Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

Sodium 3-(allyloxy)-2-hydroxypropanesulphonate

EC Number: 258-004-5
CAS Number: 52556-42-0

CLH-O-0000007154-78-01/F

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

Adopted

15 September 2022

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

sodium 3-(allyloxy)-2-hydroxypropanesulphonate

EC Number: 258-004-5

CAS Number: 52556-42-0

Index Number: NA

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Version number: 02 **Date: July 2021**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON [SODIUM 3-(ALLYLOXY)-2-HYDROXYPROPANESULPHONATE]

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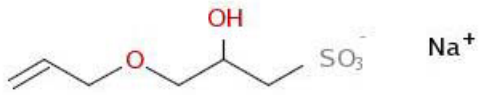
1 IDENTITY OF THE SUBSTANCE

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)	Sodium 3-(allyloxy)-2-hydroxypropanesulphonate
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	*none*
EC number (if available and appropriate)	258-004-5
EC name (if available and appropriate)	sodium 3-(allyloxy)-2-hydroxypropanesulphonate
CAS number (if available)	52556-42-0
Other identity code (if available)	/
Molecular formula	C6H12O5S.Na
Structural formula	
SMILES notation (if available)	/
Molecular weight or molecular weight range	218.203
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*[If the substance structure demonstrates stereo-isomerism the ratio of these stereo-isomers should be specified. If the ratio is unknown it should be stated as such. For optical isomers a measure of optical activity (specific rotation) should be specified.]*
Description of the manufacturing process and identity of the source (for UVCB substances only)	*[In the case of UVCB substance a full manufacturing process description should be provided including the identity of the source or starting materials and their ratio. Any relevant process parameters should also be specified.]*
Degree of purity (%) (if relevant for the entry in Annex VI)	> 80% w/w

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 3.1 (CLP)**	**Current self-classification and labelling (CLP)**

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Sodium 3-(allyloxy)-2-hydroxypropanesulphonate	>80 % (w/w)	No harmonised classification	Skin Irrit. 2 – H315 Eye. Dam. 1 – H318 Repro. 2 – H361 STOT SE 3 – H335

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

Confidential information

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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 harmonised classification										
Dossier submitters proposal	tbd	Sodium 3-(allyloxy)-2-hydroxypropanesulphonate	258-004-5	52556-42-0	Eye Dam. 1 Repr. 1B	H318 H360F	GHS08 Danger	H318 H360F			
Resulting Annex VI entry if agreed by RAC and COM	tbd	Sodium 3-(allyloxy)-2-hydroxypropanesulphonate	258-004-5	52556-42-0	Eye Dam. 1 Repr. 1B	H318 H360F	GHS08 Danger	H318 H360F			

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	data conclusive but not sufficient for classification	No
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases	hazard class not applicable	No
Gases under pressure	hazard class not applicable	No
Flammable liquids	hazard class not applicable	No
Flammable solids	data conclusive but not sufficient for classification	No
Self-reactive substances	data conclusive but not sufficient for classification	No
Pyrophoric liquids	hazard class not applicable	No
Pyrophoric solids	data conclusive but not sufficient for classification	No
Self-heating substances	data conclusive but not sufficient for classification	No
Substances which in contact with water emit flammable gases	data conclusive but not sufficient for classification	No
Oxidising liquids	hazard class not applicable	No
Oxidising solids	data conclusive but not sufficient for classification	No
Organic peroxides	hazard class not applicable	No
Corrosive to metals	Data lacking	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	**harmonised classification proposed: Eye Dam. 1 – H318**	Yes
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	**harmonised classification proposed: Repr. 1B – H360F**	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 for this substance.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Concerning classification for toxicity on reproduction:

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

Concerning classification for Eye damage:

Justification that action is needed at Community level is required.

There are differences in self-classification for this endpoint according to ECHA website (25 June 2020):

- Eye Dam 1 – H318: 6/53 self-classifications
- Eye Irrit. 2 – H315: 38/53 self-classifications
- Not classified for this endpoint: 9/53 self-classifications

5 IDENTIFIED USES

The substance is manufactured and/or imported in the European Economic area in 1000 – 10 000 tonnes per year. It is used in formulation or re-packing, at industrial sites and in manufacturing (ECHA, 2020). According to US-EPA, the substance is included in the following product or use categorisations: “manufacturing, chemical”, “consumer use”, “manufacturing, plastics”, “manufacturing, raw material”, “paint”, surface treatment”. Industrials uses consists in corrosion inhibitors and anti-scaling agent, intermediate and solid separation agent. Consumer uses consist in adhesives and sealants, paints and coatings, resin products and water treatment product (EPA dashboard, 2020; Pubchem, 2020).

6 DATA SOURCES

Data are issued from the registration dossier (public ECHA website, IUCLID ECHA and CSR) and from literature research (June 2020). Lead registrant was contacted in July 2020 in order to obtain the study reports for the endpoints concerned. Study reports for the BCOP study (Anonymous, 2012a), the 28-day study (Anonymous, 2007), the 90-day study (Anonymous, 2016b), the OECD 414 study (anonymous, 2017a) and the OECD 421 study (without the individual data) (Anonymous, 2013) were obtained between February and March 2021.

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid at 20°C and 101.3 kPa	ECHA (2020)	

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Property	Value	Reference	Comment (e.g. measured or estimated)
Melting/freezing point	No melting point or melting range of the test item sodium	ECHA (2020)	The substance decomposes (177°C) before melting
Boiling point	The key value cannot be determined	ECHA (2020)	The substance decomposes (177°C) before boiling
Relative density	1.5 at 20°C	ECHA (2020)	OECD 109 guideline study
Vapour pressure	0.0002 Pa at 20°C	ECHA (2020)	EU A.4 using the effusion method (weight loss).
Surface tension	71.31 mN/m at 20°C at 1 mg/L	ECHA (2020)	OECD 115 guideline study. Plate method
Water solubility	781.1g/L at 20°C	ECHA (2020)	OECD Guideline 105 Measured with HPLC-UV
Partition coefficient n-octanol/water	Log Kow (Log Pow): -1.51 at 25°C at pH of 0	ECHA (2020)	OECD 107 guideline study. Shake flask at 20°C and then quantification by ion chromatography.
Flash point	Not relevant	ECHA (2020)	Substance is a solid
Flammability	non flammable	ECHA (2020)	OECD 107 guideline study
Explosive properties	no chemical groups associated with explosive properties present in the molecule	ECHA (2020)	statement
Self-ignition temperature	The study has not to be conducted, for solids, if the substance has a melting point < 160°C. The test item decomposed at 177 °C before melting. Therefore it could be expected that sodium 3-(alloxy)-2-hydroxypropane-1-sulfonate is not self igniting and a testing is not necessary.	ECHA (2020)	statement
Oxidising properties	Based on structural considerations for sodium 3-(alloxy)-2-hydroxypropane-1-sulfonate, oxidizing properties are expected to be highly unlikely based on the chemical structure.	ECHA (2020)	statement
Granulometry	the study does not need to be conducted because the substance is marketed or used in a non solid or granular form: substance is usually used and sold in aqueous formulation	ECHA (2020)	statement
Stability in organic solvents and identity of relevant degradation products	No data	ECHA (2020)	Not critical
Dissociation constant	pKa at 20°C: 11.004	ECHA	OECD 112.

Property	Value	Reference	Comment (e.g. measured or estimated)
		(2020)	
Viscosity	Not relevant	ECHA (2020)	Substance is a solid

8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

There is no experimental toxicokinetics study available for the substance.

Information can be estimated from structure and physicochemical properties. The substance contains sulphonate group which is potentially ionisable. The molecular weight is 218.21 g/mol and the substance is soluble (moderate to high) in water (781.1 g/L). These properties are favourable for absorption. In contrast, the log Pow is not very favourable for absorption (-1.51). The substance has a low volatility with a vapour pressure of about $2x10^{-4}$ Pa.

According to the DK QSAR Toolbox (June 2020), the absorption from the gastrointestinal tract for 1 mg and 1000 mg doses is estimated at 5%. The skin absorption is estimated to be 0.00382 mg/cm^2/event. The log brain/blood partition coefficient is -1.3463. It is not estimated to be a substrate of CYP2C9 and 2D6.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

Not assessed in this report.

10.2 Skin corrosion/irritation

Not assessed in this report.

10.3 Serious eye damage/eye irritation

Table 6: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
BCOP test OECD 437 GLP	Sodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS) Aqueous solution (see confidential annex for composition)	Undiluted test item incubated on the cornea for 10 minutes Negative and positive controls included.	IVIS = 150.293	Anonymous (2012a) Disseminated registration dossier (2020)

10.3.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

A Bovine Corneal Opacity and Permeability (BCOP) Test (OECD TG 437) is available with Sodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS) (Anonymous, 2012a). Aqueous solution of HAPS, was incubated on the cornea for 10 minutes at 32±1 °C. According to study report, since the test item is a non-surfactant liquid, it was tested directly, without dilution or preparation of a solution. After removal of the test item and two hours post-incubation, opacity and permeability values were measured. Three replicates were included for each treatment groups: negative control (physiological sodium chloride solution: 0.9% NaCl), positive control (sodium hydroxide, 10% NaOH dissolved in 0.9% sodium chloride solution) and test item.

The *in vitro* irritancy score (IVIS) (defined in the study report as opacity difference + (15 x corrected OD_{490} value)) was calculated to be 150.293. The negative and positive controls were considered valid.

Other data regarding irritative potential of sodium 3-(allyloxy)-2-hydroxypropanesulphonate are available:

The substance (applied as powder in water) was tested in an *in vitro* skin corrosion assay on human skin model Epiderm™ (OECD TG 431: In vitro skin corrosion: reconstructed human epidermis (RhE) test method). Based on the absorbance values reported, the substance is not considered corrosive to skin in this *in vitro* assay (Anonymous, 2012b).

The substance (applied as powder wetted with DPBS-buffer) was tested in an *in vitro* skin irritation assay on human skin model Epiderm™ (OECD TG 439: In vitro skin irritation: reconstructed human epidermis (RhE) test method). Based on tissue viability reported, the substance is not considered irritant to skin in this *in vitro* assay (Anonymous, 2012c).

In an *in vivo* acute dermal toxicity study (OECD TG 402), erythema was noted from 24 hours post-dose in all tested rats (semi-occlusive; substance applied as aqueous solution) and was fully reversible within 7 days. Scabs were noted in all animals from 48 hours post-dose and remained on day 14 in all animals. (Anonymous, 2012d).

10.3.2 Comparison with the CLP criteria

According to OECD TG 437, a substance needs to be classified as Eye Damage category 1 if the IVIS is ≥ 55. Since the IVIS of sodium 3-(allyloxy)-2-hydroxypropanesulphonate is 150.293, the substance fulfils criteria for classification as Eye Dam. 1 according to CLP regulation.

10.3.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the BCOP test, Sodium 3-(allyloxy)-2-hydroxypropanesulphonate fulfils criteria for Eye. Dam 1 – H318 according to CLP regulation.

RAC evaluation of eye irritation and corrosion

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed harmonised classification and labelling of sodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS) as Eye Dam. 1 based on an OECD TG 437 Bovine Corneal Opacity and Permeability (BCOP) test where the undiluted test item (aqueous solution) incubated on the cornea for 10 minutes yielded an *in vitro* irritancy score (IVIS) of 150.293.

Comments received during consultation

One MSCA requested clarity on the constituents of the test substance used in the toxicity studies by specifically referring to the composition provided in the confidential Annex to the CLH report and the REACH registration dossier. They inquired as to whether NaOH has been added to the test material used in the BCOP study and if rather a mixture has been tested. The DS explained that NaOH is a starting material to form the sodium salt (HAPS) and considered an impurity from the manufacturing process, furthermore it was understood that NaOH is spontaneously formed due to dissolution of HAPS in aqueous solution.

Assessment and comparison with the classification criteria

The DS presented an *in vitro* study on eye damage/irritation, a BCOP test performed according to GLP and OECD TG 437. The study was conducted with an aqueous solution of the test substance (composition indicated in the confidential Annex of the CLH report). The study was reported as done in 2012, thus performed according to the original OECD TG 437 (2009), as the recent updated guideline was adopted only in 2020. The study and its results are described in Annex I of the CLH report. The IVIS (defined as opacity difference + (15 x corrected OD_{490} value)) was calculated for HAPS to be 150.293 based on three replicates, which were in similar range to the positive control (PC) 10% NaOH. The validity criteria for negative control and positive control were fulfilled. A mean IVIS of 150.293 corresponds to an ICCVAM[1] classification as 'very severe eye irritant'. According to the OECD TG 437, a substance that induces an IVIS ≥ 55 calculated based on OP-KIT formula is defined as a corrosive or severe irritant and identified as Category 1 according to GHS. According to the CLP guidance, a substance can be considered causing serious eye damage (Category 1) based on positive results in the BCOP test.

Additional skin data have been summarized by the DS. HAPS was tested in an OECD TG 431 *in vitro* skin corrosion test and in the OECD TG 439 *in vitro* skin irritation test using the human skin model Epiderm™ and measuring the cell viability following the two different protocols. HAPS was not considered corrosive and not irritant in accordance with these tests. In an *in vivo* acute dermal toxicity study, erythema was observed 24 hours post-dose in all animals and was totally reversible on day 7, but scabs were noted in all animals from 48 hours post-exposure remaining on day 14 in all animals.

In view of these data, RAC takes note of the confidential comment submitted by one MSCA regarding the composition of the substance used in the BCOP study and whether NaOH is spontaneously formed in aqueous solution, or should rather be considered an additive which has been purposely added to the solution and consequently a mixture would have been tested in the BCOP instead of a substance.

HAPS is manufactured and registered as a solid material. The BCOP study summary in the disseminated REACH registration dossier specifically reports NaOH as an additive (not as an impurity) in the test material, and the latter being an aqueous solution. It is also

[1] Interagency Coordinating Committee on the Validation of Alternative Methods

stated that the test item was tested "pure". This introduces confusion whether NaOH has been added to the substance or test material and whether the test item supplied to the testing facility was a solid or an aqueous solution. For the reproductive study summaries in the REACH dossier, the same composition is provided as for the BCOP study, however, in this case the test substance solution has been intentionally neutralised with acid before administration to neutralise the strong basic pH (see REACH registration dossier and also Annex to CLH report, e.g. section 3.2.1.1: "*Aqueous solution with 35.2% concentration of HAPS ...The raw solution also contained 3.7% NaOH increasing the pH up to 13.2, causing severe corrosive effects during application*").

The information overall is not clear, but RAC considers it unlikely that NaOH has been added to the test solution and also considers that no pH neutralisation took place. Furthermore, RAC shares the same understanding as the DS on this issue.

According to the DS, NaOH is used as a starting material to manufacture the sodium salt of 3-(allyloxy)-2-hydroxypropanesulphonate, i.e. HAPS. HAPS is likely isolated by filtration and some NaOH may remain in the solid material. RAC agrees that NaOH in this case is an impurity, originating from the starting material and manufacturing process. Furthermore, it is expected that the pH of the aqueous solution is basic because the sodium salt will dissociate upon dissolution of HAPS in water, forming NaOH due to spontaneous reversion to the starting material. Thus RAC understands that HAPS as is marketed, even if pure, will always contain NaOH when used as an aqueous solution and consequently may exert corrosivity once a water solution is prepared.

In the context of REACH, an additive is a compound that has been intentionally added during the manufacturing process to stabilise the substance. In any case, additives and impurities are to be considered in the same way for the purpose of classification. In contrast, in the case suggested by the MSCA, NaOH would purposely have been added to the aqueous solution and thus a mixture would have been tested. However, this does not seem supported by the available information. During the RAC 62 working group meeting no further information became available when discussing with the DS. According to the DS, lengthy discussions have been taken place during the proposal preparation and the registrant was contacted but no further information was obtained at that time.

RAC thus concludes that the test substance (containing and dissociating to NaOH), and not a mixture, has been tested to be corrosive in the BCOP study.

In the eye and skin corrosion/irritation studies, different materials or preparations were used. In the BCOP study and in the acute dermal toxicity study, an aqueous solution of HAPS was tested. In the OECD TG 431 on skin corrosion, the disseminated REACH dossier specifies the test substance is a solid powder that was grounded and applied together with H_2O. For the skin irritation study the test material is described as well as solid and it has been wetted with DPBS-buffer for the application. It appears thus that in the negative *in vitro* skin irritation and corrosion studies a solid material was tested that was only moistened to improve the contact to the Epiderm™ skin model. This difference in the preparation of the test item is important, as the aqueous solution is expected to contain NaOH due to dissolution and spontaneous formation of NaOH, which is corrosive

(Skin Corr. 1A, H314, SCL: Eye Irrit. 2; H319: 0,5 % ⩽ C < 2 %, Annex VI CLP).

Further to this, it is important to determine whether NaOH should be mentioned as an impurity in the Annex VI entry as contributing to the classification, according to Annex VI CLP, 1.1.1.4 (*"containing ⩾ xx % impurity"). *Impurities, additives, and minor constituents are normally not mentioned in the Annex VI entry unless they *contribute significantly* to the classification. In this particular case, the substance placed on the market and used under realistic conditions, i.e. as an aqueous solution, even if pure, is expected to contain NaOH and may exert corrosivity due to spontaneous formation / reversion upon dissolution. This despite any unintended or intended constituent of the substance in the form of impurities or additives originating from the manufacturing process. The impact of substance purity, whether NaOH in the form of impurity or additive, on the intrinsic hazardous properties and classification is not obvious based on the available information. It has not been shown that such presence leads to different classification. The available information does not allow to provide reference to impurities in the Annex VI entry for HAPS with the minimum concentration in NaOH (*⩾ xx %)* contributing to its classification. Therefore, RAC does not recommend including a reference to NaOH as an impurity in the Annex VI entry. This recommendation is in line with the ECHA paper on impurities (2018).

In conclusion, RAC recommends classification of sodium-3-(allyloxy)-2-hydroxypropanesulphonate (HAPS) for causing **Serious Eye Damage category 1, H318 (Eye Dam. 1)**.

10.4 Respiratory sensitisation

Not assessed in this report.

10.5 Skin sensitisation

Not assessed in this report.

10.6 Germ cell mutagenicity

Not assessed in this report.

10.7 Carcinogenicity

Not assessed in this report.

10.8 Reproductive toxicity

10.8.1 Adverse effects on sexual function and fertility

Table 7: 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
Reproduction/ Developmental Toxicity Screening Test OECD TG 421 GLP Male and female Wistar rats (12/sex/dose)	Aqueous solution with a concentration of 35.2% HAPS (see confidential annex for further information on composition) Dose levels: 0, 62.5, 250 and 1000 mg/kg bw/day by gavage. Dosing referred to the effective content of the active ingredient (HAPS). Due to several fatalities in the high dose group, satellite groups were included in the study (at 0 or 1000 mg/kg bw/day) Males were dosed daily for 42 to 57 days (two weeks of dosing prior to mating and continued throughout the mating period until approximately four weeks post-mating). Females were dosed at least 47 days to 55 days (two weeks prior to mating, covering at least two complete oestrous cycles, the variable time to conception, the duration of pregnancy and at least four days after delivery, up to and including the day before scheduled termination of the in-life phase)	LOAEL (P0 and F1) = 62.5 mg/kg bw/day (nominal) P0: - clinical signs (mild discomfort) in males and mortality at 1000 mg/kg bw/day - minimal to slight ovarian hypertrophy/ hyperplasia at 1000 mg/kg bw/day characterised by the presence of many, partly cystic corpora lutea, several tertiary follicles and increase in the number of interstitial cells[2] Reproductive performance: 11/12, 5/12, 0/12, 0/12, 0/12 animals achieved pregnancies (control, low, medium, high and satellite groups, respectively) F1 generation: Offspring was present only in the low dose group and in the control group.	Anonymous, 2013 Key study Klimisch score 2

Table 8: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
28-day study in accordance with the "28-day repeated-dose toxicity study using	Aqueous solution of HAPS (see confidential annex for composition)	male and female Crj:CD(SD) rats (5/sex/dose) 0, 25, 150 or 1000 mg/kg bw/day for 28 days.	Uterine cyst in the muscle layer in one animal of the 25 mg/kg bw/day group. Other effects mainly reported in the 1000 mg/kg bw/day group: clinical signs (loose faeces and salivation), decreased amount of ultromotivity and hyperplasia of the squamous epithelium at the edge of the anterior stomach.	Anonymous, 2007

[2] The ovaries were only examined at the highest tested dose.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
mammals" (Japan) GLP		Gavage, daily	The NOAEL = 150 mg/kg bw/day.	
14-day range finding study GLP	Aqueous solution of HAPS (no further information on disseminated dossier)	male and female Wistar rats (5/sex/dose) 0, 100, 300 or 1000 mg/kg bw/day for 14 days. Gavage, daily	No treatment-related effect identified. Regarding reproductive organs: slight or moderate hydrometra was noted for one female animal of each group. No histopathology performed.	Anonymous, 2016a
90-day toxicity study OECD TG 408; GLP	Aqueous solution of HAPS (see confidential annex for composition)	male and female Wistar rats (10/sex/dose) 0, 100, 300 or 1000 mg/kg bw/day for 90 days daily by gavage	No adverse effect reported. Regarding reproductive organs: slight or moderate hydrometra in the uterus in some female animals in all groups, with highest incidence at 1000 mg/kg bw/day. In one male animal at 300 mg/kg bw/day, larger than normal testis (left side). It consisted in focal granulomatous inflammation. Dilatation of the uterine horns in control and high dose group. NOAEL = 1000 mg/kg bw/day.	Anonymous, 2016b

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

In a Reproduction/Developmental Toxicity Screening Test according to OECD TG 421, male and female Wistar rats were gavaged with aqueous solution of HAPS at 62.5, 250 and 1000 mg/kg body weight/day (Anonymous, 2013). The test item has been delivered as a 35.2% aqueous solution and a relative density of 1.17 g/cm^3. Dosing referred to the effective content of the active ingredient (HAPS). The doses were chosen based on a range-finding study where the test item was administered in escalating doses up to 1000 mg/kg bw/day over a time period of 22 days without acute toxic effects in the test animals. Males were dosed over a time period of 42 to 57 days (two weeks of dosing prior to mating and continued throughout the mating period until approximately four weeks post-mating) and females were dosed at least 47 days (14 days pre-mating, up to 14 days until mating, an average of 21 days of gestation, and between 8 and 14 days of lactation). Due to several fatalities in the high dose group, a satellite group was added with 24 animals (12 males/ 12 females) treated identically to the high dose group (1000 mg/kg body weight) and supplementary included into the study on day 32. These animals were dosed for either 42 (males) or 47 (females) days. Further 24 animals (12 male/ 12 female) served as vehicle control.

Mild discomfort throughout the whole application period was observed for the male animals treated with the high dose of the test item (wiping of nose and mouth through the cage bedding, salivation after application, bleeding of mucous membranes at nose and mouth, respiratory sounds). Mortality (3 males and 5 females) occurred within the animals treated with the high dose. Death could be a result of reflux after gavage dosing leading to an accidental aspiration of dose formulation. Due to the high incidence and the exclusive occurrence within the animals treated with the high dose, a test item related effect could not be excluded. However, only one female died in the satellite group also exposed to 1000 mg/kg bw/day, suggesting that the observed deaths were not treatment-related. There were no treatment-related effects on body weight and food consumption during pre-mating phase. During post-mating, the body weight of the female animals treated with the high and the medium doses slightly increased between days 0 and 7 and decreased between days 7 and 20 of gestation, indicating that pregnancies of these animals were aborted between days 7 and 14. An increased water intake of all animals (male and female) treated at 1000 mg/kg bw/day could be observed throughout the whole in-life phase. There was no treatment-related changes in absolute and relative weights for testes and epididymis. A statistically significant increase of the mean weight of ovaries and uterus was detected for all test item dose groups compared to the vehicle control group. These differences result with high probability from the physiological changes the organs passed during pregnancy. No test item related prevalent findings were observed during gross necropsy, neither for male nor for female animals. The ovaries, testes, and epididymis from a total of 51 adult rats (high dose: 10 males / 6 females; satellite group: 11 females; vehicle control: 12 males / 12 females) and all other organs showing macroscopic lesions were submitted to histopathological examination. A minimal to slight ovarian hypertrophy/hyperplasia characterised by the presence of many, partly cystic, corpora lutea, several tertiary follicles and an increase in the number of interstitial cells was noted in the treated females.

The tested substance prevented or significantly reduced the achievement of pregnancy in all tested dose levels. Offspring was only present in the control group (11/12 females achieved pregnancy) and in the low dose group (5/12 females achieved pregnancy). At a dosage of 62.5 mg/kg bw/day, 5 of 12 females were able to achieve pregnancy but only 2 of these animals had a normal litter size and development. One animal gave birth to 5 pups. One animal gave birth to 1 pup (runt) that could not be found on day 4 post-partum. One animal gave birth to at least 2 pups, but one was found dead the next day. There was no pregnancy at 250 and 1000 mg/kg bw/day (including satellite group). Nonetheless, based on the results of the study, these effects cannot be associated with general toxicity. According to the study report, the presence of corpora lutea about 24 days after first pairing in the satellite group indicated that an implantation of the zygote took place, but embryonic development did not occur or was aborted during the first days of gestation. It was suggested that the absence of corpora lutea about 24 days after second pairing (females showing no evidence of copulation were re-mated for a second mating phase, during which dosing was continued) in the medium and high dose groups then indicated that, with prolonged dosing the implantation of the zygote or the ovarian maturation were impaired by the test item. Based on the results of the study, a specific physiological cause of

the toxic effect could not be identified. Potential effects on the spermatogenesis may not have had a sufficient time to be observed (such as reduced sperm counts affecting the fertility), as chemical exposure did not cover a complete cycle of spermatogenesis in male test animals (about 53 days). Therefore, and due to the lack of pregnancies in the test item treated female animals, an effect of the test item on the spermatogenesis could not be excluded.

Based on this study, the LOAEL for parental toxicity was set by the registrants at the lowest dose of 62.5 mg/kg (lack of achieving pregnancy; target organs: ovary, uterus; treatment-related). No NOAEL can be identified. According to the registrants, the reproductive LOAEL is set at 62.5 mg/kg bw/day (nominal) (reproductive effects in the absence of other toxic effects, treatment related). No NOAEL can be identified. For F1, the LOAEL is also 62.5 mg/kg bw/day (nominal) (male/female; viability). No NOAEL can be identified.

Other studies, that can bring additional information regarding reproductive toxicity potential of Sodium 3-(allyloxy)-2-hydroxypropanesulphonate, are available:

A short-term repeated dose toxicity study was performed according to the "28-day repeated-dose toxicity study using mammals" specified in the "Guideline for Toxicity Testings of New Chemical Substances (Japan) (Anonymous, 2007). In this study, male and female Crj:CD(SD) rats (5/sex/dose) were exposed daily by gavage to aqueous solution of HAPS at dose levels of 0, 25, 150 or 1000 mg/kg bw/day for 28 days. Recovery groups were provided in the 1000 mg/kg bw/day group and vehicle control group. Regarding reproductive organs: testes, epididymides and ovaries were weighted, and histopathology included examination of testes, epididymides, prostate, seminal vesicles, ovaries, uterus and vagina. Uterine cyst in the muscle layer in one animal of the 25 mg/kg bw/day group was the only reported effect in reproductive organs. The other effects were mainly reported in the 1000 mg/kg bw/day groups and included clinical signs in both sexes (loose faeces and salivation), decreased amount of ultromotivity at 30 to 40 minutes in males and hyperplasia of the squamous epithelium at the edge of the anterior stomach in both sexes. These effects were not found in the recovery group. The NOAEL was set by the registrants at 150 mg/kg bw/day.

A 14-day study was performed in male and female Wistar rats (5/sex/dose) to define the dose to be tested in a subsequent subchronic toxicity test (Anonymous, 2016a). Animals were exposed daily by gavage to aqueous solution of HAPS at dose levels of 0, 100, 300 or 1000 mg/kg bw/day. The substance was well tolerated by animals, with no treatment-related effect identified. Regarding reproductive organs: slight or moderate hydrometra was noted for some female animal in the control (1/5), at 100 mg/kg bw/day (1/5) and at 1000 mg/kg bw/day (1/5). No histopathology was performed.

A subchronic toxicity study was performed according to OECD TG 408 (Anonymous, 2016b). In this study, male and female Wistar rats (10/sex/dose) were exposed daily by gavage to aqueous solution of HAPS at dose levels of 0, 100, 300 or 1000 mg/kg bw/day for 90 days (doses determined on the basis of HAPS content in the product). No mortality occurred during the course of the treatment period. There was no adverse sign reported in the examined parameters (clinical observation, body weight, body weight gain, food consumption, ophthalmological, haematological and biochemistry examinations, gross pathology and histopathology). Regarding reproductive organs, testes, epididymides, uterus with fallopian tubes and ovaries were weighted. Full histological examinations were performed on preserved organs and tissues of the animals from both the control and high dose groups. Additionally, the testes and epididymides of one animal at 300 mg/kg bw/day were also processed histologically based on the necropsy observation in one side testis. The following effects were reported on reproductive organs. Slight or moderate hydrometra in the uterus was observed in some female animals in all groups, i.e. in the control (2/10), at 100 mg/kg bw/day (1/10), at 300 mg/kg bw/day (2/10) and at 1000 mg/kg bw/day (6/10) at the termination of the treatment period. Although the incidence of hydrometra was the highest in 1000 mg/kg bw/day, in the lack of pathological or inflammatory lesions, it was not considered to be toxicologically significant by the laboratory. In one male animal at 300 mg/kg bw/day, larger than normal testis was seen on the left side. It consisted in focal granulomatous inflammation. Dilatation of uterine horns was observed in 2/10 controls and 6/10 animals from the high dose group. Based on the study report, no effects were attributed to the treatment or judged as biologically significant. The NOAEL was set by the registrants at 1000 mg/kg bw/day.

In conclusion, the repeated-dose toxicity studies (from 14 days to 90 days of exposure) do not identify reproductive organs as a target of HAPS toxicity. In contrast, the well-conducted OECD TG 421 study reports clear fertility effects since the tested substance prevented or significantly reduced the achievement of pregnancy in all tested dose levels, in the absence of general toxicity.

10.8.3 Comparison with the CLP criteria

According to CLP: *"Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or 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 in this ***Category 1A*** *is largely based on evidence from humans."*

There is no human data with HAPS. Therefore, classification as Repr. 1A is not fulfilled.

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 or 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.

There is clear evidence of an adverse effect on fertility in the absence of other toxic effects in a reproduction/developmental toxicity screening test according to OECD TG 421. There was no offspring produced at the tested doses of 250 and 1000 mg/kg bw/day, and only 2/12 dams with normal litter at 62.5 mg/kg bw/day. The doses of 62.5 and 250 mg/kg bw/day were not associated with general toxicity that can be evidenced by clinical signs, mortality, body weight changes or histopathological examination. At the highest tested dose, clinical signs and minimal to slight ovarian hypertrophy/hyperplasia were reported as possibly treatment-related. In this context, the reduction / absence of litter – which is a severe adverse effect - cannot be considered secondary to the (no or) minimal general toxicity reported. It is not clear if the numerous mortalities reported in the high dose group are related to the treatment since only one female died in the satellite group also exposed to 1000 mg/kg bw/day.

In addition, it can be noted that the OECD 421 guideline study is a screening assay. According to the OECD guideline, this protocol is only "*designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition*". Therefore, the fact that reproductive effects are clearly observed in this type of study supports that they must be considered as a frequent and severe toxicity.

Therefore, HAPS fulfils criteria for classification as Repr. 1B – H360 for fertility based on clear evidence of toxicity on reproduction.

However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in ***Category 2*** *may be more appropriate.*

Clear evidence of adverse effect on fertility that cannot be considered as secondary to general toxicity is reported in a well-conducted OECD TG 421 study, and there is no mechanistic information raising doubt about the relevance of these effects for humans. Thus, classification as Repr. 2 is not appropriate.

10.8.4 Adverse effects on development

Table 9: 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**
OECD TG 414 GLP Pregnant female Wistar rats 24/dose	Aqueous solution with a concentration of 38.2% HAPS (see confidential annex for further information on composition) Dose levels: 0, 100, 300 and 1000 mg/kg bw/day by gavage. Dosing referred to the effective content of the	No treatment related effects on clinical signs, mortality, body weight and gross pathology in dams. No treatment related effects on pre- and post-implantation losses, number of viable foetuses, sex distribution, malformation and variations. NOAEL (maternal and foetuses) =	Anonymous, 2017a Key study Klimisch score 2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	active ingredient (HAPS). Duration: GD6 to GD19	1000 mg/kg bw/day	
Dose-range finding study for OECD TG 414 GLP Pregnant female Wistar rats 5-6/dose	Aqueous solution containing 38.2% concentration of HAPS Dose levels: 0, 10, 37.5, 125 and 500 mg/kg bw/day by gavage. Dosing referred to the effective content of the active ingredient (HAPS). Duration: GD5 to GD19	No treatment related effects in dams and foetuses NOAEL (maternal and foetuses) = 500 mg/kg bw/day	Anonymous, 2017b

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

HAPS, as aqueous solution at 38.2%, was administered by gavage to pregnant female Wistar rats (24/dose) at dose levels of 0, 100, 300 or 1000 mg/kg bw/day (dose levels refer to HAPS quantity in the dosing solutions calculated with 38.2 wt% in mixture) from gestation day 6 to 19 (Anonymous, 2017a). The doses were selected based on a dose-range finding study showing no treatment-related effects in dams and foetuses up to the highest tested dose of 500 mg/kg bw/day.

In the main study, there was no maternal toxicity in any of the groups. The treatment did not increase the pre and post-implantation loss and had no effect on viability and sex distribution. There was no treatment related effect on foetal and placental weights.

The number of litters with malformed foetuses was 2 in the 100 and 3 in the 1000 mg/kg bw/day groups, none at 300 mg/kg bw/day.

There were 3 foetuses with external / skeletal malformations in the 100 mg/kg bw/day group and 3 in the 1000 mg/kg bw/day group. In the high dose group, one foetus was found with bent scapula (bilateral), bent ulna (unilateral) and slightly shorter femur (unilateral). Another foetus had bent scapula (bilateral). A third foetus had a bipartite thoracic vertebra with dumb-bell shaped cartilage. In the 100 mg/kg bw/day dose group, two foetuses were found with short tail and one of them with hypoplastic pollex (not proved at skeletal examination). Both of these foetuses had multiple malformed vertebrae and in addition one of them had fused ribs. In this group a third foetus had also fused ribs and multiple malformations of the thoracic vertebrae. These malformations occurred with a low incidence or without dose response. There was no malformation found at visceral examination.

There was no treatment-related variations at external and visceral examinations. There was a statistically significant increase of markedly incomplete ossification of one or more skull bones in the 300 and 1000 mg/kg bw/day dose group as well as wavy ribs in the low and high dose. These effects did not reach statistical significance (if litter incidence was evaluated) and/or were within historical control level.

The NOAEL for maternal and developmental toxicity is set by the registrants at 1000 mg/kg bw/day.

10.8.6 Comparison with the CLP criteria

According to CLP: *"Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or 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 in this **Category 1A** is largely based on evidence from humans."*

There is no human data with HAPS. Therefore, classification as Repr. 1A is not fulfilled.

*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 or 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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in **Category 2** may be more appropriate.*

One prenatal developmental toxicity study according to OECD TG 414 is available with HAPS. Malformations were reported in some foetuses without dose-response relationship. Thus, no classification is required for HAPS regarding developmental toxicity.

10.8.7 Adverse effects on or via lactation

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

There is no study with HAPS that provide information on effects on or via lactation.

10.8.9 Comparison with the CLP criteria

There is no study with HAPS that provide information on effects on or via lactation. Thus, no classification can be proposed for this endpoint.

10.8.10 Conclusion on classification and labelling for reproductive toxicity

Classification as Repr. 1B – H360 for fertility is required based on a clear effect on fertility characterized by reduction / absence of pregnancy in a reproduction/developmental toxicity screening test according to OECD TG 421, not associated with other general toxicity.

No classification is required for developmental toxicity based on a prenatal developmental toxicity study (OECD TG 414).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS presented a Reproduction / Developmental Screening study (OECD TG 421) and three repeated dose toxicity studies, including a 28-day, 14-day range finder and 90-day (OECD TG 408) study.

In the OECD TG 421 study, clear fertility effects were reported as the test substance prevented or significantly reduced the achievement of pregnancy in all tested dose levels, in the absence of excessive general toxicity for the low and mid dose. In the high dose, excessive mortality was observed immediately or soon after dosing, but it was not clear whether this was directly treatment related or indirectly due to mis-dosing. Due to several fatalities in the high dose group, satellite groups were included in the study (at 0 or 1000 mg/kg bw/day). In the mid and high dose no pregnancy was achieved at all, corpora lutea was absent after the second mating, while in the shorter dosed satellite high dose group corpora lutea was present after the first pairing but no pregnancy was achieved either. In the low dose only two dams had normal litter size and development.

The repeated dose toxicity studies did not identify the reproductive organs as a target organ.

As to developmental toxicity, an OECD TG 414 study and a range finder study were presented. No treatment related effects on pre- and post-implantation losses, number of viable foetuses, sex distribution, malformation and variations were reported. Malformations were reported in some foetuses without dose-response.

In conclusion, the DS proposed classification as Repr. 1B for fertility based on the reduction and prevention of pregnancy in the absence of general toxicity.

Comments received during consultation

Comments from two MSCAs were submitted supporting the classification as Repr. 1B for fertility. No classification for developmental toxicity was supported by one MSCA. While the OECD TG 414 study did not reveal any developmental toxic effects up to the limit dose, the other MSCA raised the uncertainty that developmental toxicity cannot be fully excluded based on the screening study. This was related to methodological sensitivity issues in the detection of corpora lutea and possible implantation sites, and slight increases in percentage of stillborn pups and pup survival at post-natal day (PND) 4. The DS agreed there are some uncertainties, but overall considered that the data point to a fertility effect due to the high reduction / absence of implants, histopathological effects on ovaries of infertile females, absence of *corpora lutea* after the second mating and no developmental effects in the OECD TG 414 study.

Assessment and comparison with the classification criteria

Sexual function and fertility

Reproduction / developmental toxicity screening study

HAPS was tested as a neutralised aqueous solution (35.2%) in Wistar Han rats (12/sex/dose) in an OECD TG 421 screening study at dose levels of 62.5, 250, and 1000 mg/kg bw/d by oral gavage. Males were dosed daily for 42 to 57 days, this included two weeks of dosing prior to mating and continued throughout the mating period until approximately four weeks post-mating. Females were dosed two weeks prior to mating, covering at least two complete oestrous cycles, the variable time to conception, the duration of pregnancy and at least four days after delivery. The study duration therefore was 14 days pre-mating, an additional up to 14 days until mating occurred, an average of 21 days of gestation, and between 8 and 14 days of lactation. Females showing no evidence of copulation were re-mated for a second mating phase, during which dosing was continued. Those animals were dosed daily for 67 to 76 days. A satellite group was introduced during the study on day 32 with additional 12 males and females to prevent study cancellation due to high dose mortality. The satellite group was treated identically to the high dose with a duration of 42 (males) and 47 (females) days. The mortality in the high-dose group was not considered treatment-related, 8 animals (3 males / 5 females) died in the high dose group (days 4, 5, 6, 12, 16, 18, 42, 45), five of these animals died immediately after application or half an hour after application indicating the mortality was non-treatment-related but due to miss-gavage or reflux. One female, only, in the satellite group died on day 42, raising doubts whether mortalities might have been treatment related. No mortalities occurred in other groups.

General toxicity: During post-mating, body weights of high and medium dose group females slightly increased between days 0 and 7 and decreased between days 7 and 20 of gestation, this was suggested to be attributed to the pregnancy losses of these animals that aborted between days 7 and 14. Four Animals in the low dose group had body weights slightly increased during gestation phase due to being pregnant after first pairing (only a graphical presentation is provided in the Annex to CLH, no numbers or indication of statistical significance), however, the mean body weight for the group remained lower than the control. The different number of females achieving pregnancies may have masked body weight effects after the mating weeks due to variances in body weights. Some fluctuations in water and food consumption were observed, the water intake of all high dose animals increased during treatment (6-40%).

Reproductive organ weights and histopathological findings: A statistically significant increase of the mean ovaries and uterus weights was reported for all dosed groups (compared to the vehicle control group in the Annex to CLH report) and was attributed by the DS to the physiological changes the organs went through during pregnancy. RAC notes that such weight changes would also be observed in the control group's pregnant females and that no pregnancies were achieved in the mid and high dose groups, thus the explanation appears not plausible and the relevance of these findings uncertain. No effects were observed for testis and epididymis.

Overall, ovaries, testes and epididymis and other organs showing macroscopic lesions from 51 rats (high dose and control) were subjected to histopathology. The morphology of the ovaries of the infertile treated females (high dose in main and satellite group) was slightly different from control females. In the main group, a minimal to slight ovarian hypertrophy/hyperplasia characterised by the presence of many, partly cystic corpora lutea, several tertiary follicles and an increase in the number of interstitial cells was noted. This may be due to the infertile state and oestrus cycle, but a relationship to the treatment with the test item could not be excluded. RAC notes that in the main high dose group corpora lutea were markedly diminished (mean 1.5 vs 12.6 in the control), the

"*many, partly cystic*" corpora lutea reported seem contradicting.

Reproduction function and performance: No effect on spermatogenesis was evidenced. As chemical exposure did not cover a complete cycle of spermatogenesis in males, effects of the spermatogenesis may not have had an adequate time to become evident (such as reduced sperm counts affecting the fertility).

Treatment prevented pregnancies with 11/12, 5/12, 0/12, 0/11 pregnancies in the control, low, mid and high dose group, respectively. Females not achieving pregnancy in the first mating were mated a second time. The control and low dose each achieved one pregnancy in the second mating.

No pregnancies were achieved in the satellite high dose group, 0/9 pregnancies achieved in the satellite high dose group.

***Table**: Results OECD TG 421 screening study on HAPS*

Observations	Values				
Dosage (units)	High dose	High dose [SG]	Medium dose	Low dose	Vehicle
	1000 mg/kg BW	1000 mg/kg BW	250 mg/kg BW	62,5 mg/kg BW	-
Pairs started (N)	9	11	12	12	12
1st mating					
Females showing evidence of copulation (N)	7	10	11	8	9
Females achieving pregnancy (N)	0	0	0	4	10[1]
Conceiving days 1 - 5 (N)	7	10	8	6	8
Conceiving days 6 - . . .([1]) (N)	0	0	3	2	1
2nd mating					
Females showing evidence of copulation (N)	7	0	10	7	2
Females achieving pregnancy	0	0	0	1	1
Conceiving days 1 – 5 (N)	7	0	10	7	2
Conceiving days 6 - … ([1])(N)	0	0	0	0	0
Totals 1st and 2nd mating					
Females achieving pregnancy (N)	0	0	0	5	11
Conceiving days 1 – 5 (N)	14[2]	10[2]	18[2]	13[2]	9
Conceiving days 6 - … (N)	0	0	3	2	1
Pregnancy ≤ 21 days (N)	0	0	0	1	0
Pregnancy = 22 days (N)	0	0	0	0	3
Pregnancy = 23 days (N)	0	0	0	4	5
Dams with live young born (N)	0	0	0	4	10
Dams with live young at day 4pp (N)	0	0	0	3	10
Corpora lutea/dam (mean)	1,5	9,7	3,3	7,6	12,6
Implants/dam (mean)	0,0	0,0	0,3	3,6	8,8
Live pups/dam at birth (mean)	n.a.	n.a.	n.a.	5,4	8,6
Live pups/dam at day 4 (mean)	n.a.	n.a.	n.a.	5,2	8,5
Litter weight at birth (mean)	n.a.	n.a.	n.a.	35,8	54,7
Litter weight at day 4 (mean)	n.a.	n.a.	n.a.	63,9	91,9
Pup weight at birth (mean)	n.a.	n.a.	n.a.	6,5	6,7
Pup weight at day 4 (mean)	n.a.	n.a.	n.a.	12,3	11,6
No. of pups					
Live pups born day 0 (count)	0	0	0	27	95
Stillborn (count)	0	0	0	2	5
Total of pups born day 0 (count)	0	0	0	29	100
Stillborn (%)	n.a.	n.a.	n.a.	6,90	5,00
Pups alive day 4	0	0	0	26	94
Sex ratio					
Sex Ration day 0 (total numbers M/F)	0/0	0/0	0/0	12/15	43/52
Sex ratio day 0 (mean)	n.a.	n.a.	n.a.	0,80	0,83
Sex ration day 4 (total numbers M/F)	0/0	0/0	0/0	13/13[3,4]	43/51[4]
Sex ratio day 4 (mean)	n.a.	n.a.	n.a.	1,00	0,84

[1] individual animals delivered although no sperm plug was detected

[2] number higher than pairs started as values are given for both mating periods

[3] minor differences due to errors at sexing [4] differences to previous total numbers due to post-natal losses

Observations	Values				
Dosage (units)	High dose	High dose [SG]	Medium dose	Low dose	Vehicle
	1000 mg/kg BW	1000 mg/kg BW	250 mg/kg BW	62,5 mg/kg BW	-
ABNORMAL PUPS					
Dams with 0	0	0	0	4	10
Dams with 1	0	0	0	0	0
Dams with ≥ 2	0	0	0	0	0
LOSS OF OFFSPRING					
Pre-implantation (corpora lutea minus implantations)					
Dams with pre-implantation loss (count)	2	10	6	8	8
Pre-implantation loss (mean/group)	1,5	9,7	3,1	4,0	4,1
Females with 0	4	1	6	4	3
Females with 1	0	0	0	1	0
Females with 2	1	0	1	0	2
Females with ≥3	1	10	5	7	6
Pre-natal/post-implantations (implantations minus live birth)					
Dams with pre-natal loss (count)	0	0	0	4	6
Pre-natal loss (mean/group)				3,0	1,0
Females with 0	0	0	0	1	5
Females with 1	0	0	0	1	4
Females with 2	0	0	0	0	1
Females with ≥3	0	0	0	3	1
Post-natal (live births minus alive at post natal day 4)					
Dams with post-natal loss (count)	0	0	0	1	1
Post natal loss (mean pups/group)				0,2	0,1
Females with 0	0	0	0	4	10
Females with 1	0	0	0	1	1
Females with 2	0	0	0	0	0
Females with ≥3	0	0	0	0	0

The mean number of corpora lutea was dose-dependently decreased for the main groups with 12.6, 7.6, 3.3 and 1.5 for the control, low, mid, and high dose group, respectively. For the satellite high dose group, having only one mating, 9.7 was reported, the reason is likely the shorter exposure duration compared to the main groups experiencing second mating. In the satellite high dose group, corpora lutea was detected about 24 days after first pairing. This may indicate an implantation took place in the satellite high dose group however, the time from copulation to necropsy was considered too long to detect implantation sites in the main or the satellite high dose females, according to the CLH report. Thus, it is not fully clear whether implantations took place and a subsequent effect on the embryo development contributed to the complete lack of pregnancy. However, the absence of corpora lutea detected about 24 days after the second pairing, in the main group at mid and high doses, suggest a fertility effect. The increasing dosing duration likely impaired zygote implantation or ovarian maturation.

Offspring data: No offspring was produced in the mid and high dose groups. For the control and low dose, 10/11 and 4/5 pregnant dams, respectively, gave birth to live

pups with 8.6 and 5.4 live pups/dam, respectively. 5/100 control and 2/29 low dose pups were stillborn (5% versus 6.9%). For the low dose, 3/5 dams had live pups at PND4, both the control and low dose lost 1 pup each until PND4 resulting in 94/100 and 26/29 pups alive on PND4 for the control and low dose, respectively (i.e. 94% versus 89.7% survival index). Post-implantation loss was reported to be slightly higher in the low dose (4/5 low dose versus 6/11 control; pregnant dams had post-implantation losses with a mean/group of 3.0 versus 1.0, respectively). Upon request of RAC, it has been clarified by the DS, after consulting the registrant for further information, that the number of mean implantation sites of 3.6 per dam (as reported in the CLH report) referred to all females including non-pregnant, while it was 8.4 for pregnant females only. The post-implantation loss of 3.0 was calculated based on the 8.4 mean implantations/dam and the mean number of pups alive of 5.4 per dam.

Repeated Dose toxicity studies

Repeated dose toxicity studies that could provide insight into adverse effects on reproductive organs have been summarized by the DS in the CLH report, and include a 90-day OECD TG 409 study in rats (10 Wistar rats/sex/dose, 0, 100, 300, 1000 mg/kg bw/d, gavage), a "28-day repeated dose toxicity study using mammals" in rats (Anonymous, 2007; 5 Crj_CD(SD) rats/sex/dose, 0, 25, 150, 1000 mg/kg bw/d, gavage), and a 14-day range finder study (5 Wistar rats/sex/dose treated with 0, 100, 300, 1000 mg/kg bw/d, gavage), see table 8 CLH report. In summary, the repeated dose toxicity studies did not identify reproductive organs as target of HAPS, no effects were detected or attributed to treatment or judged as biologically significant in the study reports.

Conclusion on classification as sexual function and fertility

According to the CLP regulation, *"the classification of a substance in this Category 1A is largely based on evidence from humans*." There is no human data with HAPS. Therefore, classification as Repr. 1A is not fulfilled.

"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 or 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".

RAC agrees with the DS that HAPS induced adverse findings on female reproductive performance and fertility that warrant classification as Repr. 1B for fertility. This is based on a dose-dependent decrease of the Female Mating Index for the first mating, reaching 50% at the lowest dose and complete impairment (zero pregnancies) at the mid and high dose group (250 and 1000 mg/kg bw/d, respectively). Corpora lutea were dose-dependently reduced in the control, low, mid and high dose groups (mean corpora lutea/dam 12.6, 7.6, 3.3, 1.3). In addition, adverse effects on litter size and pup survival were shown from the low dose of 62.5 mg/kg bw/day.

The low and mid dose groups were not associated with any general toxicity. At the high dose minimal to slight ovarian hypertrophy/hyperplasia was reported that might be related to the infertile state or possibly directly to treatment. In addition, some uncertainty is noted due to the mortality observed in the high dose groups. However, these findings cannot explain the substance interference with pregnancy rate at the low

and mid dose. Therefore, RAC concludes that **classification of HAPS as Repr. 1B, H360F is warranted.**

The limited dosing regime in males as regards to spermatogenic cycles and the lack of investigations of spermatogenesis does not allow any conclusions as to effects on male fertility.

Developmental toxicity

In an OECD TG 414 prenatal developmental toxicity study (2007, GLP, Klimisch 2), HAPS aqueous solution (38.2%) was tested in pregnant female Wistar rats (24/dose) from gestation day (GD) 6 to 19 at dose levels of 0, 100, 300 and 1000 mg/kg bw/d by oral gavage (the formulation was adjusted to pH 6.0-7.0 before administration).

Maternal toxicity: No mortalities, no adverse effects on body weights and no gross-pathological findings were reported. Some temporary decreases in food consumption in the high dose were considered not adverse (although statistically significant on GD6-9, -6%). No effects on pregnancy duration, abortion or total litter loss were reported.

Intrauterine mortality: The substance had no statistically significant and no dose-dependent effects on the percentage of post-implantation loss, early and late embryonic death, dead foetuses, or total intrauterine mortality.

Offspring and malformations: No effects on foetal weights, number of live offspring, no changes in sex-ratio, no changes in litter size and weights were reported.

Malformations: The number of litters with malformed foetuses were 0/19 (0%), 2/22 (9.1%), 0/23 (0%) and 3/22 (13.6%) in the control, low, mid and high dose, respectively.

No visceral malformations were detected.

Skeletal malformations were observed in the high dose: one foetus was found with bent scapula (bilateral), bent ulna (unilateral) and slightly shorter femur (unilateral). Another foetus had bent scapula (bilateral). The third foetus had a bipartite thoracic vertebra with dumb-bell shaped cartilage. In the low dose, two foetuses (in a common litter) were found with short tail and both of these had multiple malformed vertebrae and in addition one of them had fused ribs. A third foetus had fused ribs and multiple malformations of the thoracic vertebrae. In summary, malformations occurred in low incidence and without dose-response.

Variations: Statistically significant increase in the incidence of markedly incomplete ossification of one or more skull bones in the 300 ($p<0.05$) and the 1000 mg/kg bw/day ($p<0.01$) dose group was reported, as well as a statistically significantly higher incidence of wavy ribs in the low ($p<0.05$) and high dose ($p<0.01$) group but within the historical control level.

In the OECD TG 414 range finder study, 5-6 Wistar rats per dose of 0, 10, 37.5, 125 and 500 mg/kg bw/d during GD5-19, no treatment related adverse effects were reported. Two malformations were found at skeletal examination in the 37.5 mg/kg bw/day group (one foetus with split and misaligned sternum and another foetus with bent ulna) but not attributed to treatment as they were considered as an isolated finding at the mid dose.

In conclusion, no adverse effects on developmental toxicity were observed in the OECD TG 414 main and range-finder studies.

As regards to the marginal findings in the OECD TG 421 study including the percentage of stillborn pups (6.9% vs 5% for low dose vs control), total pup survival (89.7% vs 94% for low dose vs control) and post-implantation loss (3.0 vs 1.0 for low dose vs control), RAC agrees with the DS that based on these minor changes observed for one dose level only (due to complete lack of pregnancies at higher dose levels) no classification is warranted.

RAC concludes on **no classification for developmental toxicity**.

Adverse effects on or via lactation

The CLH report does not include a proposal for classification for adverse effects on or via lactation and no data is available in the CLH report allowing an assessment by RAC.

RAC recommends **no classification for adverse effects on or via lactation**.

10.9 Specific target organ toxicity-single exposure

Not assessed in this report.

10.10 Specific target organ toxicity-repeated exposure

Not assessed in this report.

10.11 Aspiration hazard

Not assessed in this report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this report.

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this report.

13 ADDITIONAL LABELLING

Not assessed in this report.

14 REFERENCES

ECHA, European Chemicals Agency (2020)

Information on Chemicals - Registered Substances

Online: https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13109

https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID6028028#exposure (June 2020)

https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-3-_allyloxy_-2-hydroxypropanesulphonate#section=Industry-Uses (June 2020)

http://qsar.food.dtu.dk/ (June 2020)

See Confidential Annex

Additional references
ECHA 2018: Impurities and (degree of) purity in CLP and in the CLH process.

15 ANNEXES

ANNEX I to the CLH report

ANNEX CONFIDENTIAL to the CLH report