Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

6-[(C10-C13)-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (tetra-PSCA)

EC Number: -
CAS Number: 2156592-54-8

CLH-O-0000006924-66-01/F

Adopted

10 December 2020

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

10 December 2020

CLH-O-0000006924-66-01/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: **6-[(C10-C13)-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid**

EC Number: **-**

CAS Number: **2156592-54-8**

The proposal was submitted by **Austria** and received by RAC on **9 January 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Austria 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 **24 February 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **24 April 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Bjørge**

Co-Rapporteur, appointed by RAC: **Stine Husa**

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 **10 December 2020** 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	6-[(C10-C13)-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid	-	2156592-54-8	Repr. 1B Eye Irrit. 2	H360FD H319	GHS08 GHS07 Dgr	H360FD H319		Repr. 1B; H360FD: C ≥ 0,03%	
RAC opinion	TBD	6-[(C10-C13)-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid	-	2156592-54-8	Repr. 1B Eye Irrit. 2	H360FD H319	GHS08 GHS07 Dgr	H360FD H319			
Resulting Annex VI entry if agreed by COM	TBD	6-[(C10-C13)-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid	-	2156592-54-8	Repr. 1B Eye Irrit. 2	H360FD H319	GHS08 GHS07 Dgr	H360FD H319			

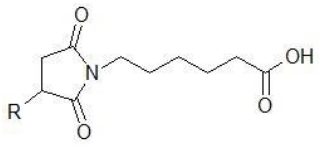
GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The weak acid 6-[(C10-C13)-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (hereafter **tetra-PSCA**) is structurally related to the acid 6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (hereafter **penta-PSCA**) the salt 6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium (hereafter **penta-PSCA Na-TEA**).

Read across assessment

An analogue read-across approach between penta-(polypropenylsuccinimido)-caproic acid (penta-PSCA), tetra-PSCA (see Figure below) and penta-PSCA Na-TEA has been proposed by the Dossier Submitter (DS) based on similarities in structure, ions release in biological media and expected similar toxicity. All substances (source and target substances) belong to the group of 2,5 dioxo-pyrrolidin hexanoates. They differ only in the number of C-atoms of the alkyl side chain (branched, unsaturated) at position 3 of the ring structure. Tetra PSCA is also a UVCB substance.



R=C10-13-alkenyl-(even and odd,branched, unsaturated); mainly C12

Figure: chemical structure of tetra-PSCA

Three Member States Competent Authorities (MSCA) agreed with the read-across approach during the general consultation whereas two other MSCAs requested additional justifications. One commenting MSCA did not support the proposed read across for local toxicity due to uncertainties in the composition of the test substance. The DS argued that read across could be applied and clarified that additional information is available in a confidential annex to the CLH report. One MSCA noted that tetra-PSCA and penta-PSCA belong to a homologous series of (Polypropenylsuccinimido)-caproic acid (PSCA) and can thus be considered to belong to a "chain length category". The substances have a high structural similarity.

RAC agrees with the grouping approach and read across proposed by the DS for STOT RE and reproductive toxicity. The assessment of reproductive toxicity was based on studies performed with penta-PSCA Na-TEA. For the assessment of STOT RE a 28-day study with tetra-PSCA was assessed in addition to a dose-range finding study to an OECD TG 422 study and the main OECD TG 422 study with exposure to penta-PSCA Na-TEA. For local effects RAC considers that the classification should be based on data on tetra-PSCA.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

For the evaluation of skin corrosion/irritation the DS included one study performed according to OECD TG 404 and GLP where 3 female New Zealand White rabbits were exposed to 0.5 mL tetra-PSCA on shaved skin under semi-occlusive conditions for 4 hours (Anonymous, 2012a). Signs of erythema and oedema were recorded 1, 24, 48 and 72 hours after patch removal. The recorded scores were zero for erythema as well as oedema. On this basis, the DS did not propose any classification for skin corrosion/irritation.

Comments received during consultation

Three commenting MSCAs supported the proposal by the DS for no classification for skin corrosion/irritation.

Assessment and comparison with the classification criteria

According to the CLP criteria a classification as Skin Irrit. 2 is warranted if the mean scoring value is between 2,3 and 4 for erythema/eschar or for oedema in at least 2of 3 tested animals. The study presented by the DS showed a score for erythema and oedema of zero for 1, 24, 48 and 72 hours after patch removal.

On this basis, RAC is of the opinion that **no classification for skin corrosion/irritation is warranted for tetra-PSCA**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

For the evaluation of serious eye damage/eye irritation the DS included one study according to OECD TG 405 and GLP where two female New Zealand White rabbits were exposed to 0.1 mL tetra-PSCA (Anonymous, 2012b). The treated eye was not rinsed 24 hours after application. The observation period was prolonged to 18 days to assess reversibility. Conjunctival redness, chemosis, discharge, corneal effects and lesion were observed in all animals. All the observed effects were fully reversible within 18 days. The scores are presented in the table below.

Table: *Scoring from OECD TG 405*

	Mean score (24, 48, 72 h)	**Max score**
Corneal opacity	1.8 (1.6; 2)	2
Iris score	1 (1; 1)	1
Conjunctivae score	2.33 (1.6; 3)	3
Chemosis score	2.5 (1; 4)	4

On this basis the DS proposed a classification for eye irritation with Eye Irrit. 2, H319.

Comments received during consultation

Three commenting MSCAs supported the proposed classification as Eye Irrit. 2, H319.

Assessment and comparison with the classification criteria

According to the CLP criteria a classification for eye irritation (Eye Irrit. 2) is warranted if in at least 2 of 3 tested animals a positive response of corneal opacity $\geqslant$ 1 and/or iritis $\geqslant$ 1, and/or conjunctival redness $\geqslant$ 2 and or conjunctival oedema (chemosis) $\geqslant$ 2 calculated as the mean scores following grading at 24 48 and 72 hours after installation of the test material and which is fully reversible within 21 days.

The study presented by the DS showed a mean score $\geqslant$ 1 for corneal opacity and iritis, and a mean score $\geqslant$ 2 for conjunctival redness and oedema (chemosis) for the two animals tested. These effects were fully reversible within 18 days.

Consequently, RAC is of the opinion that a **classification for eye irritation in category 2 (Eye Irrit. 2, H319) is warranted for tetra-PSCA**.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

For the assessment of STOT RE the DS included a 28-day study (OECD TG 407) in CD(SD) rats with exposure to tetra-PSCA (Anonymous, 1995). In addition, the DS included an OECD TG 422 (combined repeated dose toxicity study with the reproduction/developmental toxicity screening study) and an associated dose range finding (DRF) study to the OECD TG 422 with exposure to penta-PSCA Na-TEA in a read across assessment (Anonymous, 2012c; Anonymous, 2013a). Both studies were performed in Han Wistar rats and according to GLP, and some studies with TEA (2,2',2''-nitrilotriethanol).

In the 28-day study rats were exposed to 0, 8, 40, 200 and 1000 mg/kg bw/d tetra-PSCA. The main target organ following exposure to tetra-PSCA was the liver in both sexes and the kidney and forestomach in male rats. The effects observed at 200 mg/kg bw/d were not considered by the DS sufficiently severe (moderate and low incidence of eosinophilic bodies in the kidneys of males, increased relative liver weight in females (+10%), no significant changes in haematology and clinical chemistry) to warrant a classification for STOT RE 2.

In the OECD TG 422 study rats (11/sex/group) were exposed to 0, 40, 200 and 1000 mg/kg bw/d and in the associated dose range finding study (3/sex/group) to 0, 100, 300 and 1000 mg/kg bw/d penta-PSCA Na-TEA (28 days (m) to 49 days (f)). The effects reported in rats were not considered sufficiently severe (loss of appetite, reduction in body weight, and at higher levels moderate toxicity such as reduced body temperature, reduced locomotor activity) to warrant a classification for STOT RE 2.

Relevant studies with TEA

The DS clarified that the toxicity of penta-PSCA Na-TEA was not related to exposure to TEA) by assessing the following three repeated dose toxicity studies with TEA:

A 90-day oral repeated dose toxicity study in Cox CD rats (20/sex/group) with exposure to 0, 250, 500 and 1000 mg/kg bw/d TEA in feed (Anonymous, 1989). No dose-response-related systemic effects of TEA were reported up to the highest dose tested, 1000 mg/kg bw/d.

A 28-day inhalation study in rats (Gamer, 2008) with exposure to 0, 0.02, 0.1, 0.5 mg TEA/L (aerosol). In this study only local irritating effects in the submucosa of the larynx region of the rats were reported. No systemic effects were reported.

A dermal 90-day study in Fisher rats (Anonymous, 1987 with exposure to 0, 125, 250, 500, 1000 or 2000 mg TEA/kg bw/d, vehicle acetone). The main exposure related effects reported were inflammation of the skin and acanthosis, from 250 mg/kg bw/d in male rats and from 500 mg/kg bw/d in female rats. No dose-response related systemic effects of TEA were reported up to the highest dose tested, 2000 mg/kg bw/d.

Overall, the DS was of the opinion that the oral repeated dose toxicity studies with penta-PSCA Na-TEA, tetra-PSCA and TEA (ion) indicate that there is no need for a classification for STOT RE for any of these substances. Therefore, based on the results from the 28-day study with tetra-PSCA and a read across assessment from penta-PSCA Na-TEA to tetra-PSCA, no classification for STOT RE for tetra-PSCA was proposed.

Comments received during consultation

Comments were received from three MSCAs. Two MSCAs supported no classification for STOT RE since the observed effects below the Guidance Value for classification were insufficient for a classification as STOT RE. One MSCA noted that very limited data on the substance was available for the assessment of STOT RE.

Assessment and comparison with the classification criteria

In the 28-day study (OECD TG 407) study Wistar rats (6/sex/dose) were exposed to 0, 8, 40, 200 and 1000 mg/kg bw/d of tetra-PSCA (purity 90%) by oral gavage (Anonymous, 1995). The study included a recovery period of 14 days for animals exposed to 200 and 1000 mg/kg bw/d tetra-PSCA.

No deaths were reported. Further, no effects were reported on body weight, food consumption and urine analysis. Clinical signs reported included salivation in males and females from 200 mg/kg bw/d, decreased spontaneous movement and decreased respiratory rate in males at 200 mg/kg bw/d. At 1000 mg/kg bw/d in males and females a decrease in spontaneous movement, decrease in respiratory rate, soiling around the nose and mouth, hunchback posture, soiling around the anus and depilation in the lower neck region were reported. No clinical effects were seen at the end of the recovery period. Relative kidney weight was increased in females from 200 mg/kg bw/d and males at 1000 mg/kg bw/d. Male and female liver weight was increased in the 1000 mg/kg bw/d group (24% and 35% respectively). After recovery liver weight in the high dosed group was still increased with 9% in males and 11% in females. Histopathological examinations showed swelling of hepatocytes in males and females at 1000 mg/kg bw/d as well as granulation tissue accompanied by calcification. In addition, effects on forestomach (mucosa degeneration) and kidney (eosinophilic bodies) in males as well as haematological and clinical alteration were observed in males and females. Most of the effects were reversible within the recovery period. A LOAEL of 200 mg/kg bw/d was derived.

In the DRF toxicity study (Anonymous, 2013a) Han Wistar rats were exposed to 0, 100, 300 and 1000 mg/kg bw/d penta-PSCA Na-TEA (3/sex/dose). Male rats were dosed 14 days during pre-mating and 14 days during mating (total 28 days). Females were dosed during pre-mating, mating and 14 days during gestation (total 42 days). During the treatment bedding in mouth was noted in all dose groups (m, f) in a dose-dependent manner. Further, salivation was noted in the high dose group, and these findings were considered to be treatment related.

Differences in mean food consumption of males at 100, 300 and 1000 mg/kg bw/d compared to the control animals were, respectively: -8%, -8% and -29% during the pre-mating period and 0%, -9% and -17% after the mating period. Differences in mean food consumption of females at the dose levels of 100, 300 and 1000 mg/kg bw/d compared to the control animals were,

respectively: -6%, -12% and -29% during the pre-mating period and -5%, -5% and -19% during the gestation period. Differences in mean body weight gain of males at the dose levels of 100, 300 and 1000 mg/kg bw/d were, respectively: +14%, +13% and +4% during the pre-mating period, +4%, +2% and +2% during the mating period and +9%, +7% and +8% after the mating period. Differences in mean body weight gain of females at the dose levels of 100, 300 and 1000 mg/kg bw/d were, respectively: +9%, +9% and +4% during the pre-mating period and +25%, +29% and +20% during the gestation period.

No macroscopic findings were noted in males and females. Clinical chemistry investigations showed statistically significant lower relative haematocrit value (0.4 compared to 0.44 in control) and lower albumin concentration (45.72 g/L compared to 53.99 g/L in control) in females in the high dose group. No further test item related changes in haematology or clinical biochemistry parameters were noted in males or females at any dose level. No organ parameters were examined. A effective dose of 1000 mg/kg bw/d can be derived based on statistically significant reduction in food consumption, reduction of body weight and body weight gain at 1000 mg/kg bw in males and females as well as significant changes in clinical biochemistry in females.

In the OECD TG 422 study Wistar rats (11/sex/dose) were exposed to 0, 40, 200 and 1000 mg/kg bw/d penta-PSCA Na-TEA (purity > 90%) by oral gavage (Anonymous, 2012c). The male rats were exposed for 28 days in total and the female rats for 14 days prior to mating, through the mating and gestation periods until the F1 generation reached day 4 post-partum (in total for approx. 42 days).

The NOAEL for parental toxicity was 40 mg/kg bw/d based on reduced body weight gain and reduced food consumption (see table below). Further from 200 mg/kg bw/d reduced locomotor activity (m, f) and increased salivation (m, f) were reported. At 1000 mg/kg bw/d significantly reduced body temperature (m, f), significantly increased liver weight (m) and liver hypertrophy (m, f), reduced testis and epididymis weights (without histopathological findings) (m), hyaline droplets in kidneys (m), squamous hyperplasia in the forestomach (m, f), follicular cell hypertrophy in the thyroid gland (m, f) were reported. A higher kidney weight to body weight ratio in males in the high dose group as well as a higher brain weight to body weight ratio in high dosed females were considered to be due to the lower body weights.

Table: *Parental toxicity from OECD TG 422.*

	Dose (mg/kg bw/d)	**Males**		**Females**		
Period		Pre-mating	Mating**	Pre-mating	gestation	lactation
Food consumption (g/animal/day)	0	26.1		19.5	26.1	30.3
	40	25.4 (-2.7%)		18.8 (-3.6%)	23.7 (-9.2%)*	27.6 (-8.9%)
	200	23.4 (-10.3%)*		17.2 (-11.8%)*	22.4 (-14.2%)*	20.0 (-34.0%)*
	1000	17.9 (-31.4%)*		13.5 (-30.8%)*	19.0 (-27.2%)*	15.9 (-47.5%)*
Body weight gain (%)	0	+11%	+6%	+9%	+57%	+5%
	40	+8%	+8%	+6%	+55%	+6%
	200	+7%*	+7%	+6%	+49%	+4%
	1000	-1%*	+9%*	+2%*	+33%*	±0%*

Fisher's Exact Test: statistically significant different from controls; ** food consumption not reported.

Further, RAC agrees with the DS that the toxicity of penta-PSCA Na-TEA was not related to exposure to the TEA, in particular from the 90-day oral study with exposure up to 1000 mg/kg bw/d where no systemic effects related to the exposure to TEA was observed.

In summary, RAC supports the assessment of the repeated dose toxicity studies performed by the DS. In the 28-day study in rats with tetra-PSCA, effects were reported from 200 mg/kg bw/d

(within the GV of 30 < C ⩽ 300 mg/kg bw for STOT RE 2). However, the effects observed at 200 mg/kg bw/d were not considered sufficiently severe (moderate and low incidence of eosinophilic bodies in the kidneys of males, increased relative liver weight in females, no significant changes in haematology and clinical chemistry). RAC therefore supports the DS and concludes that no classification for STOT RE is justified based on the CLP criteria.

In the OECD TG 422, the effects induced following exposure to penta-PSCA Na-TEA was observed from of 200 mg/kg bw/d (within the GV of 30 < C ⩽ 300 mg/kg bw (males, 28d) and just at the GV of 20 < C ⩽ 200 mg/kg bw (females, 42d) for a STOT RE 2). The effects reported in the rats were not considered sufficiently severe (loss of appetite, reduction in body weight, and at higher levels moderate toxicity such as reduced body temperature, reduced locomotor activity). RAC therefore supports the DS and concludes that no classification for STOT RE is justified based on the CLP criteria.

In conclusion, based on the data available for tetra-PSCA and the read across from penta-PSCA Na-TEA to tetra-PSCA, RAC is of the opinion that **no classification for STOT RE is warranted for tetra PSCA** according to the CLP criteria.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

For the assessment of effects on sexual function and fertility the DS included an OECD TG 422 study (Anonymous, 2012c) and an associated DRF study (Anonymous, 2013a) in Han Wistar rats with exposure to penta-PSCA Na-TEA in a read across assessment to tetra-PSCA. Both studies were in compliance with GLP.

In the OECD TG 422 study penta-PSCA Na-TEA induced adverse effects on fertility in the absence of marked parental toxicity. The fertility parameters that were affected included the birth index and the pup viability index. These parameters were already significantly altered at 40 mg/kg bw/d. In addition, increased pre-implantation loss, reduced litter size and reduced fertility index were reported in a dose dependant manner. Further, in the high dose group a high incidence of post-implantation loss was reported, and all pregnant females had a total litter loss. These effects were not considered as secondary non-specific consequences of parental toxicity.

The DS clarified that the toxicity of penta-PSCA Na-TEA was not related to exposure to the TEA moiety by assessing a reproduction/developmental screening test (OECD TG 421) (Anonymous, 2010).

Based on the adverse effects reported on sexual function and fertility the DS proposal was to classify tetra-PSCA as Repr. 1B; H360F.

Developmental toxicity

For the assessment of developmental toxicity following exposure to penta-PSCA Na-TEA the DS included an OECD TG 422 screening study in Han Wistar rats (11/sex/group) with exposure to 0, 40, 200 and 1000 mg/kg bw/d (Anonymous, 2012c) and a developmental toxicity study in Wistar rats (5/females/group) with exposure to 0, 8, 40 and 200 mg/kg bw/d from gestation day 6-20 (Anonymous, 2013b). An additional, prenatal developmental toxicity (PNDT) study was performed according OECD TG 414 but with a reduced number of animals. This reduction was chosen as the study was designed to clarify whether the effects found in the OECD TG 422

originated from fertility impairment or was related to fetotoxicity. Both studies were with exposure to penta-PSCA Na-TEA by oral gavage.

In the OECD TG 422 screening study penta-PSCA Na-TEA induced adverse effects on development including a statistically significant increase in post-implantation loss at all dose levels as well as a decrease in the viability index at 40 and 200 mg/kg bw/d. Further, in the high dose group (1000 mg/kg bw/d), all pregnant females had a total litter loss. These effects were not considered to be a secondary non-specific consequence of parental toxicity. In the developmental toxicity study foetotoxic effects such as cleft palate formation, visceral abnormalities (small spleen) and skeletal abnormalities were observed from the lowest dose tested (8 mg/kg bw/d). The effects were seen at doses that were not associated with a maternal toxicity.

No developmental toxicity study in rats or rabbits (i.e. OECD TG 414) was presented by the DS on TEA.

Based on the adverse developmental effects reported the DS proposal was to classify tetra-PSCA as Repr. 1B; H360D.

Adverse effects on or via lactation

No data were presented by the DS in the CLH report.

Setting of specific concentration limits (SCLs)

For tetra-PSCA no data on reproductive toxicity was available. However, one OECD TG 414 study and one OECD TG 422 combined repeated dose toxicity study with the reproduction/developmental screening with the read across substance penta-PSCA Na-TEA were available.

The DS considered that post-implantation loss reported in the OECD TG 422 screening study and small spleen reported in the developmental toxicity study were the leading effects for reproductive toxicity following exposure to penta-PSCA Na-TEA. The resulting ED_{10} values for penta-PSCA Na-TEA for these effects are summarised in the table below.

Table: *ED_{10} values from OECD TG 422*

	Statistically modelling	
Effect	**Linear response**	**Sigmoidal response**
Post-implantation loss	107.7 mg/kg bw/d	117.6 mg/kg bw/d
Small spleen	23.3 mg/kg bw/d	7.8 mg/kg bw/d

The lowest ED_{10} value from the two reproductive toxicity studies for effects warranting classification determined the overall ED_{10} of the substance. For a preliminary potency evaluation, the boundaries according CLP guidance are summarised in the table below.

Table: *Potency boundaries for SCL setting*

Potency group	**Boundaries**
High potency group	ED_{10} value ≤ 4 mg/kg bw/d
Medium potency group	4 mg/kg bw/d < ED_{10} value < 400 mg/kg bw/d
Low potency group	ED_{10} value ≥ 400 mg/kg bw/d

Based on the potency boundaries and the calculated ED_{10} values a medium potency was assumed for penta-PSCA Na-TEA by the DS. In addition, the CLP Guidance (ECHA, 2017, version 5 point 3.7.2.6.5), state that other factors, so called modifying factors, should be taken into account to establish whether the preliminary calculated potency needs to be modified. These factors, and the conclusion on each of them with regards to the potency of penta-PSCA Na-TEA, were assessed by the DS and are summarised below.

- *Type and severity of the effect*: The type of effects observed in the reproductive toxicity studies following exposure to penta-PSCA Na-TEA (the source substance) included beside others post-implantation loss and small spleen at low doses and were considered as severe. As the lowest ED_{10} is close to the boundary of a higher potency group a change of the potency group was considered.
- *Data availability*: The data available for penta-PSCA Na-TEA (OECD TG 422 and OECD TG 414 study, full reports available) were considered adequate. However, only LOAELs could be derived based on the available data. The PNDT study was performed according OECD TG 414 but with a reduced number of animals. This reduced design was chosen as the study aimed to clarify whether the effects found in the OECD TG 422 originated from fertility impairment or fetotoxicity.
- *Dose-response relationship*: The lowest ED_{10} (7.8 mg/kg bw/d, small spleen) of penta-PSCA Na-TEA was similar to the LOAEL of 8 mg/kg bw/d.
- *Mode or mechanism of action:* No information was available.
- *Toxicokinetic:* No information was available.
- *Bio-accumulation*: penta-PSCA Na-TEA was not considered to be bioaccumulating based on the REACH registration data.

In addition, according to the DS, it had to be considered that the studies were conducted with the salt penta-PSCA Na-TEA and the reprotoxic effects may be due to the dissolving product penta-PSCA and also considered relevant for tetra-PSCA. The dissolved UVCB comprises about only 55% penta-PSCA. For the pure substance (acid) even lower effect levels can be assumed.

Conclusion on modifying factors

Based on the available data, penta-PSCA-Na-TEA is considered as a medium potency toxicant. As the ED_{10} is closed to the high potency group and the reported developmental toxicity effects are severe with a LOAEL at 8 mg/kg bw/d a shift into the high potency group can be considered. No additional modifying factors are considered to influence the potency.

Conclusion on concentration limit

Small spleen was the most sensitive adverse effect reported from 8 mg/kg bw/d, the lowest dose tested with an ED_{10} of 7.8 mg/kg bw/d. Other adverse effects reported (increased number of supernumerary ribs and skeletal abnormalities) were reported following exposure from 40 mg/kg bw/d of penta-PSCA Na-TEA. The potency of penta-PSCA Na-TEA was considered as a borderline case between medium and high potency. When considering the severity of the effects and the nature of the test substance penta-PSCA Na-TEA (UVCB, including 55% penta-PSCA) a specific concentration limit of 0.03% for penta-PSCA was proposed by the DS and also considered relevant for tetra-PSCA.

Relevant studies with TEA

It was clarified that the toxicity of penta-PSCA Na-TEA was not related to exposure to the TEA moiety by assessing an OECD TG 421 study with TEA:

In a reproduction/developmental screening test (OECD TG 421) Wistar rats exposed via oral gavage to 0, 100, 300 or 1000 mg/kg bw/d TEA. Males were exposed for 2 weeks premating, 2 weeks mating and 1 week post-mating, females for 2 weeks premating, max 2 weeks mating, gestation and up to lactation day 4. General toxicity: Transient salivation was reported in most high-dose animals and one low-dose animal for a few minutes immediately after exposure, however, was likely to be induced by the unpleasant taste of TEA or by local irritation of the upper digestive tract, and not considered to be a sign of systemic toxicity. A slightly lower body weight gain in the 1000 mg/kg bw/d females during gestation was likely caused by an increase in post-implantation loss rather than a systemic toxic effect of the TEA. Reproductive toxicity: In

the low- and mid-dose group no adverse effects on reproductive performance or fertility were reported. In the high-dose group, a lower mean number of implantation sites (about 20% below control levels), increased post-implantation loss (19.4%* [*p≤0.05] vs. 3.7% in control) and a lower average litter size (about 33% below control) was reported. No further details were available. No test substance-related adverse findings were reported in F1 pups.

Based on the effects reported for maternal systemic toxicity a NOAEL of > 1000 mg/kg bw/d was set. For developmental toxicity a NOAEL of 300 mg/kg bw/d was set.

The pronounced effects reported at low doses with penta-PSCA Na-TEA showed that the effects reported were attributed to exposure to penta-PSCA, which is systemically available when dissolved in biological fluids and not to the presence of TEA. Further, TEA was assessed in the REACH substance evaluation process and it was concluded that toxicity for reproduction was not identified as an initial or as an additional concern (UK, 2014).

Based on thorough analysis of all available information a read-across approach for the endpoint reproductive toxicity is considered appropriate for penta-PSCA Na-TEA to tetra-PSCA.

Comments received during consultation

Comments were received from five MSCAs all supporting a classification as Repr. 1B; H360FD based on a read across from penta-PSCA Na-TEA.

As regards the setting of a SCL, one MSCA asked for a calculation of the ED_{10} for tetra-PSCA taking into account the molecular ratio correction between tetra-PSCA and penta-PSCA Na-TEA considering that this could support a SCL for tetra-PSCA of 0.03%. One MSCA did not support a SCL of 0.03% and considered that the GCL should be applied. The MSCA considered that a SCL should only be set when available data allow to set a SCL and this was maybe not appropriate for an UVCB substance since they are comprised of variable components. Further, the MSCA included that the reproductive toxicity assessment was based read-across from penta-PSCA Na-TEA and that this further added to the uncertainty of the data for the potency determination of tetra-PSCA. One MSCA asked for a reflection on setting separated SCL for developmental toxicity and effects on fertility and sexual function since the CLP Guidance (paragraph 3.7.2.6.6.1) describes that "concentration limits have to be determined separately for the two main types of reproductive toxic effects". Two MSCA supported the SCL of 0.03% since the OECD TG 422 study performed with penta-PSCA Na-TEA comprised only 55% penta-PSCA. One of the MSCA noted that in addition to the ED_{10} values calculated by the DS for effects on development an ED_{10} value affecting fertility specifically e.g. the fertility index should have been be calculated. The DS provided additional elements to calculate the ED_{10} as well as the effects of concern that trigger the potency determination.

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

For the assessment of effects on sexual function and fertility following exposure to tetra-PSCA no studies were available. The DS therefore included for the assessment an OECD TG 422 study (Anonymous, 2012c) and associated DRF study to the OECD TG 422 study (Anonymous, 2013a) in Han Wistar rats and in compliance with GLP. No historical control data for the effects on sexual function and fertility were included in the CLH dossier.

Based on the results from the OECD TG 422 DRF study with exposure to 0, 100, 300 and 1000 mg/kg bw/d of penta-PSCA Na-TEA (purity 90%) showing a decreased fertility index and birth index in the high dose group in the presence of decreased body weight gain and reduced food

consumption, the doses for the main OECD 422 study were 0, 40, 200 and 1000 mg/kg bw/d (purity > 90%).

In the OECD 422 study rats 11/sex/dose group were orally dosed by gavage. Males were exposed for 4 weeks and females for approximately 7 weeks.

The percentage of mating was 100% assessed by the presence of copulation plug or sperm in all dose-groups. No effects were reported on the gestation length and corpora lutea as well as implantations. A reduction in the fertility index and gestation index was reported in the high dose group and a reduction in the birth index and viability index in all dose-groups, see table below.

Table: *Reproductive parameters*

Dose (mg/kg bw/d)	**0**	**40**	**200**	**1000**
Fertility index (%)	100.0	90.9	90.9	72.7
Gestation index (%)	100.0	100.0	90.0	0.0*
Birth index (%)	96.7	84.3*	68.8*	0.0*
Viability index (%)	99.2	91.6*	69.3*	na

*Fisher's Exact Test, significant at 1%; na, not applicable.

Further, and increase in post-implantation loss at all dose levels (mean incidence per dam: 0.4, 2.0*, 3.8* and 8.5* at 0, 40, 200 and 1000 mg/kg bw/d), a reduction of litter size (mean number of living pups per dam 11.9, 10.7, 8.8 and 0* respectively), in viability index in the low- and mid-dose groups (no live pups in the high dose group were reported), see table below. It should be noted that in male rats a statistically significant decrease in the left and right testis and epididymis weight was reported, however, no histopathological changes were found.

Table: *Breeding parameters*

Dose (mg/kg bw/d)	**0**	**40**	**200**	**1000**
Number of litters	11	10	10	8
Post-implantation loss (total/mean)	4/0.4	20**/2.0##	34**/3.8#	68*/8.5##
Living pups at birth (total/mean)	131/11.9	107/10.7	88/8.8	0##/0
Dead pups at birth (total/litters affected)	0/0	5/4*	24/4*	7/2
Living pups on PND 4 (total/mean)	130/11.8	98/9.8	61/6.1#	0/0.0##
Postnatal loss PND 0-4 (total/litters affected)	1/1	9**/1	27**/5	0/0

Steel test, significant at 5% (#), 1% (##); Fischer´s Exact test, statistically significant at 5% (*), 1% (**); PND postnatal day

Parental toxicity: Salivation was noted in the high dose group in males and females. Reduced body weight gain and food consumption in the mid- and high-dose group was reported, see table below.

Table: *Food consumption and body weight gain in males and females*

	Dose (mg/kg bw/d)	**Males**		**Females**		
Period		Pre-mating	Mating**	Pre-mating	gestation	lactation
Food consumption (g/animal/day)	0	26.1		19.5	21.6	30.3
	40	25.4 (-2.7%)		18.8 (-3.6%)	23.7 (-9.2%)*	27.6 (-8.9%)
	200	23.4 (-10.3%)*		17.2 (-11.8%)*	22.4 (-14.2%)*	20.0 (-34.0%)*
	1000	17.9 (-31.4%)*		13.5 (-30.8%)*	19.0 (-27.2%)*	15.9 (-47.5%)*
Body weight gain (%)	0	+11%	+6%	+9%	+57%	+5%
	40	+8%	+8%	+6%	+55%	+6%
	200	+7%*	+7%	+6%	+49%	+4%
	1000	-1%*	+9%*	+2%*	+33%*	±0%*

* Fisher's Exact Test: statistically significant different from controls; ** food consumption not reported.

The DS clarified that the toxicity of penta-PSCA Na-TEA was not related to exposure to the TEA moiety by assessing an OECD TG 421 study with TEA. In this study some reproductive toxicity parameters were altered only at the highest dose tested (1000 mg/kg bw/d) without maternal toxicity.

The pronounced effects reported at low doses with penta-PSCA Na-TEA showed that the effects reported were attributed to exposure to penta-PSCA, which is systemically available when dissolved in biological fluids and not to the presence of TEA.

Summary

In the OECD TG 422 study, penta-PSCA Na-TEA induced adverse effects on fertility. The fertility parameters affected included a decrease in the birth index and the gestation index. These parameters were already statistically significantly altered at 40 mg/kg bw/d. In addition, a reduced litter size and reduced fertility index was reported in a dose dependent manner. Further, in the high dose group with a high incidence of post implantation loss all pregnant females had a total litter loss. RAC considers that the effects on reproduction reported are considered not to be a secondary non-specific consequence of parental toxicity.

Based on thorough analysis of all available information including an OECD TG 421 study on TEA with some reproductive parameters affected at 1000 mg/kg bw/d, RAC considers that a read-across approach for the endpoint reproductive toxicity from penta-PSCA N-TEA is considered appropriate for tetra-PSCA.

RAC supports the DS and is of the opinion that based on the clear evidence of adverse effects reported on sexual function and fertility following exposure to penta-PSCA Na-TEA and read across to tetra-PSCA **classification of tetra-PSCA as Repr. 1B; H360F is warranted**.

Developmental toxicity

For the assessment of developmental toxicity following exposure to tetra-PSCA no studies were available. The DS therefore included for the assessment two studies with oral exposure to penta-PSCA Na-TEA. The first study was an OECD TG 422 screening study in Han Wistar rats (11/sex/group) with exposure to 0, 40, 200 and 1000 mg/kg bw/d (purity > 90%). The second developmental toxicity study (OECD TG 414) was conducted in Wistar rats (5/females/group) with exposure to 0, 8, 40 and 200 mg/kg bw/d from gestation day 6-20. No historical control data for the developmental effects reported were included in the CLH dossier.

In the OECD TG 422 screening study the incidence of post-implantation loss was statistically significant increased from 40 mg/kg bw/d (0.4, 2.0*, 3.8* and 8.5* in the 0, 40, 200 and 1000 mg/kg bw/d dose-group, respectively). Further, a reduction of litter size was seen at all dose levels (mean number of living pups per dam 11.9, 10.7, 8.8 and 0* in the 0, 40, 200 and 1000 mg/kg bw/d, respectively). All pregnant high dose females lost their litter completely at birth. The birth index was statistically significant reduced in all dose groups (96.7%, 84.3%*, 68.8%*, 0.0%*), as well as pup mortality (days 0-4) at 40 and 200 mg/kg bw/d (total number: 1, 9**, 27**, 0 in the 0, 40, 200 and 1000 mg/kg bw/d dose group, respectively). Parental toxicity was evident as a reduction in food consumption and body weight gain in the mid- and high-dose animals. For further details of the study see the section RAC assessment of effects on fertility and sexual function.

In the developmental toxicity study, all females survived until scheduled necropsy, and no maternal toxicity was reported. In the high dose group (200 mg/kg bw/d) the food consumption and the body weight gain were slightly reduced, see the table below.

Table: *Food consumption and body weight gain in female rats*

Dose (mg/kg bw/d)	Food consumption, different from control	BW gain during treatment	Corrected BW gain* during treatment
0	0.0%	+55%	+17.9%
8	+3.3%	+51%	+12.6%
40	+3.7%	+53%	+13.1%
200	-6.6%	+52%	+14.4%

*corrected for gravid uterus weight

All female rats in the study were pregnant. No differences were reported for control and exposed rats regarding the number of corpora lutea, implantation sites and number of live foetuses, see results in the table below. During necropsy enlarged placentas were found in one control dam and in one high dosed dam and were therefore not considered as treatment related.

Table: *Reproduction data*

Dose (mg/kg bw/d)	0	8	40	200
Corpora Lutea	77	79	75	76
Pre-implantation loss	5	5	5	12
Implantation sites	72	74	70	64
Post-implantation loss	11	5	2	4
Embryonic resorptions	10	4	2	4
Foetal resorptions	1	1	0	0
Foetus total	61	69	68	60

No effects on foetal body weights or differences in the sex ratio were reported at any dose level. The foetal evaluation included external examinations of all pups per litter as well as soft tissue examinations, skeletal examinations and head examinations for half of the pups per litter, see table below.

Table: *Foetal toxicity*

Dose (mg/kg bw/d)		**0**	**8**	**40**	**200**
Foetuses examined		**N=32**	**N=36**	**N=35**	**N=32**
Spleen	Spleen small* or small severe**	1 (3%)	4 (11%)	17 (49%)	32 (100%)
	Small*	1 (3%)	4 (11%)	17 (49%)	32 (100%)
	Small severe **	0	0	1 (3%)	27 (84%)
Dose (mg/kg bw/d) Foetuses examined		**0 N=29**	**8 N=33**	**40 N=33**	**200 N=28**
Supernumerary ribs	Left	7 (24%)	8 (24%)	26 (79%)	24 (82%)
	Right	8 (28%)	5 (15%)	24 (73%)	25 (89%)

*Small spleen: approx. 75% of expected size

**Severe small spleen: approx. 50% of expected size

In the external examination two foetuses from one litter at 200 mg/kg bw/d exhibited rare abnormalities. One of the foetuses had no lower jaw, small mouth opening and possibly a cleft palate and the second foetuses had a cleft palate.

Visceral abnormalities were seen in all foetuses in the 200 mg/kg bw/d. These included small spleen and in seven foetuses incomplete fusion of nasal septum to palate was observed. Small spleen was found from 8 mg/kg bw/d (see the table above).

Skeletal abnormalities were reported in all examined foetuses (n=28) at 200 mg/kg bw/d, comprising thin skull zygomatic jugal arch, abnormal curvature of pectoral girdle clavicle. Additional findings were absent humerus deltoid tuberosity in forelimb in 24 foetuses, short mid

region of rib cage in 17 foetuses, abnormal curvature of hyoid body in 14 foetuses and abnormal spacing of zygomatic arch structures in 6 foetuses.

Variations were also noted in the 200 mg/kg bw/d dose group. These included an increased incidence of ossification/thick tympanic ring in 28 foetuses, fusion of zygomatic arch in 22 foetuses, increased ossification of scapula in pectoral gridle in 13 foetuses and slight curved or slightly bent forelimb radius in 10 foetuses.

Further, and increased number of supernumerary ribs was reported from 40 mg/kg bw/d (see table above).

In this study, clear signs of developmental toxicity were reported at doses that were not associated with maternal toxicity.

It is noted that no developmental toxicity study was available for TEA.

RAC agrees with the DS that the pronounced effects reported at low doses with penta-PSCA Na-TEA showed that the effects reported were attributed to exposure to penta-PSCA, which is systemically available when dissolved in biological fluids and not to the presence of TEA.

Summary

In the OECD TG 422 screening study penta-PSCA Na-TEA induced adverse effects on development including a statistically significant increase in post-implantation losses at all dose levels as well as pup mortality (PND0-4) at 40 and 200 mg/kg bw/d and no live pups at 1000 mg/kg bw/d. These effects were not considered to be a secondary non-specific consequence of parental toxicity. In the PNDT study (OECD TG 414), foetotoxic effects such as cleft palate formation, visceral abnormalities (small spleen) and skeletal abnormalities were observed from the lowest dose tested (8 mg/kg bw/d). These effects were seen at doses that were not associated with maternal toxicity.

Based on thorough analysis of all available information including an OECD TG 421 study on TEA a read-across approach for developmental toxicity from penta-PSCA Na-TEA is considered appropriate for tetra-PSCA. Further, it is noted that TEA was assessed in the REACH substance evaluation process and it was concluded that toxicity for reproduction was not identified as an initial or as an additional concern (UK, 2014).

RAC supports the DS and is of the opinion that based on the clear evidence of adverse foetotoxic effects reported on the developing foetuses following exposure to penta-PSCA Na-TEA and a read across to tetra-PSCA **classification of tetra-PSCA as Repr. 1B; H360D is warranted**.

Adverse effects on lactation

No data were available, therefore no assessment of adverse effects on lactation has been performed by RAC.

Setting of specific concentration limits (SCLs)

According to the CLP guidance, the setting of specific concentration limits should only be performed when adequate and reliable scientific information are available. Since the classification of tetra-PSCA is based on the read across from penta-PSCA Na-TEA, an UVCB substance of variable components, there may be uncertainties in using the data on the nature of the test substance penta-PSCA Na-TEA, an UVCB substance that includes 55% penta-PSCA, in the potency determination for tetra-PSCA.

RAC supports the DS assessment for the setting of concentration limits from the OECD TG 422 screening reproductive toxicity study and the developmental toxicity study in rats following exposure to penta-PSCA Na-TEA. During the general consultation, it was noted that separated

SCL for developmental toxicity and effects on fertility and sexual function should be set according to the CLP Guidance (paragraph 3.7.2.6.6.1). Therefore, RAC has assessed separately the SCL for effects on sexual function and fertility and for developmental toxicity.

Concentration limit for effects on fertility and sexual function

The most sensitive effects on fertility and sexual function reported in the OECD TG 422 study with penta-PSCA Na-TEA is considered to be a 10% decrease in the fertility index at 40 mg/kg bw/d, with a corresponding ED_{10} at 40 mg/kg bw/d. The DS used the decrease in post-implantation loss for deriving an ED_{10} for reproductive toxicity, however, RAC is of the opinion that this effect should be used for setting SCL for developmental toxicity, since effects on post-implantation loss is considered for a classification for developmental toxicity (CLP Guidance on setting of SCL, example No. 1). RAC considers that an assessment of modifying factors is not relevant for setting a SCL for effects on sexual function and fertility. Due to the ED_{10} value obtained it is not relevant to modify the potency group (CLP Guidance, ECHA 2017, version 5, point 3.7.2.6.5). When considering the severity of the effects and the nature of the test substance penta-PSCA Na-TEA (UVCB, including 55% penta-PSCA) RAC still considers that the general concentration limit (GCL) is supported since when taking into account that the penta-PSCA Na-TEA includes 55% penta-PSCA, penta-PSCA is still falling into the moderate potency group with a GCL (0.3% w/w).

Concentration limit for developmental effects

For penta-PSCA Na-TEA post-implantation losses and small spleen were considered as the main effects for developmental toxicity, with the resulting ED_{10} values shown in the table above in the summary of the DS proposal. Decreased expected spleen weights were not associated at low dose levels with effects on foetal body weights or at any dose level with histopathological changes. The mechanism for this effect is unknown but it may be ascribed to immunotoxicity, as proposed by the DS.

As regards the assessment of the modifying factors, RAC agrees with the DS that based on the reported effects on the spleen, penta-PSCA Na-TEA could be considered as a borderline between the medium and high potency group with an LOAEL at 8 mg/kg bw/d and ED_{10} of 7.8 mg/kg bw/d). Other adverse effects (increased number of supernumerary ribs and skeletal abnormalities) were reported following exposure from 40 mg/kg bw/d of penta-PSCA Na-TEA.

However, RAC considers that since there are no data available for the assessment of developmental toxicity following exposure to tetra-PSCA itself and consequently no ED_{10} values can be derived, and a read-across assessment is used for the classification for developmental effects, the GCL as applied for penta-PSCA Na-TEA should be used for tetra-PSCA as well.

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).