N-(1,4-DIMETHYLPENTYL)-N'-PHENYLBENZENE-1,4-DIAMINE

(CAS #3081-01-4)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: October 21, 2021

ToxServices Review Date: October 21, 2026[1]

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[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.

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GreenScreen® Executive Summary for N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine is a slightly soluble, non-volatile, and non-flammable substituted p-phenylenediamine. This chemical is a dark, brown-purple viscous liquid at standard temperature and pressure. It is used as an antioxidant/antiozonant in rubber, fuel additives, or in monomer distillation, and as a stabilizer.

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a **GreenScreen Benchmark™ Score of 1** ("Avoid – Chemical of High Concern"). This score is based on the following hazard score combinations:

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

A data gap (DG) exists for neurotoxicity (repeated dose)-Nr*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine is a GreenScreen Benchmark™ Score of 1 despite the hazard data gap.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for respiratory sensitization, persistence, and bioaccumulation, *in vitro* assays for mutagenicity, and *in vitro* high throughput endocrine activity ToxCast models. 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-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine's NAMs dataset include lack of animal experimental data and human data for respiratory sensitization and limited experimental data for the persistence and bioaccumulation endpoints. N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine's Type II (extrapolation output) uncertainties include limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism, their focusing on one or only a few types of genotoxicity events, the *in vivo* relevance of endocrine ToxCast models for identify endocrine activity, the limitation of OECD Toolbox in identifying structural alerts for respiratory sensitization without defining the applicability domain, and not accounting for non-immunologic mechanisms of respiratory sensitization.

GreenScreen® Hazard Summary Table for N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine

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**	**L**	**L**	**M**	*L*	DG	**H**	*M*	**L**	*M*	**vH**	**vH**	*H*	*L*	*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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)

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

GreenScreen® Assessment (v.1.4) Prepared By:
Name: Rachel Doerer, M.P.H.
Title: Toxicologist
Organization: ToxServices LLC
Date: October 14, 2021

Quality Control Performed By:
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Title: Senior Toxicologist
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Date: October 21, 2021

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Name: Rachel Doerer, M.P.H.
Title: Toxicologist
Organization: ToxServices LLC
Date: November 3, 2021

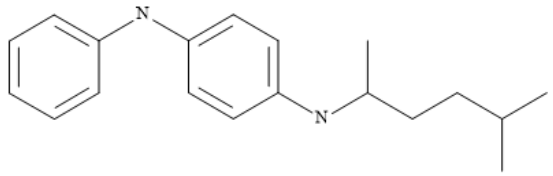
Quality Control Performed By:
Name: Bingxuan Wang, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: November 8, 2021

ToxServices Review Date: November 8, 2026[3]

Chemical Name: N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine

CAS Number: 3081-01-4

Chemical Structure(s):

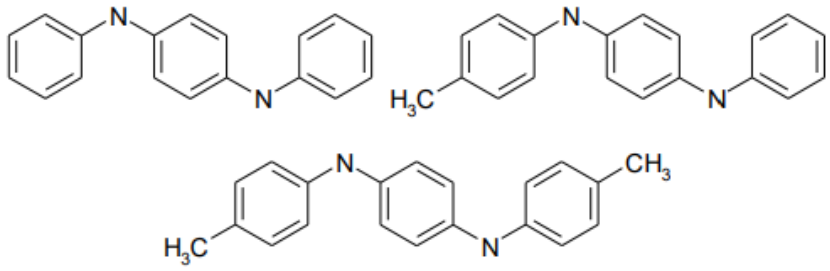


Also called:
N-(5-Methyl-2-hexyl)-N'-phenyl-p-phenylenediamine; EINECS 221-374-3; N-(1,4-Dimethylpentyl)-N'-phenyl-1,4-benzenediamine; p-Phenylenediamine, N-(1,4-dimethylpentyl)-N'-phenyl-; 1,4-Benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl-; 1,4-Benzenediamine, N1-(1,4-dimethylpentyl)-N4-phenyl-; N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine; Santoflex 14 (ChemIDplus 2021)

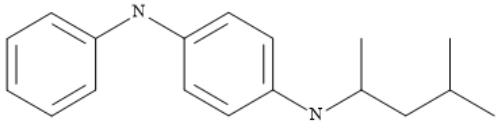
Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
A realtively complete dataset was identifed for N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine, however, some data gaps exist. N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine belongs to the United States Environmental Protection Agency (U.S. EPA)'s substituted p-Phenylenediamines category as part of the Chemical Assessment and Management Program (ChAMP) (U.S. EPA 2011), as

[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.

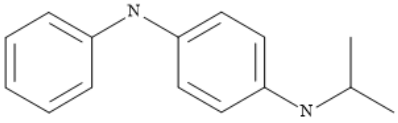
a member of the 4-aminodiphenylamone derivatives. In order to fill data gaps, ToxServices used data for other 4-aminodiphenylamine derivatives identified by the U.S. EPA including, 1,4-benzenediamine, N,N'-mixed Ph and tolyl derivatives (CAS #68953-84-4), p-phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (CAS #793-24-8), and p-phenylenediamine, N-isopropyl-N'-phenyl- (CAS #101-72-4) when data were lacking for N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine. For the bioaccumulation endpoint, ToxServices used N-(1-methylheptyl)-N'-phenylbenzene-1,4-diamine (CAS #15233-47-3) as a surrogate, which is a read-across chemical in the REACH registration dossier for the surrogate N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD).



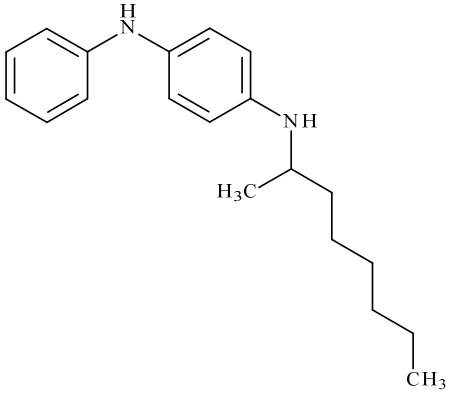
1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivatives (CAS #68953-84-4) (representative structures from U.S. EPA 2011)



p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (6PPD) (CAS #793-24-8)



p-Phenylenediamine, N-isopropyl-N'-phenyl- (IPPD) (CAS #101-72-4)



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

Identify Applications/Functional Uses: (U.S. EPA 2011, Eastman 2019)
1. Antioxidant/antiozonant in rubber, fuel additives, or in monomer distillation
2. Stabilizer

Known Impurities[4]:
Common impurities include <2% 1,4-benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl- (CAS #793-24-8) and <1.5% 4-aminodiphenylamine (CAS #101-54-2) (U.S. EPA 2011). The screen is performed on the theoretical pure substance.

GreenScreen® Summary Rating for N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine[5,6 7,8]:
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a **GreenScreen Benchmark™ Score of 1** ("Avoid – Chemical of High Concern") (CPA 2018b). This score is based on the following hazard score combinations:

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

A data gap (DG) exists for neurotoxicity (repeated dose) – Nr*. As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine is a GreenScreen Benchmark™ Score of 1 despite the hazard data gap.

Figure 1: GreenScreen® Hazard Summary Table for N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine

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**	**L**	**L**	**M**	*L*	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.

Environmental Transformation Products
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine is hydrolytically unstable at pH 4, pH 7, and pH 9, with the following degradation products identified in a GLP-compliant OECD Guideline 111 hydrolysis study: aniline, p-benzoquinone, and p-hydroquinone (pH 4); aniline, p-benzoquinone, and unknown (pH 7); and aniline and unknown (pH 9) (ECHA 2021a). Aniline, p-benzoquinone, and p-

[4] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.
[5] 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.
[6] See Appendix A for a glossary of hazard endpoint acronyms.
[7] For inorganic chemicals only, see GreenScreen® Guidance v1.4, Section 12 (Inorganic Chemical Assessment Procedure).
[8] 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.

hydroquinone are listed as an LT-1 chemical, LT-P1 chemical, and a Benchmark 1 chemical in Pharos, respectively. As N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine is already a Benchmark 1 chemical, the transformation products do not affect the Benchmark score.

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[9,10]
End of life	Hydrolysis	Aniline	62-53-3	Y	Y	LT-1
End of life	Hydrolysis	p-benzoquinone	106-51-4	Y	Y	LT-P1
End of life	Hydrolysis	p-hydroquinone	123-31-9	Y	Y	BM-1

Introduction

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine is a substituted p-phenylenediamine that is used as an antioxidant/antiozonant in rubber, fuel additives, or in monomer distillation, and as a stabilizer (U.S. EPA 2011, Eastman 2019). In 2005, N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine had an aggregated production and/or import volume of 1 million to <10 million pounds in the United States (U.S EPA 2011).

ToxServices assessed N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine 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

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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine 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),[11] 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-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine can be found in Appendix C.

[9] 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.
[10] A GreenScreen® assessment of a transformation product depends on the Benchmark score of the parent chemical (see GreenScreen® Guidance).
[11] DOT lists are not required lists for GreenScreen® List Translator v1.4. They are reference lists only.

- N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine is not listed on the U.S. DOT list.
- N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine is on the following list for multiple endpoints. Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.
    - OSPAR – Priority PBTs & Eds & Equivalent Concern – PBT – Substance of Possible Concern.

Hazard Statement and Occupational Control

No Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements were identified for N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine. H Statements reported in the ECHA REACH dossier for N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine are reported in Table 2. General personal protective equipment (PPE) recommendations are presented in Table 3, below. No occupational exposure limits (OELs) were identified.

Table 2: GHS H Statements for N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4) (ECHA 2021a)

H Statement	H Statement Details
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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Suitable gloves; safety glasses; respiratory protection unless adequate local exhaust ventilation is provided	Eastman 2019	None identified	N/A

Physicochemical Properties of N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine is a dark, brown-purple viscous liquid at standard temperature and pressure. It is slightly soluble in water and is not volatile. Its log K_{ow} of 5.17 suggests it may have the potential to be bioaccumulative.

Table 4: Physical and Chemical Properties of N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)

Property	Value	Reference
Molecular formula	$C_{19}H_{26}N_2$	ChemIDplus 2021
SMILES Notation	CC(C)CCC(C)Nc1ccc(Nc2ccccc2)cc1	ChemIDplus 2021
Molecular weight	282.428	ChemIDplus 2021
Physical state	Liquid; Oil/low melting solid	ECHA 2021a; U.S. EPA 2011
Appearance	Dark, brown-purple viscous liquid	ECHA 2021a

Table 4: Physical and Chemical Properties of N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)		
Property	**Value**	**Reference**
Melting point	29.8°C - 34.9°C	ECHA 2021a, U.S. EPA 2011
Boiling point	228°C - 232°C at 4.666 hPa; 231°C at 3.5 mm Hg	ECHA 2021a; U.S. EPA 2011
Vapor pressure	0.00000281 hPa at 25°C 7 x 10^{-7} mm Hg at 25°C (estimated)	ECHA 2021a; U.S. EPA 2011
Water solubility	0.67 mg/L at 25°C	ECHA 2021a, U.S. EPA 2011
Dissociation constant	pKa_1 = 6.7 pKa_2 = -0.65 (estimated); pKa_1 = 1.73 pKa_2 = 4.99 (estimated)	ECHA 2021a; U.S. EPA 2011
Density/specific gravity	1.01 at 20°C	ECHA 2021a
Partition coefficient	Log K_{ow} = 5.17 (estimated)	ECHA 2021a, U.S. EPA 2011

Toxicokinetics

No toxicokinetic data were identified for N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine specifically. The appearance of systemic toxicity after oral and dermal exposure in acute and repeated dose toxicity studies demonstrates N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine is bioavailable via these routes.

An examination of the hydrolysis rate in gastric juices of the surrogate p-phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl (CAS #793-24-8), 60% of the test substance hydrolyzed in the gastric juices within 48 hours, with a hydrolysis rate of -0.0188 and a half-life of 36.9 hours (ECHA 2021b).

An excretion study was conducted with male Sprague-Dawley rats administered N,N'-bis(2-methylphenyl)benzene-1,4-diamine (CAS #15017-02-4), a main constituent of the surrogate 1,4-benzenediamine, N,N'-mixed Ph and tolyl derivatives (CAS #68953-84-4), in corn administered via gavage followed by collection of urine, fecal, and bile samples at 6, 12, 24, 36, 48 and 72 hours. Approximately 75% of the dose was excreted in feces with 30% of the dose entering the GI tract via the biliary route; <2% of the dose was excreted in the urine. An analysis of the bile indicated the 95% of the metabolites excreted exhibited greater polarity than the parent compound, suggesting metabolic formation of oxidation and conjugation products of the component (ECHA 2021c).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

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

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine 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 with one surrogate and the second surrogate lacking tumor initiating or promotional activity. 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 because it is based on reliable experimental data on strong surrogates.

- 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 2021b
  - *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
  - *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
  - *In vitro: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):*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 $\geqslant$ 0.488 µg/mL resulted in $\leqslant$ 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. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
- ECHA 2021c
  - *Oral: Surrogate: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl Derivatives (CAS #68953-84-4):* 1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivatives did not have tumor initiating or promotional activity in a GLP-compliant 26/38 week study in male rats (no guideline specified). Animals were administered the test substance continuously in the diet at 1,900 ppm (>100 mg/kg/day) for up to 38 weeks. Animals were sacrificed at 26 and 38 weeks and subjected to complete gross examination and histopathological examination of the liver and bladder. 1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivatives lacked any evidence of initiating activity towards the liver and bladder. Promotional activity was not

evident as the various indices were virtually unchanged from groups that were treated with known initiators alone. The test substance was concluded to have negligible carcinogenic potential. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).

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

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Low for mutagenicity/genotoxicity based on negative *in vitro* mutagenicity studies and a negative *in vivo* clastogenicity study in rats. 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 experimental data for the target substance.

- 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 2021a
  - *In vitro:* N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was negative for mutagenicity in a GLP-compliant OECD Guideline 471 bacterial reverse mutation assay. *Salmonella typhimurium* test strains TA1535, TA1537, TA98, and TA100 were exposed to the test substance (98% purity, vehicle not reported) at concentrations of 2.5, 7.9, 25, 79 and 250 µg/plate with and without metabolic activation (S9 mix prepared from Aroclor 1254 induced male rats). Positive controls of 9-aminoacridine, 2-nitrofluorene, sodium azide, benzo(a)pyrene, and 2-aminoanthracene were used. There were no increases in the frequency of revertants reported in any strain at any concentration with or without metabolic activation. The vehicle, untreated negative, and positive controls were valid. This study is reported in the REACH dossier with a Klimisch Score 2 (reliable with restrictions).
- ECHA 2021a, U.S. EPA 2011
  - *In vitro:* N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was positive for clastogenicity in a GLP-compliant chromosome aberration assay conducted similar to OECD Guideline 473. Chinese hamster ovary (CHO) cells were exposed to the test substance (purity not reported, acetone solvent) at concentrations of 0, 7.5, 10, and 15 µg/mL with and without metabolic activation (S9 mix from Aroclor 1254-induced rat liver homogenate) in experiment one, and 7.5 and 10 µg/mL without metabolic activation in experiment two. Positive controls of cyclophosphamide and methylmethanesulfonate were used. A statistically significant increase in aberration frequency was reported at 15 µg/mL without metabolic activation and at 10 µg/mL with metabolic activation; however, a dose-response relationship was not observed. The untreated negative control and the positive control were valid; the solvent control induced a relative high number of aberrant cells in experiment one. The authors suggested that the observed effects might be secondary as they were observed in the range of cytotoxicity and due to the lack of a dose response relationship. This study is reported in the REACH dossier with a Klimisch Score 1 (reliable without restriction).
  - *In vitro:* N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was negative for mutagenicity in a non-GLP-compliant bacterial reverse mutation assay (guideline not specified). *S. typhimurium* test strains TA1535, TA1537, TA98, TA100, and TA1538, and *Saccharomyces cerevisiae* strain D4 were exposed to the test substance (96.2% purity; vehicle not reported) at concentrations of 0.001 to 5 µL/plate with and without metabolic activation (S9 mix). Positive controls of 2-acetylaminofluorene, 2-nitrofluorene, MNNG, QM, NF, Anth, AMQ, and AAF were used. There were no increases in the frequency of

revertants reported in any strain at any concentration with or without metabolic activation. The vehicle and positive controls were valid. This study is reported in the REACH dossier with a Klimisch Score 2 (reliable with restrictions).

- *In vitro:* N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was negative for mutagenicity in a GLP-compliant mammalian cell gene mutation assay (guideline not specified). CHO cells were exposed to the test substance (96.2% purity, vehicle not reported) at concentrations of 1, 3, 5, 6, and 10 µg/mL without metabolic activation and 10, 15, 20, 25, and 30 µg/mL with metabolic activation (S9 mix prepared from Aroclor 1254-induced rat liver homogenate). Positive controls of benzo(a)pyrene and ethylmethane sulfonate were used. There were no increases in the frequency of mutations reported at any concentration with or without metabolic activation. The vehicle and positive controls were valid. This study is reported in the REACH dossier with a Klimisch Score 1 (reliable without restriction).
- *In vivo:* N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was negative for clastogenicity in a GLP-compliant chromosome aberration assay conducted similar to OECD Guideline 475. Male and female Sprague-Dawley rats (5/sex/dose) were administered the test substance (96.2% purity; vehicle not reported) at 1,100 mg/kg/day via oral gavage and bone marrow samples from the femurs were collected at 6, 18 and 30 hours post treatment. A positive control of cyclophosphamide was used. Treatment with the test substance did not produce statistically significant increases in the number of aberrations or in the number of aberrant metaphases at any of the three sacrifice intervals. The vehicle, negative, and positive controls were valid. This study is reported in the REACH dossier with a Klimisch Score 2 (reliable with restrictions).

- U.S. EPA 2011
  - *In vitro:* N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was negative for mutagenicity in a mammalian cell gene mutation assay (GLP status and guideline not specified). Mouse lymphoma L5178Y cells were exposed to the test substance (>96% purity, vehicle not reported) at concentrations of 1.25 – 50 nL/mL with metabolic activation and 0.625 – 10 nL/mL without metabolic activation. The use of a positive controls was not specified. There were no increases in the frequency of mutations reported at any concentration with or without metabolic activation. Cytotoxicity was reported at 60 nL/mL with metabolic activation and 20 nL/mL without metabolic activation. A Klimisch Score is not assigned for this study.
- Based on a weight of evidence, a score of Low was assigned. *In vitro* mutagenicity studies in bacterial systems and mammalian cells were consistently negative. Although an *in vitro* clastogenicity study in CHO cells was positive, an *in vivo* chromosome aberration assay in rats was negative. Therefore, N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine is not likely to be genotoxic *in vivo*, and a score of Low can be assigned for this endpoint.

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

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine 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 studies for the target substance and strong surrogates.

- 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 2021a
    - *Oral:* In a GLP-compliant reproduction/developmental toxicity screening test conducted according to OECD Guideline 421, male and female Sprague-Dawley rats (10/sex/dose, except in the high dose group which had 15/sex/dose) were administered N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (95.5% purity) in corn oil at doses of 50, 100, and 200 mg/kg/day via gavage. Males were dosed for 14 days prior to mating, throughout mating and continuing until the day prior to euthanasia, for a total of 28 days; females were dosed for 14 days prior to mating and continuing through lactation day 13, for a total of 49 to 60 doses. Parental animals were examined for clinical signs and mortality, estrous cyclicity (females), clinical chemistry, organ weight, gross pathology, histopathology, and reproductive performance; litters were examined for viability, sex, body weight, anogenital distance, areola/nipple anlagen, thyroid hormone analysis, clinical chemistry, organ weight, and gross pathology. There was no evidence of reproductive toxicity in parental males; therefore, the authors identified a NOAEL of 200 mg/kg/day, the highest dose tested, for F0 male reproductive toxicity. Total litter loss was reported at 200 mg/kg/day and longer mean gestation length was reported at 100 and 200 mg/kg/day. Evidence of dystocia leading to the euthanasia was reported in 1 female each in the 50, 100, and 200 mg/kg/day groups. Based on these effects, the authors identified a LOAEL of 50 mg/kg/day for F0 female reproductive toxicity. This study is reported in the REACH dossier with a Klimisch Score 1 (reliable without restriction).
- U.S. EPA 2011
    - *Oral: Surrogate: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl Derivatives (CAS #68953-84-4):* In a two generation reproductive toxicity study, male and female Sprague-Dawley rats (30/sex/dose) were administered the test substance (assumed 100% pure) at doses of 0, 120, 400, and 1,500 ppm (reported as approximately 0, 8.78, 29.27 and 109.76 mg/kg/day, respectively) in the diet. The parental generation was exposed from 10 weeks prior to mating, through the 2-week mating period, 3 weeks during gestation and through weaning. The F1 generation was exposed for 10 weeks prior to mating. On postnatal day 30, litters were culled and 30 F1 generation males and females per dose group were paired and exposed for an additional 10 weeks. Pregnant rats had dystocia, which was assumed to cause prolonged gestation, increased perinatal deaths and decreased live births and increased pup weights. Polycystic lesions were reported in animals from all dose levels. Females from the high dose group had reduced body weights. In addition, treatment-related kidney lesions were also observed grossly and microscopically (not specified). Based on the dystocia, perinatal deaths, decreased live births and increased pup weights, a reproductive toxicity LOAEL of 120 ppm (8.78 mg/kg/day), the lowest dose tested, was identified for this study; a NOAEL could not be identified.
- U.S. EPA 2011, ECHA 2021b
    - *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).

- ECHA 2021b
    - *Oral:* *Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).
    - *Oral:* *Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).
    - *Oral:* *Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).

- In summary, while the target substance and the surrogates 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 for the surrogate p-phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl classified it 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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Moderate for developmental toxicity based on reduced post-natal survival, pup birth weight, body wight and body weight gain in a screening study with the target substance and incomplete ossification, decreased fetal body weights and/or increased post-implantation losses identified in rat and rabbit prenatal developmental toxicity tests with the surrogates, 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 there is limited or marginal evidence of developmental toxicity in animal studies and a GHS Category 2 classification is warranted (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for the target substance and strong surrogates.

- 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 2021a
  - *Oral:* In a GLP-compliant reproduction/developmental toxicity screening test conducted according to OECD Guideline 421, male and female Sprague-Dawley rats (10/sex/dose, except in the high dose group which had 15/sex/dose) were administered N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (95.5% purity) in corn oil at doses of 50, 100, and 200 mg/kg/day via gavage. Males were dosed for 14 days prior to mating, throughout mating and continuing until the day prior to euthanasia, for a total of 28 days; females were dosed for 14 days prior to mating and continuing through lactation day 13, for a total of 49 to 60 doses. Parental animals were examined for clinical signs and mortality, estrous cyclicity (females), clinical chemistry, organ weight, gross pathology, histopathology, and reproductive performance; litters were examined for viability, sex, body weight, anogenital distance, areola/nipple anlagen, thyroid hormone analysis, clinical chemistry, organ weight, and gross pathology. Lower postnatal survival and lower mean F1 pup birth weights, body weights, and body weight gains were observed in the 200 mg/kg/day group. Therefore, the authors identified a NOAEL of 100 mg/kg/day for F1 neonatal toxicity. This study is reported in the REACH dossier with a Klimisch Score 1 (reliable without restriction).
- U.S. EPA 2011
  - *Oral: Surrogate: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl Derivatives (CAS #68953-*

84-4): In a prenatal developmental toxicity study, pregnant Sprague-Dawley rats (25/dose) were administered the test substance (purity not reported) at doses of 0, 20, 70 and 200 mg/kg/day during gestation days (GD) 6-15 via gavage. Decreased body weight and food consumption was reported in maternal animals at the high dose. There were no adverse effects on pregnancy rates, litter sizes, number of live fetuses, uterine implantation or any gestational parameters. A dose-related decrease in fetal body weight was reported, reaching approximately 5% at the high dose. No visceral, external or skeletal abnormalities were reported in the fetuses. Based on these results, a maternal toxicity NOAEL and LOAEL of 70 and 200 mg/kg/day, respectively, and a developmental toxicity LOAEL of 20 mg/kg/day, the lowest dose tested, were established.

- *Oral*: *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* prenatal developmental toxicity study, pregnant Sprague-Dawley rats (24/dose) were exposed to IPPD by gavage in polyethylene glycol 400 at doses of 0 10, 50 or 100 mg/kg/day on GDs 6-15. Maternal animals exhibited post-dosing salivation and lethargy and a slight reduction in food consumption between GDs 6 and 9. There were no unscheduled mortality, and no treatment related effects on the developing fetus. U.S. EPA identified a NOAEL of 50 mg/kg/day and LOAEL of 100 mg/kg/day for maternal toxicity based on post-dosing salivation and lethargy, and a NOAEL of 100 mg/kg/day for developmental toxicity, which was the highest dose tested.

- U.S. EPA 2011, ECHA 2021d
  - *Oral*: *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* In a GLP-compliant developmental toxicity study conducted according to OECD Guideline 414, pregnant Sprague-Dawley rats (24/dose) received doses of 12.5, 62.5, and 125 mg/kg IPPD (97.2% purity) in polyethylene glycol (PEG) by gavage on GD 6-15. Parameters evaluated include clinical observations, body weight, food consumption, maternal examinations, uterine/implantation data, litter data, and fetal examinations. There were no treatment-related effects seen in the maternal animals according to the ECHA record, however, U.S. EPA reported slight maternal toxicity at the high dose, including reduced food intake, pre-dosing salivation and soft, dark feces. In the fetuses, there were statistically significant increases in the retardation of ossification seen in high-dose and mid-dose animals. REACH dossier authors identified a fetotoxic NOAEL of 62.5 mg/kg/day (Klimisch 1, reliable without restriction). U.S. EPA identified a NOAEL of 62.5 mg/kg/day and LOAEL of 125 mg/kg/day for maternal toxicity based on reduced food intake, pre-dosing salivation and soft, dark feces, and a NOAEL of 12.5 mg/kg/day and LOAEL of 62.5 mg/kg/day for developmental toxicity based on incomplete bone ossification.
- U.S. EPA 2011, ECHA 2021b
  - *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).

- *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions). .

- ECHA 2021b
  - *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).
  - *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).

- *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).

- *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):*
  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." This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).
- *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):*
  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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Moderate for endocrine activity based on altered female pubertal development in rats for the surrogate p-phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl.. However, there does not appear to be evidence of endocrine-mediated carcinogenicity, reproductive or developmental toxicity, or systemic toxicity that would 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 for the surrogate p-phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl. 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 it is based on reliable data from a strong surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2021b
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was predicted to be inactive for androgen receptor agonism, and binding, and active for androgen receptor antagonism using the COMPARA (consensus) model in ToxCast. It was predicted to be inactive for estrogen receptor agonism, antagonism, and binding using the CERAPP Potency Level (consensus) model in ToxCast.
- ECHA 2021a
  - *Oral:* In a GLP-compliant reproduction/developmental toxicity screening test conducted according to OECD Guideline 421, male and female Sprague-Dawley rats (10/sex/dose, except in the high dose group which had 15/sex/dose) were administered N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (95.5% purity) in corn oil at doses of 50, 100, and 200 mg/kg/day via gavage. Males were dosed for 14 days prior to mating, throughout mating and continuing until the day prior to euthanasia, for a total of 28 days; females were dosed for 14 days prior to mating and continuing through lactation day 13, for a total of 49 to 60 doses. Litters were examined for anogenital distance, areola/nipple anlagen, and thyroid hormone analysis. There were no effects to the anogenital distance and areola/nipple anlagens were unaffected in the F1 generation. Additionally, there were no test substance related effects on T4 thyroid hormone levels in the F1 males and females. This study is reported in the REACH dossier with a Klimisch Score 1 (reliable without restriction).
- ECHA 2021b
  - *Oral: <u>Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):</u>* 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. This study is reported in the REACH dossier with a Klimisch Score 1 (reliable without restriction).

- *Oral: Surrogate: p-Phenylenediamine, N-(1,3-Dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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." This study is reported in the REACH dossier with a

Klimisch Score 1 (reliable without restriction).

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): L

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Low for acute toxicity based on an oral LD_{50} of 2,100 mg/kg in rats and a dermal LD_{50} of >5,010 mg/kg in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when oral and dermal LD_{50} values are greater than 2,000 mg/kg (CPA 2018b). The confidence in the score is high has it is based on reliable data for the target substance.

- 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 2021a, U.S. EPA 2011
  - *Oral:* LD_{50} (male and female Sprague-Dawley rat) = 2,100 mg/kg (non-GLP, guideline not specified)
  - *Dermal:* LD_{50} (male and female New Zealand white rabbit) >5,010 mg/kg (non-GLP, guideline not specified)
- ECHA 2021a
  - *Oral:* LD_{50} (male and female Sprague-Dawley rat) = 2,170 mg/kg (non-GLP, guideline not specified)
  - *Dermal:* LD_{50} (male and female New Zealand white rabbit) >10,000 mg/kg (GLP status and guideline not specified)
- U.S. EPA 2011
  - *Inhalation:* 6 hour LC_{50} (male Sprague-Dawley rats) > 0.14 mg/L

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

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Low for systemic toxicity (single dose) based on the lack of specific, non-lethal toxic effects on target organs at doses below 2,000 mg/kg in acute oral and dermal toxicity studies. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (single dose) when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable data for the target substance.

- 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 2021a, U.S. EPA 2011
  - *Oral:* In a non-GLP-compliant acute oral toxicity study (guideline not specified) male and female Sprague-Dawley rats (5/dose) were administered unchanged N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (>96% purity) at doses of 1,260, 1,580, 2,000, 2,510, and 3,160 mg/kg via gavage. At 1,260, 1,580, 2,000, 2,510, and 3,160 mg/kg, 1/5, 2/5, 2/5, 3/5, and 4/5 animals died, respectively. Reduced appetite and activity was noted in survivors from all dose levels. Those that died displayed increasing weakness, collapse and death. Gross pathology of survivors was normal, while dead animals displayed hemorrhagic areas

of the lung, liver discoloration, and acute gastrointestinal inflammation. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
    - *Dermal:* In a non-GLP-compliant acute dermal toxicity study (guideline not specified) male and female New Zealand white rabbits (1-2/dose) were administered unchanged N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (purity not reported) at doses of 5,010 and 7,940 mg/kg for 24 hours. At 5,010 and 7,940 mg/kg, 0/1 and 1/2 animals died, respectively. Clinical signs included reduced appetite and activity in survivors, and increasing weakness, collapse, and death in those that died. Gross pathology of survivors was normal, while dead animals displayed hemorrhagic areas of the lung, liver, and spleen, kidney discoloration, and gastrointestinal inflammation. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
- ECHA 2021a
    - *Oral:* In a non-GLP-compliant acute oral toxicity study (guideline not specified) male and female Sprague-Dawley rats (5/dose) were administered N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (purity not reported) in corn oil at doses of 1,580, 2,000, 2,510, and 3,160 mg/kg via gavage. At 1,580, 2,000, 2,510, and 3,160 mg/kg, 1/5, 1/5, 3/5, and 5/5 animals died, respectively. Clinical signs included weakness, diarrhea, tremors and collapse (doses not specified). Gross pathology displayed inflammation of the gastric mucosa, kidney and liver discoloration, and pulmonary hyperemia (the dossier does not specify if effects were seen in surviving animals). This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
    - *Dermal:* In an acute dermal toxicity study (GLP status and guideline not specified) male and female New Zealand white rabbits (1/dose) were administered unchanged N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (purity not reported) at doses of 1,000, 1,580, 2,510, 3,980, 6,310, and 10,000 mg/kg under occlusive conditions. There were no mortalities reported. Clinical signs included lethargy for two to three days after dosing at higher dosage levels, moderate weakness, and greenish urine. The REACH dossier does not report on evaluations of body weight or histopathology. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).

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

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Moderate for systemic toxicity (repeated dose) based on an oral NOAEL and LOAEL of 101.2 and 186.9 mg/kg/day, respectively, in a 28 day study in rats. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (repeated dose) when the oral LOAEL value is between 10 and 100 mg/kg/day for 90 day studies (adjusted to 30 and 300 mg/kg/day for 28-day studies) (CPA 2018b). The confidence in the score is high as it is based on reliable data for the target substance.

- 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 2021a, U.S. EPA 2011
    - *Oral:* In a GLP-compliant subacute feeding study (guideline not specified), male and female Sprague-Dawley rats (5/sex/dose) were administered N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (96.2% purity) in the feed at 0, 500, 750, 1,500, and 3,000 ppm (reported as equivalent to 0, 36.7, 51.8, 101.2, and 186.9 mg/kg/day, respectively, for males, and 0, 39.8, 58.2, 134.3, and 199.6 mg/kg/day, respectively, for females) for 28 days. There were no mortalities and no adverse clinical signs of toxicity reported. Body weight was

decreased in high dose animals of both sexes, and reduced body weight gain and feed consumption was noted in both sexes at 750 ppm and above. No gross lesions were reported. Absolute liver weight was increased in high dose animals of both sexes (not significant). No hematology, clinical biochemistry, or histopathology examinations were conducted. Based on the effects to body and liver weights, a NOAEL and LOAEL of 1,500 and 3,000 ppm, respectively (101.2, and 186.9 mg/kg/day, respectively, for males, and 134.3, and 199.6 mg/kg/day, respectively, for females) are identified in the ECHA REACH dossier. The U.S. EPA Hazard Characterization Document identifies the NOAEL as 3,000 ppm (186.9 and 199.6 mg/kg/day in males and females, respectively) based on a lack of toxicologically significant effects. This study is reported in the REACH dosser with a Klimisch Score of 2 (reliable with restrictions).

- *Due to the 28-day duration of this study, guidance values were tripled (i.e., 100 mg/kg/day * 3 = 300 mg/kg/day) as 28 days is approximately 1/3 the duration of 90 day studies.*

- ECHA 2021a
  - *Oral:* In the previously described GLP-compliant reproduction/developmental toxicity screening test conducted according to OECD Guideline 421, male and female Sprague-Dawley rats (10/sex/dose, except in the high dose group which had 15/sex/dose) were administered N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (95.5% purity) in corn oil at doses of 50, 100, and 200 mg/kg/day via gavage. Males were dosed for 14 days prior to mating, throughout mating and continuing until the day prior to euthanasia, for a total of 28 days; females were dosed for 14 days prior to mating and continuing through lactation day 13, for a total of 49 to 60 doses. Parental animals were examined for clinical signs and mortality, estrous cyclicity (females), clinical chemistry, organ weight, gross pathology, histopathology, and reproductive performance. High dose males had statistically significantly reduced body weights, increased liver weights and decreased thymus weights. High dose females had increased liver and adrenal gland weights and adrenal cortical hypertrophy. Therefore, the authors identified a NOAEL of 100 mg/kg/day for systemic toxicity for this study (LOAEL of 200 mg/kg/day). This study is reported in the REACH dossier with a Klimisch Score 1 (reliable without restriction).
    - *Due to the 28-day duration of this study for male animals, guidance values were tripled (i.e., 100 mg/kg/day * 3 = 300 mg/kg/day) as 28 days is approximately 1/3 the duration of 90-day studies.*

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): *L*
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Low for neurotoxicity (single dose) based on a lack of signs of neurotoxicity in standard acute toxicity studies. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (single dose) when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is low as no specific neurotoxicity examinations were conducted.

- 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 2021a, U.S. EPA 2011
  - *Oral:* In a non-GLP-compliant acute oral toxicity study (guideline not specified) male and female Sprague-Dawley rats (5/dose) were administered unchanged N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (>96% purity) at doses of 1,260, 1,580, 2,000, 2,510, and 3,160 mg/kg via gavage. At 1,260, 1,580, 2,000, 2,510, and 3,160 mg/kg, 1/5, 2/5, 2/5, 3/5,

and 4/5 animals died, respectively. Reduced appetite and activity was noted in survivors from all dose levels. Those that died displayed increasing weakness, collapse and death. Gross pathology of survivors was normal, while dead animals displayed hemorrhagic areas of the lung, liver discoloration, and acute gastrointestinal inflammation. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).

  - *Dermal:* In a non-GLP-compliant acute dermal toxicity study (guideline not specified) male and female New Zealand white rabbits (1-2/dose) were administered unchanged N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (purity not reported) at doses of 5,010 and 7,940 mg/kg for 24 hours. At 5,010 and 7,940 mg/kg, 0/1 and 1/2 animals died, respectively. Clinical signs included reduced appetite and activity in survivors, and increasing weakness, collapse, and death in those that died. Gross pathology of survivors was normal, while dead animals displayed hemorrhagic areas of the lung, liver, spleen and kidney discoloration, and gastrointestinal inflammation. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).

- ECHA 2021a
  - *Oral:* In a non-GLP-compliant acute oral toxicity study (guideline not specified) male and female Sprague-Dawley rats (5/dose) were administered N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (purity not reported) in corn oil at doses of 1,580, 2,000, 2,510, and 3,160 mg/kg via gavage. At 1,580, 2,000, 2,510, and 3,160 mg/kg, 1/5, 1/5, 3/5, and 5/5 animals died, respectively. Clinical signs included weakness, diarrhea, tremors and collapse (doses not specified). Gross pathology displayed inflammation of the gastric mucosa, kidney and liver discoloration, and pulmonary hyperemia. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
  - *Dermal:* In an acute dermal toxicity study (GLP status and guideline not specified) male and female New Zealand white rabbits (1/dose) were administered unchanged N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine (purity not reported) at doses of 1,000, 1,580, 2,510, 3,980, 6,310, and 10,000 mg/kg under occlusive conditions. There were no mortalities reported. Clinical signs included lethargy for two to three days after dosing at higher dosage levels, moderate weakness, and greenish urine. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): DG
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Data Gap for neurotoxicity (repeated dose) based on a lack of data identified for this endpoint.

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

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): H
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of High for skin sensitization based on the surrogates being classified as GHS Category 1A dermal sensitizers. GreenScreen® criteria classify chemicals as a High hazard for skin sensitization when a GHS Category 1A classification is warranted (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for strong surrogates.

- 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 2021a
    - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was not sensitizing to human skin in a modified Draize sensitization study with human volunteers (n=80). There was no evidence of allergic contact sensitization to Santoflex 14 at 1% in petrolatum. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
    - ECHA dossier authors stated that there is ample evidence for multiple chemical substances structurally similar to the target chemical that skin sensitization is a common feature, so sensitization was not conducted in the early time periods of REACH, before *in vitro* tests were available, to avoid needless testing on animals when the lead registrant was comfortable in classifying the substance as a category 1 sensitizer.
- U.S. EPA 2011, ECHA 2021c
    - *Surrogate: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl Derivatives (CAS #68953-84-4):* 1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivatives was dermally sensitizing to the skin in a GLP-compliant guinea pig maximization test conducted similar to OECD Guideline 406. Male and female Hartley guinea pigs (20/dose) were intradermally induced with 0.1 mL of Freund's adjuvant, 5% test substance in 0.5% acetone in propylene glycol, and the test substance plus Freund's adjuvant at opposite sites from the dorsal midline, followed 7 days later by a topical induction under occlusive conditions after a 24-hour test site exposure to sodium lauryl sulfate. Challenge exposures were performed on day 21 with 25% and 100% test substance, and repeated on day 28. After the first challenge with 25% test substance, 5/20 animals displayed erythema at 24 hours and 3/20 animals displayed edema and/or desquamation at 48 hours. After the first challenge with 100% test substance, 0/20 animals displayed positive reactions at 24 hours and 5/20 animals displayed desquamation at 48 hours. After rechallenge with 25% test substance, 15/20 animals displayed desquamation, edema, and/or blanching at 24 hours, and 11/20 animals displayed these effects at 48 hours. After rechallenge with 100% test substance, 15/20 animals displayed desquamation, edema, and/or blanching at 24 hours, and 15/20 animals displayed these effects at 48 hours. The test substance was considered to be a contact sensitizer under the conditions this study. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).
- U.S. EPA 2011
    - *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* p-Phenylenediamine, N-isopropyl-N'-phenyl- was sensitizing to human skin in a repeated insult patch test. Human volunteers (n=50) were exposed to 50% test substance (>96% purity) in dimethyl phthalate for 24 hours and repeated for 15 applications given every other day. A challenge patch was applied after a two-week rest period. The test substance was reported to be sensitizing to humans in this study.
    - *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* p-Phenylenediamine, N-isopropyl-N'-phenyl- was sensitizing to human skin in a repeated insult patch test. Human volunteers (n=82) were exposed to 1% test substance (>96% purity) in petrolatum 3 times per week for 3 weeks. A challenge patch was applied after an unspecified rest period. The test substance was reported to be sensitizing to humans in this study.
    - *Surrogate: p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-Phenyl (CAS #793-24-8):* p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl was not sensitizing to human skin in a repeated insult patch test. Human volunteers (n=93) were exposed to 1% test substance (purity not specified) in petrolatum 3 times per week for 3 weeks. A challenge patch was applied after an unspecified rest period. The test substance was reported to be not

sensitizing to humans in this study.

- *Surrogate: p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-Phenyl (CAS #793-24-8):* p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-phenyl was sensitizing to human skin in a repeated insult patch test. Human volunteers (n=50) were exposed to 50% test substance (purity not specified) in dimethyl phthalate for 3 weeks, followed by a challenge phase. Five of the 50 subjects displayed skin reactions following the induction phase and 5 of the 50 subjects shower skin reactions in the challenge phase. The test substance was reported to be sensitizing to humans in this study.

- ECHA 2021b
  - *Surrogate: p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 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 $\geqslant$ 30% animals at $\leqslant$ 0.1% intradermal doses (UN 2021).
  - *Surrogate: p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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. This study is reported in the REACH dossier with a Klimisch Score of 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.
  - *Surrogate: p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-Phenyl (CAS #793-24-8):* p- 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. This study is reported in the REACH dossier with a Klimisch Score of 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).
    - *Surrogate: p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 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).
- ECHA 2021d
    - *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* In a guinea pig maximization test conducted according to Magnusson & Kligman (1969) (GLP compliance not specified), IPPD (purity not reported) was applied to female Hartley guinea pigs (20/dose) at 1% in Vaseline under intradermal and epicutaneous conditions. The first induction was 0.5% intracutaneously, the second induction was 1% epicutaneously, and the animals were challenged at 0.05% and 0.5% epicutaneously. IPPD was categorized as a strong sensitizer, with 90% of animals having positive reactions. The authors classified IPPD as a GHS Category 1 sensitizer (Klimisch 2, reliable with restrictions).
        - Based on 90% of the animals responding following an intradermal dose of 0.5%, IPPD 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).
    - *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* In a mouse local lymph node assay (LLNA) according to Yamano et al. (2003) (GLP compliance not specified), IPPD (purity not reported) was applied to female Balb/c mice (4/dose) at concentrations of 0, 0.01, 0.03, 0.1, and 0.3% (vehicle not reported). IPPD was found to be a skin sensitizer under the conditions of the study, with stimulation indices (SIs) of 1, 1.3, 1.68, 3.23, and 3.99. Therefore, the authors classified IPPD as a GHS Category 1 sensitizer (Klimisch 2, reliable with restrictions).
        - While the EC3 was not reported, the threshold SI of 3 was exceeded at the concentration of 0.1%, indicating that the EC3 would be less than 0.1%. This is less than the GHS guidance value of 2% for Category 1A classification (UN 2021).
    - *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* In a mouse LLNA conducted according to a non-specified guideline (GLP compliance not specified), IPPD (purity not reported) was applied to female Balb/c mice (3/dose) at concentrations of

0, 0.1, 0.5, 1, and 2% in acetone/olive oil (4:1 v/v). IPPD was found to be a skin sensitizer under the conditions of the study, with SIs of 1.5, 3.85, 2.39, and 1.42. Therefore, the authors classified IPPD as a GHS Category 1 sensitizer (Klimisch 2, reliable with restrictions).

- ToxServices could not determine an EC3 for this study as no dose response was observed.

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): *M*

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Moderate for respiratory sensitization based on the presence of a structural alert for respiratory sensitization and positive dermal sensitization data for the surrogates. GreenScreen® criteria classify chemicals as a Moderate hazard for respiratory sensitization when a GHS Category 1B classification is warranted (CPA 2018b). The confidence in the score is low as no specific data are available for respiratory sensitization.

- 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 2021
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine contains a structural alert for respiratory sensitization; Pro-Michael Addition >> Pro-quinone and related >> Phenylenediamine (Appendix D).
- No data were identified for the target compound or surrogates for this endpoint. Therefore, ToxServices attempted to evaluate the respiratory sensitization potential of N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine 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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine contains a structural alert for respiratory sensitization (Pro-Michael Addition >> Pro-quinone and related >> phenylenediamine) (OECD 2021). Additionally, it is expected to be a skin sensitizer based on positive experimental data for the surrogates. According to the ECHA guidance, the positive skin sensitization results in animals and presence of a structural alert indicates that this chemical may be a respiratory sensitizer. Therefore, a low confidence score of Moderate was assigned.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): L

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Low for skin irritation/corrosivity based on it not being classified as a dermal irritant in two acute irritation studies in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for the target substance.

- 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 2021a, U.S. EPA 2011
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was not irritating to the skin in a non-GLP-compliant dermal irritation study (guideline not specified). New Zealand white rabbits (n=6) were administered unchanged test substance (>96% purity) to intact and

abraded skin for 24 hours under semiocclusive conditions. A slight defatting effect was noted, and skin flaked off in seven to ten days. The mean 24, 48, 72 hour erythema and edema scores were both 0, and the overall irritation score was 0/8. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).

- ECHA 2021a
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was a mild irritant to the skin in a non-GLP-compliant dermal irritation study (guideline not specified). Albino rabbits (n=3) were administered unchanged test substance (purity not reported) to shaved skin for 24 hours under occlusive conditions. Slight edema and erythema were reported at 24 hours (overall score of 2.3/8), and slight redness was reported at 48 and 72 hours (overall scores of 1.6/8 and 1/8, respectively). Effects were fully reversible within 120 hours. The authors classified the test substance as a mild irritant. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
    - *The REACH dossier authors did not consider this study to be sufficient for classification as a dermal irritant.*
- Based on a weight of evidence, a score of Low was assigned. GHS criteria define skin irritants as chemicals that produce mean scores of ≥1.5 for erythema/eschar or for edema in at least 2 of 3 tested animals from gradings at 24, 48, and 72 hours (UN 2021). Due to limited data reporting it is not possible to determine if individual scores meet this criteria in the second summarized study. However, based on overall irritation scores of 2.3/8, 1.6/8 and 1/8 at 24, 48 and 72 hours, respectively, individual scores are unlikely to be above the GHS threshold for classification. Thus ToxServices agrees with the REACH dossier authors and did not classify N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine as a dermal irritant based on this study. Furthermore, the Key study in the REACH dossier reports mean 24, 48, 72 hours erythema and edema scores of 0, providing further evidence N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine is not likely to be a dermal irritant.

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): *M*
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine 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:* Not present on any screening lists for this endpoint.
- ECHA 2021a, U.S. EPA 2011
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was not irritating to the eyes in a non-GLP-compliant ocular irritation study (guideline not specified). The eyes of New Zealand white rabbits (n=6) were instilled with unchanged test substance (>96% purity) and observed for 7 days. Slight discomfort was noted immediately after instillation, and slight erythema, very slight edema, and copious discharge were noted at 1 hour after instillation. All effects were fully reversible within 72 hours, and the mean 24, 48, 72 hour overall irritation score was 3.5/110. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).
- ECHA 2021a
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was mildly irritating to the eyes in a non-GLP-compliant ocular irritation study (guideline not specified). The eyes of albino

rabbits (n=3) were instilled with unchanged test substance (purity not reported) and observed for 7 days. Slight discomfort was noted immediately after instillation, and mild redness, slight edema, copious discharge and slight cloudiness was noted at 1 hour after instillation. Inflammation reduced over 24 hours and discharge decreased within 48 hours. Corneal clarity was normal within 5 days with only a trace of erythema still present. The mean 24, 48, 72 hour overall irritation score was 13.1/110. This study is reported in the REACH dossier with a Klimisch Score of 2 (reliable with restrictions).

- *The REACH dossier did not consider this study to be sufficient for classification as an ocular irritant.*

- Under GHS criteria (UN 2021), 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-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine. Therefore, ToxServices conservatively classified N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine 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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Very High for acute aquatic toxicity based on L/EC_{50} values less than 1 mg/L in all three trophic levels. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when acute aquatic toxicity values are less than 1 mg/L (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for the target substance.

- 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 2021a, U.S. EPA 2011
  - 96 hour LC_{50} (*Lepomis macrochirus*, bluegill sunfish) = 0.3 mg/L (GLP, EPA-660/3-75-009, Klimisch Score 2)
  - 96 hour LC_{50} (*Oncorhynchus mykiss*, rainbow trout) = 0.42 mg/L (GLP, EPA-660/3-75-009, Klimisch Score 2)
- ECHA 2021a
  - 96 hour LC_{50} (*O. mykiss*, rainbow trout) = 0.4 mg/L (GLP, EPA-660/3-75-009, Klimisch Score 2)
  - 48 hour LC_{50} (*Daphnia magna*, daphnia) = 0.2 mg/L (GLP, EPA-660/3-75-009, Klimisch Score 2)
  - 48 hour LC_{50} (*D. magna*, daphnia) = 0.21 mg/L (GLP, EPA-660/3-75-009, Klimisch Score 2)
- U.S. EPA 2011
  - 96 hour LC_{50} (*Pimephales promelas*, fathead minnow) = 1.10 mg/L
  - 96 hour LC_{50} (*P. promelas*, fathead minnow) = 0.06 mg/L
  - 96 hour EC_{50} (*Pseudokirchneriella subcapitata*, green algae) = 0.7 mg/L (biomass and growth rate)

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): vH
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Very High for chronic aquatic toxicity based on chronic aquatic toxicity values less than 0.1 mg/L for the surrogates. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when chronic aquatic toxicity values are less than 0.1 mg/L (CPA 2018b). The confidence in the score is high as it is based on reliable data for strong surrogates.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2011, ECHA 2021c
  - *Surrogate: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl Derivatives (CAS #68953-84-4):* 14-day NOEC (*Cyprinus carpio*, carp) = 0.28 mg/L (GLP, similar to OECD 204, Klimisch Score 1)
  - *Surrogate: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl Derivatives (CAS #68953-84-4):* 14-day NOEC (*O. mykiss*, rainbow trout) = 0.14 mg/L (GLP, similar to OECD 204, Klimisch Score 1)
- U.S. EPA 2011
  - *Surrogate: p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 28-day LC_{50} (*P. promelas*, fathead minnow) = 0.15 mg/L
- ECHA 2021c
  - *Surrogate: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl Derivatives (CAS #68953-84-4):* 21-day NOEC (*D. magna*, daphnia) = 0.016 mg/L (GLP, OECD 211, Klimisch Score 1)
  - *Surrogate: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl Derivatives (CAS #68953-84-4):* 72-hour NOEC (*P. subcapitata*, green algae) = 0.013 mg/L (GLP, OECD 201, Klimisch Score 2)
- ECHA 2021b
  - *Surrogate: p-Phenylenediamine, N-(1,3-dimethylbutyl) N'-Phenyl (CAS #793-24-8):* 30-day NOEC (*Oryzias latipes*, Japanese rice fish) = 0.004 mg/L (GLP, OECD 210, Klimisch Score 1)
- ECHA 2021d
  - *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* 72-hour growth rate NOECb (*P. subcapitata*, green algae) = 2 mg/L (GLP, EU C.3, Klimisch Score 1)
  - *Surrogate: p-Phenylenediamine, N-Isopropyl-N'-phenyl- (CAS #101-72-4):* 72-hour growth rate NOECr (*P. subcapitata*, green algae) = 4 mg/L (GLP, EU C.3, Klimisch Score 1)

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *H*
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of High for persistence based on the predicted half-life of 75 days in soil, its major compartment. GreenScreen® criteria classify chemicals as a High hazard for persistence when soil is the major compartment and the half-life is >60 to 180 days (CPA 2018b). The confidence in the score is low as it is based on a modeled half-life.

- 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 2021a, U.S. EPA 2011
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was not biodegradable in a Shake Flask Carbon Dioxide Evaluation assay (draft ASTM E35.24) (GLP status not specified). In

this assay, 20 mg/L N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine was exposed to aerobic soil, raw sewage, and non-adapted, activated sludge for 35 days. After 35 days, the test substance degraded 0%. This study is reported in the REACH dossier with a Klimisch Score of 2.

- U.S. EPA 2017
    - The BIOWIN modeling Ready Biodegradable Predictor indicates that N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine is not expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 68% will partition to soil with a half-life of 75 days, 22.4% will partition to sediment with a half-life of 337.5 days, and 9.58% will partition to water with a half-life of 37.5 days (Appendix E).

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

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine 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:* Environment Canada (EC) – CEPA Domestic Substances List (DSL) - Bioaccumulative.
- U.S. EPA 2017
    - BCFBAF predicts a BCF of 1,199 using the regression-based model based on a modeled log K_{ow} of 5.17, and a BAF of 492.7 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix E).
- U.S. EPA 2011
    - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine has an estimated BAF of 493.
- CCR 2021
    - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine is listed as bioaccumulative on EC's CEPA DSL based on the predicted log K_{ow} of 5.17.
- ECHA 2021b
    - <u>*Surrogate: N-(1-Methylheptyl)-N'-phenylbenzene-1,4-diamine (CAS #15233-47-3)*</u>: 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.
- Based on the weight of evidence, a score of High was assigned. N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine has a predicted log K_{ow} of 5.17 and is listed on EC's CEPA DSL as bioaccumulative, which corresponds to a score of Very High. The listing on the DSL is based on the predicted log K_{ow}. Modeling using the U.S. EPA's EPI Suite™ predicts a BAF of 492.7 using the Arnot-Gobas model for the upper trophic level. The use of BAF is more appropriate over BCF when the log K_{ow} is greater than 5 (U.S. EPA 2012). However, measured data on the strong surrogate N-(1-methylheptyl)-N'-phenylbenzene-1,4-diamine trump modeled data.

<u>Physical Hazards (Physical)</u>

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

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Low for reactivity based on its NFPA instability rating of 0 and HMIS physical hazard rating of 0. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when they are not GHS classified (CPA 2018b). The confidence in the score is low as no measured data were identified.

- 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 2021a
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine does not contain a chemical moiety suggesting an oxidizing potential.
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine does not contain a chemical moiety suggesting a potential for explosivity.
- Eastman 2019
  - A safety data sheet for N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine reports an instability rating of 0 from the National Fire Protection Association (NFPA) ("Normally stable, even under fire exposure conditions, and is not reactive with water") and physical hazard rating of 0 from Hazardous Materials Identification System (HMIS) ("Materials that are normally stable, even under fire conditions, and will not react with water, polymerize, decompose, condense, or self-react. Non-explosives").

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

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine was assigned a score of Low for flammability based on its flash point of 195.5℃ and it not being classified as a flammable liquid. GreenScreen® criteria classify chemicals as a Low hazard for flammability when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on measured data for the target substance.

- 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 2021a
  - N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine has a flash point of 195.5℃ in a GLP-compliant EU Method A.9 assay. This study is reported in the REACH dossier with a Klimisch Score of 1 (reliable without restriction).
    - *Based on the flash point of 195.5 ℃, ToxServices did not classify N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine as a flammable liquid under GHS (UN 2021).*

Use of New Approach Methodologies (NAMs)[12] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for respiratory sensitization, persistence, and bioaccumulation, *in vitro* assays for mutagenicity, and *in vitro* high throughput assays and ToxCast models for endocrine activity. 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-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine's NAMs dataset include lack of sufficient animal experimental data and human data for respiratory sensitization and limited experimental data for the persistence and bioaccumulation endpoints, as well as a lack of validated test methods for respiratory sensitization. N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine's Type II (extrapolation output) uncertainties include limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism, their focusing on one or only a few types of genotoxicity events, the *in vivo* relevance of endocrine ToxCast models for identify endocrine activity, the limitation of OECD Toolbox in identifying structural alerts for respiratory sensitization without defining the applicability domain, and not accounting for non-immunologic mechanisms of respiratory sensitization. Some of N-(1,4-dimethylpentyl)-N'-phenylbenzene-1,4-diamine'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. **Persistence:** Limited experimental data are available; a draft ASTM guideline was utilized and the GLP status was not specified. **Bioaccumulation:** No measured data identified.
Type II Uncertainty: Extrapolation Output	**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[13].

[12] 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).

[13] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427

	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).[14] The mammalian cell gene mutation assay (as defined in OECD Guideline 490) cannot reliably detect aneugens, 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)[15]. 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[16]. 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[17]. **Endocrine activity:** ToxCast models don't define applicability domain. **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.	

Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	N	
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay
Reproductive toxicity	N	

[14] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[15] https://www.oecd-ilibrary.org/docserver/9789264264908-en.pdf?expires=1622037214&id=id&accname=guest&checksum=F0669770FC98B49A32E3AFBA1A4D86F5
[16] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352
[17] https://www.oecd.org/env/ehs/testing/Guidance-Document-on-the-in-vitro-Syrian-Hamster-Embryo-Cell-Transformation-Assay.pdf

Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: ToxCast models
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	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Persistence	Y	*In silico* modeling: EPI Suite™ Non-animal testing: ASTM E35.24 Biodegradation test
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

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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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)

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	N-(1,4-Dimethylpentyl)-N'-	1/4/3081	L	L	H	M	M	L	L	M	*L*	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
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-	1

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

Table 6

Chemical Name	Final GreenScreen® Benchmark Score
N-(1,4-Dimethylpentyl)-N'-phenylbenzene-	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-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)

3081-01-4
1,4-BENZENEDIAMINE, N-(1,4-DIMETHYLPENTYL)-N'-PHENYL-(9CI)
ALSO CALLED 1,4-Benzenediamine, N1-(1,4-dimethylpentyl)-N4-phenyl-, N-(1,4-dimethylpentyl)-N'-phenyl-1,4-benzene...
View all synonyms (7)

Share Profile

Hazards | Properties | Functional Uses | 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	-	-	-	-	-	pC	-	-	-	-	pC	-	pC	-	pC	-	-	-	vH	-	-	pC	U	-	-	R

Hazard Lists

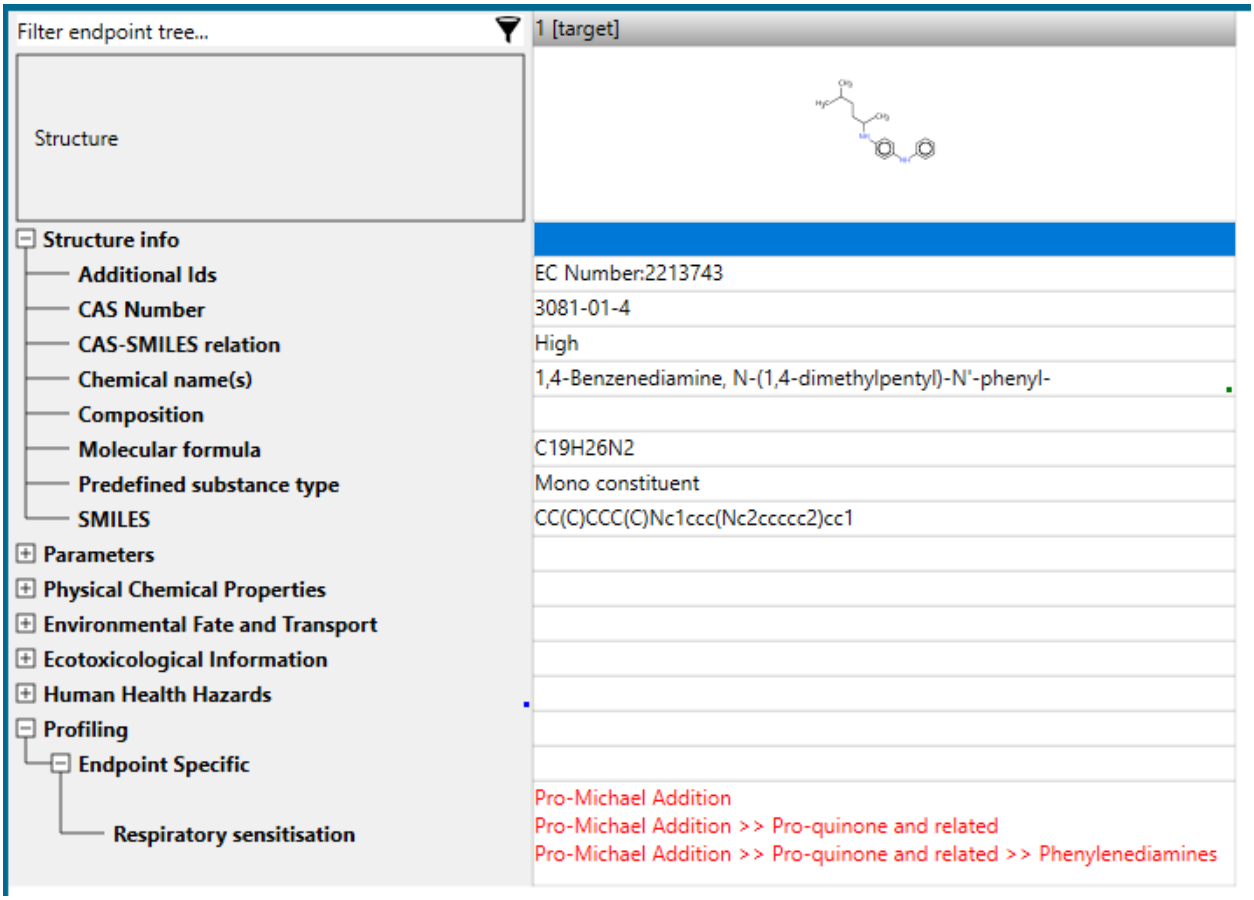
Download Lists

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Acute Mammalian Toxicity	pC	NoGS	EU - Manufacturer REACH hazard submissions	H302 - Harmful if swallowed (unverified) [Acute toxicity (oral) - Category 4]	
Skin Sensitization	pC	NoGS	DK-EPA - Danish Advisory List	Skin Sens. 1 - May cause an allergic skin reaction (modeled)	+1
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H317 - May cause an allergic skin reaction (unverified) [Skin sensitization - Category 1]	
Skin Irritation/Corrosivity	pC	NoGS	EU - Manufacturer REACH hazard submissions	H315 - Causes skin irritation (unverified) [Skin corrosion/irritation - Category 2]	
Acute Aquatic Toxicity	pC	NoGS	DK-EPA - Danish Advisory List	Aquatic Acute1 - Very toxic to aquatic life (modeled)	+2
	pC	NoGS	DK-EPA - Danish Advisory List	Aquatic Chronic1 - Very toxic to aquatic life with long lasting effects (modeled)	
	pC	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	
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	PBT - Substance of Possible Concern	
T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	pC	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]	

Restricted Substance Lists (2)

- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment
- MDH - Chemicals of High Concern and Priority Chemicals: Chemicals of High Concern

APPENDIX D: OECD Toolbox Respiratory Sensitization Results for N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)



APPENDIX E: EPI Suite™ Modeling Results for N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine (CAS #3081-01-4)

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

CAS Number: 3081-01-4
SMILES : N(c(ccc(Nc(cccc1)c1)c2)c2)C(CCC(C)C)C
CHEM : 1,4-Benzenediamine, N-(1,4-dimethylpentyl)-N -phenyl-
MOL FOR: C19 H26 N2
MOL WT : 282.43
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : 230.00
Melting Point (deg C) : 32.40
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): 0.67
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 5.17

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 381.27 (Adapted Stein & Brown method)
Melting Pt (deg C): 129.78 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 0.0633 (Modified Grain method)
VP (Pa, 25 deg C) : 8.44 (Modified Grain method)
Subcooled liquid VP: 0.0737 mm Hg (25 deg C, Mod-Grain method)
: 9.83 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 1.677
log Kow used: 5.17 (estimated)
melt pt used: 32.40 deg C

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 0.86359 mg/L

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

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 4.46E-009 atm-m3/mole (4.52E-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: 3.511E-002 atm-m3/mole (3.557E+003 Pa-m3/mole)
VP: 0.0633 mm Hg (source: MPBPVP)

WS: 0.67 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 5.17 (KowWin est)
Log Kaw used: -6.739 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 11.909
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.2737
Biowin2 (Non-Linear Model) : 0.0467
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.3271 (weeks-months)
Biowin4 (Primary Survey Model) : 3.2284 (weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : -0.1009
Biowin6 (MITI Non-Linear Model): 0.0069
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.8787
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): 9.83 Pa (0.0737 mm Hg)
Log Koa (Koawin est): 11.909
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 3.05E-007
Octanol/air (Koa) model: 0.199
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 1.1E-005
Mackay model : 2.44E-005
Octanol/air (Koa) model: 0.941

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 227.9058 E-12 cm3/molecule-sec
Half-Life = 0.047 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 0.563 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
1.77E-005 (Junge-Pankow, Mackay avg)
0.941 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 4.201E+004 L/kg (MCI method)

Log Koc: 4.623 (MCI method)
Koc : 5245 L/kg (Kow method)
Log Koc: 3.720 (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 = 3.079 (BCF = 1199 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.0901 days (HL = 1.23 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.687 (BCF = 487)
Log BAF Arnot-Gobas method (upper trophic) = 2.693 (BAF = 492.7)
log Kow used: 5.17 (estimated)

Volatilization from Water:
Henry LC: 4.46E-009 atm-m3/mole (estimated by Bond SAR Method)
Half-Life from Model River: 2.206E+005 hours (9192 days)
Half-Life from Model Lake : 2.407E+006 hours (1.003E+005 days)

Removal In Wastewater Treatment:
Total removal: 82.41 percent
Total biodegradation: 0.71 percent
Total sludge adsorption: 81.70 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0157	1.13	1000
Water	9.58	900	1000
Soil	68	1.8e+003	1000
Sediment	22.4	8.1e+003	0

Persistence Time: 1.81e+003 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0157	1.13	1000
Water	9.58	900	1000
water	(8.95)		
biota	(0.0662)		
suspended sediment	(0.564)		
Soil	68	1.8e+003	1000
Sediment	22.4	8.1e+003	0

Persistence Time: 1.81e+003 hr

Level III Fugacity Model: (EQC Default)

	Mass Amount	Half-Life	Emissions

	(percent)	(hr)	(kg/hr)
Air	0.0146	1.13	1000
Water	8.51	900	1000
water	(7.75)		
biota	(0.0573)		
suspended sediment	(0.705)		
Soil	63.5	1.8e+003	1000
Sediment	28	8.1e+003	0

Persistence Time: 1.94e+003 hr

APPENDIX F: Change in Benchmark Score

Table 6 provides a summary of changes to the GreenScreen® Benchmark™ for N-(1,4-dimethyl pentyl)-N'-phenylbenzene-1,4-diamine. There has been one round of update which did not change the Benchmark score.

Table 6: Change in GreenScreen® Benchmark™ for N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
October 21, 2021	BM-1	v. 1.4	New assessment
November 5, 2021	BM-1	v. 1.4	Hazard score for bioaccumulation is updated from Low to High due to introduction of a new surrogate

Licensed GreenScreen® Profilers

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine GreenScreen® Evaluation Prepared and Updated by:

SIGNATURE BLOCK

Rachel Doerer, M.P.H.
Toxicologist
ToxServices LLC

N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine GreenScreen® Evaluation QC'd by:

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N-(1,4-Dimethylpentyl)-N'-phenylbenzene-1,4-diamine GreenScreen® Update QC'd by:

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