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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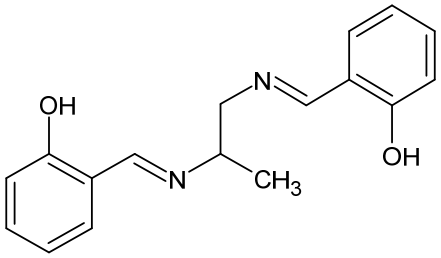
CONTENTS

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.

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

Property	Value	Reference	Comment (e.g. measured or estimated)
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 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) modified version of the traditional Ames test, i.e. the Ames II Assay (microtiter version). GLP: no Reliability:	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 protocol 0; 4; 20; 100; 500; 2500, 5000 µg/mL Triplicate plates per dose, control chemical or vehicle Positive controls: yes	→ 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
3 (screening test)				
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 experiments with two cultures each Positive controls: yes	→ 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 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	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
			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 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	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
			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 sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. Positive control: yes	→ 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. For details see Annex I.	Study report, 2013 reported from ECHA Dissemination (2021) Only study record available, key Study reported in detail in 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.

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.

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

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Classification as a 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 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	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
		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 Reliability: 1	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 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	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
		(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 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.

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

- 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. 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.	Study report, 2013 reported from ECHA Dissemination (2021) Study: 001, key, reported in section "toxicity to reproduction" Study reported in detail in Annex I
Screening for reproductive / developmental	α,α'-propylenedinitrilodi-o-cresol	**Effects on pups:** - The number of stillborn pups was increased in treatment group (34 stillborn vs. 5 in control) (indicated by reduced live birth	Study report, 2014 reported from ECHA

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
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	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.	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.	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) Sexual function and fertility

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) Developmental toxicity

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.**

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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ECHA, European Chemicals Agency (2017)

Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017

Helsinki, Finland. https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5

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