Committee for Risk Assessment

RAC

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

Adopted

15 September 2022

15 September 2022

CLH-O-0000007154-78-01/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: **Sodium 3-(allyloxy)-2-hydroxypropanesulphonate**

EC Number: **258-004-5**

CAS Number: **52556-42-0**

The proposal was submitted by **France** and received by RAC on **9 July 2021.**

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

PROCESS FOR ADOPTION OF THE OPINION

France 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 **4 October 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 December 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ruth Moeller**

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 **15 September** 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	Sodium 3-(allyloxy)-2-hydroxypropanesulphonate	258-004-5	52556-42-0	Repr. 1B Eye Dam. 1	H360F H318	GHS08 Dgr	H360F H318			
RAC opinion	TBD	Sodium 3-(allyloxy)-2-hydroxypropanesulphonate	258-004-5	52556-42-0	Repr. 1B Eye Dam. 1	H360F H318	GHS08 GHS05 Dgr	H360F H318			
Resulting Annex VI entry if agreed by COM	TBD	Sodium 3-(allyloxy)-2-hydroxypropanesulphonate	258-004-5	52556-42-0	Repr. 1B Eye Dam. 1	H360F H318	GHS08 GHS05 Dgr	H360F H318			

GROUNDS FOR ADOPTION OF THE OPINION

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.

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

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 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"). I*mpurities, 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)**.

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 available for 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 that **no classification for developmental toxicity** is warranted.

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**.

Additional references

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

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).