Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

α,α'-propylenedinitrilodi-*o*-cresol

EC Number: 202-374-2
CAS Number: 94-91-7

CLH-O-0000007246-73-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 March 2023

CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name:

α,α'-propylenedinitrilodi-o-cresol

EC Number: **202-374-2**

CAS Number: **94-91-7**

Index Number: **604-RST-VW-Y**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON
α,α'-PROPYLENEDINITRILODI-*O*-CRESOL

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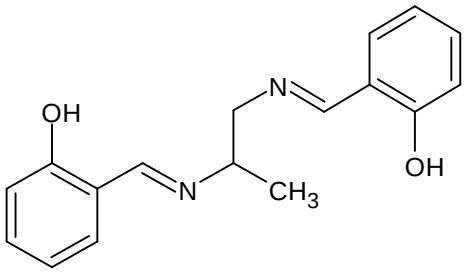
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON
α,α'-PROPYLENEDINITRILODI-*O*-CRESOL

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,2'-{propane-1,2-diylbis[azanylylidene-(methanylylidene]}diphenol
Other names (usual name, trade name, abbreviation)	2,2'-[propane-1,2-diylbis(nitrilomethylylidene)]diphenol Bis(salicylidene)propylenediamine Cuvan 80 DMD Keromet MD Metal Deactivator 2 N,N'-1,2-Propylenebis(salicylideneamine) N,N'-Disalicylidene-1,2-diaminopropane N,N'-Disalicylidene-1,2-propanediamine N,N'-Disalicylidene-1,2-propylenediamine N,N'-Propylenebis(salicylideneimine) Phenol,2,2'-[(1-methyl-1,2-ethanediyl)bis(nitrilomethylidyne)] bis-(9CI) Tenamene 60 o-Cresol, .alpha.,.alpha.'-(propylenedinitrilo)di- (6CI, 7CI, 8CI)
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	202-374-2
EC name (if available and appropriate)	α,α'-propylenedinitrilodi-o-cresol
CAS number (if available)	94-91-7
Other identity code (if available)	-
Molecular formula	$C_{17}H_{18}N_2O_2$
Structural formula	
SMILES notation (if available)	CC(CN=CC1=CC=CC=C1O)N=CC2=CC=CC=C2O
Molecular weight or molecular weight range	282.34 g/mol

Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Substance not optically active
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)***
α,α'-propylenedinitrilodi-o-cresol CAS number: 94-91-7 EC number: 202-374-2	Confidential information, see confidential Annex	No harmonised classification available	Acute Tox. 4, H302 Skin Sens. 1, H317 Repro 1B, H360 Aquatic Chronic 3, H412

* ECHA Dissemination (2021), Information on Chemicals - Registered Substances, European Chemicals Agency. Online: http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

α,α'-Propylenedinitrilodi-o-cresol is a mono-constituent substance (CAS number: 94-91-7). The current self-classification by the registrants is given in Table 2. The frequency of hazard classifications among all notifications was retrieved from PubChem on 25/06/2021 and is given below. In total, 1075 companies provided notifications with hazard classifications (14 aggregated notifications).

One company reported α,α'-propylenedinitrilodi-o-cresol as not meeting CLP hazard criteria.

Hazard classifications occurring in at least 10% of notifications:

Hazard code	**Hazard statement**	**% of notifications**
H226	Flammable liquid and vapor	36.9
H302	Harmful if swallowed	92.9
H315	Causes skin irritation	13.3
H317	May cause an allergic skin reaction	96.3
H319	Causes serious eye irritation	50.3
H360	May damage fertility or the unborn child	49.7
H411	Toxic to aquatic life with long lasting effects	39.2
H412	Harmful to aquatic life with long lasting effects	55.6

The test substance is α,α'-propylenedinitrilodi-o-cresol in all studies where the test substance was explicitly stated. The purity is given in the study records below if available.

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Confidential information, see confidential Annex.				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No information on additives available.					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: **For substance with no current entry in Annex VI of CLP**

	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	604-RST-VW-Y	α,α'-propylenedinitrilodi-o-cresol	202-374-2	94-91-7	Repr. 1B	H360FD	GHS08 Dgr	H360FD			

Table 6: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of 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	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data lacking	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	hazard class not assessed in this dossier	No
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 α,α'-propylenedinitrilodi-o-cresol. The substance has not been included in former activities on harmonised classification.

RAC general comment
α,α'-propylenedinitrilodi-*o*-cresol is used as a fuel and lubricant additive, as a process chemical and as a lubricant in high-energy open processes at industrial and professional sites. The substance is also used in fuels relevant for consumers. It has no current entry in Annex VI of the CLP Regulation. The Dossier Submitter (DS) proposed the following hazard classes for RAC evaluation: germ cell mutagenicity and reproductive toxicity with the proposal for harmonised classification and labelling as Repr. 1B, H360FD.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

The substance has CMR properties (reproductive toxicity). Harmonised classification and labelling for CMR properties is a community-wide action under article 36 of the CLP regulation.

5 IDENTIFIED USES

According to ECHA disseminated database (ECHA Dissemination, 2021) the substance is used at industrial and professional sites as a fuel and lubricant additive, as a process chemical, and as a lubricant in high energy open processes.

The substance is also used in fuels relevant for consumers.

6 DATA SOURCES

Systematic searches for publications and other relevant data were performed based on the following databases:

- U.S. National Library of Medicine, Pubmed.gov
- TOXNET, ChemIDplus, IPCS, eChemPortal
- Medline, SciSearch, Biosis, PQscitech, Chemical Abstracts (HCA), Embase (at host STN International)

The REACH registration dossier for α,α'-propylenedinitrilodi-o-cresol (last modified: 15 June 2020), publicly available from ECHA's disseminated database (ECHA Dissemination, 2021), has been analysed for study references, which then have been considered as data sources for this CLH report. Additionally, the confidential registration dossier was available for evaluation as well as several original study reports.

No relevant reviews and monographs with toxicological risk assessments on α,α'-propylenedinitrilodi-o-cresol were identified.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101.3 kPa	solid	ECHA Dissemination (2021)	visual observation
Melting/freezing point	53 °C	ECHA Dissemination (2021)	measured at 1 atm
Boiling point	-	ECHA Dissemination (2021)	Registration dossier: the study does not need to be conducted because the substance is a solid which decomposes before boiling
Density	1161.7 kg/m³	ECHA Dissemination (2021)	measured at 25 °C
Vapour pressure	0.000011 hPa	ECHA Dissemination (2021)	measured at 50 °C
Surface tension	-	ECHA Dissemination (2021)	Registration dossier: the study does not need to be conducted because based on structure, surface activity is not expected or cannot be predicted
Water solubility	190 mg/L	ECHA Dissemination (2021)	measured at 20°C at pH 8.3
Partition coefficient n-octanol/water	3.6	ECHA Dissemination (2021)	measured at 23 °C and pH 7
Flash point	229.5 °C	ECHA Dissemination (2021)	measured at 1 atm
Flammability	non flammable	ECHA Dissemination (2021)	measured
Explosive properties	-	ECHA Dissemination (2021)	Registration dossier: the study does not need to be conducted because there are no chemical groups present in the molecule which are associated with explosive properties
Self-ignition temperature	-	ECHA Dissemination (2021)	Registration dossier: the study does not need to be conducted because the substance is a solid having a melting point <= 160°C
Oxidising properties	-	ECHA Dissemination (2021)	Registration dossier: the study does not need to be conducted because the substance is incapable of reacting exothermically with combustible materials
Granulometry	-	ECHA Dissemination (2021)	Registration dossier: the study does not need to be conducted because the substance is marketed or used in a non-solid or granular form
Stability in organic solvents and identity of relevant degradation products	-	ECHA Dissemination (2021)	Registration dossier: the study does not need to be conducted because the stability of the substance is not considered to be critical
Dissociation constant	Not determined	ECHA Dissemination (2021)	Registration dossier: titration of the substance with acid or base

Property	Value	Reference	Comment (e.g. measured or estimated)
			resulted in inconsistent titration curves and content calculations. The determination of the dissociation constant of an aqueous preparation of the substance is therefore not applicable
Viscosity	-	ECHA Dissemination (2021)	Registration dossier: the study does not need to be conducted because the substance is a solid

8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
No toxicokinetic studies available.			

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

In the absence of toxicokinetic studies information on possible toxicokinetic properties of α,α'-propylenedinitrilodi-o-cresol is based on the summary provided in the registration dossier.

Absorption

According to the registrant systemic availability of the parent substance after oral absorption is not likely but bioavailability of the respective hydrolysis products can be assumed. According to the registrant α,α'-propylenedinitrilodi-o-cresol will readily hydrolyse to salicylaldehyde and propylenediamine.

The registrant elaborates: "*…once the chemical comes in contact with the digestive fluids of the stomach, hydrolysis reactions will occur. Due to the reduced molecular weight (< 200 g/mol) of the two hydrolysis products, it is possible that they directly cross the gut epithelial by passing through aqueous pores or through membranes by bulk transport of water."*

Oral LD_{50} values for the substance range between 1350 and 2250 mg/kg bw and only local effects on the gastrointestinal (GI) tract without systemic effects were observed. The registrant further explains that "*…no definite signs of systemic toxicity were observed in a 14 day dose range finder and a subacute combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test."* The registrant concluded that based on these findings it appears that no toxicologically relevant amounts enter the systemic circulation after oral intake. The authors of the CLH report do not support this assumption. The results of the two reproduction/developmental toxicity screening tests described in detail in section 10.10 show effects in pregnant females (death during parturition) and pups (stillborn or death shortly after birth) that cannot be explained by local effects in the GI tract.

Based on the very low vapour pressure and the physical form as highly viscous molten mass at room temperature inhalation is not considered a relevant exposure route. In the absence of reliable inhalation studies, no further information is available.

Physicochemical properties like log Pow, molecular weight and water solubility of α,α'-propylenedinitrilodi-o-cresol and its metabolites do favour dermal absorption. Results from skin sensitisation assays in guinea pigs showed that at least small amounts of the substance or its respective hydrolysis product are systemically available after dermal exposure.

Distribution

The registrant further elaborates *"…it is expected that the hydrolysis products are distributed within the blood stream.… access of the water soluble products to the central nervous system or the testes is likely to be restricted by the blood-brain and blood-testes barriers … Based on the low BCF value, the parent compound and the hydrolysis products have a negligible potential to bioaccumulate in the human body."*

Metabolism

The substance α,α'-propylenedinitrilodi-o-cresol can be hydrolysed to salicylaldehyde which may be further metabolised by phase I enzymes to salicylic acid. According to the registrant metabolism to toxic metabolites cannot be excluded. *"…Phase II conjugation reactions are likely to occur which covalently link an endogenous substrate (such as glycine, glucuronic acid etc) to the salicylaldehyde product or the Phase I metabolites in order to ultimately facilitate excretion."* The second metabolite resulting from the hydrolysis of α,α'-propylenedinitrilodi-o-cresol is propylenediamine. This substance may be degraded enzymatically via diamine oxidase followed by phase II modifications to facilitate excretion.

Excretion

According to the registrant the most likely excretion route is urine. This assumption is based on the molecular weight of α,α'-propylenedinitrilodi-o-cresol and the expected biotransformation products.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Evaluation not performed for this substance.

10.2 Acute toxicity - dermal route

Evaluation not performed for this substance.

10.3 Acute toxicity - inhalation route

Evaluation not performed for this substance.

10.4 Skin corrosion/irritation

Evaluation not performed for this substance.

10.5 Serious eye damage/eye irritation

Evaluation not performed for this substance.

10.6 Respiratory sensitisation

Evaluation not performed for this substance.

10.7 Skin sensitisation

Evaluation not performed for this substance.

10.8 Germ cell mutagenicity

Table 9: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Non-mammalian experimental systems				
in vitro gene mutation study in bacteria according to OECD TG 471 (Ames test) GLP: yes Reliability: 1	α,α'-propylenedinitrilodi-o-cresol purity: >99 corr. area % Vehicle: DMSO	*S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* WP2 uvr A With and without S9 mix 1. experiment: 0; 33; 100; 333; 1000; 2500 and 5000 µg/plate, standard plate test (SPT) with all strains with and without S9 mix 2. experiment: 0; 1; 3.3; 10; 33; 100 and 333 µg/plate, SPT with *Salmonella* strains with and without S9 mix 3. experiment: 0; 1; 3.3; 10; 33; 100 and 333 µg/plate (*Salmonella* strains), 0; 10; 33; 100; 333; 1000 and 2500 µg/plate (*E. coli* WP2uvrA), preincubation test (PIT) with and without S9 mix For each experiment 3 test plates per dose or per control Positive controls: yes	→ Negative with and without metabolic activation The test substance did not lead to a relevant increase in the number of revertant colonies either with or without S9 mix in all three experiments. Cytotoxicity (SPT): *Salmonella*: from about 100 µg/plate onward. *E.coli* WP2uvrA: observed from about 1000 µg/plate onward. Cytotoxicity (PIT): *Salmonella/E.coli*: depending on strain and test conditions from about 33 µg/plate onward. Controls were valid. For details see Annex I.	Study report, 2012 reported from ECHA Dissemination (2021) Study: 001, key Study reported in detail in Annex I
in vitro gene mutation study in bacteria similar to OECD TG 471 (Ames test)	α,α'-propylenedinitrilodi-o-cresol purity: not provided in the study report reference made to an analytical report Vehicle: DMSO	*S. typhimurium* TA 98, TA 7001, TA 7002, TA 7003, TA 7004, TA 7005 and TA 7006 With and without S9 mix Assy performed in microwell plates using a modified fluctuation test	→ Negative with and without metabolic activation Cytotoxicity in all strains from 2500 µg/mL onwards Controls were valid	Study report, 1999 reported from ECHA Dissemination (2021) Study: 003, supp.

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
modified version of the traditional Ames test, i.e. the Ames II Assay (microtiter version). GLP: no Reliability: 3 (screening test)		protocol 0; 4; 20; 100; 500; 2500, 5000 µg/mL Triplicate plates per dose, control chemical or vehicle Positive controls: yes		
Mammalian Cells				
in vitro gene mutation study in mammalian cells according to OECD TG 476 GLP: yes Reliability: 1	α,α'-propylenedinitrilodi-o-cresol purity: >99 corr. area % Vehicle: DMSO	Target gene: HPRT (hypoxanthine-guanine phosphoribosyl transferase) Cell line: Chinese hamster lung fibroblasts (V79) With and without phenobarbital/β-naphthoflavone induced rat liver S9 mix Pre-experiment: maximum concentration 2820 µg/mL (approx. 10 mM) Main experiment: 1. Experiment: exposure duration was 4 hours with and without metabolic activation. Concentrations: 0.0; 11.0; 22.0; 44.0; 88.0; 132.0; 176.0 (without S9 mix), 0.0; 11.0; 22.0; 44.0; 88.0; 176.0; 264.0 µg/mL (with S9 mix) 2. Experiment: exposure duration of 4 hours with and 24 hours without metabolic activation Concentrations: 0.0; 1.4; 2.8; 5.5; 11.0; 22.0; 33.0 (without S9 mix), 0.0; 22.0; 44.0; 88.0; 176.0; 264.0; 352.0 µg/mL (with S9 mix) Two independent	→ No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximum concentration. The mutant frequency generally did not exceed the historical range of solvent controls. A single increase of the induction factor exceeding three times the mutation frequency of the corresponding solvent control was observed in the first culture of the 2. experiment without metabolic activation at 2.8 µg/mL. However, the increase was based on a rather low mutation frequency of the solvent control of just 4.8 colonies per 10^6 cells. Furthermore, the effect was not reproduced in the parallel culture. Therefore, the increase of the induction factor was judged as biologically irrelevant fluctuation. Precipitation: 1.experiment: at 88.0 µg/mL with metabolic activation. However, the precipitate was probably denatured protein	Study report, 2012 reported from ECHA Dissemination (2021) Study: 002, key Study reported in detail in Annex I

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
		experiments with two cultures each Positive controls: yes	rather than test item per se as there was no precipitation in 2. experiment at comparable or even higher concentrations. 2. experiment: no precipitation Cytotoxicity: 1. experiment: relevant cytotoxic effects indicated by a relative cloning efficiency I or cell density below 50% in both parallel cultures occurred ≥ 88.0 µg/mL without metabolic activation and at 264 µg/mL with metabolic activation 2. experiment: in the second experiment cytotoxic effects as described above occurred at 176 µg/mL and above with metabolic activation. Controls were valid. For details see Annex I.	
in vitro cytogenicity / chromosome aberration study in mammalian cells according to OECD TG 473 GLP: yes Reliability: 1	α,α'-propylenedinitrilodi-o-cresol purity: >99 corr. area % Vehicle: DMSO	Cell line: Chinese hamster lung fibroblasts (V79) With and without S9 mix Pre-experiment: maximum concentration 2820 µg/mL (approx. 10 mM, due to molecular weight of the test item) Preliminary test with and without metabolic activation (concludingly used as main test): 0.0; 11.0; 22.0; 44.1; 88.1; 176.3; 352.5; 705.0; 1410.0; and 2820.0 µg/mL Exposure period: 4 Recovery: 14 h Preparation interval: 18 h 100 metaphases per culture were evaluated for structural chromosome aberrations 2 independent parallel cultures Positive controls: yes	→ Positive with and without metabolic activation Clastogenicity was observed in the absence of S9 mix after treatment with 22.0, 44.1 and 88.1 µg/mL (13.5, 12.5, 14.0 % aberrant cells, excluding gaps) and in the presence of S9 mix after treatment with 22.0, 44.1, 88.1 and 176.3 µg/mL (10.5, 7.0, 10.0 and 20.5 % aberrant cells, excluding gaps) clearly exceeding the range of the historical control data of 0.0 - 4.0 % aberrant cells, excluding gaps. No relevant increase in polyploid metaphases was found. No relevant increase in endomitotic metaphases was found Pre-experiment: At the selected concentration	Study report, 2012 reported from ECHA Dissemination (2021) Study: 004, supp. Study reported in detail in Annex I

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
			no relevant influence on solubility, pH value, or osmolarity was detected. Main test: Visible precipitation of the test item in the culture medium was observed at 352.5 µg/mL and above in the absence and presence of S9 mix. Cytotoxicity, indicated by reduced mitotic indices was observed at 352.5 µg/mL and above in the absence of S9 mix and at 176.3 µg/mL and above in the presence of S9 mix. Controls were valid. For details see Annex I.	

Table 10: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
in vivo mammalian somatic cell study: micronucleus assay according to OECD TG 474 GLP: yes Reliability: 1	α,α'-propylenedinitrilodi-o-cresol purity: >99 corr. area % Vehicle: in PEG 400	7 male NMRI mice (exposure group), 5 male NMRI mice (vehicle and positive controls) 24 h preparation interval: 0; 500; 1000; and 2000 mg/kg bw 48 h preparation interval: 0; 2000 mg/kg bw Single oral application via gavage (10 mL/kg bw) 2000 polychromatic erythrocytes (PCE) per animal were analysed for micronuclei. To investigate a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same	→ Negative In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment were below or near to the value of the vehicle control group. All values in dose groups were very well within the laboratory's historical vehicle control data. After treatment with the test item at 48 h preparation interval the number of PCEs per 2000 erythrocytes was not substantially decreased as compared to the vehicle control. Controls were valid.	Study report, 2013 reported from ECHA Dissemination (2021) Only study record available, key Study reported in detail in Annex I

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. Positive control: yes	For details see Annex I.	

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Four *in vitro* studies and one *in vivo* study are available for the assessment of germ cell mutagenicity of α,α'-propylenedinitrilodi-o-cresol. No human data were identified.

The studies are summarised in Table 9 and Table 10. *In vitro* data are available from two Bacterial Reverse Mutation Assays (one Ames Test according to OECD TG 471 and one microtiter Ames version), a Mammalian Cell Gene Mutation Test (according to OECD TG 476) and a Mammalian Chromosomal Aberration Test (according to OECD TG 473). The *in vivo* study is a Mammalian Erythrocyte Micronucleus Test (according to OECD TG 474).

In the reliable Bacterial Reverse Mutation Assay according to OECD TG 471, performed under GLP conditions (reliability 1) α,α'-propylenedinitrilodi-o-cresol was tested in three experiments with and without metabolic activation. The *S. typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* WP2 uvr A were exposed to concentrations of 0 - 5000 µg/plate in a standard plate test and 0-2500 µg/plate in a preincubation test. The test substance did not lead to a relevant increase in the number of revertant colonies either with or without S9 mix in all three experiments (For details on results see Annex I).

A second Ames test supports the negative findings. The test was performed according to a modified protocol in microwell plates (Ames II Screening Assay, microtiter version). Due to this modified protocol a reliability of 3 was assigned. In this test the *S. typhimurium* strains TA 98, TA 7001, TA 7002, TA 7003, TA 7004, TA 7005 and TA 7006 were tested with and without S9 mix in microwell plates using a modified fluctuation test protocol. The "TA mix" (TA 7001 – TA 7006) is used for the detection of base pair substitutions and TA 98 is used to detect frameshift mutations. Concentrations of 0-5000 µg/mL were tested and results throughout all strains were negative.

The potential of α,α'-propylenedinitrilodi-o-cresol to induce gene mutations at the HPRT locus in Chinese hamster V79 cells was studied in an *in vitro* gene mutation study performed according to OECD TG 476 (GLP conditions, reliability 1). In two independent experiments with and without metabolic activation a variety of concentrations (two cultures for each concentration in both experiments) was tested with an exposure duration of 4 h (experiment 1 and experiment 2) or 24 h (experiment 2, without metabolic activation only). No relevant and reproducible increase in mutant colony numbers per 10^6 cells was observed up to the maximum concentration (for details see Annex I). In the second experiment a single increase of the induction factor exceeding three times the mutation frequency of the corresponding solvent control was observed at 2.8 µg/mL in one culture without metabolic activation. However, the mutation frequency of the solvent control was rather low (4.8 colonies per 10^6 cells). Furthermore, the effect was not reproduced in the parallel culture. Therefore, the increase of the induction factor was judged as a biologically irrelevant fluctuation by the study authors.

In an *in vitro* chromosome aberration assay according to OECD TG 473 (GLP conditions, reliability 1), Chinese hamster lung fibroblasts (V79) were exposed to α,α'-propylenedinitrilodi-o-cresol for 4 hours with or without metabolic activation. The subsequent expression duration was 14 h, therefore, the preparation interval was 18 h. Test concentrations ranged from 0 to 2820.0 µg/mL in two independent parallel cultures (0.0; 11.0; 22.0; 44.1; 88.1; 176.3; 352.5; 705.0; 1410.0; and 2820.0 µg/mL). One hundred metaphases per culture were evaluated for structural chromosome aberrations. Cytotoxicity, indicated by reduced mitotic

indices, was observed at 352.5 µg/mL and above in the absence of S9 mix and at 176.3 µg/mL and above in the presence of S9 mix. In the absence of S9 mix concentrations showing clear cytotoxicity were not scorable for cytogenetic damage, therefore the highest concentration evaluated was 176.3 µg/mL. In the presence of S9 mix the highest concentration that could be evaluated despite cytotoxic effects was 176.3 µg/mL. In the absence of S9 mix clastogenicity was observed after treatment with 22.0, 44.1 and 88.1 µg/mL (13.5, 12.5, 14.0 % aberrant cells, excluding gaps) and in the presence of S9 mix after treatment with 22.0, 44.1, 88.1 and 176.3 µg/mL (10.5, 7.0, 10.0 and 20.5% aberrant cells, excluding gaps). These percentages of aberrant cells clearly exceeded the range of the historical control data of 0.0 - 4.0% aberrant cells, excluding gaps. No relevant increase in polyploid metaphases or endomitotic metaphases was found after treatment with the test item. For details see Annex I.

The positive results from the *in vitro* chromosome aberration test were not confirmed in an *in vivo* mammalian erythrocyte micronucleus assay performed according to OECD TG 474 (GLP conditions, reliability 1). Seven male NMRI mice (exposure groups) or 5 male mice (vehicle and positive controls) were exposed one-time via gavage to either 0, 500, 1000 and 2000 mg/kg bw (24 h preparation interval) or 0 and 2000 mg/kg bw (48 h preparation interval). For each animal 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei. There was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of α,α'-propylenedinitrilodi-o-cresol. The mean values of micronuclei observed after treatment were below or near to the value of the vehicle control group and well within the laboratory's historical vehicle control data. To investigate a potential cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. After treatment with the test item at the 48 h preparation interval the number of PCEs per 2000 erythrocytes was not substantially decreased compared to the vehicle control.

10.8.2 Comparison with the CLP criteria

For potential classification on germ cell mutagenicity, criteria from the CLP Regulation (EC, 2008)[1] were applied:

Comparison with Category 1 criteria

- *The classification in Category 1A is based on positive evidence from human epidemiological studies (EC, 2008)*

There are no epidemiological data to support classification of α,α'-propylenedinitrilodi-o-cresol in Category 1A.

- *The classification in Category 1B is based on positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals (EC, 2008)*

No *in vivo* studies with heritable germ cell are available.

- *Classification in Category 1B can also be based on "positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells". (EC, 2008)*

One *in vivo* mammalian erythrocyte micronucleus assay (performed according to OECD TG 474) is available. The study has a reliability of 1 and was negative at all doses tested and both preparation intervals (24 and 48 h). In addition, no data showing that the substance has the potential to cause mutations in germ cells is available. Thus, classification in Category 1B is not supported. This *in vivo* study overrules the positive results obtained in an *in vitro* chromosome aberration test.

[1] REGULATION (EC) No 1272/2008 considering all ATPs published until June 2021

Comparison with Category 2 criteria

- *Classification in category 2 is based on:*

  — positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

  — somatic cell mutagenicity tests in vivo, in mammals; or

  — other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays. (EC, 2008)

These criteria are also not met because the only available in *in vivo* somatic cell genotoxicity tests in mammals for α,α'-propylenedinitrilodi-o-cresol is negative.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Classification as a germ cell mutagen is not warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported the following *in vitro* mutagenicity/genotoxicity studies (cf. Table 9 of the CLH report and information regarding study design in Annex I to the CLH report):

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Results	Reliability	Reference
Non-mammalian experimental system					
In vitro gene mutation study in bacteria GLP study According to OECD TG 471 (Ames test)	α,α'-propylenedinitrilodi-*o*-cresol, Purity: > 99 corr. area % Vehicle: DMSO Sterility controls: yes Vehicle controls: yes Positive controls:	Strains: *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100*; E. coli WP2 uvr A* Target gene: HIS/TRP Metabolic activation system: S9-mix Experiment 1: 0; 33; 100; 333;	**Genotoxicity**: Negative (± metabolic activation) (experiment 1-3). No increase in revertant colonies (SPT or PIT) Negative control data and positive control data = within historical control data. No test substance	1	Study: 001, key study Study report, 2012 reported from ECHA Dissemination (2021)

	yes	1000; 2500 and 5000 µg/plate, SPT with all strains (± S9 mix) Experiment 2: 0; 1; 3.3; 10; 33; 100 and 333 µg/plate, SPT with Salmonella strains (± S9 mix); **Reason**: bacteriotoxicity was observed in the standard plate test Experiment 3: 0; 1; 3.3; 10; 33; 100 and 333 µg/plate (Salmonella strains), 0; 10; 33; 100; 333; 1000 and 2500 µg/plate (E. coli WP2uvrA), PIT (±S9 mix); **Reason**: no mutagenicity was observed in the standard plate test Number of plates: 3/dose (control)	precipitation (± S9 mix) **Cytotoxicity (SPT)**: *Salmonella* strains: ≥ 100 µg/plate onward. *E. coli WP2uvrA*: observed ≥ 1000 µg/plate onward **Cytotoxicity (PIT)**: *Salmonella/E.coli* : depending on the strain and test conditions ≥ 33 µg/plate onward.		
In vitro gene mutation study in bacteria GLP: no Similar to OECD TG 471 (Ames test) the Ames II Assay (microtiter version), a modified version of the Ames	α,α'-propylenedinitrilodi-*o*-cresol, Purity: not provided Vehicle: DMSO Positive controls: yes	Strains: *S. typhimurium* TA 98, TA 7001, TA 7002, TA 7003, TA 7004, TA 7005 and TA 7006 Metabolic activation system: S9 mix Assay performed in microwell plates using a modified fluctuation test protocol (± metabolic activation). Doses: 0; 4; 20; 100; 500; 2500,	**Genotoxicity**: Negative (± metabolic activation) **Cytotoxicity**: In all strains: from about 2500 µg/ml onward. Negative control data and positive control data = within historical control data.	3 (screening test)	Study: 003, supporting study Study report, 1999 reported from ECHA Disseminatio n (2021)

test		5000 µg/mL Number of plates: 3 per dose, control or vehicle			
Mammalian Cells					
In vitro gene mutation study in mammalian cells GLP: yes according to OECD TG 476	α,α'-propylenedinitrilodi-*o*-cresol Purity: > 99 corr. area % Vehicle: DMSO, the final concentration of DMSO in the culture medium was 0.5% v/v Untreated negative controls: no Negative solvent / vehicle controls: yes True negative controls: no Positive controls: yes	**Cell line**: Chinese hamster lung fibroblasts (V79) **Target gene**: HPRT (hypoxanthine-guanine phosphoribosyl transferase) ± phenobarbital/β-naphthoflavone induced rat liver S9 mix Pre-experiment: 22.0; 44.0; 88.0; 132.0; 176.0;352.5;705.0; 1410.0; 2820.0 µg/mL (approx. 10 mM) Main experiment: Experiment I: 4h ± metabolic activation. Concentrations: 0.0; 11.0; 22.0; 44.0; 88.0; 132.0; 176.0 (without S9 mix), 0.0; 11.0; 22.0; 44.0; 88.0; 176.0; 264.0 µg/mL (with S9 mix) Experiment II: exposure duration-4h with and 24h metabolic activation Concentrations: 0.0; 1.4; 2.8; 5.5;	**Genotoxicity**: Negative. The mutant frequency generally was not ↑ compared with historical range of solvent controls. A single increase of the induction factor (x3 mutation frequency in solvent control) (first culture of second experiment without metabolic activation at 2.8 µg/mL) However, the increase was based on a rather low mutation frequency of the solvent control of just 4.8 colonies per 10^6 cells. Furthermore, the effect was not reproduced in the parallel culture. Linear regression analysis showed a significant dose-dependent trend of the mutation frequency ($p<0.05$) (only in the second culture of the II	1	Study: 002, key study Study report, 2012 reported from ECHA Disseminatio n (2021)

		11.0; 22.0; 33.0 (without S9 mix), 0.0; 22.0; 44.0; 88.0; 176.0; 264.0; 352.0 µg/mL (with S9 mix) Two independent experiments with two cultures each	experiment without metabolic activation). This trend was not reproduced in the parallel culture under identical experimental conditions. **Precipitation**: Pre-experiment: at ≥ 1410 µg/mL (±metabolic activation) (4 or 24 h) Experiment I: at 88.0 µg/mL with metabolic activation. Experiment II: no precipitation **Cytotoxicity**: Pre-experiment: at ≥ 176.3 µg/mL (metabolic activation (4h treatment) – relative suspension growth below 50. Complete inhibition ≥ 176 µg/mL (no metabolic activation) (4h) and ≥ 44.1 µg/mL (24h) Experiment I: relevant cytotoxic effects - relative cloning efficiency I or cell density below 50% in both parallel cultures ≥ 88.0 µg/mL without metabolic activation and at 264 µg/mL with		

			metabolic activation Experiment II: cytotoxic effects ≥ 176 µg/mL with metabolic activation. Controls were valid = range of the historical control data.		
In vitro cytogenicity / chromosome aberration study in mammalian cells GLP: yes according to OECD TG 473	α,α'-propylenedinitrilodi-*o*-cresol Purity: > 99 corr. area % Vehicle: DMSO Untreated negative controls: no Negative solvent / vehicle controls: yes, with DMSO True negative controls: no Positive controls: yes	**Strains**: Chinese hamster lung fibroblasts (V79) ± metabolic activation with phenobarbital/ /β-naphthoflavone induced S9 mix Pre-experiment: maximum concentration 2820 µg/mL (approx. 10 mM, due to molecular weight of the test item) Preliminary test ± metabolic activation (concludingly used as main test): 0.0; 11.0; 22.0; 44.1; 88.1; 176.3; 352.5; 705.0; 1410.0; and 2820.0 µg/mL Exposure period: 4 h Recovery: 14 h Preparation interval: 18 h 100 metaphases/ culture were evaluated for structural chromosome aberrations 2 independent	**Genotoxicity**: Positive (± metabolic activation) **Clastogenicity** - in the absence of S9 mix - 22.0, 44.1 and 88.1 µg/mL (13.5, 12.5, 14.0 % aberrant cells, excluding gaps) and in the presence of S9 mix 22.0, 44.1, 88.1 and 176.3 µg/mL (10.5, 7.0, 10.0 and 20.5 % aberrant cells, excluding gaps); historical control data (0.0 - 4.0 % aberrant cells, excluding gaps). No effect in polyploid metaphases (2.1 - 3.6 %) compared with the solvent controls (2.5 - 3.1 %) No effect in endomitotic metaphases **Cytotoxicity** (↓mitotic indices): ≥	1	Study: 004, supporting study Study report, 2012 reported from ECHA Dissemination (2021)

		parallel cultures Evaluation of cytotoxicity: mitotic index Determination of polyploidy: yes Determination of endoreplication: yes	352.5 µg/mL in the absence of S9 mix and at ≥ 176.3 µg/mL in the presence of S9 mix. **Precipitation**: ≥ 352.5 µg/mL visible precipitation of the test item in the culture (± metabolic activation)		

Note: Standard plate test – SPT; Preincubation test - PIT;

The DS reported the following *in vivo* mutagenicity/genotoxicity test in mammalian somatic cells (cf. Table 10 of the CLH report and information regarding study design in Annex I to the CLH report):

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Results**	**Reliability**	**Reference**
In vivo mammalian somatic cell study: micronucleus assay GLP: yes according to OECD TG 474	α,α'-propylenedinitrilodi-*o*-cresol Purity: > 99 corr. area % **Vehicle**: polyethylene glycol (PEG) 400 (the administered volume was 10 mL/kg bw including test substance) **Positive control**: cyclophosphamide (40 mg/kg bw)	**Test animals**: male NMRI mice, 8-9 weeks old (7 males per group for the test groups and 5 males per group for control groups (vehicle and positive control) **Administration**: single oral administration via gavage **Doses**: 24h preparation interval: 0; 500; 1000 and 2000 mg/kg bw 48 h preparation interval: 0; 2000	**Genotoxicity**: negative No increase in the micronuclei frequency compared to corresponding vehicle controls. The mean values of micronuclei observed after treatment ≤ vehicle control group. **Toxicity**: mortality in the top dose animal **Controls**	1	Only study record available, key study Study report, 2013 reported from ECHA Dissemination (2021)

		mg/kg bw **Tissues and cell types examined**: 2000 polychromatic erythrocytes (PCE)/animal were analysed for micronuclei. **Cytotoxicity**: the ratio between polychromatic and normochromatic erythrocytes and expressed in polychromatic erythrocytes per 2000 erythrocytes	**(vehicle and positive)** were valid and within the historical control data.		

For the assessment of germ cell mutagenicity of α,α'-propylenedinitrilodi-*o*-cresol there were four *in vitro* studies, one *in vivo* study, and no human data available.

Among the *in vitro* studies, there were two Bacterial Reverse Mutation Assays; one Ames Test according to OECD TG 471 (a key study) and one microtiter Ames version (a supporting study), a Mammalian Cell Gene Mutation Test according to OECD TG 476 (a key study) and a Mammalian Chromosomal Aberration Test according to OECD TG 473 (a supporting study). There was only one *in vivo* study, a Mammalian Erythrocyte Micronucleus Test, a key study according to OECD TG 474. Except for the Mammalian Chromosomal Aberration Test, all the other *in vitro* and *in vivo* tests were negative.

The key study 001 is a Bacterial Mutation Assay conducted according to OECD TG 471, under GLP conditions with a reliability of 1. The bacterial strains *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* WP2 uvr A were treated with increased concentrations of α,α'-propylenedinitrilodi-o-cresol 0-5000 µg/plate (SPT) and 0-2500 µg/plate (PIT) with and without metabolic activation with S9 mix. All the tests were performed in triplicates and no increase in the number of revertant colonies was observed in any of the test conditions.

Study 003 is a second Ames test performed using a modified protocol, Ames II Screening Assay, that supports the negative results of the key study 001. The reliability of the study was assigned as 3 due to the use of the modified protocol. The bacterial strains *S. typhimurium* TA 98, TA 7001, TA 7002, TA 7003, TA 7004, TA 7005 and TA 7006 were exposed to concentrations of 0-5000 µg/mL α,α'-propylenedinitrilodi-o-cresol with and without metabolic activation using S9 mix in microwell plates applying a fluctuation test protocol. The samples were worked in triplicates and all the results were negative.

The key study 002 evaluated the potential of α,α'-propylenedinitrilodi-*o*-cresol to induce gene

mutations in HPRT locus in Chinese hamster cells. It is an *in vitro* study according to OECD TG 476 conducted under GLP conditions and evaluated as being of reliability 1. There were 2 main experiments performed in duplicate in which the cells were exposed for 4 h to 0.0; 11.0; 22.0; 44.0; 88.0; 132.0; 176.0 (without S9 mix), 0.0; 11.0; 22.0; 44.0; 88.0; 176.0; 264.0 µg/mL α,α'-propylenedinitrilodi-*o*-cresol (with S9 mix) (experiment I) and 0.0; 1.4; 2.8; 5.5; 11.0; 22.0; 33.0 (without S9 mix), 0.0; 22.0; 44.0; 88.0; 176.0; 264.0; 352.0 µg/mL α,α'-propylenedinitrilodi-o-cresol (with S9 mix) (experiment II) and for 24 h to 0.0; 1.4; 2.8; 5.5; 11.0; 22.0; 33.0 µg/mL α,α'-propylenedinitrilodi-*o*-cresol (experiment II). Up to the maximum concentration, no relevant and reproduced increase in mutant colonies/10^6 cells was observed. All the mutant colonies were in the range of historical control for the solvent controls. At the concentration of 2.8 µg/mL in the second experiment without metabolic activation, a three-fold increase in the mutation frequency was observed compared with the solvent control. However, this effect was seen only in the first culture and could not be reproduced in the parallel culture and the effect was not dose-dependent. Therefore, it was considered as a biologically irrelevant fluctuation.

Study 004 is the only *in vitro* study that showed positive results for the potential of α,α'-propylenedinitrilodi-*o*-cresol to induce gene mutations. It is a chromosome aberration assay according to OECD TG 473 performed under GLP conditions with a reliability of 1. The Chinese hamster lung fibroblasts (V79) were exposed to the test substance for 4 h with or without metabolic activation with S9 mix. The cells had a recovery period for subsequent expression duration of 14 h, with a total preparation interval of 18 h. The cells were exposed to 0.0; 11.0; 22.0; 44.1; 88.1; 176.3; 352.5; 705.0; 1410.0; and 2820.0 µg/mL α,α'-propylenedinitrilodi-*o*-cresol with or without metabolic activation. The structural chromosomal aberrations were evaluated in one hundred metaphases per culture, while the cytotoxicity was evaluated by the determination of mitotic index. All the samples were worked in duplicates. Cytotoxicity translated into a reduced mitotic index that was observed at concentrations of 352.5 µg/mL and above in the absence of S9 mix and at concentrations of 176.3 µg/mL and above in the presence of S9 mix. The precipitation in the culture was identified at concentrations of 352.5 µg/mL and above in the absence of S9 mix and at 176.3 µg/mL and above in the presence of S9 mix. Clastogenicity was observed in the absence of S9 mix after treatment with 22.0, 44.1 and 88.1 µg/mL (13.5, 12.5, 14.0 % aberrant cells, excluding gaps) and in the presence of S9 mix after treatment with 22.0, 44.1, 88.1 and 176.3 µg/mL (10.5, 7.0, 10.0 and 20.5 % aberrant cells, excluding gaps) clearly exceeding the range of the historical control data (0.0 - 4.0 % aberrant cells, excluding gaps). No relevant increase in polyploid metaphases (2.1-3.6%) compared with the solvent controls (2.5-3.1%) and no relevant increase in endomitotic metaphases was found after treatment at any concentration.

The *in vivo* mammalian somatic cell study does not support the positive results of *in vitro* study 004. The *in vivo* study is a mammalian erythrocyte micronucleus assay performed according to OECD TG 474 under GLP conditions with a reliability of 1. The test animals were male NMRI mice, 7 per group in the test groups and 5 per group in the control groups (vehicle and positive control). The animals received a single oral dose by gavage and were evaluated after 24 h for concentrations of 0; 500; 1000 and 2000 mg/kg bw or after 48 h for concentrations 0 and 2000 mg/kg bw. From each animal 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei, while the cytotoxic effect was evaluated by the ratio between polychromatic and normochromatic erythrocytes and expressed as polychromatic erythrocytes per 2000 erythrocytes. In comparison to the corresponding vehicle controls, there was no statistically significant or biologically relevant increase in the frequency of the detected

micronuclei at any preparation interval and dose level after administration of the test item. Additionally, no dose-dependent increase in the frequency of detected micronuclei was observed with increasing dosages and all values in dose groups were well within the laboratory's historical vehicle control data. Toxicity was observed only in the top-dose animals where mortality was reported. No cytotoxicity was observed in any dose or period of evaluation. Taking into consideration that the ratio between polychromatic and normochromatic erythrocytes was not affected, RAC also assessed whether there were data showing that the substance reached the bone marrow. The CLH report provided emphasises that there were no toxicokinetic studies made with α,α'-propylenedinitrilodi-*o*-cresol. The only available information came from the summary provided in the REACH registration dossier, where the registrant presented the following explanation for the distribution of the substance: "it is expected that the hydrolysis products are distributed within the bloodstream … access of the water-soluble products to the central nervous system or the testes is likely to be restricted by the blood-brain and blood-testes barriers … Based on the low BCF value, the parent compound and the hydrolysis products have a negligible potential to bioaccumulate in the human body". This information does not demonstrate that α,α'- propylenedinitrilodi-*o*-cresol is distributed in the bone marrow. However, it is also acknowledged that systemic effects were observed in the high dose in the *in vivo* study mammalian somatic cell study. In Annex 1 of the CLH report it is specified that only one animal exposed to the high dose died after 24 h from exposure and before death showed "reduction of spontaneous activity, abdominal position, eyelid closure and ruffled fur", while in other animals no clinical signs of toxicity were observed. Analysing this available information, RAC concluded that the available information does not support the conclusion that the chemical reaches bone marrow.

Comments received during consultation

Three comments were received during the consultation, two from Member State Competent Authorities (MSCAs) and one from Company/Manufacturer. All agreed with the DS conclusion that there was not sufficient evidence to warrant classification of α,α'-propylenedinitrilodi-*o*-cresol for germ cell mutagenicity.

Assessment and comparison with the classification criteria

In this case, there are no human epidemiological data to support the classification of α,α'-propylenedinitrilodi-*o*-cresol in Category 1A for germ cell mutagenicity.

There are no *in vivo* studies with heritable germ cells available.

There is an *in vivo* mammalian erythrocyte micronucleus assay according to OECD TG 474 performed under GLP conditions with a reliability of 1 that showed no genotoxicity in somatic cells at any dose or interval (24 and 48 h) tested. Also, there is no evidence that the chemical has potential to cause mutations in germ cells. Thus, classification in Category 1B is not supported.

There are no *in vivo* studies with germ cells from humans available.

The only available *in vivo* mammalian somatic cell study, a micronucleus assay according to OECD TG 474, under GLP conditions with reliability of 1 is negative. Thus, the criteria for Category 2 are not met either.

Overall conclusion: There are *in vivo* data from a mammalian somatic cell study that showed negative results for somatic cell mutagenicity for α,α'-propylenedinitrilodi-*o*-cresol, 3 negative *in vitro* tests (two Bacterial Mutation Assays and one *in vitro* gene mutation study in mammalian cells) and only one chromosome aberration study in mammalian cells positive for clastogenicity both in the presence and absence of metabolic activation with S9 mix. These pieces of evidence are not enough to warrant classification of α,α'-propylenedinitrilodi-*o*-cresol for germ cell mutagenicity.

In view of the available information, RAC agrees with DS proposal that **classification of α,α'-propylenedinitrilodi-*o*-cresol as germ cell mutagen is not warranted.**

10.9 Carcinogenicity

No studies available.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 11: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Screening for reproductive / developmental toxicity according to OECD TG 422 GLP: yes Wistar rats (Crl:WI(Han)) 10 animals/sex /dose Reliability: 1	α,α'-propylenedinitrilodi-o-cresol purity: >99 corr. area % 0, 25, 75, 250 mg/kg bw/d via gavage (based on a 14-day dose-range finding study) Males: 29 days, i.e. 2 weeks prior to mating, during mating, and up to termination. Females: 42-45 days, i.e. 2 weeks prior to mating, during mating, gestation, and up to LD 4	**Effects on P0 generation:** - No substance-specific clinical signs of toxicity were noted during the observation period. - Body weights and body weight gains were statistically significantly lower in males at 75 and 250 mg/kg bw/d on day 8 of the pre-mating period and thereafter (mating days 1, 8 and 15). However, the differences to controls were slight, and values remained within the range considered normal for rats of this age and strain (normal range: 5-95% confidence interval body weight gain on mating day 15: 11-30%). Therefore, the study authors considered these differences not to be toxicologically relevant. Body weights of females were not affected (See Annex I for details). - No toxicologically relevant changes in food consumption before or after correction for body weight were noted. - At 250 mg/kg bw/d two females had a total litter loss on lactation day (LD) 1 (this is presumably postnatal day (PND) 0), resulting in a gestation index of 77.8% for this group compared to 100% for the remaining groups. The reason for the total litter loss according to study authors could not be established as part of the study. There were no indications for a poor condition of these two females, and examination of the reproductive organs of the animals that failed to deliver healthy offspring did not reveal any	Study report, 2013 reported from ECHA Dissemination (2021) Study: 001, key Study reported in detail in Annex I

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		abnormalities. - No treatment-related toxicologically significant changes were noted for mating-, fertility- and conception indices, precoital time, and numbers of corpora lutea and implantation sites. For details see Annex I. - There was a trend towards slightly lower numbers of corpora lutea and implantation sites at 250 mg/kg bw/d which was mainly attributable to two females who had 7 corpora lutea each and 5 and 7 implantation sites, respectively. Since lower numbers were also seen for a control female (8 corpora lutea and 8 implantation sites), this finding was considered of no toxicological relevance. - No signs of difficult or prolonged parturition were noted among the pregnant females. Examination of cage debris of pregnant females revealed no signs of abortion or premature birth. No deficiencies in maternal care were observed. - The assessment of the integrity of the spermatogenetic cycle did not provide any evidence of impaired spermatogenesis. Effects on pups are reported in section 10.10.4.	
Screening for reproductive / developmental toxicity (modified one-generation reproduction toxicity study) similar to OECD TG 421 and 416 only one dose level tested with additional parameters of an OECD 416 study GLP: yes Wistar rats (Crl:WI(Han)) 25 animals/sex /dose	α,α'-propylenedinitrilodi-o-cresol purity: >99 corr. area % 0, 250 mg/kg bw/d via gavage (single dose selected to further investigate results from the 422 study) Males: 2 weeks prior to mating, during mating, and about three weeks postmating Females: 2 weeks prior to mating, during mating, gestation, and up to LD 4.	**Effects on P0 generation:** - Mean body weights of animals at 250 mg/kg bw/d were generally comparable to the respective concurrent control group during the entire study period. For details see Annex I. - Body weight gain of treated males was statistically significantly reduced during pre-mating days 0-7 and postmating days 14-20. Mean body weight gain of treated females was statistically significantly reduced during GD 0-14. For details see Annex I. - Three females of treatment group died during parturition process on GD 23 (see Annex I for details). Two of them showed adverse clinical findings preceding death: - Female 1 showed apathy (GD 22-23), piloerection and a reddish, brown vaginal discharge (GD 23, respectively) - Female 2 showed apathy on GD 22 - For one further female of treatment group dystocia was recorded on GD 22. - One female of treatment group was found dead on GD 10 without showing any clinical findings which could explain the premature death. **Female reproduction and delivery data:** - Female mating index calculated after mating was 96% in both groups. - Female fertility index was 100% both in the control and in	Study report, 2014 reported from ECHA Dissemination (2021) Study: 002, key Study reported in detail in Annex I

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reliability: 1		treatment group. - Mean number of implantation sites was comparable between test substance-treated group and control (11.6 and 11.5 implants/dam in control and treatment group, respectively). Furthermore, there were no indications for test substance-induced intrauterine embryo-/fetolethality since postimplantation loss did not show any statistically significant differences (6.8% and 5.7% in control and treatment group, respectively), and the mean number of pups delivered per dam remained unaffected (10.8 and 10.9 pups/dam in control and treatment group). - Mean duration of gestation was statistically significantly prolonged in the treatment group (22.4 days ($p \leq 0.01$) vs. 22.0 days in control). - A significantly lower number of pregnant test substance-treated females (19 ($p \leq 0.05$)) had liveborn pups, in comparison to 24 pregnant females in the control. This resulted in a lower gestation index in the treatment group (79.2% in treatment group vs. 100% in the control). **Male reproduction data:** - Male mating index was 96% both in the control and treatment group. - Male fertility index was 96% in both groups. Effects on pups are reported in section 10.10.4.	

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

Two reliable studies investigating reproductive toxicity of α,α'-propylenedinitrilodi-o-cresol are available. Both studies were performed with test substance of very high purity and oral application in polyethylene glycol 400.

The first study is a screening study for reproductive/developmental toxicity performed according to OECD TG 422 under GLP conditions (reliability 1). In this study 10 Wistar rats (Crl:WI(Han)) per sex and dose group were exposed via gavage to 0, 25, 75, or 250 mg/kg bw/d. The doses were based on a 14-day dose-range finding study with 0, 300, or 800 mg/kg bw/d (Study report, 2012 reported from ECHA Dissemination (2021), repeated dose toxicity oral, study 002): At 800 mg/kg bw/d clinical signs like severe clonic spasms, muscle twitching or gasping were observed. Four of eight animals were found dead between exposure days 2-4. At necropsy, isolated to many reddish or dark-red foci were noted in the stomach of all animals. Treatment with 800 mg/kg bw/d was stopped after a maximum of 4 days, due to the high toxicity. Due to slight signs of toxicity and irritating effects in the forestomach at 300 mg/kg bw/d, 250 mg/kg bw was selected as highest dose for the reproductive/developmental screening study. Male animals were exposed for 29 days (i.e., 2 weeks prior to mating, during mating, and up to termination). Females were exposed for 42-45 days (i.e., 2 weeks prior to mating, during mating, gestation, and up to LD 4).

No substance-specific clinical signs of toxicity were noted during the observation period. Food consumption was within the normal range in all dose groups. In male animals, body weights and body weight gains were

statistically significantly lower at 75 and 250 mg/kg bw/d on day 8 of the pre-mating period and on mating days 1, 8 and 15. However, the differences to controls were only slight, and values remained within the range considered normal for rats of this age and strain (normal range: 5-95% confidence interval for body weight gain on mating day 15: 11-30%). Therefore, the study authors considered these differences not to be toxicologically relevant. Body weight of females was not affected (see Annex I for details).

Two females in the highest dose group had a total litter loss on lactation day 1 (presumably this is PND 0), resulting in a gestation index of 77.8% for this group compared to 100% for the other groups. The reason for the total litter loss could not be established as part of the study. Based on the reported data there was no evidence of poor condition in these two females, and examination of the reproductive organs of the animals revealed no abnormalities.

No treatment-related toxicologically significant changes were noted in any of the remaining reproductive parameters investigated in this study (i.e., mating, fertility and conception indices, precoital time, and numbers of corpora lutea and implantation sites), for details see Annex I. However, there was a trend towards a slightly lower number of corpora lutea and implantation sites at 250 mg/kg bw/d. This was mainly attributable to two females that had 7 corpora lutea each and 5 and 7 implantation sites, respectively. Since lower numbers were also seen for a control female (8 corpora lutea and 8 implantation sites), this finding was considered of no toxicological relevance.

No signs of difficult or prolonged parturition were noted among the pregnant females. Examination of cage debris of pregnant females revealed no signs of abortion or premature birth. No deficiencies in maternal care were observed

Spermatogenesis was not impaired.

In the second study reported for this endpoint (screening for reproductive/developmental toxicity, similar to OECD TG 421, only one dose level tested, under GLP conditions, reliability 1) 25 Wistar rats (Crl:WI(Han))/sex and dose group were exposed via gavage to 0 or 250 mg/kg bw/d. This single dose was selected to further investigate results from the OECD TG 422 study reported above. Male rats were exposed two weeks prior to mating, during mating, and about three weeks postmating, female rats for two weeks prior to mating, during mating, gestation until LD day 4.

In the exposure group mean body weights of males and females were generally comparable to the control group during the entire study period. The body weight gain of the treated males was statistically significantly reduced during pre-mating days 0-7 and postmating days 14-20. Mean body weight change of the treated females was statistically significantly reduced during GD 0-14 (up to 20% below the concurrent control).

The mean duration of gestation was statistically significantly prolonged in the treatment group (22.4 days vs. 22.0 days in control ($p \leq 0.01$)). Three females of the treatment group died during the parturition process on GD 23. Two of them showed adverse clinical findings preceding their death: Female 1 showed apathy (GD 22-23), piloerection and a reddish, brown vaginal discharge (GD 23, respectively), Female 2 showed apathy on GD 22. Pups of these females were not included in the subsequent evaluation. For one female of the treatment group dystocia was recorded on GD 22. However, this animal delivered healthy pups.

Furthermore, one female of the treatment group was found dead on GD 10 without showing any clinical findings which could explain the premature death. In addition, one control and two test substance-treated females had a complete litter loss on PND 0.

No effects were observed on mating index (96% in both groups) and fertility index (100% both groups). Implantation was not affected by the treatment since the mean number of implantation sites was comparable between the test substance-treated group and the control, taking normal biological variation into account (11.6 and 11.5 implants/dam in control and treatment group, respectively). Furthermore, there were no indications for test substance-induced intrauterine embryo-/fetolethality since the postimplantation loss did not show any statistically significant differences between the groups (6.8% and 5.7% in control and treatment group, respectively), and the mean number of pups delivered per dam remained unaffected (10.8 and 10.9 pups/dam in control and treatment group).

Overall, a significantly lower number of pregnant test substance-treated females (19 ($p \leq 0.05$)) had liveborn pups, in comparison to 24 pregnant females in the control group. This resulted in a lower gestation index in the treatment group (79.2% in treatment group vs. 100% in the control).

Male fertility was not impaired; the male mating index was 96% both in the control and treatment group, the male fertility index was 96% in both groups.

10.10.3 Comparison with the CLP criteria

For potential classification with regard to adverse effects on sexual function and fertility, criteria from CLP Regulation (EC, 2008)[2] in combination with explanations from the Guidance on the Application of the CLP criteria (ECHA, 2017) were applied. Any adverse effect of α,α'-propylenedinitrilodi-o-cresol 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. For potential classification of α,α'-propylenedinitrilodi-o-cresol, classification criteria were analysed accordingly:

Comparison with Category 1 criteria

- Known human reproductive toxicant (Cat 1A)

  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) (EC, 2008).

  There are no epidemiological data to support classification of α,α'-propylenedinitrilodi-o-cresol in Category 1A.

- Presumed human reproductive toxicant (Cat 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. (EC, 2008).

  In a modified screening study for reproductive/developmental toxicity similar to OECD TG 421 with one exposure and one control group containing 25 animals per sex respectively, death of three females during the parturition process on GD 23 was observed. The animals showed clinical sign like apathy, piloerection and a reddish, brown vaginal discharge preceding their death. For an additional female in the treatment group dystocia was recorded on GD 22. This animal survived the parturition process and delivered healthy pups. In addition, the mean duration of gestation was statistically significantly prolonged in the treatment group (22.4 days vs. 22.0 days in control). Data reported for this study do not provide an indication of general toxicity at the dose administered (250 mg/kg bw/d). The mean body weights were comparable between control and exposure group.

  Both effects (death during parturition and dystocia) were not seen in the screening study according to OECD TG 422 which also applied 250 mg/kg bw/d as the highest of three dosages. However, it has to be noted that in this study only 10 animals per sex and dose group (as required by the OECD guideline) were used. Based on the lower number of animals per dose group it is not unexpected that

[2] REGULATION (EC) No 1272/2008 considering all ATPs published until January 2021

effects with low incidences were not observed under this study design. The study did also not detect any indications of general toxicity at 250 mg/kg bw/d (no clinical signs, no effects on body weight, healthy animals at the end of exposure).

Taken together, the effects observed in the OECD TG 421 study ("death during parturition") are considered as relevant for classification and a classification in Category 1B is justified. This is supported by the observed dystocia and prolonged duration of gestation.

- 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.

  Although there were adverse effects observed in one reliable study only (modified screening study for reproductive/developmental toxicity similar to OECD TG 421, reliability 1) the severity of one of these effects ("death during parturition") is considered clear evidence of an adverse effect on sexual function and fertility and therefore relevant for classification. The evidence is sufficiently convincing to place the substance in Category 1B.

10.10.4 Adverse effects on development

Table 12: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Screening for reproductive / developmental toxicity according to OECD TG 422 GLP: yes Wistar rats 10 animals/sex /dose Reliability: 1	α,α'-propylenedinitrilodi-o-cresol purity: >99 corr. area % 0, 25, 75, 250 mg/kg bw/d via gavage Males: 29 days, i.e. 2 weeks prior to mating, during mating, and up to termination. Females: 42-45 days, i.e. 2 weeks prior to mating, during mating, gestation, and up to LD 4	**Effects on pups:** - The mean number of living pups at first litter check was significantly lower at 250 mg/kg bw/d (110, 102, 112, 58 living pups at 0, 25, 75, 250 mg/kg bw/d). - At the first litter check 15 dead pups were recorded for 4 litters in the high dose group compared to 1 pup/1 litter, 2 pups/1 litter and 0 pups for 0, 25 and 75 mg/kg bw/d, respectively. Two of these four females at 250 mg/kg bw/d had a total litter loss at first litter check with 11 and 1 dead pup, respectively. For details see Annex I. The gestation index ((Females with living pups on Day 1 / Pregnant females) * 100) in the highest dose group was therefore reduced (77.8% versus 100% in the other groups). - Incidental macroscopic findings of dead pups: beginning autolysis and/or no milk in the stomach. The only macroscopic finding in surviving pups was a missing tail apex for one animal in the control pup. - Incidental clinical symptoms of pups consisted of no milk in the stomach, missing tail apex, and wound and scabbing on the head. The nature and incidence of these clinical signs remained within the range considered normal for pups of this age, and they were therefore considered to be of no toxicological relevance.	Study report, 2013 reported from ECHA Dissemination (2021) Study: 001, key, reported in section "toxicity to reproduction" Study reported in detail in Annex I

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		For details see Annex I. - Body weights of pups were unaffected by treatment. All values remained within the normal range of biological variation. For details see Annex I. Effects on P0 generation are reported in section 10.10.1.	
Screening for reproductive / developmental toxicity (modified one-generation reproduction toxicity study) similar to OECD TG 421 and 416 only one dose level tested with additional parameters of an OECD 416 study GLP: yes Wistar rats 25 animals/sex /dose Reliability: 1	α,α'-propylenedinitrilodi-o-cresol purity: >99 corr. area % 0, 250 mg/kg bw/d via gavage Males: 2 weeks prior to mating, during mating, and about three weeks postmating Females: 2 weeks prior to mating, during mating, gestation, and up to LD 4.	**Effects on pups:** - The number of stillborn pups was increased in treatment group (34 stillborn vs. 5 in control) (indicated by reduced live birth index of 84.5% in treatment group, in comparison to 98.1% in control). - One test substance-treated dam had only stillborn pups (female no. 143 with 13 pups). For details see Annex I. - One control animal (no. 103) and one test-substance treated animal (no. 137) had a complete litter loss on PND 0 (including stillborn pups and pups that died during the first observation day). - Viability index indicating pup mortality during lactation (PND 0-4) was 88.0% ($p \leq 0.01$) in the treatment group and 95.3% in the control. A slightly higher number of decedents (cannibalized/dead pups) in the treatment group compared to the control (8 vs. 1) was observed. For details see Annex I. - Sex distribution and sex ratios of live pups on the day of birth and on PND 4 did not show significant differences between the control and the treatment group; slight differences were regarded to be spontaneous in nature. For details see Annex I. - Mean body weights of pups from test substance-treated dams were statistically significantly below the concurrent control values on PND 1 (-9%) and PND 4 (-7%). Three male and eight female runts were noted in the treatment group (definition runts: pups that weigh less than 75% of the mean weight of the respective control pups on PND 1). For details see Annex I. Effects on P0 generation are reported in section 10.10.1.	Study report, 2014 reported from ECHA Dissemination (2021) Study: 002, key reported in section "toxicity to reproduction" Study reported in detail in Annex I

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

The same two studies reported above in section 10.10.2 for effects on sexual function and fertility are also reported for effects on development. No additional studies for the endpoint developmental toxicity were identified.

In the first study (screening study for reproductive/developmental toxicity performed according to OECD TG 422 and under GLP conditions, reliability 1) with 10 Wistar rats (Crl:WI(Han)) per sex and dose group exposed via gavage to 0, 25, 75, or 250 mg/kg bw/d the mean number of living pups at first litter check was significantly lower at the highest dose group (110, 102, 112, 58 living pups at 0, 25, 75, 250 mg/kg bw/d,

respectively). The exact time of the first litter check is not given in the study report, presumably it was on PND 0; the study report does not use the term "stillborn". All pups that were born dead or died before the first litter check are added up to "dead pups". A total of 15 dead pups at first litter check were recorded for 4 litters in this dose group compared to 1 pup/1 litter, 2 pups/1 litter and 0 pups for 0, 25 and 75 mg/kg bw/d, respectively. Two of these four females at 250 mg/kg bw/d had a complete litter loss at first litter check with 11 and 1 dead pup, respectively. Based on these findings, the gestation index ((Females with living pups on Day 1 / Pregnant females) * 100) in the highest dose group was reduced (77.8% versus 100% in the other groups). As outlined in section 10.10.2 above the reason for the total litter loss could not be established as part of the study.

Incidental macroscopic findings of pups that were found dead included beginning autolysis and/or no milk in the stomach. The only macroscopic finding in surviving pups was a missing tail apex for one control pup. Incidental clinical symptoms of pups consisted of no milk in the stomach, missing tail apex, and wound and scabbing on the head. The nature and incidence of these clinical signs remained within the range considered normal for pups of this age, and they were therefore considered to be of no toxicological relevance. Body weights of pups were unaffected by treatment.

The second study is a one-generation reproductive toxicity study, similar to OECD TG 421, where only one dose level was tested (GLP conditions, reliability 1). 25 Wistar rats (Crl:WI(Han)) per sex and dose group were exposed via gavage to 0 or 250 mg/kg bw/d. This single dose was selected to further investigate results from the 422-study reported above.

In contrast to the study described above performed according to OECD TG 422, the study report of the 421 study uses the term "stillborn" for all pups found dead at first litter check. First examination of pups was done as soon as possible after birth. Pups which died before this examination were considered as stillborn. This approach implies that pups which were born alive but died within the time between birth and first examination are wrongly considered as stillborn.

The number of stillborn pups (as defined above) was increased in the treatment group (34 stillborn vs. 5 in control). This was also seen in the reduced live birth index ((number of liveborn pups at birth/total number of pups born) x 100) of 84.5% in the treatment group compared to 98.1% in the control, for details see Annex I.

Pups from dams who died during parturition (for details see section 10.10.2) were excluded from any evaluation. One animal showing dystocia delivered healthy normal-weight pups.

One test substance-treated dam (no. 143) had only stillborn pups in its litter (13 pups). One control animal (no. 103) and one test-substance treated animal (no. 137) had a complete litter loss on PND 0 (including stillborn pups and pups that died during the first observation day). The viability index (number of live pups on day 4 after birth/ number of live pups on the day of birth) x 100) indicating pup mortality during lactation (PND 0-4) varied between 88.0% ($p \leq 0.01$) in the treatment group and 95.3% in the control. A slightly higher number of decedents (cannibalized/dead pups) in the treatment group compared to the control group (8 vs. 1) was observed. For details see Annex I.

The sex distribution and sex ratios of live pups on the day of birth and on PND 4 did not show significant differences between the control and the treatment group. Mean body weights of the test substance-treated male and female pups were statistically significantly below the concurrent control values on PND 1 (-9%) and on PND 4 (-7%). Three male and eight female runts were noted in the treatment group (definition runts: pups that weigh less than 75% of the mean weight of the respective control pups on PND 1).

10.10.6 Comparison with the CLP criteria

For potential classification of adverse effects on development, criteria from the CLP Regulation (EC, 2008) supported by explanations from the Guidance on the Application of the CLP criteria (ECHA, 2017) were applied. The manifestations of developmental toxicity (death of developing organism and altered growth) were considered. For potential classification of α,α'-propylenedinitrilodi-o-cresol, classification criteria were analysed accordingly.

Comparison with Category 1 criteria

- Known human reproductive toxicant (Cat 1A)

  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 (EC, 2008).

  There are no epidemiological data to support classification of glycerol formal in Category 1A.

- Presumed human reproductive toxicant (Cat 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. (EC, 2008)

  …The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency. (EC, 2008)

  Clear evidence of effects on development ("death of developing organism") were observed in rats in two reliable studies performed according or similar to OECD test guidelines 422 and 421. At 250 mg/kg bw/d statistically significant number of pups were born dead or died shortly after birth. In addition, mean body weight of pups in the OECD TG 421 study was statistically significantly below the concurrent control values on PND 1 (-9%) and PND 4 (-7%), and runts were observed. The effects were observed in the absence of maternal toxicity.

  A classification in Category 1B is justified based on the observed 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 […] 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.

  Adverse effects on development were observed in rats in two reliable studies (both reliability 1). The severity of this effect ("death of the developing organism") is considered clear evidence of an adverse effect on development and therefore relevant for classification. The evidence is sufficiently convincing to place the substance in Category 1B.

10.10.7 Adverse effects on or via lactation

No human or animal studies are available.

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

No human or animal studies are available.

10.10.9 Comparison with the CLP criteria

For potential classification on adverse effects via lactation, criteria from CLP Regulation (EC, 2008) were applied. For potential classification of α,α'-propylenedinitrilodi-o-cresol, 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

  No human data available are available.

  (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 one or two generation studies are available which could show adverse effects in the offspring due to transfer in the milk. In addition, no toxicokinetic studies are available which could indicate that the substance in present in potentially toxic levels in breast milk.

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

10.10.10 Conclusion on classification and labelling for reproductive toxicity

a) <u>Sexual function and fertility</u>

Results from a reliable study indicate adverse effects on parturition.

Therefore, a classification for effects on sexual function and fertility (Cat. 1B, H360F) is warranted for α,α'-propylenedinitrilodi-o-cresol.

"*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).

Effects on parturition that are the basis for the classification for fertility effects were only observed in a study performed with one dose group (250 mg/kg bw/d, for details see above). Therefore, specific concentration limits based on an ED_{10} cannot be calculated. However, the generic concentration limit 0.3% ("group 2", medium potency) can be applied for the following reasons:

- At 250 mg/kg bw/d effects on parturition were observed in 3/25 animals. If dystocia is also considered 4/25 animals are affected. Expressed in percent this is 12 or 16 percent, respectively.
- Based on the data observed, effects below 4 mg/kg bw/d are not likely.
- The ED_{10} will with high certainty be located somewhere between 4 and 250 mg/kg bw/d. Therefore, the substance is assigned to "group 2", medium potency and the generic concentration limit should be applied.

b) <u>Developmental toxicity</u>

Results from reliable studies on developmental toxicity indicate adverse effects on the development of the offspring independent of maternal toxicity.

Therefore, a classification for effects on development of the offspring (Cat. 1B, H360D) is warranted for α,α'-propylenedinitrilodi-o-cresol.

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

- Significant effects ("death of pups") were observed at 250 mg/kg bw/d in two reliable studies.
- These effects were not observed at 0, 25 or 75 mg/kg bw/d. Based on this observation, effects below 4 mg/kg bw/d are not likely.
- Again, the ED_{10} will with high certainty be located somewhere between 4 and 250 mg/kg bw/d. Therefore, the substance is assigned to "group 2", medium potency and the generic concentration limit should be applied.

c) Effects via lactation

In the absence of any studies indicating effects via lactation **no classification for effects via lactation is warranted for α,α'-propylenedinitrilodi-o-cresol.**

RAC evaluation of reproductive toxicity

ADVERSE EFFECTS ON SEXUAL FUNCTION AND FERTILITY

Summary of the Dossier Submitter's proposal

The DS assessed the following *in vivo* animal studies for adverse effects on sexual function and fertility (cf. Table 11 of the CLH report and information regarding study design in Annex I to the CLH report):

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Results	Reliability	Reference
Screening study for reproductive/developmental toxicity GLP: yes according to OECD TG 422	α,α'-propylenedinitrilodi-*o*-cresol Purity: > 99 corr. area % Vehicle: polyethylene	Male and female Wistar rats (Crl:WI(Han)) 10 animals/sex/dose	**General toxicity in P0 generation**: No clinical signs of toxicity Mortality: 1 female at mid and top dose on day 9 of pre-mating period due to **gavage**	1	Study: 001, key study Study report, 2013 reported from ECHA Disseminati

	glycol (PEG)	Oral daily administration via gavage **Dosage**: 25, 75 and 250 mg/kg bw/d **Exposure period**: Males: exposed for 29 days (from 2 weeks prior to mating until termination) Females: exposed for 42-45 days (from 2 weeks prior to mating until lactation day 4) **Control animals**: yes, concurrent vehicle **No historical control** data provided **Reproductive indices assessed**: mating index (%), fertility index (%), conception index (%), gestation index (%), duration of gestation	**accident** ↓Body weights and ↓body weight gains in males (75 and 250 mg/kg bw/d) on day 8 of the pre-mating period, during the mating period (mating days 1, 8, and 15). ↓ thymus and organ weights (absolute and relative to body weight) (250 mg/kg bw/d) (both sexes) compared to controls, statistically significant only for females. **Sexual function and fertility:** ↓ corpora lutea (12.6, 14, 14.1 and 11.1 at 0, 25, 75 and 250 mg/kg bw/d, respectively) and implantation sites at top dose (11.4, 12, 12.7 and 10 at 0, 25, 75 and 250 mg/kg bw/d, respectively), no toxicological relevance. **Developmental toxicity:** Total litter loss on LD 1 in 2 females (250 mg/kg bw/d) - euthanised after.		on (2021)

			Gestation index (due to total litter loss in 2 dams): 77.8% at the top dose compared to 100% for the remaining groups. ↓ living pups at first litter check at 250 mg/kg bw/d (110, 102, 112, 58 at 0, 25, 75 and 250 mg/kg bw/d) Dead pups at first litter check (litters affected): 1/10, 1/10, 0/9 and 4/9 at 0, 25, 75 and 250 mg/kg bw/d, respectively ↑ dead pups at first litter check in 250 mg/kg bw/d (1, 2, 0, 15 at 0, 25, 75 and 250 mg/kg bw/d, respectively). Excluding the dams with total litter loss, it was a ↓ mean live litter size at 250 mg/kg bw/d as compared with the others (11.0, 10.2, 12.4 and 8.3 for 0, 25, 75 and 250 mg/kg bw/d, respectively). Incidental clinical symptoms in pups: no milk in the stomach, missing tail apex, and wound and scabbing on the		

			head (within the range considered normal for pups of this age → no toxicological relevance). Incidental macroscopic findings of pups that were found dead: beginning autolysis and/or no milk in the stomach.		
Screening study for reproductive / developmental toxicity GLP: yes similar to OECD TG 421	α,α'-propylenedinitrilodi-*o*-cresol purity: > 99 corr. area % **Vehicle**: PEG 400	Male and female Wistar rats (Crl:WI(Han) 25 animals/sex /dose Oral daily administration via gavage **Dosage**: 0 and 250 mg/kg bw/d **Exposure period**: a 2-week pre-mating and mating period in both sexes, about three weeks post-mating in males, and the entire gestation period as well as approximately 4 days of the lactation period in females with	**General toxicity in P0 generation**: Mortality: 1 female in the 250 mg/kg bw/d group found dead on GD 10 without showing any clinical findings which could explain the premature death. **Sexual function and fertility:** ↑ mean duration of gestation in 250 mg/kg bw/d group (22.4** [p≤0.01] days vs. 22.0 days in control). 4 P0 females at 250 mg/kg bw/d died during the gestation period: 3 females were unable to deliver and were found dead on GD 23 (1 female	1	Study: 002, key study Study report, 2014 reported from ECHA Dissemination (2021)

		litters, and about 3 weeks of post-mating period in non-pregnant females. **Control animals**: yes, concurrent vehicle **No historical control** data provided **Reproductive indices assessed**: mating index (%) (male and female), fertility index (%) (male and female), gestation index (%) (female), live birth index (female), post-implantation loss (female)	found dead on GD 10 without showing any clinical findings which could explain the premature death). Clinical findings preceding death of 2 females that were unable to deliver: Female 1 showed apathy (GD 22-23), piloerection and a reddish, brown vaginal discharge (GD 23, respectively) Female 2 showed apathy on GD 22 One additional female at 250 mg/kg bw showed dystocia on GD 22. **Male sexual function and fertility:** No effects. **Developmental toxicity:** One dam in 250 mg/kg bw/d group that delivered had only stillborn pups. One control animal (no. 103) and one 250 mg/kg bw/d animal (no. 137) had a complete litter loss on PND 0 (including stillborn pups and pups that died during the first observation		

			day). ↓number of pregnant females at 250 mg/kg bw (19*/24 [p≤0.05]) with liveborn pups, in comparison to controls (24/24), ↓ gestation index in the 250 mg/kg bw/d group (79.2% vs. 100% in the control). This is due to both effects on sexual function and fertility (3 dams that were unable to deliver) and developmental effects (1 dam that delivered only stillborn pups). ↓ live birth index at 250 mg/kg bw/d (84.5%) compared to control (98.1%) ↑ number of stillborn pups at 250 mg/kg bw/d (34/219 stillborn vs. 5/259 in control). ↓ viability index indicating pup mortality during lactation (PND 0-4) 88.0% (250 mg/kg bw/d) vs. 95.3% (control) ↑ number of decedents (cannibalised/dead pups) (8 in 250 mg/kg bw/d group vs.		

			1 in control) ↓mean body weights of pups in 250 mg/kg bw/d group compared to control (PND 1 (-9%) and PND 4 (-7%)). 3 males and 8 female runts (250 mg/kg bw/d) (definition of runts: pups that weigh less than 75% of the mean weight of the respective control pups on PND 1).		

LD = lactation day; GD = gestation days; PND = postnatal day

There are two studies according to / similar to the OECD test guidelines that investigated the reproductive toxicity of α,α'-propylenedinitrilodi-*o*-cresol of high purity after oral administration in PEG400, performed under GLP conditions.

In a screening study for reproductive/developmental toxicity according to OECD TG 422 with a reliability of 1, 10 Wistar rats/sex /dose were exposed to 0, 25, 75 and 250 mg/kg bw/d α,α'-propylenedinitrilodi-*o*-cresol. Males were exposed for 29 days (2 weeks prior to mating, during mating, and up to termination) and females were exposed for 42-45 days (2 weeks prior to mating, during mating, gestation, and up to LD 4). The doses were selected based on the results of a 14-day dose-range finding study in which 800 mg/kg bw/d produced clinical signs of severe clonic spasms, muscle twitching or gasping and four animals out of eight were found dead after 2-4 days of exposure. The dose of 250 mg/kg bw/d was selected as the highest dose for the reproductive/developmental screening study as at 300 mg/kg bw/d slight signs of toxicity and irritating effects in the forestomach were observed. In this study, no substance-induced clinical signs of toxicity or changes in food consumption were observed at any dose. A statistically significant decrease in body weight and body weight gain in males was identified at 75 and 250 mg/kg bw/d on day 8 of the pre-mating period and on mating days 1, 8 and 15. However, the differences from controls were only slight, and values remained within the range considered normal variation for rats of this age and strain (normal variation range with 5-95% confidence interval for body weight gain from the beginning of the exposure till mating day 15: 11-30%). However, no historical control data were available. No treatment-related changes were observed in the body weight of females.

In the high-dose group, two out of nine pregnant females had a total litter loss on lactation day 1, resulting in a gestation index of 77.8% for this group compared to 100% for the other groups (assessed under adverse effects on development). No signs of poor maternal condition in these animals were revealed and no abnormalities in the reproductive organs were found.

There were no treatment-related effects on sexual function and fertility as regards mating, fertility and conception indices, precoital time, and the number of corpora lutea and

implantation sites. The number of corpora lutea and implantation sites were slightly decreased at 250 mg/kg bw/d. This was considered to be related to two females that had 7 corpora lutea each and 5 and 7 implantation sites, respectively. As lower numbers were also seen in a control female (8 corpora lutea and 8 implantation sites), these were considered not toxicologically relevant effects. There was no sign of difficult or prolonged parturition or abortion/premature birth in any of the groups. No deficiency in maternal care was observed. In males, no spermatogenesis impairments were detected.

The second screening study for reproductive/developmental toxicity was similar to OECD TG 421, under GLP conditions with a reliability of 1 using only one dose level (250 mg/kg bw/d) in addition to controls. There were 25 animals (Wistar rats)/sex/dose and the duration of the exposure was two weeks prior to mating, during mating, and about three weeks post-mating for males and two weeks prior to mating, during mating, gestation until LD day 4 for females.

No changes in the mean body weight in either males or females were observed. The body weight gain of the treated males was statistically significantly reduced during pre-mating days 0-7 and post-mating days 14-20. The mean body weight gain of the treated females was statistically significantly reduced during GD 0-14 (up to 20% below the concurrent control).

One dam in the treatment group was found dead on GD 10 without any clinical signs that could explain the premature death.

As regards the effects on sexual function and fertility, in the treatment group, the mean duration of gestation was statistically significantly increased (22.4 days vs. 22.0 days in the control, $p \leq 0.01$). Three females in the treatment group died during the parturition process on GD 23 and they were unable to deliver. Two of them showed adverse clinical findings preceding their death consisting of apathy in one dam (GD 22-23) and piloerection and a reddish, brown vaginal discharge in the other dam (GD 23). One female in the treatment group showed dystocia on GD 22 but survived and delivered healthy pups.

No effects were observed on the female mating index (96% in both groups) and female fertility index (100% in both groups). No effect was observed on the male mating index (96% in both the control and treatment group) and on the male fertility index (96% in both the control and treatment group).

Number of implantation sites was not affected by the treatment (11.6 and 11.5 implants/dam in the control and treatment group, respectively).

As regards developmental toxicity, there were no indications for test substance-induced intrauterine embryo-/foetolethality since the post-implantation loss did not show any statistically significant differences between the groups (6.8% and 5.7% in the control and treatment group, respectively), and the mean number of pups delivered per dam remained unaffected (10.8 and 10.9 pups/dam in control and treatment group). However, one test substance-treated dam had only stillborn pups and there were 6 dams with stillborn pups as compared to 3 dams in the control. 34/219 pups delivered (15.5%) were stillborn in the treatment group as compared to 5/259 (1.9%) in the control group.

The observed significant decrease in the number of females with liveborn pups in the treatment group (19/24 pregnant dams ($p \leq 0.05$)) compared with the control group (24/24 pregnant dams) determined a lower gestational index in the treatment group (79.2% in the treatment group vs. 100% in the control). This is due to both effects on sexual function and fertility (3 dams that were unable to deliver) and developmental effects (1 dam that delivered

only stillborn pups).

Comments received during consultation

Three comments were received during the consultation, two from MSCAs and one from Company/Manufacturer and all supported the DS proposal of classifying α,α'-propylenedinitrilodi-*o*-cresol as a reproductive toxicant in Category 1B and with the generic concentration limit (GCL) of 0.3% for adverse effects on sexual function and fertility. One MSCA commented that the reproducible reduction in gestation index was demonstrated in two GLP-compliant studies (-22.2 % and -20.8 %, in the two respective studies vs. controls) with α,α'-propylenedinitrilodi-*o*-cresol, tested under the limit concentration. While in the first study the cause of the complete litter loss was not clear, in the follow-up study with a single dose of 250 mg/kg bw/d of α,α'-propylenedinitrilodi-*o*-cresol, 16 % of females (4/25) failed to deliver live-born pups, 3 of them dying during parturition and were unable to deliver. Uncertainty remains as to whether poor foetal conditions could also have an influence on parturition complications. However, this is argued against by the fact that one dam survived with a complete litter loss. One more female died on GD 10 with no apparent signs of general toxicity. One more female experienced dystocia, but delivered live/healthy pups. No apparent signs of either avert or general toxicity were observed for the females treated with 250 mg/kg bw/d of the test substance (n=35 in both studies combined) that could explain the profound effects on pregnancy outcome.

Assessment and comparison with the classification criteria

According to the CLP Regulation Annex I, section 3.7.1.3., adverse effects on sexual function and fertility include, but is not limited to, alterations to the female and male reproductive system, adverse effects on the onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

In this case, there are no human epidemiological data to support the classification of α,α'-propylenedinitrilodi-*o*-cresol in Category 1A for sexual function and fertility.

There is one modified study for reproductive/developmental toxicity similar to OECD TG 421 with one dose of 250 mg/kg bw/d and one control group, 25 animals/sex/group done under GLP conditions in which 1 female did not get pregnant in both groups and three females of the treatment group died during parturition (GD 23), one of them showing clinical signs as apathy and another presenting piloerection and a reddish, brown vaginal discharge preceding their death. One female in the treatment group presented dystocia on GD 22 but survived and delivered healthy pups. A significant decrease in the number of females with liveborn pups in the treatment group (19/24 pregnant dams ($p \leq 0.05$)) compared with the control group (24/24 pregnant dams) determined a lower gestational index in the treatment group (79.2% in the treatment group vs. 100% in the control). This is due to both effects on sexual function and fertility (3 dams that were unable to deliver) and developmental effects (1 dam that delivered only stillborn pups). Also, a statistically significant increase in the mean duration of gestation was observed in the treatment group compared with the control group (22.4 days vs. 22.00 in the control, $p \leq 0.01$). There was no sign of general toxicity as the mean body weight was

comparable between the control and exposure group. One treated female died on GD 10 without any sign that could explain the premature death.

Death during parturition and dystocia were not observed in the screening test performed according to OECD TG 422 in which the highest dose was also 250 mg/kg be/d, but it is emphasised that there were only 10 animals/sex/group in accordance with the test guideline compared to 25 animals/sex/group in the positive study so it can be expected that the effects with low incidence would not necessarily be observed. In this study also there were no clinical signs of general toxicity or effects on the body weight in any of the groups.

Taking into consideration the inability of dams to deliver (causing death during parturition), dystocia, the statistically significant increase in the mean duration of gestation and a lower gestational index, reflecting largely maternal death due to inability to deliver in the treatment group, RAC concludes that there is enough evidence for classification in Category 1B for adverse effects on sexual function and fertility.

The severity of the effects (dystocia and death during parturition due to inability to deliver) observed in one reliable study only is considered as clear evidence of adverse effects on sexual function and fertility and to justify classification in Category 1B.

Regarding the setting of a specific concentration limit, , the adverse effects on parturition that justified the classification of α,α'-propylenedinitrilodi-*o*-cresol in Category 1B were observed in only one study that tested only one dose, 250 mg/kg bw/d. In this case, no specific concentration limit based on ED_{10} can be calculated. The GCL of 0.3% ("group 2", medium potency) can be applied based on the following reasons:

- The effects on parturition (dystocia and inability to deliver) were observed at 250 mg/kg bw/d in 4/24 pregnant animals (17%).

- Based on the data obtained from this study, effects below 4 mg/kg bw/d are not likely, indicating that the ED_{10} should be somewhere between 4 and 250 mg/kg bw/d. This sets α,α'-propylenedinitrilodi-*o*-cresol in "group 2" (medium potency), and thus the GCL should be applied.

In view of this, RAC agrees with DS proposal to **classify α,α'-propylenedinitrilodi-*o*-cresol as Repr. 1B; H360F** based on the effects observed on sexual function and fertility and **apply the GCL of 0.3% (group 2 – medium potency).**

ADVERSE EFFECTS ON DEVELOPMENT

Summary of the Dossier Submitter's proposal

The DS reported and assessed two GLP-compliant *in vivo* screening animal studies for reproductive/developmental toxicity, one according to OECD TG 422 and the other similar to OECD TG 421 (cf. Table 12 of the CLH report and information regarding study design in Annex I). The same two studies were used to evaluate adverse effects on sexual function and fertility of α,α'-propylenedinitrilodi-*o*-cresol and are summarised in the table under sexual function and fertility.

In the first screening study according to OECD TG 422 (reliability 1 and GLP), 10 Wistar rats/sex/dose were exposed to 0, 25, 75 and 250 mg/kg bw/d α,α'-propylenedinitrilodi-*o*-cresol. A significant decrease in the number of live pups was observed in the first litter check at 250 mg/kg bw/d (110, 102, 112, 58 at 0, 25, 75 and 250 mg/kg bw/d, respectively). The authors of the study specified that the time of the first litter check was day 1 of lactation, so it is presumed that the first check was performed on PND 0 and then daily. They did not differentiate between the pups born dead and the ones that died before the first litter check, and both categories are included in the "dead pups". There was an increase in the number of dead pups at the first litter check in the highest dose group: 15 dead pups as compared to 1, 2, and 0 dead pups at 0, 25 and 75 mg/kg bw/d, respectively. There were 4 litters affected at 250 mg/kg bw/d, 2 dams having a total litter loss at the first litter check, leading to a decrease in the gestational index (77.8%) in the 250 mg/kg bw/d group as compared to the other groups (100%). The reason for the total litter loss was not established by the authors. However, even if excluding the dams with total litter loss, there was a decrease in the mean live litter size at 250 mg/kg bw/d as compared with other groups (11.0; 10.2; 12.4 and 8.3 for 0; 25; 75 and 250 mg/kg bw/d, respectively). Incidental macroscopic findings of pups that were found dead included beginning autolysis and/or no milk in the stomach. The only macroscopic finding among surviving pups was a missing tail apex for one control pup and wound and scabbing on the head that was in the range considered normal variation and of no toxicological relevance. No changes in the pup's body weight were observed.

In the second screening study similar to OECD TG 421 (reliability 1, GLP) performed on 25 animals (Wistar rats)/sex/dose, only one dose of 250 mg/kg bw/d was tested. In this study, the term stillborn is used to define the pups found dead at the first litter check and the first check was performed as soon as possible after birth. Thus, there is the possibility that a pup that was born alive and died within the time between birth and first examination is wrongly called stillborn. However, from a classification perspective there is no difference if the pup died prenatally or on LD 0, both are developmental effects. In this study, a significant increase in the number of stillborn pups in the treatment group compared to the control (34 vs. 5) and a significant reduction in the live birth index (84.5% in the treatment group vs. 98.1% in the control group) were observed. In this analysis the offspring of dams that died during parturition were not considered. One test substance-treated dam had only stillborn pups in its litter and one further animal in this group had complete litter loss on PND 0 (including stillborn pups and pups that died during PND 0). One control animal also had complete litter loss on PND 0 (including stillborn pups and pups that died during PND 0). In 250 mg/kg bw/d group the viability index indicating pup mortality during lactation (PND 0-4) was significantly decreased compared with the control group (88.0% vs. 95.3%). In the 250 mg/kg bw/d group there was a higher number of decedents (cannibalised/dead pups) compared to the control (8 vs. 1). No difference was seen in the sex distribution and sex ratio of live pups on the day of birth and PND 4. Mean body weights of pups from test substance-treated dams were statistically significantly below the concurrent control values on PND 1 (-9%) and PND 4 (-7%). Three male and eight female runts were noted in the treatment group (definition of runts: pups that weigh less than 75% of the mean weight of the respective control pups on PND 1.

Comments received during consultation

Three comments were received during the consultation, two from MSCAs and one from Company/Manufacturer and all supported the DS proposal of classifying α,α'-

propylenedinitrilodi-*o*-cresol in Category 1B and with the GCL of 0.3% for adverse effects on development.

Assessment and comparison with the classification criteria

In the CLP Regulation Annex I, section 3.7.1.4, a developmental toxicant is defined as any substance that have the potential to interfere 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. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

In this case, there are no human epidemiological data to support the classification of α,α'-propylenedinitrilodi-*o*-cresol in Category 1A for development.

Both reliable studies (reliability 1, GLP), one performed according to OECD TG 422 and the other similar to OECD TG 421, showed that 250 mg/kg bw/d α,α'-propylenedinitrilodi-*o*-cresol induced a significant increase in the number of pups born dead or that died shortly after birth. Moreover, the study similar to OECD TG 421 revealed that the mean pup body weight in the treatment group was significantly below the concurrent control values on PND 1 (-9%) and PND 4 (-7%) and also an increase in the number of runts was observed in this group in the absence of maternal toxicity. Based on this evidence, classification in Category 1B for development is justified.

For development toxicity, the GCL of 0.3% ("group 2", medium potency) can be applied based on the following reasons:

- Two reliable studies showed evidence of significant developmental effects ("death of pups") at 250 mg/kg bw/d

- No effects were observed at 0, 25 or 75 mg/kg bw/d, so no effects below 4 mg/kg bw/d are likely to occur, which indicates that the ED_{10} is somewhere between 4 and 250 mg/kg bw/d. Thus, the substance is assigned to "group 2" of medium potency and the GCL should be applied according to CLP Regulation.

In the view of the available evidence, RAC agrees with DS proposal to **classify α,α'-propylenedinitrilodi-*o*-cresol as Repr. 1B; H360D** based on the effects observed on development and to **apply the GCL of 0.3%.**

Effects on or via lactation

In the absence of any studies indicating effects on or via lactation RAC agrees with the DS that no classification for effects on or via lactation is warranted for α,α'-propylenedinitrilodi-o-cresol.

10.11 Specific target organ toxicity-single exposure

Evaluation not performed for this substance.

10.12 Specific target organ toxicity-repeated exposure

Evaluation not performed for this substance.

10.13 Aspiration hazard

Evaluation not performed for this substance.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance.

12 ADDITIONAL LABELLING

Not relevant

13 ANNEXES

Please see separate documents for Annex I and confidential Annex I.

14 REFERENCES

EC, European Community (2008)

REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

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