CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

Reaction products of diphenylamine with nonene, branched

EC Number[1]:

CAS Number[1]:

Index Number: Not listed in Annex VI of CLP

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)
14 rue Pierre Marie Curie
F-94701 Maisons-Alfort Cedex
classification.clp@anses.fr

Version number: 2 **Date: January 2024**

[1] No EC or CAS number allocated. To be specified when allocated.

CONTENTS

Tables

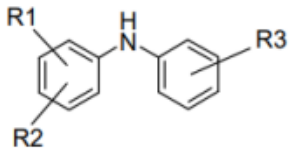
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

The present proposal for harmonised classification and labelling relates to the substance **Reaction products of diphenylamine with nonene, branched (table 1).**

The identity of the analogue substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (EC 270-128-1, CAS 68411-46-1) is reported in table 2 to assist the read across approach used in support in this CLH report. This substance is not covered by this CLH proposal.

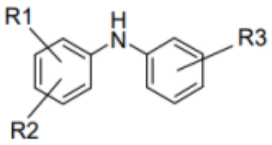
Table 1: Substance identity and information related to molecular and structural formula of the substance Reaction products of diphenylamine with nonene, branched

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Reaction products of diphenylamine with nonene, branched; Reaction products of Benzeneamine, N-phenyl- with nonene (branched); bis(nonylphenyl)amine
Other names (usual name, trade name, abbreviation)	Naugard 438L; YALUB DND
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	
EC name (if available and appropriate)	
CAS number (if available)[2]	
Other identity code (if available)	-
Molecular formula	$C_{21}H_{29}N$ - $C_{30}H_{47}N$ (main constituents)
Structural formula	 R1 = H or iso-nonyl R2 = iso-nonyl R3 = iso-nonyl
SMILES notation (if available)	-
Molecular weight or molecular weight range	≥ 295.5 ≤ 421.7
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity	Confidential information → See confidential Annex II

[2] No EC or CAS number allocated. To be specified when allocated.

of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

Table 2: Substance identity and information related to molecular and structural formula of the analogue substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (EC 270-128-1, CAS 68411-46-1).

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene; ALKLATED DIPHENYLAMINES
Other names (usual name, trade name, abbreviation)	Irganox® L 57; THANOX 5057; YALUB BODPA
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	270-128-1
EC name (if available and appropriate)	
CAS number (if available)	68411-46-1
Other identity code (if available)	-
Molecular formula	$C_{16}H_{19}N$ - $C_{28}H_{43}N$ (main constituents)
Structural formula	 R1 = H or tert-butyl or iso-octyl R2 = tert-butyl or iso-octyl R3 = tert-butyl or iso-octy
SMILES notation (if available)	-
Molecular weight or molecular weight range	≥ 225.3 ≤ 393.6
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	Confidential information → See confidential Annex II
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

1.2 Composition of the substance

The present proposal for harmonised classification and labelling relates to the substance **Reaction products of diphenylamine with nonene, branched. Information on the constituents present in the composition of this UVCB substance is provided in tables 3 and 4. There is no available information on additives.**

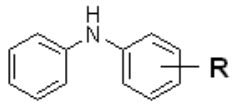
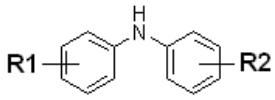
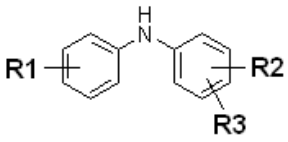
The identity of the analogue substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (EC 270-128-1, CAS 68411-46-1) will be reported is reported in tables 5 and 6 to assist the read across approach used in support in this CLH report. There is no available information on additives.

This substance is not covered by this CLH proposal.

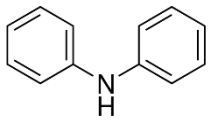
Reaction products of diphenylamine with nonene, branched

The data originate from the substance identity part of the publically disseminated lead Registration Dossier (ECHA, 2023)[3]. Two boundary compositions are presented according to the content in Diphenylamine (DPA).

Table 3: Registered boundary composition of Reaction products of diphenylamine with nonene, branched (BNPA) with 0.25% ≤ DPA < 2.5%

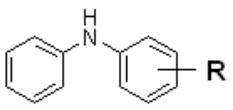
Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	Structure
N-phenyl-(ar-nonyl, branched)aniline (MNDPA) : C9DPA [$C_{21}H_{29}N$] MW = 295.5 EC no.: / CAS no.: /	Confidential information; see confidential Annex II	-	-	
N-phenyl-bis(ar-nonyl, branched)aniline (DNDPA) : C9C9DPA [$C_{30}H_{47}N$] MW = 421.7 EC no.: / CAS no.: /	Confidential information; see confidential Annex II	-	-	
N-phenyl-tris(ar-nonyl, branched)aniline (TNDPA): C9C9C9DPA [$C_{39}H_{65}N$] MW = 547.9	Confidential information; see confidential Annex II	-	-	

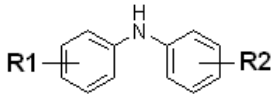
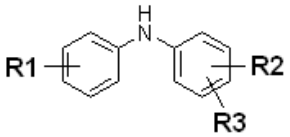
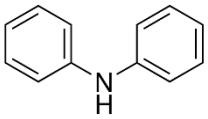
[3] Accessed on 01/02/2023

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**Structure**
EC no.: / CAS no.: /				
Other constituents [$C_{22}H_{31}N$ - $C_{29}H_{45}N$] MW = 309.5 - 407.7 EC no.: / CAS no.: /	Confidential information; see confidential Annex II	-	-	
Diphenylamine (DPA) [$C_{12}H_{11}N$] MW = 169.22 EC no.: 204-539-4 CAS no.: 122-39-4	≥ 0.25% - < 2.5% w/w	Acute Tox. 3 *, H301 Acute Tox. 3 * H311 Acute Tox. 3 * H331 STOT RE 2*, H373** Aquatic Acute 1, H400 Aquatic chronic 1, H410	Acute Tox. 3, H301 Acute Tox. 3, H311 Acute Tox. 3, H331 Eye Irrit. 2, H319 Eye Dam. 1, H318 Carc. 2, H351 STOT SE 1, H370 STOT SE 3, H335 STOT RE 2, H373 Aquatic Acute 1, H400 Aquatic chronic 1, H410	

Due to the presence of diphenylamine at a concentration range between 0.25% and 2.5%, this boundary composition is classified Aquatic Chronic 3, H412 by application of EC 1272/2008 (CLP).

Table 4: Registered boundary composition of Reaction products of diphenylamine with nonene, branched (BNPA) with DPA < 0.25%

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**Structure**
N-phenyl-(ar-nonyl, branched)aniline (MNDPA): C9DPA [$C_{21}H_{29}N$] MW = 295.5 EC no.: / CAS no.: /	Confidential information; see confidential Annex II	-	-	

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**Structure**
N-phenyl-bis(ar-nonyl, branched)aniline (DNDPA): C9C9DPA [$C_{30}H_{47}N$] MW = 421.7 EC no.: / CAS no.: /	Confidential information; see confidential Annex II	-	-	
N-phenyl-tris(ar-nonyl, branched)aniline (TNDPA): C9C9C9DPA [$C_{39}H_{65}N$] MW = 547.9 EC no.: / CAS no.: /	Confidential information; see confidential Annex II	-	-	
Constituents [$C_{22}H_{31}N$ - $C_{29}H_{45}N$] MW = 309.5 - 407.7 EC no.: / CAS no.: /	Confidential information; see confidential Annex II	-	-	
Diphenylamine (DPA) [$C_{12}H_{11}N$] MW = 169.22 EC no.: 204-539-4 CAS no.: 122-39-4	≥ 0% - < 0.25% w/w	Acute Tox. 3 *, H301 Acute Tox. 3 * H311 Acute Tox. 3 * H331 STOT RE 2*, H373** Aquatic Acute 1, H400 Aquatic chronic 1, H410	Acute Tox. 3, H301 Acute Tox. 3, H311 Acute Tox. 3, H331 Eye Irrit. 2, H319 Eye Dam. 1, H318 Carc. 2, H351 STOT SE 1, H370 STOT SE 3, H335 STOT RE 2, H373 Aquatic Acute 1, H400 Aquatic chronic 1, H410	

According to ECHA C&L inventory[4], 16 notifiers self-classify the UVCB as Aquatic chronic 3, H412 and 32 notifiers do not classify the substance.

[4] Accessed on 01/02/2023

The following self-classifications have been provided by the registrants for the Reaction products of diphenylamine with nonene, branched (according to ECHA dissemination website, 2023). This classification is included in the notifications indicated above.

For a concentration of 0.25% ≤ DPA < 2.5%:

- Aquatic chronic 3, H412

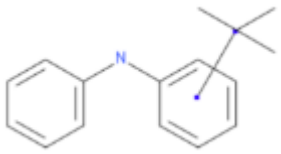
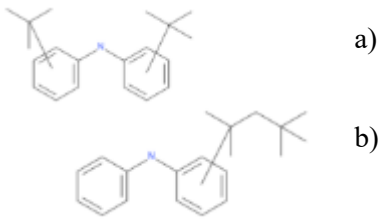
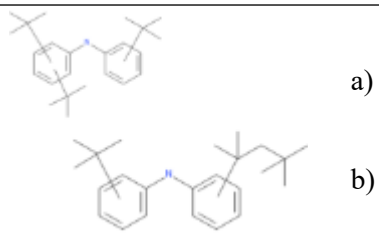
For a concentration of DPA < 0.25%:

- No classification.

Analogue substance: Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, CAS 68411-46-1, EC 270-128-1

The data originate from the substance identity part of the publically disseminated Registration Dossier (ECHA dissemination website, 2023)[5]. Two boundary compositions are presented according to the content in Diphenylamine (DPA).

Table 5: Registered boundary composition of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene with with 0.25% ≤ DPA < 2.5%

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**Structure**
N-phenyl-(ar-tert-butyl)aniline [$C_{16}H_{19}N$] Monobutyldiphenylamine: C4DPA	Confidential information; see confidential Annex II	-	-	
N-phenyl-bis(ar-tert-butyl)aniline and N-phenyl-(ar-1,1,3,3-tetramethyl-butyl)aniline [$C_{20}H_{27}N$] a) Dibutyl diphenylamine: C4C4DPA b) Monooctyl diphenylamine: C8DPA	Confidential information; see confidential Annex II	-	-	 a) b)
N-phenyl-tris(ar-tert-butyl)aniline and N-phenyl-(ar-1,1,3,3-tetramethyl-butyl)-(ar-tert-butyl)aniline [$C_{24}H_{35}N$]	Confidential information; see confidential Annex II	-	-	 a) b)

[5] Accessed on 01/02/2023

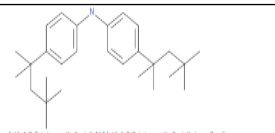
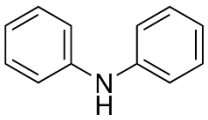
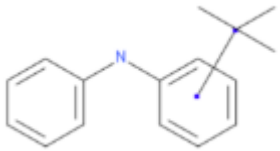
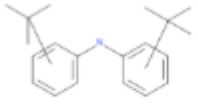
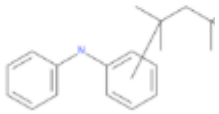
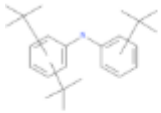
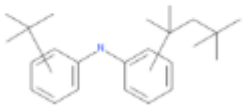
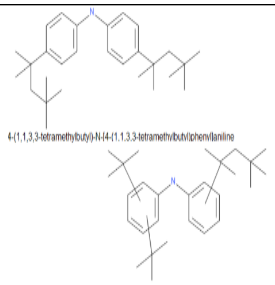
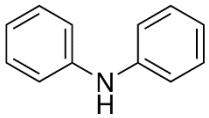
Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**Structure**
a)Tributyldiphenyl amine: C4C4C4DPA b) Monobutylmono octyldiphenyl amine: C4C8DPA				
N-phenyl-(ar-1,1,3,3-tetramethyl-butyl)-bis(ar-tert-butyl)aniline, N-phenyl-tetra(ar-tert-butyl)aniline and N-phenyl-bis(ar-1,1,3,3-tetramethyl-butyl)aniline [$C_{28}H_{43}N$] a) Dioctyldiphenyl amine: C8C8DPA b)Dibutylmonooctyldiphenylamine: C4C4C8	Confidential information; see confidential Annex II	-	-	a)  b)
Diphenylamine (DPA) [$C_{12}H_{11}N$] MW = 169.22 EC no.: 204-539-4 CAS no.: 122-39-4	≥ 0.25% - < 2.5% w/w	Acute Tox. 3 *, H301 Acute Tox. 3 * H311 Acute Tox. 3 * H331 STOT RE 2*, H373** Aquatic Acute 1, H400 Aquatic chronic 1, H410	Acute Tox. 3, H301 Acute Tox. 3, H311 Acute Tox. 3, H331 Eye Irrit. 2, H319 Eye Dam. 1, H318 Carc. 2, H351 STOT SE 1, H370 STOT SE 3, H335 STOT RE 2, H373 Aquatic Acute 1, H400 Aquatic chronic 1, H410	

Table 6: Registered boundary composition of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene with with DPA < 0.25%

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**Structure**
N-phenyl-(ar-tert-butyl)aniline [$C_{16}H_{19}N$] Monobutyldiphenylamine: C4DPA	Confidential information; see confidential Annex II	-	-	
N-phenyl-bis(ar-tert-butyl)aniline and N-phenyl-(ar-1,1,3,3-tetramethyl-butyl)aniline [$C_{20}H_{27}N$] a) Dibutyl diphenylamine: C4C4DPA b) Monooctyl diphenylamine: C8DPA	Confidential information; see confidential Annex II	-	-	 a)  b)
N-phenyl-tris(ar-tert-butyl)aniline and N-phenyl-(ar-1,1,3,3-tetramethyl-butyl)-(ar-tert-butyl)aniline [$C_{24}H_{35}N$] a)Tributyldiphenyl amine: C4C4C4DPA b) Monobutylmono octyldiphenyl amine: C4C8DPA	Confidential information; see confidential Annex II	-	-	 a)  b)
N-phenyl-(ar-1,1,3,3-tetramethyl-butyl)-bis(ar-tert-butyl)aniline, N-phenyl-tetra(ar-tert-butyl)aniline and N-phenyl-bis(ar-1,1,3,3-tetramethyl-butyl)aniline [$C_{28}H_{43}N$] a) Dioctyldiphenyl:C8C8DPA amine b)Dibutylmonooctyldiphenylamine: C4C4C8	Confidential information; see confidential Annex II	-	-	 a) b)
Diphenylamine (DPA) [$C_{12}H_{11}N$]	≥ 0% - < 0.25% w/w	Acute Tox. 3 *, H301 Acute	Acute Tox. 3, H301 Acute Tox. 3, H311	

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**Structure**
MW = 169.22 EC no.: 204-539-4 CAS no.: 122-39-4		Tox. 3 * H311 Acute Tox. 3 * H331 STOT RE 2*, H373** Aquatic Acute 1, H400 Aquatic chronic 1, H410	Acute Tox. 3, H331 Eye Irrit. 2, H319 Eye Dam. 1, H318 Carc. 2, H351 STOT SE 1, H370 STOT SE 3, H335 STOT RE 2, H373 Aquatic Acute 1, H400 Aquatic chronic 1, H410	

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 7: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Reaction products of diphenylamine with nonene, branched			Repr. 1B Aquatic Chronic 1	H360FD H410	GHS08 GHS09 Dgr	H360FD H410		M = 10	

Table 8: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	Harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification and labelling available for Reaction products of diphenylamine with nonene, branched. The substance has not been included in former activities on harmonised classification.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The available data on Reaction products of diphenylamine with nonene, branched, as presented in this report, support the following classifications :

- Repr 1B –H360FD for Substance
    - There is no requirement for justification for this hazard class.
- Aquatic chronic 1, H410
    - Reason for a need for action at Community level: disagreement of DS with current self-classification and differences in self-classification between different notifiers in the C&L Inventory are observed. There are no harmonized classification for this hazard category although data justify classification as Aquatic chronic 1, H410.

5 IDENTIFIED USES

Table 9: Summary of uses for Reaction products of diphenylamine with nonene, branched (ECHA dissemination website, 2023)

USES	
	Use(s)
Manufacture	Manufacturing of the substance.
Formulation and re-packing	Formulation of mixtures and formulation in materials. This substance is used in the following products: metal working fluids, lubricants and greases and hydraulic fluids.
Uses at industrial sites	This substance is used in the following products: lubricants and greases, metal working fluids, hydraulic fluids and heat transfer fluids. This substance is used for the manufacture of plastic products. Use in processing aids at industrial sites and of substances in closed systems with minimal release and indoor use as processing aid.
Uses by professional workers	This substance is used in the following products: lubricants and greases, metal working fluids, hydraulic fluids and heat transfer fluids. Indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters), outdoor use in close systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in

	motor oil and break fluids) and outdoor use.
Consumer uses	This substance is used in the following products: lubricants and greases. Indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters), outdoor use in close systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids) and outdoor use as processing aid.
Article service life	Outdoor use in long-life materials with low release rate (e.g. metal, wooden and plastic construction and building materials) and indoor use in long-life materials with low release rate (e.g. flooring, furniture, toys, construction materials, curtains, foot-wear, leather products, paper and cardboard products, electronic equipment). This substance can be found in products with material based on plastic.

6 DATA SOURCES

All the data available in the registration dossier (Last modification on IUCLID taken in account : 01/12/2022) and study reports provided by the registrants have been used in the CLH report.
A bibliographic search was performed based on Pubmed, Scopus and Toxcast databases from March 2021 to February 2023.

For toxicokinetics and health hazards, a generic search was carried out with the terms: “Reaction products of diphenylamine with nonene, branched OR bis(nonylphenyl)amine”, “alkyldiphenylamines” and “substituted diphenylamines” without targeted endpoints. The date of the requests was 05-09-2022 with no limit of the timeframe. Considering the paucity of retrieve articles and in the absence of any relevant ones for toxicity on reproduction no further focussed search was performed.

For environmental hazards, a generic search was performed with the following keywords: “Reaction products of diphenylamine with nonene, branched OR bis(nonylphenyl)amine”, “alkyldiphenylamines”, “substituted diphenylamines”, SDPA, SDPAs, CAS 27177-41-9, EC 701-385-4.

7 PHYSICOCHEMICAL PROPERTIES

Table 10: Summary of physicochemical properties for diphenylamine with nonene, branched

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Slightly yellow liquid (whole substance)	ECHA dissemination website, 2023	
Melting/freezing point	Glass-transition temperature at - 43 °C at 101.3 kPa. (whole substance)	ECHA dissemination website, 2023	Melting point was determined according to OECD 102 using the Differential Scanning Calorimetry method
Boiling point	No boiling found up to	ECHA dissemination	Boiling point was determined

Property	Value	Reference	Comment (e.g. measured or estimated)
	300°C (whole substance)	website, 2023	according to method OECD 103 using the method of Siwoloboff.
Relative density	970 kg/m^3 at 20 °C (whole substance)	ECHA dissemination website, 2023	Density was determined according to OECD 109 using the oscillating densitometer method.
Vapour pressure	Calculated <0.0001 Pa at 25°C (for the whole substance based on the vapour pressure of individual representative components of test substance: Di-alkyl-diphenylamine isomer and Mono-nonyl-diphenylamine isomer generated by calculation)	ECHA dissemination website, 2023	The vapour pressure was measured and extrapolated using measured values by modified static method. The extrapolated value has been considered to be inaccurate as the measurement was carried out at high temperature 170°C. Thus, the vapour pressure was additionally calculated using recommended program SPARC v4.6. The calculation have been generated on the individual representative components of test substance and resulted as follows: 1.7 E-8 Pa for Di-alkyl-diphenylamine isomer 6.8 E-5 Pa for Mono-nonyl-diphenylamine isomer It can be suggested that the vapour pressure of test substance was calculated to be <0.0001 Pa at 25°C.
Surface tension	-	ECHA dissemination website, 2023	Based on structure, surface activity is not expected and the water solubility is below 1mg/L at 20°C respectively
Water solubility	<5 µg/L at 20 °C at pH 6.1 (whole substance)	ECHA dissemination website, 2023	Water solubility was determined according to OECD 105 using the column elution method.
Partition coefficient n-octanol/water	Log Pow ≥7.5 at 25 °C for the substance (whole substance) LogPow = 11.87 for the substance Di-nonyl-diphenylamine (C9C9DPA) LogPow = 7.58 for the substance Mono-nonyl-diphenylamine (C9DPA)	ECHA dissemination website, 2023	Since the substance is an UVCB, partition coefficient was calculated with program KOWWIN based on the molecule's structure.
Flash point	> 200 °C at 999 mBar. (whole substance)	ECHA dissemination website, 2023	Flash point was determined in accordance with the test method ISO 22719.
Flammability	Non flammable	ECHA dissemination	The flammability of a liquid is

Property	Value	Reference	Comment (e.g. measured or estimated)
	(whole substance)	website, 2023	derived from flash point and was determined to be greater than 200°C.
Explosive properties	Not explosive (whole substance)	ECHA dissemination website, 2023	There are no chemical groups associated with explosive properties present in the molecule
Self-ignition temperature	490°C at 1013 hPa. (whole substance)	ECHA dissemination website, 2023	Auto-ignition termperature was determined in accordance with the test method EU Method A.15 (Auto-Ignition Temperature (Liquids and Gases).
Oxidising properties	No oxidising properties (whole substance)	ECHA dissemination website, 2023	The substance is an organic chemical which does not contain oxygen and halogen atoms.
Granulometry	-	ECHA dissemination website, 2023	The substance is marketed or used in a non solid form.
Stability in organic solvents and identity of relevant degradation products	-	ECHA dissemination website, 2023	The stability of the substance is not considered to be critical.
Dissociation constant	-	ECHA dissemination website, 2023	The substance is insoluble in water.
Viscosity	4 980 mPa · s (dynamic) at 20 °C (whole substance)	ECHA dissemination website, 2023	The measurement was carried out by rotational viscometer.

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this CLH-proposal.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No experimental toxicokinetic data regarding the absorption, distribution, metabolism and excretion of the substance “Reaction products of diphenylamine with nonene, branched” or its constituents are available. No experimental toxicokinetic data is neither available on the analogue “Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene” or its constituents.

Oral exposure

Reaction products of diphenylamine with nonene, branched caused systemic toxicity upon oral application in various repeated dose toxicity studies demonstrating its bioavailability.

Reaction products of diphenylamine with nonene, branched is a substituted diphenylamine (SDPA). It is an UVCB substances consisting in a mixture of mono-, di- and tri-nonylated diphenylamines.

In an OECD report dedicated to SDPAs (OECD, 2016a), water solubility and LogKow of the different constituents have been modelled using Epi Suite V4.11 - WSKOWWIN v1.42 and EPI Suite v4.11 - KOWWIN v1.68 respectivelly (Table 11). The estimated LogKow values of the constituents increase with chain length and number of substitutions.

In this OECD report, toxicokinetic predictions were also generated for representative structures using ACD Percepta PK Explorer (ACD 2012) in order to compare kinetic parameters for the members of the subgroups. The estimated bioavailability of the constituents is positively correlated to their water solubility and negatively correlated with their molecular weight, LogKow and bulkiness (Table 11). It is noteworthy that the PK Explorer model does not provide reliability or applicability domain information and the LogKow observed for the training set may not cover some of the most lipophilic SDPAs. As a consequence, OECD concluded that the reliability in the quantitative values generated was considered low for the model results but the results were useful for a comparative analysis (OECD, 2016a). In the case of Reaction products of diphenylamine with nonene, the estimated bioavailability of the constituent MonononylDPA is far higher than the estimated ones of the other constituents.

Table 11: Physicochemical properties and toxicokinetic parameters – modelled ACD Percepta 2012 (OECD, 2016a) of the different constituents of Reaction products of diphenylamine with nonene, branched.

UVCB	Constituents	PM	LogKow	Water solubility	* Oral bioavailability(%F)	Cmax*	Tmax*	AUC_{0-inf}*
				mg/L		µg/ml	h	µg.h/ml
Reaction products of diphenylamine with nonene ? R = highly branched nonyl-	Monononyl DPA	295.5	7.6	$4.7\ 10^{-3}$	21.53	0.13	6.26	1.62
	Dinonyl DPA	421.7	11.9	$1.6\ 10^{-7}$	0.06	0.0002	9.38	0.006
	Trinonyl DPA	547.9	16.2	$5.5\ 10^{-9}$	no data	no data	no data	no data
	Constituents C22H31N - C29H45N	309.5-407.7	no data	no data	no data	no data	no data	no data

* Each SDPA was modelled using an oral dose of 5 mg/kg bw (70kg human) with Percepta PK Explorer (OECD, 2016a)

Metabolism

In the OECD report on SDPA, a metabolic simulator (OASIS TIMES (v2.27.5) in vivo Rat Metabolism Simulator (v5.05)) was used to predict the metabolism of individual representative SDPA which gave the following results:

- Both DPA ring-hydroxylation and side-chain hydroxylation are predicted.
- Oxidative metabolism at the secondary amine is not predicted for any of the SDPAs.
- The mono alkylated SDPAs are predicted to undergo aromatic C-hydroxylation at the unoccupied para position to the amine. Subsequent quinone imine formation is predicted followed by imine hydrolysis to liberate a primary aromatic amine. The model indicates that both the probability and reliability of this occurrence is low due to other competing and more probable metabolic transformations.
- Coupling in phase II metabolism would result in more soluble metabolites that could be eliminated both via bile and kidney.

All SDPAs were considered within the property domain of the model but all were out of domain when atom centered fragments were considered. As a result, the confidence in the predictions was considered low by OECD.

Regarding oxidative metabolism at the secondary amine, contrary to OASIS estimation, the Registrants mentioned in the CSR that reversible oxidation at the nitrogen is part of the technical function as an antioxidant in engine oils. The degree of alkylation has no relevance for non-enzymatic antioxidant mechanisms.

Additional considerations

The metabolome profile in plasma of fasted Wistar rats treated with Reaction products of diphenylamine with nonene, branched (300 and 1000 mg/kg bw/day) for 28 days was investigated and compared to the metabolome of Wistar rats trated with treated with the anaologue SDPA Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (125 and 300 mg/kg bw/day) (Unpublished Report, 2014a, **- Study 8**). The plasma metabolome (225 endogenous plasma components such as amino acids, hormones, sugar, fatty acids, etc) was investigated with regard to changes relative to the control group. It was found that a majority of metabolome changes was similar for both compounds in terms of significance and direction of change. Applying a Pearson-based statistical correlation of the whole plasma metabolome, these two SDPAs were the most similar compounds in terms of metabolome changes out of a data base consisting of more than 750 substances. Results showed that mainly lipid metabolism was affected with increased complex lipids, fatty acids and derivatives. However, the substances did not show matches (with those of compounds present in MetaMap®Tox 1) which would give a clear indication for a certain toxicological mode of action.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not evaluated in this CLH proposal.

10.2 Acute toxicity - dermal route

Not evaluated in this CLH proposal.

10.3 Acute toxicity - inhalation route

Not evaluated in this CLH proposal.

10.4 Skin corrosion/irritation

Not evaluated in this CLH proposal.

10.5 Serious eye damage/eye irritation

Not evaluated in this CLH proposal.

10.6 Respiratory sensitisation

Not evaluated in this CLH proposal.

10.7 Skin sensitisation

Not evaluated in this CLH proposal.

10.8 Germ cell mutagenicity

Not evaluated in this CLH proposal.

10.9 Carcinogenicity

Not evaluated in this CLH proposal.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Reaction products of diphenylamine with nonene, branched as well as Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are SDPAs. This group has been studied as a case study for grouping and read-across by OECD (2016a), by Health Canada (ECCC, 2017) and in collaborative approach (COLLA) pilot project (ECHA, 2018). The read-across approach for reproductive toxicity has been evaluated by the DS **(refer to section 10.10.11)** in line with the ECHA Read-Across Assessment Framework (RAAF) (ECHA, 2017a) and is considered acceptable with high confidence from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) to Reaction products of diphenylamine with nonene, branched (target substance).

Table 12: Summary table of animal studies on adverse effects on sexual function and fertility.

All reported changes are statistically significant, unless otherwise specified (in italic).

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference, Reliability
Study with Reaction products of diphenylamine with nonene, branched			
Reproduction/Developmental Toxicity Screening Test **OECD TG 421 (2016)** GLP-compliant Rat Wistar Crl:Wl(Han) N = 10/sex/dose group **10-week premating period** ***Deviations:*** *Additional investigations: sperm and spermatid examinations, determination of organ weights of brain, heart, kidneys, liver, spleen and thymus, several organ or tissue fixations, and histopathology of liver.* ***NB:*** *Thyroid histopathology not performed (optional in OECD TG 421)* *Thyroid hormones measured in adult M only and PND13 M and F pups in line with OECD*	**Reaction products of diphenylamine with nonene, branched** EC: [6] *CAS:- (old 36878-20-3)* Batch 00 16046440 Purity: UVCB **By diet** **Diet :** 0, 500, 1500 and 5000 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance Eq. to: 0, 40/44, 122/133 and 407/443 mg/kg bw/d in M/F Recovery group : 0, 5000 ppm P0: **10-week premating period** F1: terminated at	**Effects on sexual function and fertility P0** **5000 ppm (407/443 mg/kg bw/d)** *Non-statistically significant ↑estrous cycles length (4.3 days vs. control 4.0 days) and ↑ of mean nb of days in diestrous stage.* ↓ nb of implantation sites (-31%) and consequently ↓ nb of pups delivered (-31%). ↓ ovaries absolute (-40%) and relative (-31%) weights. Recovery group: ↓ ovaries absolute weight (-14%). **1500 ppm (122/133 mg/kg bw/d)** ↓ nb of implantation sites (-24%) and consequently ↓ nb of pups delivered (-19%). ↓ ovaries absolute (-18%) weight. **500 ppm (40/44 mg/kg bw/d)** No effect. **General/systemic toxicity P0** **5000 ppm (407/443 mg/kg bw/d)** ↓ Final body weight (BW) in M (-12%) and ↓ BW in F at the end of the premating period (-11%), at the end of gestation (-19%), at the beginning of lactation (-18%) and termination (-17%) compared to controls. ↓ Food consumption in F from study day 7 onwards (-12% during the premating period, -20% during gestation ad -24% during lactation). Recovery group: ↓ BW weight in M (-13%) and F (-17%) and ↓ food consumption in F (-16%). Liver effects: ↑ rel liver weight in M (+27%) and F (+25%), ↑ centrilobular hypertrophy (minimal to moderate) in all M&F. Fatty changes (minimal to moderate) midzonal/macrovesicular in 7/10 M and periportal/microvesicular in 7/10 F. Single cell necrosis (minimal) in 7/10 M. Associated with biochemical signs of liver damage (↑alkaline phosphatase (ALP) activities in M&F, ↓ albumin (ALB), ↓ total proteins (TP), ↑ triglycerides (TRIG) ↑ cholesterol	Unpublished study report, 2020a **Key study for adverse effects on sexual function and fertility** **Bridging study** **Reliability:** 1, reliable without restriction Details available under "Study 1" in Annex I (3.10.1.1)

[6] No EC or CAS number allocated. To be specified when allocated.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference, Reliability
TG 421.	PND14 Recovery group (not mated): 10-week exposure + 2-week recovery period	(CHOL) ↑γ-glutamyl transferase (GGT) activities in F. ↑alanine aminotransferase (ALT) and ↓ total bilirubin (TBIL) in M. Recovery group: Centrilobular hypertrophy in both sexes and the single cell necrosis/apoptosis in M completely regressed after the recovery period. Minimal fatty changes in 3/10 M and 3/10 F persisted as well as some biochemistry changes. Thyroid effects: ↑ relative thyroid weight in M (+17%) no histopathological examination performed; *Non-statistically significant ↑ TSH in M (+45%).* **F not investigated.** **1500 ppm (122/133 mg/kg bw/d)** ↓ BW in F compared to controls at the end of gestation (- 9%), *at the beginning of lactation (-6%)* and at termination (-7%). No effect on BW during the premating period. Liver effects: ↑ relative liver weight in M (+12%) and F (+12%), ↑ centrilobular hypertrophy in 5/10 M (minimal to slight) and 3/10 F (minimal), single cell necrosis (minimal) in 7/10 M. Biochemistry: ↓ALB , ↓TP, ↑ TRIG in F. ↓TBIL in M. Thyroid effects: *Non-statistically significant ↑ TSH in M (+44%).* **500 ppm (40/44 mg/kg bw/d)** No effect on BW. Liver effect limited to centrilobular hypertrophy (minimal) in 3/10 M. In F: ↓ALB (-4%), *non-statistically significant ↑ of TRIG (+140%) and ALP (+104%).* Thyroid effects: *Non-statistically significant ↑ TSH in M (+9.5%).* **F1 pups** **5000 ppm (407/443 mg/kg bw/d)** ↓ mean terminal pup BW on PND13 (-18.8 % both sexes combined) compared to controls. ↓ mean pup BW changes PND 4-13 (up to -22.5% both sexes combined) compared to controls. **1500 ppm (122/133 mg/kg bw/d) and 500 ppm (40/44 mg/kg bw/d)** No effect.	
Studies with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene			
Reproduction/Developmental Toxicity Screening Test **OECD TG 421 (2016)**	**Benzenamine, N-phenyl-, reaction products with 2,4,4-**	**Effects on sexual function and fertility P0** **3000 ppm (260/271 mg/kg bw/d)** ↑estrous cycles length (4.7 days vs. control 4.0 days). ↑ mean nb of days in diestrous stage.	Unpublished study report, 2020b

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference, Reliability
GLP-compliant Rat Wistar Crl:Wl(Han) N = 10/sex/dose group **10-week premating period** ***Deviations:*** *Only 6 F pregnant in the low dose group while 8 per group is the minimum acceptable number according to the TG.* *Additional investigations: sperm and spermatid examinations, determination of organ weights of brain, heart, kidneys, liver, spleen and thymus, several organ or tissue fixations, and histopathology of liver.* ***NB:*** *Thyroid histopathology performed in M and F (optional in OECD TG 421).* *Thyroid hormones measured in adult M and F (optional) and PND13 M and F pups.*	**trimethylpentene** CAS: 68411-46-1 Batch: 50116118D Purity: UVCB **By diet** **Diet :** 0, 300, 1000 and 3000 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance Eq. to: 0, 26/28, 87/95 and 260/271 mg/kg bw/d in M/F F0: **10-week premating period** F1: terminated at PND13	↓ nb of implantation sites (-36%) and consequently ↓ nb of pups delivered (-34%). ↓ ovaries absolute weight (-25%). **1000 ppm (87/95 mg/kg bw/d)** *Non statistically significant ↓ nb of implantation sites (-14%).* **300 ppm (26/28 mg/kg bw/d)** No effect. **<u>General/systemic toxicity P0</u>** **3000 ppm (260/271 mg/kg bw/d)** ↓ Food consumption in F during gestation and lactation periods (-23% and -20% respectively). ↓food consumption in M on study days 0 to 14 (up to -8.8%) and on study days 42 to 49 (-22.5%). ↓ Final BW in M (-9%) and ↓ BW in F at the end of the premating period (-9%), at the end of gestation (-17%), at the beginning of lactation (-9%) and at termination PND14 (-15%) compared to controls. <u>Liver effects:</u> ↑ abs/rel liver weights in M (+28%/43%) and rel weight in F (+30%),↑ centrilobular hypertrophy (minimal to moderate) in all M and F, fatty changes (minimal to mild) periportal in 6/10 M and in 5/10 F, focal necrosis (minimal) in 1/10 male. Biochemistry: ↑ALP, and TRIG, ↓ALB and total bile acid values in M&F. ↑ CHOL and GGT in F ↓ TBIL in M. <u>Thyroid effects</u>: ↑ rel thyroid weight in M (+ 32%). Hypertrophy/hyperplasia of follicular cells of 9/10 M (minimal to moderate) and of 6/10 F (minimal to mild) in combination with altered colloid. ↓ T4 (-26%) and ↑ TSH (+136%) in M. **1000 ppm (87/95 mg/kg bw/d)** ↓ Food consumption in F during gestation period (-12%). ↓ BW in F at the end of the premating period (-7%), at the end of gestation (-8%), *at the beginning of lactation (-4%)* and at termination PND14 (-8%). <u>Liver effects:</u> ↑ relative liver weight in M (+14%) and F (+16%), ↑ centrilobular hypertrophy (minimal to mild) in all M and 8 F (minimal), fatty changes (minimal) midzonal in 4/10 M, single cell necrosis/apoptosis in 6/10 M (minimal), focal necrosis (minimal) in 1/10 M. Biochemistry: ↑ ALP and ↓ total bile acid values in M&F. ↑ TRIG and in F. <u>Thyroid effects</u>: Hypertrophy/hyperplasia of follicular cells of 4/10 M and of 2/10 F (minimal) in combination with altered colloid. ↓ T4 (-11%) in M. **300 ppm (26/28 mg/kg bw/d)**	**Bridging study** **Reliability:** Reliability: 1, reliable without restriction supporting study Details available under "Study 2" in Annex I (3.10.1.2)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference, Reliability
		Liver effects: ↑ centrilobular hypertrophy (minimal to mild) in 2/10 M and single cell necrosis/apoptosis in 2/10 M (minimal). Biochemistry: ↑ALP in F. Thyroid effects: Hypertrophy/hyperplasia of follicular cells of 3/10 M and of 2/10 F (minimal to mild) in combination with altered colloid. **F1 pups** **3000 ppm (260/271 mg/kg bw/d)** ↓ mean terminal pup BW on PND13 (-26% both sexes combined) and ↓ mean pup BW changes on PND 1-13 (-32% both sexes combined) compared to controls. ↑incidence of nipple development (100% vs. 79.6% in control) and number of nipples per animal (5.2 to control 2.5) on PND13. **1000 ppm (87/95 mg/kg bw/d)** ↓ mean terminal pup BW on PND13 (-8% both sexes combined) and ↓ mean pup BW changes on PND 1-13 (-10% both sexes combined) compared to controls. **300 ppm (26/28 mg/kg bw/d)** No effect.	
EOGRTS **OECD TG 443 (2018)** GLP-compliant Rat Wistar Crl:Wl(Han) P0: 25/sex/dose F1C1A: 20/sex/dose F1C1B (= P1): 25/sex/dose, F1C2A: 10/sex/dose F1C2B: 10/sex/dose **10-week premating period**	**Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** CAS: 68411-46-1 Batch: 50116118D Purity: UVCB **By diet** **Diet :** 0, 200, 600 and 1800 ppm ; half dose during lactation phase to maintain dams at desired target dose of the	**Effects on sexual function and fertility P0, P1(F1C1B) and F1C1A** **1800 ppm (167/166 mg/kg bw/d)** P1:↑estrous cycles length (4.3 days vs. control 4.0 days). ↑ mean nb of days in diestrous stage. No effect in P0 and F1C1A females. ↓ nb of implantation sites (P0: -15%, P1: -17%) and consequently ↓ nb of pups delivered (P0:-20%, P1:-18%). ↓ Ovaries absolute weight (P0: - 13%, P1: -12%). No effect on ovary weight in F1C1A females. **600 ppm (54 mg/kg bw/d)** P1 *non-statistically significant ↓ nb of implantation sites (-9%)* and consequently ↓ nb of pups delivered (-10%). **200 ppm (18 mg/kg bw/d)** No effect. **General/systemic toxicity P0, P1(F1C1B), F1C1A and F2C2A** No mortality or clinical signs attributed to the test substance at any dose levels in any generations.	Unpublished study report, 2021 **Key study for adverse effects on sexual function and fertility** **Reliability:** 1, reliable without restriction **2 (Reliable with restriction for some DNT parameters)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference, Reliability
Deviations: **Cohort F1C1B: No histopathology performed** Since suspected to be ED, histopathology of cohort 1B should have been performed. Since liver and thyroid are identified as target organs they should also have been analysed **F2 pups:** Thyroid hormones not measured **DNT:** No historical control data (HCD) no positive control, statistical analysis not appropriate for motor activity, auditory startle response and morphometrics.	test substance Eq. to: 0, 18, 54 and 167/166 mg/kg bw/d in M/F	**1800 ppm (167/166 mg/kg bw/d)** ↓ Food consumption in P0 F during gestation and lactation periods (-13%) and in P1 F during the premating, gestation and lactation periods (-7%, -14% and -17% respectively). No effect on other treated animals. No effect on BW in P0 M. ↓ final BW compared to controls in P1 M (-6%), F1C1A M (-8%) and F1C2A M (-8%). ↓ BW compared to controls in P0 F (-8%, -12%, -11% and -9% at the end of the premating period, at the end of gestation and at the beginning and end of lactation respectively) and in P1 F (-9%, -13%, 14% and -12% at the end of the premating period, at the end of gestation, at the beginning and end of lactation respectively). ↓ Final BW compared to controls in F1C1A F (-6%). Liver effects: **P0:** ↑ absolute/relative liver weight in M (115%/119%) and F (123%/136%). Slight to moderate hepatocellular centrilobular hypertrophy in 20 M with minimal to extreme fatty change in 14 M. Slight to moderate diffuse hepatocellular hypertrophy in 6 F and minimal to moderate centrilobular hypertrophy in 12 F. ↑ ALP, ↑ TRIG and ↓ ALB in both sexes. ↑ALT and ↓ TP in M. ↑GGT, globulin (GLOB) and cholesterol (CHOL) values in F. **P1**: ↑abs/rel liver weight in M (-/114%) and F (119%/132%). **F1C1A:** ↑ abs/rel liver weight F (121%/131%). Mild to moderate hepatocellular centrilobular hypertrophy in 20 M with minimal to mild fatty change in 10 M. Minimal to moderate centrilobular hypertrophy in 18 F. ↑ ALP, ↓ ALB and TBIL in both sexes. ↓ TP and GLOB in M. ↑ GGT, GLOB, CHOL and TRIG in F. Thyroid effects: **P0:** ↑ abs/rel thyroid weight in P0 M (124%/127%) and rel thyroid weight in P0 F (121%). Hypertrophy/hyperplasia of follicular cells of 15 M (minimal to moderate) and 12 F (minimal to slight) in combination with altered colloid. ↓ T4 and *non-statistically significant* ↑ *TSH* in M. ↑ TSH (+90%) in F. **F1C1A:** ↑ abs/rel thyroid weight in P1 F (113%/122%). Hypertrophy/hyperplasia of follicular cells of 7 M (minimal to slight) and 8 F (minimal to slight) in combination with altered colloid. ↓ T4 *and non-statistically significant* ↑ *TSH* in M. ↑ TSH in F. Haematology: In P0 and F1C1A M&F slight (<5%) ↓haemoglobin and haematocrit values. **600 ppm (54 mg/kg bw/d)** No significant effect on BW and food consumption in any generations. Liver effects: **P0**: ↑ rel liver weight in P0 M (108%) and ↑ abs/rel liver weight in P0 F (107%/108%). Minimal to slight hepatocellular centrilobular hypertrophy in 20 M with minimal to moderate fatty change in 19 M. Minimal to slight centrilobular hypertrophy in 5 F. ↑ ALP and TRIG in both sexes. ↓ ALB in F. **F1C1A:** ↑ rel liver weight in F (106%). Minimal to mild hepatocellular centrilobular hypertrophy in 19 M with minimal to moderate fatty change in 7 M. Minimal centrilobular hypertrophy in 4 F. ↑ ALP and ↓ ALB in both sexes. ↓ TP and GLOB in M. Thyroid effects: **P0:** ↑ abs/rel thyroid weight in P0 M (124%/127%) and rel thyroid weight in P0 F (121%). Hypertrophy/hyperplasia of follicular cells of 10 M (minimal) and 7 F (minimal) in combination with altered colloid. ↓ T4 and *non-statistically significant* ↑ *TSH* in M. ↑ TSH in F.	Details available under "Study 3" in Annex I (3.10.1.3)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference, Reliability
		F1C1A: ↑ abs/rel thyroid weight in P1 F (113%/114%). Hypertrophy/hyperplasia of follicular cells (minimal) in 2 M and 3 F. ↓ T4 in M&F. ↑ TSH in F. **200 ppm (18 mg/kg bw/d)** Liver effects: **P0**: Minimal hepatocellular centrilobular hypertrophy in 4 M and 3 F. ↑ ALP (+26%/+67%) in M/F and ↓ ALB (-7%) in F. **F1C1A:** Minimal hepatocellular centrilobular hypertrophy in 2 M. ↑ ALP (+43%/+24%) in M/F and ↓ ALB (-5%). Thyroid effects: **P0:** Minimal follicular cell hypertrophy/hyperplasia in the thyroid of 7 M and 2 F. ↓ T4 and *non-statistically significant ↑ TSH* in M. ↑ TSH in F. **F1C1A:** Minimal follicular cell hypertrophy/hyperplasia in the thyroid of 4 M. **F1 and F2 pups** **1800 ppm (167/166 mg/kg bw/d)** ↓ Mean number of F1 and F2 pups delivered/dam (consequence of the lower nb of implants). No effect on live birth, viability and lactation indices. ↓ F1 and F2 pups BW PND7-PND21 in M, F and M&F combined (at weaning: -12.5% F1, -16% F2) compared to controls. No effect on sex ratio, anogenital distance in F1 or F2 pups. Nipple retentions: F1: at PND20, 2 pups from the same litter with 2 nipple/areola anlagen (vs 0 in controls and HCD). F2: ↑ mean nipple number at PND13 (No persistence: 0 nipple/areola at PND20). In F1 delay to reach preputial separation (43.5 vs 42.1 days in control) and vaginal opening (31.8 vs 31.0 days in control). Considered secondary to delayed general development (weight at puberty onset similar in all groups) Thyroid hormones measurements: F1PND4: only 2 animals (due to smaller litter sizes). PND22: ↑ TSH in F1 pups (M&F). **600 ppm (54 mg/kg bw/d)** ↓ Mean number F2 pups delivered/dam (consequence of the lower nb of implants). ↓ F1 pups BW PND7-PND21 M&F combined (at weaning: -5.4%) compared to controls. Thyroid hormones measurements: F1PND4: *non-statistically significant ↓ T4 F1 pups (M&F)*. PND22: ↑ TSH in F1 pups (M&F). **200 ppm (18 mg/kg bw/d)** No effect. **Developmental neurotoxicity (DNT)**	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference, Reliability
		1800 ppm (167/166 mg/kg bw/d) Axonal degeneration in cohort 2A M (thoracic spinal cord and tibial nerve) and 2A F (lumbar spinal cord and sciatic nerve), ↑corpus callosum width in 2A M&F (MD and LD not performed) and slight ↓ brain length 2A M. ASR: ↓mean maximal amplitude in M (-19%) an ↓ *habituation in M (-57%) and M & F combined (-46%) statistical analysis not performed.* **600 ppm (54 mg/kg bw/d)** ASR: ↓mean maximal amplitude in M (-12%) and ↓ *habituation in M (-40%) and M & F combined (-42%) statistical analysis not performed.* **200 ppm (18 mg/kg bw/d)** No effect.	
Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test **OECD TG 422 (1996)** GLP-compliant Rat Wistar Crl:Wl(Han) N = 10/sex/dose group **2-week premating period** **Several deviations compared to current OECD TG 422 (2016):** Pups terminated before PND 13, AGD and nipple retention not investigated. Estrous cycle not monitored. ***NB**:* *In P0: TSH, T3 and T4 measured in 5 animal/sex/dose* *Thyroid histopathology performed in M and F.*	**Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** CAS: 68411-46-1 Batch: 40401913D Purity: UVCB **By gavage** Doses: 0, 25, 75 and 225 mg/kg bw/day Vehicle: corn oil Exposure duration: P0: 28 days (M), 53 days (F) F1: terminated at PND5-7	**Effects on sexual function and fertility P0** **225 mg/kg bw/d** *Non-statistically significant ↓ of mean number of implantation sites (-16%).* No effect at **75 mg/kg bw/d** and **25 mg/kg bw/d**. **General/systemic toxicity P0** No effects on clinical signs and BW at any dose levels. **225 mg/kg bw/d** Liver effects: ↑ relative liver weight in M (+54%), hepatocellular hypertrophy in 8/8 M (2 minimal, 6 mild) and 6/6 F (2 minimal, 4 mild), hepatocellular vacuolation of zone 1 (periportal) and/or zone 2 (midzonal) in 8/8 M (3 minimal, 5 mild) and 6/6 F (3 minimal, 1 mild, 2 moderate). Biochemistry: ↑ of ALP (M&F), TBIL (M&F), CHOL (F) and ↓ ALB (M&F), TP (F), bile acids (M) and inorganic phosphate (M&F). Thyroid effects: Follicular cell hypertrophy in 5/5 M (minimal). ↑TSH and ↓T4 for all groups (both sexes, not always statistically significant and no clear dose-response relationship). **75 mg/kg bw/d** Liver effects: ↑ relative liver weight in M (+24%) and F (+17%), hepatocellular hypertrophy in 6/6 M (6 minimal) and 1/5 F (1 minimal), Hepatocellular vacuolation of zone 1 (periportal) and/or zone 2 (midzonal) of the liver was noted in 2/5 F (2 minimal). Biochemistry: ↑ of ALP (F), TBIL (M&F), CHOL (F) and ↓ ALB (M&F), TP (F) and bile acids (M). Thyroid effects: Follicular cell hypertrophy in 5/5 M (2 minimal, 3 mild) bw/d. ↑TSH and ↓T4 for all groups (both	Unpublished study report, 2014b **Reliability:** 1, reliable without restriction **Supporting study** (screening) Details available under "Study 4" in Annex I (3.10.1.4)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference, Reliability
Thyroid hormones measured in adult M and F.		sexes, not always statistically significant and no clear dose-response relationship). **25 mg/kg bw/d** Liver effects: ↑ rel liver weight in M (+20%) no correlated histopathological and biochemical findings. Thyroid effects: Follicular cell hypertrophy in 4/5 M (2 minimal, 2 mild), compared to 1/5 M (minimal) of the control group. ↑TSH and ↓T4 for all groups (both sexes, sexes, *not always statistically significant* and no clear dose-response relationship). **F1 pups** **225 mg/kg bw/d** ↓ nb of pups delivered/live born pups (-28%) *as a consequence of non-statistically significant ↓ of mean number of implantation sites (-16%) and ↑ post-implantation loss (12.7% vs 0% in controls).* ↑ in postnatal loss (8 in 3 litters versus 0 in controls) and correspondingly↓ viability index (88.7% vs 100% in controls). No effect at **75 mg/kg bw/d and 25 mg/kg bw/d.**	

Table 13: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD TG 408 (1998)	**Reaction products of diphenylamine with nonene, branched**	Rat (Wistar) male/female oral: gavage (daily) 10/sex/dose Gavage : 0, 100, 300, 1000 mg/kg/d GLP compliant	No effect on weight or histopathological findings in reproductive organs at any dose levels. Target organs: Liver and thyroid	Unpublished study report, 2013, ECHA Dissemination (2023) Reliability: 1, reliable without restriction supporting study Details available under "Study 7" in Annex I (3.10.2.1)

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Reaction products of diphenylamine with nonene, branched has been tested in a reproduction and developmental toxicity screening tests (OECD TG 421, Unpublished study report, 2020a).

A reproduction and developmental toxicity screening tests (OECD TG 421, Unpublished study report, 2020b), an extended one-generation reproductive toxicity study (EOGRTS OECD TG 443, Unpublished study report, 2021), and a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422, Unpublished study report, 2014b) performed in rats with the source substance **Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** are also considered relevant in a read-across approach since the read-across approach for reproductive toxicity from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) to Reaction products of diphenylamine with nonene, branched (target substance) is considered acceptable with high confidence (refer to 10.10.11). Especially, in the absence of an EOGRTS performed with Reaction products of diphenylamine with nonene, the outcomes observed in the EORGTS performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene have been taken into consideration in a weight of evidence approach pursuant to CLP Annex I, 1.1.1.

All these four studies are considered reliable and discussed for adverse effects on sexual function and fertility. The original study reports were made available to the dossier submitter. Detailed information of these studies can be found in Annex 1.

The two reproduction and developmental toxicity screening tests (OECD TG 421), representing bridging studies and the EOGRTS OECD TG 443 were performed by the same laboratory.

The effects related to developmental toxicity observed in those four studies are discussed in section 10.10.5.

Additional studies investigating substances Reaction products of diphenylamine with nonene, branched or its analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene have not identified in the literature.

Studies performed with Reaction products of diphenylamine with nonene, branched

OECD TG 421 study- cf Annex I, study 1

The reproduction and developmental toxicity screening test (OECD TG 421, GLP compliant), with a reliability of 1 reported (Unpublished study report, 2020a) is considered as a bridging study for effects on sexual function and fertility and development. It also represents the key study for effects on sexual function and fertility in combination with the EOGRTS with the analogue applying a read-across approach. Reaction products of diphenylamine with nonene (branched) was administered via diet to groups of 10 male and 10 female Wistar rats (P0 animals) at concentrations of 0 ppm, 500 ppm (eq. to 40/44 mg/kg bw/d in

males/females), 1500 ppm (eq. to 122/133 mg/kg bw/d in males/females) and 5000 ppm (eq. to 407/443 mg/kg bw/d in males/females). The duration of treatment covered a 10-week premating period and a 2-week mating period in both sexes as well as the entire gestation period and 13 days of lactation period in females up to one day prior to the day of scheduled sacrifice of the animals. Additional treated but not mated animals (recovery animals) of 10 male and 10 female animals at nominal doses of 0 and 5000 ppm (eq. to 367/430 mg/kg bw/d in males/females) was maintained for a subsequent period of at least 14 days of no test substance administration in order to observe reversibility of the findings. Dose selection is considered adequate.

Fertility, sexual function and delivery data

There was no test substance-related effect on male mating index or male fertility index.

Estrous cycle: At the high dose level, a slight non-statistically significant increase of estrous cycles length (4.3 days vs. control 4.0 days) associated with a slight increased in the mean percentage of days in diestrous stage (35% vs 28% in control).

There was no test substance-related effect on female mating index, female fertility index or gestation index.

The mean number of implantation sites was significantly lower in the high-dose group (-31%) and in the mid-dose group (-24%) with a mean number of implantation sites of 9.9 and 10.9 respectively (concurrent controls 14.4). The values of all tested groups were in the range of the provided historical control data HCD (9.8-14.2) while the value of the concurrent control was slightly higher. However, the range of HCD for the number of implantation sites from 2008 to 2018 period provided in the study report of the OECD TG 443 (Unpublished study report, 2021) performed with the analogue by the same laboratory, was 11.1 - 15.3 sites and 11.2-15.3 when considering a more appropriate timeframe (2015-2018). When considering the later HCD, the value of the concurrent control is well within the HCD range while the values of the mid-dose and high-dose groups are outside (Table 14). HCD were largely based on studies conducted by gavage while the present study was performed in diet (only 2/40 in HCD of OECD 421 or 422).

Nevertheless, the concurrent control group is the most relevant comparator, historical control data cannot be used to dismiss statistically significant, consistent and dose-related findings.

Considering statistical significance (despite the low number of animal per group in a screening study) and dose-response relationship, these changes are considered to be treatment-related and adverse considering their magnitude.

Consequently to the decreased number of implantation sites, the mean number of delivered F1 pups per dam was significantly decreased (-19%) in the high-dose group with 8.7 pups per litter and in the mid-dose group (-31%) with 10.2 pups per litter. The historical control range provided in the FSR is 9.0-13.2 pups delivered per litter, while the HCD range from the EOGRTS is 10.3-14.9 and 10.9-14.9 when considering a more appropriate timeframe (2015-2018).

Considering the statistical significance and the dose-response relationship, these changes are clearly considered to be treatment-related. Considering their nature and magnitude, they are considered adverse.

Table 14: Mean number of implantation sites and delivered pups (Reaction products of diphenylamine with nonene, branched, OECD TG 421)

		Control	**500 ppm** **Eq. to: 44 mg/kg bw/d**	**1500 ppm** **Eq. to: 133 mg/kg bw/d**	**5000 ppm** **Eq. to: 443 mg/kg bw/d**	*HCDa from FSR of OECD 421*	*HCDb from FSR of OECD 443*	*HCDc from FSR of OECD 443*
Pregnant females	N	9	10	9	10			
Total number of litters	N	9	10	9	10			
With live born pups	N	9	10	9	10			
	%	100	100	100	100			
With stillborn pups	N	0	2	0	2			

Implantation sites	N	130	131	98	99			
	Mean	14.4	13.1	**10.9****	**9.9****	*9.8- 14.2*	*11.1-15.3*	*11.2-15.3*
	Sd	1.9	2.1	1.6	2.3			
Pups delivered	N	113	129	92	87			
	Mean	12.6	12.9	**10.2***	**8.7****	*9.0- 13.2*	*10.3-14.9*	*10.9-14.9*
	Sd	2.2	2.1	1.7	2.1			

* p≤0.05, ** p ≤0.01,
a: from 40 OECD 421 or 422 studies 2015-2017 (32 by gavage, 2 by inhalation, 3 via diet and 3 via drinking water)
b: from 26 reproductive toxicity studies (not precised OECD 416 or OECD 443) period 2008-2018
c: from 10 reproductive toxicity studies (not precised OECD 416 or OECD 443) period 2015-2018

Reproductive organ weights and histopathology

For males, examination of reproductive organs and sperm analysis did not reveal specific effects on male reproductive function.

For females (see Table 15), a dose-related and significant decrease in absolute ovarian weights was observed from the mid-dose group. The relative weight was also impacted from the mid-dose level but statistical significance was only reached at the high dose level. This decrease in absolute ovarian weight seemed to be attenuated in the recovery group (-14%) but was still statistically significant. No histopathologic findings were noted in the ovaries.
Considering the statistical significance (despite the low number of animal per group in a screening study) and the dose-response relationship, the decrease of ovary weight is considered to be treatment-related and adverse from the mid dose.

Table 15: Absolute and Relative ovarian weights (Reaction products of diphenylamine with nonene, branched, OECD TG 421

	0 ppm	500 ppm	1500 ppm	5000 ppm	HCD range
Absolute weight (g)	110.8	104.4	**91.0***	**66.9****	*82.7 – 142.0*
% control	100	94	**82**	**60**	
Relative Weight (%)	0.048	0.045	***0.041***	**0.033****	*0.035 – 0.062*
% control	100	93	***85***	**69**	
Recovery group Absolute weight (g)	120.4			**104.0**	*82.7 – 142.0*
% control	100			**86****	

* : p <= 0.05, **: p <= 0.01
HCD from 44 OECD TG 422 studies 2015-2018 (43 by gavage while the current study is by diet)

Systemic toxicity

There were no mortalities nor any treatment-related clinical findings.

At the high-dose a significantly lower body weight was observed at the end of the premating period (-11% in males and females compared to controls) and at termination (-12% and -17% in males and females respectively compared to controls) as well as a significant decrease in food consumption in females (-11.6% from study day 0 to 70).

In the mid-dose, decreased mean body weight was limited to females at the end of the gestation period and during the lactation period (-7% compared to controls) associated to non-significant minor adverse alterations of the food consumption. No effect on body weight was observed during the premating period.

The main target organ was the liver. At the high-dose, increased relative liver weight (males 27% and females 25% over concurrent controls) was noted, corroborated by histopathological findings (minimal to moderate centrilobular hypertrophy in all animals and minimal to moderate fatty change in 7/10 males and 7/10 females, minimal single cell necrosis in 7/10 males) and changes in biochemical parameters related to liver functions (increased alkaline phosphatase activities, increased γ-glutamyl transferase activities, increased triglyceride values, decreased albumin and total protein). The same pattern of liver effects was observed at the mid-dose however at lower incidence and/or severity as compared to the high dose group. In the low-dose group, hepatic effects were limited to centrilobular hypertrophy (minimal) in 3/10 males while in females a decreased albumin level as well as a non-statistically significant increase of triglycerides (+140%) and alkaline phosphatase activities (+104%) were observed.

In high-dose males, thyroid effects were also observed, consisting in a significantly increased relative weight (17%) while a non-statistically significant increase of thyroid stimulating hormone (TSH) levels was observed in both mid and high-dose males. Thyroid histopathology was not performed and thyroid hormones were not investigated in females (not required in standard OECD TG 421).

OECD TG 408 study - cf Annex I, study 7

In a reliable sub-chronique toxicity, Reaction products of diphenylamine with nonene (branched) was administered by gavage to groups of 10 male and 10 female Wistar rats at dose levals of 0, 100, 300, 1000 mg/kg bw/d in corn oil during 90-days (Unpublished study report, 2013). There were no treatment-related changes regarding the weight or histopathological examination of the reproductive organs at any dose levels. Body weight (-14.6% on day 91) and body weight gain (-24.2% on day 91) were affected in high dose males and liver and thyroid were the target organs in both sexes.

Studies performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene

OECD TG 421 study - cf. Annex I, study 2

The reproduction and developmental toxicity screening test (OECD TG 421, GLP compliant), with a reliability of 1 (Unpublished study report, 2020b) along with the OECD TG 421 performed with Reactionwith Reaction products of diphenylamine with nonene, branched (study 1), is considered as a bridging study for effects on sexual function and fertility and development. It was used as a dose range-finding study for the EOGRTS. Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene was administrated via diet to groups of 10 male and 10 female Wistar rats (P0 animals) at concentrations of 0 ppm, 300 ppm (eq. to 26/28, mg/kg bw/d in males/females), 1000 ppm (eq. to 87/95 mg/kg bw/d in males/females) and 3000 ppm (eq. to 260/271 mg/kg bw/d in males/females). The duration of treatment covered a 10-week premating period and a 2-week mating period in both sexes as well as the entire gestation period and 13 days of lactation period in females up to one day prior to the day of scheduled sacrifice of the animals. In addition to the standard guideline, thyroid histopathology was performed in males and females as well as thyroid hormones measurements in adult females. Dose selection is considered adequate.

Fertility, sexual function and delivery data

There was no test substance-related effect on male mating index or male fertility index.

Estrous cycle: At the high dose level, a statistically significant increase of estrous cycles length (4.7 days vs. control 4.0 days) was noted as well as an increased mean percentage of days in diestrous stage (37% vs 26% in control).

There was no test substance-related effect on female mating index, female fertility index or gestation index.

The mean number of implantation sites was significantly lower (-36%) in the high-dose group compared to the concurrent control group (8.8 vs 13.8 respectively). In the mid-dose group, while non-statistically significant, a decrease of 14% as compared to control group was already observed (Table 16).

Considering the statistical significance at the high-dose (despite the low number of animal per group in a screening study) and the dose-response relationship, these changes are considered to be treatment-related and adverse in view of their magnitude.

Consequently to the decreased number of implantation sites, the mean number of delivered F1 pups per dam was significantly decreased (-34%) in the high-dose group with 8.0 pups per litter. The historical control range provided in the FSR is 9.0-13.2 pups delivered per litter, while the HCD range from the EOGRTS is 10.3-14.9 and 10.9-14.9 when considering a more appropriate timeframe (2015-2018).

Considering the statistical significance and the dose-response relationship, these changes are clearly considered to be treatment-related. Considering their nature and magnitude, they are considered adverse.

Table 16: Mean number of implantation sites and delivered pups (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, OECD TG 421)

		Control	300 ppm Eq to: 28 mg/kg bw/d	1000 ppm Eq to: 95 mg/kg bw/d	3000 ppm Eq to: 271 mg/kg bw/d	*HCDa from FSR of OECD 421*	*HCDb from FSR of OECD 443*	*HCDc from FSR of OECD 443*
Pregnant females	N	9	6	8	9			
Number of litters	N	9	6	8	9			
With liveborn pups	N	9	6	8	9			
	%	100	100	100	100			
With stillborn pups	N	0	0	1	0			
Implantation sites	N	124	84	95	79			
	Mean	13.8	14.0	***11.9***	**8.8****	*9.8- 14.2*	*11.1-15.3*	*11.2-15.3*
	Sd	2.0	1.1	1.4	1.6			
Pups delivered	N	110	84	92	72			
	Mean	12.2	14.0	*11.9*	**8.0****	*9.0- 13.2*	*10.3-14.9*	*10.9-14.9*
	Sd	3.2	1.1	1.7	2.1			

* p≤0.05, ** p ≤0.01,
a : from 40 OECD 421 or 422 studies 2015-2017 (32 by gavage, 2 by inhalation, 3 via diet and 3 via drinking water)
b: from 26 reproductive toxicity studies (not precised OECD 416 or OECD 443) period 2008-2018
c: from 10 reproductive toxicity studies (not precised OECD 416 or OECD 443) period 2015-2018

Reproductive organ weights and histopathology

For males, examination of reproductive organs and sperm analysis did not reveal specific effects of the Substance on male reproductive function.

In females, a significant decrease (-25%) in absolute ovarian weights was observed at the high-dose level, while not statistically significant, the relative weight was also decreased (-12%) (Table 17).
Considering the statistical significance (despite the low number of animal per group in a screening study), the decrease in ovary weight is considered to be treatment-related and adverse at the high-dose level.

Table 17: Absolute and Relative ovarian weights (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene], OECD TG 421)

	0 ppm	300 ppm	1000 ppm	3000 ppm	*HCD range*
Absolute weight (g)	107.0	110.7	104.7	**80.3****	*82.7 – 142.0*
% control	100	103	98	**75**	
Relative Weight (%)	0.045	0.049	0.048	***0.04***	*0.035 – 0.062*
% control	100	108	108	***88***	

**: p <= 0.01
HCD from 44 OECD TG 422 studies 2015-2018 (**43 by gavage while the current study is by diet**)

Systemic toxicity

There were no mortalities nor any treatment-related clinical findings.

At the high-dose, a significantly lower mean body weight was observed at the end of the premating period (-9% in males and females compared to controls) and at the end of gestation (-17% compared to controls) and lactation (-15% compared to controls) periods. It was associated with as a significant decrease in food consumption in females during gestation and lactation periods.

In the mid-dose group, a significantly lower mean body weight was observed in females at the end of the premating period (-7% compared to controls), at the end of gestation (-8% compared to controls) and at termination PND14 (-8% compared to controls), associated to decreased food consumption in during gestation period (-12%).

The main target organ was the liver. At the high-dose, increased absolute and relative liver weights were noted in males (+28% and +43% respectively) as well as increased relative liver weight in females (+30%) over concurrent controls), corroborated by histopathological findings (minimal to moderate centrilobular hypertrophy in all animals and minimal to slight fatty change in 6/10 males and 5/10 females, minimal single cell necrosis in 10/10 males) and changes in biochemical parameters related to liver functions (increased alkaline phosphatase activities, increased γ-glutamyl transferase activities, increased triglyceride and cholesterol values, decreased albumin and total protein). The same pattern of liver effects was observed at the mid-dose however at lower incidence and/or severity as compared to the high dose group. In the low-dose group, hepatic effects were limited to minimal centrilobular hypertrophy and minimal single cell necrosis/apoptosis in 2/10 males while in females an increase of alkaline phosphatase activities (+78%) was observed.

Thyroid effects were observed from the low dose levels (minimal hypertrophy/hyperplasia of follicular cells of 3/10 males and 3/10 females in combination with altered colloid). In high-dose animals, thyroid effects consisted in a significantly increased relative weight (17%) in males, corroborated by hypertrophy/hyperplasia of follicular cells of 9/10 males (minimal to moderate) and of 6/10 females (minimal to mild) in combination with altered colloid. In males, thyroxine levels (T4) was significantly reduced from the mid-dose group and TSH level statistically increased in the high-dose group while no alteration of hormones levels was observed in dams and PND13 pups.

OECD TG 443 study (EOGRTS) - cf. Annex I, study 3

The Extended One-Generation Reproductive Toxicity Study (OECD TG 443, GLP compliant), with a reliability of 1 reported, except for the developmental neurotoxicity part (auditory startle response, motor activity, morphometrics) presenting some limitations and triggering a reliability of 2 (Unpublished study report, 2021), is considered as the key study for effects on sexual function and fertility in combination with the OECD TG 421 studies performed withthe two analogues]. Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene was administrated via diet to groups of 10 male and 25 female Wistar rats (P0 animals) at concentrations of 0 ppm, 200 ppm (eq. to 18 mg/kg bw/d in males/females), 600 ppm (eq. to 54 mg/kg bw/d in males/females) and 1800 ppm (eq. to 167/166 mg/kg bw/d in males/females). P0 animals were treated at least for 10 weeks prior to mating to produce a litter (F1 generation). Mating pairs were from

the same dose group. Pups of the F1 litter were selected (F1 rearing animals) and assigned to 4 different cohorts (1A, 1B, 2A and 2B) which were subjected to specific post weaning examinations (1A and 1B dedicated to reproductive endpoints and 2A and 2B to developmental toxicity (DNT) endpoints). Cohort 1B (= P1 generation parental animals) was selected to produce F2 pups. P1 animals selected for breeding were continued in the same dose group as their parents.

With regards to dose selection, the same dose levels as those tested in the OECD TG 421 study (DRF study) could have been tested in the EOGRTS since no overt toxicity (no mortality and no clinical signs) was observed up to 3000 ppm.

HCD have been provided, however several shortcomings limit their reliability. The collection period generally exceeds the recommended 5-years encompassing the year of the study. The protocol of the studies is not always clearly indicated (e.g. whether OECD TG 416 or TG 443 where followed, the route of administration not indicated for all studies). The studies included in HCD are different according to the parameters considered, which further limits the transparency and readability of those data. In view of the limitations of the provided HCD, they were not be given much weight compared to the concurrent control group, which anyway represent the most relevant comparator for determining treatment-related effects if the concurrent control is not aberrant.

Fertility, sexual function and delivery data

There was no test substance-related effect on male mating index or male fertility index and sperm analysis did not reveal specific effects of the Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene on male reproductive function.

Estrous cycle: while estrous cycle data from P0 females and F1C1A females did not revealed any treatment-related effect, in estrous cycle data from P1 female, the mean estrous cycle duration was: 4.0 / 4.0 / 4.0 and 4.3** (**:p<=0.01) days in control, low-dose, mid-dose and high-dose groups respectively. The slightly prolonged average in the high-dose group is mainly driven by an increased mean number of days in diestrous stage (41% vs 33% in control).

There was no test substance-related effect on female mating index, female fertility index gestation length or gestation index.

In P0 high-dose females, the mean number of implantation sites was statistically significantly below (-15%) the concurrent control values (10.7 versus 12.6) and below the provided historical control range (HCD = 11.1 - 15.3 implants/dam). As a consequence of the lower number of implants, the mean number of F1 pups delivered per dam (average litter size) was statistically significantly below (-20%) the concurrent control values in the high-dose group (9.6 vs 12.0) and outside the provided historical control range (HCD = 10.3 - 14.9 pups/dam) (Table 18).

In P1 high-dose females, the mean number of implantation sites was statistically significantly below (-17%) the concurrent control values (10.2 vs 12.3) and below the HCD range. As a consequence of the lower number of implants, the mean number of F2 pups delivered per dam (average litter size) was statistically significantly below (-18%) the concurrent control values (9.8 vs 12). For both parameters the values were outside the provided historical control range.

In P1 mid-dose females the mean number of implantation sites was below (-9%) the concurrent control values (11.2 vs 12.3) without reaching statistically significance. As a consequence of the lower number of implants, the mean number of F2 pups delivered per dam (average litter size) was statistically significantly below (-10%) the concurrent control values (10.8 vs 12). For both parameters the values were close to the lowest value of the provided historical control data and when considering a more appropriate timeframe (2015-2018). Considering the low reliability of the HCD, and the clear dose-response relationship, the effects are considered treatment-related from the mid-dose in P1 females.

Table 18: Mean number of implantation sites and pups delivered pups (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, OECD TG 443)

	Control	**200 ppm** **Eq to: 18 mg/kg**	**600 ppm** **Eq to: 54**	**1800 ppm** **Eq to: 166**	***HCDb from FSR of OECD***	***HCDc from FSR of OECD***

			bw/d	mg/kg bw/d	mg/kg bw/d	*443 2008-2018*	*443 2015-2018*
P0 generation							
Pregnant females	N	24	24	25	25		
Number of litters	N	24	23a	25	25		
With liveborn pups	N	24	23	25	25		
	%	100	100	100	100		
With stillborn pups	N	2	1	0	2		
With all pups stillborn	N	0	0	0	0		
Implantation sites	N	302	276	298	267		
	Mean	12.6	11.5	11.9	**10.7****	*11.1-15.3*	*11.2-15.3*
	Sd	1.6	2.6	2.1	1.5		
Pups delivered	N	288	261	288	239		
	Mean	12	11.3	11.5	**9.6****	*10.3-14.9*	*10.9-14.9*
	Sd	1.9	1.3	2.1	2.2		
P1 generation							
Pregnant females	N	23	24	24	25		
Number of litters	N	23	24	24	25		
With liveborn pups	N	23	24	24	25		
	%	100	100	100	100		
With stillborn pups	N	1	1	1	3		
With all pups stillborn	N	0	0	0	0		
Implantation sites	N	283	282	270	255		
	Mean	12.3	11.8	***11.2***	**10.2****	*11.1-15.3*	*11.2-15.3*
	Sd	2.3	2.2	1.9	1.9		
Pups delivered	N	277	270	260	244		
	Mean	12	11.2	**10.8***	**9.8****	*10.3-14.9*	*10.9-14.9*
	Sd	2.2	2.3	1.8	1.9		

* p≤0.05, ** p ≤0.01,
a: One sperm positive low-dose female did not deliver F1 pups but had implants in the utero.
b: from 26 reproductive toxicity studies (not precised OECD 416 or OECD 443) period 2008-2018
c: from 10 reproductive toxicity studies (not precised OECD 416 or OECD 443) period 2015-2018

Reproductive organ weights and histopathology

For males, examination of reproductive organs and sperm analysis did not reveal specific effects of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene on male reproductive function.

In females, a statistically significant decrease in absolute ovarian weights was observed in high-dose P0 females (-13%) and in high-dose P1 females (-12%). No correlated histopathological findings were detected in P0 females (while histopathology was not performed in P1 females) (Table 19).

Table 19: Absolute and Relative ovarian weights (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, OECD TG 443)

	0 ppm	200 ppm	600 ppm	1800 ppm
P0 generation				
Absolute weight (g)	118.8	118.8	117.2	**103.9****
% control	100	100	99	**87**
P1 generation				
Absolute weight (g)	111.6	112.1	114.6	**98.1****
% control	100	100	103	**88**

**: p <= 0.01

Onset of puberty

In high-dose F1 females, the mean number of days to reach vaginal opening was slightly but significantly higher than in control group (31.8 vs 31.0).
In high-dose F1 males, the mean number of days to reach preputial separation was also significantly higher than in control group (43.5 vs 42.1).
The delay in puberty onset was observed in both sexes of the high-dose group, which does not suggest an endocrine mode of action. Furthermore, a decreased body weight was noted at weaning in high-dose animals (-12%) while the weight at puberty onset is similar in all groups (Table 20 and Table 21). The delay in sexual maturation observed at the high dose level is therefore considered as a consequence of the delayed general development (lower pup weights).

Table 20: Age and weight at vaginal opening of F1 females (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, OECD TG 443)

Dose levels (ppm)		0 ppm	200 ppm	600 ppm	1800 ppm	*HCD range 2008-2018*
Days at vaginal opening	Mean	31.0	31.1	31.3	**31.8***	*29.5- 38.8*
	S.d.	1.1	1.6	1.4	2.0	
	N	55	55	55	54	
Weight at vaginal opening	Mean	96.0	97.1	94.3	92.2	*84.7-105.8*
	S.d.	8.1	10.4	10.1	9.7	
	N	55	55	55	54	

* p≤0.05, ** p≤0.01

Table 21: Age and weight at prepitial separation of F1 males (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, OECD TG 443)

Dose levels (ppm)		0 ppm	200 ppm	600 ppm	1800 ppm	*HCD range 2008-2018*
Days at preputial separation	Mean	42.1	41.5	42.3	**43.5****	*40.1-45.2*
	S.d.	2.1	1.7	1.5	1.9	
	N	55	55	55	55	

Weight at preputial separation	Mean	181.2	176.7	180.9	175.3	*158.2-221.1*
	S.d.	14.5	13.3	13.8	15.1	
	N	55	55	55	55	

* p≤0.05, ** p≤0.01

Systemic toxicity

There were no mortalities nor any treatment-related clinical findings.

Effects on the mean body weight compared to controls were limited to the high-dose groups P0 females (-8.2%, -12.1% and -9.4% at the end of the premating period, gestation and lactation respectively), P1 males during major parts of the study (-6.2% below control at termination) and P1 females during major parts of the premating period and the entire gestation and lactation period (-9%, -13% and -12% below control at the end of the premating period, gestation and lactation respectively). Food consumption was not altered in P0 and P1 males while it was decreased in P0 high-dose females during gestation and lactation periods and in P1 high-dose females during premating, gestation and lactation periods. Slight haematological findings were also observed in the high-dose (1800 ppm) animals with haemoglobin and haematocrit values marginally but significantly decreased (less than 5% decrease).

The target organs were the liver and the thyroid.

At the high-dose, increased absolute and relative liver weights were noted in P0 males (+15%/+19%), P0 females (+23%/+36%) and P1 females (+19%/+32%) as well as increased relative liver weight in P1 males (+14 %%) over concurrent controls. Histopathological correlates in P0 were: minimal to moderate centrilobular hypertrophy in all males and in 12/20 females, fatty change in 14/20 (minimal to severe) and diffuse hepatocellular hypertrophy in 6/20 females (slight to moderate) associated with changes in biochemical parameters related to liver functions (increased alkaline phosphatase activities, increased γ-glutamyl transferase activities, increased triglyceride and cholesterol values, decreased albumin and total protein). The same pattern of liver effects was observed at the mid-dose however at lower incidence and/or severity as compared to the high dose group. In the low-dose group, hepatic effects were limited to minimal centrilobular hypertrophy 4/20 P0 males and 3/20 P0 females as well as an increase of alkaline phosphatase activities in both sexes and a slight decreased of albumin level in females.

Thyroid effects were observed from the low-dose group (minimal hypertrophy/hyperplasia of follicular cells of 7/20 P0 males and 2/20 P0 females in combination with altered colloid). In high-dose animals, thyroid effects consisted in significantly increased absolute and relative weights in P0 males (+24%/+27%) and increased relative weight in P0 females (+21%), corroborated by hypertrophy/hyperplasia of follicular cells of 15/20 males (minimal to moderate) and of 12/20 females (minimal to slight) in combination with altered colloid. In P0 males, T4 were significantly reduced from the low-dose group while TSH levels were statistically increased from the low-dose group in P0 females.

OECD TG 422 study - cf. Annex I, study 4

The combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422, GLP compliant, Unpublished study report, 2014b), with a reliability of 1 is considered as a supporting study for effects on sexual function and fertility and development.

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene was administrated to groups of 10 male and 10 female Wistar rats (P0 animals) by oral gavage at dose levels of 0, 25, 75 and 225 mg/kg/d formulated in corn oil. The duration of treatment covered a 2-week premating period and a 2-week mating period in both sexes as well as the entire gestation period and 4 days of lactation period in females up to one day prior to the day of scheduled sacrifice of the animals. Dose selection is considered adequate.

Fertility, sexual function and delivery data

There was no test substance-related effect on male mating index or male fertility index.

Estrous cycle was not investigated (not required in OECD TG 422 1996).

There was no test substance-related effect on female mating index, female fertility index or gestation index.

While not statistically significant, the mean number of implantation sites was decreased (-16%) in the high-dose group (Table 22).

Table 22: Mean number of implantation sites and pups delivered pups (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, OECD TG 422)

		Control	25 mg/kg bw/d	75 mg/kg bw/d	225 mg/kg bw/d
Pregnant females	N	10	10	10	9
Number of litters	N	10	10	10	9
Implantation sites	Mean	10.9	11.5	10.5	***9.2***
	Sd	2.4	2.2	1.7	2.3

* p≤0.05, ** p ≤0.01,

Reproductive organ weights and histopathology

Examination of reproductive organs did not reveal specific effects of the Substance on males or females.

Systemic toxicity

There were no mortalities, no effects on body weight nor any treatment-related clinical findings.

Significant increased relative liver weight was observed from the low-dose level in males and from the mid-dose in females, associated with histopathological findings (hepatocellular hypertrophy and hepatocellular vacuolation) and changes in biochemical parameters related to liver functions (increased alkaline phosphatase activities, triglyceride and cholesterol values, decreased albumin and total protein and bile acids) from the mid-dose level. Thyroid effects were observed from the low dose level (minimal to slight hypertrophy of follicular cells) in males. Decreased T4 and increased TSH levels were observed in all treated groups (both sexes, not always statistically significant and no clear dose-response relationship).

It is noteworthy that no effect was observed at histopathological examination of the spinal cord (cervical, thoracic and lumbar) and the sciatic nerve in male and female adults.

Overall, the available dataset consistently reports across studies the following effects relevant for sexual function and fertility:

- **Lower mean number of implantation sites and consequently smaller litter sizes (main critical effect).** The litter size (mean number of delivered pups) was significantly reduced by 19% and 31% in the mid-dose (1500 ppm eq. 133 mg/kg bw/d) and high-dose dose (5000 ppm eq. 443 mg/kg bw/d) dams exposed to Reaction products of diphenylamine with nonene, branched.
  For the analogue, the litter size was reduced by 34% in high-dose dose (3000 ppm eq. 271 mg/kg bw/d) dams of the OECD TG 421 study, by 20% and 18% in high-dose dose (1800 ppm eq. 166 mg/kg bw/d) P0 and P1 dams respectively and by 10% in mid-dose dose (600 ppm eq. 54 mg/kg bw/d) P1 dams of the EOGRTS and by 28% in high-dose dose (225 mg/kg bw/d) dams of the OECD TG 422 study.
  This effect was dose-related and observed from dose levels (mid-dose groups) where no effects on body-weight and food consumption were observed during the premating periods.

- **Decreased ovary weights**. Dose-related and significant decrease in absolute ovarian weights was observed in dams exposed to Reaction products of diphenylamine with nonene, branched (by 18% and 40% at mid- and high dose level respectively) in the OECD TG 421 study. The relative weight was also impacted from the mid-dose level but statistically significantly only at the high dose level. While a statistically significant decrease (-14%) in absolute ovarian weight was also observed in the recovery group of this study (not mated females exposed to 5000 ppm during 10 weeks with a 2-week recovery period) no effect on ovary weight was noted in females up to 1000 mg/kg bw/d of

Reaction products of diphenylamine with nonene, branched by gavage in corn oil in a GLP-compliant 90-day toxicity study (Unpublished study report, 2013**- Study 7**).
Absolute ovary weight was also significantly reduced by 25% in high-dose dams exposed to the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, in the OECD TG 421 study and by 13% and 12% in P0 and P1 high-dose dams respectively.
While this effect was consistently observed in functional reproductive studies, no histopathological correlate was reported in any of the studies.

- **Effects on cyclicity.** Increase of estrous cycles length associated with an increased mean percentage of days in diestrous stage was observed in high-dose dams exposed to Reaction products of diphenylamine with nonene, branched or Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. Statistical significance was reached only with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene in in high-dose females of the OECD TG 421 and in high-dose P1 females of the EOGRTS.

In the EOGRTS, a slight delay on puberty onset was observed in both sexes of the high-dose group while the weight at puberty onset is similar in all groups. A decreased body weight was noted at weaning in high-dose animals (-12%). The delay in sexual maturation observed at the high dose level is therefore considered as a consequence of the delayed general development (lower pup weights).

10.10.3 Comparison with the CLP criteria

CLP Regulation in combination with explanations from the Guidance on the Application of the CLP criteria (ECHA, 2017b) were applied. Any adverse effect of Reaction products of diphenylamine with nonene and its analogue on the female and male reproductive system, on the onset of puberty, gamete production and transport, reproductive cycle, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems were considered.

Comparison with Category 1 criteria as laid down in CLP Regulation:

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility […] in humans, or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.

The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

- *Known human reproductive toxicant (1A)*

The classification of a substance in Category 1A is largely based on evidence from humans.

- *Presumed human reproductive toxicant (1B)*

The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility […] in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

- *Suspected human reproductive toxicant (Cat 2)*

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility […] and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

There are no human data to support classification of Reaction products of diphenylamine with nonene, branched in Category 1A.

Animal studies provide clear evidence of an adverse effect on fertility. Indeed, in the four reliable guideline and GLP-compliant studies addressing explicitly reproduction (one OECD TG 421 with the substance itself and one OECD TG 421, one OECD TG 422 and one OECD TG 443 with the analogue), Reaction products of diphenylamine with nonene, branched and its analogue **consistently induced lower numbers of implantation sites with subsequently smaller litter sizes** as compared to control animals. **This main critical effect** was dose-related in all the studies, statistically significant even in screening OECD 421 TG studies with a small number of animals (10/sex/dose) and was repeated in both generations of the EOGRTS.

At the top doses tested, the litter size was reduced by 31% and 36% in dams exposed to 5000 ppm of Reaction products of diphenylamine with nonene, branched and 3000 ppm of the analogue respectively, which **is considered a severe impact on fertility/reproductive function (a decrease of one third in the progeny).** At those dose levels the systemic toxicity was not marked (i.e.: no lethality, no dramatic reduction in absolute body weight, no coma), body weights at the end of the premating period were decreased by 10% and liver and thyroid effects were observed. However, a direct correlation between systemic toxicity and reduced implantation sites could not be established based on the data available and lower numbers of implantation sites with subsequently smaller litter sizes were already observed at lower dose levels where body weight was not affected.

Other supportive effects relevant for fertility/sexual function classification noted with both Reaction products of diphenylamine with nonene and its analogue were statistically significant decrease of absolute ovary weights (without histopathological correlates) and increased estrous cycles length associated with a slight increase in the mean percentage of days in diestrous stage (reaching statistically significance only in high-dose dams of the OECD TG 421 and in the high-dose dams of the EOGRTS performed withthe analogue).

The consistency of the effects observed with the two structural analogues through the available studies further strengthens the causality between exposure to two SDPAs and the observed reproductive outcomes.

The reliability of the available studies compliant to GLP and current OECD TGs, make the quality of evidence convincing.

Therefore, laying down the criteria of CLP Regulation, classification of the Reaction products of diphenylamine with nonene, branched with Category 1B for effects on fertility (H360F), is considered warranted.

10.10.4 Adverse effects on development

Table 23: Summary table of animal studies on adverse effects on development

All reported changes are statistically significant, unless otherwise specified (in italic).

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal developmental toxicity studies			
Study with Reaction products of diphenylamine with nonene, branched			
Prenatal Developmental Toxicity Study OECD TG 414 (2001) GLP-compliant Wistar IGS Crl: WI (Han) rats N = 24 mated females/dose	**Reaction products of diphenylamine with nonene, branched** EC: *CAS:- (old 36878-20-3)* Batch 240312/k7 Purity: UVCB **By gavage** Dose levels: 0, 50, 150, 500 mg/kg bw/d Vehicle: corn oil Exposure duration: from GD6 to GD19	**Effects on development** No effect on post-implantation survival at any dose level. **500 mg/kg bw/d** *Non statistically significant ↑ number of small fetuses (fetal weight < 2.7 g) 10 in 3 litters vs 2 in 2 litters in controls.* Visceral and skeletal examination of fœtus : no effect. **50 and 150 mg/kg bw/d** No effect. **Maternal toxicity** **500 mg/kg bw/d** ↓ Final body BW (-7%) Final corrected BW (-6%) and corrected BW gain GD6-GD20 (-52%) compared to controls. ↓ Food consumption (up to 22%) in F from GD9. **150 mg/kg bw/d** ↓ Final corrected BW (-4%) and corrected BW gain GD6-GD20 (-31%). **50 mg/kg bw/d** No effect.	Unpublished study report, 2014c **Key study for adverse effects on development** **Reliability:** 1, reliable without restriction Details available under "Study 5" in Annex I (3.10.1.5)
Dose-range-finding study in pregnant rats Non-GLP Wistar IGS Crl: WI (Han) rats	**Reaction products of diphenylamine with nonene, branched** EC:	**Effects on development** Litter data : only uterus weight performed, no differences between groups. Examination of the fetus : not realised. **Maternal toxicity**	Unpublished study report, 2014 **Supporting study** Reliability: 2 reliable with restrictions

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
N = 10 mated females/dose	*CAS:- (old 36878-20-3*) Batch 240312/k7 Purity: UVCB **By gavage** Dose levels: 0, 300, 1000 mg/kg bw/d Vehicle: corn oil Exposure duration: from GD6 to GD19	**1000 mg/kg bw/d** No mortality. ↓ Terminal BW(-16%) compared to controls, corrected BW (-10%) and corrected BW gain (-68%) compared to controls. ↓ Food consumption (from gestation Day 9 until the end of study : up to 66% of the control values). Liver effects: ↑ rel weight (+15%), pale colour in 4 animals, abnormal clear fluid in the abdominal cavity, Biochemistry changes: ↑ ALP, ALT, TRIG, CHOL, APTT and ↓ CREA, TP, ALB and GLOB. **300 mg/kg bw/d** Biochemistry changes: ↑ TRIG and ↓ ALB.	
Prenatal Developmental Toxicity Study OECD TG 414 (2018) GLP-compliant New Zealand White rabbits N = 25 mated females/dose	**Reaction products of diphenylamine with nonene, branched** EC: *CAS:- (old 36878-20-3*) Purity: UVCB **By gavage** Dose levels: 0, 10, 30, 100 mg/kg bw/d Vehicle: CMC (carboxymethyl cellulose) - 0.5% CMC suspension in deionized water Exposure duration: from GD6 to GD28	**Effects on development** No effect on post-implantation survival at any dose level. **100 mg/kg bw/d** Abortion : **4 vs 2 in controls** at the end of the gestation period. ↓ Fœtus weight (-12%), ↓ males Fœtus weight (-13%), *non-statistically significant ↓ females fœtus weight (-11%)* Delays of ossification *Non-statistically significant ↑ of total external malformations due to 4 fetuses **in one single litter** with multiple external malformations.* **10 and 30 mg/kg bw/d** No effect. **Maternal toxicity** **100 mg/kg bw/d** *↓ defecation in 20 dams (vs in 4 dams in controls).* ↓ food consumption from GD 7-23 (up to -59%). *Non-statistically significant ↓ food consumption (-31% during the treatment period GD 6-28).* ↓ transitory mean BW (d14-d25 up to 5%) and ↓ transitory body weight gain (BWC) (d9-d11), ↓ *corrected BWG not statistically significant (-421.0 g) in comparison to the concurrent control (-322.2 g) (-30%).*	Unpublished study report, 2019a **Key study for adverse effects on development** **Reliability:** 1, reliable without restriction Details available under "Study 6" in Annex I (3.10.1.6)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		30 mg/kg bw/d ↓ *defecation in 11 dams.* **10 mg/kg bw/d** No effect.	
Dose-range-finding study in pregnant rabbits Non-GLP New Zealand White rabbits N = 5 mated females/dose	**Reaction products of diphenylamine with nonene, branched** EC: *CAS:- (old 36878-20-3*) Purity: UVCB **By gavage** Dose levels: 0, 30, 50, 100 mg/kg bw/d Vehicle: 0.5% CMC Exposure duration: from GD6 to GD28	**<u>Effects on development</u>** Abortion: 1/5 vs 0 in controls at 50 and 100 mg/kg bw/d. Litter data : only uterus weight performed, no differences between groups. Examination of the fetus : not realised. **<u>Maternal toxicity</u>** **100 mg/kg bw/d** ↓ Mean food consumption (-44%) along with a lower net body weight change. **50 mg/kg bw/d** ↓ Mean food consumption (-13%) along with *a lower net body weight change.*	Unpublished study report, 2019b **Supporting study** Reliability: 2 reliable with restrictions
Dose-range-finding study in non-pregnant rabbits Non-GLP New Zealand White rabbits N = 3 non pregnant females/dose	**Reaction products of diphenylamine with nonene, branched** EC: *CAS:- (old 36878-20-3*) Purity: UVCB **By gavage** Dose levels: 0,	• Toxicity was characterized by a marked reduction of food consumption (up to 93%) and significant body weight loss at 200 and 300 mg/kg bw/d. ↓ terminal bw compared to control (-13% and -14% respectively). • At 100 mg/kg bw/d similar effects were seen but less severe. • Dose levels of 30, 50 and 100 mg/kg bw/d were chosen to be used in the maternal toxicity range finding study in pregnant rabbits.	Unpublished study report, 2019c **Supporting study** Reliability: 2 reliable with restrictions

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	100, 200, 300 mg/kg bw/d Vehicle: 0.5% CMC Exposure duration: 21 days		
Generational studies			
Study with Reaction products of diphenylamine with nonene, branched			
Reproduction/Developmental Toxicity Screening Test **OECD TG 421 (2016)** GLP-compliant Rat Wistar Crl:Wl(Han) N = 10/sex/dose group **10-week premating period** ***Deviation:*** *Additional investigations: sperm and spermatid examinations, determination of organ weights of brain, heart, kidneys, liver, spleen and thymus, several organ or tissue fixations, and histopathology of liver.* ***NB:*** *Thyroid histopathology not performed (optional in OECD TG 421)* *Thyroid hormones measured in*	**Reaction products of diphenylamine with nonene, branched** EC: *CAS:- (old 36878-20-3)* Batch 00 16046440 Purity: UVCB **By diet** **Diet :** 0, 500, 1500 and 5000 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance Eq. to: 0, 40/44, 122/133 and 407/443 mg/kg bw/d in M/F Recovery group : 0, 5000 ppm	**F1 pups** **5000 ppm (407/443 mg/kg bw/d)** ↓ Mean number of F1 pups delivered/dam **(consequence of the lower nb of implants).** No effect on post-implantation loss and live birth, viability and survival PND4-13 indices. ↓ mean terminal pup BW from PND 7 in both sexes on PND13 (-19 % both sexes combined), ↓ mean pup BW changes PND 4-13 (up to -22.5% both sexes combined) **1500 ppm (122/133 mg/kg bw/d) and 500 ppm (40/44 mg/kg bw/d)** ↓ Mean number of F1 and F2 pups delivered/dam **(consequence of the lower nb of implants)**. No effect on BW **500 ppm (40/44 mg/kg bw/d)** No effect. **General/systemic toxicity P0** **5000 ppm (407/443 mg/kg bw/d)** ↓ Final body weight (BW) in M (-12% compared to controls) and ↓ BW in F at the end of the premating period (-11%), at the end of gestation (-18%) and at the end of lactation (-17%). ↓ Food consumption in F from study day 7 onwards (-12% during the premating period, -20% during gestation ad -24% during lactation) compared to controls. Liver and thyroid effects. **1500 ppm (122/133 mg/kg bw/d)** ↓ BW in F at the end of gestation (- 9%) and at the end of lactation (-7%) compared to controls. No effect on BW	Unpublished study report, 2020a **Key study for adverse effects on sexual function and fertility** **Bridging study** **Reliability:** 1, reliable without restriction Details available under "Study 1" in Annex I (3.10.1.1)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
adult M only and PND13 M and F pups in line with OECD TG 421.	P0: **10-week premating period** F1: terminated at PND14 Recovery group (not mated): 10-week exposure + 2-week recovery period	during the premating period. Liver effects. **500 ppm (40/44 mg/kg bw/d)** Slight liver effects.	
Studies with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene			
Reproduction/Developmental Toxicity Screening Test **OECD TG 421 (2016)** GLP-compliant Rat Wistar Crl:Wl(Han) N = 10/sex/dose group **10-week premating period** ***Deviations:*** *Only 6 F pregnant in the low dose group while 8 per group is the minimum acceptable number according to the TG.* *Additional investigations: sperm and spermatid examinations, determination of organ weights of brain, heart, kidneys, liver, spleen and thymus, several organ or tissue fixations, and histopathology*	**Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** CAS: 68411-46-1 Batch: 50116118D Purity: UVCB **By diet** **Diet :** 0, 300, 1000 and 3000 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance Eq. to: 0, 26/28, 87/95 and 260/271 mg/kg bw/d in M/F F0: **10-week premating period**	**F1 pups** **3000 ppm (260/271 mg/kg bw/d)** ↓ Mean number of F1 pups delivered/dam **(consequence of the lower nb of implants).** No effect on post-implantation loss and live birth, viability and survival PND4-13 indices. ↓ mean terminal pup BW on PND13 (-26% both sexes combined) and ↓ mean pup BW changes on PND 1-13 (-32% both sexes combined) compared to controls. ↑incidence of nipple development (100% vs. 79.6% in control) and number of nipples per animal (5.2 to control 2.5) on PND13. **1000 ppm (87/95 mg/kg bw/d)** ↓ mean terminal pup BW on PND13 (-8% both sexes combined) and ↓ mean pup BW changes on PND 1-13 (-10% both sexes combined) compared to controls. **300 ppm (26/28 mg/kg bw/d)** No effect. **General/systemic toxicity P0** **3000 ppm (260/271 mg/kg bw/d)** ↓ Food consumption in F during gestation and lactation periods (-23% and -20% respectively). ↓food consumption in M on study days 0 to 14 (up to -8.8%) and on study days 42 to 49 (-22.5%). ↓ Final BW in M (-9% compared to controls) and ↓ BW in F at the end of the premating period (-9%), at the end	Unpublished study report, 2020b **Bridging study** **Reliability:** Reliability: 1, reliable without restriction supporting study Details available under "Study 2" in Annex I (3.10.1.2)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
of liver. ***NB:*** *Thyroid histopathology performed in M and F (optional in OECD TG 421).* *Thyroid hormones measured in adult M and F (optional) and PND13 M and F pups.*	F1: terminated at PND13	of gestation (-17%) and at termination PND14 (-15%) compared to controls. Liver and thyroid effects **1000 ppm (87/95 mg/kg bw/d)** ↓ Food consumption in F during gestation period (-12%). ↓ BW compared to controls in F at the end of the premating period (-7%), at the end of gestation (-8%) and at termination PND14 (-8%). Liver and thyroid effects **300 ppm (26/28 mg/kg bw/d)** Slight liver and thyroid effects.	
EOGRTS **OECD TG 443 (2018)** GLP-compliant Rat Wistar Crl:Wl(Han) P0: 25/sex/dose F1C1A: 20/sex/dose F1C1B (= P1): 25/sex/dose, F1C2A: 10/sex/dose F1C2B: 10/sex/dose **10-week premating period** **Deviations:** **Cohort F1C1B: No histopathology performed** Since suspected to be ED, histopathology of cohort 1B should have been performed. Since liver and thyroid are identified as target organs they should also been analysed **F2 pups:** Thyroid hormones	**Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** CAS: 68411-46-1 Batch: 50116118D Purity: UVCB **By diet** **Diet :** 0, 200, 600 and 1800 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance Eq. to: 0, 18, 54 and 167/166 mg/kg bw/d in M/F	**<u>F1 and F2 pups</u>** **1800 ppm (167/166 mg/kg bw/d)** ↓ Mean number of F1 and F2 pups delivered/dam (**consequence of the lower nb of implants**). No effect on post-implantation loss and live birth, viability and lactation indices. ↓ F1 and F2 pups BW PND7-PND21 in M, F and M&F combined (at weaning: -12.5% F1, -16% F2) compared to controls. No effect on sex ratio, anogenital distance in F1 or F2 pups Nipple retentions: F1: at PND20, 2 pups from the same litter with 2 nipple/areola anlagen (vs 0 in controls and HCD). F2: ↑ mean nipple number at PND13 (No persistence: 0 nipple/areola at PND20) In F1 delay to reach preputial separation (43.5 vs 42.1 days in control) and vaginal opening (31.8 vs 31.0 days in control). Considered secondary to delayed general development (weight at puberty onset similar in all groups) Thyroid hormones measurements: F1PND4: only 2 animals (due to smaller litter sizes). PND22: ↑ TSH in F1pups (M&F). **600 ppm (54 mg/kg bw/d)** ↓ Mean number F2 pups delivered/dam **(consequence of the lower nb of implants).** No effect on post-implantation loss and live birth, viability and lactation indices. ↓ F1 pups BW PND7-PND21 M&F combined (at weaning: -5.4%)) compared to controls. Thyroid hormones measurements: F1PND4: *non-statistically significant ↓ T4 F1pups (M&F)*. PND22: ↑ TSH in F1 pups (M&F).	Unpublished study report, 2021 **Key study for adverse effects on sexual function and fertility** **Reliability:** 1, reliable without restriction **2 (Reliable with restriction for some DNT parameters)** Details available under "Study 3" in Annex I (3.10.1.3)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
not measured **DNT:** No historical control data (HCD) no positive control, statistical analysis not appropriate for motor activity, auditory startle response and morphometrics.		**200 ppm (18 mg/kg bw/d)** No effect. **<u>Developmental neurotoxicity (DNT)</u>** **1800 ppm (167/166 mg/kg bw/d)** Axonal degeneration in cohort 2A M (thoracic spinal cord and tibial nerve) and 2A F (lumbar spinal cord and sciatic nerve), ↑corpus callosum width in 2A M&F (MD and LD not performed) and slight ↓ brain length 2A M. ASR: ↓mean maximal amplitude in M (-19%) and *↓habituation in M (-57%) and M & F combined (-46%)(statistical analysis not performed).* **600 ppm (54 mg/kg bw/d)** ASR: ↓mean maximal amplitude in M (-12%) and *↓habituation in M (-40%) and M & F combined (-42%) (statistical analysis not performed).* **200 ppm (18 mg/kg bw/d)** No effect. **<u>General/systemic toxicity P0, P1(F1C1B), F1C1A and F2C2A</u>** **1800 ppm (167/166 mg/kg bw/d)** ↓ Food consumption in P0 F during gestation and lactation periods (-13%) and in P1 F during the premating, gestation and lactation periods (-7%, -14% and -17% respectively). No effect on other treated animals. No effect on BW in P0 M. ↓ final BW in P1 M (-6%), F1C1A M (-8%) and F1C2A M (-8%) compared to controls. ↓ BW compared to controls in P0 F (-8%, -12% and -9% at the end of the premating period, gestation and lactation respectively) and in P1 F (-9%, -13% and -12% at the end of the premating period, gestation and lactation respectively). ↓ Final BW of F1C1A F (-6%). Liver and thyroid effects, slight ↓ (<5%) ↓haemoglobin and haematocrit values. **600 ppm (54 mg/kg bw/d)** No significant effect on BW and food consumption in any generations. Liver and thyroid effects **200 ppm (18 mg/kg bw/d)**	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Slight liver and thyroid effects	
Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test **OECD TG 422 (1996)** GLP-compliant Rat Wistar Crl:Wl(Han) N = 10/sex/dose group **2-week premating period** **Several deviations compared to current OECD TG 422 (2016):** Pups terminated before PND 13, AGD and nipple retention not investigated. Estrous cycle not monitored. ***NB:*** *In P0: TSH, T3 and T4 measured in 5 animal/sex/dose* *Thyroid histopathology performed in M and F.* *Thyroid hormones measured in adult M and F.*	**Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** CAS: 68411-46-1 Batch: 40401913D Purity: UVCB **By gavage** Doses: 0, 25, 75 and 225 mg/kg bw/day Vehicle: corn oil Exposure duration: P0: 28 days (M), 53 days (F) F1: terminated at PND5-7	**<u>F1 pups</u>** **225 mg/kg bw/d** ↓ nb of pups delivered/live born pups (-28%) *as a consequence of non-statistically significant ↓ of mean number of implantation sites (-16%) and ↑ post-implantation loss (14% vs **0% in controls**)* ↑ in postnatal loss (8 in 3 litters versus 0 in controls) and correspondingly↓ viability index (88.7% vs 100% in controls). No effect at **75 mg/kg bw/d and 25 mg/kg bw/d** **<u>General/systemic toxicity P0</u>** No effects on clinical signs and BW at any dose levels. **225 mg/kg bw/d** Liver and thyroid effects **75 mg/kg bw/d** Liver and thyroid effects **25 mg/kg bw/d** Slight thyroid effects in M.	Unpublished study report, 2014b **Reliability:** 1, reliable without restriction **Supporting study** (screening) Details available under “Study 4” in Annex I (3.10.1.4)

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Regarding prenatal development, one Prenatal Developmental Toxicity Study (PNDTS) in rats (Unpublished study report, 2014) and another one in rabbits (Unpublished study report, 2019) performed with the substance **Reaction products of diphenylamine with nonene, branched** as well as their respective DRF studies are available.

The studies mentioned in chapter 10.10.1 (i.e. OECD TG 421 studies with the substance **Reaction products of diphenylamine with nonene**, branched and its analogue **Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** and the EOGRTS and the OECD TG 422 with analogue) also addressed effects on postnatal development and have been considered for developmental toxicity.

The studies performed with the analogue are considered relevant in a read-across approach since the read-across for reproductive toxicity from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) to Reaction products of diphenylamine with nonene, branched (target substance) is considered acceptable with high confidence (refer to 10.10.11). Especially, in the absence of an EOGRTS performed with Reaction products of diphenylamine with nonene, the outcomes observed in the cohorts of the EOGRTS performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene have been taken into consideration in a weight of evidence approach pursuant to CLP Annex I, 1.1.1.

All these studies are considered reliable and discussed for adverse effects on development. The original study reports were made available to the dossier submitters. Detailed information of these studies can be found in Annex I.

Additional studies investigated **Reaction products of diphenylamine with nonene, branched** or its analogue **Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** in the literature were not identified.

- **Prenatal development**

Reaction products of diphenylamine with nonene, branched

PNDTS in rats - cf Annex I, study 5

In the GLP-compliant PDTS in rat, OECD TG 414 (Unpublished study report, 2014c), carried out with Reaction products of diphenylamine with nonene, branched, by gavage at dose levels of 0, 50, 150 and 500 mg/kg bw/d, maternal toxicity was characterized by a slight, but statistically significant, decrease in corrected body weight at 500 mg/kg bw/day (-6%), and corrected body weight gain (-52%) compared to controls. Statistically significant decrease (up to -22%) in food consumption was observed, starting from Day 9 post coitum until the end of the study. At 150 a very slight (-4%), but statistically significant, decrease in corrected body weight compared to controls was also observed. However, the differences of corrected body weight at 150 mg/kg bw/d versus control group was minor (258 g versus 270 g) and therefore the effect is not considered adverse at this dose level.

Concerning developmental data, an increased number of smaller fetuses (fetal weight < 2.7 g) was observed at the top dose 10 fetuses vs 2 in controls. It is noteworthy that the higher number of small fetuses at the top dose was mainly due to one litter (7 fetuses from female no. 147). Systemic toxicity was particularly marked in this female (corrected terminal BW -21% as compared to controls and negative corrected BW gain, hunched posture and piloerection at GD20). The increased number of small fetuses in the high-dose group is therefore rather considered as secondary to the poor condition of female no.147 than a specific landmark of developmental toxicity. One fetus in the high dose group showed malrotation of the hindlimb, which was considered as incidental. There were no relevant changes recorded at the external, skeletal and visceral examination of fetuses in treated groups, compared to control.

A range finding study preceded this study and showed marked maternal toxicity (Unpublished study report, 2014) at the highest dose (1000 mg/kg bw/d) decreased final body weight (-16%) corrected body weight (-

10%) and food consumption was significantly reduced from gestation GD9 until termination (up to -66%). Dose levels of 0, 50, 150, 500 mg/kg bw/d were chosen to be used in the definitive prenatal developmental toxicity study in rat.

PNDTS in rabbits - cf Annex I, study 6

In the GLP-compliant PDTS in New Zealand White rabbit, OECD TG 414 (Unpublished study report, 2019a) carried out with the Substance Reaction products of diphenylamine with nonene, branched by gavage at dose levels of 0, 10, 30 and 100 mg/kg bw/d, the general toxicity concerns mainly the digestive system; in total, reduced defecation was observed in 4 control, 2 low-dose, 11 mid-dose and 20 high-dose females. In comparison to the control group, the mean food consumption of the does in high-dose group (100 mg/kg bw/d) was distinctly and statistically significantly reduced from GD 7-23 (up to -59% in comparison to the control). Overall, the high-dose does consumed 31% less food than the concurrent control does during the treatment period (GD 6-28) and had a non-statistically significant decrease of corrected BW at termination (-4% versus control group) (Table 24). While, there were no test substance-related effects on pre- and the post-implantation losses, numbers of resorptions and viable fetuses, the abortion rate was twice at the highest dose compared to control (2 abortions in control, 4 at the highest doses). The increase was not statistically significant however from the provided HCD in the study report based on 14 studies (2014-2017), only 4 abortions out 350 dams (1, 1%) were reported (the incidence by study was not reported). Furthermore, 1 abortion out of 5 does was also noted in the mid and high doses of the range finding study. However, several studies on effects of caloric restriction alone during pregnancy in rabbit have shown that abortions may occur due to markedly reduced food consumption (Matsuzawa, 1981; Cappon, 2005; Matsuoka, 2006; Lopez-Tello, 2019). Therefore, the drop in food consumption may at least partly contribute to the abortions observed in high-dose group.

Table 24: Effects in dams in the PNDTS in rabbits

Dose mg/kg bw/d	**0**	**10**	**30**	**100**
Mean food consumption/d GD6-28 (g)	118.2	123	114.1	81.6 (-31%)
Mean BW GD6 (g)	3932	3914	3908	3910
Mean BW GD14 (g)	4036	4022	4020	3888*
Mean BW GD28 (g)	4030	4071	4018	3864
Mean corrected BW GD28 (g)	3610.3	3663.4	3591.9	3473.3 (-4%)
Mean BW change GD6-28 (g)	104.4	156.9	110.4	-24
Mean corrected BW change GD6-28 (g)	-322.2	-250.2	-316	-421
Pregnant females N	23	24	24	24
Abortion	2	0	0	**4**

* : p≤ 0.05

A significant decrease in mean fetus weight was observed at the highest dose (-12% males and females combined) associated with a delay in ossification (Table 25).

Two skeletal variations related to a delay in ossification reached statistical significance between the control and the treated groups, i.e. irregular ossification of interparietal (increased and outside the historical control range in the mid- and high-dose groups) and unossified talus (increased and outside the historical control range in the mid- and high-dose groups).

At the high-dose level there was an increase of total external malformations. While not statistically significant, this increase exceeded the HCD range when both expressed as fetal incidence and incidence of affected fetuses/litter. This increase was driven by four fetuses in one single litter (high-dose doe No. 76) having multiple craniofacial malformations, i.e. domed head, cleft palate and small tongue. One of these four fetuses had also a hydrocephaly (visceral malformation) and another one severely malformed skull bone (skeletal malformation). All of them had paw hyperflexion (variation) and empty stomach (unclassified soft

tissue observation). The clustered appearance in a single litter and the similar spectrum of findings in all those four fetuses may suggest a genetic origin although the HCD report only one fetus per litter for each malformation individually or multiple external malformations. Regarding maternal toxicity, food consumption from GD6-28 (107g/d), body weight gain from GD6-28 (+253g) and corrected BW at GD28 (38854.4 g) of doe No. 76 were similar to mean values of controls.

Table 25: Effects in fetuses in the PNDTS in rabbits

Dose mg/kg bw/d		**0**	**10**	**30**	**100**	**HCD % range**
Fetal weights						
Mean fetal weight males and females combined (g)		38.3	38.6	36.3	**33.6***	
Mean fetal weight males (g)		39.1	39.2	36	**33.9***	
Mean fetal weight females (g)		37.6	37.3	36	33.6	
Total external malformations						
Litter Fetuses	N N	20 174	24 189	24 205	20 179	
Fetal incidence	N (%)	1 (0.6)	1 (0.5)	0	**5** (2.8)	0.0 -1.1
Litter incidence	N (%)	1 (5.0)	1 (4.2)	0	2 (10)	0.0 - 9.1
Affected fetuses/litter	Affected fetuses/litter mean%	0.5	0.4	0	**2.4**	0.3 -0.9
Individual fetal external malformations						
Domed head	Pup N (%) Litter N (%) Affected fetuses/litter mean%	1 (0.6)# 1 (5.0) 0.5	0 (0) 0 (0) 0	0 (0) 0 (0) 0	**4** (2.2)## 1 (5) 1.5	Not reported
Cleft palate	Pup N (%) Litter N (%) Affected fetuses/litter mean%	0 (0) 0 (0) 0	0 (0) 0 (0) 0	0 (0) 0 (0) 0	**4** (2.2)## 1 (5) 1.5	0.0-0.6 0.0-4.8 0.0-0.4
Small tongue	Pup N (%) Litter N (%) Affected fetuses/litter mean%	0 (0) 0 (0) 0	0 (0) 0 (0) 0	0 (0) 0 (0) 0	**4** (2.2)## 1 (5) 1.5	Not reported
Umbilical hernia	Pup N (%) Litter N (%) Affected fetuses/litter mean%	0 (0) 0 (0) 0	0 (0) 0 (0) 0	0 (0) 0 (0) 0	1 (0.6) 1 (5.0) 0.4	0.0-0.5 0.0-4.2 0.0-0.5
Open eye	Pup N (%) Litter N (%) Affected fetuses/litter mean%	0 (0) 0 (0) 0	1 (0.5) 1 (4.2) 0.4	0 (0) 0 (0) 0	0 (0) 0 (0) 0	0.0-0.4 0.0-4.5 0.0-0.4
Multiple external malformations	Pup N (%) Litter N (%) Affected fetuses/litter mean%	1 (0.6)# 1 (5.0) 0.5	0 (0) 0 (0) 0	0 (0) 0 (0) 0	4 (2.2)## 1 (5) 1.5	0.0-1.1 0.0-9.1 0.3-0.9
# Same fetus ## Same 4 fetuses from the same doe						
Individual skeletal variations						
Irregular ossification of interparietal	Pup N (%) Litter N (%) Affected fetuses/litter mean%	1 (0.6) 1 (5.0) 0.6	4 (2.1) 3 (13) 1.6	6 (2.9) **6 (25)** **2.9***	6 (3.4) **6 (30)*** **2.6***	0.0-2.2 0.0-18.2 0.0-1.7
Unossified talus; cartilage present	Pup N (%) Litter N (%) Affected fetuses/litter mean%	0 (0) 0 (0) 0	2 (1.1) 2 (8.3) 0.8	0 (0) 0 (0) 0	10 (5.6) 6 (30)* **4.4****	0.0-2.7 0.0-18.2 0.0-2.6

HCD from the study report based on 14 studies (2014-2017)

Generational studies - cf Annex I, study 1 (Reaction products of diphenylamine with nonene, branched) and studies 2, 3, 4 (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene)

- **Prenatal development**

In the OECD TG 421 performed with the substance itself as well as in the OECD TG 421 and EOGRTS performed with its analogue, there were no treatment-related effect on post-implantation loss, number of stillbirth, sex ratio or birth weight. The decreased mean litter size at birth observed in all these studies result from the decreased number of implantation sites. This effect is addressed in the above section dedicated to effects to sexual function and fertility.

In the OECD TG 422 performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, the mean number of delivered F1 pups per dam (litter size) was significantly decreased (-28%) in the high-dose group resulting from both a decreased number of implantation sites and to an increase of post- implantation loss (14.1% vs 0% in controls). It should be noted that the post-implantation in control group is particularly low (0%).

Table 26: Mean number of implantation sites and pups delivered pups and calculated post-implantation loss (Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, OECD TG 422)

		Control	25 mg/kg bw/d	75 mg/kg bw/d	225 mg/kg bw/d
Pregnant females	N	10	10	10	9
Number of litters	N	10	10	10	9
Litter with liveborn pups	N	9	10	10	9
	%	90	100	100	100
Litter with stillborn pups	N	0	1	0	0
Implantation sites	Mean	10.9	11.5	10.5	***9.2***
	Sd	2.4	2.2	1.7	2.3
Pups delivered	N	98	105	94	71
	Mean	10.9	10.5	9.4	**7.9***
	Sd	2.6	2.5	2.0	1.8
Post-implantation loss		0	9	10	14

* p≤0.05
** Caculated by the DS as mean number of implantations – mean number of pups delivered/ mean number of implantations x 100 (no statistical analysis performed)

Postnatal development

Pups viability

No treatment-related effect was observed on live birth, viability and lactation indices the OECD TG 421 up to 5000 ppm (eq. to 443 mg/kg bw/d) performed with]the substance or in the OECD TG 421 up to 3000 ppm (eq. to 271 mg/kg bw/d) and in the EOGRTS up to 1800 ppm (eq. to 166 mg/kg bw/d) performed with the analogue.

In the OECD TG 422 carried out with the analogue, an increase in postnatal loss (8 pups versus 0 in controls) and a correspondingly lower viability index (88.7%** versus 100% in controls) were seen in high-dose females (at 225 mg/kg bw/d) (Table 27). While the majority of the postnatal loss was attributable to one female (5 pups), two other litters were also affected and a possible effect of treatment could not be excluded.

Table 27: Offspring viability in the OECD TG 422 performed Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene

Dose levels mg/kg bw/d	0	25	75	225
Postnatal loss (%)	0	1	0	11.3**
Litters affected	0	1	0	3
Total number of died pups	0	1	0	8
Mean/litter	0	0.3	0	1.7
N of pregnant dams	9	10	10	9
Viability index (%)	100	99	100	88.7**

** p <=0.01

Pups growth (Table 28)

In the OECD TG 421 study (Unpublished study report, 2020a) performed with the substance at 5000 ppm, while birth weight was not altered by treatment, a significant decrease of mean pup body weight was observed from PND 7 to termination (-19% versus controls both sexes combined on PND13) and a significant decrease of mean pup body weight changes from PND1-13 (-22.5% both sexes combined). In dams, at this dose level during the lactation period, a significant decrease of food consumption (-23.5% d1 -> 13) and a significant decrease of body weight were also observed (-17% at termination compared to controls). At 1500 ppm a transitory decrease of mean pup body weight changes was observed during PND 4-7 (up to -13.2% both sexes combined compared to controls) but no significant effect was observed in terminal body weight and on BW changes during PND 1-13.

In the OECD TG 421 study (Unpublished study report, 2020b) performed with the analogue, while birth weight was not altered by treatment, at both mid and high-dose levels, a significant decrease of mean pup body weight was observed from PND 7 to termination (-8% and -26% compared to controls both sexes combined on PND13 at 1000 ppm and 3000 ppm respectively) as well as a significant decrease of mean pup body weight changes. In dams at these dose levels during the lactation period, a significant decrease of food consumption and a significant decrease of body weight were also observed (-8% and -17% at termination compared to controls, at 1000 ppm and 3000 ppm respectively).

In the EOGRTS study (Unpublished study report, 2021) performed with the analogue, pup body weight development of the high-dose offspring (1800 ppm) was affected from PND7 of lactation, as these offspring weighed about 12% and 16% less than control (for F1 and F2 pups respectively) on PND21. The F1 mid-dose offspring (600 ppm) was also affected, though at a lower extent (-6% at PND21), while the F2 mid-dose offspring remained unaffected. Body weights and body weight change of 1800 ppm female P0 and P1 were also affected during the lactation period (body weight of -9% and -12% less than control at the end of lactation in P0 and P1 respectively). No significant effect on body weight was noted in lower dose levels.

Table 28: summary of pups body weight during lactation in the OECD TG 421 studies performed with the substance and its analogue, and the EORGTS performed with the analogue

		Males				Females			
Study performed with Reaction products of diphenylamine with nonene, branched									
OECD 421	**Dose levels (ppm)**	**0**	**500**	**1500**	**5000**	**0**	**500**	**1500**	**5000**
F1 pups	**PND1 BW (g)**	6.8	6.6	6.6	6.6	6.6	6.2	6.3	6.3
	Deviation vs control (%)		-3	-3	-3		-5	-4	-4
	PND13 BW (g)	33.0	31.8	30.4	**26.8****	32.5	31.3	30.2	**26.4****
	Deviation vs control (%)		-4	-8	**-19**		-4	-7	**-19**
Studies performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene									

OECD 421	Dose levels (ppm)	0	300	1000	3000	0	300	1000	3000
F1 pups	**PND1 BW (g)**	7.1	6.4	6.9	6.8	6.8	6.0*	6.5	6.6
	Deviation vs control (%)		-11	-3	-5		-12	-4	-2
	PND13 BW (g)	33.5	31.7	**30.6***	**24.6****	32.5	30.8	**29.8***	**24.0****
	Deviation vs control (%)		-5	**-9**	**-27**		-4	**-8**	**-26**
EOGRTS	**Dose levels (ppm)**	**0**	**200**	**600**	**1800**	**0**	**200**	**600**	**1800**
F1 pups	**PND1 BW (g)**	7.0	7.1	6.7	6.8	6.7	6.8	6.4	6.5
	Deviation vs control (%)		1	-5	-3		1	-5	-3
	PND7 BW (g)	17.1	17.2	**16.0***	**15.3****	16.60	16.70	**15.4***	**15.0****
	Deviation vs control (%)			**-6**	**-11**			**-7**	**-10**
	PND14 BW (g)	32.4	32.2	31.4	**28.4****	31.9	31.9	30.5	**27.9****
	Deviation vs control (%)		0	-3	**-12**		0	-5	**-13**
	PND21 BW (g)	51.4	52.1	49.0	**45****	50.1	50.7	**47.3***	**44.2****
	Deviation vs control (%)		1.0	-5.0	**-12**		1	**-6.0**	**-12**
F2 pups	**PND1 BW (g)**	6.8	7.1	6.8	6.4	6.5	6.7	6.6	6.1
	Deviation vs control (%)		4	1	-6		4	1	-5
	PND7 BW (g)	16.7	17.7	16.7	**14.7****	16.2	17.1	16.2	**14.2****
	Deviation vs control (%)		6	0	**-12**		5	0	**12**
	PND14 BW (g)	32.4	34.1	31.6	**27****	31.7	33.3	30.7	**24.6****
	Deviation vs control (%)		5	-3	**-17**		5	-3	**-17**
	PND21 BW (g)	51.1	53.2	49.7	**42.7****	49.6	51.2	48.1	**41.9****
	Deviation vs control (%)		4	-3	**-16**		3	-2	**-16**

* $p \leq 0.05$, ** $p \leq 0.01$

Nipple retention /areola anlagen (Table 29)

In the OECD TG 421 study (Unpublished study report, 2020a) performed with the substance, the number and percentage of male pups having areolae on PND13, was not impacted by the test substance while in the OECD TG 421 study (Unpublished study report, 2020b) performed with the analogue, an increased incidence of nipple development (100% vs. 79.6% in control) and number of nipples per animal (5.2 to control 2.5) was observed in high-dose males on PND13. The study author considered that this effect could be related to the delay of general development in male pups at this dose level. This statement was further supported by the individual data where the male pups with the highest number of nipples (n=8) had the lowest body weights (19.9 to 22.2 g vs mean body weight of 24.6 g).

In the EOGRTS (Unpublished study report, 2021) performed withthe analogue, on PND13, the percentage of F1 male pups having nipple retention was not influenced by the test substance. Although there was no difference in the percentage of high-dose F2 male pups having nipples/areolae, the mean nipple number was statistically significantly above the concurrent (4.1* vs 2.6 in control group).

Owing to the high background incidence of areola retention on PND13 in this laboratory, all male pups are routinely re-examined for residual areolae on PND20. At this time point, two F1 high-dose male pups of one litter had two nipple/areola anlagen while no nipples were detected in any F2 male pup. Despite the absence of nipple retention on PND20 in the HCD on PND 20, the study author considered the remaining nipple in the two F1 high-dose pups to be a spontaneous event.

No alteration of other sensitive endpoints related to antiandrogenic potential (no effect on sex ratio, anogenital index, sperm parameters, and male reproductive organs in any of the studies) was noted in any of the three studies. Therefore, the increased of nipple number on PND13 observed in high-dose pups in the

OECD TG 421 and in high-dose F2 pups in the EOGRTS is considered to be rather a consequence of a general delay of pup development than a specific effect on hormonal homeostasis.

Regarding the 2 pups exhibiting nipple retention on PND20, one of them was sacrificed on PND22 (precluding any conclusion on whether the effect was transient or permanent) and for the other raised (cohort 2A), nipple retention was not reported for the day prior to necropsy.

Table 29: Nipple retention in males pups

		F1 male pups				F2 male pups				
Study performed with Reaction products of diphenylamine with nonene, branched										
OECD 421	**Dose levels (ppm)**	**0**	**500**	**1500**	**5000**					
PND13	% males with nipple	71.4	65.6	52.8	62.8					
	Affected per litter %	27.9	32.5	46.7	**32.4**					
	Mean nipple number	**0.5**	1	1	**1**					
Studies performed with the anaologue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene										
OECD 421	**Dose levels (ppm)**	**0**	**300**	**1000**	**3000**					**HCDa**
PND13	% males with nipple	80.0	88.0	79.0	100.0					0-97.1
	Affected per litter %	79.6	87.5	80.6	100*					0-97.6
	Mean nipple number	2.5	2.9	3.2	5.2**					0-4.7
EOGRTS	**Dose levels (ppm)**	**0**	**200**	**600**	**1800**	**0**	**200**	**600**	**1800**	**HCDb**
PND 13	% males with nipple	67.0	57.0	79.0	78.0	79.0	63.0	76.0	86.0	8.6-95
	Affected per litter %	66.80	57	76	79	77	59	74	86	8.7-84
	Mean nipple number	2.0	1.5	2.70	2.40	2.60	1.80	2.90	**4.1***	-
PND 20	% males with nipple	0.0	0.0	0	**2**	0.0	0.0	0	0	0-0
	Affected per litter %	0.0	0.0	0.0	**1.4**	0.0	0.0	0.0	0.0	0-0
	Mean nipple number	0.0	0	0	0	0	0	0	0	-

* $p \leq 0.05$, ** $p \leq 0.01$
HCDa from 40 studies (mainly OECD 422 by gavage) 2015-2017
HCDb from 23 studies 2007-2018

- **Development neurotoxicity - cf Annex I, study 3**

In the EOGRTS (Unpublished study report, 2020), cohorts F1C2A (10 animals/sex/dose sacrificed on PND77) and F1C2B (10 animals/sex/dose sacrificed on PND22) were subject to developmental neurotoxicity testing.
No statistically significant or biologically relevant changes were noted in C2A animals during detailed clinical observations, functional observational battery (FOB) and in motor activity measurements.

Auditory startle response (ASR) performed on PND24 in C2A animals

The ASR test presented some limitations: poor reporting of the apparatus used, statistical analysis not in line with the NAFTA guidance (i.e. no mention, or results presented for testing for interactions of sex, trial blocks and treatment) for maximal amplitude and latency as well as a complete absence of any statistical testing for habituation (a variable required under OECD 443). Furthermore, the lack of HCD and positive control increases the possibility the risk of false negative findings taking into account the low statistical

power in DNT investigations. Therefore, the effects from the mid-dose level on the mean maximal amplitude in males as well as habituation (proxy calculation mean Block1 minus mean Block 5) from the mid-dose in males and in males and females combined are considered biologically relevant considering their magnitude in the absence of appropriate statistical analysis (testing for interactions of sex, trial blocks and treatment) and positive controls (Table 30).

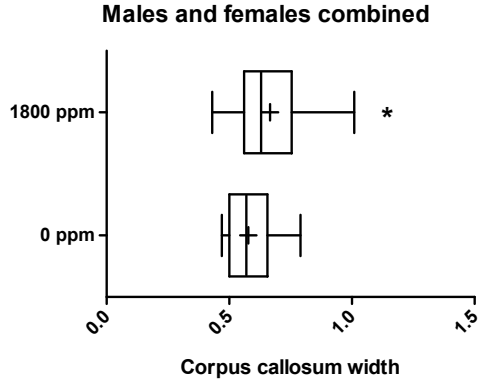
Table 30: Synthesis findings from the ASR expressed as % controls(EOGRTS, Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene)

	Males			Females			Males and females combined		
	200	**600**	**1800**	**200**	**600**	**1800**	**200**	**600**	**1800**
Mean max amplitude Block 1 to Block 5	120	**88**	**81**	112	95	109	116	91	94
Habituation (calculated as Block1 - Block 5)	181	**60**	**43**	54	55	65	118	**58**	**54**

Morphometrics

There was a slight but statistically significant brain length reduction (-3.2%) in high dose C2A males.

No statistically significant changes were reported for the other morphometric measurements. However, the corpus callosum width was non-significantly increased in the high dose group by 17 and 16%, in males and females, respectively (low- and mid-dose levels not analysed). This is a rather large and biologically significant changes in the size of a brain region. Furthermore, a two way anova (sex, treatment) statistical analysis of the data performed by the DS showed that the effect is actually statistically significant. It is noteworthy that the corpus callosum is the principal inter-hemispheric myelinated tract (white matter) and histopathological findings linked to myelin degeneration in the cord white matter were observed in C2A animals.



Histopathological findings

Increased incidence of axonal degeneration in the thoracic cord of male animals (9/10, minimal to mild) was observed in high-dose C2A males with presence of gitter cells (macrophages with foamy cytoplasm interpreted as ingested myelin debris) in 5/10 animals. This finding was further confirmed by an internal fully-blinded peer review (Table 31). This lesion was characterized by digestion chambers with occasional pyknotic nuclei and presence of gitter cells.

According to the study author, the pathogenesis of this finding might be linked to thyroid and suggested that axonal degeneration observed in this study might be an exacerbation of the spontaneous finding as the repair mechanism controlled by thyroid hormones might be impaired.

While not discussed in the study report and not graded, axonal degenerations were also slightly increased in other areas (2 vs 0 tibial nerve degeneration in high-dose males; lumbar cord axonal degeneration and sciatic nerve degeneration in 2 high-dose females vs 0 in controls).

Table 31: Neurohistopathological findings in C2A animals (EOGRTS, Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene)

		Males				**Females**			
	Dose level (ppm)	**0**	**200**	**600**	**1800**	**0**	**200**	**600**	**1800**
	No. of animals	10	10	10	10	10	10	10	10
Thoracic cord	Degeneration, axonal	2	5	4	**9**	4	3	1	4
	• Grade 1	2	5	4	**8**	4	3	1	4
	• Grade 2	1	0	0	1	0	0	0	0
	Gitter cells	1	2	1	**5**	2	1	0	0
	• Present	1	2	1	**5**	2	1	0	0
Lumbar cord	Degeneration, axonal	1	–	–	0	0	–	–	**2**
Prox. sciatic nerve	Degeneration, axonal	1	–	–	1	0	–	–	**2**
Prox. tibial nerve	Degeneration, axonal	**0**	–	–	**2**	**1**	–	–	**1**

A follow-up analysis of thoracic spinal cord of C2B males (sacrificed on PND22) has been performed and no incidence of axonal degeneration was found. Other areas in C2B males were not examined and C2B females were not investigated.

In the absence of effects in C2B males, the study author interpreted the axonal degeneration in thoracic cord of C2A males to be a chronic toxic effect rather than a developmental effect. However, exposure during the developmental period could have contributed to the delayed effects observed in C2A animals on PND77 even if not observed at an earlier time point (PND22). According to RAC note (RAC/62/2022/05) addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes, adverse effects on the nervous system **investigated or detected at any point in the life span** of the organism exposed during the developmental period, covering both prenatal and postnatal development until sexual maturation (determined by preputial separation and vaginal opening), **should be addressed under developmental toxicity (DNT**), even if the exposure had also continued after sexual maturation. Furthermore, in the single available study (i.e. OECD TG 422) where the spinal cord and the sciatic nerve of adults (not exposed during developmental phases) were processed for histopathological investigation, no axonal degeneration was observed in males or females which further supports the involvement of developmental exposure in the occurrence of this lesion.

Thyroid hormones

T4 and TSH values in PND13 male and female pups were not affected by treatment in the OECD TG 421 performed with Reaction products of diphenylamine with nonene, branched or its analogue.

In the EORGTS, T4 and TSH values in PND4 male and female pups were not statistically significantly changed (Table 32). However, in high-dose group, hormone values of only 2 pups of each sex could be measured (due to small litter size) and therefore were not included in the statistical analysis. In both sexes, T4 values from the mid-dose group were decreased as compared to the concurrent controls and outside the HCD range.

TSH values in male and female PND22 pups from the mid-dose group were significantly increased. While TSH values of all test groups were included in the HCD ranges, the clear dose-response relationship strongly support a treatment related finding.

T4 and TSH measurements were not performed in F2 pups which is not in line with OECD TG 443 requirements.

Table 32: Thyroid hormones levels in F1 pups (EOGRTS, Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene)

		Males					Females				
		0	**200**	**600**	**1800**	***HCD range***	**0**	**200**	**600**	**1800**	***HCD range***
PND4 T4 [nmol/L] day 4	Mean	16.02	15.76	14.12	10.14	*18.36 - 36.79*	18.74	17.95	14.89	15.71	*17.88 - 34.51*
	S.d.	5.36	2.78	2.30	2.50		4.62	5.00	2.66	5.49	
	N	9	7	9	**2**		10	8	9	**2**	
	Median	16.76	14.89	13.71	10.14		17.70	17.01	15.37	15.71	
	Deviation vs control [%]		-1.61	**-11.88**	**-36.68**			-4.19	**-20.54**	**-16.16**	
PND4 TSH [µg/L] day 4	Mean	4.43	4.50	4.47	3.98	*3.19 - 5.25*	4.46	4.72	4.74	3.83	*3.05 - 6.36*
	S.d.	0.33	0.44	0.42	0.32		0.41	0.81	0.60	0.81	
	N	9	7	9	2		10	8	9	2	
	Median	4.44	4.35	4.56	3.98		4.52	4.66	4.70	3.83	
	Deviation vs control [%]		1.46	0.83	-10.29			5.96	6.30	-14.07	
PND22 T4 [nmol/L] day 4	Mean	53.52	49.56	54.46	56.49	*50.57 - 71.39*	52.85	49.21	58.31	55.84	*44.85 - 73.70*
	S.d.	10.26	6.66	9.35	9.57		7.22	5.60	13.55	9.29	
	N	10	10	10	10		10	10	10	10	
	Median	53.72	47.84	52.26	57.46		52.89	49.21	52.78	57.71	
	Deviation vs control [%]		-7.39	1.77	5.55			-6.89	10.34	5.67	
PND22 TSH [µg/L] day 4	Mean	3.51	3.78	**4.19***	**4.86****	*3.40 - 4.87*	3.57	3.99	**4.05***	**4.15****	*2.92 - 5.13*
	S.d.	0.57	0.64	0.71	0.63		0.45	0.81	0.52	0.36	
	N	10	10	10	10		10	10	10	10	
	Median	3.54	3.71	4.12	4.88		3.49	3.92	3.91	4.18	
	Deviation vs control [%]		7.78	**19.45**	**38.44**			11.58	**13.23**	**16.23**	

* : $p \leq 0.05$, ** : $p \leq 0.01$
HCD from 10 studies 2015-2019

Overall, from the EOGRTS there are indications supporting developmental neurotoxicity especially in males. In high-dose C2A animals, clear neurohistopathological and neuromorphometric findings were observed. Regarding functional tests, the decrease in maximal amplitude and habituation observed in the ASR from the mid-dose in males are considered biologically relevant in the absence of appropriate statistical analysis and positive controls.

The available data suggest that these neurodevelopment effects could be linked to decreased thyroid hormones (THs) since indications of an alteration of THs levels were noted in pups at the same dose levels and it is well established that THs are essential for fetal and post-natal nervous system development. Due to central nervous system immaturity at birth in rats compared to humans and accelerated postnatal development (i.e.: myelination, glial cell proliferation, synapse formation and axonal spouting begin after birth in rats (Pagnin, 2021)), exposure during postnatal period is also critical in this species as regard DNT.

Regarding dams, at termination T4 levels were not affected by the test substance administration but TSH levels were significantly increased from the low dose and the mid dose in P0 and F1C1A respectively (indicating an alteration of the hypothalamo-pituitary-thyroid-axis, and previous T4 level decrease). However, since no TH measurements during gestation are included in the design of EOGRTS, no final conclusion on thyroid hormone status of pregnant rats can be drawn.

In respect to the observed effects related to nervous system development in this EOGRTS study, a recent review has highlighted the crucial role of THs in myelinisation process in both humans and rodents (Pagnin, 2021). In rodent models, developmental hypothyroidism interferes with neuronal migration, differentiation, and myelination and thyroid hormone regulates genes that control formation of the corpus callosum and neuronal migration (Goodman and Gilber, 2007). Hypothyroxinemia during critical windows may lead to cognitive and hearing deficits (Noyes, 2019).

10.10.6 Comparison with the CLP criteria

CLP Regulation in combination with explanations from the Guidance on the Application of the CLP criteria (ECHA, 2017b) were applied. Any adverse effect of Reaction products of diphenylamine with nonene, branched and of its analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene on development of the offspring, i.e. any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency (Annex I: 3.7.1.4 of CLP Regulation). For potential classification of, classification criteria were analysed accordingly:

Comparison with Category 1 criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect […] on development in humans, or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.

The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

- *Known human reproductive toxicant (1A)*

The classification of a substance in this Category 1A is largely based on evidence from humans.

- *Presumed human reproductive toxicant (1B)*

The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect […] on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Comparison with Category 2 criteria

- *Suspected human reproductive toxicant*

Substances are classified in Category 2 for development when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect […] on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects (CLP Regulation).

There are no human data to support classification in Category 1A.

Effects from the available data set relevant for development classification:

(1) Death of the developing organism: Post-implantation loss and fœtal viability were not affected by treatment with Reaction products of diphenylamine with nonene, branched, in both rat and rabbit PNDTSs. In the main study in rabbits, increased number of abortions (4 vs. 2 in control) was observed at the high dose level (1 abortion out of 5 does was also noted in the mid and high doses of the range finding study). While a direct effect cannot be excluded, the severe drop in food consumption GD 7-23 (up to -59% in comparison to the control group) may partly be involved, as supported by studies on caloric restriction during pregnancy in rabbit (Matsuzawa, 1981; Cappon, 2005; Matsuoka, 2006; Lopez-Tello, 2019).

In the generational studies performed with Reaction products of diphenylamine with nonene, branched and its analogue, the decreased litter size at birth observed in all studies results from the decreased number of implantation sites; this effect is addressed in the section dedicated to effects to sexual function and fertility. Post-implantation loss and fœtal viability were not affected in any of

the generational studies except in the OECD TG 422 performed with the analogue where the mean post-implantation loss in the high-dose dams was increased compared to controls (14% vs 0%). It is noteworthy that the value in controls of the study was particularly low.

Furthermore, the viability index was significantly reduced in this OECD TG 422 at the high-dose level (88.7% at 225 mg/kg bw/d versus 100% in controls). However, no treatment-related effect was observed on live birth, viability and lactation indices in the OECD TG 421 performed with Reaction products of diphenylamine with nonene, branched up to 5000 ppm (eq. to 443 mg/kg bw/d) or in the OECD TG 421 up to 3000 ppm (eq. to 271 mg/kg bw/d) and in the EOGRTS up to 1800 ppm (eq. to 166 mg/kg bw/d) performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces death of the developing organism.

→ While post-implantation viability was not affected in the PNDTSs in rats and rabbits, the increased number of abortions in rabbits provide **slight evidence that Reaction products of diphenylamine with nonene, branched could induce death of the developing organism**, however these abortions may be partly related to the severe drop in food consumption observed at this dose level, as demonstrated in published studies on caloric restriction during pregnancy in rabbit.

Based on the available data, there is **slight evidence that Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** induces death of the developing organism based on reduction of pre- and postnatal viability in the high-dose animals of the OECD TG 422, but these effects were not reproduced in the other generational studies performed with the substance and its analogue.

(2) Structural abnormality: Reaction products of diphenylamine with nonene, branched was not teratogenic in rat. In rabbit, an increase of external malformations was observed at the top dose (four fetuses in one single litter with multiple external malformations). The clustered appearance limited to one litter with similar spectrum of findings (i.e. craniofacial malformations consisting in domed head, cleft palate and small tongue in all those four fetuses) suggest rather a genetic origin than a treatment-related effect. Statistically significant increases of two skeletal variations (i.e. irregular ossification of interparietal and unossified talus) were also observed at this dose-level as well as a general delay in ossification.

In the high-dose C2A animals of the EOGRTS performed with the analogue, neurohistopathological findings (increased incidence of axonal degeneration in the thoracic cord 9/10 males as well as slight increased incidence of axonal degeneration in other area in males and females) and neuromorphometric changes (decreased brain length in males and increased corpus callosum width in both males and females) were observed. Such parameters were not investigated for Reaction products of diphenylamine with nonene, branched.

The increased of nipple number on PND13 observed in high-dose pups in the OECD TG 421 and in high-dose F2 pups on PND13 in the EOGRTS performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene is considered to be rather a consequence of a general delay of pup development than a specific effect on hormonal homeostasis in the absence of alteration in other sensitive endpoints related to antiandrogenic potential.

→ Based on the available data, **there is clear evidence that the anologue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces abnormalities in the central nervous system** in animals exposed during the developmental period. Such parameters were not investigated for Reaction products of diphenylamine with nonene, branched.
In the PNDTS in rabbits performed with **the substance, delay in ossification was noted in the presence of maternal toxicity**. Regarding the cluster of four fetuses with multiple common malformations from a single litter of the high-dose group, a genetic origin is considered more likely than a treatment-related effect.

(3) Altered growth:

In rabbits exposed to Reaction products of diphenylamine with nonene, branched, a significant decrease in fetus weight (-12%) was observed at the highest dose (100 mg/kg bw/d) associated with delays of ossification. At this dose level, does consumed 31% less food than the concurrent control does during the treatment period (GD6-28), showed marked reduced defecation and had slight reduced corrected body weight.

Postnatal growth was also altered from PND7 up to weaning in the high-dose groups of the generational studies performed with the substance (OECD TG 421) or its analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EOGRTS, OECD TG 421). At these dose levels, effects on body weight of similar magnitude were observed in females at the end of the lactation period.

→ Based on the available data, **there is evidence that both Reaction products of diphenylamine with nonene, branched and its analogue alter growth of the developing organism** at dose levels also affecting maternal/parental weight.

(4) Functional deficiency:

In the EOGRTS performed with the analogue, despite limitations of the auditory startle response test, effects from the mid-dose level on mean maximal amplitude in males as well as decreased habituation from the mid-dose in males and in males and females combined are considered biologically relevant in the absence of appropriate statistical analysis (testing for interactions of sex, trial blocks and treatment) and positive controls.

→ Based on the available, **there is some evidence that Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces functional deficiency**. However, the deficiencies of the test limit the reliability on the effects observed in auditory startle response. Such parameters were not investigated for Reaction products of diphenylamine with nonene, branched.

Overall, **the main critical effects** are those linked to **neurodevelopmental toxicity** observed in C2A animals (especially in males) of the EORGTS performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. In high-dose C2A animals, **clear severe neurohistopathological findings** (increased incidence of axonal degeneration in the thoracic cord 9/10 males as well as slight increased incidence of axonal degeneration in other area in males and females) and neuromorphometric changes (decreased brain length in males and increased corpus callosum width in both males and females) were observed. No axonal degeneration was found in C2B males (sacrificed on PND22). However, exposure during the developmental period could have contributed to the delayed effects observed in C2A animals on PND77 even if not observed at an earlier time point (PND22). Furthermore, in the available OECD TG 422, no axonal degeneration was observed in males or females (not exposed during developmental phases) which further supports the involvement of developmental exposure in the occurrence of this lesion.

The reliability of the neurohistopathological findings from the EOGRTS compliant to GLP and to current OECD TG, make the quality of evidence convincing for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene to induce developmental neurotoxicity.

Despite limitations of the auditory startle response test, effects from the mid-dose level on mean maximal amplitude in males as well as decreased habituation from the mid-dose in males and in males and females combined are considered biologically relevant and further support neurodevelopmental toxicity.

In the absence of specific neurodevelopmental testing with Reaction products of diphenylamine with nonene, branched and considering the read-across for reproductive toxicity from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) to Reaction products of diphenylamine with nonene, branched (target substance) acceptable with high confidence as detailed in section 10.10.11, the evidence coming from the source Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene substance is also considered convincing for Reaction products of diphenylamine with nonene.

The available data suggest that neurodevelopment effects could be linked to decreased thyroid hormones (THs). The similarity of thyroid effects induced by both substances further support a read-across approach for neurodevelopmental effects.

With respect to the severity of the above mentioned neurodevelopmental effects category 1B is considered triggered.

Other effects of concern (death of developing organism) consist in the increased number of abortions observed in rabbits exposed to Reaction products of diphenylamine with nonene, branched; while ,a direct effect cannot be excluded, the severe drop in food consumption might be partly involved. Regarding the increased post-implantation and post-natal losses observed at the high dose level of the OECD TG 422 performed with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, the fact that such effects were not reproduced in any of the other generational studies (including an EOGRTS), **makes the evidence less convincing**.
Regarding malformations, the cluster of four fetuses with multiple common malformations from a single litter of the high-dose group in the rabbit PNDTS, **a genetic origin is considered more likely than a treatment-related effect.**
Other supportive findings, consist in effects on fetal growth (-12%) in the PNDTS in rabbit performed with the substance associated with delay in ossification in the presence of maternal toxicity. Postnatal growth was also altered from PND7 up to weaning in high-dose groups of the generational studies performed with the substance and its analogue. However, with respect to the nature of those effects and the concurrent decreased body weight of similar magnitude in lactating dams, these effects are considered of less concern.

Therefore, laying down the criteria of CLP Regulation, classification of Reaction products of diphenylamine with nonene, branched with Category 1B for effects on development (H360D) is considered warranted primarily based on the neurodevelopmental effects.

10.10.7 Adverse effects on or via lactation

No human or animal dedicated studies are available.

The results of the studies performed with Reaction products of diphenylamine with nonene, branched (OECD TG 421 study) and with the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (OECD TG 421 study and the EOGRTS) are available in Table .

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

There are no experimental data specifically related to adverse effects on or via lactation nor specific data showing that the Substances or their constituents may be present in breast milk. However, transfer in milk can be suspected based on PC data (high lipophilia).

Some information can be derived from the OECD TG 421 studies and the EOGRTS (see sections above). While fetal weight was not affected by treatment, decreased body weight of pups were found in high dose groups of all the three studies from PND7, supporting effects on or via lactation. However, no data on transfer in the milk or on the quality of the milk is available. Furthermore, significant decreases of body weight in dams during the lactation period were also observed at the same dose levels which may indirectly impair milk production as a nonspecific secondary effect.

Regarding developmental neurotoxicity (DNT), based on the EOGRTS design (continues exposition during prenatal and postnatal periods), it cannot be distinguished if the observed adverse effects were caused by gestational exposure and/or by lactation exposure.

10.10.9 Comparison with the CLP criteria

CLP Regulation in combination with explanations from the Guidance on the Application of the CLP criteria (ECHA, 2017b) were applied. For potential classification of Reaction products of diphenylamine with nonene, branched, classification criteria were analysed accordingly:

> *…"However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the:*
>
> *(a) human evidence indicating a hazard to babies during the lactation period; and/or*
>
> *(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or*
>
> *(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."*

No human data are available.

While adverse effects in offspring were observed (decreased body weight) in the generational studies no specific data are available to conclude there are due to transfer in the milk or adverse effect on the quality of the milk and no ADME studies are available. Regarding DNT effects, due to the EOGRTS design it cannot be distinguished if the observed adverse effects result from gestational exposure and/or by lactation exposure.

Therefore, no additional labelling of the two substances for "adverse effects on or via lactation" is considered warranted.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

- **Sexual function and fertility**

Based on effects on fertility (i.e. lower numbers of implantation sites with subsequently smaller litter sizes, decreased ovary weight and altered cyclicity) consistently observed through the reliable dataset, a classification for effects on sexual function and fertility (Cat. 1B, H360F) is considered warranted for Reaction products of diphenylamine with nonene, branched.
"*Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous*" (EC, 2008).

For the endpoint "sexual function and fertility" the generic concentration limit of 0.3% ("group 2", medium potency) can be applied for the following reasons:

No effects on fertility were observed in the low-dose group of any of the available studies (lowest dose tested 200 ppm equivalent to 18 mg/kg bw/d in the EOGRTS) meaning that no effect were noted at doses below 4 mg/kg bw/d (i.e., effective dose with a 10% effect level above the background (ED_{10}) is above 4 mg/kg bw/d).

Effects on fertility were observed from 1500 ppm (eq. to 133 mg/kg bw/d) to 5000 ppm (eq. to 443 mg/kg bw/d) of Reaction products of diphenylamine with nonene and from 600 ppm (eq. to 54 mg/kg bw/d) to 3000 ppm (eq. to 271 mg/kg bw/d) of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene.

Therefore, the ED_{10} is between 4 and 400 mg/kg bw/d and the substance is assigned to "group 2", medium potency and the generic concentration limit should be applied.

- **Developmental toxicity**

Based on the severe neurodevelopmental effects (i.e. neurohistopathological and neuromorphometric findings supported by changes in auditory startle response) observed in the EOGRTS performed with the analogue and to a lesser extent effects on viability (less convincing evidence coming from the rabbit PNDTS (abortion) and OECD TG 422 with the nalogue) effects on pups growth in all the generational studies (less concern) a classification for effects on development (Cat. 1B, H360D), is considered warranted for Reaction products of diphenylamine with nonene, branched

No effects on development were observed in the low-dose group of any of the available studies (lowest dose tested 10 mg/kg bw/d of Reaction products of diphenylamine with nonene, in the rabbit PNDTS and 200 ppm equivalent to 18 mg/kg bw/d of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene in the EOGRTS) meaning that no effect were noted at doses below 4 mg/kg bw/d (i.e., effective dose with a 10% effect level above the background (ED_{10}) is above 4 mg/kg bw/d).

Effects on development were observed from 100 mg/kg bw/d (rabbit PNDTS) to 5000 ppm (eq. to 443 mg/kg bw/d, OECD TG 421) of Reaction products of diphenylamine with nonene, the sunstance and from 600 ppm (eq. to 54 mg/kg bw/d, EOGRTS) to 3000 ppm (eq. to 271 mg/kg bw/d, OECD 421) of the analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene.

Therefore, the ED_{10} is between 4 and 400 mg/kg bw/d and the two substances are assigned to "group 2", medium potency and the generic concentration limit should be applied.

- **Lactation**

In the absence kinetic data and specific studies allowing to distinguish if the observed adverse effects in offspring were caused by gestational exposure and/or by lactation exposure, **no additional labelling of the the substance for "adverse effects on or via lactation" is considered warranted.**

10.10.11 Assessment of the reliability of the read-across (in line with the ECHA Read-Across Assessment Framework, RAAF)

The SDPAs have been studied as a case study for grouping and read-across by OECD (OECD, 2016a). based on a previous work carried out by Health Canada (ECCC, 2017).

OECD has defined four different subgroups:

- Subgroup 1: Monoalkylated SDPAs
- **Subgroup 2: SDPAs with variable number of alkyl substitutions including both Reaction products of diphenylamine with nonene, branched and its analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene**
- Subgroup 3: Dialkylated SDPAs
- Subgroup 4: SDPAs with variable number of phenyl substitutions
- SDPA mixture with variable number of alkyl and phenyl substitutions are not considered part of any subgroup.

Collaborative approach (COLLA) pilot project for SDPA **concluded that for human health-related aspects of the assessment, the read-across was possible only among SDPAs that belong to the same OECD subgroup (ECHA, 2018)**.

Reaction products of diphenylamine with nonene, branched, is currently under Reach evaluation in the context of the CORAP 2021 (FR being the evaluating Member State Competent Authority (the eMSCA)). In the registration dossier, a read-across from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, was initially proposed by the registrants to fulfil REACH Annex X, Section 8.7.3., before the registrants submitted a testing proposal ((17/12/2021)) for an EOGRTS performed with the substance itself. The eMSCA does not support the need to carry out further testing considering, that available data are adequate to support a classification for reproductive toxicity Category 1B forReaction products of diphenylamine with nonene, branched, based on studies with the substance itself and read-across across

approach from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene as formerly proposed by the registrant.

An assessment of the reliability of the read-across in line with the ECHA RAAF has been further performed by the dossier submitter in the context of this CLH report.

An analogue approach has been selected by the DS since this read-across concerns two structurally similar substances. Furthermore, the read-across hypothesis is based on different compounds with qualitatively similar properties (RAAF scenario 2)[7].

In the context of this CLH report, the following read-across applied to **endpoints related to reproductive toxicity**:

- Bridging Reproduction/Developmental Toxicity Screening Tests (OECD TG 421) performed with both Reaction products of diphenylamine with nonene, branched and Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene from the same laboratory reported the same pattern of effects, indicating the same biological targets after oral exposure and are strongly supporting the read-across approach for reproductive toxicity.
- The extended one-generation reproductive toxicity study (EOGRTS) performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) can be used to predict effects that would be observed in a study with Reaction products of diphenylamine with nonene, branched (target substance) if it was to be conducted.

Assessment elements (AE) for scenario 2 of ECHA's read-across assessment framework were investigated.

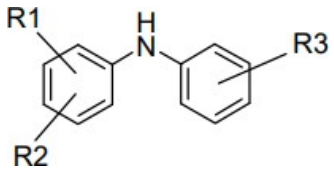
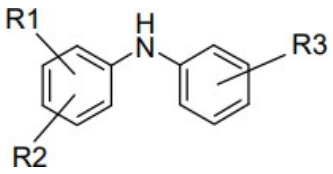
1- Characterisation of the two substances

Both the target and the source substances are organic UVCB substances. They are mixture of mono-, di- and tri-alkylated diphenylamines. The molecular mass distribution and structures of their inherent constituents are reported are in Table 33.

Table 33: Identity of the two substances

	Target substance	Source substance
Name	Reaction products of diphenylamine with nonene, branched	Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene
Trade name	Irganox L67	Irganox L57
CAS	-	68411-46-1
EC	-	270-128-1
Molecular mass distribution	>=295.5<=421.7	>=225.3<=393.6
Molecular formula	C21H29N - C30H47N (main constituents)	C16H19N - C28H43N (main constituents)

[7] In the registration dossier, the registrants initially proposed a read-approach based on analogue approach, Scenario 1 (i.e. hypothesis based on (bio) transformation to common compound(s)), while no emprirical data are availble on metabolism in mammals to support this scenario. Since brindging OECD TG 421 studies are available, Scenario 2 (i.e. hypothesis based on different compounds which have the same type of effect(s)), has been considered more appropriate by the DS.

Structural formula	 R1 = H or iso-nonyl R2 = iso-nonyl R3 = iso-nonyl	 R1 = tert-butyl or iso-octyl R2 = H or tert-butyl or iso-octyl R3 = H or tert-butyl or iso-octyl
Typical concentration	Complete (100%) reaction product of Benzenamine- N-phenyl- with nonene (branched)	Complete (100%) reaction product of Benzenamine- N-phenyl, with 2,4,4-trimethylpentene

Manufacture

The manufacture and processes are detailed in the confidential Annex.

Impurities

The constituents of the two substances and the compositions of the two substances are presented in the tables 2, 3, 4 and 5 of the confidential Annex There are no impurities that have been identified that would lead to substance classification for reproduction. As the manufacturing process is similar for both substances, and the starting material is equivalent, it is expected that the impurities profile are comparable.

2- Link of structural similarity and differences with the proposed prediction

Reaction products of diphenylamine with nonene, branched and its analogue Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene are both UVCB mixtures of mono-, di- and tri-alkylated diphenylamine. Alkyl-groups are highly branched and have a length of four to nine carbon atoms. The major fractions are mono, di and tri-alkylated DPA. It is reported that alkylation is frequently, yet not exclusively, occurring at the para-position. The substances share the following functional groups:

- phenylamine group
- dialkyl-diphenyl core
- mono-alkyl-diphenyl core
- terminal tert-butyl or isobutyl groups

There are no other functional groups present in any constituents of both substances which are known to exert toxicological effects.

3- Reliability and adequacy of the source studies

The EOGRTS with the source substance (reliability 1) has been performed according to the current version of OECD TG 443 and under GLP conditions. The test material used in this study represents the substance as described in terms of purity and impurities

The same considerations also apply to the two comparative OECD TG 421 tests performed with Reaction products of diphenylamine with nonene, branched (target substance) and Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) and to the OECD TG 422 performed with the source substance.

Overall studies are considered adequate for the purpose of classification and labelling.

4- Compounds the test organism is exposed to

No experimental toxicokinetic data regarding the absorption, distribution, metabolism and excretion of any of two substances or their constituents are available.

Table 34: Physicochemical data of the two substances

	Target substance Reaction products of diphenylamine with nonene, branched **(CAS -, EC)**	**Source substance** Reaction products of diphenylamine with 2,4,4-trimethylpentene **(CAS 68411-46-1, EC 270-128-1)**
Grouping SDPAs	UVCB with number of alkyl substitutions. Subgroup 2 (OECD, 2016a)	UVCB with variable number of alkyl substitutions. Subgroup 2 (OECD, 2016a)
Molecular formula	$C_{21}H_{29}N$ - $C_{30}H_{47}N$ (main constituents)	$C_{16}H_{19}N$ - $C_{28}H_{43}N$ (main constituents)
Molecular weight range (g/mol)	≥ 295.5 ≤ 421.7	≥ 225.3 ≤ 393.6
Water solubility	<5 µg/L at 20 °C at pH 6.1	No reliable measured value
LogKow (calculated with Program KOWWIN based on their constituents)	Log Pow ≥7.5	log Pow ≥ 5

Comparison of the available physicochemical data shows that the physicochemical properties of the target and the source substance are similar. Indeed, both substances have a molecular weight range of > 225 <425 grams/mol and a logPow > 5. In addition, the two substances are characterized by a low water solubility. It is noteworthy that the measured value for Reaction products of diphenylamine with 2,4,4-trimethylpentene, available in the registration dossier is not reliable since the method implemented is not appropriate and far lower water solubility is expected based on the water solubility of its constituents.

In the OECD report on SDPA (OECD, 2016a) comparative predictions were generated for the different constituents using ACD Percepta PK Explorer (ACD 2012).The constituents monoalkylDPA (butyl-, octyl- and nonyl-) as well as the dibutylDPA have lower molecular weight, lower LogKow and higher water solubility compared to the other constituents; the bioavailability estimated in the OECD report (2016a) was 39.5%, 28.88%, 21,53% and 21.8% for butyl-, octyl- nonyl- and dibutylDPA, respectively, while the estimated bioavailability of the other constituents was far lower. Based on their respective composition (refer to the confidential annex , for the boundary compositions of the two substances) and the comparative estimated oral bioavailability data of the constituents, Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene might be slightly more bioavailable than Reaction products of diphenylamine with nonene, branchedSubstance. In the bridging studies OECD TG 421, similar magnitude of effects was observed at a dose level slightly lower : 3000 ppm [eq.to 260/271 mg/kg bw/d in M/F] of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene versus 5000 ppm [eq.to 407/443 mg/kg bw/d in M/F] of Reaction products of diphenylamine with nonene, branched.While the minimal difference may reflect a slightly higher bioavailibility of the source substance, the dose difference is very small and the bioavialibilty may vary due to UVCB nature of the two substances.

Table 35: Modelled LogKow, water solubility and toxicokinetic parameters of the different constituents of two UVCBs (OECD, 2016a).

UVCB	**Constituents**	**PM**	**LogKow**	**Water solubility**	*** Oral bioavailability(%F)**	**Cmax***	**Tmax***	**AUC_{0-inf}***
				mg/L		**µg/ml**	**h**	**µg.h/ml**
Target susbtance Reaction products of diphenylamine with nonene, branched R = highly branched nonyl-	Monononyl DPA	295.5	7.6	$4.7\ 10^{-3}$	21.53	0.13	6.26	1.62
	Dinonyl DPA	421.7	11.9	$1.6\ 10^{-7}$	0.06	0.0002	9.38	0.006
	Trinonyl DPA	547.9	16.2	$5.5\ 10^{-9}$	no data	no data	no data	no data
	Constituents C22H31N - C29H45N	309.5-407.7	no data	no data	no data	no data	no data	no data

Source susbtance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene R = tert-butyl or tert. Octyl	Monobutyl DPA	225.3	5.2	1.17	39.35	0.43	5.87	5.71
	Dibutyl DPA	281.4	7.1	0.014	28.88	0.2	6.33	2.88
	Monooctyl DPA				21.8	0.15	6.46	2.17
	Monobutyl monooctyl DPA	337.5	9	1.5×10^{-4}	11.04	0.07	8.05	1.67
	Tributy DPA				no data	no data	no data	no data
	Dioctyl DPA	393.6	10.8	1.93×10^{-6}	0.43	0.002	9.6	0.06
	Dibutyl monooctyl DPA				no data	no data	no data	no data

* Each SDPA was modelled using an oral dose of 5 mg/kg bw (70kg human) with Percepta PK Explorer (OECD, 2016a)

In the OECD report on SDPA, a metabolic simulator (OASIS TIMES (v2.27.5) in vivo Rat Metabolism Simulator (v5.05)) was used to predict the metabolism of the different constituents which supports that similar breakdown products and metabolites are expected for both substances.

In conclusion, there is no large difference expected regarding toxicokinetics. Based on the similar structure and functional groups, similar breakdown products and metabolites are expected.

5- Common underlying mechanism, qualitative aspects

Similar results on fertility and reproductive performance have been observed in the OECD TG 421 studies, performed with boththe source and the target substances. The same pattern of effects were observed with the two substances consisting in lower numbers of implants with subsequently smaller litter sizes, decreased ovary weight and altered cyclicity. The same effects were reproduced in the EOGRTS carried with the source substance despite lower doses tested compared to those in OECD TG 421 study (Table 34).

Effects observed for other properties were also similar with both substances. The main target organs being liver and thyroid.

The metabolome profile in plasma of fasted Wistar rats treated with Reaction products of diphenylamine with nonene, branched and Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene was similar for both compounds in terms of significance and direction of change. Applying a Pearson-based statistical correlation of the whole plasma metabolome, the two substances were the most similar compounds in terms of metabolome changes out of a data base consisting of more than 750 substances. Results showed that mainly lipid metabolism was affected with increased complex lipids, fatty acids and derivatives. However, the substances did not show matches which would give a clear indication for a certain toxicological mode of action (profile comparison to the established specific metabolite patterns present in MetaMap® Tox).

When screened for alerting groups with DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus software (DEREK Nexus 2023 (v6.2.1) from the LHASA Group, the different constituents of Reaction products of diphenylamine with nonene, branched and Reaction products of diphenylamine with 2,4,4-trimethylpentene highlighted no alerts for any of endpoints including development and reproductive toxicity (DART) ones when stated by the model at a reasoning level of at least equivocal.

Profilers from OECD (Q) SAR Toolbox v4.5 2023 did not find DART alert for any of the different constituents of the two substances.

While mechanistic explanation is lacking, the two substances induced the same effects on fertility in the bridging studies. Regarding other properties, both the two substances induced liver and thyroid toxicity with very similar pattern. No other specific effects have been found from the available dataset nor alert in QSAR analysis of the different constituents.

Regarding neurodevelopmental effects induced bythe source substance, data suggest that decreased TH levels could be involved. While DNT was not investigated for the target substance, neurodevelopmental effects could be expected in respect to the similar effects on thyroid observed with the two substances.

Regarding neurodevelopmental effects induced by the source substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene , data suggest that decreased TH levels could be involved. While DNT was not investigated for the source Reaction products of diphenylamine with nonene, branched substance, neurodevelopmental effects could be expected in respect to the similar effects on thyroid observed with the two substances.

Table 36: Available data on reproductive and prenatal developmental toxicity

	Target substance Reaction products of diphenylamine with nonene, branched **(CAS -, EC)**	**Source substance** Reaction products of diphenylamine with 2,4,4-trimethylpentene **(CAS 68411-46-1, EC 270-128-1)**
	Bridging studies	
Method	**OECD TG 421 / GLP (10-week pre-mating treatment) - cf. Annex I, study 1**	**OECD TG 421 / GLP (10-week pre-mating treatment) - cf. Annex I, study 2**
Dosages	0, 500, 1500, 5000 ppm Eq. to: 0, 40/44, 122/133 and 407/443 mg/kg bw/d in M/F	0, 300, 1000, 3000 ppm Eq. to: 0, 26/28, 87/95 and 260/271 mg/kg bw/d in M/F
Study duration	10 weeks pre-mating, during gestation, PND 13 recovery group maintained for additional 14 days	10 weeks pre-mating, during gestation, PND 13
Species	rat (Wistar)	rat (Wistar)
Result	<u>Fertility</u> 5000 ppm ↓ nb of implantation sites (-31%) and consequently ↓ nb of pups delivered/live born pups (-31%) ↓ ovaries absolute weight (- 40%) ↑ *Non-statistically significant estrous cycles length (4.3 days vs control 4.0 days).* ↑ *of mean nb of days in diestrous stage* 1500 ppm ↓ nb of implantation sites (-24%) and consequently ↓ nb of pups delivered (-19%) ↓ ovaries absolute (-18%) weight 500 ppm No effect <u>Development</u> 5000 ppm ↓ mean terminal pup BW on PND13 (-19 % compared to controls both sexes combined) 1500 ppm No effect 500 ppm No effect <u>Systemic toxicity</u> 5000 ppm ↓ Final BW in M (-12% compared to controls) and ↓ BW in F at the end of the premating period (-11% compared to controls) 1500 ppm ↓ BW compared to controls in F at the end of gestation (- 9% compared to controls), *at the beginning of lactation (-6%)* and at termination (-7%). No effect on	<u>Fertility</u> 3000 ppm ↓ nb of implantation sites (-36%) and consequently ↓ nb of pups delivered/live born pups (-34%) ↓ ovaries absolute weight (-25%) ↑estrous cycles length (4.7 days vs. control 4.0 days). ↑ of mean nb of days in diestrous stage 1000 ppm *Non-statistically significant ↓ nb of implantation sites (-14%)* 300 ppm No effect <u>Development</u> 3000 ppm ↓ mean terminal pup BW on PND13 (-26% compared to controls both sexes combined) ↑incidence of nipple development 1000 ppm ↓ mean terminal pup BW on PND13 (-8% compared to controls both sexes combined) 300 ppm No effect <u>Systemic toxicity</u> 3000 ppm ↓ Final BW in M (-9% compared to controls) and ↓ BW in F at the end of the premating period (-9%) at the end of gestation (-17%), at the beginning of lactation (-9%) and at termination (-15%) compared to controls. 1000 ppm

	BW during the premating period. 500 ppm No effect on BW Target organs: liver and thyroid	↓ BW compared to controls in F at the end of the premating period (-7%), at the end of gestation (-8%), *at the beginning of lactation (-4%)* and at termination (-8%). 300 ppm No effect on BW Target organs: liver and thyroid
Reference	Unpublished study report, 2020a	Unpublished study report, 2020b
Extended one-generation reproductive toxicity study (EOGRTS)		
Method	**Read-across**	**OECD TG 443/GLP - cf. Annex I, study 3**
Dosages		0, 200, 600, 1800 ppm Concentrations were reduced to 50% during lactation to keep doses consistent Eq. to: 0, 18, 54 and 167/166 mg/kg bw/d in M/F
Study duration		P0 animals: 10 weeks pre-mating, 2 weeks mating, PND21 P0: 17 weeks (♂), 19 weeks (♀) F1: 3 weeks (Cohort 2B), 11 weeks (Cohort 2A), 13 weeks (Cohort 1A), 19-25 weeks (Cohort 1B) F2: 3 weeks
Species		rat (Wistar)
Result		<u>Fertility</u> 1800 ppm ↓ nb of implantation sites (-15% in P0, -17% in P1) and consequently ↓ nb of pups delivered/live born pups (-20% in P0, -18% in P1) ↓ ovaries absolute weight (-13% in P0 -12% in P1) ↑estrous cycles length (4.3 days vs. control 4.0 days) in P1 ↑ of mean nb of days in diestrous stage. 600 ppm P1 *non-statistically significant ↓ nb of implantation sites (-9%)* and consequently ↓nb of pups delivered (-10%). 200 ppm No effect <u>Development</u> 1800 ppm ↓ mean number of F1 and F2 pups delivered/dam (consequence of the lower nb of implants). No effect on live birth, viability and lactation indices. ↓ F1 and F2 pups BW compared to controls PND7-PND21 in M, F and M&F combined (at weaning: -12.5% F1, -16% F2) Nipple retentions: F1: at PND20, 2 pups from the same litter with 2 nipple/areola anlagen (vs 0 in controls and HCD). F2: ↑ mean nipple number at PND13 (No persistence: 0 nipple/areola at PND20) In F1 delay to reach preputial separation (43.5 vs 42.1 days in control) and vaginal opening (31.8 vs 31.0 days in control). Considered secondary to delayed

		general development (weight at puberty onset similar in all groups) ↓ mean terminal pup BW on PND13 (-26% both sexes combined) ↑incidence of nipple development 600 ppm ↓ Mean number F2 pups delivered/dam (consequence of the lower nb of implants). ↓ F1 pups BW compared to controls PND7-PND21 M&F combined (at weaning: -5.4%) 200 ppm No effect <u>DNT</u> 1800 ppm Axonal degeneration in cohort 2A M (thoracic spinal cord and tibial nerve) and 2A F (lumbar spinal cord and sciatic nerve), ↑corpus callosum width in 2A M&F (MD and LD not performed) and slight ↓ brain length 2A M. *ASR:* ↓mean maximal amplitude in M and ↓ *habituation in M and M & F combined (statistical analysis not performed)* 600 ppm *ASR:* ↓mean maximal amplitude in M and ↓ *habituation in M and M & F combined (statistical analysis not performed)* 200 ppm No effect <u>Systemic toxicity</u> 1800 ppm No effect on BW in P0 M. ↓ final BW compared to controls in P1 M (-6%). ↓ BW compared to controls in F at the end of the premating period (-8% in P0 and -9% in P1), at the end of gestation (-12% in P0 and –13% in P1), at the end of lactation (-9% in P0 and –12% in P1) 600 and 200 ppm No effect on BW in any generations Target organs: liver and thyroid
Reference		Unpublished study report, 2021
Reproductive screening assay		
Method	**Read-across**	**OECD TG 422 / GLP – cf. Annex I, study 4**
Dosages		0, 25, 75, 225 mg/kg bw/d
Study duration		28 days (males), ca 53 days (females)
Species		rat (Wistar)
Result		<u>Fertility</u> 225 mg/kg bw/d *non-statistically significant ↓ of mean number of implantation sites (-16%)* 75 and 25 mg/kg bw/d No effect

		Development 225 mg/kg bw/d ↓ nb of pups delivered/live born pups (-28%) ↑ *post-impost-implantation loss (14% vs 0% in controls)* ↑ in postnatal loss (8 in 3 litters versus 0 in controls) and correspondingly↓ viability index (88.7% vs 100% in controls). 75 and 25 mg/kg bw/d No effect Systemic toxicity No effect on BW or food consumption at any dose levels. Target organs: liver and thyroid
Reference		Unpublished study report, 2014b
Prenatal developmental toxicity		
Method	**OECD TG 414 / GLP – – cf. Annex I, study 5**	**No data**
Dosages	0, 50, 150, 500 mg/kg bw/d	
Study duration	Gestation days 6-19	
Species	rat (Wistar)	
Result	Development Development No effect on post-implantation survival at any dose level. 500 mg/kg bw/d *Non-statistically significant* ↑ *number of small fetuses* (fetal weight < 2.7 g) 10 in 3 litters vs 2 in 2 litters in controls Visceral and skeletal examination of fœtus : no effect 50 and 150 mg/kg bw/d No effect Maternal toxicity 500 mg/kg bw/d ↓ Final body BW (-7%) Final corrected BW (-6%) and corrected BW gain GD6-GD20 (-52% compared to controls). ↓ Food consumption (up to 22%) in F from GD9 150 mg/kg bw/d ↓ Final corrected BW (-4%) and corrected BW gain GD6-GD20 (-31% compared to controls). 50 mg/kg bw/d No effect	
Reference	Unpublished study report,, 2014c	
Method	**OECD TG 414 / GLP – cf Annex I, study 6**	**No data**

Dosages	0, 10, 30, 100 mg/kg bw/d	
Study duration	Gestation days 6 - 28	
Species	rabbit (NZW)	
Result	<u>Development</u> No effect on post-implantation survival at any dose level. 100 mg/kg bw/d Abortion : 4 vs 2 in controls at the end of the gestation period ↓ Fœtus weight (-12%), Delays of ossification *Non-statistically significant ↑ of external malformations due to 4 fetuses in one single litter with multiple external malformations.* 10 and 30 mg/kg bw/d No effect <u>Maternal toxicity</u> 100 mg/kg bw/d ↓ food consumption from GD 7-23 (up to -59%). ↓ defecation ↓ *mean BW (d14-d25 up to 5%) compared to controls* 30 mg/kg bw/d ↓ defecation in 11 dams 10 mg/kg bw/d No effect	
Reference	Unpublished study report,, 2019a	

6- Common underlying mechanism, quantitative aspects

The comparison of the effects described for fertility and other properties available in the data matrix was consistent and showed the same toxicity profiles for both substances. Similar magnitude of effects were obtained with 5000 ppm (407/443 mg/kg bw/d) of Reaction products of diphenylamine with nonene, branched versus 3000 ppm (260/271 mg/kg bw/d) of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene.

While the minimal difference in dose levels may reflect a slightly higher bioavailability of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, the dose difference is very small (less than a factor 2) and the bioavailability may vary due to UVCB nature of the two substances.

Overall, no major quantitative difference is observed based on the bridging studies (Table 36).

7- Exposure to other compounds than to those linked to the prediction

There are no impurities that have been identified that would lead to substance classification for reproduction. As the manufacturing process is similar for both the target and source substances, and the starting material Diphenylamine (DPA) is equivalent, it is expected that the impurities profile are comparable. The content of Diphenylamine is also similar in both substances (0.25% ≤ DPA < 2.5%).

As mentioned above, based on the similar structure and functional groups, similar breakdown products and metabolites are expected.

8- Occurrence of other effects than covered by the hypothesis and justification

Effects observed for other properties than DART were also similar with both substances. By oral route LD50 of both substances exceeds 5000 mg/kg BW. The two substances are not irritating to the skin, to the eye and no skin sensitizing potential was observed for both substances.

Regarding repeated dose toxicity, the main target organs being liver with consistent histopathological findings (centrilobular hypertrophy and fatty change) and consistent changes in biochemical parameters related to liver functions (e.g. increased alkaline phosphatase activities, increased triglyceride values, decreased albumin and total protein) and thyroid with follicular cell hypertrophy associated with thyroid hormone level changes.

When screened for alerting groups with DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus software (DEREK Nexus 2023 (v6.2.1) from the LHASA Group, the different constituents of the two substances highlighted no alerts for any of endpoints at a reasoning level of at least equivocal.

The two substances induced the same effects in the bridging studies. They both induced liver and thyroid toxicity with very similar pattern. No other specific effects have been found from the available dataset nor alert in QSAR analysis of the different constituents.

9- Bias that influences the prediction

Reaction products of diphenylamine with nonene, branched and Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene both belong to Subgroup 2 (i.e. SDPAs with variable number of alkyl substitutions) as defined by OECD (2016a). Collaborative approach (COLLA) pilot project for SDPA concluded that for human health-related aspects of the assessment, the read-across was possible only among SDPAs which belong to the same OECD subgroup (ECHA, 2018). Therefore, the reproductive studies available in their respective registration dossiers were considered.

Another SDPA belonging to sub group 2 cited in OECD (2016a) and Health Canada (ECCC, 2017), Benzenamine, N-phenyl-, reaction products with isobutylene and ,4,4-trimethylpentene (CAS no : 184378-08-3, EC no: 606-029-0) is not registered under REACH.

A Combined Repeated Dose Toxicity Study with the Reproduction/Developmental, OECD TG 422 in Sprague-Dawley rats, (Unpublished study report, 2006) by gavage (doses tested 0, 5, 25, and 125 mg/kg bw/d with a 2-week premating exposure) was reported in OECD 2016 and Health Canada (ECCC, 2017). In this study no effect on the number of implantation sites was noted. However, the exposure during the premating period was limited to two weeks and the highest dose tested was 125 mg/kg bw/d which can explain the discrepancy of those results compared those obtained with Reaction products of diphenylamine with nonene, branched and Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. Shorter gestation lengths, lower viability indices, and slightly lower mean offspring weights were observed at 125 mg/kg bw/d while liver effects were observed from 25 mg/kg bw/d.

Since Benzenamine, N-phenyl-, reaction products with isobutylene and ,4,4-trimethylpentene (CAS no : 184378-08-3, EC no: 606-029-0) is not registered under REACH Regulation, detailed composition is not available precluding an appropriate comparison with the two substances under consideration.

The above mentioned considerations justify not to consider this substance in the read-across approach.

10- Conclusion

After examining all assessment elements for Scenario 2 of the analogue approach, it can be concluded that the provided scientific data seem adequate and reliable for read-across. Read-across approach for reproductive toxicity is considered acceptable with high confidence from Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene to Reaction products of diphenylamine with nonene, branched. Therefore the EOGRTS performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (source substance) can be used to predict effects that would be observed in an EOGRTS with Reaction products of diphenylamine with nonene, branched (target substance) if it was to be conducted.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH proposal.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this CLH proposal.

10.13 Aspiration hazard

Not evaluated in this CLH proposal.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Reaction products of diphenylamine with nonene, branched is a UVCB substance consisting of three main constituents (isomers) with nonene (branched) –monoalkylated (C9DPA), -dialkylated (C9C9DPA) or –trialkylated (C9C9C9DPA) substituted diphenylamine.

Table 37: Summary of relevant information on rapid degradability for the UVCB substance and its constituents

Method	Results	Remarks	Reference
Test type: Aerobic mineralisation in surface water - simulation biodegradation test with radio labelled test substance	Tested concentrations: 2 and 10µg/L Duration: 60 days	2 (reliable with restrictions) - total recovery <90% - identification and quantification of the	Unpublished study report (2020c) Details available in

Method	Results	Remarks	Reference
Test system: natural water: freshwater (aerobic) OECD Guideline 309 Biodegradation in water: simulation testing on ultimate degradation in surface water GLP compliant	DT50 (50% disappearance time) at 12°C and 10 µg/L: C9DPA 0.3 d C9C9DPA 4.2 d No significant mineralization (max 0.6% after 60d)	transformation products was not possible Key study Test material: Reaction products of Benzeneamine, N-phenyl- with nonene (branched) experimental result	Annex I Study 1 (4.1.3)
Test type: Inherent biodegradability: activated sludge (predominantly domestic sewage) and mixed population of soil microorganisms (natural) (aerobic) OECD Guideline 302D Draft Inherent Biodegradability - Concawe Test GLP compliant	Tested concentration: 23.4 mg/L Duration: 56 days Not inherently biodegradable No degradation of the test material based on carbon dioxide production was observed after 56 days 3% degradation after 56 days based on the results of the compound specific analyses	1 (reliable without restriction) Supporting study Test material: C9DPA constituent. Reaction products of Benzenamine, N-phenyl, and Nonene, branched EC 253-249-4; CAS no.: 36878-20-3, followed by purification (distillation) with a 96% mono-alkyl content experimental result	Unpublished study report (2003a) Details available in Annex I Study 2 (4.1.4)
Catalogic model predictions v.11.15 Estimation of ready biodegradability as measured in OECD Guideline 301 C	Not readily biodegradable C9DPA: BOD 28 days = 24% Primary DT50 = 4.75 d Ultimate DT50 = 2m 11d C9C9DPA: BOD 28 days = 31% Primary DT50 = 2.41 d Ultimate DT50 = 2m 21d	C9C9C9DPA out of applicability domain Supporting information	Unpublished study report (2019d)
EPI suite BIOWIN model predictions v.4.10	Not readily biodegradable Based on BIOWIN models 1, 2, 5 & 6 for the 3 main constituents	Supporting information	

11.1.1 Ready biodegradability

No experimental screening studies for ready biodegradability is available for the UVCB substance.

Quantitative estimation method (QSAR) for estimating the degree of biodegradability of organic substances may be used to predict that a substance is not rapidly degradable, or be used in a weight of evidence approach (ECHA, 2017b).

The DS made estimations using BIOWIN (v4.10) models 1, 2, 5 & 6 to calculate the probability score that the three main constituents of the UVCB substance (C9DPA, C9C9DPA & C9C9C9DPA) will be rapidly or

readily biodegradable in the environment under aerobic conditions with mixed cultures of microorganisms, according to CLP guidance (ECHA, 2017b).

Table 38: EPI suite probability of rapid and ready degradation (Biowin models v.4.10) for the substance main constituents

C9DPA SMILES : CCCC(C)CC(C)(C)c1ccc(Nc2ccccc2)cc1	C9C9DPA SMILES : CCCC(C)CC(C)(C)c1ccc(Nc2ccc(C(C)(C)CC(C)CCC)cc2)cc1	C9C9C9DPA SMILES : CCCC(C)CC(C)(C)c1ccc(Nc2ccc(C(C)(C)CC(C)CCC)cc2C(C)(C)CC(C)CCC)cc1
Biowin1 : 0.3173	Biowin1 : -0.0548	Biowin1 : -0.2989
Biowin2 : 0.0466	Biowin2 : 0.0002	Biowin2 : 0.0000
Biowin5 : -0.0838	Biowin5 : -0.3007	Biowin5 : -0.5177
Biowin6 : 0.0112	Biowin6 : 0.0014	Biowin6 : 0.0002

The results for these 4 models are < 0.5 and the substance should be regarded as not rapidly degradable (BIOWIN 1 and BIOWIN 2) and not readily biodegradable (BIOWIN 5 and BIOWIN 6). The constituents have a molecular weight (MW) of 295-548 g/mol and they are included in the MW range (31-698) of the training set compounds. Thus, the results are considered to be in the applicability domain of the models for all constituents.

Catalogic 301C model predictions on ready biodegradability for the three main constituents of the UVCB substance (C9DPA, C9C9DPA & C9C9C9DPA) were also evaluated. The results for the main constituents are summarized below.

Table 39: Biochemical oxygen demand (BOD) and number of degradation products for the main constituents (please refer to table 38 for the SMILES used in model predictions) for the substance main constituents.

	BOD 28 days MITI (OECD 301C)	Ready biodegradable (RDB)	Degradation products	Applicability domain
C9DPA	0.24 ± 0.0260 Primary DT50 = 4.75 d Ultimate DT50 = 2m 11d	Not RBD	113	In domain*
C9C9DPA	0.31 ± 0.0298 Primary DT50 = 2.41 d Ultimate DT50 = 2m 21d	Not RBD	137	In domain*
C9C9C9DPA	0.09 ± 0.0500 Primary DT50 = 25.59 d Ultimate DT50 = 7m 1d	Not RBD	135	The chemical is out of the interpolation structural space (80%)

* in parametric, structural (fragments in correctly predicted training chemicals – 100.00%) and metabolic domain

The BOD predictions of the two main constituents (C9DPA and C9C9DPA) are considered reliable as they are within the applicability domain of the Catalogic model used.

CATALOGIC as well as an alternative software for predicting pathways, EAWAG PPS, predict that the substance could be hydroxylated at various positions as a first step. Various transformation products, over than 100 for each one of the constituents, are predicted with CATALOGIC.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

No study available. The substance has a low solubility in water, therefore hydrolysis test is not applicable. In water, the substance is expected to be hydrolytically stable as the substance does not present functional groups that result in significant hydrolysis.

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

One inherent biodegradability – Concawe test (according to the OECD TG 302D draft) performed on one constituent of the UVCB substance is included in the registration dossier. This GLP study was realized with the test material “Reaction products of Benzenamine, N-phenyl, and Nonene, branched” (EC 253-249-4; CAS 36878-20-3), followed by purification (distillation) with a 96% C9DPA content (EC 248-295-7; CAS 27177-41-9). This study meets the validity critera (RI 1), can be use in the weight of evidence for rapid degradation decision and is considered as a supporting study in the absence of a ready biodegradability test.

The test material, at a nominal concentration of 23.4 mg/L, was exposed to a mixed population of soil microorganisms (natural) (aerobic) and activated sewage sludge (predominantly domestic sewage) with culture medium. The sealed culture vessels were placed in the dark at 21°C for 56 days. The inoculum used in the biodegradation test was pre-exposed to the test material in order to enhance the biodegradative potential of the inoculum. The test material is poorly soluble (water solubility=0.0113 mg/L), therefore the test material was dissolved in diethylether. An aliquot of the solvent stock solution containing the test material was applied to a glass fiber filter paper, which was added to the test medium after evaporation of the solvent.

The degradation of the test material was assessed by the determination of carbon dioxide produced once a week for 56 days, and by compound specific analyses (HPLC) on days 0, 28 and 56. A standard control with n-Hexadecane and a toxicity control were used for validation purposes. Compound-specific analyses conducted on day 0, 28 and 56 indicated that the test material attained 3% degradation after 56 days. The pass level (more than 70 % degradation) was not reached in this inherent biodegradability test. No degradation of the test material based on carbon dioxide production was observed after 56 days.

In principle, the test conditions of the inherent biodegradability - Concawe test (302D draft) should be very favourable for degradation. Nevertheless, no significant degradation occurred, both on basis of mineralization and on basis of disappearance of parent compound. This indicates that the substance is not inherently biodegradable and supports the decision to consider the UVCB substance as not rapidly degradable.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

One simulation testing on ultimate degradation in surface water according to OECD TG 309 (GLP compliant, 2020c) was performed for the UVCB substance and mentioned in the registration dossier. This reliable study (RI 2) is considered a key study to assess the rapid degradation of the UVCB substance.

The test system was collected from a small stream west of Schifferstadt Germany (surface water along with surface sediment as suspended solids). The test, using the radiolabeled UVCB substance, was performed in closed vessels for 60 days at two different concentrations (2 µg/L and 10 µg/L). The test vessels were closed with lids impermeable to air and CO2 and were incubated in dark conditions at a temperature range of 11.7 °C to 12.4 °C. Replicates of test substance were sampled at the start of exposure (Day 0) and on Day 7, Day 14, Day 27, Day 49, Day 55 and at the end of exposure (Day 60). The test included a blank control, an abiotic sterile control, a control assay with reference substance aniline (10µg/L) and a solvent control (ethanol 10.8 µL). The results were obtained and analysed for the two main constituents (C9DPA and C9C9DPA) of the UVCB substance.

Different extraction methods were used. The UVCB substance and its constituents are considered highly adsorptive and a significant part of the substance might have been adsorbed to the laboratory material. Thus, only a reduced recovery of the total applied radioactivity was feasible leading to low mass balance recovery at the end of the experiment (approximately 75% for the highest concentration of 10 µg/L and approximately 89% for the lowest concentration of 2 µg/L). These values are below the acceptable range of 90-110% recommended in the guideline.

The calculated DT50 (50% disappearance time) values were 0.3 d for C9DPA and 4.2 d for C9C9DPA at 12°C for the highest concentration of 10 µg/L. It was not possible to distinguish between primary degradation and adsorption in this study. Primary degradation cannot be excluded and might have occurred to a certain extent. However, no significant ultimate degradation occurred as indicated by the $^{14}CO_2$ radioactivity at the end of the study (< 1% of the total applied radioactivity after 60d). Identification or quantification of the degradation products was not possible. Thus, following the decision scheme in relation to rapid degradability, in the absence of information on degradation products and their related toxicity, the substance could not be considered as rapidly degradable (ECHA, 2017a, p.498).

Since no significant mineralization was observed, the surface water simulation degradation study provides evidence that the UVCB substance and its main constituents do not exhibit ultimate biodegradation in surface water and are not rapidly degradable.

11.1.4.4 Photochemical degradation

No relevant information is available regarding phototransformation in air, water nor soil.

11.2 Environmental fate and other relevant information

Based on Environment Canada's assessment (2017) of Substituted diphenylamine antioxidants (SDPAs), it was concluded that SDPAs as a group (containing several of the C4, C8 and C9 multiple branched alkyl chains) persist in soil and sediment, and most of them persist in water. It was argued that this group "are known to degrade slowly and their structure sterically impede the degradation of the diphenylamine core". Although some transformation is expected to occur in the water-soluble fractions, as we have seen in the case of the simulation study described above, the biodegradation rate is expected to be minimal. Based on the document, "most SDPAs contain structural features that are not easily biodegradable (e.g., C=C bonds)". Therefore, considering the models and empirical results (including Reaction products of diphenylamine with nonene, branched or analogs) as well as the structural features, Environment Canada concluded that there is sufficient evidence to indicate that the biodegradation mineralization half-life of most SDPAs is ≥ 182 days in water.

11.3 Bioaccumulation

Table 40: Summary of data on bioaccumulation for substance Reaction products of diphenylamine with nonene, branched and its constituents

Method	Results	Remarks	Reference
Test type: Bioaccumulation in aquatic species:fish Common carp (Cyprinus carpio) under flow-through conditions for 42 days According to the testing methods for New Chemical Substances (1974, amended 1998), comparable to OECD Guideline 305 (Bioaccumulation in Fish: Aqueous Exposure) GLP compliant	Tested concentrations: 10 and 100 µg/L Duration: exposure 42 days and depuration 42 days Measured BCF (L/kg whole body w.w.): Based on steady state: C9DPA (10 µg/L)=1730 C9DPA (100 µg/L)=411 Based on bcmfR calculation(BCF_{KLip}): C9DPA (10µg/L)=2219	2 (reliable with restrictions) - method comparable to OECD TG305 -use of dissolvent Key study experimental result Test material: C9DPA constituent Ar-nonyldiphenylanil ine	Unpublished study report (2000) Details available in Annex I Study 3 (4.2.1)
EPI suite KOWWIN model predictions v1.68	Estimated log Kow: C9DPA=7.58 C9C9DPA=11.87 C9C9C9DPA=16.17	Supporting information	
Catalogic model predictions OASIS v5.11.19 (Bioaccumulation base-line model v.02.09)	Estimated BCF (L/kg w.w.): C9DPA=831.76 C9C9DPA=7.58	Supporting information C9C9C9DPA is out of the applicability domain of the model	Unpublished study report (2017)
EPI suite BCFBAF model predictions v3.01	Estimated BCF (L/kg w.w.): Regression-based method C9DPA=6893 Arnot-Gobas method (upper trophic) C9DPA=721.6	Supporting information C9C9DPA and C9C9C9DPA are out of the applicability domain of the model	

11.3.1 Estimated bioaccumulation

According to CLP guidance (ECHA, 2017a), when no experimental data of high quality are available, validated Quantitative Structure Activity Relationships (QSARs) for log Kow may be used in the classification process. KOWWIN software program (v.1.68) was used to predict the log Kow values of the main constituents (C9DPA, C9C9DPA and C9C9C9DPA). The results are presented in the table 42 below. The constituents have a log Kow ≥ 4 and thus the substance is considered to meet the criterion for a potential for bioaccumulation.

Information from model predictions are available for the constituents. The registrants have provided Catalogic BCF and Diamax predictions. The model BCFBAF (v3.01) of EPI Suite (US EPA, 2012) was also run by the DS to provide quantitative estimates. Results of model predictions are summarised in the table below and are presented as supporting information for a bioaccumulative potential of the substance.

Table 41: Catalogic and EPI Suite bioaccumulation models prediction for the constituents of UVCB substance (please refer to table 38 for the SMILES used in model predictions).

	C9DPA	C9C9DPA	C9C9C9DPA
Molecular weight (Da)	295	422	548
Log Kow (KOWWIN EPI suite)	7.58	11.87	16.17
Catalogic Diamax average (Å)	16.6	20	20.8
Log BCF L/kg corrected Catalogic 5.11.19 (Bioaccumulation baseline model v2.09)	2.92 ± 0.404 BCF 831.76	0.88 ± 0.109 BCF 7.58	0.89 ± 0.0922 * BCF 7.76
BCF L/kg BCFBAF v3.01 regression-based method Arnot-Gobas method (upper trophic) Biotransformation half-life	 6893 721.6 17.74 days	 54.4* 1.7* 1394 days*	 3.2* 0.9* 2.732 E04 days*

* Out of the applicability domain of the model

According to Catalogic, C9C9C9DPA is out of the applicability domain of the model for BCF prediction. According to EPI Suite BCFBAF model prediction v.3.01, constituents that have a log Kow value greater than 11.26 are considered out of the applicability domain of the model and BCFBAF model may be highly uncertain for chemicals that have estimated log Kow values > 9. Thus, the prediction is relevant for C9DPA constituent only. Based on the physicochemical properties of the constituents, the results also indicate that C9DPA is the most likely to have a potential for bioaccumulation. The calculated LogKow values increase with the number of substitution. C9C9DPA and C9C9C9DPA which are less water soluble and also more bulky with a LogKow > 10 are expected to be poorly absorbed. Finally, based on Catalogic and BCFBAF predictions, C9DPA indicates a BCF value ≥500, thus the substance is considered to meet the criterion for a potential for bioaccumulation in aquatic organisms.

11.3.2 Measured partition coefficient and bioaccumulation test data

No experimental data evaluating the bioaccumulative properties are available for the UVCB substance "Reaction products of diphenylamine with nonene, branched".

The bioaccumulation potential in aquatic species of one constituent of the UVCB substance (C9DPA) was examined in a study. The study follows the guideline of the test methods designated for New Chemical Substances (1974, amended 1998) under Chemical Substances Control Law of Japan (MITI). The study was realised on *Cyprinus carpio* in continuous flow-through system for 42 days of exposure followed by additional 42 days of depuration duration. The C9DPA constituent was prepared by addition of test substance to HCO-30 surfactant which was then dissolved in 2-methoxyethanol and fish were exposed at two nominal concentrations: 100 µg/L and 10 µg/L. The higher concentration was well above the water solubility of the test item (11.3 µg/L) and was not considered in this assessment. Although some elements deviated

from the standard OECD TG 305 (use of surfactant and dissolvent, measurements were made for a group of 2 fish instead of individually), the study is well conducted, follows GLP principles and is reliable for use under CLP in the absence of data on the UVCB substance.

The bioconcentration factors at steady state (BCF_{SS}) were used to evaluate the potential of bioaccumulation. At a concentration of 10 µg/L, a BCF for the whole body of 1730 L/kg w.w. was calculated by the authors (BCF=411 L/kg w.w. for high exposure dose). The DS revised the calculation using the R-package "bcmfR" to estimate the BCF of the low exposure dose (10µg/L) using kinetic approach and taking into account lipid normalisation. The new BCF calculated was BCF_{KLip}=2219 L/kg.

Considering the estimated log Kow≥4 for the constituents and the measured BCF≥500 for the C9DPA, it is therefore concluded that the substance has a potential for bioaccumulation in aquatic species.

11.4 Acute aquatic hazard

Table 42: Summary of relevant information on acute aquatic toxicity for substance Reaction products of diphenylamine with nonene, branched and its constituents

Method	Species	Test material	Results	Remarks	Reference
OECD Guideline 203 (Fish, Acute Toxicity Test) freshwater Semi-static 96h	Rainbow trout *Oncorhynchus mykiss*	Test material: C9DPA constituent Reaction products of Benzenamine, N-phenyl, and Nonene, branched EC 253-249-4; CAS no.: 36878-20-3, followed by purification (distillation) with a 96% mono-alkyl content.[8] Tested concentration: 0.0113 mg/L nominal	No mortality, no sub-lethal effects observed LC50 (96h): >0.0013 mg/L test mat. (dissolved fraction) (**meas. (TWA)**) based on the concentration of centrifuged test media to give a "worst case" scenario) LC50 (96h): >0.0113 mg/L test mat. (total fraction) (**nominal**)	GLP compliant 2 (reliable with restrictions) - marked decline in measured concentrations after 24 h (30-47% of nominal) experimental result	Unpublished study report (2003b)
Japanese Industrial Standard(JIS) Method "K0102-1993, Industrial Waste Water Testing Method, 71, Acute toxicity study using fish. freshwater Semi-static 96h	Japanese Medaka *Oryzias latipes*	Test material: C9DPA constituent Reaction products of Benzenamine, N-phenyl, and Nonene, branched EC 253-249-4; CAS no.: 36878-20-3, followed by purification (distillation) with a 96% mono-alkyl content[5]. Tested concentration: 0, 10, 20, 40, 80 mg/L nominal	**Effects on mortality** Sublethal effects not mentionned LC50 (96h) 52 mg/L (**nominal**)	GLP compliant 3 (non reliable) use of a solvent and a dispersant 10% mortality in control group No analytical measurements reported	Unpublished study report (2000) Details available in Annex I Study 4 (4.3.1)
OECD Guideline 202 (*Daphnia* sp. Acute	*Daphnia magna*	Test material: Reaction products of Benzeneamine, N-phenyl- with nonene (branched)	No effect EL50 (48h) >100 mg/L test mat. (**nominal** loading rate, WAF) based on mobility	GLP compliant 2 (reliable with restrictions) measured concentrations	Unpublished study report (2004)

[8] Those identifiers are mentionned in the publically disseminated lead Registration Dossier but are incorrect as they are corresponding to a substance without branched alkyl chain.

Test) freshwater static 48h		Tested concentration: 100 mg/L nominal	EL50 (24h) >100 mg/L test mat. (**nominal** loading rate WAF) based on mobility	in test medium samples <LOQ (0.0024 mg/L) experimental result	
OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) freshwater static 48h	*Daphnia magna*	Test material: C9DPA constituent Reaction products of Benzenamine, N-phenyl, and Nonene, branched EC 253-249-4; CAS no.: 36878-20-3, followed by purification (distillation) with a 96% mono-alkyl content[9]. Tested concentration: 0.0113 mg/L nominal	No effect EC50 (48h): >0.0014 mg/L test mat. (dissolved fraction) (**meas. (TWA)**) based on mobility EC50 (48h): >0.0113 mg/L test mat. (total fraction) (**nominal**) based on mobility	GLP compliant 2 (reliable with restrictions) -marked decline in measured concentrations after 48 h (53-69% of nominal) experimental result	Unpublished study report (2003c)
OECD Guideline 201 (Alga, Growth Inhibition Test) freshwater static 72h	*Pseudokirchneriella subcapitata*	Test material: Reaction products of Benzeneamine, N-phenyl- with nonene (branched) Tested concentration: 100 mg/L nominal	No toxic effect E_rL50 (72h): >100 mg/L (**nominal** loading rate WAF) based on growth rate E_yL50 (72h): >100 mg/L (**nominal** loading rate WAF) based on yield	GLP compliant 2 (reliable with restrictions) - rapid decrease in the measured concentration <LOQ (1 µg/L) after 72h experimental result	Unpublished study report (2020f)
OECD Guideline 201 (Alga, Growth Inhibition Test) EPA OTS 797.1050 (Algal Toxicity, Tiers I and II) freshwater static 96h	*Pseudokirchneriella subcapitata (Selenastrum capricornutum)*	Test material: Reaction products of Benzeneamine, N-phenyl- with nonene (branched) Tested concentrations: 0, 0.3, 3.3, 33, 330, 3300 mg/L nominal	**Effects on biomass and growth rate** EL50 (72h): 600 mg/L test mat. (**nominal** loading rate WAF) based on growth rate (95% CL: 330 - 3300 mg/L) EL50 (96h): 870 mg/L test mat. (**nominal** loading rate WAF) based on growth rate (95% CL: 330 - 3300 mg/L) EL50 (96h): 220 mg/L test mat. (**nominal** loading rate WAF) based on cells/mL (95% CL: 330 - 3300	GLP compliant 2 (reliable with restrictions) - no measured concentration experimental result	Unpublished study report (1997) Details available in Annex I Study 5 (4.3.3)

[9] Those identifiers are mentionned in the publically disseminated lead Registration Dossier but are incorrect as they are corresponding to a substance without branched alkyl chain.

			mg/L)		
OECD Guideline 201 (Alga, Growth Inhibition Test) freshwater static 72h	*Scenedesmus subspicatus*	Test material: C9DPA constituent Reaction products of Benzenamine, N-phenyl, and Nonene, branched EC 253-249-4; CAS no.: 36878-20-3, followed by purification (distillation) with a 96% mono-alkyl content[10]. Tested concentration: 0.0113 mg/L nominal	No toxic effect EC50 (72h): >0.00222 mg/L test mat. (dissolved fraction) (**meas. (TWA)**) based on growth rate and biomass EC50 (72h): >0.0113 mg/L test mat. (total fraction) (**nominal**) based on growth rate and biomass	GLP compliant 2 (reliable with restrictions) - marked decline in measured concentrations after 72 h (28-32% of nominal) experimental result	Unpublished study report (2003d)

11.4.1 Acute (short-term) toxicity to fish

Experimental data are not available for short-term toxicity to fish for the substance Reaction products of diphenylamine with nonene, branched.

Additional data on the constituents to be considered in the weight of evidence

In the absence of data for the UVCB substance, one reliable short-term study on fish species *Oncorhynchus mykiss* is available (OECD TG 203, GLP compliant, 2003b) for constituent C9DPA and considered in the weight of evidence. The water solubility of the constituent was determined to be 0.0113 mg/L, thus the test material was dissolved in dimethylformamide and the maximum nominal concentration employed in the study would not be exceeding this value. This study was realised in semi-static condition (daily renewal) and the fish (two groups of ten) were exposed at a single test concentrations (0.0113 mg/L) for 96 hours. A control and a solvent control (100µL/L dimethylformamide) are reported in the study. No adverse effects, mortality nor sublethal effects were observed. Thus the 96-Hour LC50 based on nominal test concentrations was greater than 0.0113 mg/L. The test preparations were observed to be clear, colourless solutions throughout the test. However, the results of the preliminary dosing trials indicated that a significant proportion of undissolved/dispersed test material would be present. Samples taken from the test preparations were therefore analysed untreated and after centrifugation in order to give an indication of the dissolved and hence bioavailable test material concentration. It was observed a decline in the measured concentrations in the media over time and at a greater extent following centrifugation (in the 24h old media at 0, 24, 48, 72h, from 30% to 47% of nominal concentrations for the uncentrifuged samples and from 3% to 19% for the centrifuged samples). This decline in test concentrations could be due to loss of material due to adsorption to particulates, dispersed material or the test vessel. The calculation of the results were therefore based on the time-weighted mean measured test concentrations of the centrifuged test media to give a "worst case" analysis of the data. The LC50(96 h) >0.0013 mg/L was calculated based on the time-weighted mean measured test concentrations of the centrifuged test media.

In addition, one bioaccumulation study conducted with C9DPA constituent was included in the registration dossier (Unpublished study report, 2000). The first part of the study consisted in an acute toxicity test (according to the Japanese Industrial Standard (J I S) Method "K0102-1993, Industrial Waste Water Testing Method, 71, Acute toxicity study using fish."). The study is GLP compliant and was conducted for 96 h

[10] Those identifiers are mentionned in the publically disseminated lead Registration Dossier but are incorrect as they are corresponding to a substance without branched alkyl chain.

using Japanese Medaka (*Oryzias latipes*). Five different nominal concentrations (0 (control), 10, 20, 40 and 80 mg/L) and 10 fish/groups were tested. The LC50 (96 h) was calculated to be 52 mg/L (nominal). This study is considered non reliable: 10% mortality was observed in the control group at the end of the 96 hours and no analytical measurements of the test concentration were reported. Thus the validity criteria of the study are not met and the study is not considered reliable for use under CLP.

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

One reliable (RI 2) short-term study on *Daphnia magna* is available for the UVCB substance (OECD 202, GLP compliant, 2004). Four replicates of five daphnids each were tested for a duration of 48h for the control and the single test concentration. The test solution was prepared following a water accomodated fraction (WAF) method with a unique loading rate of 100 mg/L according to the OECD Guidance Document No. 23 on Aquatic Toxicity Testing of Difficult Substance or Mixtures (OECD, 2019). No auxiliary solvent or emulsifier was used. In the control and in the WAF with the loading rate of the test item of 100 mg/L, no immobilized test organisms were observed during the test period of 48h. No remarkable observations were noted on the appearance of the test medium during the test period. The analytically measured test item concentration in the test medium samples (WAF with loading rate of 100 mg/L) was, at the start and at the end of the test, below the limit of quantification of 0.0024 mg/L. All biological results are then related to the loading rate of the WAF. Therefore, the 48-hour NOEC was reported in the study to be at least the loading rate of 100 mg/L. The 48-hour ELC50 was determined to be higher than the loading rate of 100 mg/L. This value could not be quantified since no toxic effects was observed in this study.

Additional data on the constituents to be considered in the weight of evidence

Additionaly, one reliable (RI 2) short-term study on aquatic invertebrates species *Daphnia magna* is available (OECD TG 202, GLP compliant, 2003c) for the constituent C9DPA. The water solubility of the constituent was determined to be 0.0113 mg/L, thus the test material was dissolved in dimethylformamide and the maximum nominal concentration employed in the study would not be exceeding this value. This study was realised in static condition and the daphnids (4 replicates of 10 animals) were exposed at a single test concentrations (0.0113 mg/L) for 48 hours. A control and a solvent control (100 µL/L dimethylformamide) are reported in the study. No immobilisation or adverse reactions to exposure were observed, thus the 48-Hour EC50 based on nominal test concentrations was greater than 0.0113 mg/L. The test preparations were observed to be clear, colourless solutions throughout the test. However, the results of the preliminary dosing trials indicated that a significant proportion of undissolved/dispersed test material would be present. Samples taken from the test preparations were therefore analysed untreated and after centrifugation in order to give an indication of the dissolved and hence bioavailable test material concentration. It was observed a decline in the measured concentrations in the media over time and at a greater extent following centrifugation (after 48 hours, 53% to 69% of nominal concentrations for the uncentrifuged samples and 6% for the centrifuged samples). This decline in test concentrations could be due to loss of material due to adsorption to particulates, dispersed material or the test vessel. The calculation of the results were therefore based on the time-weighted mean measured test concentrations of the centrifuged test media to give a "worst case" analysis of the data. The EC50 (48 h) >0.0014 mg/L was calculated based on the time-weighted mean measured test concentrations of the centrifuged test media.

11.4.3 Acute (short-term) toxicity to algae or other aquatic plants

Two valid and reliable algae studies are available for the UVCB substance Reaction products of diphenylamine with nonene, branched. The first study (OECD TG 201, GLP compliant, 2020f) on *Pseudokirchneriella subcapitata* assessed the effects on growth rate and yield over a period of 72 hours. The

study was conducted under static conditions and a water accommodated fraction (WAF) was prepared with a nominal loading rate of the test item of 100 mg/L. Six replicates were used for the limit loading rate and the control. The limit loading rate and the control were analytically verified by LC-MS/MS at the start and the end of the exposure. Only the monoalkylated isomers (C9DPA) could be quantified. The measured concentrations of the water accommodated fraction (WAF) of the test item in the fresh media were in the range of < LOQ (1 µg test item/L) to 8.6 µg/L monoalkylated isomers. At the end of exposure after 72 hours, all measured concentrations were < LOQ. No inhibition was found after 72 hours exposure to the substance.The WAF was checked via laser beam (Tyndall effect) for undissolved test item and the Tyndall effect was negative. In the absence of toxic effects observed in the study, the evaluations were based on the nominal loading rates. Thus, the ErL10 (72h) >100 mg/L and ErL50 (72h) >100 mg/L were determined.

The second study (OECD TG 201, GLP compliant, 1997) was also realized on the same species, *Pseudokirchneriella subcapitata*. The test was performed at 24°C under static conditions. No detail was given regarding the identity of the test substance in the study report. The test substance is mentioned in the registration dossier as Reaction products of Benzeneamine, N-phenyl- with nonene (branched). **This study is the only acute test for the UVCB substance, and wich is considered reliable for the use under CLP, showing adverse effects.** The study does not mention the use of a dissolvent or solubiliser. No insoluble material was noted during the test that might explain physical effects. The test was performed for 96 hours under static condition with a control (0 mg/L) and five nominal test concentrations (WAF): 0.3, 3.3, 33, 330, 3300 mg/L. No measured concentration of the test substance was available, thus the results are based on nominal concentrations. A 72 hour EC50 corresponding to 200 mg/L was calculated using the number of cells/mL (96 hour EC50=220 mg/L). A 72 hour EC50 corresponding to 600 mg/L was calculated using the average specific growth rate (96 hour EC50=870 mg/L). Finally, a NOEC(96h) of 33 mg/L defined as the highest concentration of test substance that allowed at least 90% of control growth was obtained both for the number of cell/mL and the specific growth rate.

The study report indicated that, at the conclusion of the test, a sample of test media was used to determine wheter toxic effects were algicidal or algistatic. After 168h of incubation, it was reported that the effect of the test material was algistatic rather than algicidal.

Additional data on the constituents to be considered in the weight of evidence

An additional study on aquatic algae species *Desmodesmus (Scenedesmus) subspicatus* realised with test material C9DPA is available (OECD TG 201, GLP compliant, 2003d). The water solubility of the constituent was determined to be 0.0113 mg/L, thus the test material was dissolved in dimethylformamide and the maximum nominal concentration employed in the study would not be exceeding this value. This study was realised in static condition and the algae (6 replicates flasks) were exposed at a single test concentrations (0.0113 mg/L) for 72 hours. A control and a solvent control (100µL/L dimethylformamide) are reported in the study. No statistically significant differences (P≥0.05) neither on growth or biomass was observed between the test and control groups, thus the 72-hour EC50 based on nominal test concentrations was greater than 0.0113 mg/L. At 0h, the control and test preparations were clear colourless solutions. However, the results of the preliminary dosing trials indicated that a significant proportion of undissolved/dispersed test material would be present. Samples taken from the test preparations were therefore analysed untreated and after centrifugation in order to give an indication of the dissolved and hence bioavailable test material concentration. It was observed a decline in the measured concentrations in the media over time and at a greater extent following centrifugation (after 72 hours, 28% and 32% of nominal concentrations for the uncentrifuged samples and from 3% and 10% for the centrifuged samples). This decline in test concentrations could be due to loss of material due to adsorption to particulates, dispersed material or the test vessel. The calculation of the results were therefore based on the time-weighted mean measured test concentrations of the centrifuged test media to give a "worst case" analysis of the data. The EC50(72h) >0.00222 mg/L was calculated based on the time-weighted mean measured test concentrations of the centrifuged test media.

In conclusion, only one reliable study presented in the data package indicated an acute aquatic toxicity. This OECD TG 201 (1997) is contradicted by a more recent and more detailed study (OECD TG 201, 2020f). Considering that the study from 2020 has a more comprehensive reporting and included analytical measurments, the DS considers that the study from 2020 should be given more weight in the decision of the application of the criteria. This decision is supported by Ecosar data on the constituents of the UVCB substance indicating that, based on LogKow values >5, no effects at saturation are expected for aquatic acute toxicity in fish, daphnid and green algae. Nevertheless, considering the poor solubility of the substance and the discrepancies between nominal and measured concentrations reported in studies where the substance was analysed, it is noted that the results may have under-estimated the acute toxicity of the substance.

11.4.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.5 Long-term aquatic hazard

Table 43: Summary of relevant information on chronic aquatic toxicity for substance Reaction products of diphenylamine with nonene, branched and its constituents

Method	Species	Test material	Results	Remarks	Reference
OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test) freshwater Semi-static 34d	*Danio rerio*	Test material): Reaction products of Benzeneamine, N-phenyl- with nonene (branched) Tested concentration: 10 mg/L nominal	**No effect** NOELR (34d) 10mg/L test mat. (**nominal** loading rate WAF)	GLP compliant 2 (reliable with restrictions) - rapid decrease in the measured concentration <LOQ (1 µg/L)-5.35 µg/L after 24h Supporting study experimental result	Unpublished study report (2020d)
OECD Guideline 211 (*Daphnia magna* Reproduction Test) freshwater semi-static 21d	*Daphnia magna*	Test material: Reaction products of Benzeneamine, N-phenyl- with nonene (branched) Tested concentrations: 1.98, 2.96, 4.45, 6.67, 10.0 mg/L nominal	**Effects on reproduction and adult mortality** NOELR (21d) 4.45 mg/L test mat. (**nominal** loading rate WAF) based on reproduction ELR10 (21d) 4.12 mg/L test mat. (**nominal** loading rate WAF) based on reproduction ELR50 (21d) >10mg/L test mat. (**nominal** loading rate WAF) based on	GLP compliant 2 (reliable with restrictions) -rapid decrease in the measured concentration <LOQ (1 µg/L)-1.31 µg/L after 24h **key study** experimental result	Unpublished study report (2020e) Details available in Annex I Study 6 (4.4.4)

			reproduction **LOEC (21d) 1.73 µg/L test mat. (measured) based on reproduction** **NOEC (21d) 1.28 µg/L test mat. (measured) based on reproduction**		
OECD Guideline 201 (Alga, Growth Inhibition Test) freshwater static 72h	*Pseudokirchneriella subcapitata*	Test material: Reaction products of Benzeneamine, N-phenyl- with nonene (branched) Tested concentration: 100 mg/L nominal	No toxic effects E_rL10 (72h): >100 mg/L (**nominal** loading rate WAF) based on growth rate E_yL10 (72h): >100 mg/L (**nominal** loading rate WAF) based on yield	GLP compliant 2 (reliable with restrictions) - rapid decrease in the measured concentration <LOQ (1 µg/L) after 72h Supporting study experimental result	Unpublished study report (2020f)
OECD Guideline 201 (Alga, Growth Inhibition Test) EPA OTS 797.1050 (Algal Toxicity, Tiers I and II) freshwater static 96h	*Pseudokirchneriella subcapitata (Selenastrum capricornutum)*	Test material: Reaction products of Benzeneamine, N-phenyl- with nonene (branched) Tested concentrations: 0, 0.3, 3.3, 33, 330, 3300 mg/L nominal	**Effects on biomass and growth rate** NOELR (96h): 33 mg/L test mat. (**nominal loading rate WAF**) based on: growth rate	GLP compliant 2 (reliable with restrictions) - no measured concentrations Supporting study experimental result	Unpublished study report (1997)
OECD Guideline 201 (Alga, Growth Inhibition Test) freshwater static 72h	*Scenedesmus subspicatus*	Test material: C9DPA constituent Reaction products of Benzenamine, N-phenyl, and Nonene, branched EC 253-249-4; CAS no.: 36878-20-3, followed by purification (distillation) with a 96% mono-alkyl content. Tested concentration: 0.0113 mg/L nominal	No toxic effects NOEC (72h): 0.0022 mg/L test mat. (dissolved fraction) (**meas. TWA**) based on: growth rate and biomass NOEC (72h): 0.0113 mg/L test mat. (total fraction) (**nominal**) based on: growth rate and biomass	GLP compliant 2 (reliable with restrictions) - marked decline in measured concentrations after 72 h (28-32% of nominal) Supporting study experimental result	Unpublished study report (2003c)

11.5.1 Chronic toxicity to fish

Experimental data for the UVCB substance on long-term toxicity to fish (*Danio rerio*) is available (OECD TG 210, GLP compliant, 2020). 80 eggs of *Danio rerio* (4 replicates of 20 eggs each) were exposed to the limit loading rate (WAF) of 10 mg/L. The fish were exposed for 34 days with a daily renewal in a proportion of 75% of the total test media. Based on the results of the pre-test, the analytics (LC-MS/MS) were confined to the detection of the C9DPA constituent. The measured concentrations in the fresh media were either extremely low (1.1 – 18.1 µg/L) or generally even below the limit of quantification (<1 µg/L). In the old media, the measured concentrations were <LOQ up to 5.35 µg/L. The WAF was checked via laser beam (Tyndall effect) for undissolved test item and the Tyndall effect was negative. No statistically significant differences on hatchability, mortality, fry growth length and weight were detected between the dilution water control and the limit loading rate of 10 mg/L of the test item. As a consequence, results of the study were based on the nominal loading rates initially prepared and a NOELR (34d) of 10 mg/L was proposed.

11.5.2 Chronic toxicity to aquatic invertebrates

For the UVCB substance, one long-term study on *Daphnia magna* indicated toxic effects of the substance (OECD TG 211, GLP compliant, 2020). A significant reduction in the reproduction per female parent animal inserted at the start of the exposure was observed at the nominal loading rates of 6.67 and 10.0 mg/L (NOELR of 4.45 mg/L). A significant trend in mortality was observed reaching 50% mortality at the highest loading rate tested (NOELR adult mortality: 6.67 mg/L). The calculated ELR10 (21d) for the Substance based on the nominal loading rate was 4.12 mg/L.

In this key study on *Daphnia magna*, the test was conducted as a semi-static limit test at nominal loading rates (1.98 - 2.96 - 4.45 - 6.67 - 10.0 mg/L). The chemical specific analysis showed that only one of the two main constituents (the C9DPA) could be determined, suggesting that C9DPA is the most water-soluble and bioavailable constituent. The concentration of dialkylated isomers (C9C9DPA) was below the Lowest Calibration Level (LCL) of 0.1 µg/L in every sample in the preliminary range finding, thus not determined in the final test. The measured concentrations of C9DPA in the fresh media, at the beginning of the exposure-renewal interval, were in the range of < LOQ (1 µg test item/L) to 4.65 µg/L. At the end of an exposure-renewal interval (24 hours), most of the measured concentrations were < LOQ. The decline in test concentrations could be due to adsorption to glassware, waste material in the test vessels or dispersed material. The WAFs were checked for any undissolved or emulsified material by Tyndall effect, which was negative.

As indicated in the section on poorly water soluble substances of the Guidance R7b (ECHA, 2017b) “summary of difficult substance testing issues” in table R.7.8-3 (p.80) :

“Toxicity may be observed at concentrations nominally in excess of water solubility, or below the detection limit of the analytical method. Such data are not automatically invalid since the original solubility estimate may be uncertain, and the solution may have been prepared appropriately (e.g. provided any undissolved substance is removed prior to testing). If physical effects are not obvious, then as a realistic worst case, the lowest effect concentration may be based on either the water solubility limit or detection limit of the analytical method, whichever is the lower.”

Taking into account the measured concentrations of the constituent C9DPA, the effects on reproduction occurred at concentrations below the water solubility limit of this constituent, which is < 5 µg/L (OECD TG 105). Thus, based on this study, the LOEC for the substance (according to the C9DPA measured concentrations) should be based on the LOQ of 1 µg/L (which is lower than water solubility of the substance <5 µg/L).

According to Guidance R.10 (Table R.10-1, ECHA, 2008), a LOEC (lowest observed effect concentration) stands for the lowest concentration where an effect has been observed. It may therefore not be used as a NOEC. In case only a LOEC is given in the report (or extrapolated in this case), it can be used to derive a NOEC with the following procedures:

- LOEC > 10 and < 20% effect: NOEC can be calculated as LOEC/2.

- If the effect percentage of the LOEC is unknown no NOEC can be derived.

Considering the available data, the magnitude of the effects (decrease in mean of cumulative offspring per introduce parent at 21d) that were observed at the lowest effects concentration (nominal concentration of 6.67 mg/L) is outside the range of effect (>20%). Thus the NOEC cannot be calculated as LOEC/2 (= 0.5 µg/L).

In addition, the DS recalculated the treatment dose, based on the available measured concentration (Day 0, Day 7 and Day 14), and following the OECD TG 211 for the calculation of the time-weighted mean concentration. Where measurements indicated <LOQ, the DS considered the approach mentioned in the Guidance on the Biocidal Products Regulation Vol IV B+C (ECHA, 2017d, p. 109 & p.183)[11] and used a value corresponding to LOQ/2=0.5µg/L. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance (p.76) also mentioned that "when the substance is detected but not quantified, it is good practice to use half of the limit of quantification" (ECHA, 2017c). There seems to be a slight trend to have higher dissolved concentrations of C9DPA in the test media with higher loading rates when calculating the average based on the 3 measurements from fresh solutions. The corresponding treatment doses (measured) are indicated in the table below.

Table 44: NOEC and LOEC estimations based on nominal and time-weighted mean measured concentration.

Treatment based on nominal concentration of the UVCB substance (mg/L)	Treatment based on time-weighted mean measured concentration of the C9DPA constituent (µg/L)	Mean offspring per introduced parent after 21d (% effect relative to control)	
Control	Control	96.9	
1.98	1.13 (average meas. fresh)	92.4 (-4.6)	
2.96	1.46 (average meas. fresh)	100.4 (+3.6%)	
4.45	1.28 (average meas. fresh)	84.7 (-12.6%)	NOEC
6.67	1.73 (average meas. fresh)	74.5* (-23.1%)	LOEC
10.0	1.74 (average meas. fresh)	76.1* (-21.5%)	

[11] EC (1999). Study on the Prioritisation of Substances Dangerous to the Aquatic Environment: II Assessment of Options of the Statistical Treatment and Evaluation of Monitoring Data within the COMMPS Procedure. Office for Official Publications of the EC, Luxembourg.

* Significants effects p(J)>α=0.05 (Step-down Jonckheere-Terpstra Test Procedure)

The DS also used REGTOX_EV7.0.7 to calculated an EC10 value based on the time-weighted mean concentration of the C9DPA constituent.

Table 45: EC10 estimations and confidence intervals (REGTOX_EV7.0.7)

Calc. Parameters	**Parameters values**			**Confidence intervals**	
HILL	**Optimal**	**Average**	**Median**	**< alpha =5**	**> alpha =5**
Control	93.57	94.60	94.53	87.03	103.15
Hill number	29.61	55.01	28.95	3.07	242.52
EC10	1.69	1.65	1.69	1.21	1.74

The EC10 1.69 µg/L (CI_{95} 1.21-1.74) is considered by DS. Confronting the data obtained from these different methods, it can be assumed that the values for LOEC, EC10 or NOEC are in the same range of concentration. This EC10 value (1.69 µg/L) being close to NOEC 1.28 µg/L, and considering that the statistical analysis from the study is adequate, the DS determined that a NOEC of 1.28 µg/L and a LOEC of 1.73 µg/L based on measured concentrations for the C9DPA will be used for further considerations.

11.5.3 Chronic toxicity to algae or other aquatic plants

Two studies were selected to assess the toxicity of the UVCB substance on aquatic algae. These studies are also described in the section 11.4.3 "acute (short-term) toxicity to algae or other aquatic plants studies" above.

The key study (OECD TG 201, GLP compliant, 1997) was realised on *Pseudokirchneriella subcapitata* and a NOEC (96h) of 33 mg/L (nominal) based on growth rate was reported. In the second study (OECD TG 201, GLP compliant, 2020) also on *Pseudokirchneriella subcapitata*, no toxic effect were identified and an ErL10 (72h) of >100 mg/L (nominal loading rate WAF) based on growth rate was reported.

Additional data on the constituents to be considered in the weight of evidence

An additional study (OECD TG 201, GLP compliant, 2003) on aquatic algae species *Desmodesmus(Scenedesmus) subspicatus* was realised with one constituent of the UVCB substance (C9DPA). The results show no effect on growth. There were no differences between the control, solvent control and the 0.0113 mg/L test group in this study. Therefore, the NOEC (72h) was 0.0113 mg/L based on nominal concentration and 0.0022 mg/L based on measured dissolved fraction for both growth rate and biomass.

11.5.4 Chronic toxicity to other aquatic organisms

No data available.

11.6 Comparison with the CLP criteria

For aquatic hazards, it is possible to use the water solubility as the value to compare with the CLP criteria where effects are observed in excess of the water solubility (CLP Guidance Annex I I.4.2 a-d). The DS have considered this approach in the assessment, although this approach was not prefered for the proposal on classification. For long-term aquatic hazard the NOELR of 1.28 µg/L used for classification is lower than, although in the same range as, the water solubility of the substance (<5 µg/L OECD TG 105). For acute aquatic hazard, the water solubility of the substance approach could apply. However, considering the weight of evidence, and as explained in section 11.4.3, this approach was not retained.

11.6.1 Acute aquatic hazard

At least one acceptable study is available for each category of aquatic organisms. However, the interpretation of several studies is limited by the absence of measured concentration and/or uncertainties on the solubilisation of the test substance. Two studies showed adverse effects in regards to the substance. One study conducted with C9DPA constituent using Japanese Medaka (*Oryzias latipes*) without analytical measurments and using a solvent reported a LC50 (96 h) of 52 mg/L (nominal). This study is considered non reliable for use under CLP. The second study is an algea growth inhibition test (Unpublished study report, 1997). In this OECD TG 201 on *Pseudokirchneriella subcapitata,* in the absence of measured concentrations, a 72 hour EC50 corresponding to 200 mg/L (nominal concentration) was calculated using the number of cells/mL (96 hour EC50=220 mg/L). In addition, a 72 hour EC50 corresponding to 600 mg/L was calculated using the average specific growth rate (96 hour EC50=870 mg/L). The growth rate is the recommended endpoint in the determination of aquatic toxicity to algae, therefore the chosen endpoint is the value of 600 mg/L for algae and it does not fulfil the criteria for an aquatic acute classification under the CLP regulation. Considering the poor solubility of the substance and the discrepancies between nominal and measured concentrations in studies where it was analysed, it is however noted that the use of nominal concentration may largely have under-estimated the acute toxicity of the substance. The available experimental acute data on fish, invertebrates and algae indicate that the substance or constituents are not toxic up to its solubility limit of 5 µg/L.

	Criteria for acute environmental hazards	**Substance Reaction products of diphenylamine with nonene, branched**	**Conclusion**
Acute Aquatic Toxicity	Cat. 1: LC50/EC50/ErC50 ≤ 1 mg/L	Fish: 96h-LC50= >0.0013 mg/L (*Oncorhynchus mykiss*) No effect at saturation Invertebrates: 48h-EC50= >0.0014 mg/L (*Daphnia magna*) No effect at saturation Algae: 72h-EC50= 600 mg/L (*Pseudokirchneriella subcapitata*)	**No classification required**

11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Considering the results of all models and empirical data on the substance, there is a consistent line of evidence to suggest that the main constituents (C9DPA, C9C9DPA and C9C9C9DPA) of "Reaction products

of diphenylamine with nonene, branched" do not undergo a rapid rate of biodegradation in water. Moreover, the model predictions and data on the substance show a slow rate for ultimate degradation.

Collected information supports the potential for bioaccumulation of the substance and its main constituents (Log Pow > 4 and BCF > 500).

At least, one acceptable toxicity study is available for each category of aquatic organisms although the interpretation of some studies is limited by the absence of measured concentration and/or uncertainties on the solubilisation of the test substance.

Two studies showed adverse effects in regards to the substance.

The study on *Daphnia magna* indicated toxic effects of the substance (OECD TG 211, GLP compliant, 2020). The DS revised the NOEC value based on measured concentration of C9DPA constituent, which was the only constituent that could have been detected and measured in this test with the UVCB substance, and determined a NOEC of 1.28 µg/L.

The algae study (OECD TG 201, GLP compliant, 1997) was realised on *Pseudokirchneriella subcapitata* and a NOEC (96h) of 33 mg/L (nominal) based on growth rate was reported. No analytical measurements of the substance was reported in the study. Considering the poor solubility of the substance and the discrepancies between nominal and measured concentrations in studies where it was analysed, it is however noted that the use of nominal concentration may largely have under-estimated chronic toxicity of the substance.

	Criteria for long-term environmental hazards	**Substance Reaction products of diphenylamine with nonene, branched**	**Conclusion**
Rapid degradation	Half-life hydrolysis < 16 days	Not applicable	**Not rapidly degradable**
	Readily biodegradable in a 28-day test for ready biodegradability (> 70 % DOC removal or > 60 % theoretical oxygen demand, theoretical carbon dioxide)	Not available, one inherent degradability study <70% degradation in 56 days	
	Primary degradation: half-life < 16 days (if degradation products do not fulfil criteria for classification as hazardous to the aquatic environment)	OECD 309 non reliable for primary degradation, no information on degradation products, no significant ultimate degradation	
Bioaccumulation	BCF ≥ 500	Based on bcmfR calculation(BCF_{KLip}): C9DPA (10µg/L)=2219	**Bioaccumulative** (potential for bioconcentration in the aquatic environment)
Chronic Aquatic Toxicity	Not rapidly degradable substances: Cat. 1: NOEC ≤ 0.1 mg/L Cat. 2: NOEC ≤ 1 mg/L (based on Table 4.1.0 (b) (i) of the CLP Regulation)	Invertebrate: 21d-NOEC= 1.28 µg/L (0.00128 mg/L) (*Daphnia magna*)	Aquatic Chronic 1 (based on invertebrate-NOEC) M-factor=10

	Surrogate approach in absence of appropriate chronic toxicity reference data (based on Table 4.1.0 (b) (iii) of the CLP Regulation): Not rapidly degradable substances and/or bioaccumulative substances: Cat. 1: E/LC50 ≤ 1 mg/L Cat. 2: E/LC50 > 1 to ≤ 10 mg/L Cat. 3: E/LC50 > 10 to ≤ 100 mg/L	Not applicable	**Not applicable**

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on the CLP regulation, a classification for aquatic acute hazards is not justified for the substance.

Based on the CLP regulation, a classification in category 1 - H410 for aquatic chronic hazards is justified for the substance according to the criteria given in Table 4.1.0(b)(i) and considering the chronic data on toxicity for *Daphnia magna* NOEC(21d) 1.28µg/L. A Mfactor=10 should apply.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

ACD/Percepta (2012) [PK Explorer]. c1997-2012. Toronto (ON): Advanced Chemistry Development. Available from: www.acdlabs.com/products/percepta/

Cappon, G. D., Fleeman, T. L., Chapin, R. E., & Hurtt, M. E. (2005). Effects of feed restriction during organogenesis on embryo-fetal development in rabbit. Birth defects research. Part B, Developmental and reproductive toxicology, 74(5), 424–430. https://doi.org/10.1002/bdrb.20058

ECHA (2008) Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment.

ECHA (2017a). Read-Across Assessment Framework (RAAF). European Chemicals Agency. Rf: ECHA-17-R-01-EN. ISBN: 978-92-9495-758-0. DoI: 10.2823/619212. March 2017. URL: https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/614e5d61-891d-4154-8a47 -87efebd1851a

ECHA (2017b) Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0 - July 2017. Helsinki, Finland. https://echa.europa.eu/documents/10162/2324906/clp_en.pdf/

ECHA (2017c) Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7b: Endpoint specific guidance. Version 4.0 June 2017.

ECHA (2017d) Guidance on the Biocidal Products Regulation. Volume IV Environment – Assessment and evaluation (Parts B+C) Version 2.0 October 2017.

ECHA (2018): Collaborative approach pilot projects : March 2017–March 2018 : final report, European Chemicals Agency, 2018, https://data.europa.eu/doi/10.2823/224234

ECHA (2022): Advice on dose-level selection for the conduct of reproductive toxicity studies (OECD TGs 414, 421/422 and 443) under REACH. https://echa.europa.eu/documents/10162/17220/211221_echa_advice_dose__repro_en.pdf/27159fb1-c31c-78a2-bdef-8f423f2b6568?t=1640082455275

Environment and Climate Change Canada (ECCC), Health Canada (HC) (2017): Screening Assessment for Substituted Diphenylamines. https://www.canada.ca/content/dam/eccc/documents/pdf/pded/sdpas/English%20Screening%20Assessment%20for%20Substituted%20Diphenylamines1.pdf

Goodman, J. H., & Gilbert, M. E. (2007). Modest thyroid hormone insufficiency during development induces a cellular malformation in the corpus callosum: a model of cortical dysplasia. Endocrinology, 148(6), 2593–2597. https://doi.org/10.1210/en.2006-1276

Lopez-Tello, J., Arias-Alvarez, M., Gonzalez-Bulnes, A., & Sferuzzi-Perri, A. N. (2019). Models of Intrauterine growth restriction and fetal programming in rabbits. Molecular reproduction and development, 86(12), 1781–1809. https://doi.org/10.1002/mrd.23271

Matsuoka, T., Mizoguchi, Y., Serizawa, K., Ishikura, T., Mizuguchi, H., & Asano, Y. (2006). Effects of stage and degree of restricted feeding on pregnancy outcome in rabbits. The Journal of toxicological sciences, 31(2), 169–175. https://doi.org/10.2131/jts.31.169

Matsuzawa, T., Nakata, M., Goto, I., & Tsushima, M. (1981). Dietary deprivation induces fetal loss and abortion in rabbits. Toxicology, 22(3), 255–259. https://doi.org/10.1016/0300-483x(81)90088-3

Moser V, Bailey F, Bowers W, Raffaele K, Crofton K, Gilbert M (2016). North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (TWG) Developmental Neurotoxicity Study Guidance Document. https://www.epa.gov/sites/production/files/201702/documents/developmental_neurotoxicity_study_internal_guidance_document_final_0.pdf

Noyes PD, Friedman KP, Browne P, Haselman JT, Gilbert ME, Hornung MW, Barone SJr, Crofton KM, Laws SC, Stoker TE, Simmons SO, Tietge JE and Degitz SJ (2019). Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with in Vitro Testing and Adverse Outcome Pathway Approaches. *Environmental health perspectives*, 127(9), pp. 95001-95020. https://doi.org/10.1289/EHP5297.

OECD (2016a): Case study on the use of integrated approaches for testing and assessment for repeat dose toxicity of substituted diphenylamines (SDPA). OECD Series on Testing & Assessment, No. 252. ENV/JM/MONO(2016)50.

https://one.oecd.org/document/ENV/JM/MONO(2016)50/en/pdf

OECD (2016) Guidance document to OECD TG 305 2nd DRAFT Drafted by the Lead Countries DE, UK, NL 4/12/2016

OECD (2019) Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals series on testing and assessment no. 23 (second edition). Environment directorate joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology env/jm/mono(2000)6/rev1.

Pagnin, M., Kondos-Devcic, D., Chincarini, G., Cumberland, A., Richardson, S. J., & Tolcos, M. (2021). Role of thyroid hormones in normal and abnormal central nervous system myelination in humans and rodents. Frontiers in neuroendocrinology, 61, 100901. https://doi.org/10.1016/j.yfrne.2021.100901

Unpublished Study Report (1997) OECD Guideline 201 (Alga, Growth Inhibition Test) [before 23 March 2006] ; according to guideline EPA OTS 797.1050 (Algal Toxicity, Tiers I and II), Acute toxicity of the water accommondated fraction (WAF) to the freshwater alga, *Selenastrum carpicornutum*.

Unpublished Study Report (2000) Bioaccumulation in aquatic species: fish Cyprinus carpio [fish] aqueous flow-through, According to test methods designated for New Chemical Substances (1974, amended 1998) under Chemical Substances Control Law of Japan (MITI).

Unpublished Study Report (2003a) Inherent biodegradability: activated sludge (predominantly domestic sewage) and mixed population of soil microorganisms (natural) (aerobic) according to guideline OECD Guideline 302 D Draft (Inherent Biodegradability - Concawe Test)

Unpublished Study Report (2003b) Oncorhynchus mykiss (previous name: Salmo gairdneri) freshwater short-term toxicity to fish according to guideline EU Method C.1 (Acute Toxicity for Fish) ; according to guideline OECD Guideline 203 (Fish, Acute Toxicity Test).

Unpublished Study Report (2003c) OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test). Acute toxicity to *Daphnia magna.*

Unpublished Study Report (2003d) OECD Guideline 201 (Alga, Growth Inhibition Test).

Unpublished Study Report (2004) OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test). Acute toxicity to *Daphnia magna* in a 48-hour immobilization test.

Unpublished Study Report (2013) Reaction products of benzeneamine, N-phenyl with nonene (branched) - Repeated-dose 90-Day Toxicity Study in Wistar Rats Administration by Gavage (study report)

Unpublished Study Report (2014a) Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene Metabolome analysis conducted for a screening study in Wistar rats Administration by gavage for 29 days.

Unpublished Study Report (2014b) Combined oral repeated dose toxicity study and reproduction/developmental toxicity screening test.

Unpublished Study Report (2014c). Oral prenatal developmental toxicity study in rats

Unpublished Study Report (2014d) Reaction products of benzeneamine, N-phenyl with nonene (branched). Preliminary oral prenatal developmental toxicity study in rats

Unpublished Study Report (2017) Estimation of bioaccumulation using CATALOGIC v5.11.17 (BCF base-line model v02.09) (model calculation)

Unpublished Study Report (2019a) Reaction products of benzeneamine, N-phenyl with nonene (branched) Prenatal Developmental Toxicity Study in New Zealand White Rabbits Oral Administration (Gavage)

Unpublished Study Report (2019b) Summary of Results - Reaction products of benzeneamine, N-phenyl with nonene (branched) Maternal Toxicity Study in New Zealand White Rabbits (Range-Finding) Oral Administration (Gavage)

Unpublished Study Report (2019c) Summary of Results - Test study in female non pregnant New Zealand White Rabbits Oral Administration (Gavage)

Unpublished Study Report (2019d) OASIS Catalogic 5.13.1.156 CATALOGIC 301C v11.15 CAS 36878-20-3C9C9dialkylated (QSAR QPRF)

Unpublished Study Report (2020a) Reaction products of benzeneamine, N-phenyl with nonene (branched), Reproduction/Developmental Toxicity Screening Test in Wistar Rats Administration via the Diet and recovery period of 2 weeks.

Unpublished Study Report (2020b) Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene Reproduction/Developmental Toxicity Screening Test in Wistar Rats Administration via the Diet.

Unpublished Study Report (2020c) Biodegradation in water: simulation testing on ultimate degradation in surface water: Test system: natural water: freshwater (aerobic) according to guideline OECD Guideline 309.

Unpublished Study Report (2020d) OECD Guideline 210: Early-Life Stage Toxicity Test with Zebrafish (Danio rerio).

Unpublished Study Report (2020e) OECD Guideline 211 (Daphnia magna Reproduction Test), Daphnia magna Reproduction Test, Semi-static, 21 days.

Unpublished Study Report (2020f) OECD Guideline 201 (Alga, Growth Inhibition Test), Alga, Growth Inhibition Test with Pseudokirchneriella subcapitata, 72 hours.

Unpublished Study Report (2021) Report Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene Extended One-Generation Reproduction Toxicity Study in Wistar Rats Administration via the Diet.

15 ANNEXES

Please see separate documents for:

- *Non-confidential Annex I with the details of the studies*
- *Confidential Annex II containing confidential information regarding the identities of the substance and its analogue and the list of confidential references.*