

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene

EC Number: 270-128-1

CAS Number: 68411-46-1

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

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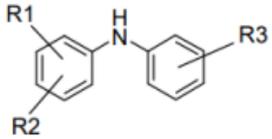
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

The present proposal for harmonised classification and labelling relates to the substance **Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (EC 270-128-1, CAS 68411-46-1) (table 1).**

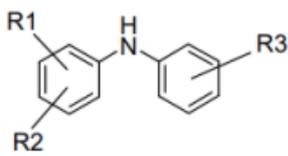
The identity of the analogue substance **Reaction products of diphenylamine with nonene, branched** is reported in table 2 to assist the read across approach used in support in this CLH report. This substance is not covered by this CLH proposal.

Table 1: Substance identity and information related to molecular and structural formula of the substance 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	 <p>R1 = H or tert-butyl or iso-octyl R2 = tert-butyl or iso-octyl R3 = tert-butyl or iso-octyl</p>
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

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Table 2: Substance identity and information related to molecular and structural formula of the analogue 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)	1
EC name (if available and appropriate)	
CAS number (if available)	
Other identity code (if available)	-
Molecular formula	C ₂₁ H ₂₉ N - C ₃₀ H ₄₇ N (main constituents)
Structural formula	 <p>R1 = H or iso-nonyl R2 = iso-nonyl R3 = iso-nonyl</p>
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 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 No EC or CAS number allocated. To be specified when allocated.

1.2 Composition of the substance

The present proposal for harmonised classification and labelling relates to the substance **Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (EC 270-128-1, CAS 68411-46-1)**. 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.

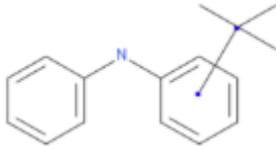
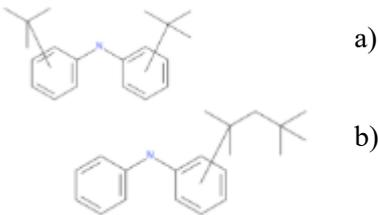
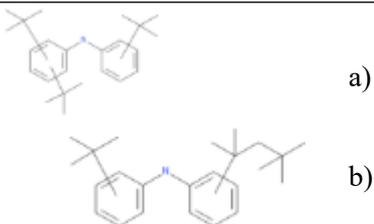
The composition of the analogue substance Reaction products of diphenylamine with nonene, branched is reported in tables 5 and 6 to assist the read across approach used in support in this CLH report. There is no available information on additives.

This substance is not covered by this CLH proposal.

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

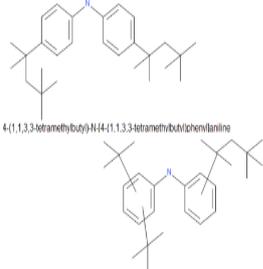
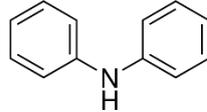
The data originate from the substance identity part of the publically disseminated lead Registration Dossier (ECHA dissemination website, 2023)². Two boundary compositions are presented according to the content in Diphenylamine (DPA).

Table 3: Registered boundary composition of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene with with $0.25\% \leq \text{DPA} < 2.5\%$

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex V I 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) Monoocetyl diphenylamine: C8DPA	Confidential information; see confidential Annex II	-	-	
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) Tributyldiphenylamine: C4C4C4DPA	Confidential information; see confidential Annex II	-	-	

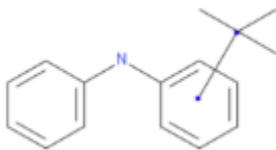
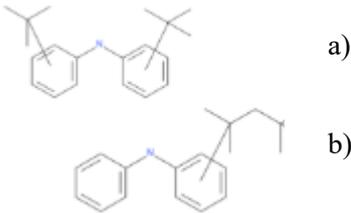
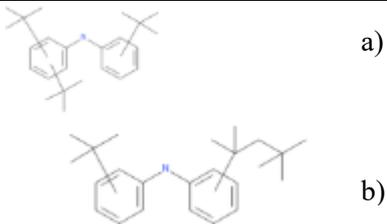
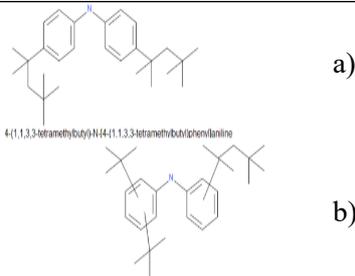
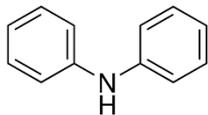
² Accessed on 01/02/2023

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Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex V I Table 3.1 (CLP)	Current self-classification and labelling (CLP)	Structure
b) Monobutylmono octyldiphenyl amine: C4C8DPA				
<p>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</p> <p>[C₂₈H₄₃N]</p> <p>a) Dioctyldiphenyl amine: C8C8DPA b) Dibutylmono octyldiphenylamine: C4C4C8</p>	Confidential information; see confidential Annex II	-	-	 <p>a)</p> <p>b)</p>
<p>Diphenylamine (DPA)</p> <p>[C₁₂H₁₁N]</p> <p>MW = 169.22</p> <p>EC no.: 204-539-4 CAS no.: 122-39-4</p>	≥ 0.25% - < 2.5% w/w	<p>Acute Tox. 3 *, H301 Acute Tox. 3 *, H311 Acute Tox. 3 *, H311 Acute Tox. 3 *, H331 Eye Irrit. 2, H319 Eye Dam. 1, H331 STOT H318 RE 2*, H373** Aquatic Acute 1, H400 Aquatic chronic 1, H410</p>	<p>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</p>	

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Table 4: 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 V I 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) Monoctyl diphenylamine: C8DPA	Confidential information; see confidential Annex II	-	-	
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) Tributyldiphenylamine: C4C4C4DPA b) Monobutylmono octyldiphenylamine: C4C8DPA	Confidential information; see confidential Annex II	-	-	
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) Dioctyldiphenylamine: C8C8DPA b) Dibutylmono octyldiphenylamine: C4C4C8	Confidential information; see confidential Annex II	-	-	
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	

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Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex V I 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	

According to ECHA C&L inventory, 2023³, on the 878 C&L notifications :

- 611 classifications Aquatic chronic 3, H412
- 178 no classification
- 111 classifications Repr.2, H361f
- 23 classifications Aquatic chronic 2, H411
- 6 classifications Aquatic acute 1, H400
- 6 classifications Eye Irrit. 2, H319
- 4 classifications STOT RE 2, H373

The following self-classifications have been provided by the registrants for the Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (according to ECHA dissemination, 2023). This classification is included in the notifications indicated above.

For a concentration of $0.25\% \leq \text{DPA} < 2.5\%$:

- Repr.2, H361f
- Aquatic chronic 3, H412

³ Accessed on 01/02/2023

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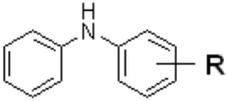
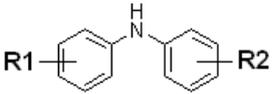
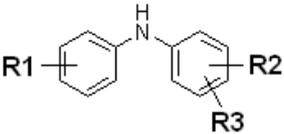
For a concentration of DPA < 0.25%:

- Repr.2, H361f

Analogue substance: Reaction products of diphenylamine with nonene, branched

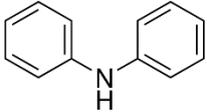
The data originate from the substance identity part of the publically disseminated Registration Dossier (ECHA, 2023)⁴. Two boundary compositions are presented according to the content in Diphenylamine (DPA).

Table 5: Registered boundary composition of Reaction products of diphenylamine with nonene, branched (BNPA) with $0.25\% \leq \text{DPA} < 2.5\%$

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 EC no.: / CAS no.: /	Confidential information; see confidential Annex II	-	-	
Other constituents [C ₂₂ H ₃₁ N - C ₂₉ H ₄₅ N]	Confidential information; see confidential Annex II	-	-	

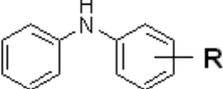
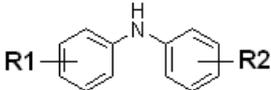
⁴ Accessed on 01/02/2023

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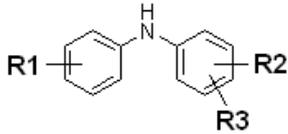
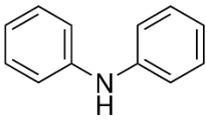
Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	Structure
MW = 309.5 - 407.7 EC no.: / CAS no.: /				
Diphenylamine (DPA) [C ₁₂ H ₁₁ N] MW = 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 6: 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-onyl, 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-onyl, branched)aniline (DNDPA): C9C9DPA [C ₃₀ H ₄₇ N]	Confidential information; see confidential Annex II	-	-	

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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 classification self- and labelling (CLP)	Structure
MW = 421.7 EC no.: / CAS no.: /				
N-phenyl-tris(arynonyl, 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	

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	benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene	270-128-1	68411-46-1	Repr. 1B Aquatic Chronic 2	H360FD H411	GHS08 GHS09 Dgr	H360FD H411			

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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, as presented in this report, support the following classifications:

- Repr 1B- H360FD
 - o There is no requirement for justification for this hazard class.
- Aquatic chronic 2, H411
 - o 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 2, H411.

5 IDENTIFIED USES

Table 9: Summary of uses for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (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	<p>This substance is used in the following products: lubricants and greases, metal working fluids, hydraulic fluids and heat transfer fluids.</p> <p>This substance is used for the manufacture of plastic product. This substance is used for the manufacture of chemicals.</p> <p>Industrial use in the production of articles and in processing aids at industrial sites.</p>
Uses by professional workers	<p>This substance is used in the following products: lubricants and greases, adhesives and sealants, hydraulic fluids, metal working fluids and heat transfer fluids.</p> <p>Indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), outdoor use, indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters) and outdoor use in close systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids).</p>

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Consumer uses	<p>This substance is used in the following products: lubricants and greases.</p> <p>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.</p>
Article service life	<p>Industrial abrasion processing with low release rate (e.g. cutting of textile, cutting, machining or grinding of metal). 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 (e.g. food packaging and storage, toys, mobile phones).</p>

6 DATA SOURCES

All the data available in the registration dossier (Last modification on IUCLID taken in account : 14/12/2022) 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: “Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene OR 68411-46-1”, “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 retrieved 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: “Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene OR 68411-46-1”, “alkyldiphenylamines”, “substituted diphenylamines”, SDPA, SDPAs, EC 270-128-1 and CAS 68411-46-1.

7 PHYSICOCHEMICAL PROPERTIES

Table 10: Summary of physicochemical properties for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene [2]

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Physical state at 20°C and 1013 hPa: liquid Form: liquid viscous Colour: yellow (whole substance)	ECHA dissemination website, 2023	
Melting/freezing point	No melting temperature	ECHA dissemination	The melting/freezing point was

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Property	Value	Reference	Comment (e.g. measured or estimated)
	was found between -100°C and 50 °C. (whole substance)	website, 2023	carried out according to OECD Guideline 102.
Boiling point	>300°C (whole substance)	ECHA dissemination website, 2023	The determination was conducted by Siwoloboff method according to OECD Guideline 103.
Relative density	969 kg/m ³ at 20°C (whole substance)	ECHA dissemination website, 2023	The density was determined by pycnometer according to OECD Guideline No.109.
Vapour pressure	Measured 0.0015 Pa at 20°C (whole substance) Calculated <0.01 Pa at 25°C (for the whole substance based on vapour pressure data on the individual components generated by calculation)	ECHA dissemination website, 2023	The vapour pressure was measured by the thermogravimetry method (diffusion controlled evaporation) In addition, the vapour pressure was calculated using recommended program SPARC v4.6. The calculation have been generated on the individual components of test substance and it can be summarized that the vapour pressure was calculated to be <0.01 Pa at 25°C
Surface tension	-	ECHA dissemination website, 2023	Based on structure, surface activity is not expected.
Water solubility	The solubilities of the UVCB (whole substance) at 20°C and at pH 5.6 were found as follows: 1.6 mg/l with a loading rate of ca. 1 g/L 2.3 mg/l with a loading rate of ca. 10 g/L 4 µg/L at 20°C and pH 6.6 for one isomer of the constituent DTBDA C4C4DPA or C8DPA)	ECHA dissemination website, 2023	The determinations of the solubility of the UVCB were carried out by flask method in accordance with the OECD Guideline 105. The quantification was analyzed by TOC. The measured values for the UVCB are not considered reliable by DS. The method implemented (i.e. flask method quantification by TOC) is not appropriate according to the OECD Guideline 105 for poorly water soluble substances which is the case of the UVCB. The water solubility of the UVCB is expected to be far lower considering the water solubility of 4 µL/ for C4C4DPA (a major constituent) measured with the appropriate method (i.e. elution method). This is further supported by the estimated values of water solubility of the different constituents using Epi Suite V4.11 - WSKOWWIN v1.42.

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Property	Value	Reference	Comment (e.g. measured or estimated)
			The determination of the solubility of one isomer of the DTBDA was carried out by column elution method according to OECD Guideline 105. The concentration was quantified by LC-MS.
Partition coefficient n-octanol/water	log Pow ≥ 5 at 25°C. (For the whole substance based on the log Kow of individual constituents generated by calculation) log Pow= 6.7 at 23°C, pH 6.7 (Partition coefficient of one isomer of the constituent DTBDA)	ECHA dissemination website, 2023	Since the substance is a UVCB thus the log Pow values on representative selection constituents have been individually calculated using recommended software program KOWWIN module (v.1.68) of software EPI Suite (v.4.11). The determination of the log Pow of the constituent DTBDA was conducted by slow-stirring method according to OECD Guideline 123.
Flash point	154°C at 981 mBar (whole substance)	ECHA dissemination website, 2023	The determination was performed according to DIN 51758.
Flammability	-Not highly flammable upon ignition - No pyrophoric properties - Does not liberate flammable gases on contact with water. (whole substance)	ECHA dissemination website, 2023	The flammability of a liquid is derived from flash point and was determined to be greater than 100°C. - Based on chemical structure and experience in handling and use of substance the pyrophoric properties and the flammability in contact with water are not to be expected. It can be concluded that the substance is not considered to be classified as a flammable material.
Explosive properties	No explosive properties (whole substance)	ECHA dissemination website, 2023	The substance does not contain chemical groups associated with explosive properties present in the molecule. The substance is not to be considered having potential explosive properties. Thus, it is not to be classified as an explosive material.
Self-ignition temperature	490 °C (whole substance)	ECHA dissemination website, 2023	The determination was performed according to DIN 51794

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Property	Value	Reference	Comment (e.g. measured or estimated)
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. The criteria above are fulfilled for the substance and which is not to be considered having potential oxidizing properties. Thus, it is not to be classified as an oxidizing material.
Granulometry	-	ECHA dissemination website, 2023	The substance is a liquid.
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 test substance is a complex mixture (UVCB) of individual substances with different solubilities therefore the pKa values could not be determined experimentally. Additionally, the pKa values were calculated using recommended program SPARC v4.6. The calculation have been generated on the individual components of test substance. It can be concluded that the pKa values of test substance was estimated in the range of 1.5 to 2.
Viscosity	352.7 mm ² /s at 40°C (whole substance)	ECHA dissemination website, 2023	The determination was carried out in Ubbelohde capillary viscometer according to the DIN 51562 -1. Due to the very high viscosity of the test item at 20 °C all the measurements were carried out at 40 °C

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

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Oral exposure

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene caused systemic toxicity upon oral application in various repeated dose toxicity studies demonstrating its bioavailability.

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene is a substituted diphenylamine (SDPA). It is an UVCB substances consisting in a mixture of mono-, di- and tri-alkylated 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 respectively (Table 10). 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 10). 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).

Table 11: Modelled LogKow, water solubility and toxicokinetic parameters of the different constituents of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (OECD, 2016a).

UVCB	Constituents	PM	LogKow	Water solubility mg/L	* Oral bioavailability(%F)	Cmax* µg/ml	Tmax* h	AUC _{0-inf} * µg.h/ml
Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene 270-128-1 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
	Mono-octyl DPA				21.8	0.15	6.46	2.17
	Monobutyl mono-octyl DPA	337.5	9	1.5 x 10 ⁻⁴	11.04	0.07	8.05	1.67
	Tributyl DPA				no data	no data	no data	no data
	Di-octyl DPA				0.43	0.002	9.6	0.06
	Dibutyl mono-octyl DPA	393.6	10.8	1.93 x 10 ⁻⁶	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, 2016)

** Measured value for dibutylDPA was also available from the registered dossier 0.004 mg/L at 20°C, pH 6.6 (OECD TG 105, column elution method)

Metabolism

In the OECD report on SDPAs (2016), 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.

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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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (125 and 300 mg/kg bw/day) for 28 days was investigated and compared to the metabolome of Wistar rats treated with Reaction products of diphenylamine with nonene, branched (analogue SDPA) (300 and 1000 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 substances 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 substances 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

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene as well as Reaction products of diphenylamine with nonene, branched 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 Reaction products of diphenylamine with nonene, branched (source substance) to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (target substance).

10.10.1 Adverse effects on sexual function and fertility

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 of exposure	Results	Reference, Reliability
Studies with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene			
<p>Reproduction/Developmental Toxicity Screening Test OECD TG 421 (2016)</p> <p>GLP-compliant</p> <p>Rat Wistar Crl:WI(Han) N = 10/sex/dose group</p> <p>10-week pre mating period</p> <p><i>Deviations:</i> Only 6 F pregnant in the low dose group while 8 per group is the minimum acceptable number according to the TG.</p> <p><i>Additional investigations:</i> 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.</p> <p>NB: Thyroid histopathology performed in M and F (optional in OECD TG 421). Thyroid hormones measured in adult M and F (optional) and</p>	<p>Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene</p> <p>CAS: 68411-46-1</p> <p>Batch: 50116118D</p> <p>Purity: UVCB</p> <p>By diet</p> <p>Diet : 0, 300, 1000 and 3000 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance</p> <p>Eq. to: 0, 26/28, 87/95 and 260/271 mg/kg bw/d in M/F</p> <p>F0: 10-week pre mating period</p> <p>F1: terminated at PND13</p>	<p>Effects on sexual function and fertility P0</p> <p>3000 ppm (260/271 mg/kg bw/d)</p> <p>↑ estrous cycles length (4.7 days vs. control 4.0 days). ↑ mean nb of days in diestrous stage.</p> <p>↓ nb of implantation sites (-36%) and consequently ↓ nb of pups delivered (-34%).</p> <p>↓ ovaries absolute weight (-25%).</p> <p>1000 ppm (87/95 mg/kg bw/d)</p> <p><i>Non statistically significant ↓ nb of implantation sites (-14%).</i></p> <p>300 ppm (26/28 mg/kg bw/d)</p> <p>No effect</p> <p>General/systemic toxicity P0</p> <p>3000 ppm (260/271 mg/kg bw/d)</p> <p>↓ 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%).</p> <p>↓ Final BW in M (-9%) and ↓ BW in F at the end of the pre mating period (-9%), at the end of gestation (-17%), at the beginning of lactation (-9%) and at termination PND14 (-15%) compared to controls.</p> <p>Liver effects: ↑ 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.</p> <p>Thyroid effects: ↑ 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.</p> <p>1000 ppm (87/95 mg/kg bw/d)</p> <p>↓ Food consumption in F during gestation period (-12%).</p> <p>↓ BW in F at the end of the pre mating period (-7%), at the end of gestation (-8%), <i>at the beginning of lactation (-4%)</i> and at termination PND14 (-8%).</p>	<p>Unpublished study report, 2020b</p> <p>Bridging study</p> <p>Reliability: Reliability: 1, reliable without restriction supporting study</p> <p>Details available under "Study 2" in Annex I (3.10.1.2)</p>

CLH REPORT FOR BENZENAMINE, N-PHENYL-, REACTION PRODUCTS WITH 2,4,4-TRIMETHYLPENTENE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of exposure	Results	Reference, Reliability
<p><i>PND13 M and F pups.</i></p>		<p><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.</p> <p><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.</p> <p>300 ppm (26/28 mg/kg bw/d)</p> <p><u>Liver effects:</u> ↑ centrilobular hypertrophy (minimal to mild) in 2/10 M and single cell necrosis/apoptosis in 2/10 M (minimal). Biochemistry: ↑ALP in F.</p> <p><u>Thyroid effects:</u> Hypertrophy/hyperplasia of follicular cells of 3/10 M and of 2/10 F (minimal to mild) in combination with altered colloid.</p> <p>F1 pups</p> <p>3000 ppm (260/271 mg/kg bw/d)</p> <p>↓ 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.</p> <p>↑ incidence of nipple development (100% vs. 79.6% in control) and number of nipples per animal (5.2 to control 2.5) on PND13.</p> <p>1000 ppm (87/95 mg/kg bw/d)</p> <p>↓ 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.</p> <p>300 ppm (26/28 mg/kg bw/d)</p> <p>No effect.</p>	
<p>EOGRTS OECD TG 443 (2018) GLP-compliant Rat Wistar CrI:WI(Han) P0: 25/sex/dose F1C1A: 20/sex/dose F1C1B (= P1): 25/sex/dose,</p>	<p>Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene CAS: 68411-46-1 Batch: 50116118D Purity: UVCB By diet</p>	<p><u>Effects on sexual function and fertility P0, P1(F1C1B) and F1C1A</u></p> <p>1800 ppm (167/166 mg/kg bw/d)</p> <p>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.</p> <p>↓ nb of implantation sites (P0: -15%, P1: -17%) and consequently ↓ nb of pups delivered (P0: -20%, P1: -18%).</p> <p>↓ Ovaries absolute weight (P0: -13%, P1: -12%). No effect on ovary weight in F1C1A females</p> <p>600 ppm (54 mg/kg bw/d)</p>	<p>Unpublished study report, 2021</p> <p>Key study for adverse effects on sexual function and</p>

CLH REPORT FOR BENZENAMINE, N-PHENYL-, REACTION PRODUCTS WITH 2,4,4-TRIMETHYLPENTENE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of exposure	Results	Reference, Reliability
<p>F1C2A: 10/sex/dose F1C2B: 10/sex/dose</p> <p>10-week prematuring period</p> <p>Deviations:</p> <p>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</p> <p>F2 pups: Thyroid hormones not measured</p> <p>DNT: No historical control data (HCD) no positive control, statistical analysis not appropriate for motor activity, auditory startle response and morphometrics.</p>	<p>Diet : 0, 200, 600 and 1800 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance</p> <p>Eq. to: 0, 18, 54 and 167/166 mg/kg bw/d in M/F</p>	<p>P1 <i>non-statistically significant</i> ↓ <i>nb of implantation sites (-9%)</i> and consequently ↓ <i>nb of pups delivered (-10%)</i>.</p> <p>200 ppm (18 mg/kg bw/d)</p> <p>No effect.</p> <p>General/systemic toxicity P0, P1(F1C1B), F1C1A and F2C2A</p> <p>No mortality or clinical signs attributed to the test substance at any dose levels in any generations.</p> <p>1800 ppm (167/166 mg/kg bw/d)</p> <p>↓ Food consumption in P0 F during gestation and lactation periods (-13%) and in P1 F during the prematuring, gestation and lactation periods (-7%, -14% and -17% respectively). No effect on other treated animals.</p> <p>No effect on BW in P0 M. ↓ final BW compared to controls in P1 M (-6%), F1C1A M (-8%) and F1C2A M (-8%).</p> <p>↓ BW compared to controls in P0 F (-8%, -12%, -11% and -9% at the end of the prematuring 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 prematuring period, at the end of gestation, at the beginning and end of lactation respectively). ↓ Final BW compared to controls in F1C1A F (-6%).</p> <p>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%).</p> <p>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.</p> <p>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 <i>non-statistically significant</i> ↑ <i>TSH</i> 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 <i>non-statistically significant</i> ↑ <i>TSH</i> in M. ↑ TSH in F.</p> <p>Haematology: In P0 and F1C1A M&F slight (<5%) ↓haemoglobin and haematocrit values.</p> <p>600 ppm (54 mg/kg bw/d)</p> <p>No significant effect on BW and food consumption in any generations.</p> <p>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</p>	<p>fertility</p> <p>Reliability: 1, reliable without restriction</p> <p>2 (Reliable with restriction for some DNT parameters)</p> <p>Details available under “Study 3” in Annex I (3.10.1.3)</p>

CLH REPORT FOR BENZENAMINE, N-PHENYL-, REACTION PRODUCTS WITH 2,4,4-TRIMETHYLPENTENE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of exposure	Results	Reference, Reliability
		<p>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.</p> <p><u>Thyroid effects:</u> 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 <i>non-statistically significant</i> ↑ TSH in M. ↑ TSH in F. 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.</p> <p style="text-align: center;">200 ppm (18 mg/kg bw/d)</p> <p><u>Liver effects:</u> 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%).</p> <p><u>Thyroid effects:</u> P0: Minimal follicular cell hypertrophy/hyperplasia in the thyroid of 7 M and 2 F. ↓ T4 and <i>non-statistically significant</i> ↑ TSH in M. ↑ TSH in F. F1C1A: Minimal follicular cell hypertrophy/hyperplasia in the thyroid of 4 M.</p> <p><u>F1 and F2 pups</u></p> <p style="text-align: center;">1800 ppm (167/166 mg/kg bw/d)</p> <p>↓ 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</p> <p>↓ F1 and F2 pups BW PND7-PND21 in M, F and M&F combined (at weaning: -12.5% F1, -16% F2) compared to controls</p> <p>No effect on sex ratio, anogenital distance in F1 or F2 pups</p> <p>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)</p> <p>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)</p> <p>Thyroid hormones measurements: F1PND4: only 2 animals (due to smaller litter sizes). PND22: ↑ TSH in F1 pups (M&F)</p> <p style="text-align: center;">600 ppm (54 mg/kg bw/d)</p> <p>↓ Mean number F2 pups delivered/dam (consequence of the lower nb of implants)</p> <p>↓ F1 pups BW PND7-PND21 M&F combined (at weaning: -5.4%) compared to controls</p>	

CLH REPORT FOR BENZENAMINE, N-PHENYL-, REACTION PRODUCTS WITH 2,4,4-TRIMETHYLPENTENE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of exposure	Results	Reference, Reliability
		<p>Thyroid hormones measurements: F1PND4: <i>non-statistically significant</i> ↓ T4 F1 pups (M&F). PND22: ↑ TSH in F1 pups (M&F).</p> <p>200 ppm (18 mg/kg bw/d)</p> <p>No effect.</p> <p>Developmental neurotoxicity (DNT)</p> <p>1800 ppm (167/166 mg/kg bw/d)</p> <p>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.</p> <p>ASR: ↓ mean maximal amplitude in M (-19%)</p> <p>↓ <i>habituation in M (-57%) and M & F combined (-46%) statistical analysis not performed</i></p> <p>600 ppm (54 mg/kg bw/d)</p> <p>ASR: ↓ mean maximal amplitude in M (-12%)</p> <p>↓ <i>habituation in M (-40%) and M & F combined (-42%) statistical analysis not performed</i></p> <p>200 ppm (18 mg/kg bw/d)</p> <p>No effect.</p>	
<p>Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test</p> <p>OECD TG 422 (1996)</p> <p>GLP-compliant</p> <p>Rat Wistar Crl:WI(Han) N = 10/sex/dose group</p> <p>2-week pre-mating period</p> <p>Several deviations compared to current OECD TG 422 (2016): Pups terminated before PND 13, AGD and nipple retention not investigated. Estrous cycle not monitored.</p>	<p>Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene</p> <p>CAS: 68411-46-1</p> <p>Batch: 40401913D</p> <p>Purity: UVCB</p> <p>By gavage</p> <p>Doses: 0, 25, 75 and 225 mg/kg bw/day</p> <p>Vehicle: corn oil</p> <p>Exposure duration: P0: 28 days (M), 53 days (F) F1:</p>	<p>Effects on sexual function and fertility P0</p> <p>225 mg/kg bw/d</p> <p><i>Non-statistically significant</i> ↓ of mean number of implantation sites (-16%).</p> <p>No effect at 75 mg/kg bw/d and 25 mg/kg bw/d.</p> <p>General/systemic toxicity P0</p> <p>No effects on clinical signs and BW at any dose levels.</p> <p>225 mg/kg bw/d</p> <p><u>Liver effects:</u> ↑ 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).</p> <p><u>Thyroid effects:</u> 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).</p>	<p>Unpublished study report, 2014b</p> <p>Reliability: 1, reliable without restriction</p> <p>Supporting study (screening)</p> <p>Details available under “Study 4” in Annex I (3.10.1.4)</p>

CLH REPORT FOR BENZENAMINE, N-PHENYL-, REACTION PRODUCTS WITH 2,4,4-TRIMETHYLPENTENE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of exposure	Results	Reference, Reliability
<p>NB:</p> <p><i>In P0: TSH, T3 and T4 measured in 5 animal/sex/dose</i></p> <p><i>Thyroid histopathology performed in M and F.</i></p> <p><i>Thyroid hormones measured in adult M and F.</i></p>	<p>terminated at PND5-7</p>	<p>75 mg/kg bw/d</p> <p><u>Liver effects:</u> ↑ 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).</p> <p><u>Thyroid effects:</u> Follicular cell hypertrophy in 5/5 M (2 minimal, 3 mild) bw/d. ↑TSH and ↓T4 for all groups (both sexes, not always statistically significant and no clear dose-response relationship).</p> <p>25 mg/kg bw/d</p> <p><u>Liver effects:</u> ↑ rel liver weight in M (+20%) no correlated histopathological and biochemical findings.</p> <p><u>Thyroid effects:</u> 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, <i>not always statistically significant</i> and no clear dose-response relationship).</p> <p>F1 pups</p> <p>225 mg/kg bw/d</p> <p>↓ 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).</p> <p>↑ in postnatal loss (8 in 3 litters versus 0 in controls) and correspondingly ↓ viability index (88.7% vs 100% in controls).</p> <p>No effect at 75 mg/kg bw/d and 25 mg/kg bw/d.</p>	
<p>Study with the analogue Reaction products of diphenylamine with nonene, branched</p>			
<p>Reproduction/Developmental Toxicity Screening Test</p> <p>OECD TG 421 (2016)</p> <p>GLP-compliant</p> <p>Rat Wistar CrI:Wl(Han) N = 10/sex/dose group</p> <p>10-week prematuring period</p>	<p>Reaction products of diphenylamine with nonene, branched</p> <p>EC:</p> <p>CAS:- (old 36878-20-3)</p> <p>Batch 00 16046440</p> <p>Purity: UVCB</p> <p>By diet</p> <p>Diet : 0, 500, 1500 and 5000 ppm ; half</p>	<p>Effects on sexual function and fertility P0</p> <p>5000 ppm (407/443 mg/kg bw/d)</p> <p><i>Non-statistically significant ↑estrous cycles length (4.3 days vs. control 4.0 days) and ↑ of mean nb of days in diestrous stage.</i></p> <p>↓ nb of implantation sites (-31%) and consequently ↓ nb of pups delivered (-31%).</p> <p>↓ ovaries absolute (-40%) and relative (-31%) weights.</p> <p>Recovery group: ↓ ovaries absolute weight (-14%).</p> <p>1500 ppm (122/133 mg/kg bw/d)</p> <p>↓ nb of implantation sites (-24%) and consequently ↓ nb of pups delivered (-19%).</p>	<p>Unpublished study report, 2020a</p> <p>Key study for adverse effects on sexual function and fertility</p> <p>Bridging study</p> <p>Reliability: 1,</p>

CLH REPORT FOR BENZENAMINE, N-PHENYL-, REACTION PRODUCTS WITH 2,4,4-TRIMETHYLPENTENE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of exposure	Results	Reference, Reliability
<p>Deviations:</p> <p><i>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.</i></p> <p>NB:</p> <p><i>Thyroid histopathology not performed (optional in OECD TG 421)</i></p> <p><i>Thyroid hormones measured in adult M only and PND13 M and F pups in line with OECD TG 421.</i></p>	<p>dose during lactation phase to maintain dams at desired target dose of the test substance</p> <p>Eq. to: 0, 40/44, 122/133 and 407/443 mg/kg bw/d in M/F</p> <p>Recovery group : 0, 5000 ppm</p> <p>P0: 10-week pre-mating period</p> <p>F1: terminated at PND14</p> <p>Recovery group (not mated): 10-week exposure + 2-week recovery period</p>	<p>↓ ovaries absolute (-18%) weight.</p> <p>500 ppm (40/44 mg/kg bw/d)</p> <p>No effect.</p> <p>General/systemic toxicity P0</p> <p>5000 ppm (407/443 mg/kg bw/d)</p> <p>↓ Final body weight (BW) in M (-12%) and ↓ BW in F at the end of the pre-mating 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 pre-mating period, -20% during gestation and -24% during lactation).</p> <p>Recovery group: ↓ final BW weight in M (-13%) and F (-17%) and ↓ food consumption in F (-16%).</p> <p><u>Liver effects:</u> ↑ rel liver weight in M (+27%) and F (+25%), ↑ centrilobular hypertrophy (minimal to moderate) in all M&F. Fatty changes (minimal to moderate) midzonal/macrovacular 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 (CHOL) ↑γ-glutamyl transferase (GGT) activities in F. ↑alanine aminotransferase (ALT) and ↓ total bilirubin (TBIL) in M.</p> <p>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.</p> <p><u>Thyroid effects:</u> ↑ relative thyroid weight in M (+17%) no histopathological examination performed; <i>Non-statistically significant</i> ↑ TSH in M (+45%). F not investigated.</p> <p>1500 ppm (122/133 mg/kg bw/d)</p> <p>↓ BW in F at the end of gestation (- 9%), <i>at the beginning of lactation (-6%)</i> and at termination (-7%) compared to controls. No effect on BW during the pre-mating period.</p> <p><u>Liver effects:</u> ↑ 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.</p> <p><u>Thyroid effects:</u> <i>Non-statistically significant</i> ↑ TSH in M (+44%).</p> <p>500 ppm (40/44 mg/kg bw/d)</p> <p>No effect on BW.</p> <p>Liver effect limited to centrilobular hypertrophy (minimal) in 3/10 M. In F: ↓ALB (-4%), <i>non-statistically significant</i> ↑ of TRIG (+140%) and ALP (+104%).</p>	<p>reliable without restriction</p> <p>Details available under “Study 1” in Annex I (3.10.1.1)</p>

CLH REPORT FOR BENZENAMINE, N-PHENYL-, REACTION PRODUCTS WITH 2,4,4-TRIMETHYLPENTENE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of duration exposure	Results	Reference, Reliability
		<p>Thyroid effects: <i>Non-statistically significant</i> ↑ TSH in M (+9.5%).</p> <p>F1 pups</p> <p>5000 ppm (407/443 mg/kg bw/d)</p> <p>↓ mean terminal pup BW on PND13 (-18.8 % both sexes combined) compared to controls.</p> <p>↓ mean pup BW changes PND 4-13 (up to -22.5% both sexes combined) compared to controls.</p> <p>1500 ppm (122/133 mg/kg bw/d) and 500 ppm (40/44 mg/kg bw/d)</p> <p>No effect.</p>	

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

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene has been tested in 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), all performed in rats.

A reproduction and developmental toxicity screening tests (OECD TG 421) in rats performed with the source substance **Reaction products of diphenylamine with nonene, branched** is also available (Unpublished study report, 2020a) and is also considered relevant in a read-across approach since the read-across approach for reproductive toxicity from Reaction products of diphenylamine with nonene, branched (source substance) to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (target substance) is considered acceptable with high confidence (refer to 10.10.11).

All these four studies are considered 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 I.

The two reproduction and developmental toxicity screening tests (OECD TG 421) 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 **Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene** or its analogue **Reaction products of diphenylamine with nonene, branched** have not been identified in the literature.

Studies performed with 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) was used as a dose range-finding study for the EOGRTS (study 3) and as a bridging study along with the OECD TG 421 performed with Reaction products of diphenylamine with nonene, branched (study 1), for effects on sexual function and fertility and development. It.

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

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260/271 mg/kg bw/d in males/females). The duration of treatment covered a 10-week pre-mating 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 13).

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 14: 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)

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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 14).

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

In the mid-dose group, significant lower mean body weight compared to controls was observed in females at the end of the pre-mating period (-7%), at the end of gestation (-8%) and at termination PND14 (-8%), 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) were 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. Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene was administered 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 were 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 15).

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 statistical significance. As a consequence of the lower number of

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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 16: 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 bw/d	600 ppm Eq to: 54 mg/kg bw/d	1800 ppm Eq to: 166 mg/kg bw/d	HCD _b from FSR of OECD 443 2008-2018	HCD _c from FSR of OECD 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,

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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 16).

Table 17: 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 17 and Table 18). 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 18: 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 19: Age and weight at preputial 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 pre-mating 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 pre-mating period and the entire gestation and lactation period (-9%, -13% and -12% below control at the end of the pre-mating 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 pre-mating, 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 administered 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 pre-mating 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.

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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 19).

Table 20: 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.

Studies performed with the analogue 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. 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 pre-mating 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.

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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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene 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 20). 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 21: 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			

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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 of the analogue on male reproductive function.

For females (see Table 21), 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 22: 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 mean body weight was observed at the end of the pre-mating 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 pre-mating 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

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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 levels 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.

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 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. The mean number of implantation sites in high-dose (225 mg/kg bw/d) dams of the OECD TG 422 study was decreased by 16 % without reaching statistical significance.
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 the analogue Reaction products of diphenylamine with nonene, branched.
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 pre-mating periods.
- **Decreased ovary weights.** Absolute ovary weight was significantly reduced by 25% in high-dose dams exposed to 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 in the EOGRTS.
Dose-related and significant decrease in absolute ovarian weights was observed in dams exposed to the analogue 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 the analogue by gavage in corn oil in a GLP-compliant 90-day toxicity study (Unpublished study report, 2013- **Study 7**).
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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and Reaction products of diphenylamine with nonene, branched. Statistical significance was reached in high-dose females exposed to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene in the OECD TG 421 and in high-dose P1 females exposed in 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene 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 (i.e. one OECD TG 422 and one OECD TG 443 performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and one OECD TG 421 performed with the analogue Reaction products of diphenylamine with nonene), the two substances **consistently induced lower numbers of implantation sites with subsequently smaller litter sizes** as compared to control animals. **This main critical effect** was dose-related, 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 36% and 31% in dams exposed to 3000 ppm of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene respectively and 5000 ppm of the analogue respectively, which **is considered a severe impact on fertility/reproductive function (a decrease**

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of one third in the progeny). At this dose level 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 pre-mating 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and its analogue, were a 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 statistical significance in high-dose dams of the OECD TG 421 and in the high-dose dams of the EOGRTS performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene). Such effects were also noted in the OECD TG 421 performed with the analogue Reaction products of diphenylamine with nonene, branched.

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

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

Therefore, laying down the criteria of CLP Regulation, classification of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene 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 the analogue Reaction products of diphenylamine with nonene, branched			
Prenatal Developmental Toxicity Study OECD TG 414 (2001) GLP-compliant Wistar IGS CrI: WI (Han) rats N = 24 mated females/dose	Reaction products of diphenylamine with nonene, branched EC: <i>CAS:- (old 36878-20-3)</i> 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	<u>Effects on development</u> No effect on post-implantation survival at any dose level. 500 mg/kg bw/d <i>Non statistically significant ↑ number of small fetuses (fetal weight < 2.7 g) 10 in 3 litters vs 2 in 2 litters in controls.</i> Visceral and skeletal examination of foetus : no effect. 50 and 150 mg/kg bw/d No effect. <u>Maternal toxicity</u> 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 CrI: WI (Han) rats	Reaction products of diphenylamine with nonene, branched EC:	<u>Effects on development</u> Litter data : only uterus weight performed, no differences between groups Examination of the fetus : not realised <u>Maternal toxicity</u>	Unpublished study report, 2014 Supporting study Reliability: 2 reliable with

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
<p>N = 10 mated females/dose</p>	<p>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</p>	<p>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.</p>	<p>restrictions</p>
<p>Prenatal Developmental Toxicity Study OECD TG 414 (2018) GLP-compliant New Zealand White rabbits N = 25 mated females/dose</p>	<p>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</p>	<p>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. ↓ Foetus weight (-12%), ↓ males Foetus weight (-13%), <i>non-statistically significant</i> ↓ females foetus weight (-11%) Delays of ossification <i>Non-statistically significant</i> ↑ 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%). <i>Non-statistically significant</i> ↓ 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), ↓ <i>corrected BWG not statistically significant</i> (-421.0 g) in comparison to the concurrent control (-322.2 g) (-30%).</p>	<p>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)</p>

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
		<p>30 mg/kg bw/d ↓ defecation in 11 dams.</p> <p>10 mg/kg bw/d No effect</p>	
<p>Dose-range-finding study in pregnant rabbits Non-GLP New Zealand White rabbits N = 5 mated females/dose</p>	<p>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</p>	<p>Effects on development 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</p> <p>Maternal toxicity 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</p>	<p>Unpublished study report, 2019b</p> <p>Supporting study Reliability: 2 reliable with restrictions</p>
<p>Dose-range-finding study in non-pregnant rabbits Non-GLP New Zealand White rabbits N = 3 non pregnant females/dose</p>	<p>Reaction products of diphenylamine with nonene, branched EC: CAS:- (old 36878-20-3) Purity: UVCB By gavage Dose levels: 0, 100,</p>	<ul style="list-style-type: none"> • 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. 	<p>Unpublished study report, 2019c</p> <p>Supporting study Reliability: 2 reliable with restrictions</p>

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
	200, 300 mg/kg bw/d Vehicle: 0.5% CMC Exposure duration: 21 days		
Generational studies			
Studies with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene			
<p>Reproduction/Developmental Toxicity Screening Test OECD TG 421 (2016)</p> <p>GLP-compliant Rat Wistar CrI:WI(Han) N = 10/sex/dose group</p> <p>10-week pre-mating period</p> <p>Deviations: <i>Only 6 F pregnant in the low dose group while 8 per group is the minimum acceptable number according to the TG.</i></p> <p><i>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.</i></p> <p>NB:</p>	<p>Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene</p> <p>CAS: 68411-46-1 Batch: 50116118D Purity: UVCB</p> <p>By diet</p> <p>Diet : 0, 300, 1000 and 3000 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance</p> <p>Eq. to: 0, 26/28, 87/95 and 260/271 mg/kg bw/d in M/F</p> <p>F0: 10-week pre-mating period</p> <p>F1: terminated at PND13</p>	<p>F1 pups</p> <p>3000 ppm (260/271 mg/kg bw/d)</p> <p>↓ 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.</p> <p>↓ 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.</p> <p>↑incidence of nipple development (100% vs. 79.6% in control) and number of nipples per animal (5.2 to control 2.5) on PND13.</p> <p>1000 ppm (87/95 mg/kg bw/d)</p> <p>↓ 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.</p> <p>300 ppm (26/28 mg/kg bw/d)</p> <p>No effect.</p> <p>General/systemic toxicity P0</p> <p>3000 ppm (260/271 mg/kg bw/d)</p> <p>↓ 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%).</p> <p>↓ Final BW in M (-9% compared to controls) and ↓ BW in F at the end of the pre-mating period (-9%), at the end of gestation (-17%) and at termination PND14 (-15%) compared to controls.</p> <p>Liver and thyroid effects</p>	<p>Unpublished study report, 2020b</p> <p>Bridging study</p> <p>Reliability: Reliability: 1, reliable without restriction supporting study</p> <p>Details available under “Study 2” in Annex I (3.10.1.2)</p>

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
<p><i>Thyroid histopathology performed in M and F (optional in OECD TG 421).</i></p> <p><i>Thyroid hormones measured in adult M and F (optional) and PND13 M and F pups.</i></p>		<p>1000 ppm (87/95 mg/kg bw/d)</p> <p>↓ Food consumption in F during gestation period (-12%).</p> <p>↓ BW compared to controls in F at the end of the pre-mating period (-7%), at the end of gestation (-8%) and at termination PND14 (-8%).</p> <p>Liver and thyroid effects</p> <p>300 ppm (26/28 mg/kg bw/d)</p> <p>Slight liver and thyroid effects.</p>	
<p>EOGRTS</p> <p>OECD TG 443 (2018)</p> <p>GLP-compliant</p> <p>Rat Wistar CrI:WI(Han)</p> <p>P0: 25/sex/dose</p> <p>F1C1A: 20/sex/dose</p> <p>F1C1B (= P1): 25/sex/dose,</p> <p>F1C2A: 10/sex/dose</p> <p>F1C2B: 10/sex/dose</p> <p>10-week pre-mating period</p> <p>Deviations:</p> <p>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 be analysed</p> <p>F2 pups: Thyroid hormones not measured</p> <p>DNT: No historical control data (HCD) no positive</p>	<p>Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene</p> <p>CAS: 68411-46-1</p> <p>Batch: 50116118D</p> <p>Purity: UVCB</p> <p>By diet</p> <p>Diet : 0, 200, 600 and 1800 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance</p> <p>Eq. to: 0, 18, 54 and 167/166 mg/kg bw/d in M/F</p>	<p><u>F1 and F2 pups</u></p> <p>1800 ppm (167/166 mg/kg bw/d)</p> <p>↓ Mean number of F1 and F2 pups delivered/dam (consequence of the lower nb of implants).</p> <p>No effect on post-implantation loss and live birth, viability and lactation indices.</p> <p>↓ F1 and F2 pups BW PND7-PND21 in M, F and M&F combined (at weaning: -12.5% F1, -16% F2) compared to controls.</p> <p>No effect on sex ratio, anogenital distance in F1 or F2 pups</p> <p>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)</p> <p>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).</p> <p>Thyroid hormones measurements: F1 PND4: only 2 animals (due to smaller litter sizes). PND22: ↑ TSH in F1 pups (M&F).</p> <p>600 ppm (54 mg/kg bw/d)</p> <p>↓ Mean number F2 pups delivered/dam (consequence of the lower nb of implants).</p> <p>No effect on post-implantation loss and live birth, viability and lactation indices.</p> <p>↓ F1 pups BW PND7-PND21 M&F combined (at weaning: -5.4%) compared to controls.</p> <p>Thyroid hormones measurements: F1 PND4: <i>non-statistically significant</i> ↓ T4 F1 pups (M&F). PND22: ↑ TSH in F1 pups (M&F).</p> <p>200 ppm (18 mg/kg bw/d)</p> <p>No effect.</p>	<p>Unpublished study report, 2021</p> <p>Key study for adverse effects on sexual function and fertility</p> <p>Reliability: 1, reliable without restriction</p> <p>2 (Reliable with restriction for some DNT parameters)</p> <p>Details available under “Study 3” in Annex I (3.10.1.3)</p>

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
control, statistical analysis not appropriate for motor activity, auditory startle response and morphometrics.		<p>Developmental neurotoxicity (DNT)</p> <p>1800 ppm (167/166 mg/kg bw/d)</p> <p>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.</p> <p>ASR: ↓ mean maximal amplitude in M (-19%) and ↓ habituation in M (-57%) and M & F combined (-46%) (statistical analysis not performed).</p> <p>600 ppm (54 mg/kg bw/d)</p> <p>ASR: ↓ mean maximal amplitude in M (-12%) and ↓ habituation in M (-40%) and M & F combined (-42%) (statistical analysis not performed).</p> <p>200 ppm (18 mg/kg bw/d)</p> <p>No effect.</p> <p>General/systemic toxicity P0, P1(F1C1B), F1C1A and F2C2A</p> <p>1800 ppm (167/166 mg/kg bw/d)</p> <p>↓ Food consumption in P0 F during gestation and lactation periods (-13%) and in P1 F during the pre-mating, gestation and lactation periods (-7%, -14% and -17% respectively). No effect on other treated animals.</p> <p>No effect on BW in P0 M. ↓ final BW in P1 M (-6%), F1C1A M (-8%) and F1C2A M (-8%) compared to controls.</p> <p>↓ BW compared to controls in P0 F (-8%, -12% and -9% at the end of the pre-mating period, gestation and lactation respectively) and in P1 F (-9%, -13% and -12% at the end of the pre-mating period, gestation and lactation respectively). ↓ Final BW of F1C1A F (-6%).</p> <p>Liver and thyroid effects, slight ↓ (<5%) ↓ haemoglobin and haematocrit values.</p> <p>600 ppm (54 mg/kg bw/d)</p> <p>No significant effect on BW and food consumption in any generations.</p> <p>Liver and thyroid effects</p> <p>200 ppm (18 mg/kg bw/d)</p> <p>Slight liver and thyroid effects</p>	
Combined Repeated Dose Toxicity Study with the Reproduction/Developmental	Benzenamine, N-phenyl-, reaction products with	F1 pups	Unpublished study report, 2014b

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
<p>Toxicity Screening Test OECD TG 422 (1996) GLP-compliant Rat Wistar CrI:WI(Han) N = 10/sex/dose group 2-week pre mating 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: <i>In P0: TSH, T3 and T4 measured in 5 animal/sex/dose</i> <i>Thyroid histopathology performed in M and F.</i> <i>Thyroid hormones measured in adult M and F.</i></p>	<p>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</p>	<p>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</p> <p>General/systemic toxicity P0 No effects on clinical signs and BW at any dose levels.</p> <p>225 mg/kg bw/d Liver and thyroid effects</p> <p>75 mg/kg bw/d Liver and thyroid effects</p> <p>25 mg/kg bw/d Slight thyroid effects in M.</p>	<p>Reliability: 1, reliable without restriction Supporting study (screening) Details available under “Study 4” in Annex I (3.10.1.4)</p>
<p>Study with Reaction products of diphenylamine with nonene, branched</p>			
<p>Reproduction/Developmental Toxicity Screening Test OECD TG 421 (2016) GLP-compliant Rat Wistar CrI:WI(Han) N = 10/sex/dose group 10-week pre mating period</p>	<p>Reaction products of diphenylamine with nonene, branched EC: CAS:- (old 36878-20-3) Batch 00 16046440 Purity: UVCB By diet Diet : 0, 500, 1500</p>	<p>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)</p> <p>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</p>	<p>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</p>

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results	Reference
<p>Deviation:</p> <p><i>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.</i></p> <p>NB:</p> <p><i>Thyroid histopathology not performed (optional in OECD TG 421)</i></p> <p><i>Thyroid hormones measured in adult M only and PND13 M and F pups in line with OECD TG 421.</i></p>	<p>and 5000 ppm ; half dose during lactation phase to maintain dams at desired target dose of the test substance</p> <p>Eq. to: 0, 40/44, 122/133 and 407/443 mg/kg bw/d in M/F</p> <p>Recovery group : 0, 5000 ppm</p> <p>P0: 10-week pre-mating period</p> <p>F1: terminated at PND14</p> <p>Recovery group (not mated): 10-week exposure + 2-week recovery period</p>	<p>500 ppm (40/44 mg/kg bw/d)</p> <p>No effect.</p> <p>General/systemic toxicity P0</p> <p>5000 ppm (407/443 mg/kg bw/d)</p> <p>↓ Final body weight (BW) in M (-12% compared to controls) and ↓ BW in F at the end of the pre-mating 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 pre-mating period, -20% during gestation and -24% during lactation) compared to controls.</p> <p>Liver and thyroid effects.</p> <p>1500 ppm (122/133 mg/kg bw/d)</p> <p>↓ BW in F at the end of gestation (-9%) and at the end of lactation (-7%) compared to controls. No effect on BW during the pre-mating period.</p> <p>Liver effects.</p> <p>500 ppm (40/44 mg/kg bw/d)</p> <p>Slight liver effects.</p>	<p>Annex I (3.10.1.1)</p>

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

In the absence of Prenatal Developmental Toxicity Study (PNDTS) performed with Benzenamine, N-phenyl, reaction products with 2,4,4-trimethylpentene, a weight of evidence determination has been applied pursuant to CLP Annex I, 1.1.1.

The read-across approach from Reaction products of diphenylamine with nonene, branched to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene has been assessed by the DS following an analogue approach of compounds with qualitatively similar properties (RAAF scenario 2) and is considered acceptable with high confidence (refer to 10.10.11). Therefore the available PNDTSs performed with the analogue Reaction products of diphenylamine with nonene, branched (i.e. one PNDTS in rats (Unpublished study report, 2014) and another one in rabbits (Unpublished study report, 2019) as well as their respective DRF studies) have been considered relevant and taken into consideration for developmental classification of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene in a weight of evidence approach pursuant to CLP Annex I, 1.1.1.

The studies mentioned in chapter 10.10.1 (i.e. the OECD TG 421 study, the EOGRTS and the OECD TG 422 performed with the substance itself as well as the OECD TG 421 performed with its analogue) also addressed effects on postnatal development and have been considered for developmental toxicity.

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 the substance or its analogue in the literature were not identified.

➤ Prenatal Developmental Toxicity Study

Studies performed with the analogue Reaction products of diphenylamine with nonene, branched

PNDTS in rats - cf Annex I, study 5

In the GLP-compliant PNDTS 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

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In the GLP-compliant PNDTS in New Zealand White rabbit, OECD TG 414 (Unpublished study report, 2019a) carried out with 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 23). 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

* : $p \leq 0.05$

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 24).

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.

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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	N	20	24	24	20	
Fetuses	N	174	189	205	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 (%)	1 (0.6)#	0 (0)	0 (0)	4 (2.2)##	Not reported
	Litter N (%)	1 (5.0)	0 (0)	0 (0)	1 (5)	
	Affected fetuses/litter mean%	0.5	0	0	1.5	
Cleft palate	Pup N (%)	0 (0)	0 (0)	0 (0)	4 (2.2)##	0.0-0.6
	Litter N (%)	0 (0)	0 (0)	0 (0)	1 (5)	0.0-4.8
	Affected fetuses/litter mean%	0	0	0	1.5	0.0-0.4
Small tongue	Pup N (%)	0 (0)	0 (0)	0 (0)	4 (2.2)##	Not reported
	Litter N (%)	0 (0)	0 (0)	0 (0)	1 (5)	
	Affected fetuses/litter mean%	0	0	0	1.5	
Umbilical hernia	Pup N (%)	0 (0)	0 (0)	0 (0)	1 (0.6)	0.0-0.5
	Litter N (%)	0 (0)	0 (0)	0 (0)	1 (5.0)	0.0-4.2
	Affected fetuses/litter mean%	0	0	0	0.4	0.0-0.5
Open eye	Pup N (%)	0 (0)	1 (0.5)	0 (0)	0 (0)	0.0-0.4
	Litter N (%)	0 (0)	1 (4.2)	0 (0)	0 (0)	0.0-4.5
	Affected fetuses/litter mean%	0	0.4	0	0	0.0-0.4
Multiple external malformations	Pup N (%)	1 (0.6)#	0 (0)	0 (0)	4 (2.2)##	0.0-1.1
	Litter N (%)	1 (5.0)	0 (0)	0 (0)	1 (5)	0.0-9.1
	Affected fetuses/litter mean%	0.5	0	0	1.5	0.3-0.9
# Same fetus						
## Same 4 fetuses from the same doe						
Individual skeletal variations						
Irregular ossification of interparietal	Pup N (%)	1 (0.6)	4 (2.1)	6 (2.9)	6 (3.4)	0.0-2.2
	Litter N (%)	1 (5.0)	3 (13)	6 (25)	6 (30)*	0.0-18.2
	Affected fetuses/litter mean%	0.6	1.6	2.9*	2.6*	0.0-1.7
Unossified talus; cartilage present	Pup N (%)	0 (0)	2 (1.1)	0 (0)	10 (5.6)	0.0-2.7
	Litter N (%)	0 (0)	2 (8.3)	0 (0)	6 (30)*	0.0-18.2
	Affected fetuses/litter mean%	0	0.8	0	4.4**	0.0-2.6

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

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

Prenatal development

In the OECD TG 421 and EOGRTS performed with the substance itself as well as in the OECD TG 421 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

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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 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

* $p \leq 0.05$

** Calculated by the DS as $\frac{\text{mean number of implantations} - \text{mean number of pups delivered}}{\text{mean number of implantations}} \times 100$ (no statistical analysis performed)

Postnatal development

The effects observed postnatally in the available generational studies (i.e. the OECD TG 421, the EOGRTS and the OECD TG 422 performed with with the substance itself as well as the OECD TG 421 performed with its analogue) are reported here after.

Pups viability

In the OECD TG 422 carried out with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, 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 26). While the majority of the postnatal loss was attributable to one female (5 pups), two other litters were 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

No treatment-related effect was observed on live birth, viability and lactation indices 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene or in the OECD TG 421 up to 5000 ppm (eq. to 443 mg/kg bw/d) performed with its analogue.

Pups growth (Table 27)

In the OECD TG 421 study (Unpublished study report, 2020b) performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, 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 (Unpublished study report, 2021) performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, 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.

In the OECD TG 421 study performed with the analogue (Unpublished study report, 2020a) 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.

Table 28: summary of pups body weight during lactation in the OECD TG 421 study and the EOGRTS performed with the substance and in the OECD TG 421 study performed with the analogue

	Males	Females
Studies performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene		

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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	
Study performed with the analogue 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	

* p ≤ 0.05, ** p ≤ 0.01

Nipple retention /areola anlagen (Table 28)

In the OECD TG 421 study (Unpublished study report, 2020a) performed with the analogue, 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, 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), 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).

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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				HCD
Studies performed with 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	-
Study performed with the analogue 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	0.9	1.2	0.7					

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

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) performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, cohorts F1C2A (10 animals/sex/dose sacrificed on PND77) and F1C2B (10 animals/sex/dose sacrificed on PND22) were subject to developmental neurotoxicity testing.

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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 29).

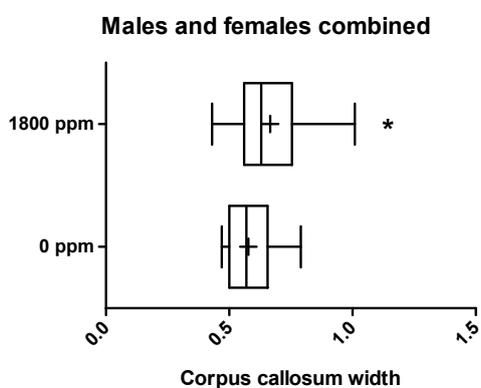
Table 30: Synthesis findings from the ASR expressed as % controls

	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

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interpreted as ingested myelin debris) in 5/10 animals. This finding was further confirmed by an internal fully-blinded peer review (Table 30). 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

		Males				Females			
		0	200	600	1800	0	200	600	1800
	Dose level (ppm)								
	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 the substance or its analogue.

In the EORGTS, T4 and TSH values in PND4 male and female pups were not statistically significantly changed (Table 31). 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,

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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 in the EOGRTS

		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 myelination 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and of its analogue Reaction products of diphenylamine with nonene, branched 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 foetal viability were not affected by treatment with the analogue 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene 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 foetal viability were not affected in any of the generational studies except in the OECD TG 422 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 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene or in the OECD TG 421 up to 5000 ppm (eq. to 443 mg/kg bw/d) performed with its analogue Reaction products of diphenylamine with nonene, branched.

→ 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.

Post-implantation viability was not affected in the PNDTSs in rats and rabbits with the analogue. 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.

- (2) Structural abnormality: the analogue 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, 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.

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 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene induces abnormalities in the central nervous system** in animals exposed during the developmental period.

In the PNDTS in rabbits performed with **the analogue, 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 the analogue 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EOGRTS, OECD TG 421) or its analogue (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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and its analogue alter growth of the developing organism** at dose levels also affecting maternal/parental weight.

(4) Functional deficiency:

In the EOGRTS, 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 its analogue.

Overall, **the main critical effects** are those linked to **neurodevelopmental toxicity** observed in C2A animals (especially in males) of the EORGTS performed with 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, **makes 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** of Benzenamine, N-phenyl-, reaction products. The available data suggest that neurodevelopment effects could be linked to decreased thyroid hormones (THs) that were observed.

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 post-implantation and post-natal losses observed at the high dose level of the OECD TG 422 performed with 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 the **increased number of abortions observed in rabbits** exposed to the analogue, **a direct effect cannot be excluded but the severe drop in food consumption might be partly involved**.

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 analogue 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (EOGRTS, OECD TG 421) and its analogue (OECD TG 421). 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (OECD TG 421 study and the EOGRTS) and with its analogue (OECD TG 421 study) are available in Table 11.

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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, 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 with the substance itself and supported by the the same findings observed with its analogue, a classification for effects on sexual function and fertility (Cat. 1B, H360F) is considered warranted for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene.

“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 (ED₁₀) is above 4 mg/kg bw/d).

Effects on fertility were observed 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 ED₁₀ 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

Primarily based on the severe neurodevelopmental effects (i.e. neurohistopathological and neuromorphometric findings supported by changes in auditory startle response) observed in the EOGRTS study performed with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and to a lesser extent on effects on viability (in the OECD TG 422 and abortions in the rabbit PNDTS with the analogue) and effects on pups growth in all the generational studies (less concern), a classification for effects on development (Cat. 1B, H360D) of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene is considered warranted.

No effects on development 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 of Benzenamine, N-phenyl-, reaction products with 2,4,4-

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trimethylpentene in the EOGRTS and 10 mg/kg bw/d of its analogue Reaction products of diphenylamine with nonene, branched in the rabbit PNDTS) 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 (ED₁₀) is above 4 mg/kg bw/d).

Effects on development were observed from 600 ppm (eq. to 54 mg/kg bw/d, EOGRTS) to 3000 ppm (eq. to 271 mg/kg bw/d, OECD 421) of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and from 100 mg/kg bw/d (rabbit PNDTS) to 5000 ppm (eq. to 443 mg/kg bw/d, OECD TG 421) of its analogue Reaction products of diphenylamine with nonene, branched. Therefore, the ED₁₀ is between 4 and 400 mg/kg bw/d and Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene 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 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and its analogue Reaction products of diphenylamine with nonene, branched**
- 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 SDPAs **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).**

In the registration dossier of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, a read-across approach is proposed from Reaction products of diphenylamine with nonene, branched to fulfil the REACH information requirements of Annex IX, section 8.7.2 and Annex X, section 8.7.2 (prenatal developmental toxicity studies).

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)⁵.

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

⁵ In the registration dossier, the registrants proposed a read-approach based on analogue approach, Scenario 1 (i.e. hypothesis based on (bio) transformation to common compound(s)), while no empirical data are available on metabolism in mammals to support this scenario. Since binding 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.

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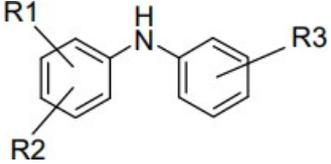
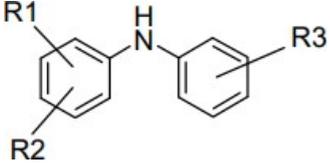
- Bridging Reproduction/Developmental Toxicity Screening Tests (OECD TG 421) performed with both substances from the same laboratory reported the same pattern of effects, indicating the same biological targets after oral exposure strongly support the read-across approach for reproductive toxicity.
- The prenatal developmental toxicity studies (PNDTS) performed with Reaction products of diphenylamine with nonene, branched (source substance) **can be used to predict developmental effects that would be observed in studies with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, (target substance) if they were to be conducted.**

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

1- Characterisation of the two substances

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (EC 68411-46-1) and Reaction products of diphenylamine with nonene, branched are both 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 32.

Table 33: Identity of the two substances

	Target substance	Source substance
Name	Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene	Reaction products of diphenylamine with nonene, branched
CAS	68411-46-1	
EC	270-128-1	
Molecular mass distribution	$\geq 225.3 \leq 393.6$	$\geq 295.5 \leq 421.7$
Molecular formula	C16H19N - C28H43N (main constituents)	C21H29N - C30H47N (main constituents)
Structural formula	 <p>R1 = tert-butyl or iso-octyl R2 = H or tert-butyl or iso-octyl R3 = H or tert-butyl or iso-octyl</p>	 <p>R1 = iso-nonyl R2 = H or iso-nonyl R3 = H or iso-nonyl</p>
Typical concentration	Complete (100%) reaction product of Benzenamine- N-phenyl, with 2,4,4-trimethylpentene	Complete (100%) reaction product of Benzenamine- N-phenyl- with nonene (branched)

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

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The two substances 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 PNDTS with Reaction products of diphenylamine with nonene, branched (source substance) in rats and rabbits (reliability 1) have been performed according to the current version of OECD TG 414 and under GLP conditions. The test materials used in each of these studies represent 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (target substance) and Reaction products of diphenylamine with nonene, branched (source substance).

In conclusion, the available 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 the two substances or their constituents are available.

Table : Physicochemical data of the two substances

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

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.

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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, branched Substance. 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 bioavailability, the dose difference is very small and the bioavailability may vary due to UVCB nature of the two substances.

Table 34: 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 substance 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 monoocetyl DPA	337.5	9	1.5 x 10 ⁻⁴	11.04	0.07	8.05	1.67
	Tributyl DPA				no data	no data	no data	no data
	Diocetyl DPA	393.6	10.8	1.93 x 10 ⁻⁶	0.43	0.002	9.6	0.06
	Dibutyl monoocetyl DPA				no data	no data	no data	no data
Source substance 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

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

In the OECD report on SDPA (OECD, 2016a), 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 both Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and Reaction products of diphenylamine with nonene, branched . 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 Benzenamine,

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N-phenyl-, reaction products with 2,4,4-trimethylpentene despite lower doses tested compared to those in OECD TG 421 study (Table 34).

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

Furthermore, the metabolome profile in plasma of fasted Wistar rats treated with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and Reaction products of diphenylamine with nonene, branched was similar for both compounds in terms of significance and direction of changes. 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and Reaction products of diphenylamine with nonene, branched 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 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.

Table 35: Available data on reproductive and prenatal developmental toxicity.

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

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

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	<p>lactation (-9%) and at termination (-15%) compared to controls. 1000 ppm ↓ BW in F at the end of the pre-mating period (-7%), at the end of gestation (-8%), <i>at the beginning of lactation (-4%) and at termination PND14 (-8%).</i> 300 ppm No effect on BW Target organs: liver and thyroid</p>	<p><i>lactation (-6%) and at termination (-7%).</i> No effect on BW during the pre-mating period. 500 ppm No effect on BW Target organs: liver and thyroid</p>
Reference	Unpublished study report, 2020b	Unpublished study report, 2020a
Extended one-generation reproductive toxicity study (EOGRTS)		
Method	OECD TG 443/GLP - cf. Annex I, study 3	No data
Dosages	<p>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</p>	
Study duration	<p>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</p>	
Species	rat (Wistar)	
Result	<p style="text-align: center;"><u>Fertility</u></p> <p>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 <i>non-statistically significant</i> ↓ nb of implantation sites (-9%) and consequently ↓ nb of pups delivered (-10%). 200 ppm No effect</p> <p style="text-align: center;"><u>Development</u></p> <p>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)</p>	

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	<p>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</p> <p>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%)</p> <p>200 ppm No effect</p> <p style="text-align: center;"><u>DNT</u></p> <p>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)</p> <p>600 ppm ASR: ↓ mean maximal amplitude in M and ↓ habituation in M and M & F combined (statistical analysis not performed)</p> <p>200 ppm No effect</p> <p style="text-align: center;"><u>Systemic toxicity</u></p> <p>1800 ppm No effect on BW in P0 M. ↓ final BW in P1 M (-6%). ↓ BW in F at the end of the pre-mating period (-8% in P0 and -9% in P1)</p> <p>600 and 200 ppm No effect on BW in any generations Target organs: liver and thyroid</p>	
Reference	Unpublished study report, 2021	
Reproductive screening assay		
Method	OECD TG 422 / GLP – cf. Annex I, study 4	No data
Dosages	0, 25, 75, 225 mg/kg bw/d	
Study duration	28 days (males), ca 53 days (females)	
Species	rat (Wistar)	
Result	<p style="text-align: center;"><u>Fertility</u></p> <p>225 mg/kg bw/d <i>non-statistically significant ↓ of mean number of implantation sites (-16%)</i></p> <p>75 and 25 mg/kg bw/d No effect</p>	

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	<p style="text-align: center;"><u>Development</u></p> <p>225 mg/kg bw/d ↓ nb of pups delivered/live born pups (-28%) ↑ <i>post-implantation loss</i> (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).</p> <p>75 and 25 mg/kg bw/d No effect</p> <p style="text-align: center;"><u>Systemic toxicity</u></p> <p>No effect on BW or food consumption at any dose levels. Target organs: liver and thyroid</p>	
Reference	Unpublished study report, 2014b	
<i>Prenatal developmental toxicity</i>		
Method	Read-across	OECD TG 414 / GLP – cf. Annex I, study 5
Dosages		0, 50, 150, 500 mg/kg bw/d
Study duration		Gestation days 6-19
Species		rat (Wistar)
Result		<p style="text-align: center;"><u>Development</u></p> <p>No effect on post-implantation survival at any dose level. 500 mg/kg bw/d <i>Non-statistically significant</i> ↑ 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 foetus : no effect 50 and 150 mg/kg bw/d No effect</p> <p style="text-align: center;"><u>Maternal toxicity</u></p> <p>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</p>
Reference	Unpublished study report,, 2014c	
Method	Read-across	OECD TG 414 / GLP – cf. Annex I, study 6
Dosages		0, 10, 30, 100 mg/kg bw/d

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Study duration		Gestation days 6 - 28
Species		rabbit (NZW)
Result		<p><u>Development</u></p> <p>No effect on post-implantation survival at any dose level.</p> <p>100 mg/kg bw/d Abortion : 4 vs 2 in controls at the end of the gestation period ↓ Fœtus weight (-12%), Delays of ossification <i>Non-statistically significant ↑ of external malformations due to 4 fetuses in one single litter with multiple external malformations.</i></p> <p>10 and 30 mg/kg bw/d No effect</p> <p><u>Maternal toxicity</u></p> <p>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</p> <p>30 mg/kg bw/d ↓ defecation in 11 dams</p> <p>10 mg/kg bw/d No effect</p>
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 very consistent and showed the same toxicity profile for both substances. Similar magnitude of effects were obtained with 3000 ppm (260/271 mg/kg bw/d) of Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene versus 5000 ppm (407/443 mg/kg bw/d) of (Reaction products of diphenylamine with nonene, branched).

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 34).

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 ($0.25\% \leq \text{DPA} < 2.5\%$).

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 LD_{50} 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 alert 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

Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and Reaction products of diphenylamine with nonene, branched both belong to subgroup 2 (i.e. SDPAs with variable number of alkyl substitutions) as defined by OECD (2016). 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 subgroup 2 cited in OECD (OECD, 2016) and Health Canada (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.

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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 pre-mating 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 pre-mating 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene and Reaction products of diphenylamine with nonene, branched. 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 2,4,4-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 PNDTSs is considered acceptable with high confidence from Reaction products of diphenylamine with nonene, branched to Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene. Therefore the prenatal developmental toxicity studies (PNDTS) performed with Reaction products of diphenylamine with nonene, branched (source substance) can be used to predict developmental effects that would be observed in studies with Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, (target substance) if they were 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

Substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1 is a UVCB substance consisting of eight main constituents (and their isomers) with tert-butyl and/or tert-octyl (branched) –monoalkylated (C4DPA, C8DPA), -dialkylated (C4C4DPA, C4C8DPA, C8C8DPA) or -trialkylated (C4C4C4DPA, C4C8C8DPA, C8C8C8DPA) substituted diphenylamine (see Table 3 above).

Table 36: Summary of relevant information on rapid degradability for the UVCB substance and its constituents

Method	Results	Remarks	Reference
Test type: biodegradation in water: ready biodegradability	Tested concentration: 10.6 and 20.1 mg/L Duration: 28 days	2 (reliable with restrictions) - use of emulsifier (nonylphenol)	Unpublished study report (1988a)

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Method	Results	Remarks	Reference
OECD Guideline 301 B Ready Biodegradability: CO ₂ Evolution Test GLP compliant	Not readily biodegradable 0% degradation (CO ₂ evolution) after 28 days (10.6 mg/L) 1% degradation (CO ₂ evolution) after 28 days (20.1 mg/L)	Key study Test material: Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EC 270-128-1 experimental result	Details available in Annex I Study 1 (4.1.1)
Test type: Biodegradation in water: simulation study OECD Guideline 309 simulation testing on ultimate degradation in surface water GLP compliant	Tested concentrations: 4 and 40 µg/L Duration: 91 days DT50 (50% disappearance time): not determinable because of methodological limitations (poor mass recovery) Mineralization (¹⁴ CO ₂): 4 µg/L= 5% TAR 40 µg/L=1% TAR	1 (reliable without restriction) Supporting information Test material: 4-tert-butyl-N-(4-tert-butylphenyl)aniline (one constituent C4C4DPA of the UVCB substance)	Unpublished study report (2023) Details available in Annex I Study 2 (4.1.3)
EPI suite BIOWIN model predictions v.4.10	Not readily biodegradable Based on BIOWIN models 1, 2, 5 & 6 for the 5 main constituents	Supporting information	

11.1.1 Ready biodegradability

One screening study for ready biodegradability is available from the registration dossier. The ready biodegradation test (OECD TG 301B) was performed using the UVCB substance (EC 270-128-1). The UVCB substance is poorly soluble in water and a solubilising agent (nonylphenol) was used. The reference substance aniline (20 mg/L) was degraded at 94.4% in 28 days. In the test, at a concentration of 10.6 mg/L, 0% degradation (CO₂ evolution) was measured after 28 days (1% degradation after 28 days at a test concentration of 20.1 mg/L). Since the pass level (> 70 % within a 28-day period) was not reached in the biodegradation screening test, it can be concluded that the substance is not readily biodegradable. Furthermore, the absence of CO₂ formation during this test suggests that none of the constituents of the UVCB substance could be ultimately degraded under conditions of the ready biodegradation test.

The DS made estimations using BIOWIN (v4.10) models 1, 2, 5 & 6 to calculate the probability score that the UVCB substance under aerobic conditions with mixed cultures of microorganisms will be rapidly or readily biodegradable in the environment, according to CLP guidance (ECHA, 2017b).

Table 37: EPI suite probability of rapid and ready degradation (Biowin models v.4.10) for the UVCB substance main constituents.

	Biowin 1	Biowin 2	Biowin 5	Biowin 6
C8C8DPA CC(C)(C)CC(C)(C)c1ccc(Nc2ccc(C(C)(C)CC(C)(C)C)cc2)cc1	-0.4093	0.0000	-0.1775	0.0016
C4C8DPA CC(C)(C)CC(C)(C)c1ccc(Nc2ccc(C(C)(C)C)cc2)cc1	-0.1987	0.0001	-0.1285	0.0033

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C8DPA CC(C)(C)CC(C)(C)c1ccc(Nc2ccccc2)cc1	0.1400	0.0105	-0.0222	0.0119
C4C4DPA CC(C)(C)c1ccc(Nc2ccc(C(C)(C)C)cc2)cc1	0.0119	0.0018	-0.0794	0.0070
C4DPA CC(C)(C)c1ccc(Nc2ccccc2)cc1	0.3507	0.1169	0.0269	0.0249
C4C4C4DPA c1cc(ccc1Nc2ccc(cc2C(C)(C)C)C(C)(C)C)C(C)(C)C	-0.1987	0.0001	-0.1857	0.0020
C4C8C8DPA CC(C)(C)CC(C)(C)c1ccc(Nc2ccc(C(C)(C)CC(C)(C)C)cc2C(C)(C)C)cc1	-0.6200	0.000	-2.3232	0.0004
C8C8C8DPA C(C)(C)(c1c(Nc2ccc(C(C)(C)CC(C)(C)C)cc2)ccc(C(C)(C)CC(C)(C)C)c1)CC(C)(C)C	-0.8306	0.0000	-0.3329	0.0002

The results for these 4 models are < 0.5 and the UVCB 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 225-505 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.

11.1.2 BOD₅/COD

Not assessed in this dossier.

11.1.3 Hydrolysis

No study available. The substance (EC 270-128-1) 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

Not available

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

Not assessed in this dossier.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No study available

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

One recent simulation testing on ultimate degradation in surface water according to OECD TG 309 (GLP compliant, 2023) was performed for the **constituent** 4-tert-butyl-N-(4-tert-butylphenyl)aniline or C4C4DPA (CAS 4627-22-9) and mentioned in the registration dossier. This reliable study (RI 1) is considered a supporting 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 constituent, was performed in closed vessels for 91 days at two different concentrations (4 µg/L and 40 µg/L). The test vessels were closed with polypropylene matching screw cap with pouring ring impermeable to air and CO₂ and were continuously stirred and incubated in dark conditions at a temperature of 12±1. Replicates of test substance were sampled at the start of exposure (Day 0) and on Day 1, Day 7, Day 14, Day 21, Day 28 and on Day 49. Because of very low ¹⁴C₂O evolution detected within the first 49 days, the experiment was extended and additional

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samplings were made on Day 59, Day 71, Day 80 and on Day 91. The test included, in addition to the test assays (FT), blank control assay (FB), abiotic sterile control assay (FS), control assay with reference substance benzoic acid, sodium salt (550 µL) (FC), solvent control assay (50 µL acetonitrile) (FOC) and parameter control assay with test substance (FP). Additional test assay without labelled substance and solvent control assay were performed.

Direct LSC measurements in the test substance assays and reference assays were taken. Sampling of ¹⁴CO₂ for quantification of mineralization was realised in the test substance assays and reference assays. Chemical analyses for the measurement of parent and degradation products were performed for the test substance assays only. Extraction was performed with dichloromethane and the extraction phases were not analyzed due to the poor mass balance recovery after this extraction procedure which was comparable to the recovery derived from direct analysis of water phase (29.5 % for the highest concentration of 40 µg/L and 65% for the lowest concentration of 4 µg/L at day 91). At day 91, since the mass balance recovery does not reach the acceptable range recommended in the guideline (90%-100%), it was assumed that the test substance and possibly formed metabolites adhered to the walls of the test vessels. Therefore, additional extractions were conducted with various solvent (Acetone, Acetonitrile, Ethanol, Chloroform, Toluene, Heptane). Recovery was poor (1%) after each solvent step except with acetone where up to 5% of total applied radioactivity (TAR) was recovered in both concentration groups, no additional analyses were conducted on these extracts. Finally, chemical analyses for the measurement of parent and degradation products were realised.

The DT50 (50% disappearance time) values determination was not possible because of the poor mass balance recovery. The maximum mineralization (¹⁴CO₂) level was found in the low concentration test assay (5% TAR) whereas in high concentration was only 1% TAR. No organic volatiles were detected in high concentration test assays but up to 0.5% organic volatiles were observed in low concentration test assays. Three different metabolites could be detected in the samples of the test group: 1) 4-tert-Butylaniline (CAS-No. 769-92-6) was confirmed by analysis, 2) 4-tert-Butylcatechol (CAS-No. 98-29-3) was a suggested metabolite which was not confirmed by analysis and 3) one metabolite which could not be characterized.

Degradation products were detected and/or identified, thus primary degradation cannot be excluded and might have occurred to a certain extent. However, no significant ultimate degradation occurred as indicated by the ¹⁴CO₂ radioactivity at the end of the study (max 5% of the total applied radioactivity after 91d). In addition, the absence of information on DT50 prevents to conclude that the substance fulfils the criteria of rapid degradation. In this case the substance is considered to be 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 Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene (EC 270-128-1) 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 38: Summary of data on bioaccumulation for Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1 and its constituents

Method	Results	Remarks	Reference
Test type : Partition coefficient OECD Guideline 123 Partition Coefficient (1-Octanol / Water), Slow-Stirring Method GLP compliant	Measured log Kow: C4C4DPA= 6.7 (23°C, pH 6.7)	1 (reliable without restriction) Key study Experimental result Test material: C4C4DPA constituent N-phenyl-bis(ar-tert-butyl)aniline and N-phenyl-(ar-1,1,3,3-tetramethyl-butyl)aniline	Unpublished study report (2019d)
EPI suite KOWWIN model predictions v1.68	Estimated log Kow: C8C8DPA=10.82 C4C8DPA=8.96 C8DPA=7.05 C4C4DPA=7.11 C4DPA=5.2 C4C4C4DPA=9.02 C4C8C8DPA=12.73 C8C8C8DPA=14.58	Supporting information	
Catalogic model predictions OASIS v5.11.19 (Bioaccumulation base-line model v.02.09)	Estimated BCF (L/kg w.w.): C8C8DPA=7.76 C4C8DPA=23.44 C8DPA=1047.13 C4C4DPA=616.59 C4DPA=602.56	Supporting information trialkylated DPAs are out of the applicability domain of the model	Unpublished study report (2016)
EPI suite BCFBAF model predictions v3.01	Estimated BCF (L/kg w.w.): Regression-based method C8C8DPA=179.4 C4C8DPA=1452 C8DPA=12 520 C4C4DPA=11 750 C4DPA=1255 C4C4C4DPA=1360 Arnot-Gobas method (upper trophic) C8C8DPA=10.39 C4C8DPA=331.1 C8DPA=1719 C4C4DPA=4176 C4DPA=1347 C4C4C4DPA=282	Supporting information trialkylated DPAs (C4C8C8DPA and C8C8C8DPA) are out of the applicability domain of the model	

11.3.1 Estimated bioaccumulation

In addition to the measured partition coefficient (OECD TG 123), KOWWIN software program (v.1.68) was used to predict the log Kow values of the main constituents. The results are presented in the table 43 below. The main constituents have a log Kow \geq 4 and thus meet the criterion that the substance has a potential for bioaccumulation.

Experimental BCF results are not available for the registered UVCB substance. QSAR estimations provided by the registrant to estimate BCF values of relevant constituents using the BCFBAF EPISuite and the BCF base-line model of Catalogic are presented in the table below. As among all constituents, C4DPA, C8DPA and C4C4DPA are considered to have the highest potential for bioaccumulation. At least these three constituents indicates a BCF value \geq 500, thus the substance is considered to meet the criterion for a potential for bioaccumulation in aquatic organisms.

Table 39: Catalogic and EPI Suite bioaccumulation models prediction for the constituents of UVCB substance EC 270-128-1 (please refer to table 36 for the SMILES used in model predictions).

	Molecular weight (Da)	EPI Suite KOWWIN Log Kow	Catalogic Diamax average (Å)	Catalogic OASIS v5.11.19 Bioaccumulation base-line model v2.09 (BCF L/kg w.w. corrected)	EPI Suite BCFBAF v3.01 (BCF L/kg w.w.) 1) regression-based method 2) Arnot-Gobas method (upper trophic)
C8C8DPA	393.66	10.82	18.724	7.76	1) 179.4 2) 10.39
C4C8DPA	337.55	8.96	17.761	23.44	1) 1452 2) 331.1
C8DPA	281.44	7.05	15.564	1047.13	1) 12 520 2) 1719
C4C4DPA	281.44	7.11	16.572	616.59	1) 11 750 2) 4176
C4DPA	225.34	5.2	14.293	602.56	1) 1255 2) 1347
C4C4C4DPA	337.55	9.02	16.502	26.30*	1) 1360 2) 282
C4C8C8DPA	449.77	12.73	18.898	7.41*	1) 20.8* 2) 1.04*
C8C8C8DPA	508.88	14.58	18.904	7.41*	1) 3.162* 2) 0.8952*

* Out of the applicability domain of the model

11.3.2 Measured partition coefficient and bioaccumulation test data

No experimental data evaluating the bioaccumulative properties are available for the UVCB substance “Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene”.

One partition coefficient study performed according to the OECD test guideline 123 (Unpublished study report, 2019d) is available in the registration dossier. The full study report has not been reviewed by the DS. The study was performed using one constituent (C4C4DPA) of the UVCB substance. This study is a GLP study and is considered reliable without restriction according to the registrant. The method used to determine the octanol-water partition coefficient was a slow-stirring method. The analytical method showed a recovery rates of 82% of the test item demonstrating a sufficient accuracy of the test item. The partition coefficient of test substance was determined to be log Kow= 6.7 at 23°C and pH 6.7.

Considering the measured log Kow \geq 4 for C4C4DPA constituent and the estimated log Kow \geq 4 for the main constituents of the UVCB substance, it is therefore concluded that the substance has a potential for bioaccumulation in aquatic species. The BCF model predictions support this conclusion.

11.4 Acute aquatic hazard

Table 40: Summary of relevant information on acute aquatic toxicity for substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1

Method	Species	Test material	Results	Remarks	Reference
OECD Guideline 203 (Fish, Acute Toxicity Test) freshwater Static 96h	Zebrafish <i>Danio rerio</i>	Test material: Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EC 270-128-1 Tested concentrations: 10, 18, 32, 58, 100 mg/L nominal	Effect on mortality (100 mg/L) and sublethal effects were observed (\geq32 mg/L) LC0 (96h): 58mg/L (nominal) mortality (fish) LC50 (96h): >100 mg/L (nominal) mortality (fish) corresponding to >71 mg/L initial measured concentration LC100 (96h): >100 mg/L (nominal) mortality (fish)	Non-GLP 3 (not reliable) - vehicle concentration above acceptability - presence of undissolved material - high deviation of the measured concentrations (21-29% of nominal after 96h) experimental result	Unpublished study report (1988b)
OECD Guideline 202 (<i>Daphnia</i> sp. Acute Immobilisation Test) freshwater static 48h	<i>Daphnia magna</i>	Test material: Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EC 270-128-1 Tested concentrations: 4.6, 10, 22, 46, 100 mg/L nominal	All tested animal immobile after 48h at 100mg/L. toxic effects observed \geq22mg/L EL50 (48h) 51 mg/L test mat. (nominal loading rate WAF) based on mobility EL50 (24h) >100 mg/L test mat. (nominal loading rate WAF) based on mobility	GLP compliant 2 (reliable with restrictions) - high deviation of the measured concentrations. (were 0.33, 0.23, 0.83, and 0.31 mg/L for the loading rates of 10, 22, 46, and 100 mg/L at the end of the test).	Unpublished study report (2004) Details available in Annex I Study 3 (4.3.2)

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				Key study	
OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) freshwater static 24h	<i>Daphnia magna</i>	Test material: Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EC 270-128-1 Tested concentration: 100 mg/L nominal	All tested animals were immobile after 24h EL50 (24h) <100 mg/L test mat. (nominal loading rate WAF) based on mobility	GLP compliant 2 (reliable with restrictions) - no analytical measurements performed Supporting study experimental result	Unpublished study report (1998) Details available in Annex I Study 4 (4.3.2)
OECD Guideline 201 (Alga, Growth Inhibition Test) freshwater static 72h	<i>Desmodesmus subspicatus</i>	Test material: Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EC 270-128-1 Tested concentrations: 1, 10, 100 mg/L nominal	Significant effects 14% growth rate inhibition at 100 mg/L. Non-significant effects at 1 & 10 mg/L ErL50 (72h): >100 mg/L test mat. (nominal loading rate WAF) based on growth rate EbL50 (72h): >100 mg/L test mat. (nominal loading rate WAF) based on biomass	non-GLP compliant 2 (reliable with restriction) - no chemical analysis was conducted Supporting study experimental result	Unpublished study report (2006) Details available in Annex I Study 5 (4.3.3)

11.4.1 Acute (short-term) toxicity to fish

Information from one non-GLP short-term study on toxicity to fish was conducted with the UVCB substance (OECD TG 203, 1988b). In this study, 10 fishes (*Danio rerio*) per concentration and for the control group were tested at nominal concentrations 10, 18, 32, 58 and 100 mg/L in static condition for 96h. Sublethal effects and mortality were observed during the test. A LC50 of >100 mg/L was given. The measured concentrations at 0h were 53-71% of nominal concentration and after 96h the measured concentrations range from 21-29% of nominal concentration. This test presented major deficiencies. The maximum vehicle concentration in the test (950 mg/L Dimethylformamide and 0.8 mg/L alkylphenol-polyglykol-ether) is above the maximum vehicle concentration specified in OECD 203 test guideline (100 mg/L). Despite the high vehicle concentration, the measured test concentrations deviate strongly from the nominal test concentrations. The study mentioned that the test concentrations appeared turbid from the start of the test and showed evidence of undissolved material. Furthermore, in this study, a single stock WAF instead of individually prepared WAFs was used, which is not appropriate. As mentioned in the decision on substance evaluation (ECHA, 2018)⁶ "ECHA considers the study to be invalid and cannot be considered for the toxicity assessment". This study is not considered appropriate for classification purpose.

⁶ <https://echa.europa.eu/documents/10162/9979a98a-c240-83e3-3862-819b90c7a523>

11.4.2 Acute (short-term) toxicity to aquatic invertebrates

Two short-term studies on toxicity to aquatic invertebrates are available. The key study (OECD TG 202, GLP compliant 2004) was conducted in a 48-hour static test with the UVCB substance on *Daphnia magna*. WAFs with loading rates of the test item at 4.6, 10, 22, 46 and 100 mg/L, and a control were tested. At each test concentration and for the control, 20 daphnids were used and divided into four replicates of five daphnids each. No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the entire test duration. Analytical monitoring showed that the mean measured test concentrations (0h and 48h) were 0.32 mg/L (loading rate 10 mg/L), 0.30 mg/L (loading rate 22 mg/L), 0.63 mg/L (loading rate 46 mg/L), and 0.36 mg/L (loading rate 100 mg/L). Toxic effects were observed in the study and were increasing with increasing nominal concentrations as indicated in the table 46 below. The measured concentrations of the test item were approximately in the same range at all loading rates and a dose-response curve for EC50 calculation based on measured concentrations could not be determined. Therefore the results were reported with the nominal loading rates of the substance although it probably under-estimates the acute toxicity of the substance.

Table 41: Effect of the UVCB substance on the mobility of *Daphnia magna*

Loading rate (mg/L)	Immobilized daphnids after 24h		Immobilized daphnids after 48h	
	No.	%	No.	%
Control	0	0	0	0
4.6	0	0	0	0
10	0	0	0	0
22	0	0	5	25
46	2	10	6	30
100	8	40	20	100

In a supporting OECD TG 202 study (GLP compliant, 1998) a 24h test was conducted on the UVCB substance tested as a WAF (Water Accommodated Fraction) with a loading rate of 100 mg/L. 20 daphnids were tested and divided into two groups of ten animals. No auxiliary solvent or emulsifier was used. The test was stopped after 24h, since 100% mortality was observed. No remarkable observations were made concerning the appearance of test medium and the control. The test medium was a clear colored solution throughout the entire test duration. No analytical measurement was performed and the EC50 could not be determined but, as mentioned in the study, were clearly below the loading rate of 100 mg/L. Therefore, based on this study, no specific EC50 value on the acute toxicity of substance or its constituents can be given.

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

One study on toxicity to algae and aquatic plants is available. The study (OECD TG 201, non-GLP compliant, 2006) was conducted on *Desmodesmus subspicatus* with the UVCB substance at nominal concentrations (based on water accommodated fractions) of 1, 10 and 100 mg/L for 72h. Three test vessels (4 for the controls) were used for each concentrations of the tested substance. No analysis of the test concentrations were conducted. The EC50 value was >100 mg/L (nominal) based on growth rate and biomass. The determined NOEC is 10 mg/L (nominal) as a significant growth rate inhibition of 14% was observed at 100 mg/L.

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

No data available.

11.5 Long-term aquatic hazard

Table 42: Summary of relevant information on chronic aquatic toxicity for substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1

Method	Species	Test material	Results	Remarks	Reference
OECD Guideline 211 (Daphnia magna Reproduction Test) freshwater semi-static	<i>Daphnia magna</i>	Test material: Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EC 270-128-1 Tested concentrations: 0, 0.625, 1.25, 2.5, 5, 10 mg/L nominal	Reduced reproduction (all test groups) No significant mortality EL10 (21d) 1.68 mg/L test mat. (nominal loading rate WAF) based on reproduction NOELR (21d) <0.625 mg/L test mat. (nominal loading rate WAF) based on reproduction	GLP compliant 2 (reliable with restriction) – no analytic measurements performed key study experimental result	Unpublished study report (2016b) Details available in Annex I Study 6 (4.4.4)
OECD Guideline 201 (Alga, Growth Inhibition Test) freshwater static 72h	<i>Desmodesmus subspicatus</i>	Test material: Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene EC 270-128-1 Tested concentrations: 1, 10, 100 mg/L nominal	Significant effects 14% growth rate inhibition at 100 mg/L. Non-significant effects at 1 & 10 mg/L EC50 (72h): >100 mg/L test mat. (nominal loading rate WAF) based on: biomass NOERL (72h): 10 mg/L test mat. (nominal nominal loading rate) based on growth rate and biomass	non-GLP compliant 2 (reliable with restriction) - no chemical analysis conducted Supporting study experimental result	Unpublished study report (2006)

11.5.1 Chronic toxicity to fish

Experimental data are not available for long-term toxicity to fish.

11.5.2 Chronic toxicity to aquatic invertebrates

One long-term study on toxicity to *Daphnia magna* is available for the UVCB substance. This key study (OECD TG 211, GLP compliant, 2016b) was conducted in semi-static condition (daily renewal of the test solution) for 21 days at 0 (control), 0.625, 1.25, 2.5, 5 and 10 mg/L nominal concentrations. Test solutions were prepared separately for each test loading rate as a water accommodated fraction (WAF). Each test group consisted of 10 replicates containing one juvenile *Daphnia* (<24h old) each. Measured concentration data were not performed in this study. Reduced reproduction was observed in all treatment groups and followed a clear concentration response pattern as indicated in the table below. The data were sufficient to calculate EC10 values for reproduction. No mortality was observed among parent animals over the 21d exposure period.

Mortality and Reproduction Summary after 21 days

Test groups	Test Groups Nominal [mg/L]	Reproduction		Mortality	
		Mean Living Young per surviving adult	% effect ^b	Parent animals	% effect ^b
0	0 (control)	120.3 (9.5% ^a)	–	0	–
1	0.625	115.4*	4.07%*	0	–
2	1.25	110.4*	8.23%*	0	–
3	2.5	103.6**	13.9%**	0	–
4	5	100.6**	16.4%**	0	–
5	10	83.2**	30.8%**	0	–

*: p ≤ 0.05; **: p ≤ 0.01

a: Coefficient of variation for control fecundity based on surviving parent.

b: Effect relative to control. Only calculated for statistically significant effects.

The reported ELR10 of 1.68 mg/L (95% CI: 0.767-3.68) and NOELR of <0.625 mg/L is therefore based on the most sensitive endpoint (reproduction). No analytical monitoring was conducted. The solutions were visually inspected for the presence of any undissolved test substance. All test solutions were colorless clear and no undissolved test substance was visible. According to CLP guidance, although measured concentrations are preferred, classification may be based on studies where nominal concentrations are the only valid data available. Although a calculated EL10 is available, the DS used the NOELR in the application of the classification criteria. Considering a conservative approach in the absence of measured concentrations and in regards to the effects observed for substance [1] the NOELR <0.625 mg/L was kept for the application of the criteria for classification. Thus, based on NOELR of <0.625 mg/L from this test it can be concluded that the UVCB substance exert chronic toxicity to aquatic invertebrates. However, the results reported with the nominal loading rates of the substance probably under-estimates the chronic toxicity of the substance.

11.5.3 Chronic toxicity to algae or other aquatic plants

One study is available to assess the toxicity of the UVCB substance on aquatic algae. This study is also described in the section 11.4.3 “acute (short-term) toxicity to algae or other aquatic plants studies” above.

The study (OECD TG 201, non-GLP compliant, 2006) was realised on *Pseudokirchneriella subcapitata* and a NOELR (72h): 10 mg/L test mat. (nominal loading rate) based on effects on growth rate and biomass was reported.

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 I.4.2 a-d). The DS have considered this approach in the assessment, although this approach was not preferred for the proposal on classification. For long-term aquatic hazard, the NOELR of <0.625 mg/L used for classification is lower than the water solubility of the substance according to OECD TG 105 (1.6 mg/L). For acute aquatic hazard, the water solubility of the substance does not allow a classification (>1mg/L). However, uncertainties were noted on the reliability of the OECD TG 105 using the flask method. The method implemented (i.e. flask method quantification by TOC) is not appropriate according to the OECD Guideline 105 for poorly water soluble substances which is the case of the UVCB (see Table 10 above). Additionally, a OECD TG 105 performed using the column elution method, indicated that the water solubility of one isomer of the constituent DTBDA (C4C4DPA) reached 4 µg/L at 20°C and pH 6.6. This information brings suspicion that the water solubility of the UVCB substance could be over-estimated. However, the extrapolation of water solubility from one constituent to the whole UVCB substance was not considered appropriate in this case for a conclusion on acute aquatic hazard classification.

11.6.1 Acute aquatic hazard

At least one acceptable study is available for invertebrates and algae categories of aquatic organisms. However, the study on fish is considered non-reliable.

Three studies showed adverse effects for substance:

A key study (OECD TG 202, GLP compliant 2004) was conducted in a 48-hour static test with the substance on *Daphnia magna*. The relevant endpoint immobility was based on water accommodated fractions (WAFs) with different loading rates. Analytical monitoring showed that the mean measured test concentrations were 0.32 mg/L (loading rate 10 mg/L), 0.30 mg/L (loading rate 22 mg/L), 0.63 mg/L (loading rate 46 mg/L), and 0.36 mg/L (loading rate 100 mg/L). Toxic effects were observed in the study and were increasing with increasing nominal concentrations. However, the measured concentrations were approximately in the same range at all loading rates and did not allow to establish a dose-response curve for EC50 calculation. Therefore the results were reported with the nominal loading rates of the substance which correspond to an EC50 (48h) of 51 mg/L based on mobility.

In the OECD TG 202 study (GLP compliant, 1998) on *Daphnia magna* a 24h test was conducted on substance tested as a WAF (Water Accommodated Fraction) with a loading rate of 100 mg/L. After 24h, 100% mortality was observed. No analytical measurement was performed and the EC50 is stated as <100 mg/L. Therefore, based on this study, no specific EC50 value on the acute toxicity of substance or its constituents can be given.

In the OECD TG 201 (non-GLP compliant, 2006) the substance was tested on *Desmodesmus subspicatus* at nominal loading rates (based on water accommodated fractions) of 1, 10 and 100 mg/L. The EC50 value was >100 mg/L based on growth rate and biomass.

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 in daphnia and algae studies may largely have under-estimated the acute toxicity of the substance.

	Criteria for acute environmental hazards	Substance [Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1]	Conclusion
Acute Aquatic Toxicity	Cat. 1: LC50/EC50/ErC50 ≤ 1 mg/L	Fish: Non reliable study Invertebrates: 48h-EC50= 51 mg/L (<i>Daphnia magna</i>) Algae:	No classification required

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		72h-EC50= >100 mg/L (<i>Desmodesmus subspicatus</i>)	
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11.6.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Considering the results of all models and empirical data on substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1, there is a consistent line of evidence to suggest that the substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1 does 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 substance and its main constituents (Log Pow > 4 and BCF > 500).

At least one acceptable study is available for two categories of aquatic organisms (invertebrates and algae). However, no long-term study on fish is available for substance. One acute study on fish species is available in the registration dossier, however this study is not considered reliable. Thus a surrogate approach is not possible for this trophic level.

Two studies showed adverse effects for substance:

The long-term study on toxicity to *Daphnia magna* of the UVCB substance reports an EL10 of 1.68 mg/L and a NOELR of <0.625 mg/L based on nominal concentration. Considering a conservative approach in the absence of measured concentrations and in regards to uncertainties related to water solubility value of the UVCB the NOELR <0.625 mg/L was kept for the application of the criteria for classification. Thus, the NOELR <0.625 mg/L was kept for the application of the criteria for classification.

The long-term study on algae (OECD TG 201, non-GLP compliant, 2006) was realised on *Pseudokirchneriella subcapitata* and a NOEC (72h) of 10 mg/L test mat. (nominal loading rate) based on growth rate and biomass was reported.

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 in daphnia and algae studies may largely have under-estimated chronic toxicity of the substance. It is also important to mention that the registration dossiers systematically use read-across data from substance Reaction products of diphenylamine with nonene, branched in the registration dossier of substance Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1 as supporting data. These data were not discussed for each endpoints and trophic levels. However, taking into account the discussion on the read across applicability in section 10.10.11, and considering all the aquatic toxic effects observed in this grouping approach for both substances, similar observations and conclusions can emerge. *Daphnia magna* is a, if not the most, sensitive species when testing both substances in the aquatic environment. If criteria for acute toxicity was not reached in this dossier due to the absence of measurements data, the data strongly suggested that the substance exerts a level of toxicity higher than nominal value obtained. The DS also considers that, although it cannot be demonstrated by measured data, the chronic toxicity of the UVCB substance could be at least similar if not higher than the level of toxicity observed for analogue substance Reaction products of diphenylamine with nonene, branched (proposed classification H410, M factor=10).

However, considering the available data, the following classification can be proposed (see table):

	Criteria for long-term environmental hazards	Substance [Benzenamine, N-phenyl-, reaction products with 2,4,4-trimethylpentene, EC 270-128-1]	Conclusion
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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)	OECD 301B (28d) based on CO ₂ evolution: 0% degradation (10.6 mg/L) 1% degradation (20.1 mg/L)	
	Primary degradation: half-life < 16 days (if degradation products do not fulfil criteria for classification as hazardous to the aquatic environment)	Non available	
Bioaccumulation	Log Kow ≥ 4	Measured log Kow OECD 123: C4C4DPA= 6.7 (23°C, pH 6.7)	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-NOELR of <0.625 mg/L (<i>Daphnia magna</i>)	Aquatic Chronic 2 (based on invertebrate-NOELR)
	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 2 - H411 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* NOELR(21d) <0.625mg/L.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

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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 identity of the substance and its analogue and the list of confidential references.*