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Provisional Peer-Reviewed Toxicity Values for

3,4-Toluenediamine
(CASRN 496-72-0)

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

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Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA Office of Research and Development (ORD) Center for Public Health and Environmental Assessment (CPHEA) website at https://ecomments.epa.gov/pprtv.

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COMMONLY USED ABBREVIATIONS AND ACRONYMS

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

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

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 3,4-TOLUENEDIAMINE (CASRN 496-72-0)

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

3,4-Toluenediamine (3,4-TDA), also known as 3,4-diaminotoluene (CASRN 496-72-0), belongs to the class of compounds known as anilines and is an *ortho* (*o*)-substituted compound. The principal commercial use for *o*-TDAs, including 3,4-TDA, is in the production of tolyltriazoles used in corrosion and nitrification inhibitors. 3,4-TDA is also used as an intermediate in the manufacture of urethane products, dyes, corrosion inhibitors, polyols, and benzimidazole thiol antioxidants and as a starting material for a pharmaceutical intermediate (Cartolano, 2005; HSDB, 2003). It is listed on U.S. EPA's Toxic Substances Control Act's public inventory (U.S. EPA, 2015), but it is not registered with Europe's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program (ECHA, 2016).

TDA isomers, including 3,4-TDA, are produced by the catalytic hydrogenation of dinitrotoluenes under a variety of temperatures, pressures, and solvents. 3,4-TDA is then separated from *meta* (*m*)-substituted TDAs by vacuum distillation (Cartolano, 2005).

3,4-TDA is one of six TDA isomers that are components of crude or commercial-grade mixtures used as intermediates in the production of dyes and pigments for commercial products (WHO, 1987). The crude mixture contains all six isomeric forms, while the two commercial mixtures are composed primarily of two isomers each. One commercial mixture, *m*-TDA, contains the 2,4- and 2,6- isomers (80:20 or 65:35), and the other, *o*-TDA, contains the 2,3- and 3,4- isomers (40:60).

The empirical formula for 3,4-TDA is $C_7H_{10}N_2$, and its structure is shown in Figure 1. Table 1 summarizes the physicochemical properties of 3,4-TDA. The compound is a light gray to purple solid at room temperature (Cartolano, 2005). The low vapor pressure and low estimated Henry's law constant for 3,4-TDA indicate that it is unlikely to volatilize from either dry or moist surfaces. 3,4-TDA has an estimated atmospheric half-life of 0.6 hours for the reaction with hydroxyl radicals, but this is not expected to be an important fate process because the compound is not likely to partition to the atmosphere. The estimated water solubility and low soil adsorption coefficient for 3,4-TDA indicate that it has the potential to leach to groundwater or undergo runoff after a rain event. However, given its acid dissociation constant (pKa), 3,4-TDA may exist partially as a cation in the environment, and cations generally adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts. Also, aromatic amines contain highly reactive amino groups that may cause strong bonding to soil organic matter.

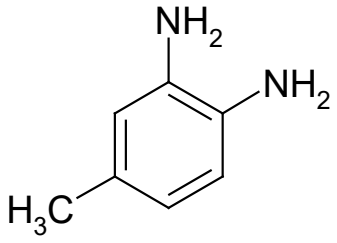


Figure 1. 3,4-Toluenediamine (CASRN 496-72-0) Structure

Table 1. Physicochemical Properties of 3,4-Toluenediamine (CASRN 496-72-0)

Property (unit)	Value[a]
Physical state	Solid[b]
Boiling point (°C)	243
Melting point (°C)	88.8
Density (g/cm^3)	1.13 (predicted)
Vapor pressure (mm Hg)	6.29×10^{-4}
pH (unitless)	NV
Acid dissociation constant (pKa) (unitless)	5.00
Solubility in water (mol/L)	2.39 (predicted)
Octanol-water partition coefficient (log K_{ow})	0.66
Henry's law constant (atm-m^3/mol)	5.59×10^{-8} (predicted)
Soil adsorption coefficient (K_{oc}) (L/kg)	31.3 (predicted)
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	1.43×10^{-10} (predicted)
Atmospheric half-life (h)	0.6 (predicted)[b]
Relative vapor density (air = 1)	NV
Molecular weight (g/mol)	122
Flash point (°C)	137 (predicted)

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard (3,4-Toluenediamine, CASRN 496-72-0. https://comptox.epa.gov/dashboard/DTXSID9024930. Accessed on April 20, 2021). All values are experimental averages unless otherwise specified.
[b]U.S. EPA (2012).

NV = not available.

No toxicity values for 3,4-TDA are available from U.S. EPA or other agencies/organizations searched, as shown in Table 2.

Table 2. Summary of Available Toxicity Values for 3,4-Toluenediamine (CASRN 496-72-0)

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

NA = not applicable; NV = not available.

Non-date-limited literature searches were conducted in November 2017 and updated in May 2020 and April 2021 for studies relevant to the derivation of provisional toxicity values for 3,4-toluenediamine (CASRN 496-72-0). Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE[1] (including TSCATS1), and Web of Science. The following resources were searched outside of HERO for health-related values: American Conference of Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (CalEPA), Defense Technical Information Center (DTIC), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), European Chemicals Agency (ECHA), U.S. EPA Chemical Data Access Tool (CDAT), U.S. EPA ChemView, U.S. EPA 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).

[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 updates from May 2020 and April 2021.

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

As shown in Tables 3A and 3B, there are no potentially relevant subchronic or chronic studies or developmental or reproductive toxicity studies of 3,4-TDA in humans or animals for noncancer and cancer endpoints following oral or inhalation exposures. WHO (1987) described a small number of occupational health surveys of male workers exposed to diaminotoluene and dinitrotoluene mixtures; however, these studies are not useful for determining the effects of 3,4-TDA because they did not discuss or otherwise verify the presence of this isomer in the mixtures. The phrase "statistical significance" and 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 3,4-Toluenediamine (CASRN 496-72-0)					
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**Reference**	**Notes**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					

ND = no data.

Table 3B. Summary of Potentially Relevant Cancer Data for 3,4-Toluenediamine (CASRN 496-72-0)					
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**Reference**	**Notes**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					

ND = no data.

2.1. HUMAN STUDIES

2.1.1. Oral Exposures

No human studies following oral exposure to 3,4-TDA have been identified.

2.1.2. Inhalation Exposures

No human studies following inhalation exposure to 3,4-TDA have been identified.

2.2. ANIMAL STUDIES

2.2.1. Oral Exposures

No animal studies following oral exposure to 3,4-TDA have been identified.

2.2.2. Inhalation Exposures

No animal studies following inhalation exposure to 3,4-TDA have been identified.

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

Toxicity data available for the 3,4-TDA isomer are limited to genotoxicity studies and a 5-day oral study with few endpoints. Additionally, studies evaluating TDA mixtures containing the 3,4-TDA isomer are available, including a reproductive toxicity study, two developmental toxicity studies, several acute lethality studies, and eye and skin irritation assays.

2.3.1. Genotoxicity

Available genotoxicity data for 3,4-TDA are summarized in Table 4. In vitro data indicate that 3,4-TDA has the potential to cause mutations in bacteria; however, evidence for mutation in mammalian cells is equivocal. In an NTP-sponsored study, 3,4-TDA was mutagenic to *Salmonella typhimurium* with, but not without, metabolic activation at concentrations as low as 333 µg/plate, with cytotoxicity reported at ≥3,333 µg/plate (Zeiger et al., 1988). Other assays showed mutagenicity in *S. typhimurium* following exposure to 3,4-TDA with (but not without) metabolic activation. The number of revertants increased, but they were not dose related and/or observed only at high concentrations (~500 µg/plate or higher) associated with >50% toxicity (Allied Chemical, 1983a, b, c, 1979a, b; Litton Bionetics, 1979a, b). 3,4-TDA was not mutagenic to *S. typhimurium* in studies using lower concentrations of ≤366 µg/plate (Watanabe et al., 1989; Florin et al., 1980); toxicity was not reported in either study. Marginal evidence for mutagenicity in Chinese hamster ovary (CHO) cells and L5178Y/TK± mouse lymphoma cells was reported at concentrations associated with cytotoxicity (Allied Chemical, 1983a, b; Litton Bionetics, 1980b; Allied Chemical, 1979a, b; Litton Bionetics, 1979a).

Available data indicate that 3,4-TDA has the potential to cause cell transformation in mammalian cells at concentrations that were often associated with cytotoxicity. A significant enhancement of viral-induced cell transformation was observed in primary Syrian hamster embryo (SHE) cell cultures following exposure to 3,4-TDA at concentrations ≥10 µg/mL either before or after inoculation with simian adenovirus SA7. Cell survival was generally <50% at all doses tested (Greene and Friedman, 1980). Evidence for induction of cell transformation in secondary SHE cell cultures was equivocal, with marginal increases in cell transformation observed in only two of five replicate assays. These findings were not dose related at noncytotoxic concentrations (≤10 µg/mL), and higher concentrations caused significant cytotoxicity (Greene and Friedman, 1980). Similarly, the number of transformed foci in Balb/3T3 cells exposed to 3,4-TDA was marginally increased only at the highest concentration (4 µg/mL), which produced 50% cytotoxicity (Litton Bionetics, 1980a, 1979a).

Limited data from in vivo studies indicate that intraperitoneal (i.p.) exposure to 3,4-TDA induces micronuclei (MN) formation in mouse bone marrow (Wild et al., 1980) and inhibits deoxyribonucleic acid (DNA) synthesis in mouse testes (Allied Chemical, 1983a, d; Greene et al., 1981; Allied Chemical, 1979a, b).

Table 4. Summary of 3,4-Toluenediamine (CASRN 496-72-0) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Genotoxicity studies in prokaryotic organisms						
Mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0, 1,000, 1,710, 2,924, 5,000 µg/plate	−	+ TA98, TA1538 − TA100, TA1535, TA1537	Plate incorporation assay. The number of revertants was increased at all doses in TA98 and TA1538 by 2- to 7-fold, but a dose-response relationship was not observed. Relative survival was <50% for TA98, TA100, and TA1538 at ≥1,000 µg/plate with or without metabolic activation and for TA1535 and TA1537 at 5,000 µg/plate without metabolic activation.	Allied Chemical (1983a); Allied Chemical (1979a); Allied Chemical (1979b); Allied Chemical (1983b); Allied Chemical (1983c)
Mutation	*S. typhimurium* TA98	0, 0.5, 5.0, 50, 500 µg/plate	Not tested	+ (rat S9 or mouse S9 pretreated with saline) − (mouse S9 pretreated with 3,4-TDA)	Plate incorporation assay. Cells were metabolically activated with rat S9 or S9 prepared from C57Bl/6xC3H mice pretreated with physiological saline or 3,4-TDA (i.p.). The number of revertants was increased 2- to 4-fold at 500 µg/plate in samples activated with rat S9 or S9 prepared from C57Bl/6xC3H mice pretreated with physiological saline. Toxicity was not reported.	Allied Chemical (1983a); Allied Chemical (1979a); Allied Chemical (1979b); Allied Chemical (1983b); Allied Chemical (1983c)
Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0, 0.5, 1.0, 10, 100, 500, 1,000, 2,500, 5,000 µg/plate	−	+ TA98, TA1538 − TA100, TA1535, TA1537	Plate incorporation assay. The number of revertants was increased 6- to 20-fold in TA98 and 5- to 7-fold in TA1538 at ≥500 µg/plate. Toxicity was observed at ≥500 µg/plate.	Litton Bionetics (1979a); Litton Bionetics (1979b)
Mutation	*S. typhimurium* TA 97, TA98, TA100, TA1535	0, 33, 100, 333, 666, 1,000, 3,333, 6,666, 10,000 µg/plate	−	+ TA97, TA98, TA100 − TA1535	Preincubation assay. The number of revertants was increased 7- to 20-fold in TA98 at ≥333 µg/plate (toxicity observed at ≥3,333 µg/plate) and at 2- to 4-fold in TA100 and TA97 at ≥1,000 µg/plate (toxicity observed at ≥6,666 µg/plate).	Zeiger et al. (1988)

Table 4. Summary of 3,4-Toluenediamine (CASRN 496-72-0) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate (366 µg/plate)	−	−	Spot test. The number of revertants was not increased by 3,4-TDA exposure. Toxicity was not reported.	Florin et al. (1980)
Mutation	*S. typhimurium* TA98	0, 10, 30 µg/plate	−	−	Plate incorporation assay. The mutagenic potency of 3,4-TDA was not enhanced by the addition of H_2O_2, indicating that oxidative products are not mutagenic. Toxicity was not reported.	Watanabe et al. (1989)
Genotoxicity studies in mammalian cells in vitro						
Mutation	CHO cells	Without S9: 0, 67, 100, 126, 149, 150, 158, 199, 223, 224, 250, 334, 447, 500 µg/mL With S9: 0, 200, 250, 299, 354, 447, 500, 669, 707, 1,000 µg/mL	(+)	(+)	3,4-TDA induced a dose-related increase in mutant frequency with or without metabolic activation in 1/4 replicate assays. The study authors note that cytotoxicity was higher without metabolic activation (no further details were provided).	Allied Chemical (1983a); Allied Chemical (1979a); Allied Chemical (1979b); Allied Chemical (1983b)
Mutation	L5178Y/TK± mouse lymphoma cells	Without S9: 0, 0.29, 4.69, 9.38, 13.8, 37.5 µg/mL With S9: 0, 0.29, 18.8, 37.5, 50, 75, 100, 150 µg/mL	+	(+)	Mutation frequency was increased 2- to 8-fold at ≥13.8 µg/mL without metabolic activation and 2-fold at ≥18.8 µg/mL with metabolic activation. Moderate to high toxicity was observed at all concentrations. Without metabolic activation, high toxicity was observed at ≥9.38 µg/mL (93−98% growth inhibition). With metabolic activation, high toxicity was observed at 150 µg/mL.	Litton Bionetics (1980b); Litton Bionetics (1979a)

Table 4. Summary of 3,4-Toluenediamine (CASRN 496-72-0) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Cell transformation assay	Primary SHE cells inoculated with simian adenovirus SA7	Exposure prior to viral inoculation: 0, 12.5, 25, 50, 100, 200 µg/mL Exposure after viral inoculation: 0, 10, 18, 20, 32, 36, 56, 63, 100, 112, 200 µg/mL	+	Not tested	3,4-TDA significantly ($p < 0.05$) enhanced viral transformation of primary SHE cells at all concentrations by 2- to 19-fold when added before or after viral inoculation. Survival was generally <50% at all doses tested, and 100% toxicity was observed at ≥100 µg/mL with exposure after viral inoculation.	Greene and Friedman (1980)
Cell transformation assay	Secondary SHE cells	0, 2.5, 5.0, 10, 15, 20, 22, 33, 50 µg/mL	(+)	Not tested	3,4-TDA induced cell transformation in two of five replicate assays. In positive replicates, 1−2 transformed loci were observed at noncytotoxic concentrations (≤10 µg/mL); findings were not dose related. The three negative replicates had "low activity" of positive control (BaP). Relative survival was decreased by approximately 50% or more at ≥15 µg/mL.	Greene and Friedman (1980)
Cell transformation assay	Balb/3T3 cells	0, 0.05, 0.5, 1, 2, 4 µg/mL	(+)	Not tested	A marginal increase in the total number of transformed foci was observed at 4 µg/mL, which produced 50% cytotoxicity.	Litton Bionetics (1980a); Litton Bionetics (1979a)
Genotoxicity studies in mammals in vivo						
Bone marrow micronucleus test	NMRI mice treated with 3,4-TDA i.p.; 2 doses separated by 24 h; bone marrow isolated 6 h after second dose	0, 122, 244, 366 mg/kg-d	+		The percentage of micronucleated polychromatic erythrocytes in bone marrow was significantly ($p \leq 0.01$) increased by 4.4−8% at ≥244 mg/kg-d, compared with controls.	Wild et al. (1980)

Table 4. Summary of 3,4-Toluenediamine (CASRN 496-72-0) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Testicular DNA synthesis inhibition test	C57Bl/6xC3H mice treated with 3,4-TDA i.p.; ^{3}H-thymidine incorporation in testicular DNA was measured	0, 500 mg/kg	+		Significant decrease in radioactivity incorporated into DNA relative to controls ($p < 0.01$ in one replicate, $p < 0.1$ in a second replicate).	Allied Chemical (1983a); Allied Chemical (1979a); Allied Chemical (1979b); Allied Chemical (1983d)
Testicular DNA synthesis inhibition test	C57Bl/6xC3H mice treated with 3,4-TDA i.p.; ^{3}H-thymidine incorporation in testicular DNA was measured	0, 200, 299, 262, 300 mg/kg	+		Significant decrease in radioactivity incorporated into DNA relative to controls at all doses ($p < 0.025$). No significant change in rectal temperature (changes in rectal temperature can affect testicular DNA synthesis).	Greene et al. (1981)

[a]+ = positive result; (+) = weak positive result; − = negative result.

BaP = benzo[a]pyrene; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; ^{3}H = hydrogen-3 isotope (tritium); H_2O_2 = hydrogen peroxide; i.p. intraperitoneal; SHE = Syrian hamster embryo; TDA = toluenediamine.

2.3.2. Short-Term and Acute Toxicity Studies

Available short-term and acute toxicity studies of 3,4-TDA or TDA mixtures containing 3,4-TDA are summarized in Table 5.

Toxicity data for 3,4-TDA alone are limited to a short-term exposure study in which female Sprague Dawley rats were administered 3,4-TDA (97% purity) twice-daily via gavage in water at a dose of 500 mg/kg (1,000 mg/kg-day) (Selye, 1973). The study was terminated on the fifth day of exposure because 7/13 exposed rats had died (no further information on time of death was provided). Gross necropsy revealed that six of the dead animals had grossly observed perforated duodenal ulcers. Severe icterus (jaundice) was also reported, although further details were not provided. The study authors stated that comparable results were observed when males and females (10/sex) were similarly exposed via gavage in water, peanut oil, dimethylsulfoxide, or propylene glycol or via i.p. or subcutaneous (s.c.) injection (no further details were provided) (Selye, 1973).

Oral median lethal dose (LD_{50}) values in rats for TDA mixtures containing 3,4-TDA range from 660−1,760 mg/kg (WIL Research, 1978; Air Products and Chemicals, 1976; Carpenter et al., 1974). An inhalation median lethal concentration (LC_{50}) value >670 ppm was reported in rats exposed to *o*-TDA; the duration of exposure was not reported (Air Products and Chemicals, 1976). Exposure to concentrated *o*-TDA vapors was lethal after >8 hours of exposure (Carpenter et al., 1974). Dermal LD_{50} values for *o*-TDA in rabbits ranged from 1,120 to >5,750 mg/kg (Air Products and Chemicals, 1976; Carpenter et al., 1974). *o*-TDA is slightly to moderately irritating to rabbit skin, and the undiluted liquid is irritating to the rabbit eye (Air Products and Chemicals, 1976; Carpenter et al., 1974). Skin sensitization was “insignificant” in guinea pigs exposed to *o*-TDA (Air Products and Chemicals, 1976).

Table 5. Short-Term and Acute Toxicity Studies for 3,4-Toluenediamine (CASRN 496-72-0)

Test	Materials and Methods	Results	Conclusions	References
Exposure to 3,4-TDA alone				
Short-term oral	Main experiment: Female Sprague Dawley rats (n = 13) were exposed to 3,4-TDA twice daily via gavage in water at 500 mg/kg per dose (1,000 mg/kg-d) for up to 5 d. The animals were observed for mortality, and gross necropsy was performed on the animals that died. Additional experiments: The main experiment was repeated with male and female rats (10/group) using water, peanut oil, dimethylsulfoxide, or propylene glycol as a vehicle, as well as with parenteral (i.p. or s.c.) exposure.	Main experiment: 7/13 rats died within the first 5 d; 6/7 dead rats had "macroscopically obvious" and "usually perforated" duodenal ulcers. Severe icterus was also reported. Additional experiments: Results following oral exposure "essentially the same as in the main experiment." Result following parenteral exposure were "only doubtfully less efficacious." No further details were provided.	Oral or parenteral exposure to high doses of 3,4-TDA caused perforating duodenal ulcers and icterus in rats. The study authors propose using 3,4-TDA to create an animal model of duodenal ulcers for testing of therapeutic interventions.	Selye (1973)
Exposure to TDA mixtures containing 3,4-TDA				
Acute oral lethality	Rats were exposed to a mixture of 2,3- and 3,4-TDA in 4% aqueous solution by gavage at 6 doses. Study was reported in tabular form with no further details.	LD_{50} = 660 mg/kg. No further details were provided.	Rat oral LD_{50} = 660 mg/kg.	Air Products and Chemicals (1976)
Acute oral lethality	Rats were exposed to a mixture of 2,3- and 3,4-TDA. No further details were provided.	LD_{50} (95% CI) = 810 (590−1,120) mg/kg. No further details were provided.	Rat oral LD_{50} = 810 mg/kg.	Carpenter et al. (1974)
Acute oral lethality	Sprague Dawley rats (5/sex/group) were exposed to two mixtures containing 2,3-, 2,4-, and 3,4-TDA and 4,4-methylenedianiline at doses of 0, 310, 630, or 1,250 mg/kg via gavage in corn oil. One formulation contained 17.9% (wt) 3,4-TDA; the other was a similar formulation, but exact percentages were not "precisely known." The animals were observed hourly for the first 6 h for signs of toxicity, and subsequently for 14 d for mortality. Gross necropsies were performed.	Most deaths occurred between 1 and 3 d postexposure. Surviving animals exhibited shallow respiration, depression, depressed righting and placement reflexes, excessive salivation, unkempt coats, piloerection, and yellowish-orange urine and mucoid diarrhea stains. All survivors were observed to be emaciated between the fourth and eighth day postexposure. Necropsies of decedents indicated congested lungs, adrenals, and kidneys; mottled livers; irritated GI tracts and peritoneal walls; and fluid-filled stomachs. Necropsies of survivors were unremarkable.	LD_{50} = 1,100−1,760 mg/kg in male rats. LD_{50} = 1,080−1,220 mg/kg in female rats.	WIL Research (1978)

Table 5. Short-Term and Acute Toxicity Studies for 3,4-Toluenediamine (CASRN 496-72-0)

Test	Materials and Methods	Results	Conclusions	References
Acute inhalation lethality	Rats were exposed to a mixture of 2,3- and 3,4-TDA. Study was reported in tabular form with no further details.	LC_{50} >670 ppm. No further details were provided.	Rat inhalation LC_{50} >670 ppm.	Air Products and Chemicals (1976)
Acute inhalation lethality	Rats were exposed to the concentrated vapors of a mixture of 2,3- and 3,4-TDA. No further details were provided.	Maximum time producing no deaths was 8 h. No further details were provided.	Exposure to the concentrated vapors of a TDA mixture is lethal after >8 h of exposure.	Carpenter et al. (1974)
Acute dermal lethality	Rabbits were exposed dermally (abraded and nonabraded skin) to a mixture of 2,3- and 3,4-TDA in a 60% aqueous paste for 24 h and observed for 14 d. Study was reported in tabular form with no further details.	LD_{50} >5,750 mg/kg. No further details were provided.	Dermal LD_{50} >5,750 mg/kg.	Air Products and Chemicals (1976)
Acute dermal lethality	Rabbits were exposed to a mixture of 2,3- and 3,4-TDA on shaved backs for 24 h. No details regarding concentration or occlusion were provided.	LD_{50} (95% CI) = 1,120 (620−2,040) mg/kg. No further details were provided.	Dermal LD_{50} = 1,120 mg/kg.	Carpenter et al. (1974)
Acute dermal irritation	Rabbits were exposed to a mixture of 2,3- and 3,4-TDA on the uncovered skin of the belly. No details regarding concentration or duration of exposure were provided.	Irritation score was 5/10.	A mixture of TDA isomers is moderately irritating to the skin of rabbits.	Carpenter et al. (1974)
Skin sensitization	Guinea pigs were exposed to a mixture of 2,3- and 3,4-TDA. The study was reported in tabular form with no further details.	“Insignificant” sensitization.	A mixture of TDA isomers is not a skin sensitizer in guinea pigs.	Air Products and Chemicals (1976)
Eye irritation	Rabbits were exposed to a mixture of 2,3- and 3,4-TDA in 5% aqueous solution in eye. The study was reported in tabular form with no further details.	No irritation after 72 h.	A diluted mixture of TDAs is not irritating to the rabbit eye.	Air Products and Chemicals (1976)
Eye irritation	Rabbits were exposed to a mixture of 2,3- and 3,4-TDA in a “suitable vehicle” in eye.	Irritation score was 7/10.	A mixture of TDA isomers is irritating to the rabbit eye.	Carpenter et al. (1974)

CI = confidence interval; GI = gastrointestinal; i.p. = intraperitoneal; LC_{50} = median lethal concentration; LD_{50} = median lethal dose; s.c. = subcutaneous; TDA = toluenediamine.

2.3.3. Reproductive/Developmental Studies of TDA Mixtures

Becci et al. (1983)

Groups of 22 pregnant Sprague Dawley rats were administered *o*-TDA (a 40:60 mixture of 2,3- and 3,4-TDA) at doses of 0, 10, 30, 100, or 300 mg/kg-day via gavage in corn oil from Gestation Days (GDs) 6−15. Observations were conducted daily for general appearance, behavior, and mortality. Body weights of the dams were recorded on GDs 0, 6, 9, 12, 15, and 20. On GD 20, all dams were sacrificed, and uterine contents were removed and examined. One-half of the fetuses were examined for soft-tissue anomalies, and the remaining fetuses were examined for skeletal anomalies. No treatment-related effects on appearance or behavior were observed in treated dams, and all dams survived the duration of the study. There was a statistically significant decrease in weight gain (−20%) during gestation for treated dams receiving 300 mg/kg-day compared with controls. No significant differences in the number of live fetuses, implantation sites, or resorption sites were indicated. Fetal effects indicative of developmental delay included significant reductions in fetal body weight (−18%) in the 300-mg/kg-day group and significant increases in skeletal variations per litter (missing sternebrae at 300 mg/kg-day and incomplete ossification of vertebrae at 100 and 300 mg/kg-day), compared with controls. No exposure-related skeletal or soft-tissue malformations were observed. No maternal or developmental effects were seen at ≤30 mg/kg-day.

Additionally, groups of 15 pregnant Dutch belted rabbits were exposed to *o*-TDA at doses of 0, 3, 10, 30, or 100 mg/kg-day via gavage in corn oil from GDs 6−18. Observations were conducted daily for general appearance, behavior, and mortality. Body weights of the does were recorded on GDs 0, 6, 9, 12, 15, 18, and 29. On GD 29, all does were sacrificed, and uterine contents were removed and examined. All of the fetuses were examined for both soft-tissue and skeletal anomalies. Appearance and behavior of does were unaffected by treatment. All does survived the duration of the study. Body-weight gain during gestation was significantly decreased (−60%) in treated does receiving 100 mg/kg-day compared with controls. Other observations at this dose included a significant 2.5-fold increase in the incidence of resorptions, a 16% decrease in the mean number of live fetuses/doe (reported as statistically significant in the text, but not in the table showing the data), and a significant 22% decrease in fetal body weight. No exposure-related skeletal or soft-tissue malformations or variations were observed. No maternal or developmental effects were seen at ≤30 mg/kg-day.

BASF (2010)

In an OECD 421 reproductive/developmental (R/D) study available only as an industry-submitted summary, groups of male and female Wistar rats (10/sex/group) were administered *o*-TDA (45:50 mixture of 2,3- and 3,4-TDA) at doses of 0, 10, 50, or 250 mg/kg-day via gavage (vehicle not reported) from premating through mating (males, at least 28 days) or premating through Postnatal Day (PND) 4 (up to 60 days for females). The pups were sacrificed and examined on PND 4 (endpoints examined at sacrifice were not reported). The available summary reported only “the most relevant results”; no statistics were provided, and the summary did not include the magnitude/incidence for many of the findings.

Clinical signs of toxicity (reduced activity, eyelid drop, salivation, and/or piloerection) were observed in males and dams at ≥50 mg/kg-day. Decreased food consumption was observed during premating in males at 250 mg/kg-day and females at ≥50 mg/kg-day; decreased food consumption was also observed in dams during gestation at 250 mg/kg-day. Body-weight gain was decreased throughout the study in high-dose males, with a decreased terminal body weight compared with controls (magnitude not reported). Decreased body weight and body-weight gains

were observed during premating and gestation in dams at 250 mg/kg-day, with a decreased terminal body weight compared with controls (magnitude not reported). In males, a decrease in the number of spermatids/g testis was reported at 250 mg/kg-day; however, the study summary did not indicate whether decreased fertility was observed. Reproductive effects observed in high-dose dams included a 39% decrease in the number of implantation sites compared with controls, a 27.4% post implantation loss (control value not reported), and a 42% decrease in the number of delivered pups/litter. In offspring, a decreased viability index of 91% was observed at 250 mg/kg-day (viability index in controls was not reported). No effects were noted in males or dams administered 10 mg/kg-day.

2.3.4. Mode-of-Action/Mechanistic Studies

Mechanistic data for 3,4-TDA are limited. Perkins and Green (1975) suggested that the duodenal ulcers observed by Selye (1973) may be a result of 3,4-TDA toxicity to Brunner's glands in the proximal duodenum. Brunner's glands function to secrete an alkaline mucoid material to protect the duodenum from the corrosive action of gastric juices. The volume of Brunner's glands' secretions was quantified in situ following single subcutaneous injections of 3,4-TDA in rats at 125 mg/kg (which caused minimal gastroduodenal damage), 350 mg/kg (which caused maximal duodenal damage and minimal mortality), and 500 mg/kg (which caused a low incidence of duodenal and a high incidence of gastric damage). The output of fluid from Brunner's glands was significantly decreased in all exposed groups compared with control, with lower inhibition at 125 mg/kg (25%) than 350 and 500 mg/kg (61 and 57%, respectively).

3. DERIVATION OF PROVISIONAL VALUES

3.1. DERIVATION OF PROVISIONAL REFERENCE DOSES

No subchronic or chronic studies have been located regarding the toxicity of 3,4-TDA to humans or animals via oral administration. Potentially relevant toxicity data for 3,4-TDA are limited to a 5-day gavage study that reported perforating duodenal ulcers in rats following exposure to 1,000 mg/kg-day (Selye, 1973). Additionally, gavage exposure studies evaluating TDA mixtures containing the 3,4-TDA isomer (approximately 50−60% 3,4-TDA) showed some evidence of potential R/D effects in rats at 250 mg/kg-day following premating through PND 4 and in rats and rabbits at ≥100 mg/kg-day following gestational exposure, primarily at doses associated with potential maternal toxicity (BASF, 2010; Becci et al., 1983). The scope and design of these studies are inadequate to support the derivation of a subchronic or chronic provisional reference dose (p-RfD) for 3,4-TDA using chemical-specific data. Instead, screening p-RfDs are derived in Appendix A using an alternative, read-across approach. Based on the overall analogue approach presented in Appendix A, 2,5-toluenediamine was selected as the most appropriate analogue for 3,4-TDA for deriving a screening subchronic and chronic p-RfD (see Table 6).

3.2. DERIVATION OF PROVISIONAL REFERENCE CONCENTRATION

The absence of relevant inhalation data precludes derivation of provisional reference concentrations (p-RfCs) for 3,4-TDA directly. An alternative read-across approach was attempted, but screening p-RfCs could not be derived due to a lack of inhalation toxicity values for analogues identified (see Appendix A).

3.3. SUMMARY OF NONCANCER PROVISIONAL REFERENCE VALUES

The noncancer provisional reference values for 3,4-TDA are summarized in Table 6.

Table 6. Summary of Noncancer Reference Values for 3,4-Toluenediamine (CASRN 496-72-0)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Screening subchronic p-RfD (mg/kg-d)	Rat/F	Increased serum AST	1×10^{-3}	NOAEL (HED)	0.32 (based on analogue POD)	300	Hill (1997) as cited in SCCP (2007) and reported by U.S. EPA (2013)
Screening chronic p-RfD (mg/kg-d)	Rat/F	Increased serum AST	1×10^{-4}	NOAEL (HED)	0.32 (based on analogue POD)	3,000	Hill (1997) as cited in SCCP (2007) and reported by U.S. EPA (2013)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

AST = aspartate aminotransferase; F = female(s); HED = human equivalent dose; NDr = not determined; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; 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 3,4-TDA (see Table 7). 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 was a *concern for potential carcinogenicity* of 3,4-TDA (see Appendix C).

Table 7. Cancer WOE Descriptor for 3,4-Toluenediamine (CASRN 496-72-0)

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, chemical-specific data in humans or animals to evaluate the carcinogenic potential of 3,4-TDA.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	No evidence of noncarcinogenicity is available.

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

3.5. DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

The absence of suitable data precludes development of cancer risk estimates for 3,4-TDA (see Table 8).

Table 8. Summary of Cancer Risk Estimates for 3,4-Toluenediamine (CASRN 496-72-0)

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 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 3,4-toluenediamine (3,4-TDA). However, some information is available for this chemical, which although insufficient to support derivation of a provisional toxicity value under current guidelines, may be of limited use to risk assessors. In such cases, the 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 deriving 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 read-across approach allows for the use of data from related compounds to calculate screening values when data for the compound of interest are limited or unavailable. Details regarding searches and methods for analogue analysis are presented in Wang et al. (2012). Three types of potential analogues (structural, metabolic, and toxicity-like) are identified to facilitate the final analogue chemical selection. The analogue approach may or may not be route specific or applicable to multiple routes of exposure. All information was considered together as part of the final weight-of-evidence (WOE) approach to select the most suitable analogue both toxicologically and chemically.

Structural Analogues

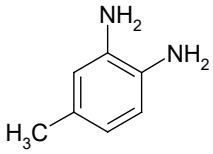
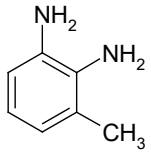
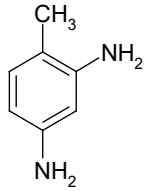
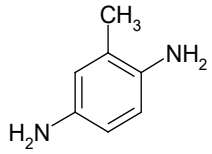
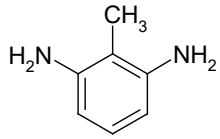
An initial analogue search focused on the identification of structurally similar chemicals with toxicity values from the Integrated Risk Information System (IRIS), PPRTV, Agency for Toxic Substances and Disease Registry (ATSDR), or California Environmental Protection Agency (CalEPA) databases to take advantage of the well-characterized chemical-class information. This was accomplished by searching structural similarity software tools, namely the National Library of Medicine's (NLM) ChemIDplus database (NLM, 2019) and Organisation for Economic Co-operation and Development (OECD) quantitative structure-activity relationship (QSAR) Toolbox (OECD, 2019). These software tools employ slightly different quantitative methods to make similarity comparisons between chemical structures based on fingerprints; ChemIDplus uses a modified Tanimoto index and the OECD Toolbox uses the Dice index. Two TDA isomers that have oral noncancer toxicity values were identified as potential structural analogues of 3,4-TDA: 2,6- (U.S. EPA, 2005b) and 2,5-TDA (U.S. EPA, 2013) (see Table A-1). In addition, 2,3- (a compound being evaluated in a separate PPRTV assessment) and 2,4-TDA were included in the read-across analysis to provide information on the potential influence of the position of the amino groups (*ortho* [*o*-], *meta* [*m*-], or *para* [*p*-]) on toxicity (note: these analogues do not have oral toxicity values; see Table A-1). Previous structure-activity relationship (SAR) analyses have suggested increased chemical reactivity and toxicity for *o*- and *p*- versus *m*-substituted aromatic amines (Bajot et al., 2010). The target and 2,3-TDA are *o*- isomers, 2,5-TDA is a *p*- isomer, and 2,4- and 2,6-TDAs are *m*- isomers.

Table A-1 summarizes the physicochemical properties and similarity scores for all analogues. 3,4-TDA and the identified analogues are aromatic amines that share a common basic structure, which consists of a benzene ring, two amino groups and a methyl group, differing only in the position of the amino functional groups. These compounds are major components of commercial grade TDA mixtures (WHO, 1987) and have physicochemical properties that are of the same relative order of magnitude; therefore, differences in the absorption and distribution between the analogues and the target are not expected to be significant or result in a preference in the selection of one analogue over another. These compounds are all weak bases and are expected to be substantially ionized at physiological pH values. Furthermore, their water solubility and their octanol-water partition coefficient (log K_{ow}) values are consistent with a high degree of hydrophilicity (see Table A-1). Additionally, all of these diamines have low volatility and are not expected to be eliminated in exhaled breath.

Structural alert (SA) predictions for relevant toxicity endpoints were generated for the TDA isomers using the OECD QSAR Toolbox [OECD (2019); see Table A-2]. These included the repeated-dose profiler based on the Hazard Evaluation Support System (HESS) database and the developmental and reproductive toxicity (DART) scheme adapted from the Wu et al. (2013) framework for identifying chemicals with structural features associated with potential reproductive/developmental (R/D) toxicants. The model predictions suggest concern for hepatotoxicity, hemolytic anemia with methemoglobinemia, and for R/D toxicity for 3,4-TDA and all analogues. The predictions are based on SAs for aniline and toluene/small alkyl toluene derivatives, respectively. The HESS model also showed concern for renal toxicity for 3,4-, 2,3-, and 2,6-TDA based on the SA for toluene.

In summary, the candidate analogues are considered suitable analogues for 3,4-TDA based on their similarities in structural and physicochemical properties and SA predictions.

Table A-1. Physicochemical Properties of 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues[a]

Property	3,4-Toluenediamine	2,3-Toluenediamine	2,4-Toluenediamine	2,5-Toluenediamine	2,6-Toluenediamine
Role	Target	Analogue	Analogue	Analogue	Analogue
Structure					
CASRN	496-72-0	2687-25-4	95-80-7	95-70-5	823-40-5
DTXSID	9024930	4027494	4020402	6029123	4027319
Molecular weight	122	122	122	122	122
ChemIDplus similarity score (%)[b]	100	70	64	75	55
OECD toolbox similarity score (%)[c]	100	67	78	78	78
Melting point (°C)	88.8	60.1	98.2	64.0	105
Boiling point (°C)	243	254	286	274	260
Vapor pressure (mm Hg)	6.29×10^{-4}	5.53×10^{-4}	1.7×10^{-4}	2.25×10^{-3} (predicted)	2.46×10^{-3}
Henry's law constant (atm-m^3/mol)	5.59×10^{-8} (predicted)	5.59×10^{-8} (predicted)	5.60×10^{-8} (predicted)	5.73×10^{-8} (predicted)	5.61×10^{-8} (predicted)
Water solubility (mol/L)	2.39 (predicted)	2.36 (predicted)	2.57 (predicted)	2.73 (predicted)	2.52 (predicted)
Octanol-water partition coefficient (Log K_{ow})	0.66	0.549 (predicted)	0.14	0.11 (predicted)	0.21 (predicted)
Acid dissociation constant (pKa) (unitless)	5.00	4.91 (predicted)[d]	5.58 (predicted)[e]	6.52 (predicted)[e]	5.28 (predicted)[e]

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard. Accessed on April 20, 2021). All values are experimental averages unless otherwise specified.
[b]ChemIDplus advanced similarity scores (NLM, 2019).
[c]OECD QSAR Toolbox, similarity scores (OECD, 2019).
[d]HSDB (2013).
[e]ChemAxon (2017).

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

Table A-2. Comparison of SAs for Relevant Endpoints for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues from the OECD QSAR Toolbox[a]

SA	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Repeated-dose toxicity (HESS)	• Hepatotoxicity (anilines) • Hemolytic anemia with methemoglobinemia (anilines) • Renal toxicity (toluene)	• Hepatotoxicity (anilines) • Hemolytic anemia with methemoglobinemia (anilines) • Renal toxicity (toluene)	• Hepatotoxicity (anilines) • Hemolytic anemia with methemoglobinemia (anilines)	• Hepatotoxicity (anilines) • Hemolytic anemia with methemoglobinemia (anilines)	• Hepatotoxicity (anilines) • Hemolytic anemia with methemoglobinemia (anilines) • Renal toxicity (toluene)
DART scheme	• Known precedent reproductive and developmental toxic potential (toluene and small alkyl toluene derivatives)	• Known precedent reproductive and developmental toxic potential (toluene and small alkyl toluene derivatives)	• Known precedent reproductive and developmental toxic potential (toluene and small alkyl toluene derivatives)	• Known precedent reproductive and developmental toxic potential (toluene and small alkyl toluene derivatives)	• Known precedent reproductive and developmental toxic potential (toluene and small alkyl toluene derivatives)

[a]OECD QSAR Toolbox (OECD, 2019).

DART = developmental and reproductive toxicity; HESS = Hazard Evaluation Support System; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; SA = structural alert.

Metabolic Analogues

Table A-3 summarizes available toxicokinetic data in experimental animals for 3,4-TDA and the structurally similar compounds identified as candidate analogues.

No toxicokinetic data has been identified for 3,4-TDA. Available information on the 2,4-, 2,5-, and 2,6-TDA analogues suggest that these compounds are rapidly and extensively absorbed following oral exposure, and are rapidly eliminated in the urine, which is a predominant route of excretion (see Table A-3). Major metabolic steps for the TDA analogues are acetylation of amino groups and ring hydroxylation, with some evidence for oxidation of the methyl groups in rats and mice exposed via intraperitoneal (i.p.) administration (see Table A-3).

In the absence of in vivo toxicokinetic data on 3,4-TDA, a selection of available software tools, specifically the in vivo and in vitro rat metabolic simulators available within the Tissue Metabolism Simulator (TIMES) program (Dimitrov et al., 2005; Mekenyan et al., 2004) and Meteor Nexus (Marchant et al., 2008) were used to predict metabolites for the target compound and analogues. Predicted metabolites for the TDA isomers are summarized in Table D-1 and additional information on the in silico analysis can be found in Appendix D. The analysis reveals some overlap in terms of metabolites for the individual TDA compounds across the different tools and when comparing predictions with experimental data from in vivo rodent studies (captured in Table A-3), which increases confidence in the in silico results (see Table D-1). Furthermore, the corresponding metabolic pathway transformations were extracted from Meteor Nexus to allow for similarity comparisons across the TDAs. This level of information was not available from other tools (i.e., TIMES). Table A-4 shows a consistent pattern of pathway transformations among the TDA compounds, and Figure A-1 confirms a high degree of similarity between 3,4-TDA and the candidate analogues with regards to the Meteor Nexus pathway predictions. There is also concordance between the in silico results (see Table A-4) and the major pathways expected for this group of compounds (*N*-acetylation, ring hydroxylation, and oxidation of methyl groups). Importantly, no outstanding differences in the predicted metabolic profiles between the target and analogues are noted. The metabolic tree for 2,4-TDA is displayed in Figure D-1 to illustrate the relationship of the predicted metabolites for this specific analogue that correspond to the pathway transformations shared among the TDAs (see Appendix D for more details).

In summary, in vivo data demonstrate toxicokinetic commonalities among the analogues, particularly with respect to metabolism pathways, and according to in silico predictions, a similar metabolism pattern is expected for 3,4-TDA. Therefore, the candidate analogues are considered suitable analogues for 3,4-TDA based on their toxicokinetic properties.

Table A-3. Comparison of Available ADME Data for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

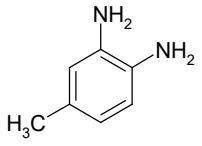
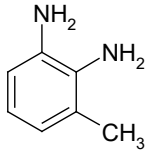
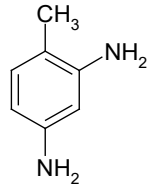
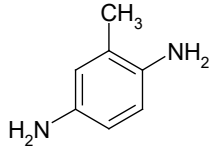
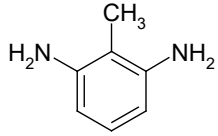
3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
				
Target	Analogue	Analogue	Analogue	Analogue
DTXSID 9024930	DTXSID 4027494	DTXSID 4020402	DTXSID 6029123	DTXSID 4027319
Absorption				
ND	ND	Rats exposed orally (single dose of 3 or 60 mg/kg): • 70% of administered dose based on recovered radioactivity in the urine and tissue/carcass over 48 h Rats exposed via i.p. injection (single 77-mg/kg dose): • Rapid absorption; levels peaked in blood and plasma 1 h after dosing	Rats exposed orally (single dose of 2.5 or 25 mg/kg): • Blood radioactivity peaked at 1 h after dosing (as sulfate) • Time to C_{max} in blood 0.5−1 h after dosing (as sulfate)	Rats exposed orally (single dose of 10 mg/animal; approximately 57 to 67 mg/kg based on a reported body weights of 150−175 g): • Rapidly and extensively absorbed

Table A-3. Comparison of Available ADME Data for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Distribution				
ND	ND	Rats exposed orally (single dose of 3 or 60 mg/kg): • 2−5% in tissue and carcass (after 48 h) Rats and mice exposed via i.p. injection (single 77-mg/kg dose): • Liver and kidney > blood > muscle; high levels also in GI tract, spleen, heart, testes, lymph nodes, eyes, and lungs • Mouse tissues showed lower level of radioactivity than rat tissues	ND	Rats exposed orally (single dose of 10 mg/animal; approximately 57 to 67 mg/kg based on a reported body weights of 150−175 g): Wide distribution following single dose (% of administered dose) • 3.6% large intestine • 1% muscle • 0.6% liver • 0.5% skin • 0.2% blood • 0.1% small intestine • >0.1% for perirenal fat, stomach contents, brain, spleen, testis, heart, and lungs
Metabolites				
ND	ND	Rats exposed orally (single 50-mg/kg dose): Urinary over 48 h (% dose excreted) • 3-Hydroxy-4-acetylamino-2-aminotoluene (18%) • 5-Hydroxy-4-acetylamino-2-aminotoluene (14%) • 5-Hydroxy-2,4-diaminotoluene (12%), 3-hydroxy-2,4-diaminotoluene (8%) and 6-hydroxy-2,4-diaminotoluene (5%) • Other (unidentified) conjugates (16−34% of all acid-labile conjugates)	Rats exposed orally to sulfate salt (single dose of 2.5 or 25 mg/kg): Urinary over 96 h • *N,N′*-Diacetyl-toluene-2,5-diamine • Two unidentified mono-*N*-acetylated metabolites Rats exposed by i.v. injection (single 2.5-mg/kg dose): Urine and feces over 96 h • 2,5-Diacetylamino toluene was a major metabolite	Rats exposed orally (single 10 mg/animal; approximately 57 to 67 mg/kg based on a reported body weights of 150−175 g): Urinary over 24 h • 3-Hydroxy-2,6-toluenediamine • 5-Hydroxy-2-acetylamino-6-aminotoluene • 2-Acetylamino-6-aminotoluene • 2,6-Diacetylamino toluene • No parent compound detected

Table A-3. Comparison of Available ADME Data for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Continued:	Continued:	Continued: <u>Rats exposed via i.p. injection (single 77-mg/kg dose):</u> Urinary over 24 h (% dose) • Free metabolites (20.9%), including 4-acetylamino-2-aminotoluene, (5.7%), 4-acetyl amino-2-amino benzoic acid (2.7%), and 2,4-diacetylamino toluene (2.6%) • Glucuronide conjugates (7.5%) • Sulfate conjugates (10.1%) • Water soluble (30.9%) <u>Mice exposed via i.p. injection (single 77-mg/kg dose):</u> Urinary over 24 h (% dose) • Free metabolites (20.2%), including 4-acetylamino-2-aminobenzoic acid (5.4%), 4-acetylamino-2-aminotoluene (2.1%), and 2,4-diacetyl aminobenzoic acid (1.1%) • Glucuronide conjugates (17.4%) • Sulfate conjugates (9.6%) • Water soluble (35.3%)	Continued:	Continued:

Table A-3. Comparison of Available ADME Data for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Excretory pattern after oral exposure (unless otherwise indicated)				
ND	ND	Rats exposed orally (single dose of 3 or 60 mg/kg): Urine (% dose): >60% (within 48 h) Feces (% dose): 23−31 (within 48 h)	Rats exposed orally (single dose of 2.5 or 25 mg/kg): Urine (% dose): >60 (within 98 h) Feces (% dose): 22−31 (within 98 h)	Rats exposed orally (single dose of 10 mg/animal; approximately 57 to 67 mg/kg based on a reported body weights of 150−175 g): Urine (% dose): 85 (within 24 h) Feces (% dose): 10 (within 72 h) Exhaled air (% dose): 0
References				
NA	NA	Timchalk et al. (1994); Grantham et al. (1979); Waring and Pheasant (1975)	Wenker (2005b), Wenker (2005a), Wenker (2005c), and Charles River Laboratories (2010) as cited in SCCS (2012), pages 50−52 and 56−57	Cunningham et al. (1989)

ADME = absorption, distribution, metabolism, and excretion; C_{max} = maximum concentration; GI = gastrointestinal; i.p. = intraperitoneal; i.v. = intravenous; NA = not applicable; ND = no data.

Table A-4. Comparison of Metabolic Pathway Transformations for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues from Meteor Nexus[a, b]

Pathway	3,4-TDA	2,3-TDA	2,4-TDA	2,5-TDA	2,6-TDA
5-Hydroxylation of 1,2,4-trisubstituted benzenes	1	0	1	1	0
Hydroxylamines from primary aromatic amines	1	1	1	1	1
Hydroxylation of methyl carbon next to an aromatic ring	1	1	1	1	1
N-Acetylation of primary aromatic amines	1	1	1	1	1
O-Sulfonation of aromatic alcohols	0	0	1	0	0
O-Sulfonation of *N*-hydroxy compounds	1	1	1	1	1
Oxidation of primary alcohols	1	1	1	1	1

[a]Meteor Nexus (Dimitrov et al., 2005; Mekenyan et al., 2004).
[b]1/0 denotes whether pathway transformation was identified/not identified.

TDA = toluenediamine.

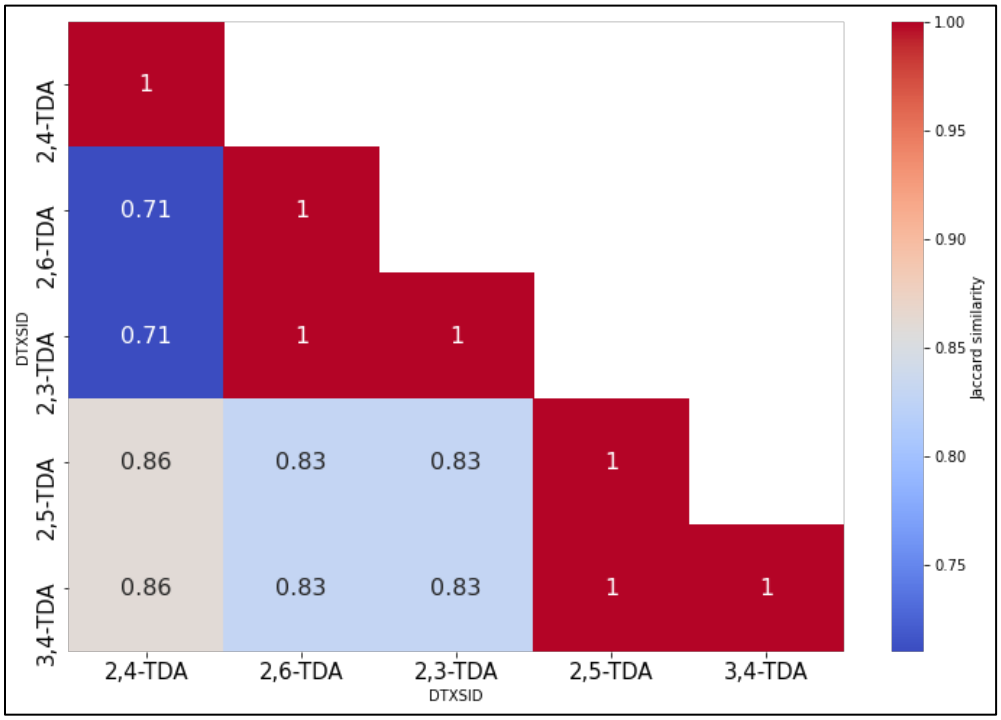


Figure A-1. Metabolic Pathway Similarities for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues. Heatmap displays Jaccard pairwise similarities rounded to two decimal places for the TDA compounds, comparing metabolic pathway transformations from Meteor Nexus (Dimitrov et al., 2005; Mekenyan et al., 2004).

Toxicity-Like Analogues

Table A-5 summarizes subchronic and chronic oral toxicity data for 3,4-TDA and the compounds identified as candidate analogues. None of the analogues had subchronic or chronic inhalation toxicity values from U.S. EPA, ATSDR, or CalEPA.

Relevant toxicity data in animals for 3,4-TDA alone comes from a single 5-day study in rats with limited information on toxicity endpoints and associated effect levels (increased mortality and incidence of perforated duodenal ulcers in dead animals at 1,000 mg/kg-day) (Selye, 1973). Oral toxicity values are available for 2,5- and 2,6-TDA. Hepatic effects were the basis for the subchronic and chronic provisional reference doses (p-RfDs) for 2,5-TDA using a point of departure (POD) of 2.5 mg/kg-day (1.4 mg/kg-day for the free base estimated for this assessment) (U.S. EPA, 2013). Thyroid and body-weight effects were the basis for the subchronic p-RfD for 2,6-TDA using a POD of 62 mg/kg-day (U.S. EPA, 2005b). The chronic p-RfD for 2,6-TDA was derived based on a POD of 25 mg/kg-day for no adverse effects (U.S. EPA, 2005b). Although thyroid toxicity was only noted following 2,6-TDA exposure, liver effects (ranging from changes in serum biomarkers and liver weight to gross and histopathological lesions) were reported after exposure to 2,4- (≥6 mg/kg-day), 2,5- (≥3 mg/kg-day), and 2,6-TDA (692 mg/kg-day) (U.S. EPA, 2013, 2005b; Criteria Group for Occupational Standards, 2001; WHO, 1987).

R/D toxicity was commonly seen with exposure to TDA compounds, but these endpoints were generally less sensitive than the systemic effects described above. Impaired male fertility and sperm damage were reported in male rats orally exposed to 2,4-TDA at 15 mg/kg-day (Criteria Group for Occupational Standards, 2001). Developmental effects were observed in laboratory animals orally exposed to 2,6- (≥100 mg/kg-day) or 2,5-TDA (≥44 mg/kg-day) during gestation, primarily at doses associated with potential maternal toxicity (U.S. EPA, 2013; WHO, 1987). Data on the *o*-TDA mixture (40:60 or 45:50 mixture of 2,3- and 3,4-TDA) showed possible evidence of R/D effects in rats and rabbits exposed to ≥100 mg/kg-day via gavage, including alterations in sperm measures (decreased spermatid number) and/or fetal viability and growth (decreased implantation sites, litter size, pup viability, and fetal weight, as well as increased post implantation loss, resorptions and skeletal variations) often accompanied by reductions in maternal body-weight gain (BASF, 2010; Becci et al., 1983). These findings suggest that the reproductive system and developing embryo/fetus may be toxicity targets of 3,4- and 2,3-TDA.

Acute lethality studies via different exposure routes were available for *o*- (2,3- and 3,4-) and *m*- (2,4- and 2,6-) TDA mixtures and individual TDA isomers (see Table A-6). The oral median lethal dose (LD_{50}) value for 2,5-TDA (102 mg/kg) in rats was lower than the oral LD_{50} values for *o*- (660 and 810 mg/kg) and *m*-TDA (270 and 300 mg/kg) mixtures. In mice, the i.p. LD_{50} values for 2,3-TDA (286 mg/kg) and a *m*-TDA mixture (240 mg/kg) were similar. Likewise, the rabbit dermal LD_{50} value for a *o*-TDA mixture (1,120 mg/kg) was similar to the rat dermal LD_{50} value for a *m*-TDA mixture (1,200 mg/kg). Central nervous system depression and methemoglobinemia were associated with high-dose, acute TDA toxicity in animals (WHO, 1987).

SAR evaluations have suggested increased chemical reactivity for *o*- and *p*-substituted aromatic amines such as 3,4-, 2,3-, and 2,5-TDA based on their oxidation potential into reactive quinones that can interact with glutathione to produce reactive oxygen species (ROS) (Bajot et al., 2010). In contrast, *m*-subsituted aromatic amines such as 2,4- and 2,6-TDA are less likely to

form quinones and are therefore expected to have decreased chemical reactivity (Bajot et al., 2010). The *o*- and *p*- substituents were also associated with enhanced, acute aquatic toxicity compared to *m*- substituents (Bajot et al., 2010). No experimental data was found to evaluate potential differences in chemical reactivity for the TDA isomers. The available evidence in animals shows consistency with respect to toxicity targets (primarily liver and R/D effects) among this group of compounds and although potency differences are apparent in some cases, there is no clear pattern with respect to the position of the amino groups.

In summary, limited toxicity data for 3,4-TDA from mixture studies reveals similarities in acute toxicity potency and R/D outcomes between the target and analogues. As such, the candidate analogues are considered suitable analogues for 3,4-TDA on the basis of toxicity similarity comparisons.

Table A-5. Comparison of Available Oral Toxicity Data and Health Reference Values for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

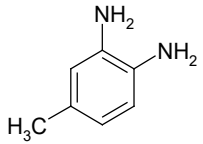
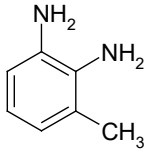
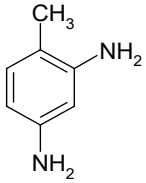
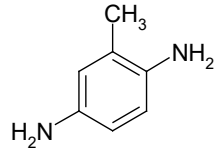
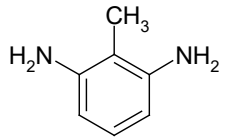
Parameter	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Role	Target	Analogue	Analogue	Analogue	Analogue
DTXSID	9024930	4027494	4020402	6029123	4027319
Structure					
Repeated-dose toxicity—short-term and subchronic studies					
Effects	In a 5-d study in rats (n = 13), exposure to 3,4-TDA (97% purity) at a dose of 1,000 mg/kg-d for 5 d resulted in 7/13 deaths; 6 animals had grossly observed perforated duodenal ulcers (refer to Table 5 in the main document for additional details).	NA	In a 14-d study in female mice (n = 6−8/group) exposed to 25, 50, or 100 mg/kg-d of 2,4-TDA (98.4% purity), increased B cells in spleens, and lower spleen weights were observed at ≥25 mg/kg-d and changes in hepatic enzymes in serum and elevated liver weights were observed at 100 mg/kg-d. Unclear whether organ-weight changes were absolute and/or relative. A 7-wk study exposed rats (n = 5/sex/group) to 0, 250, 500, 1,000, 2,000, or 3,000 ppm and mice	Groups of 15 male and female rats were gavaged with doses of 0, 2.5, 5, 10, or 20 mg/kg-d 2,5-TDA sulfate (99.7% purity) for 13 weeks. Increased serum AST in females at 5 mg/kg-d (3 mg/kg-d as free base), increased urine with decrease in specific gravity at ≥10 mg/kg-d (6 mg/kg-d as free base) and abnormally shaped pituitary glands at 20 mg/kg-d (11 mg/kg-d as free base) were observed (refer to the "Noncancer Oral Toxicity Values" section below for additional details).	A 91-d dietary exposure study in rats (n = 10/sex/group) dosed with 0, 100, 300, 1,000, 3,000, or 10,000 ppm of 2,6-TDA dihydrochloride (>99% purity). Thyroid hyperplasia in males and decreased body weight (both sexes) were observed at 192 mg/kg-d (as free base); thyroid hyperplasia was observed in females at 767 mg/kg-d (highest dose as free base). Other effects observed at the high dose (692 mg/kg-d in males and 767 mg/kg-d in females as free base) included thyroid enlargement;

Table A-5. Comparison of Available Oral Toxicity Data and Health Reference Values for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

Parameter	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Continued:	Continued:	Continued:	Continued: (*n* = 5/sex/group) to 0, 100, 200, 300, 500, 700, or 1,000 ppm of 2,4-TDA (>99.9% purity) via the diet. Decreased body weights, elevated hematopoiesis, and histopathological liver changes were observed in rats at 1,000 ppm (~75 mg/kg-d). The same diet resulted only in decreased body weights in mice.	Continued: In an accompanying range-finding study in rats (*n* = 10/sex/group) gavaged with 0, 7.5, 15, 30, or 60 mg/kg-d of 2,5-TDA sulfate, variations in serum CPK, AST, and LDH and increased absolute and relative liver weight were seen at ≥30 mg/kg-d (20 mg/kg-d as free base).	Continued: darkening of spleen, lymph nodes, liver, kidney, adrenals, and nasal turbinates; histopathological lesions in the kidney (nephrosis) and bone marrow (hyperplasia); and death. In the companion mouse study, reduced body weight was also observed; however, exposure levels were unclear. No pathological changes were noted.
Sources	Selye (1973)	NA	Bums et al. (1994) and NCI (1979) as cited in Criteria Group for Occupational Standards (2001)	Hill (1997, 1994) as cited in U.S. EPA (2013)	NTP (1980) as cited in U.S. EPA (2005b)
Repeated-dose toxicity—chronic studies					
Effects	NA	NA	A 2-yr NTP bioassay exposed groups of 20−50 rats (TWA doses of 0, 79, or 171−176 ppm) and mice (0, 100, or 200 doses) to 2,4-TDA (>99.9% purity). Reduced body weight and histopathological changes in the liver (lesions ranged from cellular alterations to severe	In a 78-wk cancer bioassay, rats and mice were exposed to 2,5-TDA toluene sulfate (>99% purity) at TWA concentrations of 0.06 or 0.2% and 0.06 or 0.1% in the diet, respectively (*n* = 50/species/sex/group); no exposure-related changes in clinical signs, survival,	In a 103-wk study in rats (*n* = 50/sex/group) exposed to 0, 250, or 500 ppm of 2,6-TDA dihydrochloride (>99%) in the diet, no adverse effects were reported up to 500 ppm (25 and 30 mg/kg-d in males and females, respectively as free base).

Table A-5. Comparison of Available Oral Toxicity Data and Health Reference Values for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

Parameter	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Continued:	Continued:	Continued:	Continued: degenerative changes in rats and hyperplasia in mice) were observed in exposed animals [at doses ~≥6 mg/kg-d in rats and ≥17 mg/kg-d in mice estimated for this analysis using U.S. EPA (1988) default values for BW and food intake]. Decreased survival, kidney histopathology (chronic renal disease) and secondary hyperthyroidism associated with renal disease were also reported in rats at same doses.	Continued: growth, or non-neoplastic histology were observed up to the highest doses tested (~95 mg/kg-d as free base).	Continued: In the companion mouse study (n = 50/sex/group) with exposures to 0, 50, or 100 ppm, no adverse effects were observed at doses up to 100 ppm 2,6-TDA dihydrochloride (10 mg/kg-d for both males and females as free base).
Source	NA	NA	NCI (1979) as cited in Criteria Group for Occupational Standards (2001) and WHO (1987)	NTP (1978) as cited in U.S. EPA (2013)	NTP (1980) as cited inU.S. EPA (2005b)

Table A-5. Comparison of Available Oral Toxicity Data and Health Reference Values for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

Parameter	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Reproductive/developmental studies					
Effects	An R/D screen in rats (*n* = 10/sex/group) exposed to a 45:50 mixture of 2,3- and 3,4-TDA (0, 10, 50, or 250 mg/kg-d doses) reported clinical signs, decreased food consumption and body weight, decreased number of spermatids/g testis, decreased number of implantation sites, increased post implantation loss, decreased litter size, and decreased pup viability at 250 mg/kg-d. Gestational exposure studies in pregnant rats (*n* = 22/group) and rabbits (*n* = 15/group) with exposure to a 40:60 mixture of 2,3- and 3,4-TDA (0, 10, 30, 100, or 300 mg/kg-d and 0, 3, 10, 30, or 100 mg/kg-d for rats and rabbits, respectively) reported increased incidences of skeletal variations at ≥100 mg/kg-d and reduced maternal body-weight gain and reduced fetal weight at 300 mg/kg-d in rats. Reduced maternal body-weight gain, reduced fetal weight, increased number of resorption sites, and decreased numbers of live fetuses/litter were observed at 100 mg/kg-d in rabbits. Refer to the "Reproductive/Developmental Studies of TDA Mixtures" section in the main document for additional study details.		Three studies evaluated male reproductive effects in rats (*n* = 8−10/group) after a 10-wk exposure in the diet containing 0, 0.01, or 0.03% 2,4-TDA (98% purity); they reported decreased fertility and inhibition of sperm production, altered serum reproductive hormones, and histological changes in the reproductive organs at the highest exposure level (~15 mg/kg-d).	Gestational exposure studies in animals (*n* = 16−30/group) gavaged with 2,5-TDA sulfate (purity unspecified) reported decreased maternal body weight at ≥50 mg/kg-d (28 mg/kg-d as free base) and increased resorptions at 80 mg/kg-d in rats (44 mg/kg-d as free base). Increased maternal and neonatal mortality were observed at 160 mg/kg-d in mice (88 mg/kg-d as free base), and no effects were observed in rabbits at doses up to 50 mg/kg-d (28 mg/kg-d as free base). Exposure doses were 0, 10, 50, or 80 mg/kg-d in rats (GD 6−15), 0, 10, 35, or 50 mg/kg-d in rabbits (GD 6−18) and 160 mg/kg-d in mice (GD 8−12).	A gestational exposure study (GDs 6−15) in rats (number of animals and 2,6-TDA purity not specified) gavaged with 0, 10, 30, 100, or 300 mg/kg-d showed increased incidence of hemorrhagic abdomens at ≥30 mg/kg-d and skeletal variations at ≥100 mg/kg-d following exposure to 2,6-TDA, with reduced maternal weight gain at 300 mg/kg-d. An accompanying study in rabbits (GDs 6−18) exposed to 0, 3, 10, 30, or 100 mg/kg-d showed reduced maternal weight, increased resorptions, decreased fetal weight, and decreased neonatal survival following gavage exposure to 2,6-TDA at 100 mg/kg-d.

Table A-5. Comparison of Available Oral Toxicity Data and Health Reference Values for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

Parameter	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Continued:	Continued:		Continued:	Continued: A 2-generation reproductive toxicity study in rats (n = 24/sex/group) exposed to 0, 5, 15, or 45 mg/kg-day 2,5-TDA sulfate (98.2% purity) reported no effects in clinical signs, body-weight gain, food consumption, male and female fertility or fetal growth and survival up to the highest dose (~25 mg/kg-d as free base).	Continued:
Source	BASF (2010); Becci et al. (1983)		Varma et al. (1988); Thysen et al. (1985a) and (1985b) as cited in Criteria Group for Occupational Standards (2001)	Bornatowicz (1986), Osterburg (1982), Seidenberg et al. (1986), and Kavlock et al. (1987) as cited in U.S. EPA (2013)	Knickerbocker et al. (1980) as cited in WHO (1987)
Health reference values—subchronic					
POD (mg/kg-d)	NA	NA	NA	2.5 (as 2,5-TDA sulfate); 1.4 (as free base estimated for this assessment)	62 (as free base)
POD type	NA	NA	NA	NOAEL	NOAEL
UF_C	NA	NA	NA	1,000 (UF_A, UF_D, UF_H)	1,000 (UF_A, UF_D, UF_H)
p-RfD (mg/kg-d)	NA	NA	NA	3×10^{-3} (as 2,5-TDA sulfate); 2×10^{-3} (as free base)[a] *Note:* screening value owing to use of secondary source.	6×10^{-2} (as free base)

Table A-5. Comparison of Available Oral Toxicity Data and Health Reference Values for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

Parameter	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Critical effects	NA	NA	NA	Increased serum AST in females.	Thyroid hyperplasia (males) and decreased body weight (both sexes).
Species	NA	NA	NA	Rat	Rat
Duration	NA	NA	NA	13 wk (91 d)	91 d
Route (method)	NA	NA	NA	Oral (gavage)	Oral (diet)
Source	NA	NA	NA	Hill (1997) as cited in U.S. EPA (2013)	NTP (1980) as cited in U.S. EPA (2005b)
Health reference values—chronic					
POD (mg/kg-d)	NA	NA	NA	2.5 (as 2,5-TDA sulfate); 1.4 (as free base estimated for this assessment)	25 (as free base)
POD type	NA	NA	NA	NOAEL	NOAEL
UF_C	NA	NA	NA	10,000 (UF_A, UF_D, UF_H, UF_S)	1,000 (UF_A, UF_D, UF_H)
p-RfD (mg/kg-d)	NA	NA	NA	3×10^{-4} (as 2,5-TDA sulfate); 2×10^{-4} (as free base)[a] *Note:* screening value owing to the use of secondary source and UF_C >3,000.	3×10^{-2} (as free base)
Critical effects	NA	NA	NA	Increased serum AST in females.	None
Species	NA	NA	NA	Rat	Rat
Duration	NA	NA	NA	13 wk	103 wk

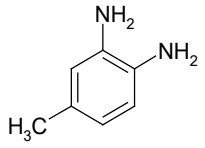
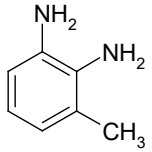
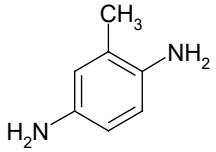
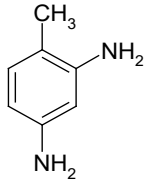
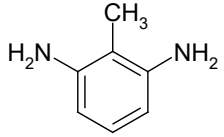
Table A-5. Comparison of Available Oral Toxicity Data and Health Reference Values for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

Parameter	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,4-Toluenediamine CASRN 95-80-7	2,5-Toluenediamine CASRN 95-70-5	2,6-Toluenediamine CASRN 823-40-5
Route (method)	NA	NA	NA	Oral (gavage)	Oral (diet)
Source	NA	NA	NA	Hill (1997) as cited in U.S. EPA (2013)	NTP (1980) as cited in U.S. EPA (2005b)

[a]The screening p-RfD values for 2,5-TDA as free base were calculated as follows: p-RfD for 2,5-TDA sulfate × (MW of 2,5-TDA as free base [122.17] ÷ MW of 2,5-TDA sulfate [220.25] = 0.55) (U.S. EPA, 2013).

AST = aspartate aminotransferase; BW = body weight; CPK = creatine phosphokinase; GD = gestation day; LDH = lactate dehydrogenase; MW = molecular weight; NA = not applicable; NOAEL = no-observed-adverse-effect level; NTP = National Toxicology Program; POD = point of departure; p-RfD = provisional reference dose; R/D = reproductive/developmental; TDA = toluenediamine; TWA = time-weighted average; 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.

Table A-6. Comparison of Available Acute Lethality Data for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues

Parameter	3,4-Toluenediamine CASRN 496-72-0	2,3-Toluenediamine CASRN 2687-25-4	2,5-Toluenediamine CASRN 95-70-5	2,4-Toluenediamine CASRN 95-80-7	2,6-Toluenediamine CASRN 823-40-5
Role	Target	Analogue	Analogue	Analogue	Analogue
DTXSID	9024930	4027494	6029123	4020402	4027319
Structure					
Rat oral LD_{50} (mg/kg)	660 (mix of 2,3- and 3,4-); 810 (mix of 2,3- and 3,4-)		102	270 and 300 (mix of 2,4- and 2,6-)	
Mouse oral LD_{50} (mg/kg)	NV		NV	350 (mix of 2,4- and 2,6-)	
Rat i.p. LD_{50} (mg/kg)	NV		NV	230 (mix of 2,4- and 2,6-) 325 (2,4-TDA technical grade)	
Mouse i.p. LD_{50} (mg/kg)	NV	286	NV	240 (mix of 2,4- and 2,6-) 90−480 (2,4-TDA technical grade)	
Rabbit dermal LD_{50} (mg/kg)	1,120 (mix of 2,3- and 3,4-)		NV	NV	
Rat dermal LD_{50} (mg/kg)	NV		NV	1,200 (mix of 2,4- and 2,6-)	
Source	Air Products and Chemicals (1976); Carpenter et al. (1974); NLM (2019)		NLM (2019)	Izmerov et al. (1982), Weisbrod and Stephan (1983), Grantham et al. (1979), and Weisburger et al. (1978) as cited in WHO (1987)	

i.p. = intraperitoneal; LD_{50} = median lethal dose; NV = not available; TDA = toluenediamine.

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 mode of action (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.

Oral

2,5- and 2,6-TDA were identified as structural analogues of 3,4-TDA with available noncancer oral toxicity values. Two additional structural analogues were included in the read-across analysis, 2,3- and 2,4-TDA, to provide information on the potential influence of the position of the amino groups on toxicity. The analogues share a basic structure with the target compound (a benzene ring, two amino groups, and a methyl group, differing only in the position of the amino functional groups) and have similar physicochemical properties (i.e., water solubility, log K_{ow}, volatility, etc.; see Table A-1) important for bioavailability. 3,4-TDA and its analogues also showed similar SA predictions for repeated-dose toxicity and R/D endpoints (see Table A-2). Evidence from oral and i.p. exposure studies in rodents suggests that the TDA analogues are predominantly metabolized via acetylation of amino groups, ring hydroxylation, and potential oxidation of methyl groups (see Table A-3). A comparative analysis of metabolite predictions across different software tools revealed a similar metabolic profile for the target compound and analogues and confirmed observations from in vivo studies (see "Metabolic Analogues" section above and Appendix D for more details). Oral exposure studies in animals showed commonalities in target tissues for the TDA analogues, most notably, liver and R/D toxicities (see Table A-5). No adequate toxicity data is available for 3,4-TDA; however, studies evaluating TDA mixtures containing 3,4-TDA suggest similarities between the target and analogues with respect to acute toxicity potency and R/D outcomes (see Tables A-5 and A-6).

Similarities in structure, physicochemical properties, SA, and metabolite predictions and limited toxicity data support the suitability of both 2,5- and 2,6-TDA (the two analogues with available toxicity values) as analogues of 3,4-TDA. 2,5-TDA is selected as the most appropriate analogue for deriving screening p-RfDs based on mechanistic considerations and health protectiveness. Although it is unclear how the position of the amino groups could affect the repeated-dose toxicity of TDA compounds, the *o*- and *p*- isomers (3,4- and 2,5-TDA, respectively) are expected to have greater chemical reactivity (related to quinone formation) than the *m*- isomer (2,6-TDA). Furthermore, the POD values for 2,5-TDA (1.4 mg/kg-day for both the subchronic and chronic p-RfDs) are more than an order of magnitude lower than the POD values for 2,6-TDA (62 and 25 mg/kg-day for the subchronic and chronic p-RfDs, respectively).

Inhalation

None of the candidate analogues have repeated-dose inhalation toxicity values, precluding derivation of screening provisional reference concentrations (p-RfCs).

NONCANCER ORAL TOXICITY VALUES

Derivation of a Screening Subchronic Provisional Reference Dose

Based on the overall analogue approach presented in this PPRTV assessment, 2,5-TDA is selected as the most appropriate analogue for 3,4-TDA for deriving a screening subchronic p-RfD. The principal study used for the U.S. EPA screening subchronic p-RfD for 2,5-TDA was a 13-week rat study [Hill (1997) as cited in SCCP (2007) and reported by U.S. EPA (2013)]. U.S. EPA (2013) described the study as follows:

> *Hill (1997, as cited in SCCP, 2007) administered toluene-2,5-diamine sulfate (99.7% pure) via gavage in deionized water to Sprague-Dawley rats (15/sex/dose) at 0, 2.5, 5, 10, or 20 mg/kg-day for 13 weeks. The original report for this study is not available; SCCP briefly described the study. Animals were observed daily for mortality and clinical signs. Body weights and food intake were recorded weekly. Ophthalmoscopic examinations were performed on all animals before the initiation of treatment and during Week 13. Blood and urine samples were collected during Week 4 and during Week 12 or 13. Following treatment, all animals were sacrificed and necropsied. Organ weights were recorded, and tissues were subjected to microscopic examination. No dose-related changes in mortality, clinical signs, body weights, body-weight gains, or food consumption were reported (data not shown). The researchers did not consider hematological variations (not further described) to be treatment-related. Aspartate aminotransferase (AST) levels were statistically significantly ($p < 0.05$) increased in females at doses of ≥5 mg/kg-day (data not shown). Increased urine levels, associated with a statistically ($p < 0.05$) significant decrease in specific gravity, were observed at ≥10 mg/kg-day (females) or 20 mg/kg-day (males) (data not shown). Although retinopathy was observed in some animals, a pathology peer review concluded that the incidence of these effects in the treatment groups was similar to the spontaneous incidence for Sprague-Dawley rats. At 20 mg/kg-day, an increased incidence of abnormally shaped pituitary glands was reported. The SCCP (2007) identified a NOAEL of 2.5 mg/kg-day for toluene-2,5-diamine sulfate in this study based on significantly elevated AST levels at 5 mg/kg-day. However, experimental data were not presented in the summary, and the adversity of the reported effects has not been demonstrated (there was no mention of the magnitude or dose-response of the observed change in AST, or corresponding changes in other serum enzymes or liver pathology). The available description of this study lacked information to support independent evaluation of the study.*

> *The apparent NOAEL of 2.5 mg/kg-day and LOAEL of 5 mg/kg-day for increased serum AST levels in rats treated with toluene-2,5-diamine sulfate by gavage in water for 13 weeks (Hill, 1997, as cited in SCCP, 2007) can be used as the basis for derivation of screening provisional toxicity values for toluene-2,5-diamine sulfate and toluene-2,5-diamine. Based on available information, this appeared to be the most sensitive endpoint identified in the available studies. The choice of endpoint was supported by the results of the 14-day range-finding study, which reported changes in AST and other clinical chemistry measures at 30 mg/kg-day (Hill, 1994, as cited in SCCP, 2007).*

Reproductive and developmental toxicity studies reported effects only at higher doses (80−160 mg/kg-day) (Kavlock et al., 1987; Seidenburg et al., 1986; Osterberg, 1982a,b, as cited in SCCP, 2007 and reviewed in Pang, 1992).

The critical effect identified in the U.S. EPA (2013) assessment for 2,5-TDA was increased serum aspartate aminotransferase (AST) in female rats exposed for 13 weeks (Hill, 1997). A no-observed-adverse-effect level (NOAEL) of 2.5 mg/kg-day for increased AST was selected as the POD in the screening subchronic p-RfD for 2,5-TDA sulfate (U.S. EPA, 2013). The corresponding POD for the free base is calculated by multiplying the POD for the sulfate by the ratio of the molecular weights (MW of 2,5-TDA as free base [122.17] ÷ MW of 2,5-TDA sulfate [220.25] = 0.55). The resulting NOAEL of 1.4 mg/kg-day for 2,5-TDA is adopted as the POD for deriving the screening subchronic p-RfD for 3,4-TDA. The NOAEL of 1.4 mg/kg-day is not adjusted for molecular-weight differences between 3,4- and 2,5-TDA (both as free base), because the molecular weights are identical.

The NOAEL of 1.4 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 body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD from effects that are not portal-of-entry effects.

Following U.S. EPA (2011c) guidance, the POD for increased serum AST in female rats is converted to an HED through the application of 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 in a subchronic study 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 NOAEL of 1.4 mg/kg-day yields a POD (HED) as follows:

$$\begin{aligned} \text{POD (HED)} &= \text{NOAEL (mg/kg-day)} \times \text{DAF} \\ &= 1.4 \text{ mg/kg-day} \times 0.23 \\ &= 0.32 \text{ mg/kg-day} \end{aligned}$$

In deriving a screening p-RfD for 3,4-TDA, a composite uncertainty factor (UF_C) of 300 is applied, based on a 3-fold uncertainty factor value for interspecies extrapolation (interspecies uncertainty factor [UF_A], reflecting use of a dosimetric adjustment) and 10-fold uncertainty factor values for both intraspecies variability (UF_H) and database deficiencies (database uncertainty factor [UF_D], reflecting lack of adequate repeated dose toxicity information for 3,4-TDA). The screening subchronic p-RfD for 3,4-TDA is derived as follows:

Screening Subchronic p-RfD = Analogue POD (HED) ÷ UF_C
= 0.32 mg/kg-day ÷ 300
= **1×10^{-3} mg/kg-day**

Table A-7 summarizes the uncertainty factors for the screening subchronic p-RfD for 3,4-TDA.

Table A-7. Uncertainty Factors for the Screening Subchronic p-RfD for 3,4-Toluenediamine (CASRN 496-72-0)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for residual uncertainty, including toxicodynamic differences, between rats and humans following 3,4-TDA exposure. The toxicokinetic uncertainty has been accounted for by calculation of an HED through application of a DAF in extrapolating from animals to humans (U.S. EPA, 2011c).
UF_D	10	A UF_D of 10 is applied owing to the absence of adequate repeated-dose toxicity studies for 3,4-TDA alone and the use of a read-across approach to derive the screening p-RfD.
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 3,4-TDA in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a NOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic study was selected as the principal study.
UF_C	300	Composite uncertainty factor = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

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; TDA = toluenediamine; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Derivation of a Screening Chronic Provisional Reference Dose

2,5-TDA is also selected as the most appropriate analogue for 3,4-TDA for deriving the screening chronic p-RfD. U.S. EPA (2013) used the critical effect of increased AST levels in female rats and associated POD of 2.5 mg/kg-day (HED of 0.32 mg/kg-day estimated for this assessment) identified in the 13-week rat study (Hill, 1997) to derive a screening chronic p-RfD for 2,5-TDA. The principal study and calculation of the POD (HED) is described above. Although a cancer bioassay in rats and mice exposed to 2,5-TDA for 78 weeks was available (NTP, 1978), the U.S. EPA (2013) assessment concluded that the study was inadequate in scope and design for evaluating noncancer oral toxicity based on the following: (1) evaluations were limited to measures of body weight, food consumption, clinical signs, and non-neoplastic histopathology; (2) histopathological examinations were conducted after a lengthy recovery period (28−31 weeks); and (3) treatment was initiated at different times for the low- and high-dose groups (~11 months apart).

In deriving the screening chronic p-RfD for 3,4-TDA, the POD (HED) of 0.32 mg/kg-day from the 13-week rat study with 2,5-TDA is selected, applying an additional uncertainty factor of 10 to account for increased uncertainty associated with extrapolating from a subchronic to a chronic exposure (UF_S). A UF_C of 3,000 was derived, reflecting a 3-fold UF_A, and 10-fold uncertainty factor values for UF_H, UF_S, and UF_D. Finally, the screening chronic p-RfD for 3,4-TDA is derived as follows:

$$\begin{aligned} \textbf{Screening Chronic p-RfD} &= \text{Analogue POD (HED)} \div UF_C \\ &= 0.32 \text{ mg/kg-day} \div 3{,}000 \\ &= \mathbf{1 \times 10^{-4} \text{ mg/kg-day}} \end{aligned}$$

Table A-8 summarizes the uncertainty factors for the screening chronic p-RfD for 3,4-TDA.

Table A-8. Uncertainty Factors for the Screening Chronic p-RfD for 3,4-Toleuenediamine (CASRN 496-72-0)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for residual uncertainty, including toxicodynamic differences, between rats and humans following 3,4-TDA exposure. The toxicokinetic uncertainty has been accounted for by calculation of an HED through application of a DAF in extrapolating from animals to humans (U.S. EPA, 2011c).
UF_D	10	A UF_D of 10 is applied owing to the absence of adequate repeated-dose toxicity studies for 3,4-TDA alone and the use of a read-across approach to derive the screening p-RfD.
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 3,4-TDA in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a NOAEL.
UF_S	10	A UF_S of 10 is applied because a subchronic study was selected as the principal study.
UF_C	3,000	Composite uncertainty factor = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

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; TDA = toluenediamine; 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.

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 3,4-toluenediamine (3,4-TDA). 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 through 8 apply to any target chemical and are described in this appendix. Chemical-specific data for all steps in this process are summarized in Appendix C.

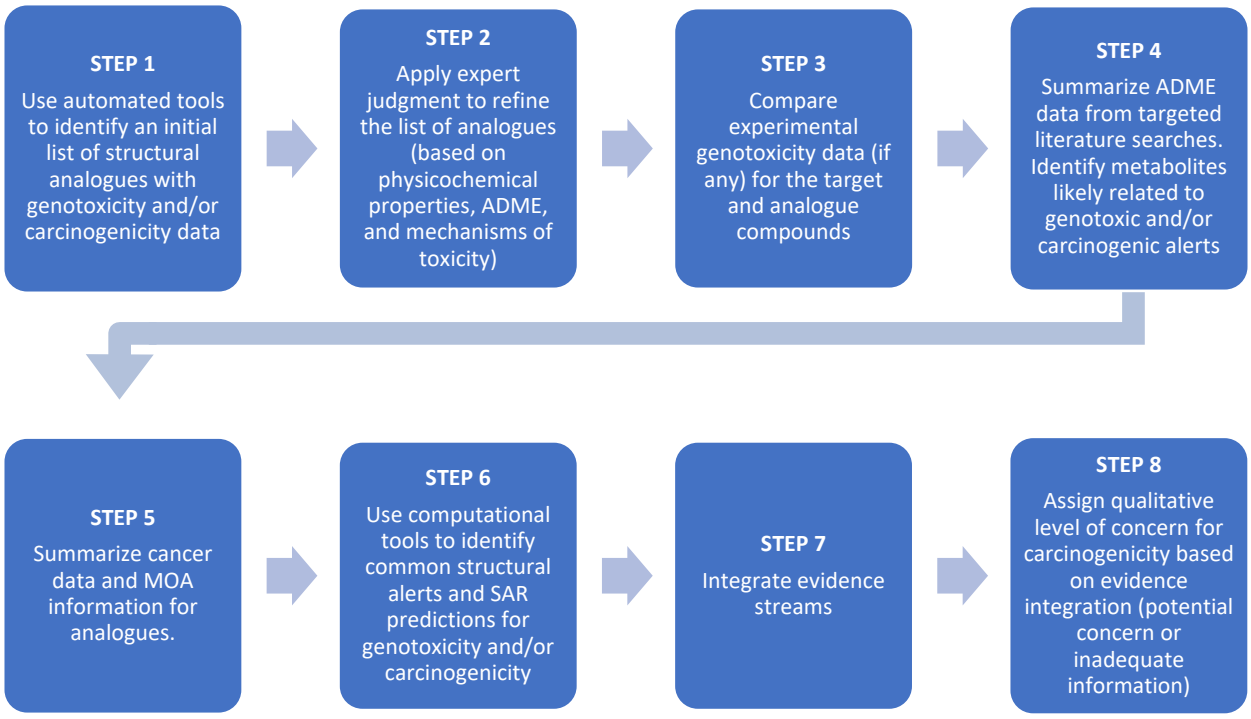


Figure B-1. Steps Used in the Screening Evaluation of Potential Carcinogenicity

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

ChemACE Clustering

The U.S. EPA's Chemical Assessment Clustering Engine [ChemACE; U.S. EPA (2011a)] is an automated tool that groups (or clusters) a user-defined list of chemicals based on chemical structure fragments. The methodology used to develop ChemACE was derived from U.S. EPA's Analog Identification Methodology (AIM) tool, which identifies structural analogues for a chemical based on common structural fragments. ChemACE uses the AIM structural fragment recognition approach for analogue identification and applies advanced queries and user-defined rules to create the chemical clusters. The ChemACE 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 clustering 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.

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.

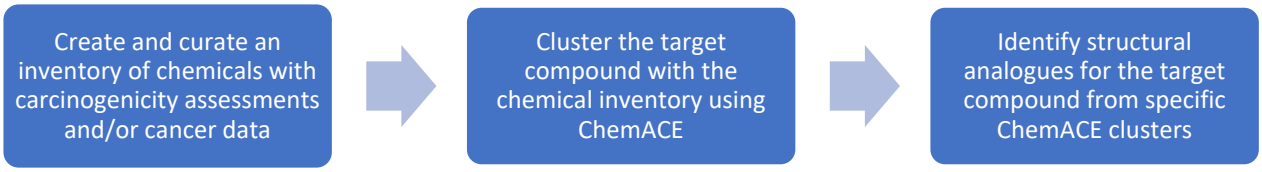


Figure B-2. Overview of ChemACE Clustering Process

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 (2016a)]
- NTP technical reports (NTP, 2017)
- Integrated Risk Information System (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, 2017)
- 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 a CASRN or unambiguous chemical identification; and
3) Substance has a unique Simplified Molecular Input Line Entry System (SMILES) notation (encoded molecular structure format used in ChemACE) that can be identified from one of these sources:
   a. Syracuse Research Corporation (SRC) and Distributed Structure-Searchable Toxicity (DSSTox) database lists of known SMILES associated with unique CASRNs (the combined lists contained >200,000 SMILES) or
   b. ChemIDplus, U.S. EPA 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 Method)

The OECD QSAR Toolbox (Version 4.1) is used to search for additional structural analogues of the target compound. There are several structural similarity score equations available in the Toolbox (Dice, Tanimoto, Kulczynski-2, Ochiai/Cosine, and Yule). Dice is considered the default equation. The specific options that are selected for the performance of 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 carcinogenicity or genotoxicity (see Table B-1).

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

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

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

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

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

[a]Descriptions were obtained from the OECD QSAR Toolbox documentation [Version 4.1; OECD (2017)].

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 3,4-TDA 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 SAs and Predict Carcinogenicity and Genotoxicity

Name	Description[a]
OECD QSAR Toolbox (Version 4.1)	Seven OECD QSAR Toolbox profiling methods were used, including: • Carcinogenicity (genotox and nongenotox) alerts by ISS (Version 2.3); updated version of the module originally implemented in Toxtree. It 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 assay 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. It 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 corresponds to 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 o Aliphatic halide (general) o Aliphatic halide (specific) o Aliphatic halogens o Aromatic amine (general) o Aromatic amine (specific) o Aromatic amines o Aromatic and aliphatic substituted primary alkyl halides o Aromatic nitro (general) o Aromatic nitro (specific) o Aromatic nitro groups o Nitroarenes o Nitro-aromatic o Primary and secondary aromatic amines o Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group • Nongenotoxic carcinogenicity o Aliphatic halogens

Table B-2. Tools Used to Identify SAs and Predict Carcinogenicity and Genotoxicity

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 Institute with automatic tools
Toxtree (Version 2.6.13)	Toxtree estimates toxic hazard by applying a decision tree approach. Chemicals were queried in Toxtree using the Benigni/Bossa rulebase for mutagenicity and carcinogenicity. If a potential carcinogenic alert based on any QSAR model or if any SA for genotoxic and nongenotoxic carcinogenicity was reported, then the prediction was recorded as a positive carcinogenicity prediction for the test chemical. The output definitions from the tool manual are listed below: • SA for genotoxic carcinogenicity (recognizes the presence of one of more SAs and specifies a genotoxic mechanism) • SA for nongenotoxic carcinogenicity (recognizes the presence of one or more SAs, and specifies a nongenotoxic mechanism) • Potential *Salmonella typhimurium* TA100 mutagen based on QSAR • Unlikely to be a *S. typhimurium* TA100 mutagen based on QSAR • Potential carcinogen based on QSAR (assigned according to the output of QSAR8 aromatic amines) • Unlikely to be a carcinogen based on QSAR (assigned according to the output of QSAR8 aromatic amines) • Negative for genotoxic carcinogenicity (no alert for genotoxic carcinogenicity) • Negative for nongenotoxic carcinogenicity (no alert for nongenotoxic carcinogenicity)

Table B-2. Tools Used to Identify SAs and Predict Carcinogenicity and Genotoxicity

Name	Description[a]
VEGA	VEGA applies several QSARs to a given chemical, as described below: • Mutagenicity (Ames test) CONSENSUS model: a consensus assessment is performed based on predictions of the VEGA mutagenicity models (CAESAR, SARpy, ISS, and *k*-NN) • Mutagenicity (Ames test) model (CAESAR): integrates 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 analysis 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 database • Carcinogenicity model (ISS): built implementing the same alerts Benigni and Bossa (ISS) implemented in the software Toxtree 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 European Union-funded project ANTARES • Carcinogenicity model (IRFMN/ISSCAN-CGX): based on a set of rules (43 SAs) extracted with the SARpy software from a data set of 986 compounds; the data set of carcinogenicity of different species was provided by Kirkland et al. (2005)

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

ANTARES = Alternative 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; 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. EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF 3,4-TDA 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 CARCINOGENICITY AND/OR GENOTOXICITY DATA

U.S. EPA's Chemical Assessment Clustering Engine (ChemACE) clustering was performed as described in Appendix B. The cluster containing 3,4-toluenediamine (3,4-TDA; less restrictive approach; Cluster 71) also contains 2,3-toluenediamine (2,3-TDA; an additional target compound being evaluated in a separate Provisional Peer-Reviewed Toxicity Value [PPRTV] document) and 13 structural analogues. The 15 cluster members all contain a benzene ring substituted with one or more amino groups (−NR_2) and one or more methyl groups (−CH_3). The methyl groups are present on the ring or the nitrogen substituent (−$N(CH_3)_2$) (see Figure C-1).

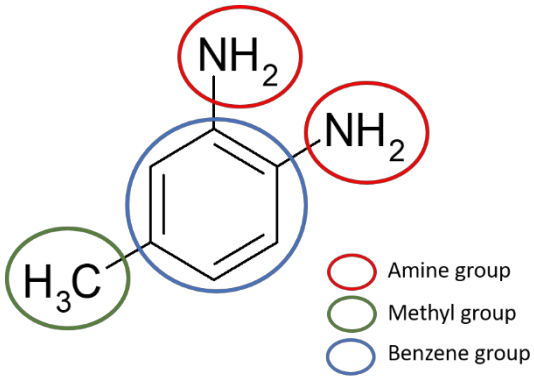


Figure C-1. Illustration of Common Fragments in Cluster 71

The Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox Profiler was used to identify structural analogues from the Dice analogue search with carcinogenicity and/or genotoxicity data (see Step 1 methods in Appendix B). This process identified an additional 49 compounds to be considered as potential analogues for 3,4-TDA. 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 (1) one aromatic ring (benzene) substituted with (2) two unsubstituted amines on the ring, in a *meta* (*m*)- or *para* (*p*)-substitution pattern, (3) a methyl group on the ring, and (4) no other functional group. Such compounds are similar to the target chemical in all attributes except for the proximity of the two amine substituents to one another on the aromatic ring. The closest analogue structurally for

3,4-TDA would be 2,3-TDA because of the similar *ortho* (*o*)-substitution pattern; however, 2,3-TDA does not have adequate experimental data for evaluating potential carcinogenicity and was not considered further as an analogue. Simple salts (e.g., hydrochlorides or sulfates) of the *m*- and *p*-substituted diamines are also considered as potential analogues.

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 the presence of polycyclic aromatics or ring systems other than toluene; lack of two amine substituents; occurrence of functional groups not present in the target chemicals (e.g., phenols, halogens, carboxylic acids); *N*-alkyl-substituted amines and acetamide derivatives of aromatic amines. In addition, nitro amines and dinitro compounds were not selected. Each of these attributes introduce significant differences in bioavailability, reactivity, and applicable metabolic pathways relative to 3,4-TDA. Additionally, ar-methyl-1,3-benzenediamine (CASRN 25376-45-8) was not selected for further review because it can exist as a mixture of two TDA isomers, in which the location of the methyl on the aromatic ring is not defined.

The remaining nine possible analogues for 3,4-TDA are listed in Table C-1. The existence of a cancer risk estimate and/or a weight-of-evidence (WOE) determination for cancer is indicated for each analogue. Compounds are grouped with their respective simple salts, which were identified by Dice only. Salts did not cluster with free acids in ChemACE because it is fragment-based; therefore, salts and free acids have different fragments and will not cluster without special treatment (i.e., modify the Simplified Molecular Input Line Entry System [SMILES] being clustered so that representative free acid structures are entered for salts). The analogue results from Dice are based on SMILES arbitrary target specification (SMARTS) substructure searching, allowing for identification of both free acid and respective salt analogues.

Table C-1. Summary of Cancer Assessment Information for Analogues of 3,4-Toluenediamine (CASRN 496-72-0)

Analogue Name (CASRN)	Cancer Risk Estimates (if available)	WOE Determinations
2,6-TDA (823-40-5)[a, b] 2,6-TDA dihydrochloride (15481-70-6)[b]	None	U.S. EPA (2005b)—*inadequate information*
2,5-TDA (95-70-5)[a, b] 2,5-TDA dihydrochloride (615-45-2)[b] 2,5-TDA sulfate (6369-59-1 and 615-50-9)[b]	U.S. EPA (2013)—screening p-OSF	U.S. EPA (2013)—*suggestive* IARC (1987)—*not classifiable*
2,4-TDA (95-80-7)[a, b] 2,4-TDA dihydrochloride (636-23-7)[b]	CalEPA (2011a)—OSF, IUR	IARC (1987)—*possibly* NTP (2016b)—*reasonably anticipated* CalEPA (2011a)—*known*
2,3-Dimethylbenzene-1,4-diamine (5306-96-7)[b]	None	None
2,5-Dimethylbenzene-1,4-diamine (6393-01-7)[b]	None	None

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

IUR = inhalation unit risk; OSF = oral slope factor; p-OSF = provisional oral slope factor; TDA = toluenediamine; WOE = weight of evidence.

2,3-Dimethylbenzene-1,4-diamine and 2,5-dimethylbenzene-1,4-diamine, which lack cancer risk estimates or WOE determinations (highlighted in gray in Table C-1), were not further considered as potential analogues for the screening evaluation of potential carcinogenicity of 3,4-TDA. Compounds selected for further consideration were 2,4-TDA, 2,5-TDA, and 2,6-TDA and their simple salts.

STEP 3. COMPARISON OF THE EXPERIMENTAL GENOTOXICITY DATA FOR 3,4-TDA AND ANALOGUES

The available genotoxicity data for 3,4-TDA are described in detail in the "Other Data" section in the main body of this report. Briefly, the data indicate that 3,4-TDA is mutagenic in bacterial systems with metabolic activation; however, evidence for mutation in mammalian cells is equivocal. 3,4-TDA induces cell transformation in mammalian cells at cytotoxic concentrations and also induces micronuclei (MN) and inhibits deoxyribonucleic acid (DNA) synthesis in vivo. A summary of the genotoxicity data for the structural analogues, 2,4-, 2,5-, and 2,6-TDA, is provided below for comparative purposes.

2,4-, 2,5-, and 2,6-TDA are mutagenic to *Salmonella typhimurium* in the presence of metabolic activation (U.S. EPA, 2013; ECHA, 2008; U.S. EPA, 2005b). Sex-linked recessive mutations were observed in *Drosophila melanogaster* exposed to 2,4-TDA (ECHA, 2008). However, 2,4-, 2,5-, and 2,6-TDA were generally nonmutagenic to mammalian cells in vitro or in vivo (U.S. EPA, 2013; ECHA, 2008; U.S. EPA, 2005b).

2,4-, 2,5-, and 2,6-TDA show evidence of in vitro clastogenicity in mammalian cells, both with and without metabolic activity. Chromosomal aberrations (CAs) were induced by 2,4- and 2,5-TDA, sister chromatid exchanges (SCEs) were induced by 2,4-TDA, and MN were induced by 2,6-TDA (U.S. EPA, 2013; ECHA, 2008; U.S. EPA, 2005b). Induction of MN in bone marrow or hepatocytes was generally not observed following in vivo exposure to 2,4-, 2,5-, or 2,6-TDA. However, weak induction of MN in bone marrow following exposure to 2,4- or 2,6-TDA was reported in some studies (Takasawa et al., 2013; U.S. EPA, 2013; ECHA, 2008; U.S. EPA, 2005b).

The majority of in vitro studies indicate that 2,4-, 2,5-, and 2,6-TDA are capable of damaging mammalian DNA. Results were most consistent with 2,4-TDA, which induced DNA damage and/or unscheduled DNA synthesis (UDS) in human skin fibroblasts, human hepatocytes, and primary rat hepatocytes, and formed DNA adducts in rat hepatocytes and purified calf thymus DNA (ECHA, 2008). 2,5-TDA also induced DNA damage in rat and hamster hepatocytes (U.S. EPA, 2013). UDS was observed in primary cultured human hepatocytes exposed to 2,6-TDA, but not primary rat hepatocytes (U.S. EPA, 2005b). Low levels of covalent binding to DNA were observed for 2,6-TDA (U.S. EPA, 2005b). DNA strand breaks and UDS were consistently reported in rodents following in vivo exposure to 2,4-TDA (ECHA, 2008), but results in rodents exposed to 2,5- or 2,6-TDA were mixed (U.S. EPA, 2013, 2005b). DNA adducts were observed in multiple organs following in vivo exposure to 2,4-, but not 2,6-TDA (ECHA, 2008; U.S. EPA, 2005b). 2,5- and 2,6-TDA induced cell transformation in hamster embryo cells (U.S. EPA, 2013, 2005b).

In summary, the available genotoxicity data suggest some commonalities between the target compound and TDA analogues. Like 3,4-TDA, the TDA analogues are mutagenic in bacterial systems with metabolic activation and show some evidence of genotoxicity in mammalian cells, including clastogenic effects and DNA damage under certain conditions.

STEP 4. TOXICOKINETICS OF 3,4-TDA AND ANALOGUES

The toxicokinetics of 3,4-, 2,4-, 2,5-, and 2,6-TDA are briefly described in Table C-2 (see additional information in Table A-3). Experimental data indicate that 2,4-, 2,5-, and 2,6-TDA are rapidly absorbed following oral exposure and excreted in the urine (see Table A-3). The primary metabolic pathways for 2,4-, 2,5-, and 2,6-TDA include acetylation of the amino groups and ring hydroxylation with some evidence of oxidation of the methyl group (see Table A-3). No toxicokinetic data are available for 3,4-TDA, but similar metabolic pathways are expected for the target compound based on a comparative in silico metabolism analysis (see section on "Metabolic Analogues" in Appendix A and Appendix D for additional details).

Table C-2. Summary of Toxicokinetic Data for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues

Compound	Absorption, Distribution, Excretion	Metabolism	References
3,4-TDA	ND	ND	NA
2,4-TDA	• Rapid and extensive absorption • Wide distribution • Primarily excreted in urine, with small amounts in feces	• Primary pathways include acetylation of the amino groups and ring hydroxylation • Primary urinary metabolites: 3-hydroxy-4-acetylamino-2-aminotoluene, 5-hydroxy-2,4-diaminotoluene, and 5-hydroxy-4-acetylamino-2-aminotoluene	Timchalk et al. (1994); Grantham et al. (1979); Waring and Pheasant (1975)
2,6-TDA	• Rapid and extensive absorption • Wide distribution • Primarily excreted in urine, with small amounts in feces	• Primary pathways include acetylation of the amino groups and ring hydroxylation • Primary urinary metabolites: 3-hydroxy-2,6-toluenediamine, 5-hydroxy-2-acetylamino-6-aminotoluene, 2-acetylamino-6-aminotoluene, and 2,6-diacetylamino-toluene	Cunningham et al. (1989)
2,5-TDA	• Rapid and extensive absorption • Wide distribution • Primarily excreted in urine, with small amounts in feces	• Primary pathways include acetylation of the amino groups and ring hydroxylation • Primary urinary metabolites: *N,N′*-diacetyl-toluene-2,5-diamine	Wenker (2005a), Wenker (2005b), Wenker (2005c) and Charles River Laboratories (2010) as cited in SCCS (2012), pages 50−52 and 56−57

NA = not applicable; ND = no data; TDA = toluenediamine.

STEP 5. CARCINOGENICITY OF 3,4-TDA ANALOGUES AND MODE-OF-ACTION DISCUSSION

U.S. EPA cancer WOE descriptors for 3,4-TDA 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 3,4-TDA. The analogue 2,5-TDA is characterized by U.S. EPA as having evidence of carcinogenic potential. Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *"Suggestive Evidence of Carcinogenic Potential"* for 2,5-TDA (U.S. EPA, 2013). The U.S. EPA has not assessed the potential carcinogenicity of 2,4-TDA (U.S. EPA, 1991); however, this compound is listed as a carcinogen by CalEPA (2011a), considered *possibly carcinogenic to humans* by IARC (1987) and *reasonably anticipated to be a human carcinogen* by NTP (2016a). The U.S. EPA determined that there is *"Inadequate Information to Assess Carcinogenic Potential"* for 2,6-TDA (U.S. EPA, 2005b). Oral slope factor (OSF) values varied by an order of magnitude, with the highest potency value calculated for 2,4-TDA (4×10^{0} [mg/kg-day]$^{-1}$) (CalEPA, 2011b) and the lowest (screening) potency value for 2,5-TDA (1.8×10^{-1} [mg/kg-day]$^{-1}$ as a sulfate) (U.S. EPA, 2013). Exposure-related increases were observed in liver tumors in male and female rats and female mice, subcutaneous fibromas in male rats, mammary tumors in female rats, and lymphoma in female mice following dietary 2,4-TDA exposure (NTP, 2016a; CalEPA, 2011a; IARC, 1987). Testicular tumors were observed in male rats and lung tumors were observed in female mice following dietary exposure to 2,5-TDA (U.S. EPA, 2013). Potential carcinogenic effects of

2,6-TDA were evaluated in rats and mice in 2-year feeding studies (U.S. EPA, 2005b; NTP, 1980). Dose-related trends for increased incidence of hepatocellular carcinomas and islet-cell adenomas of the pancreas were observed in male rats, a slight increase in vascular neoplasm of the spleen and liver and a significant trend in increased lymphomas were observed in male mice, and a significant trend for increased hepatocellular carcinomas was reported in female mice (U.S. EPA, 2005b). The study authors did not consider the neoplastic lesions observed with exposure to 2,6-TDA to be treatment related due to the absence of statistically significant effects in any treatment group compared to controls, but it was unclear whether exposure levels were adequate to assess carcinogenic potential (U.S. EPA, 2005b). The carcinogenic mode of action (MOA) has not been established for 2,4- or 2,5-TDA, although both compounds (along with 2,6-TDA and the target compound, 3,4-TDA) exhibit some evidence of genotoxicity (see "Step 3. Comparison of the Experimental Genotoxicity Data for 3,4-TDA and Analogues" above for more information).

Table C-3. Comparison of Available Oral Carcinogenicity Data for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues

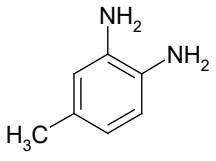
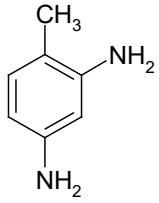
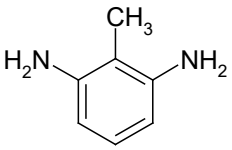
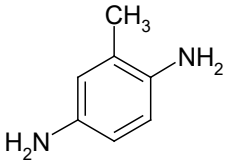
Type of Data	3,4-TDA CASRN 496-72-0	2,4-TDA CASRN 95-80-7	2,6-TDA CASRN 823-40-5	2,5-TDA CASRN 95-70-5
Role	Target	Analogue	Analogue	Analogue
Structure				
U.S. EPA WOE characterization	*"Inadequate Information to Assess Carcinogenic Potential"* (see Table 7)	NA[a]	*"Inadequate Information to Assess Carcinogenic Potential"*	*"Suggestive Evidence of Carcinogenic Potential"*
Oral slope factor $(mg/kg\text{-}d)^{-1}$	NA	4×10^{0} [b]	ND	Screening p-OSF: 1×10^{-1} (as sulfate); screening p-OSF: 1.8×10^{-1} (as free base)
Data set(s) used for slope factor derivation	NA	NTP (1978): mammary gland tumors in female F344 rats	NTP (1980) studies were considered insufficient to assess carcinogenic potential; results were not considered treatment related but doses were too low, and a maximum tolerated dose was not achieved	NTP (1978): interstitial-cell tumors of the testis in male F344 rats
Other tumors observed in animal bioassays	NA	Liver tumors in rats and mice; subcutaneous fibroma in male rats; lymphoma in female mice	NA	Lung tumors in female mice
Study doses (mg/kg-d)	NA	0, 3.2, 7.0 (M); 0, 3.95, 8.55 (F)	NA	Adjusted daily dose: 0, 47, 158 (M); 0, 55, 183 (F)

Table C-3. Comparison of Available Oral Carcinogenicity Data for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues

Type of Data	3,4-TDA CASRN 496-72-0	2,4-TDA CASRN 95-80-7	2,6-TDA CASRN 823-40-5	2,5-TDA CASRN 95-70-5
Route (method)	NA	Diet	Diet	Diet
Duration	NA	103 wk	2 yr	78 wk
POD type	NA	$BMDL_{10}$	NA	$BMDL_{10}$ (HED)
Source	NA	CalEPA (2011a); CalEPA (2009)	U.S. EPA (2005b)	U.S. EPA (2013)

[a]There is no U.S. EPA WOE descriptor for 2,4-TDA; however, this compound is listed as a carcinogen by CalEPA (2011a), considered *possibly carcinogenic to humans* by IARC (1987) and *reasonably anticipated to be a human carcinogen* by NTP (2016b).
[b]OSF derived by CalEPA (2011a).

$BMDL_{10}$ = 10% benchmark dose lower confidence limit; F = female(s); HED = human equivalent dose; M = male(s); NA = not applicable; ND = no data; OSF = oral slope factor; POD = point of departure; p-OSF = provisional oral slope factor; TDA = toluenediamine; WOE = weight of evidence.

STEP 6. STRUCTURAL ALERTS AND STRUCTURE-ACTIVITY RELATIONSHIP PREDICTIONS FOR 3,4-TDA 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 3,4-TDA and its analogue compounds are shown in Table C-4. Concerns for carcinogenicity and/or mutagenicity of 3,4-TDA and its analogues were indicated by several models within each predictive tool (see Table C-4). Table C-5 provides a list of the specific SAs that underlie the findings of a concern for carcinogenicity or mutagenicity in Table C-4.

OECD QSAR Toolbox models showed a concern for mutagenicity, CAs, MN, and protein binding for 3,4-TDA and all analogues based on SAs (see Tables C-4 and C-5). The ToxRead and VEGA models also indicated a concern for mutagenicity for 3,4-TDA and all analogues. The Toxtree tool indicated a concern for 3,4-, 2,4-, and 2,5-TDA mutagenicity in *S. typhimurium* TA100, but indicated that 2,6-TDA was unlikely to be mutagenic in *S. typhimurium* TA100. The Toxtree results for 2,6-TDA are inconsistent with positive experimental data (see "Step 3. Comparison of the Experimental Genotoxicity Data for 3,4-TDA and Analogues" above for more information) and the results of the other QSAR models.

Table C-4. Heat Map Illustrating the Structural Alert and SAR Prediction Results for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues

Tool	Model[a]	3,4-TDA	2,6-TDA	2,6-TDA dihydrochloride	2,5-TDA	2,5-TDA dihydrochloride	2,5-TDA sulfate	2,4-TDA	2,4-TDA dihydrochloride
Mutagenicity/genotoxicity alerts									
OECD QSAR Toolbox	DNA alerts for Ames by OASIS								
	DNA alerts for CA and MNT by OASIS								
	In vitro mutagenicity (Ames test) alerts by ISS								
	In vivo mutagenicity (micronucleus) alerts by ISS								
	Protein binding alerts for CA by OASIS								
ToxRead	ToxRead (mutagenicity)								
VEGA	Mutagenicity (Ames test) CONSENSUS model—assessment								
	Mutagenicity (Ames test) model (CAESAR)—assessment								
	Mutagenicity (Ames test) model (SARpy/IRFMN)—assessment								
	Mutagenicity (Ames test) model (ISS)—assessment								
	Mutagenicity (Ames test) model (*k*-NN/read-across)—assessment								
Toxtree	Potential *Salmonella typhimurium* TA100 mutagen based on QSAR								
Carcinogenicity alerts									
OECD QSAR Toolbox	Carcinogenicity (genotoxicity and nongenotoxicity) alerts by ISS								
OncoLogic	OncoLogic (prediction of the carcinogenic potential of the chemical)								
VEGA	Carcinogenicity model (CAESAR)—assessment								
	Carcinogenicity model (ISS)—assessment								
	Carcinogenicity model (IRFMN/ANTARES)—assessment								
	Carcinogenicity model (IRFMN/ISSCAN-CGX)—assessment								
Toxtree	Potential carcinogen based on QSAR								
	Nongenotoxic carcinogenicity								

Table C-4. Heat Map Illustrating the Structural Alert and SAR Prediction Results for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues

Tool	Model[a]	3,4-TDA	2,6-TDA	2,6-TDA dihydrochloride	2,5-TDA	2,5-TDA dihydrochloride	2,5-TDA sulfate	2,4-TDA	2,4-TDA dihydrochloride
Combined alerts									
ToxAlerts	Aromatic amine (general) (for genotoxic carcinogenicity, mutagenicity)								
	Aromatic amine (specific) (for genotoxic carcinogenicity, mutagenicity)								
	Aromatic amines (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; 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); DNA = deoxyribonucleic acid; IRFMN = Istituto di Ricerche Farmacologiche Mario Negri; ISS = Istituto Superiore di Sanità; ISSCAN-CGX = Istituto Superiore di Sanità Chemical Carcinogen; *k*-NN = *k*-nearest neighbor; OECD = Organisation for Economic Co-operation and Development; SAR = structure-activity relationship; QSAR = quantitative structure-activity relationship; VEGA = Virtual models for property Evaluation of chemicals within a Global Architecture.

Table C-5. SAs for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues		
SA	**Tools**	**Compounds**
Aromatic amine	OncoLogic	3,4-TDA; 2,4-TDA; 2,4-TDA dihydrochloride; 2,5-TDA; 2,5-TDA dihydrochloride; 2,5-TDA sulfate; 2,6-TDA; 2,6-TDA dihydrochloride[a]
	ToxAlerts	
Primary aromatic amine, hydroxyl amine, and its derived esters	Toxtree	
	OECD QSAR Toolbox	
Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group	ToxAlerts	
Substituted anilines	OECD QSAR Toolbox	
Single ring-substituted primary aromatic amines	OECD QSAR Toolbox	

[a]The SA in OncoLogic for 2,6-TDA dihydrochloride was reported as "marginal."

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

OECD QSAR Toolbox models showed a concern for carcinogenicity for 3,4-TDA and all analogues based on SAs (see Tables C-4 and C-5). The level of carcinogenicity concern in OncoLogic for 3,4-TDA was "high−moderate" based on structure-activity relationship (SAR) analysis only (aromatic amine with amino groups *ortho* to one another). OncoLogic indicated the level of concern for carcinogenicity as "moderate" for 2,4-TDA based on animal carcinogenicity data and SAR analysis (aromatic amine with amino groups *meta* to one another). The level of carcinogenicity concern in OncoLogic for 2,6-TDA, 2,5-TDA, 2,5-TDA hydrochloride, 2,5-TDA sulfate, and 2,4-TDA dihydrochloride was "moderate" based on SAR analysis only (aromatic amine with amino groups *meta* or *para* to one another). OncoLogic reported a "marginal" level of concern for 2,6-TDA dihydrochloride (shown as no results for models in Table C-4) based on a lack of evidence of carcinogenicity from animal studies and SAR analysis (aromatic amine with amino groups *meta* to one another). VEGA showed concern for carcinogenicity of 3,4-TDA using the CAESAR and ISS models (no data for the IRFMN/ANTARES or IRFMN/ISSCAN-CGX models). All four VEGA models showed concern for carcinogenicity for 2,4-TDA and 2,4-TDA dihydrochloride. Carcinogenicity models in VEGA produced inconsistent results for 2,5- and 2,6-TDA (and their salts). While the CAESAR model showed concern for both compounds (and their salts), the ISS model showed concern only for 2,5-TDA (and its salts), and the IRFMN/ISSCAN-CGX model did not show concern for either compound (or their salts). There were no data for the IRFMN/ANTARES model for 2,5- or 2,6-TDA (or their salts). The Toxtree tool indicated that 2,4- and 2,6-TDA were potential carcinogens based on QSAR, but that 3,4- and 2,5-TDA were not. The Toxtree tool showed there was no concern for nongenotoxic carcinogenicity for 3,4-TDA or any of its analogues.

The ToxAlerts tool showed a concern for genotoxic carcinogenicity and/or mutagenicity for 3,4-TDA and all analogues based on various SAs (see Tables C-4 and C-5). The Toxtree models also suggest a concern for genotoxic carcinogenicity for 3,4-TDA and all analogues based on SAs.

In general, SAR predictions indicate a concern for genotoxicity and carcinogenicity for 3,4-TDA and all TDA analogues across several software systems evaluated. Moreover, a clear pattern or relationship between the position of the amino groups (3,4-TDA is a *o*- isomer, 2,5- is a *p*- isomer, and 2,4- and 2,6- are *m*- isomers) and potential differences in SAR predictions are not apparent for the TDA compounds. Previous SAR evaluations have suggested enhanced chemical reactivity for the *o*- and *p*-substituted aromatic amines due to quinone formation (Bajot et al., 2010). However, based on the available experimental and in silico data discussed above, the influence of the position of the amino groups on the potential genotoxicity and carcinogenicity of the TDA compounds is unclear.

STEP 7. EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF 3,4-TDA CARCINOGENICITY

Table C-6 presents the data for multiple lines of evidence pertinent to the screening evaluation of the carcinogenic potential of 3,4-TDA.

Table C-6. Integration of Evidence for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues

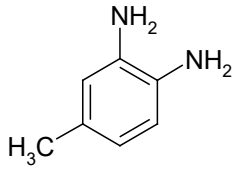
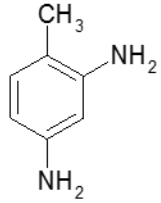
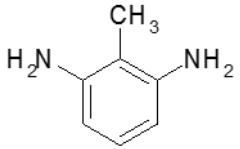
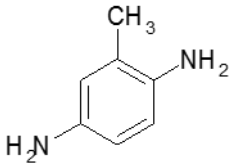
Evidence Stream	3,4-TDA CASRN 496-72-0	2,4-TDA CASRN 95-80-7	2,6-TDA CASRN 823-40-5	2,5-TDA CASRN 95-70-5
Role	Target	Analogue	Analogue	Analogue
Structure				
Analogue selection and evaluation (see Steps 1 and 2)	Target compound: contains (1) one aromatic ring (benzene) substituted with (2) two unsubstituted amines on the ring, in an *o*-substitution pattern, (3) a methyl group on the ring, and (4) no other functional group	Isomer: contains (1) one aromatic ring (benzene) substituted with (2) two unsubstituted amines on the ring, in a *m*-substitution pattern, (3) a methyl group on the ring, and (4) no other functional group	Isomer: contains (1) one aromatic ring (benzene) substituted with (2) two unsubstituted amines on the ring, in a *m*-substitution pattern, (3) a methyl group on the ring, and (4) no other functional group	Isomer: contains (1) one aromatic ring (benzene) substituted with (2) two unsubstituted amines on the ring, in a *p*-substitution pattern, (3) a methyl group on the ring, and (4) no other functional group
Experimental genotoxicity data (see Step 3)	Mutagenic in *Salmonella*; induces MN in vivo; inhibits DNA synthesis in vivo; induces cell transformation in mammalian cells	Mutagenic in *Salmonella*; clastogenic in mammalian cells; DNA damaging in mammalian cells in vitro and in vivo; forms DNA adducts in vivo	Mutagenic in *Salmonella*; clastogenic in mammalian cells; inconsistent evidence for DNA damage in mammalian cells in vitro and in vivo; induces cell transformation in hamster embryo cells	Mutagenic in *Salmonella*; clastogenic in mammalian cells; inconsistent evidence for DNA damage in mammalian cells in vitro and in vivo; induces cell transformation in hamster embryo cells
ADME evaluation (see Step 4)	ND; metabolic pathways expected to be similar to other TDA isomers based on metabolite prediction data	Common metabolic pathways with other TDA isomers (acetylation of the amino groups, ring hydroxylation and potential oxidation of methyl groups)	Common metabolic pathways with other TDA isomers (acetylation of the amino groups, ring hydroxylation and potential oxidation of methyl groups)	Common metabolic pathways with other TDA isomers (acetylation of the amino groups, ring hydroxylation and potential oxidation of methyl groups)
Cancer data and MOA (see Step 5)	ND	Liver tumors in rats and mice, subcutaneous fibromas in male rats, mammary tumors in rats, lymphoma in female mice; MOA not established	No significant evidence of carcinogenicity (but doses may have been too low); MOA not established	Testicular tumors in rats, lung tumors in mice; MOA not established

Table C-6. Integration of Evidence for 3,4-Toluenediamine (CASRN 496-72-0) and Analogues

Evidence Stream	3,4-TDA CASRN 496-72-0	2,4-TDA CASRN 95-80-7	2,6-TDA CASRN 823-40-5	2,5-TDA CASRN 95-70-5
Common SA and SAR predictions (see Step 6)	ALERTS • Aromatic amine • Primary aromatic amine, hydroxyl amine and its derived esters • Primary aromatic amine, hydroxyl amine and its derived esters or amine generating group • Substituted anilines • Single ring-substituted primary aromatic amines SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; not likely to be a carcinogen based on QSAR in Toxtree; no concern for nongenotoxic carcinogenicity in Toxtree	ALERTS • Aromatic amine • Primary aromatic amine, hydroxyl amine and its derived esters • Primary aromatic amine, hydroxyl amine and its derived esters or amine-generating group • Substituted anilines • Single ring-substituted primary aromatic amines SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; no concern for nongenotoxic carcinogenicity in Toxtree	ALERTS • Aromatic amine • Primary aromatic amine, hydroxyl amine and its derived esters • Primary aromatic amine, hydroxyl amine and its derived esters or amine-generating group • Substituted anilines • Single ring-substituted primary aromatic amines SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; no concern for carcinogenicity in two of three VEGA models and no concern for nongenotoxic carcinogenicity in Toxtree	ALERTS • Aromatic amine • Primary aromatic amine, hydroxyl amine and its derived esters • Primary aromatic amine, hydroxyl amine and its derived esters or amine-generating group • Substituted anilines • Single ring-substituted primary aromatic amines SAR PREDICTIONS: Concerns for mutagenicity and carcinogenicity in most models; no concern for carcinogenicity in one of three VEGA models; not likely to be a carcinogen based on QSAR in Toxtree; no concern for nongenotoxic carcinogenicity in Toxtree

ADME = absorption, distribution, metabolism, and excretion; DNA = deoxyribonucleic acid; *m* = *meta*; MN = micronuclei; MOA = mode of action; ND = no data; *o* = *ortho*; *p* = *para*; QSAR = quantitative structure-activity relationship; SA = structural alert; SAR = structure-activity relationship; TDA = toluenediamine; VEGA = Virtual models for property Evaluation of chemicals within a Global Architecture.

STEP 8. QUALITATIVE LEVEL OF CONCERN FOR 3,4-TDA POTENTIAL CARCINOGENICITY

A *concern for potential carcinogenicity* for 3,4-TDA is identified based on multiple lines of evidence, including similarities in structural features, in silico metabolism profiles, SAs and SAR predictions, and experimental data for carcinogenicity and/or genotoxicity for the target and analogues (see Table C-7 for additional details). 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 3,4-Toluenediamine (CASRN 496-72-0)

Level of Concern	Designation	Comments
Concern for potential carcinogenicity	**Selected**	**2,4-, 2,5-, and 2,6-TDA were identified as structural analogues of 3,4-TDA for evaluating carcinogenic potential. These compounds share a basic chemical structure (benzene ring, two amino groups, and a methyl group), differing only in the position of the amino functional groups. The analogues exhibit commonalities in toxicokinetic properties, including common metabolic pathways, which are expected to be similar for 3,4-TDA based on metabolite predictions. Two of three analogues have carcinogenic potential based on tumors observed in rodent studies (2,4- and 2,5-TDA); the third analogue (2,6-TDA) has not been adequately assessed for carcinogenicity. Although the carcinogenic MOA for 3,4-, 2,4-, 2,5- and 2,6-TDA is not known, all compounds appear to be mutagenic in bacterial systems with metabolic activation and show some evidence of genotoxicity in mammalian test models. Furthermore, the target compound and analogues have identical SAs (e.g., aromatic amine) and similar SAR predictions showing concern for carcinogenicity/genotoxicity.**
Inadequate information for assigning qualitative level of concern	Not selected	NA

MOA = mode of action; NA = not applicable; SA = structural alert; SAR = structure-activity relationship; TDA = toluenediamine.

APPENDIX D. METHODOLOGY AND RESULTS FOR IN SILICO METABOLITE ANALYSIS OF TARGET AND ANALOGUES

An in silico analysis of metabolism was conducted for 3,4-toluenediamine (3,4-TDA) and its analogues using different software tools. The main objective of this analysis is to provide a qualitative comparison of metabolite predictions for TDA compounds in the absence of experimental data for the target. The focus is on the major metabolism pathways characterized in the literature, highlighting any notable differences between the target and analogues.

Chemical structures were extracted from the U.S. EPA CompTox Chemicals Dashboard for 3,4-TDA and the identified structural analogues [2,3-, 2,4-, 2,5-, and 2,6-TDA; U.S. EPA (2019)]. The metabolite predictions for the chemicals of interest were generated using commercially available software systems, including the Tissue Metabolism Simulator (TIMES) (Dimitrov et al., 2005; Mekenyan et al., 2004) and Meteor Nexus (Marchant et al., 2008). A structure data file (SDF) was imported into the TIMES program (Version 2.29.1; http://oasis-lmc.org/products/software/times.aspx), using the in vitro rat S9 metabolic simulator (Version 11.16) and the rat in vivo metabolic simulator (Version 07.12) to make predictions of likely metabolites. The predictions were exported as a .txt file for subsequent processing.

For the Meteor Nexus predictions, the SDF was split into separate molecular data (MOL) files for batch processing in Meteor Nexus. A python script (Python; Version 3.6.5; python.org) was used to split the SDF, and a second script was used to concatenate the individual substance prediction files that were created as separate excel workbooks. Default settings were used in Meteor Nexus (Version 3.1.0) developed by Lhasa Limited (https://www.lhasalimited.org/library/publishing.htm). The settings were for a maximum depth of tree to be 3, for the maximum number of metabolites to be capped at 1,000 and for the scoring method to be Site of Metabolism Scoring (with Molecular Mass Variance). The results are described as a score that uses experimental data for compounds that match the same biotransformation, have similar molecular weights and are structurally similar around the site of metabolism to the query compound (for more details, see https://www.lhasalimited.org/products/meteor-reasoning-methodologies.htm). The prediction files were then processed further within a Jupyter notebook (jupyter.org) imported with python libraries RDKit (Version 2018.03.2.0; RDKit.org), Pandas (Version 0.23.1; pandas.pydata.org), NumPy (Version 1.14.3; numpy.org), and Matplotlib (Version 2.2.2; matplotlib.org).

The software systems provided Simplified Molecular Input Line Entry System (SMILES) representations for the predicted metabolites. These were converted into RDKit mol objects and exported as a Pandas Tools worksheet that provided depictions of chemical structure. International Union of Pure and Applied Chemistry (IUPAC) International Chemical Identifier (InChI™) keys were created using RDKit because SMILES representations are not unique. The structures of the predicted metabolites from each of the tools evaluated are presented in Table D-1, which compares metabolites identified across the different software tools and experimental data from in vivo animal studies captured in Table A-3 of the "Metabolic Analogues" section in Appendix A. Additionally, pathway transformations corresponding to the metabolite predictions were extracted from Meteor Nexus to facilitate similarity comparisons between the target and candidate analogues. Other software tools (i.e., TIMES) did not provide the same level of information. The pathway transformations for target and candidate analogues were extracted from the Meteor Nexus summary report, then grouped by substance and pivoted

to provide a representation of the TDA compounds as rows and the unique pathways as columns (see Table A-4 in Appendix A). A pairwise distance matrix was then computed using the Jaccard distance as a metric, which was then transformed to a similarity matrix (see Figure A-1 in Appendix A). A metabolic tree for the 2,4-TDA was constructed to highlight the relationships of predicted metabolites for this specific analogue that correspond to the pathway transformations shared among the TDA compounds (see Figure D-1).

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

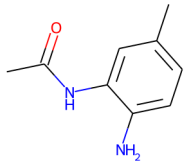
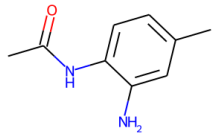
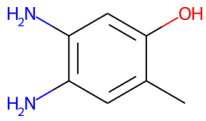
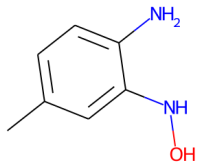
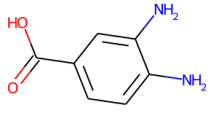
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
3,4-Toluenediamine (CASRN 496-72-0)						
	CDOUPQQJGFCACL-UHFFFAOYSA-N	CC(=O)Nc1cc(C)ccc1N	1	1	1	NDr
	JBJRVPVZADJOOX-UHFFFAOYSA-N	CC(=O)Nc1ccc(C)cc1N	1	1	1	NDr
	LXBXLRPRAMALBT-UHFFFAOYSA-N	Cc1cc(N)c(N)cc1O	1	1	1	NDr
	AMUSQFJRRCHIDJ-UHFFFAOYSA-N	Cc1ccc(N)c(NO)c1	1	0	1	NDr
	HEMGYNNCNNODNX-UHFFFAOYSA-N	Nc1ccc(C(=O)O)cc1N	1	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

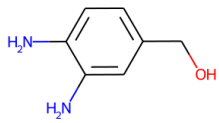
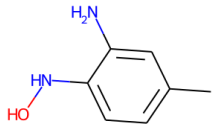
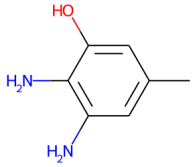
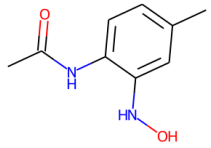
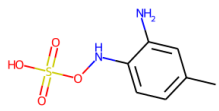
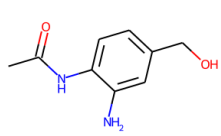
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	HMVJXTUUQJUYJI-UHFFFAOYSA-N	Nc1ccc(CO)cc1N	1	0	1	NDr
	LQKOQGZAAWXAJO-UHFFFAOYSA-N	Cc1ccc(NO)c(N)c1	1	0	1	NDr
	FQQXRJHNPCQKQB-UHFFFAOYSA-N	Cc1cc(N)c(N)c(O)c1	0	1	1	NDr
	AELQHYALRZNYNG-UHFFFAOYSA-N	CC(=O)Nc1ccc(C)cc1NO	1	0	0	NDr
	CDBLIIZYTJQRIR-UHFFFAOYSA-N	Cc1ccc(NOS(=O)(=O)O)c(N)c1	1	0	0	NDr
	CFFKPFQMLZQSMC-UHFFFAOYSA-N	CC(=O)Nc1ccc(CO)cc1N	1	0	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

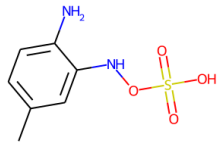
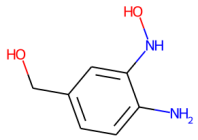
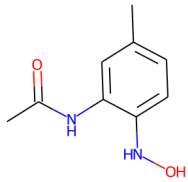
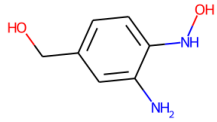
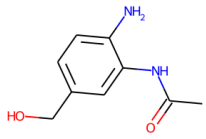
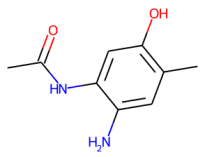
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	DMIWVGYENDrUAGJ-UHFFFAOYSA-N	Cc1ccc(N)c(NOS(=O)(=O)O)c1	1	0	0	NDr
	OYMWICMADIVGJD-UHFFFAOYSA-N	Nc1ccc(CO)cc1NO	1	0	0	NDr
	OYXHIBSVHUIIHN-UHFFFAOYSA-N	CC(=O)Nc1cc(C)ccc1NO	1	0	0	NDr
	YPMPQTKAGFCMHX-UHFFFAOYSA-N	Nc1cc(CO)ccc1NO	1	0	0	NDr
	ZSHDMSDVKOVLAA-UHFFFAOYSA-N	CC(=O)Nc1cc(CO)ccc1N	1	0	0	NDr
	CFNITIXCKKIDKK-UHFFFAOYSA-N	CC(=O)Nc1cc(O)c(C)cc1N	0	1	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

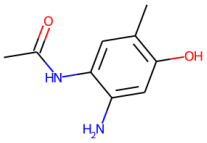
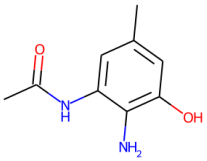
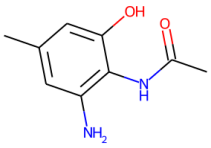
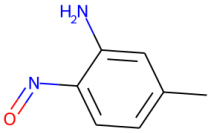
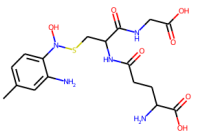
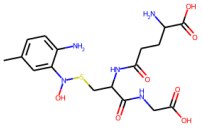
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	IHUHNLWFEHPZOR-UHFFFAOYSA-N	CC(=O)Nc1cc(C)c(O)cc1N	0	1	0	NDr
	RHCSARHFCNPXNQ-UHFFFAOYSA-N	CC(=O)Nc1cc(C)cc(O)c1N	0	1	0	NDr
	VRFLCZIWWHOCPA-UHFFFAOYSA-N	CC(=O)Nc1c(N)cc(C)cc1O	0	1	0	NDr
	AIYGLIJSQZZWPP-UHFFFAOYSA-N	Cc1ccc(N=O)c(N)c1	0	0	1	NDr
	CFDDUZIKVUIONZ-UHFFFAOYSA-N	Cc1ccc(N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)c(N)c1	0	0	1	NDr
	FGMJURDRCOTDCZ-UHFFFAOYSA-N	Cc1ccc(N)c(N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)c1	0	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

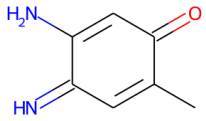
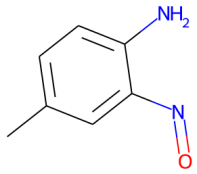
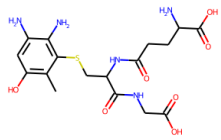
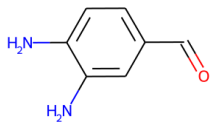
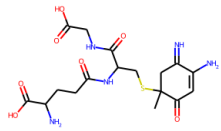
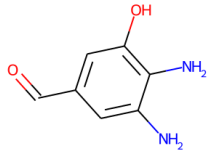
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	GMFRNXFZQNBPLZ-UHFFFAOYSA-N	CC1=CC(=N)C(N)=CC1=O	0	0	1	NDr
	GMPOLBFESRQGCU-UHFFFAOYSA-N	Cc1ccc(N)c(N=O)c1	0	0	1	NDr
	NAGMCFVTHPBBFR-UHFFFAOYSA-N	Cc1c(O)cc(N)c(N)c1SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O	0	0	1	NDr
	NSILMBMDJGSYNS-UHFFFAOYSA-N	Nc1ccc(C=O)cc1N	0	0	1	NDr
	QAFMNLZTMYPOOY-UHFFFAOYSA-N	CC1(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)CC(=N)C(N)=CC1=O	0	0	1	NDr
	UVIYTNUYRRBTME-UHFFFAOYSA-N	Nc1cc(C=O)cc(O)c1N	0	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

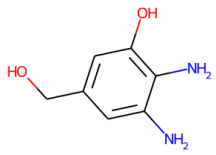
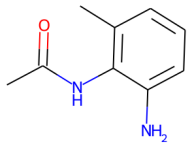
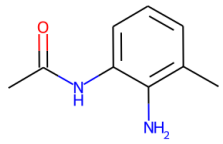
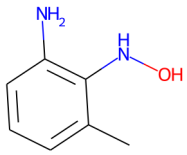
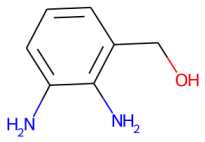
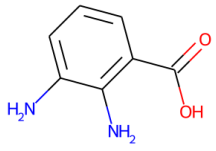
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	YXTRRMZKHFFNPW-UHFFFAOYSA-N	Nc1cc(CO)cc(O)c1N	0	0	1	NDr
2,3-Toluenediamine (CASRN 2687-25-4)						
	CDQDPNFLCSCJCH-UHFFFAOYSA-N	CC(=O)Nc1c(C)cccc1N	1	1	1	NDr
	LQAAALNVGAFVJD-UHFFFAOYSA-N	CC(=O)Nc1cccc(C)c1N	1	1	1	NDr
	FQKUNAYBPMGVRP-UHFFFAOYSA-N	Cc1cccc(N)c1NO	1	0	1	NDr
	FYUDUZRLZITSTF-UHFFFAOYSA-N	Nc1cccc(CO)c1N	1	0	1	NDr
	KKTUQAYCCLMNOA-UHFFFAOYSA-N	Nc1cccc(C(=O)O)c1N	1	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

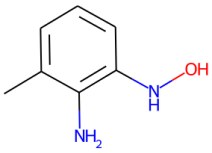
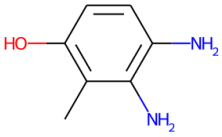
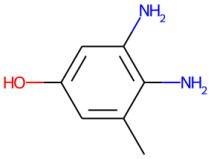
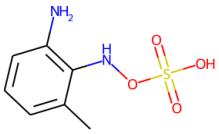
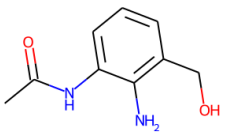
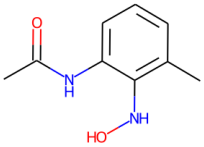
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	VYFFPFYOFVMNGG-UHFFFAOYSA-N	Cc1cccc(NO)c1N	1	0	1	NDr
	GVEXOXFTENPDOH-UHFFFAOYSA-N	Cc1c(O)ccc(N)c1N	0	1	1	NDr
	SRFOBSMZHWJDJN-UHFFFAOYSA-N	Cc1cc(O)cc(N)c1N	0	1	1	NDr
	DCYIPFHDKQUXBG-UHFFFAOYSA-N	Cc1cccc(N)c1NOS(=O)(=O)O	1	0	0	NDr
	DEYTUXOGDHQHRY-UHFFFAOYSA-N	CC(=O)Nc1cccc(CO)c1N	1	0	0	NDr
	GMTRKMQJURSNIX-UHFFFAOYSA-N	CC(=O)Nc1cccc(C)c1NO	1	0	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

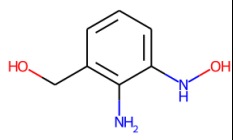
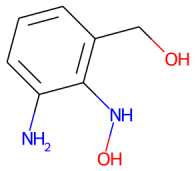
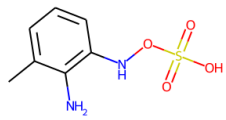
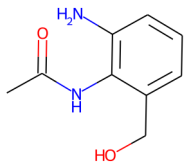
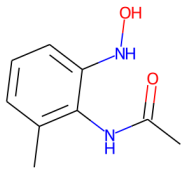
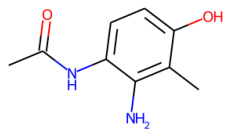
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	HRGIXVSYYVWOGJ-UHFFFAOYSA-N	Nc1c(CO)cccc1NO	1	0	0	NDr
	LFOLOGIZBFISKF-UHFFFAOYSA-N	Nc1cccc(CO)c1NO	1	0	0	NDr
	YJDFBYAXTGPZSK-UHFFFAOYSA-N	Cc1cccc(NOS(=O)(=O)O)c1N	1	0	0	NDr
	YRXPMBZXLPQXIS-UHFFFAOYSA-N	CC(=O)Nc1c(N)cccc1CO	1	0	0	NDr
	ZPZCAEZFJLLBMW-UHFFFAOYSA-N	CC(=O)Nc1c(C)cccc1NO	1	0	0	NDr
	FUVMDOMRPMZMOA-UHFFFAOYSA-N	CC(=O)Nc1ccc(O)c(C)c1N	0	1	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

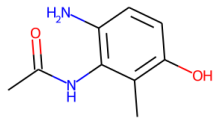
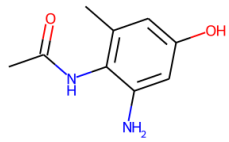
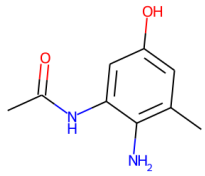
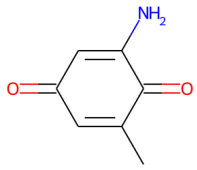
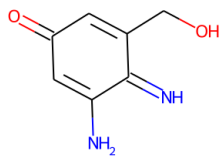
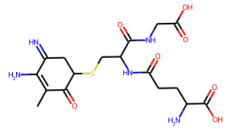
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	HBCUEGKUEMKCKO-UHFFFAOYSA-N	CC(=O)Nc1c(N)ccc(O)c1C	0	1	0	NDr
	PNMPOSDTYOCRAS-UHFFFAOYSA-N	CC(=O)Nc1c(C)cc(O)cc1N	0	1	0	NDr
	ZEBMHLAASWFAGS-UHFFFAOYSA-N	CC(=O)Nc1cc(O)cc(C)c1N	0	1	0	NDr
	ARCDFSYMTMSQRI-UHFFFAOYSA-N	CC1=CC(=O)C=C(N)C1=O	0	0	1	NDr
	CCIQDILKNGQPCA-UHFFFAOYSA-N	N=C1C(N)=CC(=O)C=C1CO	0	0	1	NDr
	FTKYIFRLYMBQDC-UHFFFAOYSA-N	CC1=C(N)C(=N)CC(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)C1=O	0	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

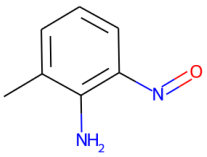
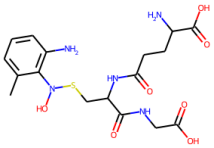
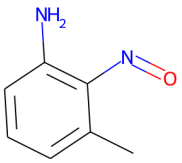
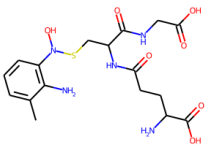
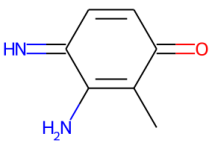
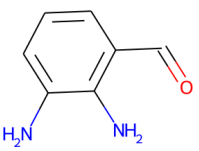
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	NAJRDVTXZXCQAM-UHFFFAOYSA-N	Cc1cccc(N=O)c1N	0	0	1	NDr
	NRERCLGIEBGCTA-UHFFFAOYSA-N	Cc1cccc(N)c1N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O	0	0	1	NDr
	PMJTXIMMLKKLKL-UHFFFAOYSA-N	Cc1cccc(N)c1N=O	0	0	1	NDr
	PYGSXASUOAHHOW-UHFFFAOYSA-N	Cc1cccc(N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)c1N	0	0	1	NDr
	RJICMTPCHYXVJA-UHFFFAOYSA-N	CC1=C(N)C(=N)C=CC1=O	0	0	1	NDr
	UOGKMJUEKOCDAX-UHFFFAOYSA-N	Nc1cccc(C=O)c1N	0	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

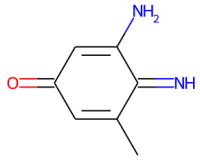
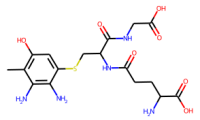
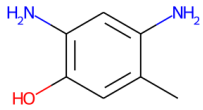
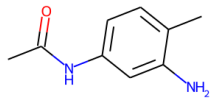
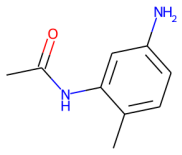
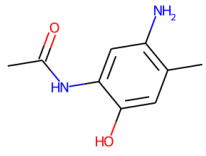
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	VVDQVHPTNDVFMR-UHFFFAOYSA-N	CC1=CC(=O)C=C(N)C1=N	0	0	1	NDr
	WWYXBGROOLIEEU-UHFFFAOYSA-N	Cc1c(O)cc(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)c(N)c1N	0	0	1	NDr
2,4-Toluenediamine (CASRN 95-80-7)						
	DPKOCFTZJRJTQL-UHFFFAOYSA-N	Cc1cc(O)c(N)cc1N	1	1	1	1
	RBQWGHBZCHFUQU-UHFFFAOYSA-N	CC(=O)Nc1ccc(C)c(N)c1	1	1	1	1
	UAZGSMMESOKKQZ-UHFFFAOYSA-N	CC(=O)Nc1cc(N)ccc1C	1	1	1	NDr
	BATBGGSVKZESGJ-UHFFFAOYSA-N	CC(=O)Nc1cc(N)c(C)cc1O	1	1	0	1

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

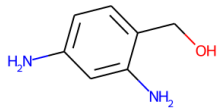
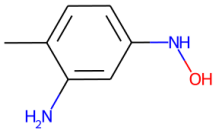
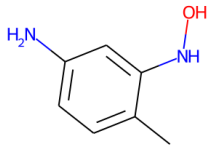
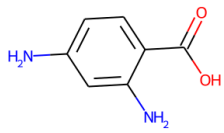
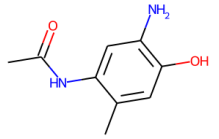
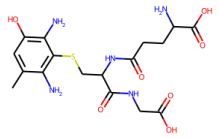
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	FADNCTVVKDWKIX-UHFFFAOYSA-N	Nc1ccc(CO)c(N)c1	1	0	1	NDr
	JCSKFCJYMXDFAD-UHFFFAOYSA-N	Cc1ccc(NO)cc1N	1	0	1	NDr
	KARRBUHHWCMGHB-UHFFFAOYSA-N	Cc1ccc(N)cc1NO	1	0	1	NDr
	LDQMZKBIBRAZEA-UHFFFAOYSA-N	Nc1ccc(C(=O)O)c(N)c1	1	0	1	NDr
	LZEMQCKKBQRKFM-UHFFFAOYSA-N	CC(=O)Nc1cc(N)c(O)cc1C	1	1	0	NDr
	CEKKEYTVRSICNQ-UHFFFAOYSA-N	Cc1cc(O)c(N)c(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)c1N	1	0	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

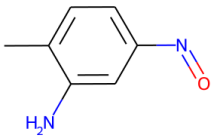
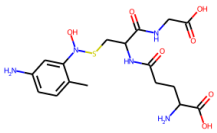
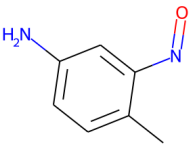
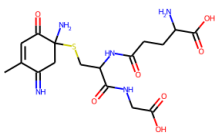
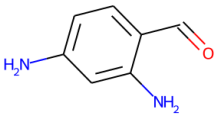
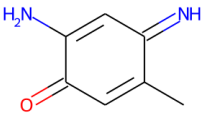
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	DYLOOKKMCKMECT-UHFFFAOYSA-N	Cc1ccc(N=O)cc1N	1	0	0	NDr
	IODXTXYTNSSKSC-UHFFFAOYSA-N	Cc1ccc(N)cc1N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O	1	0	0	NDr
	LLROHMIZNDXUDK-UHFFFAOYSA-N	Cc1ccc(N)cc1N=O	1	0	0	NDr
	RGMVILWFQAJIRO-UHFFFAOYSA-N	CC1=CC(=O)C(N)(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)CC1=N	1	0	0	NDr
	VMFJRVFZHAPENO-UHFFFAOYSA-N	Nc1ccc(C=O)c(N)c1	1	0	0	NDr
	YIJGNYPGRJIBNG-UHFFFAOYSA-N	CC1=CC(=O)C(N)=CC1=N	1	0	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

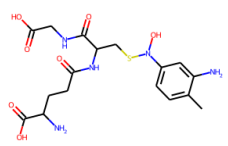
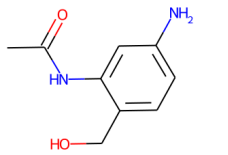
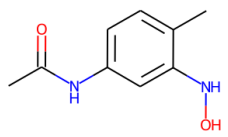
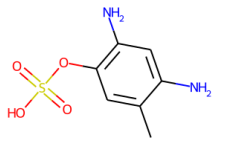
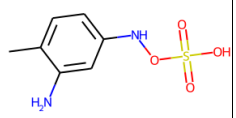
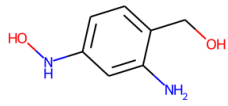
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	YLDTVWMQWTYLOW-UHFFFAOYSA-N	Cc1ccc(N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)cc1N	1	0	0	NDr
	DGBUOAIABOATGK-UHFFFAOYSA-N	CC(=O)Nc1cc(N)ccc1CO	1	0	0	NDr
	HJLRPSJGXCSTOA-UHFFFAOYSA-N	CC(=O)Nc1ccc(C)c(NO)c1	1	0	0	NDr
	NNYGTEAPVBYVOR-UHFFFAOYSA-N	Cc1cc(OS(=O)(=O)O)c(N)cc1N	0	0	1	NDr
	NTVDFUPWAZHKRU-UHFFFAOYSA-N	Cc1ccc(NOS(=O)(=O)O)cc1N	0	0	1	NDr
	OHWHMZFQZAEYNR-UHFFFAOYSA-N	Nc1cc(NO)ccc1CO	0	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

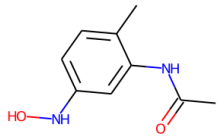
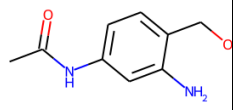
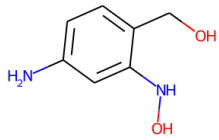
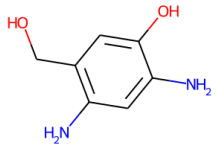
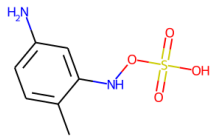
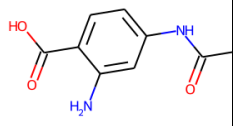
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	PQHCWNHKWOJEJH-UHFFFAOYSA-N	CC(=O)Nc1cc(NO)ccc1C	0	0	1	NDr
	ULBRTOCNNKAALW-UHFFFAOYSA-N	CC(=O)Nc1ccc(CO)c(N)c1	0	0	1	NDr
	WQJKPERQJHMQFH-UHFFFAOYSA-N	Nc1ccc(CO)c(NO)c1	0	0	1	NDr
	XKZAGZAKKCRYJI-UHFFFAOYSA-N	Nc1cc(N)c(CO)cc1O	0	0	1	NDr
	YEDHXAKCFRBZAC-UHFFFAOYSA-N	Cc1ccc(N)cc1NOS(=O)(=O)O	0	0	1	NDr
	IFQWBWVICSDDSO-UHFFFAOYSA-N	C1=C(C(=CC=C1NC(C)=O)C(=O)O)N	0	0	0	1

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

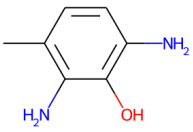
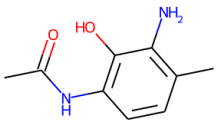
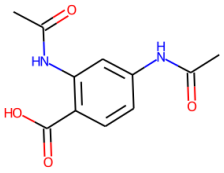
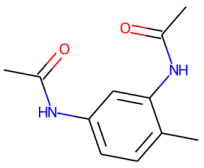
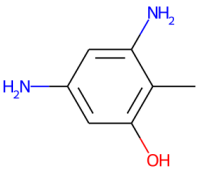
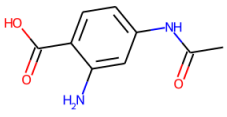
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	RMHSOFVBSGKWJZ-UHFFFAOYSA-N	C1(=C(C=CC(=C1O)N)C)N	0	0	0	1
	UZDRYZZYGHVZBF-UHFFFAOYSA-N	C1=CC(=C(C(=C1C)N)O)NC(C)=O	0	0	0	1
	UZGYKGBZSGOYCM-UHFFFAOYSA-N	C1=CC(=C(C=C1NC(C)=O)NC(C)=O)C(=O)O	0	0	0	1
	XAHUNQAOCGDSBW-UHFFFAOYSA-N	C1(=CC=C(C=C1NC(C)=O)NC(C)=O)C	0	0	0	1
	YKMOHRBCUQRNQM-UHFFFAOYSA-N	C1=C(C=C(C(=C1O)C)N)N	0	0	0	1
	IFQWBWVICSDDSO-UHFFFAOYSA-N	C1=C(C(=CC=C1NC(C)=O)C(=O)O)N	0	0	0	1

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

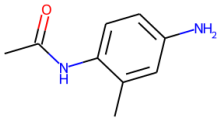
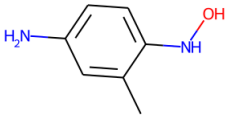
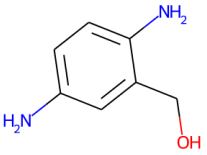
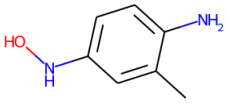
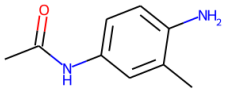
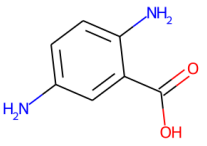
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
2,5-Toluenediamine (CASRN 95-70-5)						
	GWFPMSIIVJMYRZ-UHFFFAOYSA-N	CC(=O)Nc1ccc(N)cc1C	1	1	1	NDr
	LJHMYKUSHZQTMV-UHFFFAOYSA-N	Cc1cc(N)ccc1NO	1	0	1	NDr
	NKNCGBHPGCHYCQ-UHFFFAOYSA-N	Nc1ccc(N)c(CO)c1	1	0	1	NDr
	QNLWDEIYRQGAEE-UHFFFAOYSA-N	Cc1cc(NO)ccc1N	1	0	1	NDr
	QXWUFIZOBXUMSM-UHFFFAOYSA-N	CC(=O)Nc1ccc(N)c(C)c1	1	1	1	NDr
	UONVFNLDGRWLKF-UHFFFAOYSA-N	Nc1ccc(N)c(C(=O)O)c1	1	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

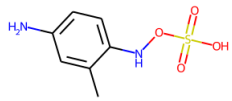
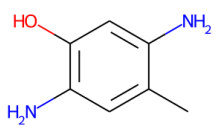
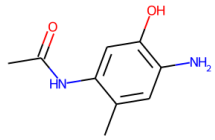
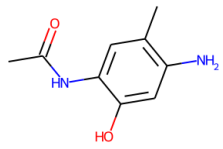
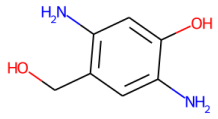
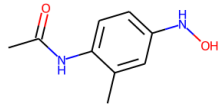
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	AHAAQFCJXQOJQM-UHFFFAOYSA-N	Cc1cc(N)ccc1NOS(=O)(=O)O	1	0	0	NDr
	BAYXOGMUGKSOIY-UHFFFAOYSA-N	Cc1cc(N)c(O)cc1N	1	0	0	NDr
	GDRJGNLVIHNOTC-UHFFFAOYSA-N	CC(=O)Nc1cc(O)c(N)cc1C	1	0	0	NDr
	JCEYYZACUKLBJT-UHFFFAOYSA-N	CC(=O)Nc1cc(C)c(N)cc1O	1	0	0	NDr
	LPVMVIHWFLVFLL-UHFFFAOYSA-N	Nc1cc(CO)c(N)cc1O	1	0	0	NDr
	OJKNEPYQGJFQCX-UHFFFAOYSA-N	CC(=O)Nc1ccc(NO)cc1C	1	0	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

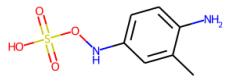
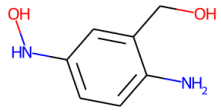
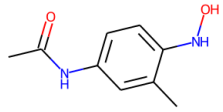
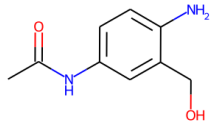
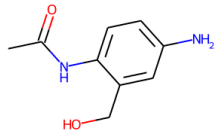
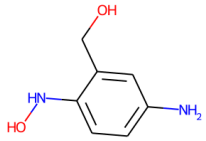
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	PJLKOWKKMQTFPM-UHFFFAOYSA-N	Cc1cc(NOS(=O)(=O)O)ccc1N	1	0	0	NDr
	PMHSKYOPGGHUCD-UHFFFAOYSA-N	Nc1ccc(NO)cc1CO	1	0	0	NDr
	QWRHGJVFZGEQMN-UHFFFAOYSA-N	CC(=O)Nc1ccc(NO)c(C)c1	1	0	0	NDr
	QWUDZFZVWQFZNJ-UHFFFAOYSA-N	CC(=O)Nc1ccc(N)c(CO)c1	1	0	0	NDr
	WJQKIKWBEAJBTM-UHFFFAOYSA-N	CC(=O)Nc1ccc(N)cc1CO	1	0	0	NDr
	YFLDRIRBIYOFLP-UHFFFAOYSA-N	Nc1ccc(NO)c(CO)c1	1	0	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

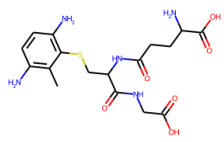
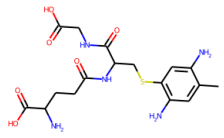
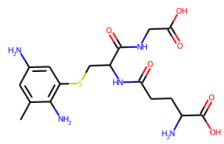
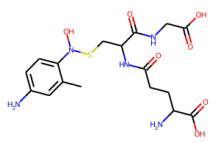
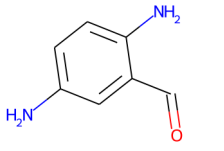
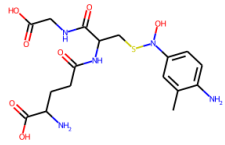
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	CJGYEDFJVQTRDO-UHFFFAOYSA-N	Cc1c(N)ccc(N)c1SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O	0	0	1	NDr
	GAANDMBIVOKAES-UHFFFAOYSA-N	Cc1cc(N)c(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)cc1N	0	0	1	NDr
	GFXJDKBBELRHKA-UHFFFAOYSA-N	Cc1cc(N)cc(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)c1N	0	0	1	NDr
	GVAJQXAWDPTTRS-UHFFFAOYSA-N	Cc1cc(N)ccc1N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O	0	0	1	NDr
	KIWGKZZMXPLHDG-UHFFFAOYSA-N	Nc1ccc(N)c(C=O)c1	0	0	1	NDr
	LDEKAEXXYZEVAI-UHFFFAOYSA-N	Cc1cc(N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)ccc1N	0	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

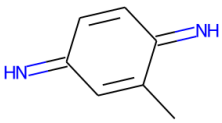
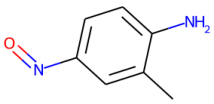
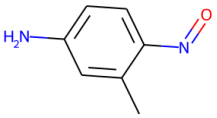
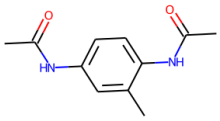
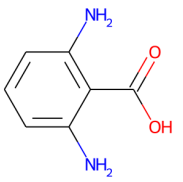
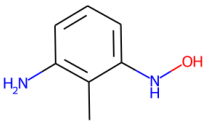
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	LFHSUMCUWFSVFL-UHFFFAOYSA-N	CC1=CC(=N)C=CC1=N	0	0	1	NDr
	UFBHQACBYVXASE-UHFFFAOYSA-N	Cc1cc(N=O)ccc1N	0	0	1	NDr
	XIRVYFRBHGMGMO-UHFFFAOYSA-N	Cc1cc(N)ccc1N=O	0	0	1	NDr
	ZYCWUKASEILJBB-UHFFFAOYSA-N	C1(=CC(=CC=C1NC(C)=O)NC(C)=O)C	0	0	0	1
2,6-Toluenediamine (CASRN 823-40-5)						
	CIEFZSDJGQKZNS-UHFFFAOYSA-N	Nc1cccc(N)c1C(=O)O	1	0	1	NDr
	NVKFKABKBGAWBK-UHFFFAOYSA-N	Cc1c(N)cccc1NO	1	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

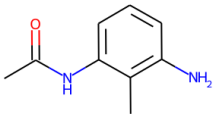
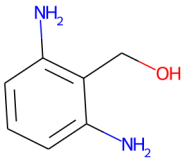
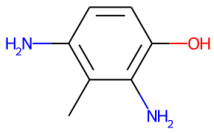
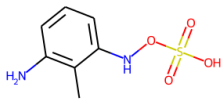
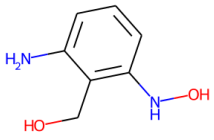
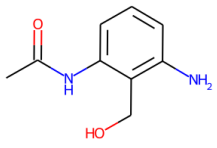
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	TZEOVCYRUCGICH-UHFFFAOYSA-N	CC(=O)Nc1cccc(N)c1C	1	1	1	1
	ZJRAQHULMYRRQG-UHFFFAOYSA-N	Nc1cccc(N)c1CO	1	0	1	NDr
	SHWMCLVULOXIMZ-UHFFFAOYSA-N	Cc1c(N)ccc(O)c1N	0	1	1	1
	BIMQURICJYBVBU-UHFFFAOYSA-N	Cc1c(N)cccc1NOS(=O)(=O)O	1	0	0	NDr
	DPFBFFAJBRRLGH-UHFFFAOYSA-N	Nc1cccc(NO)c1CO	1	0	0	NDr
	FHWODLDYCOFLFK-UHFFFAOYSA-N	CC(=O)Nc1cccc(N)c1CO	1	0	0	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

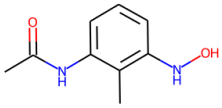
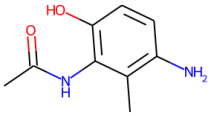
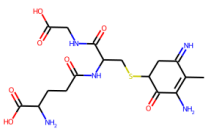
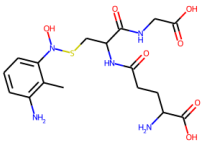
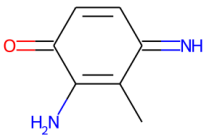
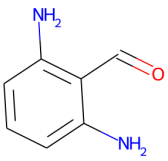
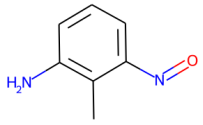
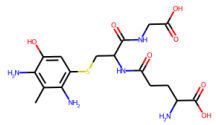
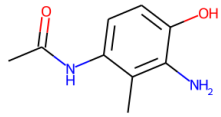
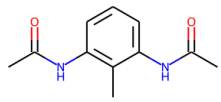
Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	LNZDEXXJPTWLTL-UHFFFAOYSA-N	CC(=O)Nc1cccc(NO)c1C	1	0	0	NDr
	POQKQLONFCRNFP-UHFFFAOYSA-N	CC(=O)Nc1c(O)ccc(N)c1C	0	1	0	NDr
	FCSVJIUNOCBBTJ-UHFFFAOYSA-N	CC1=C(N)C(=O)C(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)CC1=N	0	0	1	NDr
	KWPKZWQEAFVXHD-UHFFFAOYSA-N	Cc1c(N)cccc1N(O)SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O	0	0	1	NDr
	PXORAQVFLKLDLZ-UHFFFAOYSA-N	CC1=C(N)C(=O)C=CC1=N	0	0	1	NDr
	QWENKMCDOWMUAG-UHFFFAOYSA-N	Nc1cccc(N)c1C=O	0	0	1	NDr

Table D-1. Comparison of Metabolite Predictions for 3,4-Toluenediamine (CASRN 496-72-0) and Candidate Analogues across Software Tools and Observations from In Vivo Rodent Studies[a]

Structure	InChI Key	SMILES	Meteor Nexus[b]	TIMES_In Vivo[c]	TIMES_In Vitro[c]	Observed In Vivo[d]
	VOOYTMAMLLHBSZ-UHFFFAOYSA-N	Cc1c(N)cccc1N=O	0	0	1	NDr
	YPGMZCDVNOMVOG-UHFFFAOYSA-N	Cc1c(N)c(O)cc(SCC(NC(=O)CCC(N)C(=O)O)C(=O)NCC(=O)O)c1N	0	0	1	NDr
	KSPKSSDXPVUERG-UHFFFAOYSA-N	CC(=O)Nc1ccc(O)c(N)c1C	0	1	0	1
	NAXDRCLBFGZQSR-UHFFFAOYSA-N	C1(=C(C=CC=C1NC(C)=O)NC(C)=O)C	0	0	0	1

[a]1/0 denotes whether a metabolite was identified/not identified by a software tool or experimental animal data captured in Table A-3.
[b]Meteor Nexus (Dimitrov et al., 2005; Mekenyan et al., 2004).
[c]In Vivo/In Vitro Rat Tissue Metabolism Simulator (Dimitrov et al., 2005; Mekenyan et al., 2004).
[d]Metabolites reported from in vivo animal studies for the TDA isomers. Experimental data for 2,3- and 3,4-TDA were not available. Refer to Table A-3 for additional details.

InChI = IUPAC International Chemical Identifier; IUPAC = International Union of Pure and Applied Chemistry; NDr = not determined; SMILES = Simplified Molecular Input Line Entry System; TDA = toluenediamine; TIMES = Tissue Metabolism Simulator.

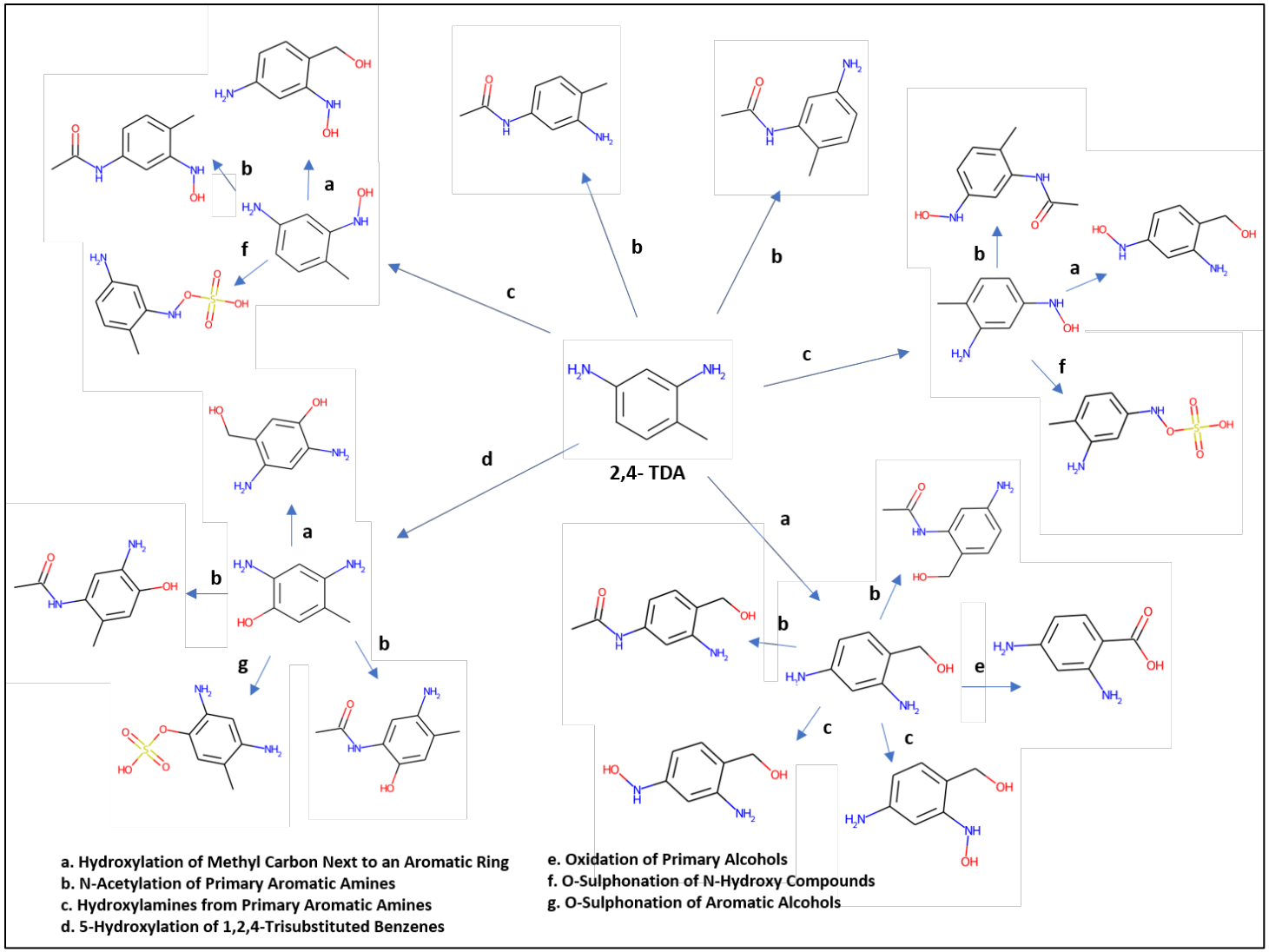


Figure D-1. Metabolic Tree for the 2,4-Toluenediamine (CASRN 95-80-7) Analogue. Diagram displays the relationship of the metabolites identified from Meteor Nexus (Dimitrov et al., 2005; Mekenyan et al., 2004) to the parent compound and notes the corresponding pathway transformations (a−g).

APPENDIX E. REFERENCES

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