows:

> *The effects of chronic oral exposure to 4-nitroaniline have been investigated in a 2-year gavage study in rats (Nair et al., 1990). Nair et al. (1990) treated groups of 60 male and 60 female Sprague-Dawley rats by daily gavage with 4-nitroaniline (purity 99.9%) in corn oil at doses of 0, 0.25, 1.5, or 9.0 mg/kg daily for 2 years. Rats were observed for mortality and clinical signs of toxicity twice daily, and were given detailed physical examinations weekly. Ophthalmoscopic examinations were conducted on all rats prior to treatment, and after 3, 12, and 24 months of treatment. Body weights and food consumption were recorded weekly for the first 14 weeks and biweekly thereafter. Hematology (MetHgb, Hgb, Hct, RBC count, reticulocyte count, WBC count with differential), serum chemistry (complete list not reported, but included serum sodium and potassium), and urinalysis (gross appearance, specific pH, protein, glucose, ketones, bilirubin, occult blood, and urobilinogen and microscopic examination of sediment) were evaluated after 6, 10, 12, 18, and 24 months of treatment in randomly selected animals (10/sex/group); blood methemoglobin levels were*

evaluated at 6, 10, 12, 18, and 24 months. Complete necropsies were conducted on all animals. Organ weights of adrenals, brain, ovaries, testes, kidneys, liver, heart, and spleen were recorded for rats surviving at 2 years. Tissue masses, gross lesions, and tissue samples (35 tissues) were examined microscopically in all control and high-dose animals. In addition, all gross lesions and tissue masses, as well as the spleen and liver, were examined microscopically in low- and mid-dose animals.

Treatment resulted in slightly increased mortality in males treated with 9.0 mg/kg-day (44 deaths), relative to control (37 deaths) (Nair et al., 1990). Although the increase was not statistically significant by pairwise comparison, Life Table analysis showed a statistically significant positive trend for the males. Weekly mean body weights for 4-nitroaniline-treated males were similar to controls throughout the study. For females, weekly mean body weights were similar to controls for the 0.25 and 1.50 mg/kg-day groups, but tended to be higher than control values in the 9.0 mg/kg-day group, with differences reaching statistical significance at various times throughout the study (data not reported). Increased food intake occurred sporadically throughout the study in rats of both sexes treated with 1.5 or 9.0 mg/kg-day (data not reported). There were no treatment-related effects on clinical observations, ophthalmoscopic examinations, clinical chemistry, or urinalysis. Significant changes in hematological parameters attributed to 4-nitroaniline after 12 and 24 months of exposure are summarized in Table 11 (data from other time points not reported). Methemoglobin levels were increased in the 1.5 and 9.0 mg/kg-day groups at both time points in a dose-related manner in both sexes. In the high-dose groups, the increases in methemoglobin were large (6−8-fold over control levels) and methemoglobin levels exceeded 2%. Small decreases in hemoglobin and red blood cell count were also seen in the high-dose groups.

In male rats, administration of 4-nitroaniline produced a dose-related increase in absolute and relative spleen weights in the 1.5 and 9.0 g/kg-day groups and increased relative liver weights in the 9.0 mg/kg-day group (see Table 12). Treatment did not affect absolute or relative organ weights in female rats. Microscopic examination revealed increased accumulations of brown pigment (probably hemosiderin) in the Kupffer cells (sinusoidal macrophages) of the liver and reticuloendothelial cells of the spleen of treated rats (see Table 12). Statistical analysis of data was not performed by the study authors. Fisher's exact tests performed for this review showed that the increases were statistically significant in the liver in the high-dose groups of both sexes and in the 1.5 mg/kg-day group in males. The incidence of hemosiderosis in the spleen was significantly increased in males of the 1.5 and 9.0 mg/kg-day groups. Due to the high incidence of splenic hemosiderosis in control females, there was no increase in overall incidence with treatment. However, the severity of splenic hemosiderosis increased with dose in both sexes. The Jonckheere-Terpstra test performed for this review showed that the increase in severity was statistically significant at ≥0.25 mg/kg-day in the female rats. The same pattern was seen in the male rats, although the increase in severity in males was not statistically significant at doses lower than 9.0 mg/kg-day. Based on increased methemoglobin in both male and female rats, and increases in spleen weights and hemosiderosis

in the liver and spleen in male rats, the NOAEL and LOAEL in this study were 0.25 mg/kg-day and 1.5 mg/kg-day, respectively.

The critical effect for this study was methemoglobinemia in male rats (U.S. EPA, 1997). U.S. EPA (2009b) used a $BMDL_{1SD}$ of 0.37 mg/kg-day, obtained by modeling the methemoglobinemia data in male rats, as the POD. As with the derivation of the screening subchronic p-RfD, the POD was not adjusted for molecular weight differences between 3,5-dinitroaniline and the analogue because the difference is less than twofold (Wang et al., 2012).

The $BMDL_{1SD}$ of 0.37 mg/kg-day is converted to an HED using a DAF by using a reference BW_a of 0.523 kg for male Sprague Dawley rats under chronic study conditions and a reference BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.29 using the same methods described earlier when deriving the screening subchronic p-RfD. Applying this DAF to the 10% benchmark dose lower confidence limit ($BMDL_{10}$) of 0.37 mg/kg-day yields a POD (HED) as follows:

$$\begin{aligned} \text{POD (HED)} &= BMDL_{1SD}\ (\text{mg/kg-day}) \times \text{DAF} \\ &= 0.37\ \text{mg/kg-day} \times 0.29 \\ &= 0.11\ \text{mg/kg-day} \end{aligned}$$

The U.S. EPA (2009b) chronic p-RfD for 4-nitroaniline was derived using a UF_C of 100, reflecting 10-fold uncertainty factors for both UF_A and UF_H variability. An uncertainty factor for database uncertainties (UF_D) is not applied because of the availability of well-designed subchronic and chronic studies in two species, as well as developmental studies in two species and a multigeneration reproduction study (U.S. EPA, 2009b). In deriving the screening chronic p-RfD for 3,5-dinitroaniline, a UF_A of 3 is used to account for uncertainty associated with extrapolating from animals to humans when cross-species dosimetric adjustment (HED calculation) is performed. A UF_H of 10 is applied to account for human-to-human variability. In addition, a UF_D of 10 is used for database uncertainties to account for the absence of any toxicity information for 3,5-dinitroaniline. Thus, the screening chronic p-RfD for 3,5-dinitroaniline is derived using a UF_C of 300 reflecting a UF_A of 3, UF_H of 10, and UF_D of 10.

$$\begin{aligned} \textbf{Screening Chronic p-RfD} &= \text{Analogue POD (HED)} \div UF_C \\ &= 0.11\text{mg/kg-day} \div 300 \\ &= \mathbf{4 \times 10^{-4}\ mg/kg\text{-}day} \end{aligned}$$

Table A-8 summarizes the uncertainty factors for the screening chronic p-RfD for 3,5-dinitroaniline.

Table A-8. Uncertainty Factors for the Screening Chronic p-RfD for 3,5-Dinitroaniline (CASRN 618-87-1)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following 3,5-dinitroanline exposure. The toxicokinetic uncertainty has been accounted for by calculating an HED through application of a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c). Dosimetric adjustment calculations were performed on the POD for the selected analogue, 4-nitroaniline.
UF_D	10	A UF_D of 10 is applied to account for the absence of toxicity data for 3,5-dinitroaniline.
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 3,5-dinitroaniline in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the analogue POD is a $BMDL_{1SD}$.
UF_S	1	A UF_S of 1 is applied because a chronic study was selected as the principal study for the chronic assessment.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

$BMDL_{1SD}$ = benchmark dose lower confidence limit, one standard deviation; DAF = dosimetric adjustment factor; 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; UF = uncertainty factor(s); 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 REFERENCE VALUES

Derivation of a Screening Subchronic Provisional Reference Concentration

Based on the overall WOE approach presented in this PPRTV assessment, 4-nitroaniline is selected as the analogue for 3,5-dinitroaniline to derive a screening subchronic p-RfC. Subchronic and chronic p-RfCs derived by U.S. EPA are available only for 4-nitroaniline; inhalation toxicity values were not available for any of the other candidate analogues. Note that there are uncertainties in the selection of 4-nitroaniline as the analogue given the lack of other analogues with published inhalation toxicity values. Additional uncertainty is introduced by the lack of toxicokinetic data to inform about route-specific toxicokinetic differences between the target and analogue chemical. The principal study used for the U.S. EPA (2009b) subchronic p-RfC for 4-nitroaniline was a 4-week rat study [Nair et al. (1986) as cited in U.S. EPA (2009b)]. Like oral exposure, inhalation exposure to 4-nitroaniline also results in increased methemoglobin in rats. U.S. EPA (2009b) described the study as follows:

> *The effects of inhalation exposure of rats to 4-nitroaniline for 4 weeks was studied by Nair et al., (1986). Groups of 10 male (204−243 g) and 10 female (204−243 g) Sprague-Dawley rats were exposed (whole body exposure) to an aerosol of 4-nitroaniline 6 hours/day, 5 days/week, for 4 weeks. 4-Nitroaniline was dissolved in isopropyl alcohol and the solution fed into a spray atomizer. Mean measured exposure concentrations for 4-nitroaniline were 0 (1500 ppm solvent only), 10, 32, and 80 mg/m^3. Particle size mass median aerodynamic diameters and geometric standard deviations (MMAD ± GSD) were 0.80 ± 5.42,*

1.37 ± 4.04 and 0.78 ± 6.42 µm for the 10, 32, and 80 mg/m^3 exposures, respectively. Endpoints monitored throughout the study include mortality, clinical signs, and body weights. A comprehensive ophthalmoscopic examination was performed on all rats before the study began and prior to termination of the study. Blood was drawn from all animals before sacrifice for hematologic and clinical chemistry determinations. At the end of the study, all rats underwent gross necropsy and the major organs were weighed. Microscopic examinations of all major organs and tissues (including nasal turbinates, trachea, and lungs) of all control and high-exposure rats, and of spleens of all rats, were performed.

No mortality or compound-related clinical signs of toxicity were observed during the study, and body weights were not different from controls (data not reported) (Nair et al., 1986). Results from the ophthalmoscopic examinations showed no treatment-related changes. Hematologic changes attributed to exposure to 4-nitroaniline were: a concentration-related increase in blood methemoglobin (MetHb) levels in male and female rats that was statistically significant at ≥32 mg/m^3; an increased incidence of morphological changes in the red blood cells (polychromasia in both sexes and anisocytosis in females) at ≥32 mg/m^3 (incidence data and statistical significance not reported); and significantly increased WBC counts in males at 80 mg/m^3 (see Table 13). Data on RBC counts were not reported. These changes in hematological parameters are consistent with 4-nitroaniline-induced methemoglobinemia and compensatory hematopoiesis. No treatment-related clinical chemistry findings or gross pathological changes were observed. Increased relative and absolute spleen weights were observed in males and females in all 4-nitroaniline groups (see Table 14). Hemosiderosis and extramedullary hematopoiesis in the spleen were observed in all groups with comparable frequency; however, the severity of the changes was concentration-related (see Table 14). Livers of the high-exposure females had a qualitatively higher degree of extramedullary hematopoiesis relative to the controls (data not reported). No compound-related histopathological changes were observed in other tissues. A LOAEL of 10 mg/m^3 was identified for increased spleen weights and severity of splenic hemosiderosis and extramedullary hematopoiesis in males and females. The corresponding human equivalent concentration (HEC) is 4.2 mg/m^3 for the systemic toxicity. A NOAEL was not identified.

The critical effect for this study was methemoglobinemia in male rats (U.S. EPA, 1997). U.S. EPA (2009b) used a $BMCL_{1SD}$ (HEC) of 1.7 mg/m^3, obtained by modeling the methemoglobinemia data in male rats, as the POD. As with the derivation of the oral toxicity values, the POD was not adjusted for molecular weight differences between 3,5-dinitroaniline and the analogue because the difference is less than twofold (Wang et al., 2012). A UF_C of 100, reflecting a 10-fold factor for UF_H and 3-fold factors for both UF_A and UF_D, was applied to the POD to obtain the subchronic p-RfC for 4-nitroaniline (U.S. EPA, 2009b). In deriving the screening subchronic p-RfC for 3,5-dinitroaniline, a full 10-fold UF_D is used to account for the absence of any toxicity information for 3,5-dinitroaniline. Thus, the screening subchronic p-RfC for 3,5-dinitroaniline is derived using a UF_C of 300 reflecting a UF_A of 3, UF_H of 10, and UF_D of 10.

Screening Subchronic p-RfC = Analogue POD (HEC) ÷ UF_C
= 1.7 mg/m^3 ÷ 300
= **6×10^{-3} mg/m^3**

Table A-9 summarizes the uncertainty factors for the screening subchronic p-RfC for 3,5-dinitroaniline.

Table A-9. Uncertainty Factors for the Screening Subchronic p-RfC for 3,5-Dinitroaniline (CASRN 618-87-1)

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 (HEC calculation) is performed.
UF_D	10	A UF_D of 10 is applied to account for the absence of toxicity data for 3,5-dinitroaniline.
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 3,5-dinitroaniline in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the analogue POD is a $BMCL_{1SD}$.
UF_S	1	A UF_S of 1 for exposure duration is applied. As noted by U.S. EPA (2009b) in describing the application of a UF_S for the 4-nitroaniline analogue compound, "based on results of subchronic oral toxicity studies, maximum blood methemoglobin levels appear to be reached within 2−7 wk of exposure to 4-nitroaniline. These levels decline and reach a plateau within 3 mo. It is unlikely that the duration-related plateau varies with route of exposure."
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

$BMCL_{1SD}$ = benchmark concentration lower confidence limit, one standard deviation; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfC = provisional reference concentration; UF = uncertainty factor(s); 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 Concentration

Based on the overall analogue approach presented in this PPRTV assessment, 4-nitroaniline is selected as the analogue for 3,5-dinitroaniline for deriving a screening chronic p-RfC. To derive the chronic p-RfC for 4-nitroaniline, U.S. EPA (2009b) used the same study [Nair et al. (1986) as cited in U.S. EPA (2009b)] and POD ($BMCL_{1SD}$ [HEC] of 1.7 mg/m^3) as was used to derive the screening subchronic p-RfC. A UF_C of 300, reflecting a 10-fold factor for UF_H and 3-fold factors for UF_A, UF_D, and UF_S, was applied to the POD to obtain the chronic p-RfC for 4-nitroaniline. Although a 4-week study would not typically be used as the basis for a chronic p-RfC, U.S. EPA (2009b) argued that methemoglobin levels are not significantly affected by exposure duration. The use of a threefold UF_S in deriving the chronic value from the 4-week study was to account for potential effects of exposure duration on other health outcomes. As stated in U.S. EPA (2009b), methemoglobin blood levels plateau after several months of exposure, and there is evidence in the nitroarene literature that methemoglobinemia and the constellation of sequelae, including splenic and hepatic effects, do not seem to significantly increase in incidence and/or severity with chronic exposures. In summary, when looking across the health effects in the previous examples, 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 applied to cover any remaining uncertainty.

In deriving the screening chronic p-RfC for 3,5-dinitroaniline, a full 10-fold UF_D is used to account for the absence of any toxicity information for 3,5-dinitroaniline. Thus, the screening chronic p-RfC for 3,5-dinitroaniline is derived using a UF_C of 1,000 reflecting a UF_A of 3, UF_H of 10, UF_D of 10, and UF_S of 3.

Screening Chronic p-RfC = Analogue POD (HEC) ÷ UF_C
= 1.7 mg/m^3 ÷ 1,000
= **2×10^{-3} mg/m^3**

Table A-10 summarizes the uncertainty factors for the screening chronic p-RfC for 3,5-dinitroaniline.

Table A-10. Uncertainty Factors for the Screening Chronic p-RfC for 3,5-Dinitroaniline (CASRN 618-87-1)

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 (HEC calculation) is performed.
UF_D	10	A UF_D of 10 is applied to account for the absence of toxicity data for 3,5-dinitroaniline.
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 3,5-dinitroaniline in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a $BMCL_{1SD}$.
UF_S	3	A UF_S of 3 ($10^{0.5}$) is applied for a less than chronic exposure duration. As noted by U.S. EPA (2009b) in describing the application of a UF_S for the 4-nitroaniline analogue compound, "based on results of subchronic oral toxicity studies, maximum blood methemoglobin levels appear to be reached within 2−7 wk of exposure. These values then decline and reach a plateau within 3 mo. It is unlikely that the duration-related plateau varies with route of exposure. However, due to lack of chronic inhalation data, it is not known if lifetime inhalation exposure to 4-nitroaniline produces adverse effects in other organs, such as the respiratory tract."
UF_C	1,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

$BMCL_{1SD}$ = benchmark concentration lower confidence limit, one standard deviation; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfC = provisional reference concentration; UF = uncertainty factor(s); 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.

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 3,5-dinitroaniline. 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.

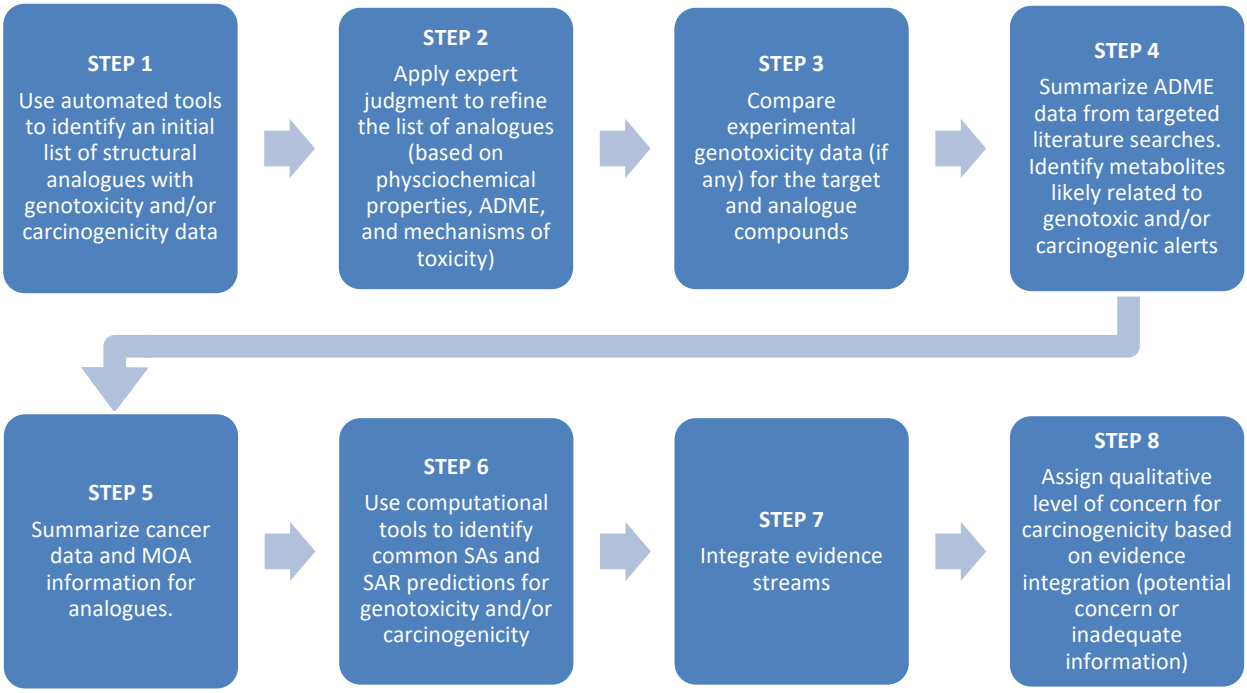


Figure B-1. Steps Used in the Screening Evaluation of Potential Carcinogenicity

STEP 1. USE OF AUTOMATED TOOLS TO IDENTIFY STRUCTURAL ANALOGUES WITH GENOTOXICITY AND/OR CARCINOGENICITY 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 cluster 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 detecting structural analogues to fill data gaps and/or perform read-across analysis.

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.

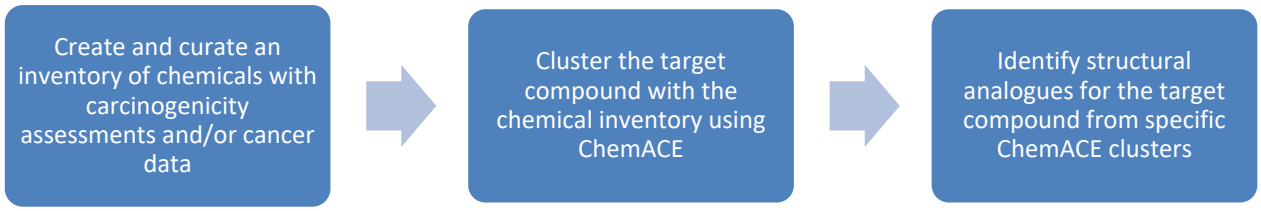


Figure B-2. Overview of ChemACE Clustering Process

The chemical inventory was populated with chemicals 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 (CalEPA) 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, 2020b)

In total, 2,123 distinct substances were identified from the sources above. For the purpose of ChemACE clustering, 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 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 CompTox 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, resulted in 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)

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 exceeded 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 genotoxicity or carcinogenicity (see Table B-1).

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

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

Database Name	Toolbox Database Description[a]
CPDB	The CPDB provides access to cancer 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 cancer 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 74 chemicals and 9,186 data points.
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.
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.

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

Database Name	Toolbox Database Description[a]
ISSMIC	The ISSMIC database provides data on the results of in vivo MN mutagenicity assays 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; ISSCTA = Istituto Superiore di Sanità Cell Transformation Assay; ISSMIC = Istituto Superiore di Sanità Micronucleus; ISSSTY = Istituto Superiore di Sanità *Salmonella typhimurium*; 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, Authorization 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 physicochemical 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 3,5-DINITROANILINE 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).

Table B-2. Tools Used to Identify Structural Alerts and Predictions for Genotoxicity and Carcinogenicity

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 is 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.

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 approved the submitted data. A list of the ToxAlerts found for the chemicals screened in the preliminary batch is below:
	• Genotoxic carcinogenicity, mutagenicity:
	  ○ Aliphatic halide (general)
	  ○ Aliphatic halide (specific)
	  ○ Aliphatic halogens
	  ○ Aromatic amine (general)
	  ○ Aromatic amine (specific)
	  ○ Aromatic amines
	  ○ Aromatic and aliphatic substituted primary alkyl halides
	  ○ Aromatic nitro (general)
	  ○ Aromatic nitro (specific)
	  ○ Aromatic nitro groups
	  ○ Nitroarenes
	  ○ Nitro-aromatic
	  ○ Primary and secondary aromatic amines
	  ○ Primary aromatic amine, hydroxyl amine, and its derived esters or amine-generating group
	• Nongenotoxic carcinogenicity
	  ○ Aliphatic halogens

Table B-2. Tools Used to Identify Structural Alerts and Predictions for Genotoxicity and Carcinogenicity

Name	Description[a]
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 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 Structural Alerts and Predictions for Genotoxicity and Carcinogenicity

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 two 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 OECD QSAR Toolbox, but the prediction is available only through OncoLogic, and alerts or decision trees were used or adapted in several models (e.g., Benigni and Bossa alerts and Toxtree decision tree).

ANTARES = Alternative Non-Testing 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); CRS4 = Center for Advanced Studies, Research and Development in Sardinia; 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.

The tool results for the target and analogue compounds are provided in Appendix C.

STEP 7. WEIGHT-OF-EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF 3,5-DINITROANILINE CARCINOGENICITY

Data identified across multiple lines of evidence from Steps 1−6 (outlined above) are integrated to determine the qualitative level of *concern for 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 GENOTOXICITY AND/OR CARCINOGENICITY DATA

U.S. EPA's Chemical Assessment Clustering Engine (ChemACE) clustering was performed as described in Appendix B. The cluster containing 3,5-dinitroaniline (less restrictive approach; Cluster 85) contains four structural analogues. All members of the cluster contain a benzene ring fragment substituted with one or more amine groups (−NH_2) and one or more nitro (−NO_2) groups; the location and number of the substituents vary. For example, the structure of the target compound shown in Figure C-1 contains one benzene ring, one amine group, and two nitro groups in the 3 and 5 (*meta*) positions.

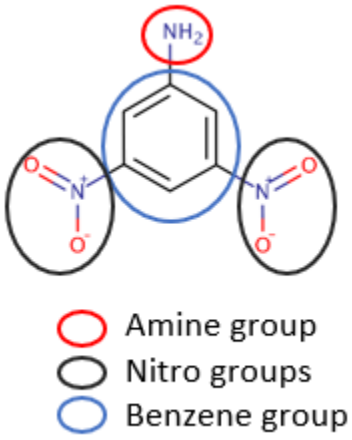


Figure C-1. Illustration of Common Fragments in Cluster 85

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 genotoxicity and/or carcinogenicity data (see Step 1 methods in Appendix B). This process identified an additional 58 compounds to be considered as potential analogues for 3,5-dinitroaniline. Two target compounds (2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene) were also identified by this search; however, these compounds are being evaluated in separate provisional toxicity value documents and were not considered potential analogues for 3,5-dinitroaniline. 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 62 potential analogues based on physicochemical properties; absorption, distribution, metabolism, and excretion (ADME); and mechanisms of toxicity.

Compounds were considered potential analogues if they had a benzene or toluene ring substituted with two or three nitro groups, amines, or hydroxylamines. As discussed in

Appendix A during selection of noncancer analogues, both *meta*- and *para*-substituted compounds were considered as potential analogues based on metabolic and toxicological considerations (the bioreactivity of *ortho*-substituted compounds is expected to be lower due to steric reasons).

Of the 62 chemicals identified as potential analogues by ChemACE clustering and the OECD Toolbox analogue selection tool (Dice), 54 were not selected for further review. Common rationales for not selecting these chemicals included substitution in the *ortho* position, ring systems other than benzene or toluene, and occurrence of functional groups absent in 3,5-dinitroaniline (e.g., phenols, halogens, carboxylic acids). Each of these attributes introduce significant differences in bioavailability, reactivity, and applicable metabolic pathways relative to 3,5-dinitroaniline. Dinitrotoluene compounds were also excluded because the primary metabolic pathway for these compounds is methyl group oxidation leading to the formation of a carboxylic acid (ATSDR, 2016), and this pathway is not possible for 3,5-dinitroaniline (see Appendix A for further details). Additionally, methyldinitrobenzene (CASRN 25321-14-6) was not selected for further review because the CASRN/name does not specify the placement of the nitro groups on the ring. It cannot be ruled out that the nitro groups are *ortho* to one another, or that this substance is a mixture that contains the *ortho* isomer.

The remaining eight possible analogues for 3,5-dinitroaniline are listed in Table C-1. The existence of a cancer risk estimate and/or a WOE determination for cancer is indicated for each analogue.

Table C-1. Summary of Cancer Assessment Information for Analogues of 3,5-Dinitroaniline (CASRN 618-87-1)[a]

Analogue Name (CASRN)	Cancer Risk Estimates (if available)	WOE Determinations
1,3-Dinitrobenzene[b] (99-65-0)	None	U.S. EPA (2005b)—*not classifiable*
1,3,5-Trinitrobenzene[b] (99-35-4)	None	None
1,4-Dinitrobenzene[b] (100-25-4)	None	U.S. EPA (2006)—*inadequate information*
3-Nitroaniline[b, c] (99-09-2)	None	U.S. EPA (2009a)—*inadequate information*
4-Nitroaniline[c] (100-01-6)	U.S. EPA (2009b)—p-OSF	U.S. EPA (2009b)—*suggestive evidence*
2,4,6-Trinitrotolulene[b] (118-96-7)	U.S. EPA (2002a)—OSF	U.S. EPA (2002a)—*possible* IARC (1996)—*not classifiable* CalEPA (2017)—*known*
2,4-Dihydroxyamino-6-nitrotoluene[b] (185376-54-9)	None	None
2-Hydroxylamino-4,6-dintrotoluene[b] (59283-76-0)	None	None

[a]Gray shading indicates that there was not a cancer risk estimate and/or a WOE determination for cancer for that analogue.
[b]Found in Dice.
[c]Found in ChemACE.

OSF = oral slope factor; p-OSF = provisional oral slope factor; WOE = weight of evidence.

Three compounds that lack a cancer risk estimate or WOE determination (highlighted in gray in Table C-1) were not further considered as a potential analogue for the screening evaluation of potential carcinogenicity of 3,5-dinitroaniline. Compounds selected for further consideration were 1,3-dinitrobenezene, 1,4-dinitrobenzene, 3-nitroaniline, 4-nitroaniline, and 2,4,6-trinitrotoluene (TNT).

STEP 3. COMPARISON OF THE EXPERIMENTAL GENOTOXICITY DATA FOR 3,5-DINITROANILINE AND ANALOGUES

The very limited genotoxicity data available for 3,5-dinitroaniline are described in the "Other Data" section in the main body of this report (summarized in Table 4). Available data indicate that 3,5-dinitroaniline is mutagenic to bacteria in vitro with and without metabolic activation. A summary of the available genotoxicity data for potential analogues is provided below. In general, data indicate that these compounds are mutagenic in bacterial systems and there is evidence of clastogenicity for some analogues.

1,3-Dinitrobenzene, 3-nitroaniline, 4-nitroaniline, and TNT were mutagenic in bacterial systems in the presence and absence of metabolic activation (U.S. EPA, 2009a, b; Bolt et al., 2006; U.S. EPA, 2006, 2005b; ATSDR, 1995a, b). Mutagenicity was generally increased with metabolic activation, and mutations were not observed in nitroreductase-deficient strains,

indicating that metabolites are the primary mutagens. Weak and inconsistent evidence of mutagenicity in bacteria was observed for 1,4-dintrobenzene (U.S. EPA, 2006).

Limited mammalian cell mutagenicity data for TNT produced inconsistent findings (i.e., positive in the mouse lymphoma assay without metabolic activation; negative in V79 Chinese hamster cells, with and without metabolic activation) (Bolt et al., 2006; ATSDR, 1995b). 4-Nitroaniline did not cause forward gene mutations in Chinese hamster ovary cells (U.S. EPA, 2009b). No data on mammalian cell mutagenicity are available for 1,3-dinitrobenzene, 1,4-dinitrobenzene, or 3-nitroaniline. Sex-linked recessive lethal mutations were not observed in *Drosophila melanogaster* larvae exposed to 4-nitroaniline (U.S. EPA, 2009b); no studies in *D. melanogaster* were identified for other analogues.

1,3-Dinitrobenene, 1,4-dinitrobenzene, and 4-nitroaniline induced chromosomal aberrations (CAs) in human peripheral lymphocytes exposed in vitro (U.S. EPA, 2009b, 2006). In hamster cells, CAs were induced by 3-nitroaniline and CAs and sister chromatid exchanges were induced by 4-nitroaniline (U.S. EPA, 2009a, b). In in vivo studies, micronuclei (MN) frequency was increased in mice exposed to 3-nitroaniline, but not in mice exposed to 4-nitroaniline or rats exposed to TNT (U.S. EPA, 2009a, b; Bolt et al., 2006; ATSDR, 1995b). TNT also did not induce CAs in in vivo studies in rats (Bolt et al., 2006; ATSDR, 1995b).

1,3-Dinitrobenzene induced deoxyribonucleic acid (DNA) damage in male rat germ cells exposed in vitro (Xu et al., 2006). Unscheduled DNA synthesis (UDS) was not observed in vitro in human fibroblasts exposed to TNT or rat liver cells exposed to 1,3-dinitrobenene, 1,4-dinitrobenzene, 3-nitroaniline, or 4-nitroaniline (U.S. EPA, 2009a, b, 2006, 2002a; ATSDR, 1995a, b). UDS was also not observed in mouse liver cells following in vivo exposure to TNT (ATSDR, 1995b). Covalent ribonucleic acid (RNA) binding was observed in human granulocytes exposed to 4-nitroaniline; however, binding to DNA was at the limit of detection (U.S. EPA, 2009b).

STEP 4. TOXICOKINETICS OF 3,5-DINITROANILINE AND ANALOGUES

The toxicokinetics of 3,5-dinitroaniline and potential analogues are briefly described in Table C-2. There are no data available regarding the toxicokinetics of 3,5-dinitroaniline or 3-nitroaniline. 1,3-Dinitrobenzene, 1,4-dinitrobenzene, 4-nitroaniline, and TNT are all well absorbed via the oral route, have low potential for accumulation in the body, and are primarily excreted in the urine (U.S. EPA, 2009b, 2006; ATSDR, 1995a, b). Experimental data for 1,3-dintrobenzene, 1,4-dinitrobenzene, and TNT indicate that metabolism occurs via common pathways, including sequential nitroreduction followed by *N*-acetylation or ring hydroxylation (U.S. EPA, 2006; ATSDR, 1995a, b; Cossum and Rickert, 1985). *N*-Acetylation may occur in the liver or in the bladder, where the acidic pH subsequently promotes formation of nitrenium ions that form DNA adducts (Sabbioni and Jones, 2002). No data on the primary metabolic pathway for 4-nitroaniline were available; however, analysis of unidentified urinary metabolites indicated that 56% consisted of two sulfate conjugates (U.S. EPA, 2009b).

Table C-2. Summary of Toxicokinetic Data for 3,5-Dinitroaniline and Candidate Analogues

Compound	Absorption, Distribution, Excretion	Metabolism	References
3,5-Dinitroaniline (target)	ND	ND	NA
1,3-Dinitrobenzene	• Well absorbed via oral route • Low potential for accumulation, ND on deposition • Primarily excreted in urine	• Primary pathway: sequential nitroreduction followed by *N*-acetylation or ring hydroxylation, forming hydroxylamine intermediates • Some of the resulting metabolites are subsequently conjugated with sulfate or glucuronic acid • Primary urinary metabolites in rats: 3-aminoacetanilide (22%), 4-acetamidophenyl sulfate (6%), 1,3-diacetamidobenzene (7%), and 3-nitroaniline-*N*-glucuronide	U.S. EPA (2006); ATSDR (1995a); Cossum and Rickert (1985)
1,4-Dintrobenzene	• Well absorbed via oral route • Low potential for accumulation, ND on deposition • Primarily excreted in urine	• Primary pathway: sequential nitroreduction followed by *N*-acetylation or ring hydroxylation • Some of the resulting metabolites are subsequently conjugated with sulfate • Primary urinary metabolites in rats: 2-amino-5-nitrophenyl sulfate (35%), S-(4-nitrophenyl)-*N*-acetylcysteine (13%), and 1,4-diacetamindobenzene (7%) • Secondary pathway: glutathione conjugation	U.S. EPA (2006)
3-Nitroaniline	ND	ND	U.S. EPA (2009b)
4-Nitroaniline	• Well absorbed via oral route • Low potential for accumulation, no preferential deposition • Primarily excreted in urine	• ND on primary metabolic pathway • Nine unidentified metabolites in rat urine; 56% consisted of two sulfate conjugates	U.S. EPA (2009b)

Table C-2. Summary of Toxicokinetic Data for 3,5-Dinitroaniline and Candidate Analogues

Compound	Absorption, Distribution, Excretion	Metabolism	References
2,4,6-Trinitrotoluene	• Well absorbed via oral route • Low potential for accumulation, no preferential deposition • Primarily excreted in urine	• Primary pathway: sequential nitroreduction followed by *N*-acetylation or ring hydroxylation, forming hydroxylamine intermediates • Additional pathways: oxidation of methyl group and benzene ring • Primary metabolites identified in human urine include 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 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 (1995b)

NA = not applicable; ND = no data.

In summary, available data for four analogues identify nitroreduction followed by *N*-acetylation or ring hydroxylation as the primary metabolic pathway. This pathway is plausible for the target compound as well as analogues lacking metabolism data.

STEP 5. CARCINOGENICITY OF 3,5-DINITROANILINE ANALOGUES AND MOA DISCUSSION

U.S. EPA cancer WOE descriptors for 3,5-dinitroaniline and its analogue compounds are shown in Table C-3. As noted in the main PPRTV document, there is inadequate information to assess the carcinogenic potential of 3,5-dinitroaniline. Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *"Suggestive Evidence of Carcinogenic Potential"* for 4-nitroaniline and *"Inadequate Information to Assess Carcinogenic Potential"* for 1,4-dinitrobenzene and 3-nitroaniline. U.S. EPA carcinogenicity assessments for TNT and 1,3-dinitrobenzene predated these guidelines, and the WOE descriptors were *"Possible Human Carcinogen (Group C)"* for TNT and *"Not Classifiable as to Human Carcinogenicity (Group D)"* for 1,3-dinitrobenzene. The Group C designation for TNT was based on increased urinary bladder tumors in female rats (U.S. EPA, 2002a). For 4-nitroaniline, the WOE of *"Suggestive Evidence"* was based on increased vascular tumors (hemangiomas and hemangiosarcomas, particularly in the liver) in male mice treated orally (U.S. EPA, 2009b). In both cases, data were sufficient to derive oral slope factor (OSF) values (provisional for 4-nitroaniline), and these are similar in magnitude. No cancer data were available for 1,3-dintrobenzene, 1,4-dinitrobenzene, or 3-nitroaniline.

The U.S. EPA (2009b) proposed a mutagenic MOA for 4-nitroaniline; however, support for this MOA is exclusively from in vitro data, and no evidence linking mutagenesis to the development of observed vascular cell tumors was available. The carcinogenic MOA has not been established for TNT, although it exhibits some evidence of genotoxicity (see Step 3).

Table C-3. Comparison of Available Oral Carcinogenicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

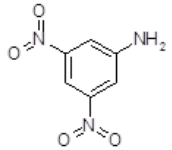
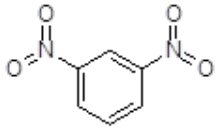
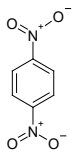
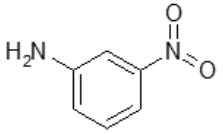
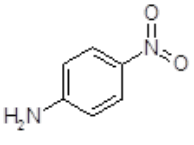
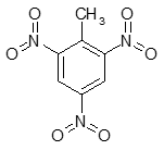
Type of Data	3,5-Dinitroaniline CASRN 618-87-1 (target)	1,3-Dinitrobenzene CASRN 99-65-0	1,4-Dintrobenzene CASRN 100-25-4	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	2,4,6-Trinitrotoluene CASRN 118-96-7
Structure						
U.S. EPA WOE characterization	*"Inadequate Information to Assess Carcinogenic Potential"* (see Table 6)	*"Not Classifiable as to Human Carcinogenicity (Group D)"*	*"Inadequate Information to Assess Carcinogenic Potential"*	*"Inadequate Information to Assess Carcinogenic Potential"*	*"Suggestive Evidence of Carcinogenic Potential"*	*"Possible Human Carcinogen (Group C)"*
OSF $(mg/kg\text{-}d)^{-1}$	NV	NV	NV	NV	2×10^{-2} (provisional)	3×10^{-2}
Data set(s) used for slope factor derivation	NV	NV	NV	NV	Hemangiomas or hemangiosarcomas (all sites) in male B6C3F1 mice	Urinary bladder tumors in female F344 rats (transitional cell papilloma and transitional squamous cell carcinomas)
Other tumors observed in animal bioassays	NV	ND	ND	ND	No additional tumors identified	Leukemia and/or malignant lymphoma of the spleen in female B6C3F1 mice
Study doses (mg/kg-d)	NV	NV	NV	NV	0, 3, 30, 100 ADD: 0, 2.1, 21.4, 71.4 HED: Not reported per dose (HED conversion done after OSF calculation)	0, 0.4, 2, 10, 50 HED: 0, 0.065, 0.325, 1.623, 8.117
Route (method)	NV	NV	NV	NV	Gavage	Diet
Duration	NV	NV	NV	NV	2 yr	24 mo

Table C-3. Comparison of Available Oral Carcinogenicity Data for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

Type of Data	3,5-Dinitroaniline CASRN 618-87-1 (target)	1,3-Dinitrobenzene CASRN 99-65-0	1,4-Dintrobenzene CASRN 100-25-4	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	2,4,6-Trinitrotoluene CASRN 118-96-7
POD type	NV	NV	NV	NV	$BMDL_{10}$ (HED)	BMDL (linearized multistage procedure, extra risk; no further details reported)
Source	NV	U.S. EPA (2005b)	U.S. EPA (2006)	U.S. EPA (2009a)	U.S. EPA (2009b)	U.S. EPA (2002a)

ADD = adjusted daily dose; BMD = benchmark dose; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., subscripted 10 = exposure concentration associated with 10% extra risk); BMR = benchmark response; HED = human equivalent dose; ND = no data; NV = not available; OSF = oral slope factor; POD = point of departure; WOE = weight of evidence.

STEP 6. STRUCTURAL ALERTS AND STRUCTURE ACTIVITY RELATIONSHIP PREDICTIONS FOR 3,5-DINITROANILINE 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 3,5-dinitroaniline and its analogue compounds are shown in Table C-4. Concerns for carcinogenicity and/or mutagenicity of 3,5-dinitroaniline 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, ToxRead, and Virtual models for property Evaluation of chemicals within a Global Architecture (VEGA) models showed a concern for mutagenicity for 3,5-dinitroaniline and all analogues based on SAs (see Table C-5). The Toxtree tool indicated that 3,5-dinitroaniline, 3-nitroaniline, and 4-nitroaniline were unlikely to be mutagenic in *Salmonella* TA100 based on QSAR. The Toxtree results for the nitroaniline compounds 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 3,5-dinitroaniline and all analogues based on SAs (see Table C-5). The level of carcinogenicity concern in OncoLogic for 3,5-dinitroaniline was “moderate” based on structure-activity relationship (SAR) predictions only (aromatic compound containing three amino/amine-generating groups, two of which are nitro groups). OncoLogic indicated the level of concern for carcinogenicity as “low-moderate” for 1,3-dinitrobenzene, 3-nitroaniline, and TNT (shown as “no data” in the heat map) and “marginal” for 1,4-dinitrobenzene and 4-nitroaniline (shown as “no data” in the heat map). VEGA showed concern for carcinogenicity of 3,5-dinitroaniline, 1,3-dinitrobenzene, 1,4-dinitrobenzene, and TNT using the Istituto Superiore di Sanità (ISS), Istituto di Ricerche Farmacologiche Mario Negri (IRFMN)/Alternative Non-Testing Methods Assessed for REACH Substances (ANTARES), and IRFMN/Istituto Superiore di Sanità Chemical Carcinogen (ISSCAN-CGX) models, but not the Computer Assisted Evaluation of industrial chemical Substances According to Regulations (CAESAR) model. For 3-nitroaniline, VEGA showed concern for carcinogenicity using the ISS and IRFMN/ISSCAN-CGX models but not the CAESAR model (no data for the IRFMN/ANTARES model). No concern for carcinogenicity was shown for 4-nitroaniline using the CAESAR, IRFMN/ANTARES, and IRFMN/ISSCAN-CGX models (no data for the ISS model). These results are inconsistent with positive carcinogenicity data for 4-nitroaniline (see Step 5). The Toxtree tool indicated that 3,5-dinitroaniline, 3-nitroaniline, and 4-nitroaniline were potential carcinogens based on QSAR. According to this tool, there was no concern for nongenotoxic carcinogenicity for 3,5-dinitroaniline or any of its analogues.

The ToxAlerts tool showed a concern for genotoxic carcinogenicity and/or mutagenicity for 3,5-dinitroaniline and all analogues based on various SAs (see Table C-5). The Toxtree models also suggest a concern for genotoxic carcinogenicity for 3,5-dinitroaniline and all analogues based on SAs (see Table C-5).

Overall, these in silico tools indicate some evidence of mutagenicity and/or carcinogenicity, as well as showing shared metabolic pathways, common SAs (aromatic nitro, nitroarenes, polynitroarenes), and SAR predictions. Although there are some inconsistencies that

varied by model system, most predictive SAR tools show concern for mutagenicity/carcinogenicity (summarized in Tables C-4, C-5, and C-6).

Table C-4. Heat Map Illustrating the SA and SAR Prediction Results for 3,5-Dinitroaniline (CASRN 618-87-1) and Analogues

Tool	Model[a]	3,5-Dinitroaniline	1,3-Dinitrobenzene	1,4-Dinitrobenzene	3-Nitroaniline	4--Nitroaniline	2,4,6-Trinitrotoluene
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 SA and SAR Prediction Results for 3,5-Dinitroaniline (CASRN 618-87-1) and Analogues

Tool	Model[a]	3,5-Dinitroaniline	1,3-Dinitrobenzene	1,4-Dinitrobenzene	3-Nitroaniline	4--Nitroaniline	2,4,6-Trinitrotoluene
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						

	Model results or alerts indicating no concern for carcinogenicity/mutagenicity.
	Model results outside the applicability domain for carcinogenicity/mutagenicity.
	Model results or alerts indicating concern for carcinogenicity/mutagenicity.

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

ANTARES = Alternative Non-Testing 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; SA = structural alert; SAR = structure-activity relationship; QSAR = quantitative structure-activity relationship; VEGA = Virtual models for property Evaluation of chemicals within a Global Architecture.

Table C-5. SAs and Chemical Mechanisms for 3,5-Dinitroaniline (CASRN 618-87-1) and Analogues

SA	Tools	Compounds
Aromatic amine	OncoLogic (includes compounds with amine generating groups)	3,5-Dinitroaniline, 1,3-dinitrobenzene,[a] 1,4-dinitrobenzene,[a] 3-nitroaniline,[a] 4-nitroaniline[a]
	ToxAlerts	3,5-Dinitroaniline, 3-nitroaniline, 4-nitroaniline
Aromatic nitro/nitro-aromatic	ToxAlerts	3,5-Dinitroaniline, 3-nitroaniline, 1,3-dinitrobenzene, 4-nitroaniline, 2,4,6-trinitrotoluene, 1,4-dinitrobenzene
	OECD QSAR Toolbox	
	Toxtree	
Nitroarenes	ToxAlerts	
	OncoLogic	2,4,6-Trinitrotoluene[a]
Polynitroarenes	OECD QSAR Toolbox	3,5-Dinitroaniline, 1,3-dinitrobenzene, 2,4,6-trinitrotoluene, 1,4-dinitrobenzene
Primary aromatic amine, hydroxyl amine and its derived esters	Toxtree	3,5-Dinitroaniline, 3-nitroaniline, 1,3-dinitrobenzene, 4-nitroaniline
	OECD QSAR Toolbox	3,5-Dinitroaniline, 3-nitroaniline, 4-nitroaniline
Primary aromatic amine, hydroxyl amine and its derived esters or amine generating group	ToxAlerts	3,5-Dinitroaniline, 3-nitroaniline, 4-nitroaniline
Nitroaniline derivative	OECD QSAR Toolbox	3,5-Dinitroaniline, 3-nitroaniline, 4-nitroaniline

[a]Identified as low–moderate or marginal alerts (shown as white cells in Table C-4, indicating results or alerts outside the applicability domain).

OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; SA = structural alert.

STEP 7. EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF 3,5-DINITROANILINE CARCINOGENICITY

Table C-6 presents the data for multiple lines of evidence pertinent to the screening evaluation of the carcinogenic potential of 3,5-dinitroaniline.

Table C-6. Integration of Evidence for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

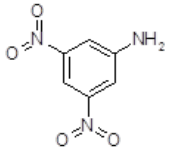
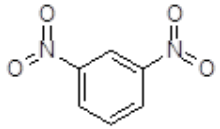
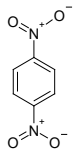
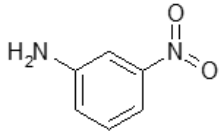
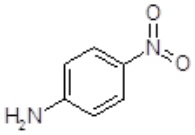
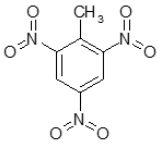
Evidence Streams	3,5-Dinitroaniline CASRN 618-87-1	1,3-Dinitrobenzene CASRN 99-65-0	1,4-Dintrobenzene CASRN 100-25-4	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	2,4,6-Trinitrotoluene CASRN 118-96-7
Structure						
Analogue selection and evaluation (see Steps 1 and 2)	Target compound; contains: (1) one aromatic ring (benzene) substituted with (2) one amine group and two nitro groups on the ring, in a *meta*-substitution pattern, and (3) no other functional group	Contains: (1) one aromatic ring (benzene) substituted with (2) two nitro groups, in a *meta*-substitution pattern, and (3) no other functional group	Contains: (1) one aromatic ring (benzene) substituted with (2) two nitro groups, in a *para*-substitution pattern, and (3) no other functional group	Contains: (1) one aromatic ring (benzene) substituted with (2) one amine group and one nitro group on the ring, in a *meta*-substitution pattern, and (3) no other functional group	Contains: (1) one aromatic ring (benzene) substituted with (2) one amine group and one nitro group on the ring, in a *para*-substitution pattern, and (3) no other functional group	Contains: (1) one aromatic ring (toluene) substituted with (2) three nitro groups on the ring, and (3) no other functional group
Experimental genotoxicity data (see Step 3)	Mutagenic in *Salmonella*; data for other endpoints not available	Mutagenic in *Salmonella*; limited evidence of clastogenicity in mammalian cells; caused DNA damage in rat germ cells in vitro; did not induce UDS in rat liver cells in vitro	Inconsistent evidence of weak mutagenicity in *Salmonella*; limited evidence of clastogenicity in mammalian cells in vitro; did not induce UDS in rat liver cells in vitro	Mutagenic in *Salmonella*; clastogenic in mammalian cells in vitro and in vivo; did not induce UDS in rat liver cells in vitro	Mutagenic in *Salmonella*; did not cause mutations in mammalian cells in vitro or *Drosophila* larvae; clastogenic in mammalian cells in vitro; did not induce UDS in rat liver cells in vitro; bound RNA in vitro	Mutagenic in *Salmonella*; limited and inconsistent findings for mutagenicity in mammalian cells; negative in rodents (in vivo) for clastogenic endpoints; did not induce UDS in mammalian cells in vitro or in vivo

Table C-6. Integration of Evidence for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

Evidence Streams	3,5-Dinitroaniline CASRN 618-87-1	1,3-Dinitrobenzene CASRN 99-65-0	1,4-Dintrobenzene CASRN 100-25-4	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	2,4,6-Trinitrotoluene CASRN 118-96-7
ADME evaluation (see Step 4)	ND; metabolic pathways identified for analogues with data are plausible	Common primary metabolic pathway with other analogues with data (nitroreduction followed by *N*-acetylation or ring hydroxylation)	Common primary metabolic pathway with other analogues with data (nitroreduction followed by *N*-acetylation or ring hydroxylation)	ND; metabolic pathways identified in analogues with data are plausible	Limited data; metabolic pathways identified in analogues with data are plausible	Common primary metabolic pathway with other analogues with data (nitroreduction followed by *N*-acetylation or ring hydroxylation)
Cancer data and MOA (see Step 5)	ND	ND	ND	ND	Vascular tumors (hemangiomas and hemangiosarcomas) in male mice Proposed MOA: Mutagenicity	Urinary bladder tumors in female rats, leukemia and/or malignant lymphoma of the spleen in female mice; MOA not established

Table C-6. Integration of Evidence for 3,5-Dinitroaniline (CASRN 618-87-1) and Candidate Analogues

Evidence Streams	3,5-Dinitroaniline CASRN 618-87-1	1,3-Dinitrobenzene CASRN 99-65-0	1,4-Dintrobenzene CASRN 100-25-4	3-Nitroaniline CASRN 99-09-2	4-Nitroaniline CASRN 100-01-6	2,4,6-Trinitrotoluene CASRN 118-96-7
Common structural alerts and SAR predictions (see Step 6)	ALERTS • Aromatic amine • Aromatic nitro/nitro-aromatic • Nitroarenes • Polynitroarenes • Primary aromatic amine, hydroxyl amine, and its derived esters • Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group • Nitroaniline derivative SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; no concern for nongenotoxic carcinogenicity in Toxtree	ALERTS • Aromatic amine generating groups • Aromatic nitro/nitro-aromatic • Nitroarenes • Polynitroarenes • Primary aromatic amine, hydroxyl amine, and its derived esters SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; low–moderate concern for carcinogenicity in OncoLogic and no concern for nongenotoxic carcinogenicity in Toxtree	ALERTS • Aromatic amine generating groups • Aromatic nitro/nitro-aromatic • Nitroarenes • Polynitroarenes SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; marginal concern for carcinogenicity in OncoLogic and no concern for nongenotoxic carcinogenicity in Toxtree	ALERTS • Aromatic amine • Aromatic nitro/nitro-aromatic • Nitroarenes • Primary aromatic amine, hydroxyl amine, and its derived esters • Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group • Nitroaniline derivative SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; low–moderate concern for carcinogenicity in OncoLogic and no concern for nongenotoxic carcinogenicity in Toxtree	ALERTS • Aromatic amine • Aromatic nitro/nitro-aromatic • Nitroarenes • Primary aromatic amine, hydroxyl amine, and its derived esters • Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group • Nitroaniline derivative SAR PREDICTIONS: Concerns for mutagenicity in most models and concerns for carcinogenicity in some models; marginal concern for carcinogenicity in OncoLogic, no concerns for carcinogenicity in three VEGA models, and no concern for nongenotoxic carcinogenicity in Toxtree	ALERTS • Aromatic nitro/nitro-aromatic • Nitroarenes • Polynitroarenes SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; low−moderate concern for carcinogenicity in OncoLogic and no concern for nongenotoxic carcinogenicity in Toxtree

ADME = absorption, distribution, metabolism, excretion; DNA = deoxyribonucleic acid; MOA = mode of action; ND = no data; RNA = ribonucleic acid; SAR = structure-activity relationship; UDS = unscheduled DNA synthesis; VEGA = Virtual models for property Evaluation of chemicals within a Global Architecture.

STEP 8. QUALITATIVE LEVEL OF CONCERN FOR 3,5-DINITROANILINE POTENTIAL CARCINOGENICITY

Table C-7 identifies the qualitative level of *concern for potential carcinogenicity* of 3,5-dinitroaniline based on the multiple lines of evidence described above. Because of the lack of information supporting carcinogenic portal-of-entry effects, the qualitative level of concern for this chemical is considered to be applicable to all routes of exposure.

Table C-7. Qualitative Level of Concern for Carcinogenicity of 3,5-Dinitroaniline (CASRN 618-87-1)

Level of Concern	Designation	Comments
Concern for potential carcinogenicity	**Selected**	**3,5-Dinitroaniline and its analogues all produce some evidence of genotoxicity, share common metabolic pathways, and have common structural alerts (aromatic nitro, nitroarenes, polynitroarenes) and SAR predictions. Only two analogues of 3,5-dinitroaniline have in vivo animal cancer data (4-nitroaniline and 2,4,6-trinitrotoluene); however, both report carcinogenic potential. Additionally, most SAR predictive tools show concern for mutagenicity and/or carcinogenicity. Based on available evidence, there is *concern for potential carcinogenicity* of 3,5-dinitroaniline.**
Inadequate information for assigning qualitative level of concern	NS	NA

NA = not applicable; NS = not selected; SAR = structure-activity relationship.

APPENDIX D. REFERENCES

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