Committee for Risk Assessment

RAC

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

Adopted

16 March 2023

16 March 2023

CLH-O-0000007246-73-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **α,α'-propylenedinitrilodi-*o*-cresol**

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

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

The proposal was submitted by **The Netherlands** and received by RAC on **23 February 2022.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **11 April 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 June 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Anca Oana Docea**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 March 2023** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	α,α'-propylenedinitrilodi-*o*-cresol	202-374-2	94-91-7	Repr. 1B	H360FD	GHS08 Dgr	H360FD			
RAC opinion	TBD	α,α'-propylenedinitrilodi-*o*-cresol	202-374-2	94-91-7	Repr. 1B	H360FD	GHS08 Dgr	H360FD			
Resulting Annex VI entry if agreed by COM	TBD	α,α'-propylenedinitrilodi-*o*-cresol	202-374-2	94-91-7	Repr. 1B	H360FD	GHS08 Dgr	H360FD			

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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: yes	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; 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	**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 precipitation (± S9 mix) **Cytotoxicity (SPT)**: *Salmonella* strains: ≥ 100 µg/plate onward. *E. coli WP2uvrA*: observed ≥ 1000 µg/plate onward **Cytotoxicity (PIT)**:	1	Study: 001, key study Study report, 2012 reported from ECHA Dissemination (2021)

		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)	*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 test	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, 5000 µg/mL Number of plates: 3 per dose, control or vehicle	**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 Dissemination (2021)
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	**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	**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	1	Study: 002, key study Study report, 2012 reported from ECHA Dissemination (2021)

	Positive controls: yes	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; 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	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 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	**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 %)	1	Study: 004, supporting study Study report, 2012 reported from ECHA Dissemination (2021)

		structural chromosome aberrations 2 independent parallel cultures Evaluation of cytotoxicity: mitotic index Determination of polyploidy: yes Determination of endoreplication: yes	No effect in endomitotic metaphases **Cytotoxicity** (↓mitotic indices): ≥ 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 mg/kg bw **Tissues and cell types examined**: 2000 polychromatic erythrocytes	**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 (vehicle and positive)** were valid and within the historical control data.	1	Only study record available, key study Study report, 2013 reported from ECHA Dissemination (2021)

		(PCE)/animal were analysed for micronuclei. **Cytotoxicity**: the ratio between polychromatic and normochromatic erythrocytes and expressed in polychromatic erythrocytes per 2000 erythrocytes			

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 a 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.**

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 glycol (PEG)	Male and female Wistar rats (Crl:WI(Han)) 10 animals/sex/dose 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 (%),	**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 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	1	Study: 001, key study Study report, 2013 reported from ECHA Dissemination (2021)

		duration of gestation	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. 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 litters, and about 3 weeks of post-mating	**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	1	Study: 002, key study Study report, 2014 reported from ECHA Dissemination (2021)

		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)	unable to deliver and were found dead on GD 23 (1 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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).