N-(1,3-DIMETHYLBUTYL)-N'-PHENYL-P-PHENYLENEDIAMINE (6PPD)

(CAS #793-24-8)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: November 8, 2021

ToxServices Review Date: November 8, 2026[1]

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING
1367 Connecticut Ave., N.W., Suite 300
Washington, D.C. 20036

[1] Although CPA's Assessment Expiration Policy (CPA 2018a) indicates that Benchmark 1 assessments have no expiration date, ToxServices strives to review BM-1s in a five-year period to ensure currency of data presented in the BM-1 GreenScreen® assessments.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

GreenScreen® Executive Summary for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) functions as an antioxidant, antiozonant, and polymer stabilizer in rubbers. It is a low melting point, brown solid under standard temperature and pressure. It is non-reactive, non-flammable, and slightly soluble in water.

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a **GreenScreen Benchmark™ Score of 1** ("Avoid—Chemical of High Concern"). This score is based on the following hazard score:

- Benchmark 1a
  - High persistence-P + High bioaccumulation-B + Very High Ecotoxicity (acute aquatic toxicity-AA and chronic aquatic toxicity-CA)
  - High P + High B + High Group I Human Health Hazard (reproductive toxicity-R)
  - High P + High B + High Group II* Human Health Hazard (skin sensitization-SnS*)
- Benchmark 1e
  - High Group I Human Health Hazard (reproductive toxicity-R)

A data gap (DG) exists for repeated dose neurotoxicity-Nr*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) meets requirements for a GreenScreen Benchmark™ Score of 1 despite the hazard data gap. In a worst-case scenario, if N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) were assigned a High score for the data gap Nr*, it would still be categorized as a Benchmark 1 Chemical.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* genotoxicity, cell transformation, and endocrine activity testing, *in silico* modeling for endocrine activity, respiratory sensitization, persistence, and bioaccumulation, and OECD Guideline 301 B and C ready biodegradation tests. The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties:

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

Type I (input data) uncertainties in N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)'s NAMs dataset include lack of experimental data for respiratory sensitization along with a lack of validated test method for respiratory sensitization. N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)'s Type II (extrapolation output) uncertainties include limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism and their focusing on one or only a few types of genotoxicity events, the limitation of OECD Toolbox in identifying structural alerts without defining the applicability domain, the uncertain relevance of the EDSP Tox 21 screening assays and *in silico* modeling of endocrine receptor binding due to lack of consideration of toxicokinetic factors including metabolism, the limitations in the examination of structural alerts for respiratory sensitization evaluation that does not account for non-immunologic mechanisms of respiratory sensitization, and the lack of guidance from the ECHA framework to subclassify respiratory sensitizers to Category 1A and 1B. Some of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)'s type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary Table for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD)

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
L	**L**	**H**	**M**	**M**	**M**		**M**	*M*	DG	**H**	*M*	**L**	**M**	**vH**	**vH**	*H*	**H**	**L**	**L**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

GreenScreen® Chemical Assessment for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)

Method Version: GreenScreen® Version 1.4
Assessment Type[2]: Certified
Assessor Type: Licensed GreenScreen® Profiler

GreenScreen® Assessment (v.1.4) Prepared By:
Name: Zach Guerrette, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: October 12, 2021, November 2, 2021

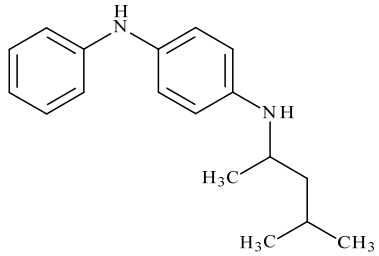
Quality Control Performed By:
Name: Bingxuan Wang, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: October 12, 2021, November 8, 2021

ToxServices Review Date: November 8, 2026[3]

Chemical Name: N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)

CAS Number: 793-24-8

Chemical Structure(s):

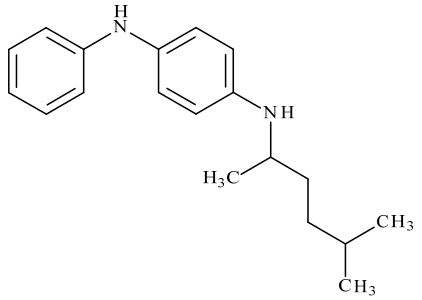


Also called:
1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl-; N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylenediamine; 6PPD; Antage 6C; Antioxidant 4020; Antioxidant CD; Antioxidant CD 13; Antozite 67; Antozite 67F; BRN 2215491; CD 13; DBDA; Diafen 13; Diafen FDMB; DMBPD; Dusantox 6PPD; EC 212-344-0; Flexzone 7F; Flexzone 7L; Forte 6C; N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine; N-1,3-Dimethylbutyl-N'-phenyl-p-phenylendiamine; N-Phenyl-N'-(1,3-dimethyl butyl)-para-phenylenediamine; N-Phenyl-N'-(1,3-dimethylbutyl)-p-phenylenediamine; NCI-C56315; Nocrac 6C; Nocrane 6C; Nocrane 7 L; Ozonon 6C; p-Phenylenediamine, N-(1,3-dimethylbutyl)-N'-phenyl-; Permanax 120; Permanax 6PPD; Santoflex 13; Santoflex 13F; Santoflex 6PPD; UOP 562; UOP 588; Vulkanox 4020; Wingstay 300; 1,4-Benzenediamine, N1-(1,3-dimethylbutyl)-N4-phenyl-; N-1,3-Dimethylbutyl-N'-phenyl-p-phenylenediamine; N1-(4-Methylpentan-2-yl)-N4-phenylbenzene-1,4-diamine; 4-N-(4-methylpentan-2-yl)-1-N-phenylbenzene-1,4-diamine; N-(1,3-dimethylbutyl)-N'-phenylbenzene-1,4-diamine; Ozonone 6C; ACMC-1BLWD; Rubber Antioxidant 6PPD; Akrochem antiozonant pd-2; 4-(1,3-Dimethylbutyl)amino-diphenylamine; 76600-84-5; 4-[(4-Methyl-2-pentyl)amino]diphenylamine; (4-anilino-phenyl)-(1,3-dimethyl-butyl)-amine; n-(1,3-dimethyl butyl)-n'-

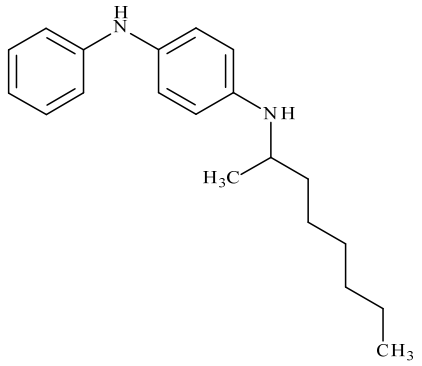
[2] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), or "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent).
[3] Although CPA's Assessment Expiration Policy (CPA 2018a) indicates that Benchmark 1 assessments have no expiration date, ToxServices strives to review BM-1s in a five-year period to ensure currency of data presented in the BM-1 GreenScreen® assessments.

phenyl-p-phenylenediamine; N-(1,3-Dimethylbutyl)-N\'-phenyl-p-phenylenediamine; 1,4-Benzenediamine,N1-(1,3-dimethylbutyl)-N1-phenyl-; N-(4-Methyl-2-pentyl)-N'-phenyl-1,4-phenylenediamine; N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine; 50809-58-0 (ChemIDplus 2021, PubChem 2021)

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
A sufficiently complete toxicological dataset was identified to assign N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) a Benchmark Score. However, ToxServices used data for the structurally related compounds 1,4-benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- (CAS #3081-01-4) and N-(1-methylheptyl)-N'-phenylbenzene-1,4-diamine (CAS #15233-47-3) in a weight of evidence approach for the environmental fate endpoints. N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and 1,4-benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- have a maximum common substructure (MCS) Tanimoto coefficient of 0.8636[4] and N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and N-(1-methylheptyl)-N'-phenylbenzene-1,4-diamine have an MCS Tanimoto coefficient of 0.8261, indicating sufficient structural similarity for both surrogates. No surrogate with data were identified for the data gap repeated dose neurotoxicity.



Surrogate: 1,4-Benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- (CAS #3081-01-4)



Surrogate: N-(1-Methylheptyl)-N'-phenylbenzene-1,4-diamine (CAS #15233-47-3)

Identify Applications/Functional Uses (HSDB 2002)**:**
1. Antioxidant, antiozonant, and polymer stabilizer in rubber.

[4] Determined using ChemMine's similarity workbench (https://chemminetools.ucr.edu/similarity/).

Known Impurities[5]:
N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) may contain < 1.5% w/w N-(1-(2-methylpropyl)-3,5-dimethylhexyl)-N′-phenyl-1,4-phenylene diamine (CAS #N/A); < 1% w/w N-(4-aminophenyl)aniline (CAS #101-54-2), unspecified concentrations of N-(1,3-Dimethylbutyl)-N′-phenyl-1,4-benzoquinone diamine; ≤ 5% 1-phenylamino-4-(1,3-dimethylbutylamino)-3,6-bis-(4-phenylaminophenylimino)-1,4-cyclohexadiene (CAS #N/A); and < 1.5% w/w N,N′-bis-(1,3-Dimethylbutyl)-N′-phenyl-1,3-phenylene diamine (CAS #N/A) (UNEP 2005). The screen is performed on the theoretical pure substance.

GreenScreen® Summary Rating for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD)[6,7 8,9]: N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a **GreenScreen Benchmark™ Score of 1** ("Avoid—Chemical of High Concern") (CPA 2018b). This score is based on the following hazard score:

- Benchmark 1a
  - High persistence-P + High bioaccumulation-B + Very High Ecotoxicity (acute aquatic toxicity-AA and chronic aquatic toxicity-CA)
  - High P + High B + High Group I Human Health Hazard (reproductive toxicity-R)
  - High P + High B + High Group II* Human Health Hazard (skin sensitization-SnS*)
- Benchmark 1e
  - High Group I Human Health Hazard (reproductive toxicity-R)

A data gap (DG) exists for repeated dose neurotoxicity-Nr*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) meets requirements for a GreenScreen Benchmark™ Score of 1 despite the hazard data gap. In a worst-case scenario, if N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) were assigned a High score for the data gap Nr*, it would still be categorized as a Benchmark 1 Chemical.

Figure 1: GreenScreen® Hazard Summary Table for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD)

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
L	**L**	**H**	**M**	**M**	**M**		**M**	*M*	DG	**H**	*M*	**L**	**M**	**vH**	**vH**	*H*	**H**	**L**	**L**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of

[5] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.
[6] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.
[7] See Appendix A for a glossary of hazard endpoint acronyms.
[8] For inorganic chemicals only, see GreenScreen® Guidance v1.4 Section 12 (Inorganic Chemical Assessment Procedure).
[9] For Systemic Toxicity and Neurotoxicity, repeated exposure data are preferred. Lack of single exposure data is not a Data Gap when repeated exposure data are available. In that case, lack of single exposure data may be represented as NA instead of DG. See GreenScreen® Guidance v1.4 Annex 2.

repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

Environmental Transformation Products

The major environmental degradation products for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylene diamine (6PPD) are 4-hydroxydiphenylamine (CAS #122-37-2, also known as p-hydroxy diphenylamine), N-phenyl-p-benzoquinone monoimine (CAS # 2406-04-4), phenylbenzoquinone imine (CAS #N/A), 1,3-dimethylbutylamine aniline (CAS #N/A), p-benzoquinone (CAS #106-51-4, also known as 1,4-benzoquinone), and 1,3-dimethylbutylamine (CAS #108-09-8) (UNEP 2005). Aniline (CAS #62-53-3) is also formed to a lesser degree. These degradation products are formed via abiotic degradation in water (e.g., hydrolysis) and/or biodegradation. Another degradation product N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone, also known as 6PPD-quinone or 6PPDQ, has been recently identified in the primary literature (Tian et al. 2021). With the exception of aniline, none of the environmental transformation products are LT-1 or BM-1 chemicals. While aniline is an LT-1 chemical, it is readily biodegradable (ECB 2004). Therefore, the Benchmark Score for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is not modified by the environmental transformation products.

Table 1: Environmental Transformation Product Summary

Life Cycle Stage	Transformation Pathway	Environmental Transformation Product	CAS #	Feasible (Yes or No)	Relevant (Yes or No)	GreenScreen® List Translator Score or GreenScreen® Benchmark™ Score[10,11]
End	Abiotic degradation in water Biodegradation	4-Hydroxydiphenylamine / p-hydroxydiphenylamine	122-37-2	Yes	Yes	LT-UNK
End	Abiotic degradation in water	N-Phenyl-p-benzoquinone monoimine	2406-04-4	Yes	Yes	Not listed in Pharos Database
End	Abiotic degradation in water Biodegradation	1,3-Dimethylbutylamine	108-09-8	Yes	Yes	LT-UNK
End	Abiotic degradation in water	Aniline	62-53-3	Yes	No	LT-1
End	Biodegradation	Phenylbenzoquinone imine	N/A	Yes	Yes	Not listed in Pharos Database
End	Biodegradation	1,3-Dimethylbutylamine aniline	N/A	Yes	Yes	Not listed in Pharos Database
End	Biodegradation	p-Benzoquinone / 1,4-benzoquinone	106-51-4	Yes	Yes	LT-P1
End	Unknown	N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone, 6PPDQ)	N/A	Yes	Yes	Not listed in Pharos Database

[10] The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to screen for GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2021) is an online list-searching tool that is used to screen chemicals against the lists in the List Translator electronically.
[11] A GreenScreen® assessment of a transformation product depends on the Benchmark score of the parent chemical (see GreenScreen® Guidance).

Introduction

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is used as an antioxidant, antiozonant, and polymer stabilizer in butadiene rubber, nitrile-butadiene rubber, styrene-butadiene rubber, and synthetic isoprene rubber (HSDB 2002). It is produced via reductive alkylation of p-phenylenediamine with methyl isobutyl ketone, reductive amination of methyl isobutyl ketone and p-aminodiphenylamine.

ToxServices assessed N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) against GreenScreen® Version 1.4 (CPA 2018b) following procedures outlined in ToxServices' SOPs (GreenScreen® Hazard Assessment) (ToxServices 2020).

U.S. EPA Safer Choice Program's Safer Chemical Ingredients List (SCIL)

The SCIL is a list of chemicals that meet the Safer Choice standard (U.S. EPA 2021a). It can be accessed at: http://www2.epa.gov/saferchoice/safer-ingredients. Chemicals on the SCIL have been assessed for compliance with the Safer Choice Standard and Criteria for Safer Chemical Ingredients (U.S. EPA 2015).

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is not listed on the U.S. EPA SCIL.

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2021) is an online list-searching tool that is used to screen chemicals against all of the lists in the List Translator electronically. ToxServices also checks the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b),[12] which are not considered GreenScreen® Specified Lists but are additional information sources, in conjunction with the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) can be found in Appendix C.

- N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is not listed on the U.S. DOT list.
- N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is on the following lists for multiple endpoints:
    - EC - CEPA DSL - Inherently Toxic in the Environment (iTE).
    - German FEA - Substances Hazardous to Waters - Class 2 - Hazard to Waters.
- Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

Hazard Statement and Occupational Control

A harmonized EU GHS classification is not available for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). The REACH registration dossier authors self-classified this chemical as a Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Category 4 acute oral toxicant (H302), a GHS Category 1 skin sensitizer (H317), a GHS Category 1B reproductive toxicant (H360), a GHS Category 1 acute aquatic toxicant (H400), and a GHS Category 1 chronic aquatic toxicant (H410) (ECHA 2021), as indicated in Table 2. General personal protective equipment (PPE)

[12] DOT lists are not required lists for GreenScreen® List Translator v1.4. They are reference lists only.

recommendations are presented in Table 3, below. No occupational exposure limits (OELs) were identified.

Table 2: GHS H Statements for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8) (ECHA 2021)	
H Statement	**H Statement Details**
H302	Harmful if swallowed
H317	May cause an allergic skin reaction
H360	May damage fertility or the unborn child
H400	Very toxic to aquatic life
H410	Very toxic to aquatic life with long lasting effects

Table 3: Occupational Exposure Limits and Recommended Personal Protective Equipment for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
Gloves, eye protection, protective clothing, respirator	ECHA 2021	None identified	

Physicochemical Properties of N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD)

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is a dark brown solid distributed as flakes or pastilles under standard temperature and pressure. It has a low vapor pressure (0.00003 mm Hg) indicating that it exists mostly in the solid phase. It is slightly soluble in water (1 mg/L) and is more soluble in octanol than in water (log K_{ow} values of 2.82-4.68).

Table 4: Physical and Chemical Properties of N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)		
Property	**Value**	**Reference**
Molecular formula	C18-H24-N2	ChemIDplus 2021
SMILES Notation	CC(C)CC(C)Nc1ccc(Nc2ccccc2)cc1 CC(C)CC(C)NC1=CC=C(C=C1)NC2=CC=CC=C2	ChemIDplus 2021 PubChem 2021
Molecular weight	268.402 g/mol	ChemIDplus 2021
Physical state	Solid	ECHA 2021
Appearance	Dark brown flakes, pastilles	ECHA 2021
Melting point	49.2°C (EU Method A.1)	ECHA 2021
Boiling point	163-165°C	ECHA 2021
Vapor pressure	0.004 Pa (0.00003 mm Hg) at 25°C (estimated)	ECHA 2021
Water solubility	1 mg/L at 50°C	ECHA 2021
Dissociation constant	pKa = 6.7 at 20°C	ECHA 2021
Density/specific gravity	0.995 g/cm^3 at 50°C	ECHA 2021
Partition coefficient	Log K_{ow} = 4.68 at 20°C (estimated) Log K_{ow} = 2.82 Log K_{ow} = 4.08 at 25°C	ECHA 2021

Toxicokinetics

- *Absorption*
  - No data are available for the *in vivo* absorption of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). Based on the toxicity identified following oral dosing, this chemical is presumed to be absorbed following oral dosing (UNEP 2005). Due to the lower oral LD_{50} obtained for rats dosed with N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in olive oil compared to dosing without a vehicle, absorption from the gastrointestinal tract may be greater when present in a lipid/fat-rich medium.
- *Distribution*
  - No data are available for the *in vivo* distribution of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD).
- *Metabolism*
  - When placed in gastric juice (origin not specified), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13) had a hydrolysis half-life of 36.9 hours. The major hydrolysis product was identified as aniline, with lesser amounts of benzoquinoneimine-N-phenyl (CAS #N/A) and N-1,3 dimethyl-butylamine p-phenol (CAS #N/A), and possibly quinone (CAS #106-51-4) and 2-amino-4-methylpentane (CAS #108-09-8) (Klimisch Score 2, reliable with restrictions) (ECHA 2021). No additional metabolism studies were identified.
- *Excretion*
  - No data are available for the excretion of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): L

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Low for carcinogenicity based on the lack of carcinogenicity identified in two chronic feeding studies in rats and negative results for cell transformation. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate negative data are available, and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2005, ECHA 2021
  - A non-GLP-compliant chronic feeding study conducted in a manner similar to OECD Guideline 451 was performed with Sprague-Dawley rats (70/sex/group) provided diets containing N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, 100% active ingredient) at 0, 50, 250, or 1,500 ppm (providing doses of 2.6, 13.5, and 84.8 mg/kg/day for males, and 3.2, 16.5, and 109.5 mg/kg/day for females, respectively) for up to two years. After 12 months, 20 rats/sex/group were sacrificed, and the remaining animals were sacrificed after 24 months. A slight, non-statistically significant increase in the incidence of thyroid follicular cell carcinoma was identified in male rats (the control, low, mid, high dose group incidences were 0/70, 0/69, 2/70, and 3/69, respectively). No such increase was identified in female rats. Reviews in the literature suggest that the increased

incidence of this neoplasm may be due to increased liver activity and disruption of thyroid-pituitary signaling and may not be relevant for humans. Therefore, the authors concluded that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is not likely to be carcinogenic (Klimisch Score 2, reliable with restrictions).

- A non-GLP-compliant feeding study was performed with Charles River (CD Outbred) rats (50/sex/group) provided diets containing N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) at 0, 100, 300, or 1,000 ppm (contributing doses of 8, 23, and 75 mg/kg/day, respectively) for 24 months. Treatment did not increase the tumor frequency or type of tumors relative to those identified in the control group (Klimisch Score 2, reliable with restrictions).
- A GLP-compliant *in vitro* cell transformation assay was performed with BALB/3T3 cells exposed to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity not specified) at 0.61-1,000 µg/mL (range finding) and 0.165-0.99 µg/mL (cell transformation assay). Exposure to ≥ 0.488 µg/mL resulted in ≤ 32.3% relative survival. Treatment did not increase the frequency of transformed foci relative to the solvent control, whereas the positive control (methylcholanthrene) produced the expected increase in transformed foci (Klimisch Score 2, reliable with restrictions).

Mutagenicity/Genotoxicity (M) Score (H, M, or L): L

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Low for mutagenicity/genotoxicity based on negative mutagenicity data *in vitro* and negative clastogenicity data *in vivo*. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative data are available for both gene mutations and chromosome aberrations, and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2005, ECHA 2021
  - Note: Due to the high volume of studies provided in the dossiers, only the key *in vitro* studies are summarized and evaluated in this assessment.
  - *In vitro*: A GLP-compliant, OECD Guideline 471/Japanese Guidelines for Screening Mutagenicity Testing Of Chemicals bacterial reverse mutation assay was performed with *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2 uvr A exposed to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (99% purity) in dimethyl sulfoxide (DMSO) at ≤ 156 µg/plate (*S. typhimurium*) or ≤ 5,000 µg/plate (*E. coli*) with and without exogenous metabolic activation (unspecified S9 mix). Treatment did not increase the mutation frequency in the presence or absence of metabolic activation but was evaluated at up to cytotoxic concentrations or guideline concentration limits. The vehicle and positive (2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide, 9-aminoacridine, and 2-aminoanthracene) controls were reported as valid (Klimisch Score 1, reliable without restriction).
  - *In vitro*: A GLP-compliant mammalian cell gene mutation assay was performed with Chinese hamster ovary (CHO) cells exposed to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) in DMSO at ≤ 5 µg/mL without and ≤ 24 µg/mL with exogenous metabolic activation (unspecified S9 mix). Treatment did not increase the mutation frequency in the presence or absence of metabolic activation when tested up to cytotoxic concentrations. The vehicle and positive

(ethylmethanesulphonate and benzo(a)pyrene) controls were reported as valid (Klimisch Score 1, reliable without restriction).

- *In vitro*: A GLP-compliant, OECD Guideline 473/Japanese Guidelines for Screening Mutagenicity Testing Of Chemicals mammalian chromosome aberration test was performed with Chinese hamster lung cells (CHL/IU) exposed to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (99% purity) in DMSO at ≤ 0.01 mg/mL without metabolic activation for continuous treatment and ≤ 0.0025 mg/mL without and ≤ 0.015 mg/mL with exogenous metabolic activation (unspecified S9 mix) for short-term treatment. Cytotoxicity was evidence at ≥ 0.02 mg/mL during the continuous exposure and at ≥ 0.005 mg/mL without and ≥ 0.03 mg/mL with metabolic activation during the short-term exposure. Treatment increased chromosome aberration frequencies during the continuous exposure at ≥ 0.005 mg/mL at 24 hours and ≥ 0.0.01 mg/mL at 48 hours. Treatment did not increase the chromosome aberration frequency during the short-term exposures in the presence or absence of metabolic activation. The vehicle, untreated negative, and positive (mitomycin C and cyclophosphamide) controls were reported as valid (Klimisch Score 1, reliable without restriction).
- *In vivo*: A GLP-compliant chromosome aberration test was performed with Sprague-Dawley rats (5/sex/group) administered single gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) in corn oil at 1,000 mg/kg and sacrificed 6, 18, or 30 hours after dosing. Femoral bone marrow was isolated for the clastogenicity assessment. Treatment did not increase the frequency of chromosome aberrations. Toxicity was evident as clinical signs of toxicity and gross pathological changes, indicating absorption of the test material from the gastrointestinal tract. The vehicle and positive (cyclophosphamide) controls were reported as valid (Klimisch Score 1, reliable without restriction).
- *In vivo*: A chromosome aberration assay was performed with male Swiss mice (5/group) administered two intraperitoneal injections of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity not specified) in 1% gum acacia at 0, 100, or 200 mg/kg/day within 24 hours of each other. The animals were sacrificed 24 hours after the second injection. Treatment did not increase the frequency of chromosome aberrations. The vehicle and positive control (cyclophosphamide) were reported as valid (Klimisch Score 2, reliable with restrictions).
- *In vivo*: A GLP-compliant micronucleus assay was performed with CD-1 mice (5/sex/group) administered single intraperitoneal injections of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Flexzone 7F and 7L, purity not specified) in corn oil at 0 or 1,000 mg/kg and sacrificed 30, 48, or 72 hours. Treatment did not increase the frequency of micronuclei in erythrocytes, but toxicity was evident based on clinical signs of toxicity. The vehicle and positive (triethylenemelamine) controls were reported as valid (Klimisch Score 2, reliable with restrictions).[13]
- *In vivo*: A micronucleus test was performed with male Swiss mice (5/group) administered two intraperitoneal injections of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity not specified) in 1% gum acacia at 0, 100, 150, or 200 mg/kg/day within 24 hours of each other. The animals were sacrificed 24 hours after the second injection and bone marrow cells were isolated for the micronuclei assessment. Treatment did not increase the frequency of micronuclei. The vehicle and positive (cyclophosphamide) controls were reported as valid (Klimisch Score 2, reliable with restrictions).

[13] Note: This study is presented in the SIDS dossier with a Klimisch Score 1 (reliable without restriction). Whenever there is a discrepancy between reliability scores, ToxServices presents the lowest score.

- In summary, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was not mutagenic in bacterial or mammalian cells. While it was clastogenic in CHL/IU cells during the continuous exposure in the absence of metabolic activation, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine treatment did not increase the frequency of chromosome aberrations or micronuclei in *in vivo* rodent studies. Therefore, ToxServices concludes that it is not likely to be genotoxic *in vivo*.

Reproductive Toxicity (R) Score (H, M, or L): H

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of High for reproductive toxicity based on ToxServices classifying it as a GHS Category 1B reproductive toxicant. GreenScreen® criteria classify chemicals as a High hazard for reproductive toxicity when they are classified as GHS Category 1B reproductive toxicants (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021
  - A GLP-compliant, OECD Guideline 443 extended one-generation reproductive toxicity study was performed with Sprague-Dawley rats (25-30/sex/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (95.7% purity) in corn oil at 7, 20, or 60 mg/kg/day. F0 males were dosed for 70 consecutive days prior to mating and through mating for a minimum of 10 weeks. F0 females were dosed for 70 consecutive days prior to mating, during mating, gestation, and lactation, and until weaning of the F1 pups. The parental animals were evaluated for clinical signs of toxicity, estrous cyclicity, sperm parameters (numbers, production rate, motility, progressive motility, and morphology), gross pathology, histopathology, and reproductive performance. Treatment did not adversely affect sperm parameters or male reproductive performance. Two and five females in the mid and high dose groups, respectively, were found dead or euthanized *in extremis* on gestation day 21 through lactation day 2. The authors attributed to deaths and moribund condition to prolonged labor and/or dystocia (difficult birth). Therefore, the authors identified a female reproductive toxicity NOAEL of 7 mg/kg/day based on the dystocia identified at 20 and 60 mg/kg/day (Klimisch Score 1, reliable without restriction).
  - A GLP-compliant reproduction / developmental toxicity screening test conducted in a manner similar to OECD Guideline 421 was used as the dose range-finding study for the OECD Guideline 443 study discussed above. Sprague-Dawley rats (15/sex/group) were administered gavage does of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (96.9% purity) in corn oil at 0, 50, 75, or 100 mg/kg/day. Males were dosed for at least 14 days prior to mating and through mating for 28 days. Females were dosed for at least 14 days prior to mating and through mating, gestation, and lactation. Over the course of the study, one female each in the low and mid dose groups were found dead and one and three females each in the low and high dose groups were euthanized *in extremis*. No treatment-related effects were identified for body weight, food consumption, thyroid hormone levels [triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH)], or histopathological findings. Treatment did not adversely affect mating, fertility, or copulation/conception indices or the mean estrous cycle lengths, but mean gestation lengths in the treatment group were greater than the concurrent control group (statistical significance not provided). Dystocia was identified for one, one, and five females in the low, mid, and high dose groups, respectively, including for the three high dose females sacrificed *in*

extremis. As this was a dose range-finding study, the authors did not identify a reproductive toxicity NOAEL (Klimisch Score 1, reliable with restrictions).

- UNEP 2005, ECHA 2021
    - A GLP-compliant, OECD Guideline 421 reproduction/developmental toxicity screening test was performed with Crj: CD(SD) rats (12/sex/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (99.4% purity) in corn oil at 0, 6, 25, or 100 mg/kg/day. Males were dosed for 48 days, and females were dosed for 14 days prior to mating until lactation/postnatal day 3. Treatment did not affect body weight gain, and food consumption rates increased intermittently in high dose males and in females in all dose groups during lactation only. Treatment did not adversely affect the copulation or fertility index or estrus cyclicity, but the gestation length was statistically significantly greater in the high dose group (22.7 days) compared to the concurrent control group (22.2 days). The authors identified a reproductive toxicity NOAEL of 100 mg/kg/day based on the lack of adverse effects on fertility (Klimisch Score 1, reliable without restrictions).
    - A non-GLP-compliant three-generation reproduction toxicity test was performed with Charles River CD rats (8 males and 15 females per group per generation) provided diets containing N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13) at 0, 100, 300, or 1,000 ppm (contributing doses of 0, 8, 23, and 75 mg/kg/day, respectively). The F0 males and females were treated for 11 weeks prior to mating, and the exposure continued through mating, gestation, and lactation for two successive litters. The mating and fertility indices, incidence of parturition, mean number of live and dead pups at birth, and number of pups weaned were comparable between the control and treatment groups. The fertility indices for mid dose F1b males and F2a females were lower than controls but the authors attributed these findings to their poor health (decreased body weights and decreased survival). The authors concluded that treatment did not adversely affect fertility in this study and identified a reproductive toxicity NOAEL of 1,000 ppm (75 mg/kg/day) the highest dose tested (Klimisch Score 2, reliable with restrictions).
- In summary, while N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) did not adversely affect fertility, several studies identified increased gestation length and/or an increased incidence of dystocia with treatment. As multiple studies identified dystocia with treatment and due to the potential adverse impacts on the health of the mother and offspring, the REACH dossier authors classified N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a GHS Category 1B reproductive toxicant. ToxServices agrees with this classification and assigned the hazard score for this endpoint based on this classification.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): M

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Moderate for developmental toxicity based on decreased fetal body weights and/or increased post-implantation losses identified in rat and rabbit prenatal developmental toxicity tests, sometimes identified at maternally-toxic doses, and altered female pubertal development in rats. GreenScreen® criteria classify chemicals as a Moderate hazard for developmental toxicity when limited or marginal evidence of developmental toxicity is available in animals (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
    - *Authoritative:*
        - MAK - Pregnancy Risk Group C ("Damage to the embryo or foetus is unlikely when the MAK value or the BAT value is observed").
    - *Screening:* Not present on any screening lists for this endpoint.

- UNEP 2005, ECHA 2021
    - A GLP-compliant prenatal developmental toxicity test conducted in a manner similar to OECD Guideline 414 was performed with pregnant female Sprague-Dawley rats (25/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, 100% purity) in corn oil at 50, 100, or 250 mg/kg/day on gestation days 6-15. The animals were sacrificed on gestation day 20. Dams in the mid and high dose groups exhibited increased incidences of salivation, diarrhea, soft stool, green staining of the anogenital fur, and green fecal discoloration. Treatment did not adversely impact maternal body weights or body weight gains. High dose dams exhibited decreased food consumption during the first three days of treatment, but food consumption increased following the exposure period. Treatment did not adversely affect the number of viable fetuses, early and late resorptions, fetal sex ratio, fetal weights, or the types and incidences of fetal malformations or variations. The authors identified a maternal toxicity NOAEL of 50 mg/kg/day based on clinical signs of toxicity and a teratogenicity NOAEL of 250 mg/kg/day based on the lack of malformations induced at up to the highest dose tested.
        - The REACH dossier assigned this study a Klimisch Score of 1 (reliable without restriction). In contrast, the SIDS dossier authors assigned it a Klimisch Score of 4 (not assignable).
    - A non-GLP-compliant prenatal developmental toxicity test was performed with pregnant female New Zealand albino rabbits (17-23/group) administered oral doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) in gelatin capsules at 0, 10, or 30 mg/kg/day on gestation days 6-18. Treatment decreased body weights in all dose groups during the dosing period, with controls and high dose animals exhibiting decreased overall body weights. Mortality rates were 5/17 (29%), 3/17 (17%), and 6/23 (26%) for the control, low dose, and high dose groups, respectively. Two dams in the low and high dose groups had terminated pregnancies. The relative resorption rates were 31.4%, 30.5%, and 38.6% for the control, low dose, and high dose groups respectively, indicating an increased resorption rate for the high dose group (statistical significance not provided). The relative number of live offspring (per 100 implantation sites) in the control, low dose, and high dose groups were 68.8%, 48.3%, and 38.6%, respectively, indicating a dose-related decrease in fetal viability. One pup in a high dose litter exhibited spina bifida, but the authors concluded the incidence was too low to attribute this finding to the treatment. No other external, visceral, or skeletal malformations were identified with treatment. The authors identified a maternal toxicity and developmental toxicity NOAEL of 30 mg/kg/day. ToxServices disagrees with the selection of the developmental toxicity NOAEL based on the reduced fetal viability at both dose groups tested. Therefore, ToxServices identified a developmental toxicity LOAEL of 10 mg/kg/day for this study based on the decreased fetal viability at ≥ 10 mg/kg/day (Klimisch Score 2, reliable with restrictions).
    - A GLP-compliant OECD Guideline 421 reproduction/developmental toxicity screening test was performed with Crj: CD(SD) rats (12/sex/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (99.4% purity) in corn oil at 0, 6, 25, or 100 mg/kg/day. Females were dosed for 14 days prior to mating until lactation/postnatal day 3. Treatment did not affect maternal body weight gain, but increased food consumption for females in all treatment groups during lactation. Treatment reduced the total number of pups born (control, low, mid, high dose: 184, 160, 149*, 131), total live pups born (183, 158, 148*, and 131), and number of live pups on postnatal day 4 (males: 81, 74, 74, and 72; females: 78, 80, 71, and 57) (* = $p < 0.05$). Pup body weights at birth and at postnatal day 4

increased in the mid and high dose groups, possibly due to the increased amount of nutrition delivered on an individual basis with the decreased litter sizes. Treatment did not increase the incidence of external malformations. The authors identified a maternal toxicity NOAEL of 6 mg/kg/day based on systemic toxicity (see the systemic toxicity section below for details) and a developmental toxicity NOAEL of 100 mg/kg/day based on the lack of teratogenicity. ToxServices identified a developmental toxicity NOAEL of 6 mg/kg/day based on statistically significantly reduced number of pups born and live pups on postnatal day 4 at ≥ 25 mg/kg/day (Klimisch Score 1, reliable without restriction).

- ECHA 2021
  - A GLP-compliant, OECD Guideline 414 prenatal developmental toxicity test was performed with pregnant female New Zealand White rabbits (24-28/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (95.7% purity) in methyl cellulose (400 cP) at 0, 25, 50, or 100 mg/kg/day on gestation days 7-28. Maternal examinations included clinical signs of toxicity, body weights, food consumption, ovaries, and uterine content. Fetal examinations included evaluation of sex, fetal body weight, crown-rump length, and the incidence of external, visceral, and skeletal malformations. Three high dose females terminated their pregnancies on gestation day 22 or 24 following marked body weight losses (12.7-18.3% decreases relative to body weight at the start of the exposure period) and reduced food consumption. One female in the low dose group was discovered dead on gestation day 28 (not considered treatment-related by the study authors, possibly resulting from a gavage error). Mid and high dose dams exhibited marked to severe decreases in food consumption and increased incidences of decreased defecation, mucoid feces (high dose only), and brown material on the facial area. The decreased food consumption correlated with decreased body weights and body weight gains in mid and high dose females during the dosing period. During the period after dosing and prior to sacrifice, mid and high dose group females exhibited mean body weight gains and food consumption comparable to or greater than the control group. At sacrifice, mid and high dose dams exhibited decreased mean net body weight changes and high dose dams exhibited decreased mean gravid uterine weight. Mid and high dose dams exhibited increased mean absolute and relative liver weights. Treatment increased mean post-implantation losses and correspondingly decreased the mean litter proportion of viable fetuses in the high dose group. Mean fetal body weights decreased 9.9% and 22.2% in the mid and high dose groups, respectively, relative to the concurrent control group. Treatment did not increase the incidence of external, visceral, or skeletal malformations. The authors identified a maternal toxicity NOAEL of 25 mg/kg/day based on the decreased body weights and food consumption and increased liver weights at ≥ 50 mg/kg/day, and a developmental toxicity NOAEL of 25 mg/kg/day based on decreased fetal body weights at ≥ 50 mg/kg/day and increased post-implantation losses at 100 mg/kg/day (Klimisch Score 1, reliable without restriction).
  - A GLP-compliant U.S. EPA OPPTS 890.1450 pubertal study was performed with juvenile female Sprague-Dawley rats (15/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (91% purity) in corn oil at 0, 250, or 500 mg/kg/day for 21 days (postnatal days 22 to 42 or 43). The animals were evaluated for clinical signs of toxicity, body weights, vaginal opening, estrous cyclicity, thyroid hormone levels (T4 and TSH), and histopathology (kidney, thyroid, ovary, and uterus). Treatment-related clinical signs of toxicity included salivation prior to dosing, clear material around the mouth approximately two hours after dosing, and yellow material around the urogenital region (high dose only). Decreased mean body weight gains were identified during the first

two (low dose) or three (high dose) days of dosing, resulting in mean body weights that were 8.73% and 14.83% less than the control group for the low and high dose groups, respectively, during the treatment period. Vaginal opening was achieved at an earlier date for the high dose group (33.2 days) than the concurrent control group (35.2 days), and lower body weights were noted for females in both dose groups at the time of vaginal opening. Treatment increased the age at first estrus for the high dose group (39.2 days) compared to the concurrent controls (36.3 days), and a lower number of animals were cycling by the end of study period relative to the control group (estrous cycle lengths could not be evaluated). Treatment in both dose groups increased serum TSH and cholesterol levels and decreased serum T4, AST, and triglyceride levels. High dose females also exhibited increased total bilirubin and gamma-glutamyl transferase (GGT) levels. High dose females exhibited decreased ovary weights, and mid and high dose females exhibited decreased uterine (blotted and unblotted) weights and increased liver, kidney, and thyroid weights. Treatment-related histopathological alterations included lower colloid area and increased follicular cell height in thyroid glands of mid and high dose females, and vacuolation of the liver, absence of corpora lutea with increased tertiary follicles in the ovaries (i.e., non-cycling), and immature uterus of high dose females. The authors postulated that the increased liver weights, alterations to thyroid gland histopathology and T4 and TSH levels were secondary to hepatomegaly, but the liver histopathology was not evaluated. The authors concluded that oral dosing with N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) produced evidence of endocrine-mediated effects on pubertal development and thyroid function in juvenal female rats (Klimisch Score 1, reliable without restriction).

- A GLP-compliant OPPTS 890.1500 Endocrine Disruption test was performed with juvenile male Sprague-Dawley rats (15/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (91.0% purity) in corn oil at 250 or 500 mg/kg/day for 30 days (postnatal days 23 to 53 or 54). The males were evaluated for clinical signs of toxicity and body weights, balanopreputial separation (beginning on postnatal day 30), serum T4, TSH, and testosterone levels, and histopathology of the kidney, thyroid, testis, and epididymis. One high dose male was euthanized *in extremis* on postnatal day 25 due to severe body weight loss. Treatment-related clinical signs of toxicity included salivation prior to dosing and red and/or clear material around the mouth approximately two hours after dosing. Decreased body weight gains were noted in both dose groups, with mean final body weights for the low and high dose group animals up to 8.69% and 22.33% lower than the control group, respectively. High dose males exhibited a delayed mean age of balanopreputial separation attainment, and lower body weights on the day of attainment of balanopreputial separation was noted for both dose groups. The authors attributed these findings to the decreased body weights for these groups. High dose males exhibited higher GGT and ALT activities, and mid and high dose males exhibited decreased T4 and testosterone and increased TSH levels. Treatment-related organ weight changes included increased liver weights and decreased testes, epididymides, prostate, and seminal vesicle/coagulating gland weights in males of both dose groups. Treatment-related histopathological changes were limited to lower colloid area and increased follicular cell height in the thyroid gland in both dose groups. The authors considered the histopathological changes in the thyroid gland, increased liver weights, TSH, ALT, and GGT levels, and decreased T4 to be secondary to hepatomegaly, although the histopathology of the liver was not evaluated. Additionally, the authors attributed the decreased testosterone levels and male reproductive organ weights to be secondary to systemic stress (decreased body weights). "Therefore, there was no clear evidence of any direct test-substance-related endocrine effects." (Klimisch Score 1, reliable without restriction)

- A GLP-compliant OPPTS 890.1450 pubertal development and thyroid function test with juvenile female Sprague-Dawley rats (15/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) in corn oil at 10, 100, or 300 mg/kg/day for 21 days is presented in the REACH dossier. However, no results are provided for this test.

Endocrine Activity (E) Score (H, M, or L): M

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Moderate for endocrine activity based on altered female pubertal development in rats. In addition, *in vitro* data from the EDSP21 program and ToxCast modeling indicate that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and/or its metabolites may be estrogen/androgen receptor antagonists/agonists. However, there does not appear to be endocrine-mediated carcinogenicity, reproductive or developmental toxicity, or systemic toxicity that warrant raising the final score to High. While the score for reproductive toxicity endpoint is High, there is no evidence that the critical reproductive effect, dystocia, is mediated via endocrine disruption. GreenScreen® criteria classify chemicals as a Moderate hazard for endocrine activity when there is evidence of endocrine activity and there are no linked health effects that warrant raising the score (CPA 2018b). The confidence in the score is high as estrogen-mediated effects on female pubertal development were observed *in vivo*.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021
  - A GLP-compliant, U.S. EPA OPPTS 890.1450 pubertal study was performed with juvenile female Sprague-Dawley rats (15/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (91% purity) in corn oil at 0, 250, or 500 mg/kg/day for 21 days (postnatal days 22 to 42 or 43). The animals were evaluated for clinical signs of toxicity, body weights, vaginal opening, estrous cyclicity, thyroid hormone levels (T4 and TSH), and histopathology (kidney, thyroid, ovary, and uterus). Vaginal opening was achieved at an earlier date for the high dose group (33.2 days) than the concurrent control group (35.2 days), and lower body weights were noted for females in both dose groups at the time of vaginal opening. Treatment increased the age at first estrus for the high dose group (39.2 days) compared to the concurrent controls (36.3 days), and a lower number of animals were cycling by the end of study period relative to the control group (estrous cycle lengths could not be evaluated). Treatment in both dose groups increased serum TSH levels and decreased serum T4 levels. High dose females exhibited decreased ovary weights, and mid and high dose females exhibited decreased uterine (blotted and unblotted) weights and increased thyroid weights. Treatment-related histopathological alterations included lower colloid area and increased follicular cell height in thyroid glands of mid and high dose females, and absence of corpora lutea with increased tertiary follicles in the ovaries (i.e., non-cycling), and immature uterus of high dose females. The authors postulated that the alterations to thyroid gland histopathology and T4 and TSH levels were secondary to hepatomegaly, but the liver histopathology was not evaluated. The authors concluded that oral dosing with N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) produced evidence of endocrine-mediated effects on pubertal development and thyroid function in juvenal female rats (Klimisch Score 1, reliable without restriction).
  - A GLP-compliant OPPTS 890.1500 Endocrine Disruption test was performed with juvenile male Sprague-Dawley rats (15/group) administered gavage doses of N-(1,3-dimethylbutyl)-

N'-phenyl-p-phenylenediamine (6PPD) (91.0% purity) in corn oil at 250 or 500 mg/kg/day for 30 days (postnatal days 23 to 53 or 54). The males were evaluated for clinical signs of toxicity and body weights, balanopreputial separation (beginning on postnatal day 30), serum T4, TSH, and testosterone levels, and histopathology of the kidney, thyroid, testis, and epididymis. High dose males exhibited a delayed mean age of balanopreputial separation attainment, and lower body weights on the day of attainment of balanopreputial separation was noted for both dose groups. The authors attributed these findings to the decreased body weights for these groups. Mid and high dose males exhibited decreased T4 and testosterone and increased TSH levels. Treatment-related organ weight changes included decreased testes, epididymides, prostate, and seminal vesicle/coagulating gland weights in males of both dose groups. Treatment-related histopathological changes were limited to lower colloid area and increased follicular cell height in the thyroid gland in both dose groups. The authors considered the histopathological changes in the thyroid gland, increased TSH levels, and decreased T4 levels to be secondary to hepatomegaly, although the histopathology of the liver was not evaluated. Additionally, the authors attributed the decreased testosterone levels and male reproductive organ weights to be secondary to systemic stress (decreased body weights). "Therefore, there was no clear evidence of any direct test-substance-related endocrine effects." (Klimisch Score 1, reliable without restriction)

- U.S. EPA 2021b
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was active in 4/6 estrogen receptor (ER) assays (positive in 4 antagonism assays, negative in two agonist assays), 7/8 androgen receptor (AR) assays (positive in 6 antagonist assays and in 1/2 agonist assays), 2/2 steroidogenesis assays, and 2/6 thyroid receptor assays (positive in 2/3 antagonist assays) performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century (Appendix D).
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was predicted to be inactive for estrogen receptor agonism but active (very weak) for estrogen receptor antagonism and binding using the CERAPP Potency Level (from literature) model. It was predicted to be inactive for estrogen receptor agonis, antagonism, and binding using the CERAPP Potency Level (consensus) model. It was also predicted to be inactive for androgen receptor agonism and binding but active for androgen receptor antagonism using the COMPARA (Consensus) model in ToxCast (U.S. EPA 2021b) (Appendix E).
- VEGA 2021
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is outside of the applicability domain for the VEGA Estrogen Receptor Relative Binding Affinity model (IRFMN) (Appendix F)
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is predicted to be non-active in the Estrogen Receptor-mediated effect (IRFMN/CERAPP) 1.0.0 model with strong reliability (global applicability domain (AD) index = 0.915, similarity index = 0.837, accuracy index = 1, concordance index = 1) (Appendix F).
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was predicted to be non-active in the Androgen Receptor-mediated effect (IRFMN/COMPARA) 1.0.0 model with strong reliability (global AD Index = 0.922, similarity index = 0.85, accuracy index = 1, concordance index = 1) (VEGA 2019) (Appendix F).
- DTU 2021
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is not predicted to bind to the estrogen receptor as it lacks hydroxyl and amine groups, but its metabolites predicted via

in vivo rat metabolism and rat liver S9 metabolism are expected to be bind strongly to the estrogen receptor as they contain hydroxyl and/or amine groups (Appendix G).
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was predicted to be negative and in domain for the model battery for estrogen receptor α-binding with the balanced training set (composed of negative and in domain results by CASE Ultra, Leadscope, and SciQSAR). Additionally, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is predicted to be negative and in domain for the model battery for estrogen receptor α activation (composed of negative and in domain results by Leadscope and SciQSAR) and by the Leadscope model, CERAPP data (*in vitro*) (Appendix G).
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was predicted to be negative and in domain for the model batteries for androgen receptor inhibition (human *in vitro*) (composed of negative and in domain results by CASE Ultra, Leadscope, and SciQSAR). Additionally, it is predicted to be negative and in domain in the Leadscope model for androgen receptor activation, CoMPARA data (*in vitro*) (Appendix G).

Group II and II* Human Health Effects (Group II and II* Human)

Note: Group II and Group II* endpoints are distinguished in the v 1.4 Benchmark system (the asterisk indicates repeated exposure). For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints. See GreenScreen® Guidance v1.4, Annex 2 for more details.

Acute Mammalian Toxicity (AT) (Group II) Score (vH, H, M, or L): M

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Moderate for acute toxicity based on oral LD_{50} values as low as 893 mg/kg in rats. GreenScreen® criteria classify chemicals as a Moderate hazard for acute toxicity when oral LD_{50} values are > 300 to 2,000 mg/kg (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – Japan - H302 - Harmful if swallowed [Acute Toxicity (oral) - Category 4].
      - Based on an oral LD_{50} of 893 mg/kg in female rats (NITE 2008).
    - GHS – Korea - H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4].
- UNEP 2005, ECHA 2021
  - *Oral*: LD_{50} (Sprague-Dawley rats) = 893 mg/kg (females), 1,005 mg/kg (males) (GLP-compliant, OECD Guideline 401) (Klimisch Score 1, reliable without restriction).
    - Corn oil was used as the vehicle with 99% purity test substance.
  - *Oral*: LD_{50} (Sprague-Dawley rats) = 3,340 (males and females) (Klimisch Score 2, reliable without restriction).
    - No vehicle was used (test substance purity not specified).
  - *Oral*: LD_{50} (Sprague-Dawley rats) = 3,580 mg/kg (males and females) (Klimisch Score 2, reliable without restriction).
    - No vehicle was used with the 95.7% purity test substance.
  - *Dermal*: LD_{50} (New Zealand White rabbits) > 7.940 mg/kg (males and females)
    - No vehicle was used with the 95.7% purity substance warmed to 115°F to liquefy and applied under semi-occlusive dressing for 24 hours.
- ECHA 2021
  - *Oral*: LD_{50} (Sprague-Dawley rats) > 5,000 mg/kg (males and females) (GLP-compliant, EPA OTS 798.1175) (Klimisch Score 1, reliable without restriction).
    - No vehicle was used with the 97.6% purity test substance.

  - *Dermal*: LD_{50} (Wistar rats) > 2,000 mg/kg (males and females) (GLP-compliant, OECD Guideline 434) (Klimisch Score 4, not assignable).
    - No vehicle was used with the test substance (Dusantox 6PPD, purity not specified) under semi-occlusive dressing for 24 hours.
  - Additional acute oral toxicity tests are presented in the REACH dossier for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). However, they were assigned Klimisch Scores of 3 (not reliable) or 4 (not assignable); therefore, ToxServices did not include the results of these studies in the current assessment.
- In summary, dermal LD_{50} values are greater than 2,000 mg/kg in rabbits and rats. Oral LD_{50} values are more variable depending on whether a vehicle was used. In the absence of a vehicle, oral LD_{50}s range from 3,340 mg/kg to > 5,000 mg/kg. In contrast, when olive oil is used as the vehicle, the oral LD_{50}s are 893-1,005 mg/kg. Therefore, it is likely that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is more bioavailable in a lipid/fat rich media due to its lipophilicity (log K_{ow} = 2.82-4.68). Consequently, ToxServices assigned the score for this endpoint based on the lower LD_{50} of 893 mg/kg.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single) (Group II) Score (vH, H, M, or L): DG

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Data Gap for systemic toxicity (single dose) based on insufficient data. Available studies either tested at doses higher than GHS guidance cutoffs or reported effects at lethal doses.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2005, ECHA 2021
  - *Oral*: In the GLP-compliant, OECD Guideline 401 acute oral toxicity test that identified oral LD_{50}s of 893-1,000 mg/kg in male and female Sprague-Dawley rats, clinical signs of toxicity included decreased fecal volume at ≥ 500 mg/kg; diarrhea, hypothermia, prone position, bradypnea (abnormally slow breathing rate), and hypoactivity at ≥ 1,000 mg/kg, and abnormal gait (males only), lacrimation (females only), and weakness of limbs (females only) at 2,000 mg/kg. At necropsy, pathological lesions were identified in the respiratory tract and digestive organs (dose levels not specified). The tested doses were 250, 500 1000, and 2,000 mg/kg. Mortality was observed at the two highest doses only. No further details were provided (Klimisch Score 1, reliable without restriction).
  - *Oral*: In the acute oral toxicity study that identified an oral LD_{50} of 3,340 mg/kg in male and female Sprague-Dawley rats, clinical signs of toxicity included collapse 15-30 minutes after dosing with subsequent recovery, and loss of appetite, salivation, severe diarrhea, and dyspnea (difficult or labored breathing). At necropsy, renal and liver congestion and inflammation of the gastric mucosa were identified (unclear what dose level these findings were identified at and whether findings were for decedents or survivors). The tested doses were 2,510, 3,160, 3,980, and 5,010 mg/kg (Klimisch Score 2, reliable without restriction).
  - *Oral*: In the acute oral toxicity test that identified an oral LD_{50} of 3,580 mg/kg in male and female Sprague-Dawley rats, clinical signs of toxicity included decreased appetite and activity lasting 2-5 days, diarrhea, weakness, ocular discharge, and collapse. At necropsy, some survivors exhibited slight discoloration of the liver. The tested doses were 2,510, 3,160, 3,980, 5,010, and 6,310 mg/kg (Klimisch Score 2, reliable without restriction).
  - *Dermal*: In the acute dermal toxicity test that identified a dermal LD_{50} > 7,940 mg/kg in male and female New Zealand White rabbits, clinical signs of toxicity included decreased

activity and appetites for 3-7 days. Treatment did not affect gross pathological findings. The tested doses were 3,160, 5,010, and 7,940 mg/kg (Klimisch Score 2, reliable without restriction).

- ECHA 2021
  - *Oral*: In the GLP-compliant, EPA OTS 798.1175 acute oral toxicity test that identified an oral LD_{50} > 5,000 mg/kg in male and female Sprague-Dawley rats, clinical signs of toxicity included reduced fecal output, piloerection, rough coat, urine/fecal stains, dark material around the facial region, and diarrhea/soft stools. Reduced body weights were identified for one surviving male and 3 surviving females, while the remaining surviving animals exhibited body weight gains. At necropsy, the most notable finding was hard (rock-like), black material in the stomach of decedents and survivors. The only dose tested was 5,000 mg/kg (Klimisch Score 1, reliable without restriction).
  - *Dermal*: No details on clinical signs of toxicity, body weights, or gross pathological findings were provided for the study that identified a dermal LD_{50} > 2,000 mg/kg in Wistar rats. The only dose tested was 2,000 mg/kg (Klimisch Score 4, not assignable).
- In summary, only one study evaluated doses less than the GHS oral guidance value of 2,000 mg/kg (UN 2021). This study identified significantly adverse clinical signs of toxicity (morbidity) at doses ≥ 1,000 mg/kg. However, mortality also occurred at ≥ 1,000 mg/kg. Section 3.8.1.1 of the GHS criteria defines classifiable toxicity for this endpoint as non-lethal toxic effects on target organs. Therefore, ToxServices did not use these observations as basis of classification for this endpoint.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*) Score (H, M, or L): M

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Moderate for systemic toxicity (repeated dose) based on ToxServices classifying it as a Category 2 specific target organ toxicant following repeated oral doses under GHS criteria for effects on the liver and blood system. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (repeated dose) when they are classified as GHS Category 2 specific target organ toxicants following repeated oral doses (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – Japan - H373 - May cause damage to organs through prolonged or repeated exposure [Specific target organs/systemic toxicity following repeated exposure - Category 2].
      - Based on hemolytic anemia identified in rats in a 13-week repeated oral toxicity test (NITE 2008).
- UNEP 2005, ECHA 2021
  - *Oral*: A GLP-compliant repeated dose toxicity test conducted according to the Japanese Guideline for 28-day Repeat Dose Toxicity Testing of Chemicals was performed with Sprague-Dawley rats (5/sex/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (99% purity) in corn oil at 0, 4, 20, or 100 mg/kg/day for 28 days. Additional groups of 5 animals/sex/group were dosed with 0 or 100 mg/kg/day for 28 days and then maintained for an additional 14 days without dosing. The animals were evaluated for body weight, clinical chemistry, hematology, urinalysis, gross pathology, and histopathology. Treatment did not affect body weights. Statistically significantly altered clinical chemistry parameters at the end of the exposure period included increased total protein and decreased inorganic phosphate in mid dose females; increased creatinine, total

protein, total cholesterol and calcium and decreased albumin/globulin in high dose males; and increased total protein and albumin and decreased inorganic phosphate in high dose females. At the end of the recovery period, high dose males exhibited statistically significantly increased triglyceride levels. Statistically significantly altered hematology parameters at the end of the exposure period included increased platelets and decreased hematocrit in high dose males, and increased platelets and decreased hemoglobin, hematocrit, mean corpuscular volume (MCV), prothrombin time, and activated partial thromboplastin time in high dose females. At the end of the recovery period, high dose males exhibited significantly decreased MCV, mean corpuscular hemoglobin (MCH), and hematocrits and increased platelets, and high dose females exhibited decreased hemoglobin, hematocrit, MCH, and MCV. High dose males and females exhibited increased urinary proteins levels (time point not specified), and increased liver weights at the end of the exposure period. The increased liver weights were still evident in high dose females at the end of the recovery period. Reversible liver enlargement was identified for two males and one female in the high dose groups, and reversible periportal fatty change was evident for mid and high dose females and high dose males. The authors identified a NOAEL of 20 mg/kg/day based on effects to the liver and hematology and clinical chemistry parameters at 100 mg/kg/day (Klimisch Score 1, reliable without restriction).

- As this study had an exposure period less than 90 days, ToxServices adjusted the GHS oral guidance values of 10 and 100 mg/kg/day (UN 2021) by a factor of 3 (28 days is approximately one-third of 90 days) to 30 and 300 mg/kg/day, respectively. Since the LOAEL of 100 mg/kg/day is between the adjusted guidance values, ToxServices classified N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a GHS Category 2 specific target organ toxicant following repeated oral dosing based on effects to the liver and blood system.

- *Oral*: A non-GLP-compliant chronic feeding study conducted in a manner similar to OECD Guideline 452 was performed with Sprague-Dawley rats (70/sex/group) provided diets containing N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (100% active ingredient as Santoflex 13) at 0, 50, 250, or 1,500 ppm (contributing doses of 2.6, 13.5, and 84.8 mg/kg/day for males and 3.2, 16.5, and 109.5 mg/kg/day for females, respectively). After 12 months, 20 rats/sex/group were sacrificed and evaluated for treatment-related effects. The surviving animals were sacrificed after 24 months. The animals were evaluated for clinical signs of toxicity, body weight, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, and histopathology. High dose males and females and mid dose females consistently exhibited decreased mean body weights. These groups exhibited increased food consumption values, suggesting a decreased food utilization for these animals. Treatment slightly altered hematology parameters in the high dose group, including decreased hemoglobin, hematocrit, erythrocyte counts (females only), MCV, and MCH, and increased platelet counts. Treatment also significantly increased cholesterol levels in high dose males and females. At necropsy, mean absolute and relative kidney weights increased in high dose males and females sacrificed after 12 months but not after 24 months. The severity but not the incidence of chronic nephropathy increased in high dose males and females sacrificed after 12 and 24 months. The mean absolute and relative liver weights increased in mid dose males and females at termination and in high dose males and females at the 12- and 24-month sacrifices. High dose females exhibited increased incidences of cytoplasmic vacuolization of the liver and pigment in the hepatocytes and reticuloendothelial cells. No histopathological alterations were identified in the livers of high dose males or mid dose males or females. Treated males had a slightly increased incidence of thyroid follicular cell hyperplasia. Treatment increased the mean

absolute and relative spleen weights of high dose males sacrificed after 12 months but not 24 months. The authors identified a NOEL/NOAEL of 50 ppm (equivalent to 2.6-3 mg/kg/day) and LOEL/LOAEL of 250 ppm (equivalent to 13.5-16.5 mg/kg/day) based on decreased body weights and increased food consumption in mid dose females and increased liver weights for both sexes.

- The REACH dossier authors assigned this study a Klimisch Score 2 (reliable with restrictions). In contrast, the SIDS dossier authors assigned this study a Klimisch Score 4 (not assignable).
- Since the exposure duration was greater than 90 days, ToxServices adjusted the GHS oral guidance values of 10 and 100 mg/kg/day by a factor of 8 (90 days is approximately eight time less than 24 months or 730 days) to 1.25 mg/kg/day and 12.5 mg/kg/day, respectively. As the LOEL/LOAEL of 13.5-16.5 mg/kg/day was greater than the adjusted guidance values, ToxServices did not classify N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a specific target organ toxicant following repeated doses based on the results of this study.

- *Oral*: A GLP-compliant, OECD Guideline 421 reproduction/developmental toxicity screening test was performed with Crj: CD(SD) rats (12/sex/group) administered gavage doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (99.4% purity) in corn oil at 0, 6, 25, or 100 mg/kg/day. Males were dosed for 48 days, and females were dosed for 14 days prior to mating until lactation/postnatal day 3. Treatment did not affect body weight gain, and food consumption rates increased intermittently in high dose males and in females in all dose groups during lactation only. In the low dose group, histopathological changes were limited to slightly cellular infiltration of lymphocytes in 4/12 males (organ not specified, presumably liver). In the mid dose group, treatment increased the incidence of salivation and vacuolar liver degeneration in males, increased absolute liver weights in females, and increased relative liver weights in males and females. In the high dose group, one dam died on gestation day 23 and both sexes exhibited increased incidences of salivation, liver enlargement, increased relative and absolute liver wights. High dose males also exhibited increased absolute and relative adrenal weights and an increased incidence of vacuolar liver degeneration. The authors identified a NOAEL of 6 mg/kg/day based on increased liver weights, salivation, and vacuolar liver degeneration identified at 25 mg/kg/day (Klimisch Score 1, reliable without restriction).
  - As the exposure duration was less than 90 days, ToxServices adjusted the GHS oral guidance values of 10 and 100 mg/kg/day (UN 2021) by a factor of 2 (48 days is approximately half of 90 days) to 20 and 200 mg/kg/day, respectively. Since the LOAEL of 25 mg/kg/day is between the adjusted guidance values, ToxServices classified N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a GHS Category 2 specific target organ toxicant following repeated oral dosing based on effects to the liver.
- *Oral*: A non-GLP-compliant chronic feeding study was performed with Charles River (CD Outbred) rats (50/sex/group) provided diets containing N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) at 0, 100, 300, or 1,000 ppm (contributing doses of 0, 8, 23, or 75 mg/kg/day) for 24 months. Treatment did not adversely affect survival, frequency of clinical signs, clinical chemistry or urinalysis parameters, or gross or histopathological findings relative to the concurrent control group. High dose rats exhibited decreased body weights and body weight gains, which correlated with decreased food consumption during the first four and two weeks of treatment for males and females, respectively. Treatment decreased erythrocyte counts in high dose males at 3 months and high dose females at 3, 6, and 12 months but not at later times points. Treatment

significantly decreased hematocrit values in high dose males at 3 and 12 months and in high dose females at 3, 6, and 12 months, and slightly increased hematocrit values in high dose males and females at 18 and 24 months. Treatment-related organ weight changes included decreased absolute and relative kidney weights in high dose females, increased absolute and relative liver weights in high dose males, and decreased absolute and relative spleen weights in high dose females. The authors identified a NOAEL of 300 ppm (23 mg/kg/day) based on altered body weights, hematology parameters, and organ weights at 1,000 ppm (75 mg/kg/day) (Klimisch Score 2, reliable with restrictions).

- *Oral*: A GLP-compliant subchronic repeated dose toxicity test was performed with Sprague-Dawley rats (25/sex/group) provided diets containing N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, 97.1% purity) in diet at 0, 250, 1,000, or 2,500 ppm (contributing doses of 15.7, 62.3, and 153.8 mg/kg/day for males and 18.5, 75.0, and 172.1 mg/kg/day for females, respectively) for 13 weeks. Treatment did not adversely affect survival, clinical signs of toxicity, ophthalmoscopic findings. High dose females and mid and high dose males exhibited decreased body weight gains from the start of the exposure period. Terminal body weights decreased in a dose-related manner, with high dose males and females having terminal body weights that were 87% and 90%, respectively, of the concurrent controls. Decreased body weight gains correlated with decreased food consumption values for mid and high dose males and high dose females. In mid and high dose males and females, treatment increased albumin, globulin, total protein, calcium, and cholesterol levels and decreased creatine, aspartate aminotransferase (AST, SGOT), alanine aminotransferase (AST, SGPT), and blood urea nitrogen (BUN). Mid and high dose males also exhibited increased bilirubin levels. Treatment induced mild anemia and thrombocytosis and decreased red blood cell counts, hemoglobin (Hb), MCV, hematocrit (HCT), MCH, mean corpuscular hemoglobin concentration (MCHC) in mid and high dose animals. Low dose females also exhibited decreased lymphocytes and white blood cell count and slight anemia during the interim sampling period only. Increased absolute liver weights were evident in high dose males and high dose females, and increased relative liver weights were identified in mid and high dose males and females in all treated groups. Absolute spleen weights significantly decreased in high dose males, while relative spleen weights increased in high dose males. High dose males also exhibited statistically significantly decreased absolute testes weights, but relative testes weights were unaffected by the treatment. Relative brain weights increased in mid and high dose males and high dose females, and relative kidney weights increased in mid and high dose females. The authors identified a NOAEL of 250 ppm (15.7-18.5 mg/kg/day) based on reduced body weight gain, mild anemia, and increased liver weights detected at 1,000 ppm (62.3-75 mg/kg/day)
    - The REACH dossier authors assigned this study a Klimisch Score 1 (reliable without restriction). In contrast, the SIDS dossier authors assigned this study a Klimisch Score 2 (reliable with restrictions).
    - As the LOAEL of 62.3-75 mg/kg/day is between the GHS oral guidance values of 10 and 100 mg/kg/day (UN 2021), ToxServices classified N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a GHS Category 2 specific target organ toxicant following repeated oral dosing based on effects to the liver and blood system.
- Additional repeated oral dose toxicity studies and one repeated inhalation exposure study are presented in the REACH and SIDS dossiers for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). However, they were assigned Klimisch Scores of 3 (not reliable)

or 4 (not assignable) and/or had limited levels of detail available. Therefore, ToxServices did not include the results of these studies in the current assessment.

- In summary, multiple repeated oral dose toxicity studies identified the liver and blood system as the target organs for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). Based on the LOAELs for the 28-day and 90-day studies and the OECD Guideline 421 study, ToxServices classified N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a Category 2 specific target organ toxicant following repeated oral doses under GHS criteria.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): *M*
N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Moderate for neurotoxicity (single dose) based on ToxServices classifying it as a Category 3 specific target organ toxicant following single exposures for narcotic effects. GreenScreen® criteria classify chemicals as a Moderate hazard for neurotoxicity (single dose) when they are classified as GHS Category 3 specific target organ toxicant following single exposures for narcotic effects (CPA 2018b). The confidence in the score is low as it is not clear if the observed effects were specific neurotoxicity or just a manifestation of general toxicity and weakness after receiving a large dose of a chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – Japan - H336 [Specific target organs/systemic toxicity following single exposure - Category 3].
      - Based on decreased locomotor activity, hind limb weakness, gait abnormality, abdominal position, fall of posture, and exhaustion of the hind foot identified in a GLP-compliant, OECD Guideline 401 acute oral toxicity test in rats (NITE 2008).
- UNEP 2005, ECHA 2021
  - *Oral*: In the GLP-compliant OECD Guideline 401 acute oral toxicity test that identified oral LD_{50}s of 893-1,000 mg/kg in male and female Sprague-Dawley rats, clinical signs of toxicity included decreased fecal volume at ≥ 500 mg/kg; diarrhea, hypothermia, prone position, bradypnea (abnormally slow breathing rate), and hypoactivity at ≥ 1,000 mg/kg, and abnormal gait (males only), lacrimation (females only), and weakness of limbs (females only) at 2,000 mg/kg (Klimisch Score 1, reliable without restriction).
  - *Oral*: In the acute oral toxicity study that identified an oral LD_{50} of 3,340 mg/kg in male and female Sprague-Dawley rats, clinical signs of toxicity included collapse 15-30 minutes after dosing with subsequent recovery, and loss of appetite, salivation, and severe diarrhea (Klimisch Score 2, reliable without restriction).
  - *Oral*: In the acute oral toxicity test that identified an oral LD_{50} of 3,580 mg/kg in male and female Sprague-Dawley rats, clinical signs of toxicity included decreased appetite and activity lasting 2-5 days, diarrhea, weakness, and collapse (Klimisch Score 2, reliable without restriction).
  - *Dermal*: In the acute dermal toxicity test that identified a dermal LD_{50} > 7,940 mg/kg in male and female New Zealand White rabbits, clinical signs of toxicity included decreased activity and appetites for 3-7 days (Klimisch Score 2, reliable without restriction).
- In summary, single oral or dermal doses of N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) produced signs of transient narcotic effects, including decreased activity, hypothermia, weakness of limbs, and abnormal gait. Therefore, ToxServices classified it as a specific target organ toxicant following single exposures for narcotic effects under GHS criteria (UN 2021).

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): DG
N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Data Gap for neurotoxicity (repeated dose) based on insufficient data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ToxServices identified no data for this endpoint.

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): H
N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of High for skin sensitization based on ToxServices classifying it as a Category 1A skin sensitizer under GHS criteria. GreenScreen® criteria classify chemicals as a High hazard for skin sensitization when they are classified as GHS Category 1A skin sensitizers (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
  - *Authoritative:*
    - MAK - Sensitizing Substance Sh - Danger of skin sensitization.
  - *Screening:*
    - GHS – Japan - H317 - May cause an allergic skin reaction [Skin sensitizer - Category 1].
      - Based on positive results in human patch tests and a guinea pig maximization test (NITE 2008).
    - GHS – Korea - H317 - May cause an allergic skin reaction [Skin sensitization - Category 1].
    - GHS - New Zealand – H317 - Contact sensitisers (Cat. 1).
      - Based on EU Risk Phrase 43 (CCID 2021).
- ECHA 2021
  - A guinea pig maximization test was performed with female Hartley guinea pigs (4/group) administered dermal doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity not specified). The induction doses were 500 ppm (0.05%) in acetone and the challenge doses were 50 or 5,000 ppm in acetone under occlusive coverage. At 48 hours after the challenge dose, 4/4 animals exhibited positive skin reactions towards the 50 and 5,000 ppm doses. Therefore, the authors concluded that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was sensitizing to the skin under the tested conditions (Klimisch Score 2, reliable with restrictions).
    - Based on 100% of the animals responding following an intradermal dose of 0.05%, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) warrants classification as a GHS Category 1A skin sensitizer. GHS criteria define Category 1A skin sensitizers as chemicals that produce positive reactions ≥ 30% animals at ≤ 0.1% intradermal doses (UN 2021).
  - A local lymph node assay was performed with female Balb/c mice administered topical applications of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity and vehicle not specified) at 0%, 0.1%, 0.3%, 1%, or 3%. The stimulation indices were 2.34 at 1% and 5.06 at 3%. As the concentrations tested were non-irritating, the authors concluded that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was sensitizing to the skin under the tested conditions (Klimisch Score 2, reliable with restrictions).
    - As the authors did not identify an EC3, ToxServices calculated the EC3 as 1.49% using the linear interpolation method of Ryan et al. (2007). The EC3 of 1.49% is

less than the GHS guidance value of 2% (UN 2021), warranting classification as a GHS Category 1A skin sensitizer.

- A GLP-compliant, OECD Guideline 429 local lymph node assay was performed with female CBA mice (4/group) administered topical applications of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Dusantox 6PPD, purity not specified) in acetone/olive oil (4:1 v/v) at 10%, 25%, or 50%. The stimulation indices were 1.55, 12.37, and 12.38 for the 10%, 25%, and 50% solutions, respectively. The authors calculated an EC3 of 12.06% and concluded that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was moderately sensitizing to the skin under the tested conditions (This study was reported in the REACH dossier without a Klimisch Score).
  - As the EC3 of 12.06% is greater than the GHS guidance value of 2% (UN 2021), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) warrants classification as a GHS Category 1B skin sensitizer.
- A non-GLP-compliant guinea pig maximization test was performed with guinea pigs (15/group, strain not specified) administered dermal doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Vulkanox 4020). The induction doses were administered as intradermal injection doses of 1% test substance in polyethylene glycol and topical applications of 2% test substance in polyethylene glycol. The challenge dose was applied as a topical application of 12.5% or 25% test substance in polyethylene glycol under occlusive dressing. Following challenge with 12.5% test substance, 6/15 and 3/15 animals exhibited positive dermal reactions at 24 and 48 hours respectively compared to 0/15 in the negative control at both time points. Following challenge with 25% test substance, 15/15, and 14/15 animals exhibited positive dermal reactions at 24 and 48 hours, respectively, compared to 2/15 negative control animals at both time points.
  - Based on 100% of the animals responding following an intradermal dose of 1%, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) warrants classification as a GHS Category 1A skin sensitizer. GHS criteria define Category 1A skin sensitizers as chemicals that produce positive reactions ≥ 60% animals at > 0.1% to ≤ 1% intradermal doses (UN 2021).

- UNEP 2005, ECHA 2021
  - A guinea pig maximization test was performed with female Hartley guinea pigs (20/group) administered dermal doses of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity nots specified). The induction doses were applied as intradermal injections of 0.5% test substance in olive oil and a topical application of 1% test substance in Vaseline. The challenge dose was applied at topical applications of 0.05% or 0.5% in Vaseline. The type of coverage was not specified. At 48 hours after the challenge dose, the 0.05% challenge produced 10/20 positive reactions compared to 0/20 for the negative control and the 0.5% challenge produced 18/20 positive reactions compared to 4/20 for the negative control. The authors concluded that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was sensitizing to the skin under the tested conditions (Klimisch Score 2, reliable with restrictions).
    - Based on 50%-90% of the animals responding following an intradermal dose of 0.5%, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) warrants classification as a GHS Category 1A skin sensitizer. GHS criteria define Category 1A skin sensitizers as chemicals that produce positive reactions ≥ 60% animals at > 0.1% to ≤ 1% intradermal doses (UN 2021).
- UNEP 2005

    - Healthy volunteers not previously exposed to test rubber formulations had at most a low rate of sensitization towards N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), whereas individuals previously sensitized to rubber samples exhibited a higher rate of sensitization towards this chemical: positive sensitization results were identified in 17/50, 16/50, 4/50, and 5/50 previously exposed individuals participating in repeated insult patch tests.
- As the results from the majority of animal tests support classification of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a GHS Category 1A skin sensitizer, ToxServices classified it as a GHS Category 1A skin sensitizer and assigned a high hazard for this endpoint.

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): *M*
N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Moderate for respiratory sensitization based on ToxServices classifying it to GHS Category 1B due to positive skin sensitization data and the presence of a structural alert for respiratory sensitization, according to ECHA (2017)'s guidance. GreenScreen® criteria classify chemicals as a Moderate hazard for respiratory sensitization when they are classified to GHS Category 1B (CPA 2018b). Confidence in the score is reduced as there are no data to subclassify the compound to Category 1A and 1B, which translate to Moderate and High scores, respectively.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2021a
    - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) contains a structural alert for respiratory sensitization (phenylenediamines) (Appendix H).
- No data were identified for the target compound for this endpoint. Therefore, ToxServices attempted to evaluate the respiratory sensitization potential of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) according to ECHA's guideline (ECHA 2017), which states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). ECHA recommended that if a chemical is not a dermal sensitizer based on high quality data, it is unlikely to be a respiratory sensitizer. ECHA also noted that this rationale does not cover respiratory hypersensitivity caused by non-immunological mechanisms, for which human experience is the main evidence of activity (ECHA 2017). N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) does contain a structural alert, and is a skin sensitizer based on multiple studies in animals. According to the ECHA guidance, this warrants classification as a respiratory sensitizer. However, ECHA did not provide guidance on subcategorization to GHS Category 1A (high potency) and 1B (low potency). Due to the lack of specific respiratory sensitization data, ToxServices classified it to GHS Category 1B with low confidence.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): L
N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Low for skin irritation/corrosivity based on ToxServices not classifying it as a skin irritant under GHS criteria. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate and negative data and no GHS classification are available (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2005, ECHA 2021

  - A non-GLP-compliant dermal irritation test was performed with New Zealand White rabbits (6 total) administered topical applications of 0.5 mL undiluted N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (95.7% purity, warmed to 46°C to liquify) to intact and abraded skin under semi-occlusive dressing for 24 hours. A 7-day observation period followed the exposure period. At 24 and 72 hours, the mean overall irritation score was 0/8, and the authors concluded that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was not irritating under the tested conditions (Klimisch Score 2, reliable with restrictions).
  - A non-GLP-compliant dermal irritation test was performed with rabbits (strain not specified, 3 total) administered topical applications of undiluted N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) under occlusive dressing for 24 hours. An observation period of 120 hours followed the exposure period. The mean overall scores were 0.6/8, 1.6/8, 1.3/8, and 1/8 at 4, 24, 48, and 72 hours respectively. All signs of dermal irritation resolved within 120 hours. The authors concluded that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was slightly irritating under the tested conditions (Klimisch Score 2, reliable with restrictions).
  - Additional skin irritation tests were presented in the REACH and SIDS dossiers. However, they were reported in the REACH dossier with Klimisch Scores of 3 (not reliable) or 4 (not assignable). Therefore, ToxServices did not include the results of these studies in the current assessment.
- GHS criteria define skin irritants as chemicals that produce mean scores ≥ 1.5 for erythema and/or edema in at least 2 of 3 animals following readings at 24, 48, and 72 hours (UN 2021). As the results of the two skin irritation tests do not indicate skin irritation above this threshold, ToxServices did not classify N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a dermal irritant under GHS criteria.

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): *M*
N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Moderate for eye irritation/corrosivity based on ToxServices classifying it as a Category 3 ocular irritant under GHS criteria. GreenScreen® criteria classify chemicals as a Moderate hazard for eye irritation/corrosivity when they are classified as GHS Category 3 eye irritants (CPA 2018b). The confidence in the score is low as scores for individual ocular irritation endpoints were not provided.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – Japan - H320 - Causes serious eye irritation [Serious eye damage / eye irritation - Category 2B].
      - Based on an average irritation score of 20.6/110 in rabbits at 24 hours (NITE 2008).
- UNEP 2005, ECHA 2021
  - A non-GLP-compliant ocular irritation test was performed with New Zealand White rabbits (6 total) administered ocular instillations of 0.1 mL undiluted N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (95.7% purity, warmed to 46°C to liquify) for 24 hours. An observation period of seven days followed the instillation. The animals exhibited slight discomfort immediately following instillation. At 24, 48, and 72 hours, the mean overall irritation score was 1.2/110. The irritation was characterized by slight erythema at 1, 24, and 48 hours and moderate to copious discharge at 1 and 24 hours. The ocular irritation resolved completely within 72 hours. The authors concluded that N-(1,3-dimethylbutyl)-N'-phenyl-

p-phenylenediamine (6PPD) was slightly irritating under the tested conditions (Klimisch Score 2, reliable with restrictions).
    - A non-GLP compliant ocular irritation test was performed with rabbits (strain not specified, 3 total) administered 0.1 mL undiluted N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity not specified) for 24 hours. An observation period of five days followed the instillation. The mean overall irritation scores were 20.6/110, 20/110, 14.6/110, 9/110, and 4.6/110 at 1, 4, 24, 48, and 72 hours, respectively. The ocular irritation, characterized as edema and erythema at 24, 48, and 72 hours, resolved completely within 120 hours. The authors concluded that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was slightly irritating under the tested conditions (Klimisch Score 2, reliable with restrictions).
    - An additional eye irritation test was presented in the REACH dossier. However, it was reported in the REACH dossier with Klimisch Scores of 4 (not assignable). Therefore, ToxServices did not include the results of this study in the current assessment.
- Under GHS criteria (UN 2019), a chemical is classified as irritating to the eyes if it produces mean scores ≥ 1 for corneal opacity, ≥ 1 for iritis, ≥ 2 for conjunctival redness, and/or ≥ 2 for chemosis in at least 2 of 3 animals following readings at 24, 48, and 72 hours, with reversibility of the irritation effects occurring within 21 days (Category 2A) or 7 days (Category 2B). The aggregate scores provided in the ocular irritation tests above do not provide sufficient data for each of the eye irritation endpoints. However, the irritation scores for both studies support slight/mild irritation following ocular instillation of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). Therefore, ToxServices conservatively classified N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a Category 3 (mild) ocular irritant under GHS criteria (UN 2021).

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): vH

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Very High for acute aquatic toxicity based on measured acute aquatic toxicity values as low as 0.028 mg/L. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when acute aquatic toxicity values are ≤ 1 mg/L (CPA 2018b). Although no reliable algal data were identified, the available data for aquatic invertebrates and fish support this classification. The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:*
        - GHS – Japan - H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1].
            - Based on a 96-hour LC_{50} of 0.028 mg/L in fish (*Oryzias latipes*) (NITE 2008).
        - GHS – Korea - H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1].
        - GHS – New Zealand – H400 – Very toxic to aquatic life.
            - Based on EU Risk Phrase R50 (CCID 2021).
        - EC - CEPA DSL - Inherently Toxic in the Environment (iTE).
            - Based on a 96-hour LC_{50} of 0.14 in steelhead trout (*Salmo gairdneri*) (OECD 2021b).
- UNEP 2005, ECHA 2021

  - 96-hout LC_{50} (*Oryzias latipes*, Japanese rice fish) = 0.028 mg/L (measured) (GLP-compliant, OECD Guideline 203) (Klimisch Score 1, reliable without restriction).
  - 48-hour mobility EC_{50} (*Daphnia magna*) = 0.23 mg/L (measured) (GLP-compliant, OECD Guideline 202) (Klimisch Score 1, reliable without restriction).
  - 48-hour mobility EC_{50} (*D. magna*) = 0.82 mg/L (nominal) (non-GLP-compliant, U.S. EPA 1975 Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians method) (Klimisch Score 2, reliable with restrictions).
  - 48-hour mobility EC_{50} (*D. magna*) = 0.51 mg/L (nominal) (non-GLP-compliant, U.S. EPA 1975 Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians method) (Klimisch Score 2, reliable with restrictions).
    - Test substance was N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13) and its degradation products.
  - 48-hour EC_{50} (*D. magna*) = 0.79 mg/L (OECD Guideline 202) (Klimisch Score 4, not assignable).
  - An algal study presented in the REACH and SIDS dossiers identified a 96-hour chlorophyll a EC_{50} of 0.6 mg/L in *Pseudokirchneriella subcapitata* (previously *Selenastrum capricornutum*). While this study is reported in the SIDS dossier with a Klimisch Score of 2 (reliable with restrictions), it is presented in the REACH dossier with a Klimisch Score of 3 (not reliable) due to "[s]ignificant methodological deficiencies" including the lack of exponential growth of the test organisms during the incubation period. Therefore, ToxServices did not include the results of this study in the current assessment.
- Tian et al. 2021
  - The environmental transformation product 6PPD-quinone has a 24-hour LC_{50} of 0.79 µg/L (0.00079 mg/L) in juvenile coho salmon (*Oncorhynchus kisutch*).

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): vH

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Very High for chronic aquatic toxicity based on a measured chronic aquatic toxicity value of 0.004 mg/L for fish. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when chronic aquatic toxicity data are ≤ 0.1 mg/L (CPA 2018b). Although no chronic aquatic toxicity data were identified for aquatic invertebrates or algae, fish was the most sensitive trophic level for acute aquatic toxicity and the chronic fish value alone supports this classification. The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
  - *Other:*
    - GHS – Japan - H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1].
      - Based on a 96-hour LC_{50} of 0.028 mg/L in fish (*Oryzias latipes*) and lack of rapid degradability (NITE 2008).
    - GHS – Korea - H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1].
    - GHS – New Zealand – H410 – Very toxic to aquatic life with long lasting effects.
      - Based on EU Risk Phrase R50 (CCID 2021).
- ECHA 2021
  - 30-day NOEC (*O. latipes*, Japanese rice fish) = 0.004 mg/L (measured) (GLP-compliant, OECD Guideline 210) (Klimisch Score 1, reliable without restriction).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *H*

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of High for persistence based on an estimated half-life of 75 days in soil, its predicted dominant environmental compartment. GreenScreen® criteria classify chemicals as a High hazard for persistence when soil is the dominant environmental compartment and the half-life in soil is > 60 to 180 days (CPA 2018b). The confidence in the score is low as it is based on modeling.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021
  - A GLP-compliant, OECD Guideline 301 C test (modified MITI test) was performed with activated sludge (adaption not specified) exposed to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (> 99% purity) at 100 mg/L for 28 days. At the end of the exposure period, the total level of degradation (mineralization) was 2% but 92% of the 6PPD degraded to intermediate chemical species. In contrast, the reference material, aniline, degraded/mineralized 64% and 71% after 7 and 14 days, respectively. Therefore, the authors concluded that no biodegradation (mineralization) occurred under the tested conditions (Klimisch Score 1, reliable without restrictions).
  - A ready biodegradability test comparable to OECD Guideline 301 B was performed with adapted, activated sludge exposed to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (80.35% purity) at 30 mg/L for 32 days. At the end of the exposure period, the level of degradation was 7.2%. Therefore, the authors concluded that no biodegradation occurred under the tested condition (Klimisch Score 2, reliable with restrictions).
  - A second OECD Guideline 301 C (modified MITI test) is presented in the REACH dossier for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). However, it was assigned a Klimisch Score of 3 (not reliable) due to "[s]ignificant methodological deficiencies"; therefore, ToxServices did not include the results of this study in the current assessment.
  - In a GLP-compliant biodegradation test similar to an aqueous Die-Away screening method, natural or sterilized water from the Mississippi river or deionized water was exposed to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity not specified) at 1,002 µg/L for 22 hours. At the end of the exposure period, the levels of primary degradation were 97%, 96%, and 88% from the active river water, sterile river water, and deionized water, respectively. The primary transformation half-life was estimated as 2.9 hours (Klimisch Score 2, reliable with restrictions).
  - In a non-GLP-compliant hydrolysis test based on OECD Guideline 111, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Vulkanox 4020, > 98% purity) was performed to evaluate degradation of this chemical under environmental conditions (pH 7, presence of light, air, and algal nutrient medium). The test substance was incubated in buffered solution at 26°C or 50°C and nutrient medium (contains trace ions of essential heavy metals) at 26°C. The hydrolysis half-lives were 14 and 5 hours for the buffered solution at 26°C and 50°C, respectively, and 8 hours for the nutrient medium (Klimisch Score 2, reliable with restrictions).
  - In a GLP-compliant hydrolysis test conducted according to EPA OTS 796.3500 (hydrolysis as a function of pH at 25°C), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)

(as Santoflex 13, purity not specified) had a hydrolysis half-life of 3.3 hours (Klimisch Score 2, reliable with restrictions).

- N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) has an estimated atmospheric half-life of 1.7 hours due to indirect photolysis with hydroxyl radicals (Klimisch Score 2, reliable with restrictions).
- In a non-GLP-compliant hydrolysis test, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) had hydrolysis half-lives of 2.9 hours in active river water, 3.9 hours in sterile river water, and 6.8 hours in sterile deionized water. After 22 hours, the degree of primary degradation for 6PPD was 96% in sterile river water and 88% in deionized water (Klimisch Score 2, reliable with restrictions).
- In a non-GLP-compliant hydrolysis test, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) had a hydrolysis half-life of < 24 hours in deionized water (Klimisch Score 2, reliable with restrictions).
- A GLP-compliant hydrolysis test was performed with N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Santoflex 13, purity not specified) incubated in deionized and well water in the presence and absence of artificial sunlight. At pH 7.0, the hydrolysis half-lives were 5.7 and 6.3 hours in light and dark deionized water, respectively, and 3.7 and 5.7 hours in light and dark well water, respectively. Benzoquinoneimine-n-phenyl and 4-hydroxydiphenylamine were identified as major and minor hydrolysis products, respectively (Klimisch Score 2, reliable with restrictions).
- Additional hydrolysis studies are presented in the REACH dossier for 6PPD. However, they were assigned Klimisch scores of 4 (not assignable). Therefore, ToxServices did not include the results of these studies in the present assessment.
- *Surrogate: 1,4-Benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- (CAS #3081-01-4)*: A GLP-compliant, OECD Guideline 307 (aerobic and anaerobic transformation in soil) biodegradation in soil test was performed with fresh soil samples (n = 4, 100 g dry weight) exposed to ^{14}C-radiolabeled 1,4-benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- (96.30% unlabeled purity, >97% radiochemical purity) at 0.25 mg per kg dry soil in metabolism flasks placed in a dark room at 12°C for up to 56 days. The extractable radioactivity decreased from 37.2-77.1% applied radioactiivty (AR) at day 0 to 4.5-9.1% AR at the end of the study. The non-extractable radioactivity increased from 26.9-59.8% AR to 76.4-84.8% AR by the end of the study. On day 56, organic matter fractionation was used to determine the partitioning of the non-extractable readioactivity: 23.3%-57.3% was bound to insoluble humins, 9.8%-30.5% was bound to humic acids, and 7.8-16.4% AR was bound to fulvic acids. The volatile radioactivity as radioactive carbon dioxide was 4.1%-8.9% AR by the end of the study, indicating ⩽ 8.9% of the applied radioactivity reached ultimate degradation (i.e., mineralization). The authors concluded that the marginal mineralization at the end of the study was likley due to the lack of bioavailability of the radioactive chemical species. The abiotic/biotic degradation half-life in soil was 0.03 days, 1.4 days, 1.4 days, and 1.9 days for the four soil samples (Klimisch Score 1, reliable without restriction).
- *Surrogate: 1,4-Benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- (CAS #3081-01-4)*: A GLP-compliant, OECD Guideline 307 (aerobic and anaerobic transformation in soil) biodegradation in soil test was performed with fresh soil samples (100 g dry weight) exposed to ^{14}C-radiolabeled 1,4-benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- (96.30% unlabeled purity, >97% radiochemical purity) at 0.225 mg/g soil dry weight in metabolim flasks at 12°C in a dark room for 4 hours (aerobic period). Subsequently, the test systems were flooded with deoxygenated water to simulate anearobic degradation and maintained for up to 120 days. The extractable radioactivity decreased from 68.4% AR at 0

hour to 36.9% AR at the end of the aerobic period (4 hours). During the anaerobic period, the level of extractable radioactivity was 23.2%, 13.1%, 13.1%, 17.7%, 19.4%, 13.5%, and 17% AR after 1, 3 7, 14, 28, 56, and 120 days, respectively. The results indicate that 1,4-benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- degrades rapidly in soil as a result of aerobic biodegradation but is stable under anaerobic conditions (Klimisch Score 1, reliable without restriction).

- U.S. EPA 2017
    - The BIOWIN modeling Ready Biodegradable Predictor indicates that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is not expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 69.1% will partition to soil with a half-life of 1,800 hours (75 days), 17.6% will partition to sediment with a half-life of 8,100 hours (337.5 days), and 13.3% will partition to water with a half-life of 900 hours (37.5 days) (Appendix I).
- Although the measured hydrolysis half-life is 14 hours at 26°C, fugacity modeling predicts that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) will mostly partition into soil and sediment due to its low water solubility. No soil degradation data were identified for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), but the surrogate 1,4-benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- exhibited aerobic and anaerobic degradation in OECD Guideline 307 tests. However, only ⩽ 8.9% of the applied radioactivity was identified as carbon dioxide, indicating that the majority of test substance did not achieve mineralization and only underwent primary degradation and/or subsequent degradation to intermediate products. Under Section A9.4.3.4 of the GHS criteria (UN 2019), "[d]ata on primary biodegradability may be used for demonstrating rapid degradability only when it can be satisfactorily demonstrated that the degradation products formed do not fulfil the criteria for classification as hazardous to the aquatic environment." Since the transformation products of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) are aquatic toxicants (6PPD-quinone for example), ToxServices concludes that the available data do not support classifying N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as readily or rapidly biodegradable. Therefore, ToxServices used the fugacity modeling to assign the score for this endpoint. It is ToxServices' internal policy to assign the hazard score for persistence based on the dominant environmental compartment(s) (ToxServices 2020).

Bioaccumulation (B) Score (vH, H, M, L, or vL): H

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of High for bioaccumulation based on measured BCFs of 1,500-1,700 for the surrogate N-(1-methylheptyl)-N'-phenylbenzene-1,4-diamine. GreenScreen® criteria classify chemicals as a High hazard for bioaccumulation when BCFs are > 1,000 to 5,000 (CPA 2018b). The confidence in the score is high as it is based on measured data for a strong surrogate.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:*
        - EC - CEPA DSL – Bioaccumulative.
            - Based on an estimated log K_{ow} of 4.68 and log BCFs ≤ 3.96 (BCFs ≤ 9,120) (OECD 2021b).
- U.S. EPA 2017
    - BCFBAF predicts a BCF/BAF of 228.6 L/kg wet-wt. using the regression-based model based on a measured log K_{ow} of 4.08, and a BCF of 206.9 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix I).

- UNEP 2005
    - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is not stable in water (see persistence section above). Therefore, the SIDS authors concluded it is not likely to bioaccumulate despite a calculated BCF of 801. The degradation products 1,3-dimethylbutylamine, N-phenyl-p-benzoquinone monoamine, and 4-hydroxydiphenylamine have experimentally obtained BCFs of ≤ 30, indicating that they are not likely to bioaccumulate in aquatic biota.
- ECHA 2021
    - *Surrogate: N-(1-Methylheptyl)-N'-phenylbenzene-1,4-diamine (CAS #15233-47-3)*: A GLP-compliant, OECD Guideline 305 E bioaccumulation test was performed with carp (*Cyprinus carpio*) exposed to N-(1-methylheptyl)-N'-phenylbenzene-1,4-diamine (purity not specified, not radiolabeled) at nominal concentrations of 1 or 10 µg/L for 28 days. The steady state BCFs on the basis of normalized lipid fractions were 1,700 and 1,500 for the 1 and 10 µg/L solutions, respectively.

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): L

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Low for reactivity based on ToxServices not classifying it as a reactive chemical under GHS criteria. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when no GHS classification is available (CPA 2018b). The confidence in the score was high as it is based on measured data for explosivity.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021
    - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (purity not specified) was not explosive in an EU Method A.14 test.
    - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) does not contain functional groups associated with oxidizing properties.
- Based on the above information, ToxServices did not classify N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) as a reactive chemical under GHS criteria (UN 2021).

Flammability (F) Score (vH, H, M, or L): L

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was assigned a score of Low for flammability based on ToxServices not classifying it as a flammable chemical under GHS criteria. GreenScreen® criteria classify chemicals as a Low hazard for flammability when no GHS classification is available (CPA 2018b). The confidence in the score was high as it is based on reliable measured data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2021
    - As a low-melting point solid, the REACH dossier authors evaluated the flammability of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) using the flash point.
    - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (98.2% purity) has a flash point of 202.5°C as identified in a GLP-compliant, EU Method A.9 closed cup test (Klimisch Score 1, reliable without restriction).

  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) does not combust in contact with water, is stable at room temperature (not pyrophoric), and does not sustain combustion without additional energy input.
- UNEP 2005
  - N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (as Vulkanox 4020, purity not specified) has a flash point of 200°C as identified in a DIN 51758 closed cup test (Klimisch Score 2, reliable with restrictions).
- Based on the above information, ToxServices did not classify N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) has a flammable solid under GHS criteria (UN 2021). GHS criteria define flammable solids as chemicals with burning times < 45 seconds or burning rates > 2.2 mm/second.

Use of New Approach Methodologies (NAMs)[14] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* genotoxicity, cell transformation, and endocrine activity testing, *in silico* modeling for endocrine activity, respiratory sensitization, persistence, and bioaccumulation, and OECD Guideline 301 B and C ready biodegradation tests. NAMs are non-animal alternative that can be used alone or in combination to provide information for safety assessment (Madden et al. 2020). At present, there is not a uniformly accepted framework on how to report and apply individual NAMs (U.S. EPA 2020, OECD 2020). The expanded application of NAMs greatly amplifies the need to communicate uncertainties associated with their use. As defined by EFSA (2018), uncertainty is "a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question." The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties (OECD 2020):

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

As shown in Table 5, Type I (input data) uncertainties in N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)'s NAMs dataset include lack of experimental data for respiratory sensitization along with a lack of validated test method for respiratory sensitization. N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)'s Type II (extrapolation output) uncertainties include limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism and their focusing on one or only a few types of genotoxicity events, the limitation of OECD Toolbox in identifying structural alerts without defining the applicability domain, the uncertain relevance of the EDSP Tox 21 screening assays and *in silico* modeling of endocrine receptor binding due to lack of consideration of toxicokinetic factors including metabolism, the limitations in the examination of structural alerts for respiratory sensitization evaluation that does not account for non-immunologic mechanisms of respiratory sensitization, and the lack of guidance from the ECHA framework to subclassify respiratory sensitizers to Category 1A and 1B. Some of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)'s type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

Table 5: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses

Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Respiratory sensitization**: No experimental data are available and there are no validated test methods.
Type II Uncertainty: Extrapolation Output	**Carcinogenicity:** Identification of morphologically transformed colonies in the *in vitro* mammalian cell transformation assay could be subjective. The mechanism leading to cell transformations is not fully understood. The test does not inform *in vivo* potency, species-specificity or tissue-specificity of cell transformations and is being validated for mono-constituent substances only[15].

[14] NAMs refers to any non-animal technology, methodology, approach, or combination thereof that inform chemical hazard and risk assessments. NAMs include *in silico*/computational tools, *in vitro* biological profiling (e.g., cell cultures, 2,3-D organotypic culture systems, genomics/transcriptomics, organs on a chip), and frameworks (i.e., adverse outcome pathways (AOPs), defined approaches (DA), integrated approaches to testing and assessment (IATA).

[15] https://www.oecd.org/env/ehs/testing/Guidance-Document-on-the-in-vitro-Syrian-Hamster-Embryo-Cell-Transformation-Assay.pdf

	Genotoxicity: The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation system does not entirely mimic *in vivo* conditions[16]. The mammalian cell gene mutation assay (as defined in OECD Guideline 476) only detects gene mutations, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e. the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[17] The *in vitro* chromosome aberration assay (OECD 473) does not measure aneuploidy and it only measures structural chromosomal aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[18]. **Endocrine activity:** ToxCast models don't define applicability domain; the *in vivo* relevance of EDSP Tox 21 screening assays as well as the *in silico* modeling of endocrine receptor binding activity is unknown due to lack of consideration of metabolism and other toxicokinetic factors. **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization. The ECHA framework did not provide guidance on how to subclassify chemicals to Category 1A and 1B.
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	Y	*In vitro* data: cell transformation assay
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: EDSP Tox 21 screening assays/ToxCast models/ Danish QSAR/VEGA

[16] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[17] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[18] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352

Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	N	
Respiratory sensitization	N	
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Persistence	Y	*In silico* modeling: EPI Suite™ Non-animal testing: OECD 301 B and C Biodegradation tests
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

References

Chemical Classification and Information Database (CCID). 2021. 1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl- (CAS #793-24-8). Environmental Protection Authority, Government of New Zealand. Available: https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/view/1CF3CA33-94D6-43F0-8097-67D55AB92016

ChemIDplus. 2021. 1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl- (CAS #793-24-8). United States National Library of Medicine. Available: https://chem.nlm.nih.gov/chemidplus/rn/793-24-8

Clean Production Action (CPA). 2018a. GreenScreen® Assessment Expiration Policy. October 2, 2018.

Clean Production Action (CPA). 2018b. The GreenScreen® for Safer Chemicals Guidance. Version 1.4 Guidance. Dated January, 2018. Available: https://www.greenscreenchemicals.org/static/ee_images/uploads/resources/GreenScreen_Guidance_v1_4_2018_01_Final.pdf

Danish Technical University (DTU). 2021. National Food Institute. Danish QSAR Database. Available: http://qsar.food.dtu.dk/.

European Chemicals Agency (ECHA). 2017. Guidance on information requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 6.0. Dated: July 2017. Available: https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

European Chemicals Agency (ECHA). 2021. REACH registration dossier for N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine (CAS #793-24-8). Available: https://echa.europa.eu/registration-dossier/-/registered-dossier/15367

European Chemicals Bureau (ECB). 2004. European Union risk assessment report for aniline (CAS #62-53-3). EUR 21092 EN. Available: https://echa.europa.eu/documents/10162/0abd36ad-53de-4b0f-b258-10cf90f90493

European Food Safety Authority (EFSA). 2018. Guidance on uncertainty analysis in scientific assessments. *EFSA J.* 16(1): e05123. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7009727/

Hazardous Substances Data Bank (HSDB). 2002. 1,4-Benzenediamine, n-(1,3-dimethylbutyl)-n'-phenyl-. United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/5755

Madden, J.C., S.J. Enoch, A. Paini, and M.T.D. Cronin. "A Review of In Silico Tools as Alternatives to Animal Testing: Principles, Resources, and Applications," *Alt. Lab. Animals* 1-27, (2020). Available: https://journals.sagepub.com/doi/pdf/10.1177/0261192920965977

National Institute of Technology and Evaluation (NITE). 2008. GHS classification result for N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (CAS #793-24-8). Incorporated Administrative

Agency, Government of Japan. Available: https://www.nite.go.jp/chem/english/ghs/08-meti-0028e.html

Organization for Economic Cooperation and Development (OECD). 2020. Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA), Series on Testing and Assessment, No. 329, Environment, Health and Safety, Environment Directorate. Available: https://www.oecd.org/chemicalsafety/risk-assessment/concepts-and-available-guidance-related-to-integrated-approaches-to-testing-and-assessment.pdf

Organisation for Economic Co-operation and Development (OECD). 2021a. OECD QSAR Toolbox for Grouping Chemicals into Categories Version 4.5. Available: http://toolbox.oasis-lmc.org/?section=download&version=latest.

Organisation for Economic Co-operation and Development (OECD). 2021b. Categorization results from the Canadian Domestic Substance List for 1,4-benzenediamine, N-(1,3-dimethylbutyl)-N -phenyl- (CAS #793-24-8). Available: https://canadachemicals.oecd.org/Search.aspx

Pharos. 2021. Pharos Chemical and Material Library entry for N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) (CAS #793-24-8). Available: http://www.pharosproject.net/material/.

PubChem. 2021. N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine. United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/compound/13101

Ryan, C.A., J.G. Chaney, G.F. Gerberick, P.S. Kern, R.J. Dearman, I. Kimber, and D.A. Basketter. 2007. Extrapolating local lymph node assay EC3 values to estimate relative sensitizing potency. *Cutan. Ocul. Toxicol*. 26(2): 135-145.

Tian Z., H. Zhao, K.T. Peter, M. Gonzalez, J. Wetzel, C. Wu, X. Hu, J. Prat, E. Mudrock, R. Hettinger, A.E. Cortina, R.G. Biswas, F.V.C. Kock, R. Soong, A. Jenne, B. Du, F. Hou, H. He, R. Lundeen, A. Gilbreath, R. Sutton, N.L. Scholz, J.W. Davis, M.C. Dodd, A. Simpson, J.K. McIntyre, and E.P. Kolodziej. 2021. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science* 371(6525):185-189.

ToxServices. 2020. SOP 1.37: GreenScreen® Hazard Assessments. Dated: February 3, 2020.

United Nations (UN). 2021. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Ninth revised edition.

United Nations Environment Programme (UNEP). 2005. SIDS initial assessment report for N-(1,3-Dimethylbutyl)-N′-phenyl-1,4-phenylenediamine (CAS #793-24-8). Available: https://hpvchemicals.oecd.org/ui/SIDS_Details.aspx?id=de355229-d6c9-43c2-b79a-f484a8a0d59d

United States Department of Transportation (U.S. DOT). 2008a. Chemicals Listed with Classification. 49 CFR § 172.101. Available: http://www.gpo.gov/fdsys/pkg/CFR-2008-title49-vol2/pdf/CFR-2008-title49-vol2-sec172-101.pdf.

United States Department of Transportation (U.S. DOT). 2008b. Classification Criteria. 49 CFR § 173. Available: http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&tpl=/ecfrbrowse/Title49/49cfr173_main_02.tpl

United States Environmental Protection Agency (U.S. EPA). 2015. Safer Choice Standard. Available: https://www.epa.gov/saferchoice/standard

United States Environmental Protection Agency (U.S. EPA). 2017. Estimation Programs Interface (EPI) Suite™ Web, v4.11, Washington, DC, USA. Available: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm.

United States Environmental Protection Agency (U.S. EPA). 2020. New Approach Methods Workplan. Office of Research and Development. Office of Chemical Safety and Pollution Prevention. EPA 615B20001. June 2020. Available: https://www.epa.gov/sites/default/files/2020-06/documents/epa_nam_work_plan.pdf

United States Environmental Protection Agency (U.S. EPA). 2021a. Safer Chemical Ingredients List (SCIL). Available: https://www.epa.gov/saferchoice/safer-ingredients

United States Environmental Protection Agency (U.S. EPA). 2021b. Endocrine Disruptor Screening Program (EDSP) Universe of Chemicals Dashboard. Available: https://comptox.epa.gov/dashboard/chemical_lists/EDSPUOC.

Virtual Models for Evaluating the Properties of Chemicals within a Global Architecture (VEGA). 2021. Predictive Model Platform version 1.1.5. Available: http://www.vega-qsar.eu/index.php.

APPENDIX A: Hazard Classification Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GREEN SCREEN FOR SAFER CHEMICALS

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human								Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
Table 2: Chemical Details									S	R *	S	R *	*	*								
Inorganic Chemical?	Chemical Name	CAS#	C	M	R	D	E	AT	STs	STr	Ns	Nr	SNS*	SNR*	IrS	IrE	AA	CA	P	B	Rx	F
No	6PPD	793-24-8	L	L	H	M	M	M		M	*M*	DG	H	*M*	L	*M*	vH	vH	*H*	H	L	L

Table 3: Hazard Summary Table

Benchmark	a	b	c	d	e	f	g
1	Yes	No	No	No	Yes		
2	STOP						
3	STOP						
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
6PPD	1

Note: Chemical has not undergone a data gap assessment. Not a Final GreenScreen™ Score

Table 6

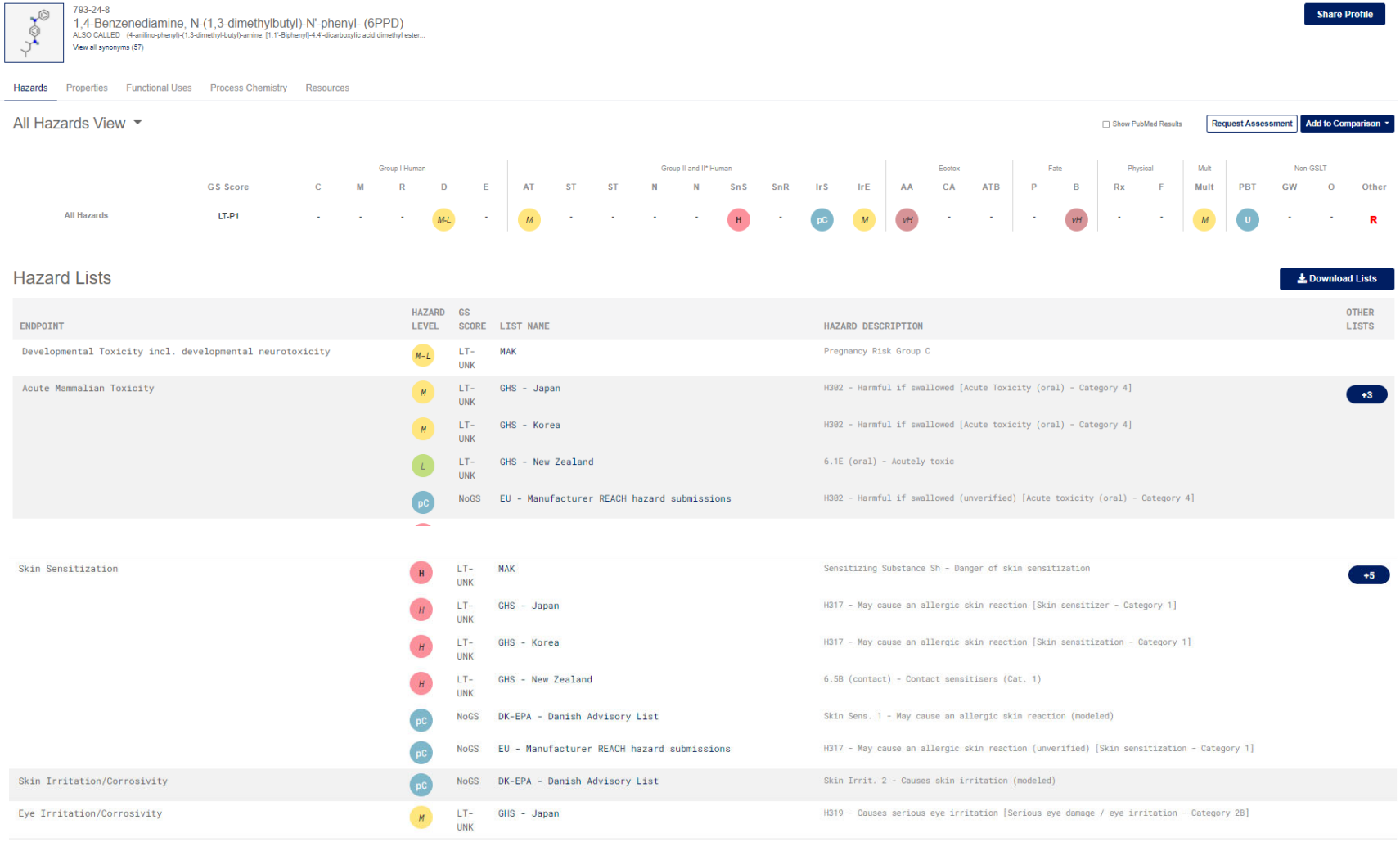
Chemical Name	Final GreenScreen® Benchmark Score
6PPD	1

After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

Table 5: Data Gap Assessment Table

Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												1
2												
3												
4												

APPENDIX C: Pharos Output for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)



793-24-8
1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl- (6PPD)
ALSO CALLED (4-anilino-phenyl)-(1,3-dimethyl-butyl)-amine, [1,1'-Biphenyl]-4,4'-dicarboxylic acid dimethyl ester...
View all synonyms (57)

Share Profile

Hazards | Properties | Functional Uses | Process Chemistry | Resources

All Hazards View

Show PubMed Results | Request Assessment | Add to Comparison

		Group I Human					Group II and II* Human								Ecotox			Fate		Physical		Mult	Non-GSLT			
	GS Score	C	M	R	D	E	AT	ST	ST	N	N	SnS	SnR	IrS	IrE	AA	CA	ATB	P	B	Rx	F	Mult	PBT	GW	O	Other
All Hazards	LT-P1	-	-	-	M-L	-	M	-	-	-	-	H	-	pC	M	vH	-	-	-	vH	-	-	M	U	-	-	R

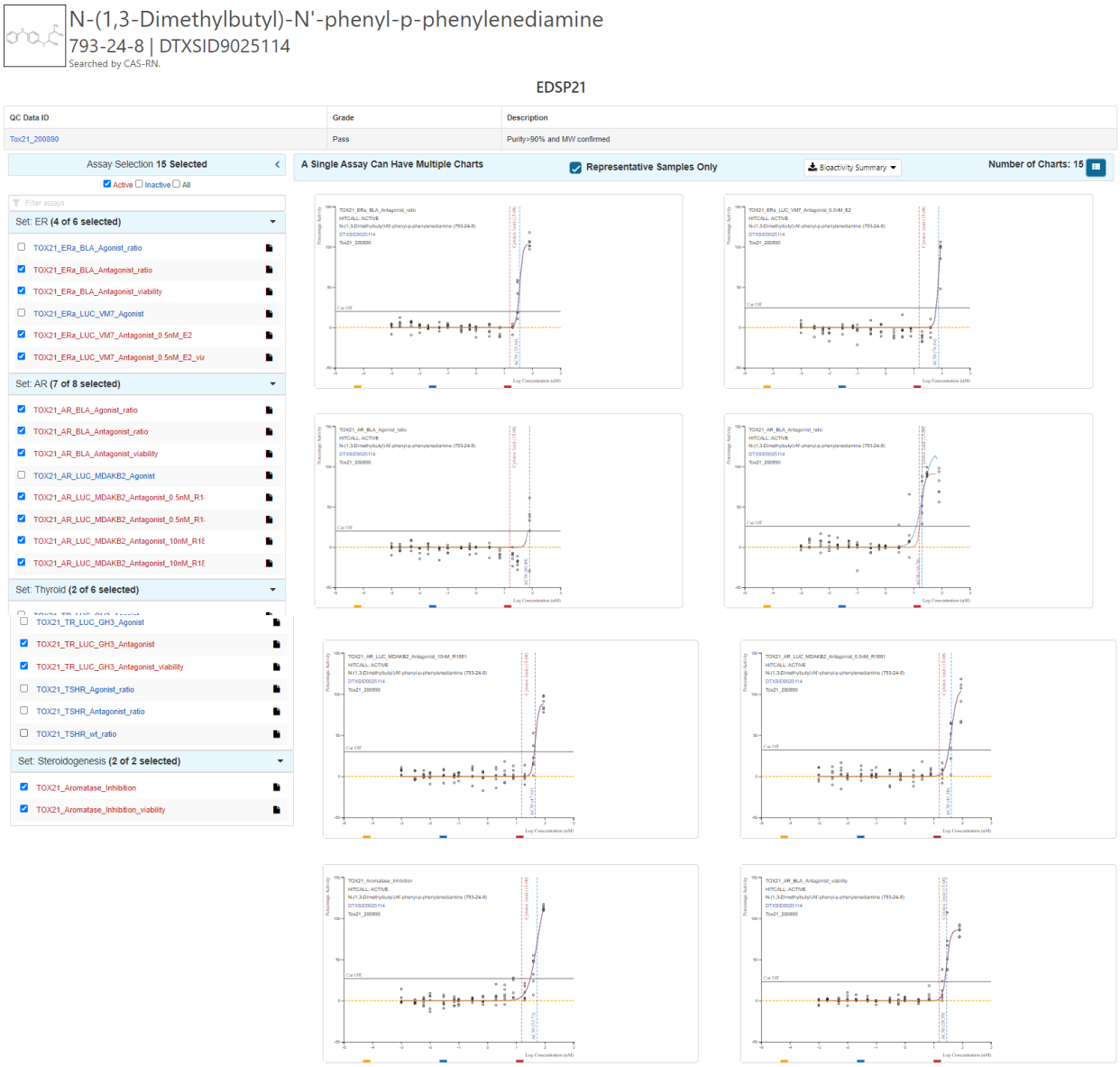
Hazard Lists

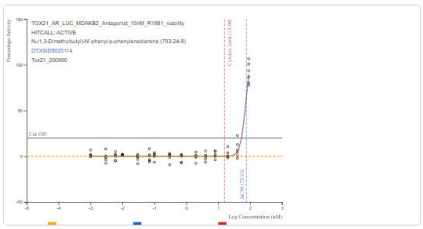
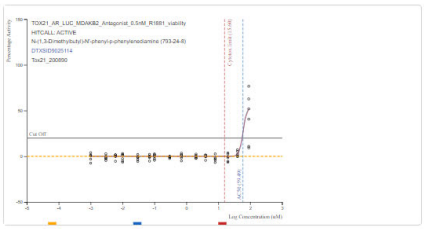
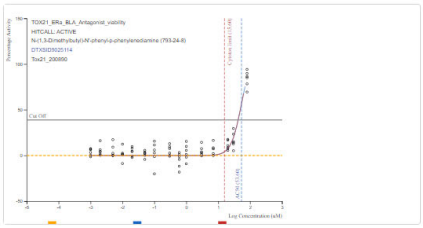
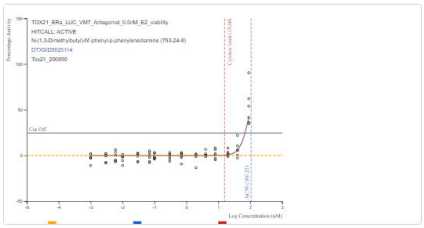
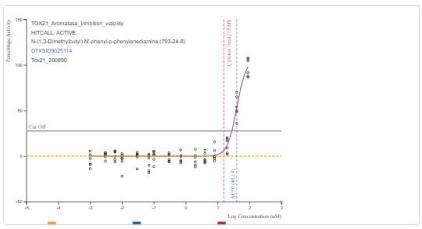
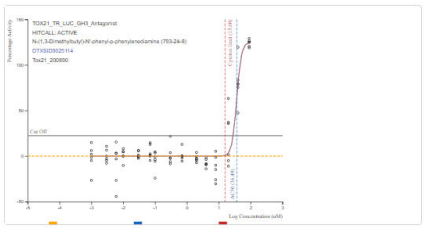
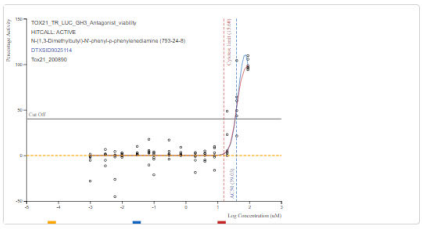
Download Lists

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Developmental Toxicity incl. developmental neurotoxicity	M-L	LT-UNK	MAK	Pregnancy Risk Group C	
Acute Mammalian Toxicity	M	LT-UNK	GHS - Japan	H302 - Harmful if swallowed [Acute Toxicity (oral) - Category 4]	+3
	M	LT-UNK	GHS - Korea	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	
	L	LT-UNK	GHS - New Zealand	6.1E (oral) - Acutely toxic	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H302 - Harmful if swallowed (unverified) [Acute toxicity (oral) - Category 4]	
Skin Sensitization	H	LT-UNK	MAK	Sensitizing Substance Sh - Danger of skin sensitization	+5
	H	LT-UNK	GHS - Japan	H317 - May cause an allergic skin reaction [Skin sensitizer - Category 1]	
	H	LT-UNK	GHS - Korea	H317 - May cause an allergic skin reaction [Skin sensitization - Category 1]	
	H	LT-UNK	GHS - New Zealand	6.5B (contact) - Contact sensitisers (Cat. 1)	
	pC	NoGS	DK-EPA - Danish Advisory List	Skin Sens. 1 - May cause an allergic skin reaction (modeled)	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H317 - May cause an allergic skin reaction (unverified) [Skin sensitization - Category 1]	
Skin Irritation/Corrosivity	pC	NoGS	DK-EPA - Danish Advisory List	Skin Irrit. 2 - Causes skin irritation (modeled)	
Eye Irritation/Corrosivity	M	LT-UNK	GHS - Japan	H319 - Causes serious eye irritation [Serious eye damage / eye irritation - Category 2B]	

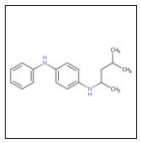
Acute Aquatic Toxicity	vH	LT-UNK	GHS - Japan	H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1]	+4
	vH	LT-UNK	GHS - Korea	H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1]	
	DG	NoGS	DK-EPA - Danish Advisory List	Aquatic Acute1 - Very toxic to aquatic life (modeled)	
	DG	NoGS	DK-EPA - Danish Advisory List	Aquatic Chronic1 - Very toxic to aquatic life with long lasting effects (modeled)	
	DG	NoGS	EU - Manufacturer REACH hazard submissions	H400 - Very toxic to aquatic life (unverified) [Hazardous to the aquatic environment (acute) - Category 1]	
Bioaccumulation	vH	LT-UNK	EC - CEPA DSL	Bioaccumulative	
Reproductive and/or Developmental Toxicity	DG	NoGS	EU - Manufacturer REACH hazard submissions	H360 - Suspected of / May damage fertility and/or the unborn child (unverified) [Reproductive toxicity - Category 1A or 1B]	
PBT [Persistence, Bioaccumulation, and any of the following: Acute Aquatic Toxicity, Chronic Aquatic Toxicity, Carcinogenicity, Mutagenicity, Reproductive Toxicity, Developmental Toxicity, Systemic Toxicity/Organ Effects repeated exposure]	U	LT-P1	OSPAR - Priority PBTs & EDs & equivalent concern	Equivalent concern - Chemical for Priority Action	
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-P1	German FEA - Substances Hazardous to Waters	Class 2 - Hazard to Waters	
T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-P1	GHS - New Zealand	9.1A (crustacean) - Very ecotoxic in the aquatic environment	+3
	U	LT-P1	GHS - New Zealand	9.1A (fish) - Very ecotoxic in the aquatic environment	
	U	LT-P1	GHS - Korea	H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]	
	DG	NoGS	EU - Manufacturer REACH hazard submissions	H410 - Very toxic to aquatic life with long lasting effects (unverified) [Hazardous to the aquatic environment (chronic) - Category 1]	
T & P and/or B [(Chronic Aquatic Toxicity and sometimes Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-P1	GHS - Japan	H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]	
Systemic Toxicity/Organ Effects [Repeated Exposure] and/or Neurotoxicity [Repeated Exposure]	M	LT-UNK	GHS - Japan	H373 - May cause damage to organs through prolonged or repeated exposure [Specific target organs/systemic toxicity following repeated exposure - Category 2]	
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	M	LT-UNK	GHS - Japan	H335 or H336 [Specific target organs/systemic toxicity following single exposure - Category 3]	
Acute aquatic toxicity; Chronic aquatic toxicity	U	LT-UNK	EC - CEPA DSL	Inherently Toxic in the Environment (iTE)	
PBT (Persistence, Bioaccumulation & Toxicity)	DG	NoGS	EU - ESIS PBT	Not fulfilling PBT & vPvB criteria	

APPENDIX D: CompTox EDSP21 Results for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)



APPENDIX E: ToxCast Model Predictions for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)



N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine

793-24-8 | DTXSID9025114

Searched by CAS-RN.

ToxCast: Models

ToxCast Model Predictions

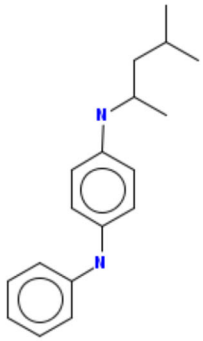
Download ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	-	-	-
ToxCast Pathway Model (AUC)	Estrogen	-	-	-
COMPARA (Consensus)	Androgen	Inactive	Active	Inactive
CERAPP Potency Level (From Literature)	Estrogen	Inactive (Inactive)	Active (Very weak)	Active (Very weak)
CERAPP Potency Level (Consensus)	Estrogen	Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)

APPENDIX F: VEGA Endocrine Activity Results for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)

1. Prediction Summary

Prediction for compound Molecule 0

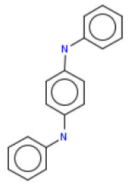


Prediction: Reliability:

Prediction is Active, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- accuracy of prediction for similar molecules found in the training set is not optimal
- some similar molecules found in the training set have experimental values that disagree with the predicted value
- some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found)

Compound: Molecule 0
Compound SMILES: c1ccc(cc1)Nc2ccc(cc2)NC(C)CC(C)C
Experimental value: -
Predicted activity: Active
Classification tree final node: 18
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

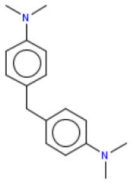
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 74-31-7
Dataset id: 595 (Training set)
SMILES: c1ccc(cc1)Nc2ccc(cc2)Nc3ccccc3
Similarity: 0.88

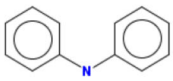
Experimental value: Active
Predicted value: Active



Compound #2

CAS: 101-61-1
Dataset id: 693 (Training set)
SMILES: c1cc(ccc1N(C)C)Cc2ccc(cc2)N(C)C
Similarity: 0.84

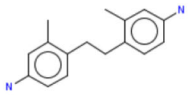
Experimental value: Inactive
Predicted value: Active



Compound #3

CAS: 122-39-4
Dataset id: 600 (Training set)
SMILES: c1ccc(cc1)Nc2ccccc2
Similarity: 0.802

Experimental value: Inactive
Predicted value: Inactive



Compound #4

CAS: 22856-62-8
Dataset id: 523 (Training set)
SMILES: Nc1ccc(c(c1)C)CCc2ccc(N)cc2C
Similarity: 0.801

Experimental value: Active
Predicted value: Active

Compound #5

CAS: 101-77-9

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.565
Explanation: the predicted compound is outside the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.859
Explanation: strongly similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 0.514
Explanation: accuracy of prediction for similar molecules found in the training set is not optimal.

Concordance for similar molecules
Concordance index = 0.514
Explanation: some similar molecules found in the training set have experimental values that disagree with the predicted value.

Model's descriptors range check
Descriptors range check = True
Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.

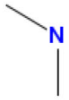
Atom Centered Fragments similarity check
ACF index = 0.85
Explanation: some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found).

Symbols explanation:

The feature has a good assessment, model is reliable regarding this aspect.
The feature has a non optimal assessment, this aspect should be reviewed by an expert.
The feature has a bad assessment, model is not reliable regarding this aspect.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

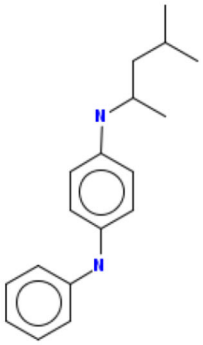
(Molecule 0) Reasoning on rare and missing Atom Centered Fragments.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:



Fragment defined by the SMILES: N(c)c
The fragment has less than 3 occurrences in the model's training set

1. Prediction Summary

Prediction for compound Molecule 0

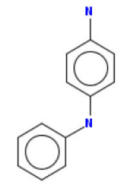


Prediction: Reliability:

Prediction is NON-active, the result appears reliable. Anyhow, you should check it through the evaluation of the information given in the following sections.
The following relevant fragments have been found: ER non-activity alert no. 4; ER possible non-activity alert no. 4

Compound: Molecule 0
Compound SMILES: c1ccc(cc1)Nc2ccc(cc2)NC(C)CC(C)C
Experimental value: -
Predicted ER-mediated effect: NON-active
No. alerts for activity: 0
No. alerts for possible activity: 0
No. alerts for non-activity: 1
No. alerts for possible non-activity: 1
Structural alerts: ER non-activity alert no. 4; ER possible non-activity alert no. 4
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values

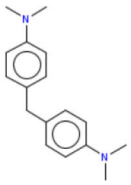


Compound #1

CAS: N.A.
Dataset id: 356 (Training set)
SMILES: Nc1ccc(cc1)Nc2ccccc2
Similarity: 0.861

Experimental value: NON-active
Predicted value: Possible NON-active

Alerts (found also in the target): ER possible non-activity alert no. 4



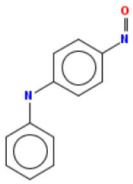
Compound #2

CAS: N.A.
Dataset id: 358 (Training set)
SMILES: c1cc(ccc1N(C)C)Cc2ccc(cc2)N(C)C
Similarity: 0.84

Experimental value: NON-active
Predicted value: Possible NON-active

Alerts (found also in the target): ER possible non-activity alert no. 4

Alerts (not found in the target): ER possible non-activity alert no. 7



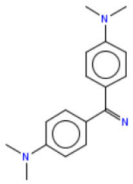
Compound #3

CAS: N.A.
Dataset id: 695 (Training set)
SMILES: O=Nc1ccc(cc1)Nc2ccccc2
Similarity: 0.816

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER possible non-activity alert no. 4

Alerts (not found in the target): ER non-activity alert no. 2



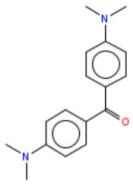
Compound #4

CAS: N.A.
Dataset id: 744 (Training set)
SMILES: N=C(c1ccc(cc1)N(C)C)c2ccc(cc2)N(C)C
Similarity: 0.809

Experimental value: NON-active
Predicted value: Possible NON-active

Alerts (found also in the target): ER possible non-activity alert no. 4

Alerts (not found in the target): ER possible non-activity alert no. 7



Compound #5

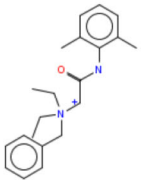
CAS: N.A.
Dataset id: 231 (Training set)
SMILES: O=C(c1ccc(cc1)N(C)C)c2ccc(cc2)N(C)C
Similarity: 0.8

Experimental value: NON-active
Predicted value: Possible NON-active

Alerts (found also in the target): ER possible non-activity alert no. 4

Alerts (not found in the target): ER possible non-activity alert no. 7; ER possible non-activity alert no. 9

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #6

CAS: N.A.
Dataset id: 1030 (Training set)
SMILES: O=C(Nc1c(cccc1C)C)C[N+](Cc2ccccc2)(CC)CC
Similarity: 0.8

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER non-activity alert no. 4; ER possible non-activity alert no. 4

Alerts (not found in the target): ER non-activity alert no. 25; ER possible non-activity alert no. 6; ER possible non-activity alert no. 9

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.915
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.837
Explanation: strongly similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 1
Explanation: accuracy of prediction for similar molecules found in the training set is good.

Concordance for similar molecules
Concordance index = 1
Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.

Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

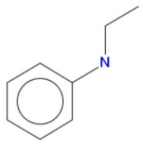
Symbols explanation:

The feature has a good assessment, model is reliable regarding this aspect.
The feature has a non optimal assessment, this aspect should be reviewed by an expert.
The feature has a bad assessment, model is not reliable regarding this aspect.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

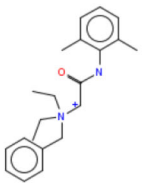
(Molecule 0) Reasoning on fragments/structural alerts - 1 of 2:

Fragment found: ER non-activity alert no. 4



Fragment related to non-activity for ER-mediated effect, defined by the SMARTS: CCNc1ccccc1

Following, the most similar compounds from the model's dataset having the same fragment.

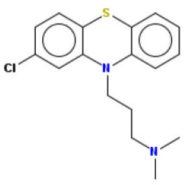


CAS: N.A.
Dataset id: 1030 (Training set)
SMILES: O=C(Nc1c(cccc1C)C)C[N+](Cc2ccccc2)(CC)CC
Similarity: 0.8

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER non-activity alert no. 4; ER possible non-activity alert no. 4

Alerts (not found in the target): ER non-activity alert no. 25; ER possible non-activity alert no. 6; ER possible non-activity alert no. 9

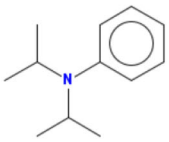


CAS: N.A.
Dataset id: 8 (Training set)
SMILES: c1ccc3c(c1)N(c2cc(ccc2S3)Cl)CCCN(C)C
Similarity: 0.789

Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER non-activity alert no. 4; ER possible non-activity alert no. 4

Alerts (not found in the target): ER possible non-activity alert no. 8



CAS: N.A.
Dataset id: 1039 (Training set)
SMILES: c1ccc(cc1)N(C(C)C)C(C)C
Similarity: 0.782

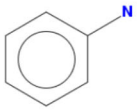
Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER non-activity alert no. 4; ER possible non-activity alert no. 4

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

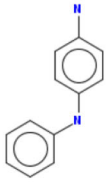
(Molecule 0) Reasoning on fragments/structural alerts - 2 of 2:

Fragment found: ER possible non-activity alert no. 4



Fragment related to possible non-activity for ER-mediated effect, defined by the SMARTS: Nc1ccccc1

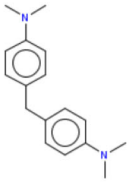
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: N.A.
Dataset id: 356 (Training set)
SMILES: Nc1ccc(cc1)Nc2ccccc2
Similarity: 0.861

Experimental value: NON-active
Predicted value: Possible NON-active

Alerts (found also in the target): ER possible non-activity alert no. 4

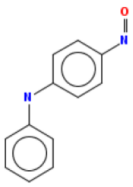


CAS: N.A.
Dataset id: 358 (Training set)
SMILES: c1cc(ccc1N(C)C)Cc2ccc(cc2)N(C)C
Similarity: 0.84

Experimental value: NON-active
Predicted value: Possible NON-active

Alerts (found also in the target): ER possible non-activity alert no. 4

Alerts (not found in the target): ER possible non-activity alert no. 7



CAS: N.A.
Dataset id: 695 (Training set)
SMILES: O=Nc1ccc(cc1)Nc2ccccc2
Similarity: 0.816

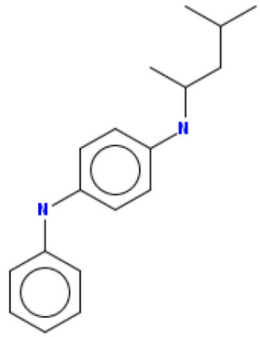
Experimental value: NON-active
Predicted value: NON-active

Alerts (found also in the target): ER possible non-activity alert no. 4

Alerts (not found in the target): ER non-activity alert no. 2

1. Prediction Summary

Prediction for compound Molecule 0

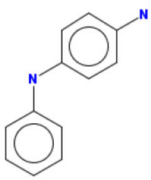


Prediction: Reliability:

Prediction is NON-active, the result appears reliable. Anyhow, you should check it through the evaluation of the information given in the following sections.

Compound: Molecule 0
Compound SMILES: c1ccc(cc1)Nc2ccc(cc2)NC(C)CC(C)C
Experimental value: -
Predicted AR binding activity: NON-active
No. alerts for binding activity: 0
No. alerts for non-binding activity: 0
Structural alerts: -
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

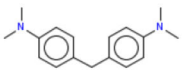
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 101-54-2
Dataset id: 847 (Training set)
SMILES: Nc1ccc(cc1)Nc1ccccc1
Similarity: 0.861

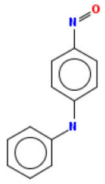
Experimental value: NON-active
Predicted value: NON-active



Compound #2

CAS: 101-61-1
Dataset id: 390 (Training set)
SMILES: CN(C)c1ccc(Cc2ccc(cc2)N(C)C)cc1
Similarity: 0.84

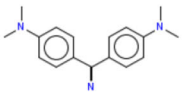
Experimental value: NON-active
Predicted value: NON-active



Compound #3

CAS: 156-10-5
Dataset id: 223 (Training set)
SMILES: O=Nc1ccc(cc1)Nc1ccccc1
Similarity: 0.816

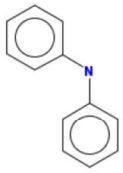
Experimental value: Active
Predicted value: NON-active



Compound #4

CAS: 2465-27-2
Dataset id: 214 (Training set)
SMILES: CN(C)c1ccc(cc1)C(=N)c1ccc(cc1)N(C)C
Similarity: 0.809

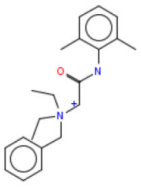
Experimental value: Active
Predicted value: NON-active



Compound #5

CAS: 122-39-4
Dataset id: 570 (Training set)
SMILES: c1ccccc1Nc1ccccc1
Similarity: 0.802

Experimental value: NON-active
Predicted value: NON-active



Compound #6

CAS: N.A.
Dataset id: 1126 (Training set)
SMILES: Cc1cccc(C)c1NC(=O)C[N+](Cc1ccccc1)(CC)CC
Similarity: 0.8

Experimental value: NON-active
Predicted value: NON-active

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.922
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.85
Explanation: strongly similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 1
Explanation: accuracy of prediction for similar molecules found in the training set is good.

Concordance for similar molecules
Concordance index = 1
Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.

Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

The feature has a good assessment, model is reliable regarding this aspect.
The feature has a non optimal assessment, this aspect should be reviewed by an expert.
The feature has a bad assessment, model is not reliable regarding this aspect.

APPENDIX G: Danish QSAR Endocrine Activity Results for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)		NEG_OUT	NEG_IN	INC_OUT	INC_OUT
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)		NEG_IN	NEG_IN	NEG_IN	NEG_IN
Estrogen Receptor α Activation (Human *in vitro*)		NEG_IN	NEG_OUT	NEG_IN	NEG_IN
Estrogen Receptor Activation, CERAPP data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition (Human *in vitro*)		NEG_IN	NEG_IN	NEG_IN	NEG_IN
Androgen Receptor Binding, CoMPARA data (*in vitro*)		N/A	N/A	INC_OUT	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)		N/A	N/A	POS_OUT	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)		N/A	N/A	INC_OUT	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)		N/A	N/A	INC_OUT	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L			42933.74	554.241	31.44841
- μM			159955.8	2064.904	117.1656
- Positive for IC_{50} ≤ 10 μM					
- Positive for IC_{50} ≤ 100 μM					
- Domain		OUT	OUT	OUT	OUT
Thyroid Receptor β Binding (Human *in vitro*)					
- mg/L			8685.582	10.07578	379.1393
- μM			32359.38	37.53877	1412.538
- Positive for IC_{50} ≤ 10 μM					
- Positive for IC_{50} ≤ 100 μM					
- Domain		OUT	OUT	OUT	OUT

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Arylhydrocarbon (AhR) Activation – Rational final model (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
Arylhydrocarbon (AhR) Activation – Random final model (Human *in vitro*)		N/A	N/A	INC_OUT	N/A
Pregnane X Receptor (PXR) Binding (Human *in vitro*)	N/A	INC_OUT	POS_OUT	POS_OUT	NEG_OUT
Pregnane X Receptor (PXR) Binding (Human *in vitro*) NEW		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Human *in vitro*)		N/A	N/A	NEG_OUT	N/A
Pregnane X Receptor (PXR) Activation (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (*in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (*in vitro*)		N/A	N/A	NEG_OUT	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (*in vitro*)		N/A	N/A	NEG_OUT	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (*in vitro*)		N/A	N/A	NEG_OUT	N/A
CYP3A4 Induction (Human *in vitro*)		N/A	N/A	INC_OUT	N/A

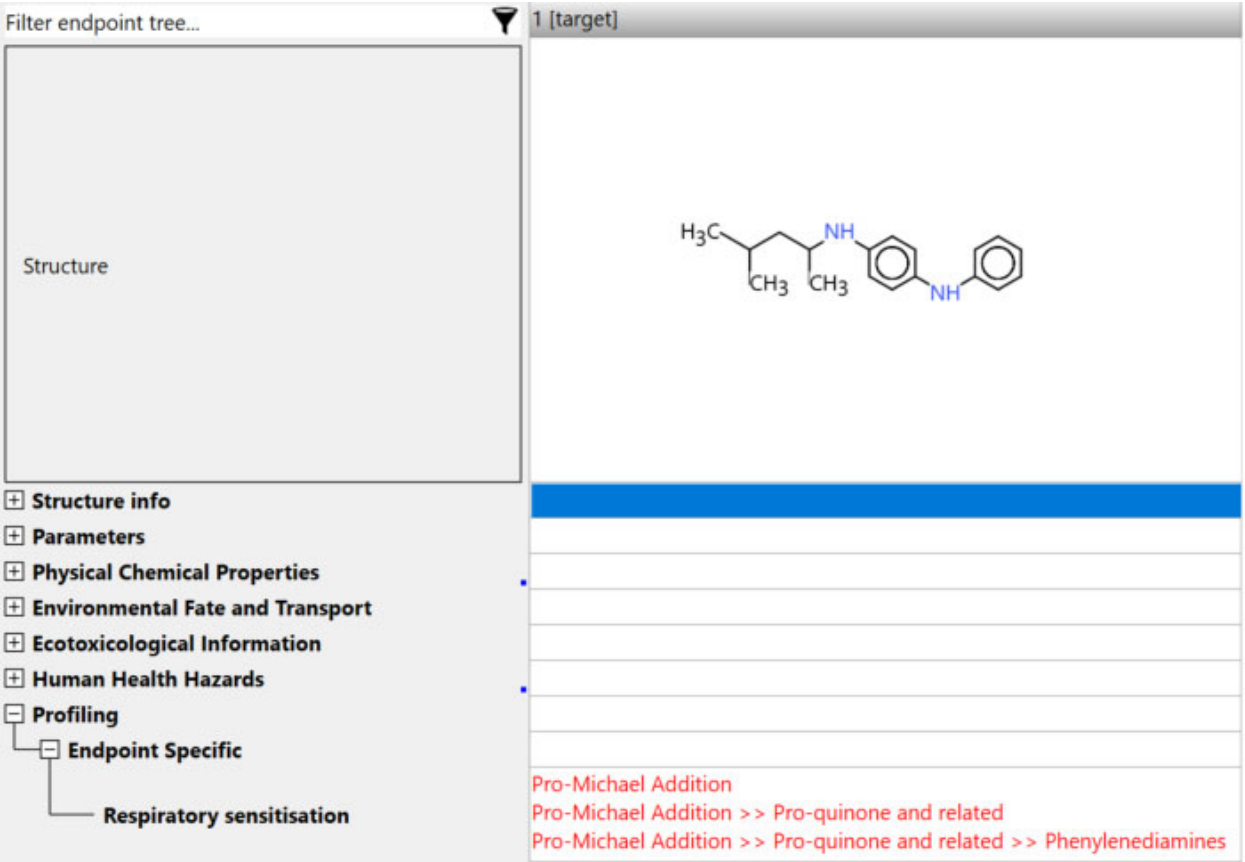
DTU-developed models

Estrogen Receptor Binding, alerts in:	
- parent only	Non binder, without OH or NH2 group
- metabolites from *in vivo* Rat metabolism simulator only	Strong binder, NH2 group; Strong binder, OH group; Moderate binder, NH2 group; Weak binder, OH group
- metabolites from Rat liver S9 metabolism simulator only	Strong binder, OH group
rtER Expert System - USEPA, alerts in:	
- parent only	No alert found
- metabolites from *in vivo* Rat metabolism simulator only	No alert found
- metabolites from Rat liver S9 metabolism simulator only	No alert found

OECD QSAR Toolbox v.4.2 profilers

Profiler predictions are supporting information to be used together with the relevant QSAR predictions

APPENDIX H: OECD Toolbox Respiratory Sensitization Results for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)



APPENDIX I: EPI Suite™ Modeling Results for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8)

(Estimated values included in the GreenScreen® are highlighted and bolded)

CAS Number: 793-24-8
SMILES: N(c(ccc(Nc(cccc1)c1)c2)c2)C(CC(C)C)C
CHEM: 1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N -phenyl-
MOL FOR: C18 H24 N2
MOL WT: 268.41
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): 4.08
Boiling Point (deg C): 163.00
Melting Point (deg C): 49.20
Vapor Pressure (mm Hg): 3E-005
Water Solubility (mg/L): 1
Henry LC (atm-m3/mole): ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 4.68
Log Kow (Exper. database match) = 4.47
Exper. Ref: SAKURATANI, Y ET AL. (2007)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 369.67 (Adapted Stein & Brown method)
Melting Pt (deg C): 121.50 (Mean or Weighted MP)
VP (mm Hg,25 deg C): 1.19 (Modified Grain method)
VP (Pa, 25 deg C): 159 (Modified Grain method)
Subcooled liquid VP: 5.21E-005 mm Hg (-999 deg C, user-entered VP)
: 0.00694 Pa (-999 deg C, user-entered VP)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 13.79
log Kow used: 4.08 (user entered)
melt pt used: 49.20 deg C

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 2.8262 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Neutral Organics

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method: 3.36E-009 atm-m3/mole (3.41E-004 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered

Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 1.060E-005 atm-m3/mole (1.074E+000 Pa-m3/mole)
VP: 3E-005 mm Hg (source: User-Entered)
WS: 1 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 4.08 (user entered)
Log Kaw used: -6.862 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 10.942
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.2804
Biowin2 (Non-Linear Model) : 0.0564
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.3581 (weeks-months)
Biowin4 (Primary Survey Model): 3.2486 (weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model): -0.1043
Biowin6 (MITI Non-Linear Model): 0.0069
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.9047
Ready Biodegradability Prediction: NO

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 0.00695 Pa (5.21E-005 mm Hg)
Log Koa (Koawin est): 10.942
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.000432
Octanol/air (Koa) model: 0.0215
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.0154
Mackay model : 0.0334
Octanol/air (Koa) model: 0.632

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 226.4928 E-12 cm3/molecule-sec
Half-Life = 0.047 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 0.567 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
0.0244 (Junge-Pankow, Mackay avg)
0.632 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 2.305E+004 L/kg (MCI method)
Log Koc: 4.363 (MCI method)
Koc : 1309 L/kg (Kow method)
Log Koc: 3.117 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Rate constants can NOT be estimated for this structure!

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 2.359 (BCF = 228.6 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -0.2335 days (HL = 0.5841 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.316 (BCF = 206.9)
Log BAF Arnot-Gobas method (upper trophic) = 2.316 (BAF = 207)
log Kow used: 4.08 (user entered)

Volatilization from Water:
Henry LC: 1.06E-005 atm-m3/mole (calculated from VP/WS)
Half-Life from Model River: 92.2 hours (3.842 days)
Half-Life from Model Lake : 1143 hours (47.64 days)

Removal In Wastewater Treatment:
Total removal: 34.16 percent
Total biodegradation: 0.35 percent
Total sludge adsorption: 33.42 percent
Total to Air: 0.39 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**0.0449**	**1.13**	**1000**
Water	**13.3**	**900**	**1000**
Soil	**69.1**	**1.8e+003**	**1000**
Sediment	**17.6**	**8.1e+003**	**0**

Persistence Time: 1.25e+003 hr

Level III Fugacity Model: (MCI Method with Water percents)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0449	1.13	1000
Water	13.3	900	1000
water	(12.8)		
biota	(0.00771)		
suspended sediment	(0.444)		
Soil	69.1	1.8e+003	1000
Sediment	17.6	8.1e+003	0

Persistence Time: 1.25e+003 hr

Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.052	1.13	1000
Water	16.2	900	1000
water	(16.1)		
biota	(0.00967)		
suspended sediment	(0.119)		
Soil	79.1	1.8e+003	1000
Sediment	4.61	8.1e+003	0

Persistence Time: 1.09e+003 hr

APPENDIX J: Change in Benchmark Score

Table 6 provides a summary of changes to the GreenScreen® Benchmark™ for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). This GreenScreen® has undergone one round of update and the benchmark score remains the same.

Table 6: Change in GreenScreen® Benchmark™ for N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD)

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
October 12, 2021	BM-1	v. 1.4	New assessment
November 8, 2021	BM-1	v. 1.4	Updated hazard score for bioaccumulation.

Licensed GreenScreen® Profilers

N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) GreenScreen® Evaluation Prepared by:

SIGNATURE BLOCK

Zach Guerrette, Ph.D., D.A.B.T.
Senior Toxicologist
ToxServices LLC

N-(1,3-Dimethylbutyl)-N'-Phenyl-p-Phenylenediamine (6PPD) GreenScreen® Evaluation QC'd by:

SIGNATURE BLOCK

Bingxuan Wang, Ph.D., D.A.B.T.
Senior Toxicologist
ToxServices LLC