Committee for Risk Assessment

RAC

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

6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts (Penta-PSCA Na-TEA)

EC Number: -
CAS Number: -

CLH-O-0000006925-64-01/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 public 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

10 December 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts

(Penta-PSCA Na-TEA)

EC Number: -

CAS Number: -

Index Number: -

Contact details for dossier submitter:

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on behalf of the Austrian Competent Authority (Austrian Federal Ministry for sustainability and tourism, Stubenring 1, 1010 Vienna, Austria)

Version number: 02 **Date: November 2019**

Note:

This chemical is a member of a group of three 2,5-dioxopyrrolidin hexanoates. Their high structural similarity justifies read-across among them for a number of hazard classes. However, their proposed harmonised classification is presented in three different dossiers as they differ in their skin and eye irritating properties.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON FOR 6-[C12-18-ALKYL-(BRANCHED, UNSATURATED)-2,5-DIOXOPYRROLIDIN-1-YL]HEXANOIC ACID, SODIUM AND TRIS(2-HYDROXYETHYL)AMMONIUM SALTS

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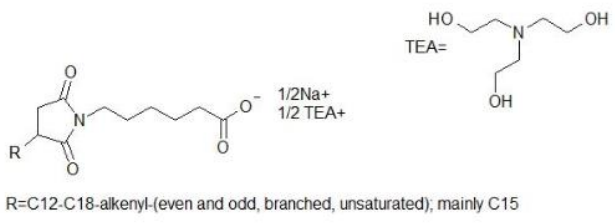
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance (source: ECHA dissemination site)

Name(s) in the IUPAC nomenclature or other international chemical name(s)	6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts
Other names (usual name, trade name, abbreviation)	Penta-PSCA Na-TEA (Pentapropenyl succinimido)-hexanoate, sodium and triethanolamine salts
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	-
EC name (if available and appropriate)	-
CAS number (if available)	-
Other identity code (if available)	-
Molecular formula	$C_{22}H_{36}NO_4.1/2Na.1/2C_6H_{16}NO_3$ - $C_{28}H_{48}NO_4.1/2Na.1/2C_6H_{16}NO_3$
Structural formula	 R=C12-C18-alkenyl-(even and odd, branched, unsaturated); mainly C15
SMILES notation (if available)	-
Molecular weight or molecular weight range	~531
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	Conf
Degree of purity (%) (if relevant for the entry in Annex VI)	Conf

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)
sodium/triethanolamine- 6-(3-C12-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoate	conf	-	-
sodium/triethanolamine- 6-(3-C13-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoate	conf	-	-
sodium/triethanolamine- 6-(3-C14-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoate	conf	-	-
sodium/triethanolamine- 6-(3-C15-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoate	conf	-	-
sodium/triethanolamine- 6-(3-C16-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoate	conf	-	-
sodium/triethanolamine- 6-(3-C17-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoate	conf	-	-
sodium/triethanolamine- 6-(3-C18-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoate	conf	-	-
Pentapropene	conf	-	-
ε-caprolactam EC 203-313-2	conf	Acute Tox. 4 *, H302 Skin Irrit. 2, H315 Eye Irrit. 2, H319 Acute Tox. 4 *, H332 STOT SE 3, H335	Acute Tox 3, H331 Acute Tox 4, H312 STOT SE 1, H370 STOT RE 1 , H372
Water EC 231-791-2	conf	-	-

* for concentration ranges see confidential Annex II

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: Classification and Labelling

	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-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts	-	-	Repr. 1B Eye Irrit. 2	H360FD H319	GHS08 GHS07 Dgr	H360FD H319			
Resulting Annex VI entry if agreed by RAC and COM	TBD	6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts	-	-	Repr. 1B Eye Irrit. 2	H360FD H319	GHS08 GHS07 Dgr	H360FD H319			

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	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*hazard class not assessed in this dossier*	No
Acute toxicity via dermal route	*hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*hazard class not assessed in this dossier*	No
Skin corrosion/irritation	*data conclusive but not sufficient for classification*	Yes
Serious eye damage/eye irritation	Eye Irrit. 2; H319	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	Repr. 1B; H360FD	Yes
Specific target organ toxicity-single exposure	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-repeated exposure	*data conclusive but not sufficient for classification*	Yes
Aspiration hazard	*hazard class not assessed in this dossier*	No

Hazard class	Reason for no classification	Within the scope of public consultation
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

No harmonized classification so far.

The current self-cassification for the substance is Skin Irrit 2, H315 and Repr. 1B, H360.

RAC general comment

The salt of the weak acid 6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts (**hereafter penta-PSCA Na-TEA**) is a salt of a weak acid, 6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (hereafter **penta-PSCA**) which is also structurally similar to 6-[(C10-C13)-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (hereafter **tetra-PSCA**). When penta-PSCA Na-TEA is dissolved in biological fluid an immediate dissociation to sodium ion, triethanolammonium ion (hereafter TEA) and penta-PSCA can be assumed.

Read across assessment

An analogue read-across approach between penta-(polypropenylsuccinimido)-caproic acid (PSCA) (source), tetra-PSCA (source) and penta-PSCA sodium-triethanolamine (Na-TEA) (target, Figure) has been proposed by the 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. The three PSCA are UVCB substances.

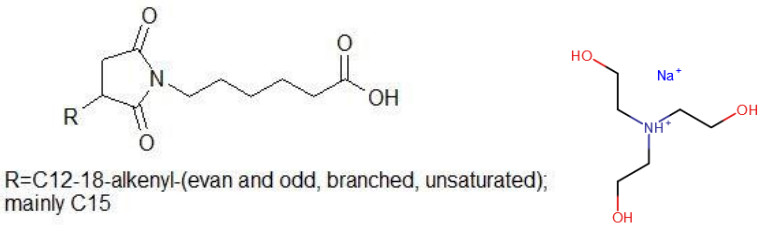


Figure: chemical structure of the salt penta-PSCA with sodium (Na^+) and triethanolammonium (TEA) ions

Three Member States Competent Authorities (MSCA) agreed with the read-across approach during the general consultation whereas two additional MSCA 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 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. The assessment of the classification for reproductive toxicity is based on studies performed with the substance itself, penta-PSCA Na-TEA. For the assessment of the STOT RE a 28-day study in rats with exposure to tetra-PSCA was included for in addition to the OECD TG 422 performed with penta-PSCA Na-TEA. For local effects RAC considers that the classification should be based on data on penta-PSCA Na-TEA supported by data on penta-PSCA and triethanolamine (TEA; 2,2',2''-nitrilotriethanol, CAS no. 102-71-6, EC no. 203-049-8).

Purity of penta-PSCA Na-TEA

Based on the structure and the molecular weight of penta-PSCA Na-TEA (ranging from 465 to 549) the DS stated that the theoretic fraction of TEA will be 13-16%.

However, in the OECD TG 422 study as well as the associated dose range finding study with penta-PSCA Na-TEA a certificate of analysis for this batch gave the following result on the composition of the UVCB:

- Pentapropylenesuccinimido-caproate: 55.0%
- Sodium: 2.9%
- TEA: 31.2%
- Water: 9.2%
- Olefins: 1.7%

For the OECD TG 414 study with penta-PSCA Na-TEA no information on detailed composition was available. However, as the study was conducted at the same laboratory in the same time period as the OECD TG 422 study and associated dose-range finding study, sponsored by the same industry, the same composition was assumed.

Triethanolamine (TEA; 2,2',2''-nitrilotriethanol, CAS no. 102-71-6, EC no. 203-049-8), a dissolving product of penta-PSCA Na-TEA, is not considered to influence the anticipated (sub)chronic toxicity as shown by data (as presented by the DS) on repeated dose toxicity and reproductive toxicity on TEA. The corrected NOAELs/LOAELs for maternal toxicity were a factor of 10-80 higher following exposure to TEA than the derived values for penta-PSCA Na-TEA and tetra-PSCA. For reproductive toxicity a factor of 20-120 applies indicating that the observed effects were due to the dissolving product penta-PSCA and not due to TEA.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

Further detail on need of action at Community level

The substance Penta-PSCA Na-TEA is a presumed human reproductive toxicant. The proposed classification is based on read-across with data from other structurally similar 2,5-dioxo-pyrrolidin hexanoates, comprising Tetra-PSCA and Penta-PSCA. A read-across justification is provided in Annex I to the CLH report. The proposed harmonised classifications of Tetra-PSCA, Penta-PSCA and Penta-PSCA Na-TEA are presented in three different CLH dossiers as they differ in their skin and eye irritating properties.

5 IDENTIFIED USES

Currently there is no active registration for this substance. Information on possible uses according to ECHA dissemination site[1] is given below.

	Use(s)	Technical function
Manufacture	Manufacturing and Filling of Penta-PSCA Na-TEA	-
Formulation	Formulation of products containing Penta-PSCA Na-TEA at industrial or dedicated professional sites	PC 17: Hydraulic fluids PC 24: Lubricants, greases, release products PC 25: Metal working fluids
Uses at industrial sites	Industrial use of Penta-PSCA Na-TEA for lubricans, grease, release products and metal working fluids	PC 17: Hydraulic fluids PC 24: Lubricants, greases, release products PC 25: Metal working fluids
Uses by professional workers	Professional use of Penta-PSCA Na-TEA for lubricans, grease, release products and metal working fluids	PC 17: Hydraulic fluids PC 24: Lubricants, greases, release products PC 25: Metal working fluids
Consumer Uses	-	-
Article service life	-	-

6 DATA SOURCES

The data included in this CLH report originate from the registration dossier submitted to ECHA and disseminated on ECHA website [https://echa.europa.eu/de/information-on-chemicals; accessed November 2018].

[1] Information on former registration of the substance can be found under the old substance ID: EC800-766-3

Original study reports of Anonymous (2012c), Anonymous (2013a) (OECD 422 study and previous dose rang finding study) and Anonymous (2013b) (OECD 414 study) have been provided by registrants and were used according REACH, Artikel 118/119.

Confidential data from IUCLID dossiers is presented in the confidential Annex II.

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	liquid	REACH registration	-
Melting/freezing point	4 ± 3 °C.	REACH registration	OECD 102
Boiling point	112 ± 13 °C at 101.9 kPa	REACH registration	OECD 103
Relative density	1.07	REACH registration	OECD 109
Vapour pressure	10^{-8}Pa	REACH registration	calculated
Surface tension	31.6 ± 0.1 mN/m at a concentration of 1g/l at 20°C	REACH registration	OECD 115
Water solubility	0.077 ± 0.039 g/L at 20°C	REACH registration	ISO 4311 The critical micelle concentration is an appropriate parameter describing water solubility of surface active materials.
Partition coefficient n-octanol/water	3.64 at 20°C	REACH registration	OECD 107
Flash point	>= 135°C	REACH registration	Pensky-Martens method
Flammability	-	-	-
Explosive properties	-	-	-
Self-ignition temperature	395 °C	REACH registration	EU A.15
Oxidising properties	-	-	-
Granulometry	-	-	-
Stability in organic solvents and identity of relevant degradation products	-	-	-
Dissociation constant	4.74 ± 0.2 at 25°C	REACH registration	Read-across to Penta-PSCA
Viscosity	42000 ± 1000 mPa.s (dynamic, 20°C), 4290 ± 110 mPa.s (dynamic, 40°C	REACH registration	OECD 114

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Penta-PSCA Na-TEA is a salt of a weak acid and is expected to dissociate immediately when dissolved in aqueous systems or in biological fluids. Manufacture and use of the substance occurs only as dissolved in aqueous systems. Therefore, the chemical species relevant for the uptake and the systemic burden is the acid form Penta-PSCA.

No experimental data on the toxicokinetic property of Penta-PSCA is available. In the registration dossier an assessment based on available toxicity data has been made.

The Penta-PSCA has a molecular mass around 420 g/mol and a log Pow of 3.64. Based on these parameters it can be concluded that the substance will be readily absorbed and distributed via lymphatic tissues.

A repeated dose study with the substances Penta-PSCA Na-TEA indicate that the liver is the target organ after oral administration. Findings like liver weight increase, hepatocellular hypertrophy, follicular cell hypotrophy in thyroid gland, prolonged bleeding time, altered values of glucose and cholesterols are indicative of an adaptive mechanism in the liver. No effects were seen after a recovery period of 14 days indicating that the chemical burden must have decreased rapidly with the cessation of treatment.

In *in vitro* genotoxicity studies with the read-across substance Tetra-PSCA the cytotoxicity with S9-mix was significantly reduced when compared to tests without S9-mix. Metabolic activation **through induction** of liver enzymes seems to be associated with detoxification.

Based on general rules of biotransformation, the most likely degradation pathway is the ß-oxidation of N-alkyl chain, followed by hydrolysis at the imine/imide moiety. The resulting metabolite, highly branched alkenyl-succinic acid, could undergo urinary excretion either as it is, or conjugated. No bioaccumulation is expected.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

No assessed

10.2 Acute toxicity - dermal route

No assessed

10.3 Acute toxicity - inhalation route

No assessed

10.4 Skin corrosion/irritation

For the evaluation of the skin irritating potential of Penta-PSCA Na-TEA one study with the substance itself, one study with the corresponding acid Penta-PSCA (source substance) and several studies with triethanolamine (TEA) are available.

Table 6: Summary table of animal studies on skin corrosion/irritation for Penta-PSCA Na-TEA (source: ECHA dissemination site).

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD guideline 404 GLP	Rabbit, New Zealand White n=3	test material contained Penta-PSCA Na-TEA by ca. 40% (other components: teak oil, oleyl alcohol, polypropylen, and water)	No vehicle (amount unknown) 4h semiocclusive Observation periode 14 d	Erythema score #1: mean (24, 48, 72h) = 2 (max. 2) #2: mean (24, 48, 72h) = 3 (max. 3) #3: mean (24, 48, 72h) = 2 (max. 2) Fully reversible within 14 days Oedema score #1: mean (24, 48, 72h) = 0.33 (max. 1) #2: mean (24, 48, 72h) = 0.33 (max. 1) #3: mean (24, 48, 72h) = 0 (max. 0) Fully reversible within 2 days	Anonymous, 1993a
OECD 404 GLP	Rabbit, New Zealand White n=3	Penta-PSCA (purity unknown)	0.5 ml (no vehicle) 4h semiocclusive Observation periode 7 d	Erythema score mean (24, 48, 72h) = 1.1 (max. 2) Fully reversible within 7 days Oedema score mean (24, 48, 72h) = 0	Anonymous, 1993b

Table 7: Summary table of animal studies on skin corrosion/irritation for Triethanolamine (CAS 102-71-6) (source: ECHA dissemination site).

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 404 non GLP	Rabbit, Vienna White 1male + 2 females	0.5 ml 85 % Triethanolamine and 15 % Diethanolamine	4h Occlusive removed 4h after exposure	Erythema Mean score (24, 48, 72h) = 0 Oedema Mean score (24, 48, 72h) = 0	Anonymous, 1983
OECD 404 non GLP	Rabbit, Vienna White	0.5 ml Triethanolamine	1, 5, 15 min and 20 hours	Erythema score (at 24h): 1min application time = 0	Anonymous, 1971a

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
	2 males (1, 5, 15 min) 2 males (20 hrs)	98 %	occlusive	5min application time = 0 15min application time = 0 20h application time = 2	
OECD 404 non GLP	Rabbit, Vienna White 2 animals (1', 5' and 15') 2 animals 20 hours	0.5 ml lutrol	1, 5, 15 min and 20 hours occlusive	application times of 1, 5 and 15min: Erythema and oedema score (mean 24, 48, 72h) = 0 20h application time: Erythema score = 1 Oedema score = 0	Anonymous, 1966a
OECD 404 non GLP	rabbit	unchanged or as 20 % solution	1,5 and 15 min occlusive	TEA did not cause skin irritation on rabbits upon short incubation periods up to 15 min neither when applied unchanged nor applied as 20 % solution at pH 10	Anonymous, 1956a
OECD 404 non GLP	Rabbit, Vienna White 2 animals (1,5,15 min) 2 animals (20 hrs)	0.5 ml Triethanolamine, technical	1, 5, 15 min and 20 hrs occlusive	Erythema score (mean 24, 48, 72h) 1min application time = 0.5 5min application time = 0.5 15min application time = 0.75 20h application time = 2 oedema score (mean 24, 48, 72h) 1min application time = 0 5min application time = 0 15min application time = 0 20h application time = 0	Anonymous, 1967a

Table 8: Dermal repeated dose toxicity of TEA after dermal application (source: ECHA dissemination site)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD 411 GLP not specified Rat, Fischer 344 n=20/sex/dose	TEA 125; 250; 500; 1000; 2000 mg/kg bw/day Vehicle: acetone 90days (5d/week)	<u>Males:</u> - Irritation at the site of application in the three highest dose groups (0.5, 1.0, and 2.0 g/kg), incidence increasing and time to onset decreasing with increasing dose level. - Scaliness at 1.0 and 2.0 g/kg levels; this was observed in 1 rat in the 1.0 g/kg group (first seen on	Anonymous, 1987

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Day 13), and in 5 rats in the 2.0 g/kg group (first seen on Day 6). - Crustiness at the site of application was recorded for 10 rats in the 2.0 g/kg dose group - Ulceration at the application site at 2.0 g/kg in two males from this group were also observed to have ulceration. <u>Females</u>: - Irritation at the 1.0 (7 rats) and 2.0 (10 rats) g/kg dose levels - Scaliness in 4 animals dosed at the 1.0 g/kg level, and 5 animals dosed at the 2.0 g/kg level - Crustiness was present in 3 female rats dosed at 2.0 g/kg. <u>Histopathology:</u> The chronic-active inflammation contained epidermal and dermal components. The epidermal component was characterized by acanthotic, hyperkeratotic, focally parakeratotic epidermis that occasionally contained rete pegs; these changes were graded separately as acanthosis. Severely affected epidermis contained focal hemorrhage, fibrin and/or mineral deposits, bacterial colonies, serum pockets, pustules, erosions and/or ulcers. The dermal component in severely affected rats was characterized bydermal fibrosis, neocapillarization, minimally distorted adnexal organs, and variably severe mixed inflammatory infiltrates consisting of histiocytes, lymphocytes, neutrophils and eosinophils. In males and females there was a dose dependent reduction in severity and incidence of skin lesions from high to no-effect dose.	
OECD 411 GLP not specified mice, B6C3F1 n=20/sex/dose	TEA 0, 250; 500; 1000; 2000; 4000 mg/kg bw/day [0, 70; 140; 280; 560; 1120 mg/ml] Vehicle: acetone 90days (5d/week)	<u>Males:</u> - Scaliness in 10 males dosed at 4.0 g/kg bw - Irritation in 10 males dosed at 4.0 g/kg bw - Discoloration in 10 males dosed at 4.0 g/kg bw - Erosion of the skin paint area in 1 males dosed at 4.0 g/kg bw <u>Females</u>: - Scaliness in 8 females dosed at 4.0 g/kg bw - Discoloration in 10 males dosed at 4.0 g/kg bw - Irritation in 7 females dosed at 4.0 g/kg bw	Anonymous, 1987

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Histopathology: The skin was examined to the lowest dose level. Gross lesions were examined in all lower dose groups. Chronic-active inflammation was seen in 4.0 g/kg male and female mice and in one 2.0 g/kg female mouse. The chronic-active inflammation contained epidermal and/or dermal components. The epidermal component included acanthotic, hyperkeratotic, focally parakeratotic epidermis; these changes were graded separately as acanthosis. Severely affected epidermis also contained focal hemorrhage, fibrin, serum pockets, pustules, erosions and/or ulcers. The dermal component in severely affected mice was characterized by dermal fibrosis, distortion of hair follicles and adnexal organs, and variably severe mixed inflammatory infiltrates consisting of histiocytes, lymphocytes and neutrophils.	
OECD 451 (Carcinogenicity study) GLP mice, B6C3F1 n= 50/sex/dose	TEA 99% Vehicle acetone Females: 0, 100, 300, 1000 mg/kg bw/day Males: 0, 200, 630, 2000 mg/kg bw/day 104 (males) or 105 (females) weeks (5 days/week)	Treatment-related clinical findings (starting at 100 mg/kg bw/day) included skin irritation with visible crusts. Microscopic examination showed epidermal hyperplasia, supparative inflammation, ulceration and dermal chronic inflammation with an incidence and severity that was related to dose.	Anonymous, 2004
OECD 451 (Carcinogenicity study) GLP Rat, Fischer 344 n= 60/sex/dose	TEA 99% Vehicle acetone Females: 0, 63, 125, or 250 mg/kg bw/day Males: 0, 32, 63, or 125 mg/kg bw/day 103 weeks (5 days/week)	Male and female rats receiving triethanolamine had irritated skin at the site of application; in dosed females, the site of application also had a crusty appearance. The number of animals in which these findings were observed increased with increasing dose. Pathology: - multiple, small, randomly located, red or brown lesions or crusts at the site of application in females - Lesions consisted of thickened epidermis (acanthosis) and ulceration with associated chronic active inflammation in dosed males and females, as well as erosion in dosed females 15-month interim evaluation: - Acanthosis and inflammation at 125 and 250mg/kg bw/day were of mild average severity - ulceration was mild in the 125 mg/kg group and moderate in the 250 mg/kg group.	Anonymous, 1999

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		2 years: - incidence of acanthosis, inflammation, and ulceration in dosed animals - Ulcers were random and multifocal and were of mild to moderate severity. Ulcers were characterized by complete segmental necrosis of epidermis, with variable erosion of the dermis and associated chronic active inflammation (neutrophils, lymphocytes, and macrophages).	

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The skin irritation potential was investigated in an OECD 404 guideline study (GLP) (Anonymous, 1993a). The test material contained Penta-PSCA Na-TEA by 40 %. Other components were teak oil, oleyl alcohol, polypropylen, and water. Self classification of oleyl alcohol varies from no classification to Skin Irrit 2 and Eye Irrit 2. Polypropylen was considered not to possess a significant irritating potential. The influence of "teak oil" could not be evaluated. The test substance was a refrigerated liquefied gas, amount applied is not known. Three rabbits were exposed (clipped, semiocclusive) for 4 hours to the test substance (no vehicle) and observed for 14 days. The individual mean erythema scores of three animals were 2, 2 and 3 respectively. Mean oedema scores were 0.33, 0.33 and 1 (see Table 6). Erythema were fully reversible within 14 days, oedema within 2 days.

REACH registrants also present a study with the acid form of the substance (Penta-PSCA) which was investigated in an OECD 404 study (Anonymous, 1993b). Three New Zealand White rabbits were exposed to 0.5 ml of Penta-PSCA under semiocclusive conditions (clipped). Animals were exposed for 4h and observed for in total 7 days. Mean erythema score (24, 48, 72h) was 1.1 with a maximum score of 2. The effect was fully reversible within 7 days. Mean oedema score (24, 48, 72h) was 0. No further details available.

When Penta-PSCA Na-TEA is dissolved in the biological fluid, an immediate dissociation is expected. The stable dissolved species are then sodium ion, triethanolammonium ion and the acid form (Penta-PSCA). When applied at dry skin no dissoziation is assumed. However the test substance was applied in a mixture with several substances and water and the presence of dissolved species can be expected under test conditions.

Penta-PSCA was slightly skin irritating (see Anonymous, 1993b) and no irritation can be assigned to sodium ion.

The animal data available for the dissolved species Triethanolamine (TEA, CAS 102-71-6) are presented in Table 7. Based on these rather old non GLP studies the substance is mild to non irritating on the skin. Triethanolamine as well as triethanolammonium compounds are self-classified as irritating (Eye Irrit 2, H319; Skin Irrit 2, H315). Repeated dose studies (with more extreme exposure conditions concerning dose and exposure duration) with TEA showed local irritating effects after dermal application at concentrations of 100 mg/kg bw/day and above. However, the

two carcinogenicity study with dermal application showed local irritating effects. In a dermal 90-day study (Anonymous, 1987) (see Table 8) Fischer rats were exposed to 0, 125, 250, 500, 1000 or 2000 mg TEA/kg bw (vehicle acetone). The main compound-related effects observed were inflammation of the skin and acanthosis, which were seen in 2000, 1000, 500, and 250 mg/kg male rats and in 2000, 1000, and 500 mg/kg female rats. In a parallel study with mice irritation scaliness and erosion were documented for the highest dose (4000 mg/kg bw/day). A carcinogenicity study in rats (Anonymous, 1999) showed local effects like acanthosis, inflammation, ulceration and epidermal erosion at 100 mg/kg bw/day and above. In a second study in mice (Anonymous, 2004) skin irritation with visible crusts, epidermal hyperplasia, supparative inflammation, ulceration and dermal chronic inflammation were seen at 100 mg/kg bw/day, the lowest concentration tested.

10.4.2 Comparison with the CLP criteria

A substance has to be classified as irritant category 2 if

(1) a mean scoring value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal was reached

or if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions

(2) Inflammation persists to the end of the observation period in at least 2 animals

An acute dermal irritation test with a test material containing about 40% Penta-PSCA Na-TEA resulted in mean score values for erythema of 2 (animal # 1 and # 3) or 3 (animal #2), and mean oedema scores of 0.33 (animal # 1 and 2) or 0 (animal #3). Effects were fully reversible within 14 days. An influence of other components of the mixture cannot be excluded.

Testing of the acid form of the substance (Penta-PSCA) resulted in a mean erythema score (24, 48, 72h) of 1.1 with a maximum score of 2. Mean oedema score (24, 48, 72h) was 0. The effect was fully reversible within 7 days. Thus, for Penta–PSCA only a mild irritation potential can be identified.

For triethanolamine only negative irritation studies are available, and repeated dose toxicity studies show an irritating potential (inflammation, acanthosis, epidermal erosion) at higher doses and longer exposure durations after dermal application.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Data on the substance Penta-PSCA Na-TEA (40%) show mild irritating properties under conditions of an OECD 404 guideline study. An influence of other components of the mixture cannot be excluded. However the classification criteria are not met and therefore no classification for skin irritation is proposed.

Dissolved species of Penta-PSCA Na-TEA, namely Penta-PSCA and TEA, which may occure in a liquid formulation or on wet skin, also only show very mild irritating properties in OECD 404 studies and therefore do not support classification.

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 according to OECD TG 404 and GLP where 3 female New Zealand White rabbits were exposed to penta-PSCA Na-TEA (40% in teak oil, oleyl alcohol, polypropylene and water) under semi-occlusive conditions for 4 hours (Anonymous, 1993a). Signs of erythema and oedema were recorded 1, 24, 48 and 72 hours after patch removal. The recorded mean score for erythema per animal was 2, 3 and 3 (mean of 24, 48, 72h gradings), fully reversible within 14 days. The mean score for oedema per animal was 0.33, 0.33 and 0 (mean of 24, 48, 72h gradings) and was fully reversible within 2 days.

The DS included one study with penta-PSCA and several studies with TEA. In a study according to OECD TG 404 and GLP, 3 female New Zealand White rabbits were exposed to 0.5 mL penta-PSCA under semi-occlusive conditions for 4 hours (Anonymous, 1993b). Signs of erythema and oedema were recorded 1, 24, 48 and 72 hours after patch removal. The recorded mean score for erythema for three rabbits was 1.1 (mean of 24, 48, 72h for all three rabbits) while the mean score for oedema was 0. The effect was fully reversible within 7 days.

The skin irritation potential of TEA was investigated in five OECD TG 404 studies, two OECD TG 411 studies and two OECD TG 451 studies.

The OECD TG 404 study by Anonymous (1993b) with three Vienna White rabbits exposed to 0.5 mL 85% TEA and 15% diethanolamine for 4 hours (occlusive) showed a mean score of 0 (all three rabbits) for erythema as well as oedema.

In the OECD TG 404 study by Anonymous (1971a) two Vienna White rabbits were exposed to 85% TEA and 15% diethanolamine for 1, 5 and 15 min (occlusive) and two Vienna White rabbits were exposed to 85% TEA and 15% diethanolamine for 20 hours (occlusive). Mean erythema score at 24 hours were 0 for 1, 5 and 15 min and 2 for 20 hours application time.

In the OECD TG 404 study by Anonymous (1966a) two Vienna White rabbits were exposed to 0.5 mL lutrol 1, 5 and 15 min (occlusive) and two Vienna White rabbits were exposed to 0.5 mL lutrol for 20 hours (occlusive). Mean erythema score at 24, 48 and 72 hours were 0 for all animals exposed for 1, 5 and 15 min and 1 (for all animals) after 20 hours application time. Mean oedema score were 0 for all animals.

In the OECD TG 404 study by Anonymous (1956a) were rabbits (unknown number) were exposed to TEA unchanged or as 20% solution for 1, 5 and 15 minutes under occlusive conditions no skin irritation were observed.

In an OECD TG 404 study by Anonymous (1967a) two Vienna White rabbits were exposed to 0.5 mL TEA 1, 5 and 15 min (occlusive) and two Vienna white rabbits were exposed to 0.5 mL TEA for 20 hours (occlusive). Mean erythema score (24, 48 and 72 hours) were 0.5, 0.5, 0.75 and 2 for 1, 5 and 15 min and 20 hours application time respectively. Mean oedema score were 0 at all time points.

Overall, the five OECD TG 404 studies (all rather old, non GLP studies) with rabbits exposed to TEA, all showed no to mild irritation of the skin.

In a dermal 90-days study (OECD TG 411) with Fisher rats dermally exposed to TEA in acetone, inflammation of the skin and acanthosis were observed from 250 mg/kg bw/d (Anonymous, 1987). In a dermal 90-days study (OECD TG 411) with mice dermally exposed to TEA in acetone, dermal scaliness and erosion were observed at the highest dose of 4000 mg/kg bw/d (Anonymous, 1987).

In a 2-year carcinogenicity study (OECD TG 451) in rats, local effects like acanthosis, inflammation, ulceration and epidermal erosion were seen at 125 mg/kg bw/d and above. (Anonymous, 1999). In a similar study in B6C3F1 mice (OECD TG 451) exposed to TEA for 2 years, skin irritation with visible crust, epidermal hyperplasia, suppurative inflammation, ulceration and dermal chronic inflammation were observed from 100 mg/kg bw/d (the lowest dose tested) (Anonymous, 2004).

Based on these studies the DS did not propose any classification for skin corrosion/irritation.

Comments received during consultation

One commenting MSCA did not support the proposed read across for local toxicity and would only take the one study on the substance itself into account. Due to uncertainties in the composition of the test substance, the commenting MSCA was of the opinion that no classification due to lack of data should be considered. The DS argued that read across to penta-PSCA could be applied since the substance is used as a liquid, and dissociation to penta-PSCA could be expected.

Another/A second MSCA supported the proposed no classification for skin corrosion/irritation.

Assessment and comparison with the classification criteria

Individual animal scores were not available for all studies in the background document. Therefore, for these studies only a qualitative comparison with the CLP criteria could be achieved. According to the CLP criteria a classification as Skin Irrit. 2 is warranted if the mean value of $\geqslant$ 2,3 - $\leqslant$ 4,0 for erythema/ eschar or for oedema is observed in at least 2 of 3 tested animals, from gradings at 24, 48 and 72 hours after patch removal.

One acute dermal irritation study (anonymous, 1993a) with penta-PSCA Na-TEA (40%) show a mean score for erythema of 2 (animal # 1 and # 3) or 3 (animal #2), and mean oedema scores of 0.33 (animal # 1 and 2) or 0 (animal #3). Effects were fully reversible within 14 days. It is noted that in this study an influence of other components of the mixture cannot be excluded.

A study (anonymous, 1993b) with the acid form of the substance (penta-PSCA) resulted in a mean erythema score (24, 48, 72h) of 1.1 with a maximum score of 2. Mean oedema score (24, 48, 72h) was 0. The effect was fully reversible within 7 days.

Several studies on TEA were also included in the assessment by the DS. These were all negative for skin irritation, however, repeated dose toxicity studies show an irritating

potential (inflammation, acanthosis, epidermal erosion) at higher doses and longer exposure durations after dermal application.

Overall, RAC concurs with the opinion of the DS that **no classification for skin corrosion/irritation is warranted for penta-PSCA Na-TEA**.

10.5 Serious eye damage/eye irritation

For the evaluation of eye irritation one study with the substance Penta-PSCA Na-TEA ist available. As immediate dissociation in biological fluids is expected also data with the acid Penta-PSCA and Triethanolamine are presented and discussed.

Table 9: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
OECD Guideline 405 GLP	Rabbit, New Zealand White n=3	test material contained the registration substance by ~40%. Other components: teak oil, oleyl alcohol, polypropylen, water	0.1 ml (no vehicle) Single application Observation 7d	cornea opacity mean score (24, 48, 72h) = 0.4 (max. 1) fully reversible within 3 days iris score mean score (24, 48, 72h) = 0.4 (max. 1) fully reversible within 3 days conjunctivae score mean score (24, 48, 72h) = 2 (max. 3) fully reversible within 7 days (individual animals cores were 1.0, 2.7, and 2.3) chemosis score mean score (24, 48, 72h) = 0.9 (max. 3) fully reversible within 3 days	Anonymous, 1993c
OECD Guideline 405 GLP	Rabbit, New Zealand White n=3	Penta-PSCA (purity unknown)	0.1 ml to the conjunctival sac (no vehicle) Single application Observation 7 days	cornea opacity mean score (24, 48, 72h) = 0.3 (max. 1) iris score mean score (24, 48, 72h) = 0.4 (max. 1)	Anonymous, 1993d

				conjunctivae score mean score (24, 48, 72h) = 1.3 (max. 3) chemosis score mean score (24, 48, 72h) = 0.6 (max. 3) All effects were fully reversible within 7 days.	

Table 10: Eye irritation data on TEA (source: ECHA dissemination site)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
OECD 405	Rabbit, New Zealand White n=6/dose	TEA 98% No vehicle	0.01, 0.03, and 0.1 ml 21 days observation	Draize Scores (mean±SE) Dose volume / days: 1: 0.01: 0±0; 0.03: 1±1; 0.1: 4±1 — 3: 0±0; 0.03: 0±0; 0.1: 2±2 — 7: 0±0; 0.03: 0±0; 0.1: 2±2 — 14: 0±0; 0.03: 0±0; 0.1: 0±0 — 21: 0±0; 0.03: 0±0; 0.1: 0±0	Griffith, 1980
OECD 405	Rabbit, Vienna white n=2 (m/f)	TEA 98% No vehicle	One dose Readings at 10 min, 1 and 24 hrs, 3 and 8 days 8 days observation	Not irritating Cornea opacity – neg. Mean conjunctive score (1h, 24h) = 1 Mean chemosis score (1h) = 1 Mean chemosis score (1d, 8d) = 0	Anonymous, 1971b
OECD 405	rabbit	TEA	0.5ml	Treatment of rabbits with the diluted test compound at pH 10 and pH 8 (neutralised with HCl) did not result in any signs of eye irritation.	Anonymous, 1956b
OECD 405	Rabbit, albino	TEA	0.05ml	Irritating mean cornea opacity score (24, 48, 72h) = 1 not fully reversible within 8 days mean conjunctivae score (24, 48, 72h) = 2 fully reversible within 8 days	Anonymous, 1967b

				mean chemosis score (24, 48, 72h) = 1.75 fully reversible within 8 days	
OECD 405	Rabbit, Vienna white n=2 (m/f)	TEA n=2	0.05ml 8 days observation	Irritating mean cornea opacity score (24, 48, 72h) = 1 not fully reversible within 8 days mean conjunctivae score = 1.08 fully reversible within 8 days mean chemosis score = 1.08 fully reversible within 8 days	Anonymous, 1966b

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

Eye irritating property was investigated in an OECD 405 study (Anonymous, 1993c) with a test material containing the substance Penta-PSCa Na-TEA by ~40% (other components: teak oil, oleyl alcohol, polypropylen, water). 0.1 ml of the test substance was applied once to the conjuctival sac of the left eye of 3 rabbits. The untreated eyes served in each case as a control. 24 hours after application treated eyes were washed out with physiological saline at approx. 37°C. The eyes were examined 1, 24, 48 and 72 hours after application of the test substance. At 24 and 72 hours as well as after 7 days, the eyes were also examined for corneal lesions under UV light after instillation of one drop of 0.01% fluorescein-sodium solution. Grading of the lesions resulted in mean scores (24, 48, 72h) of 0.4, 0.4, 2 and 0.9 for cornea opacity, iris, conjunctivae and chemosis respectively. Individual animal data for conjunctivae score are 1.0, 2.7 and 2.3. No individual scores for other endpoints are available. Most of the effects were reversible within 3 days. Conjunctivae redness was reversible within 7 days.

The acid Penta-PSCA was investigated for its eye irritation potential according to the Guideline OECD 405 (Anonymous, 1993d). 0.1 ml of Penta-PSCA (without vehicle) was instilled into the conjunctival sac of three New Zealand White rabbits. After this single application animals were observed for 7 days. The substance induced transient irritating effects that were reversible within 7 days. Mean scores (24, 48 72h) were 0.3, 0.4, 1.3 and 0.6 for corneal opacity, iris, conjunctival redness and chemosis respectively. No individual animal data are available.

Available animal data for TEA give equivocal results (Table 10). In a published study (Griffith 1980) the effect of TEA (98%) was tested in eyes of rabbits (6 per dose). The applied amounts were 0.01, 0.03 and 0.1ml TEA. And the animals were observed for 21 days. The mean draize scores are presented in Table 10 with a maximum score of 4±1 on day one after application of 0.1ml of TEA. All seen effects were reversible.

In a second study (Anonymous, 1971b) TEA was applied to the eyes of two rabbits. Slight effects are documented but all effects were reversible within the observation period of 8 days. In another study (Anonymous 1956b) 0.5 ml of TEA were instilled into rabbit eyes. No effects are documented and no further details are available.

In a study by Anonymous (1967b) 0.05 ml of TEA were applied into eyes of two rabbits. TEA shows irritating properties with mean scores (24, 48, 72h) of 1 for corneal opacity, 2 for conjunctival redness and 1.75 for chemosis. In another study (Anonymous 1966b) 0.05 ml of TEA resulted in mean scores (24, 48, 72h) of 1, 1.08 and 1.08 for corneal opacity, conjunctiva redness and chemosis respectively indicating an irritating property.

TEA show irritating properties in two out of 5 animal studies.

10.5.2 Comparison with the CLP criteria

A substance has to be classified for irreversible effects on the eye (Category 1) if, when applied to the eye of an animal, a substance produces:

- at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days
- and/ or at least in 2 of 3 tested animals, a positive response of corneal opacity ≥ 3 and/or iritis > 1,5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

A substance has to be classified for irritating to eyes (Category 2) if, when applied to the eye of an animal, a substance produces:

- at least in 2 of 3 tested animals, a positive response of corneal opacity ≥ 1 and/or iritis ≥ 1, and/or conjunctival redness ≥ 2 and/or conjunctival oedema (chemosis) ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material,
- and which fully reverses within an observation period of 21 days

One study (Anonymous, 1993c) with a test material containing ~40% of Penta-PSCA NaTEA gave mean scores (24, 48, 72h) of 0.4, 0.4, 2 and 0.9 for cornea opacity, iris, conjunctivae redness and chemosis respectively. Individual animal data for conjunctivae score are 1.0, 2.7 and 2.3. The second study (Anonymous, 1993d) investigated the effect of the acid Penta-PSCA with mean scores (24, 48, 72h) of 0.3, 0.4, 1.3 and 0.6 for corneal opacity, iris, conjunctival redness and chemosis respectively. For these studies no individual animal data are available.

Two guideline studies with TEA show a clear irritating property in rabbit eyes.

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

Testing of the substance Penta-PSCA Na-TEA (40%) (Anonymous, 1993c) resulted in a mean conjunctivae score of 2 with a maximung score of 3 and individual animals cores of 1.0, 2.7, and 2.3. A classification as Eye Irrit 2 is indicated. However, as the used mixture is not defined in detail, some uncertainty was identified.

When the substance Penta-PSCA Na-TEA is dissolved in a biological fluid, an immediate dissociation in sodium ion, triethanolammonium ion and Penta-PSCA happens. Penta-PSCA (Anonymous, 1993d) and sodium ion are not irritating. Triethanolamine as well as triethanolammonium compounds are (self-)classified as Eye Irrit 2. For TEA two studies gave clear positive results with mean scores (24, 48, 72h) of 2, 1, 1.75 (Anonymous 1967) and 1.08, 1, 1.08 (Anonymous, 1966) for conjunctiva redness, corneal opacity and chemosis respectively.

For Penta-PSCA Na-TEA, which dissolves in biological fluids, the concept of generic

concentrations limtis of ingredients of a mixture for their effects on eyes can be applied (CLP regulation, Annex I, part 3, Table 3.3.3). Based on the structure and the molecular weight of Penta-PSCA Na-TEA (ranging from 465 to 549) the fraction of TEA will be 13-16% and a classification of the mixture is indicated.

A classification as Eye Irrit 2, H319 for Penta-PSCA Na-TEA is proposed.

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 three New Zealand White rabbits were exposed to 0.1 mL penta-PSCA Na-TEA (40%) in the conjunctival sac on one eye of each test animal (Anonymous, 1993c). Other components of the test material included teak oil, oleyl alcohol, polypropylene and water. The treated eyes were washed out with physiological saline 24 hours after application. The scores are presented in the table below.

Table: *Scoring from OECD TG 405*

	Mean score (24, 48, 72 h)	**Max score**	**Reversibility (days)**
Corneal opacity	0.4	1	3
Iris score	0.4	1	3
Conjunctivae score	2	3	7
Chemosis score	0.9	3	3

In this study the mean conjunctivae score of 2 with a maximum score of 3 and individual animals scores of 1.0, 2.7, and 2.3 were reported. Based on this study a classification as Eye Irrit. 2 is indicated. It is noted that the tested mixture is not defined in detail.

Further, the DS included one study with penta-PSCA and several studies with TEA. These studies are described in the table below.

Table: *Studies with penta-PSCA and TEA*

Methods, guideline, test substance	**Species, strain, sex, no/group, dose levels, duration of exposure**	**Results**	**Reference**
OECD TG 405, GLP penta-PSCA (purity unknown)	Rabbit, New Zealand White 3 Single application Observation 7 days	Cornea opacity mean score (24, 48, 72h) = 0.3 (max. 1) Iris mean score (24, 48, 72h) = 0.4 (max. 1) Conjunctivae mean score (24, 48, 72h) = 1.3 (max. 3) Chemosis mean score (24, 48, 72h) = 0.6 (max. 3) All effects were fully reversible within 7 days.	Anonymous, 1993d
Similar to OECD TG 405 GLP unknown TEA 98% No vehicle	Rabbit, New Zealand White n=6/dose 0.01, 0.03 and	Draize scores (mean±SE) **Dose Volume** | Days 1 | 3 | 7 | 14 | 21 0.01 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0	Griffith, 1980

	0.1 mL 21 days observation	0.03 | 1±1 | 0±0 | 0±0 | 0±0 | 0±0 0.1 | 4±1 | 2±2 | 2±2 | 0±0 | 0±0	
Similar to OECD TG 405 TEA 98% No vehicle	Rabbit n=2 (m/f) 0.5 mL (single dose) Readings at 10 min, 1 and 24h, 3 and 8 days 8 days observation	Not irritating Cornea opacity = 0 Mean conjunctive score (1h, 24h) = 1 Mean chemosis score (1h) = 1 Mean chemosis score (1d, 8d) = 0	Anonymous, 1971b
Similar to OECD TG 405 TEA	Rabbit 0.5 mL	Treatment of rabbits with the diluted test compound at pH 10 and pH 8 (neutralised with HCl) did not result in any signs of eye irritation.	Anonymous, 1956b
Similar to OECD TG 405 TEA	Rabbit, albino 0.05 mL	Irritating Cornea opacity, mean score (24, 48, 72h) = 1, not fully reversible within 8 days Conjunctivae, mean score (24, 48, 72h) = 2, fully reversible within 8 days Chemosis, mean score (24, 48, 72h) = 1.75, fully reversible within 8 days	Anonymous, 1967b
Similar to OECD TG 405 TEA	Rabbit, Vienna white N=2 (m/f) 0.05 mL 8 days observation	Irritating Cornea opacity, mean score (24, 48, 72h) = 1, not fully reversible within 8 days Conjunctivae, mean score = 1.08, fully reversible within 8 days Chemosis, mean score = 1.08, fully reversible within 8 days	Anonymous, 1966b

Two studies with TEA gave clear positive results with mean scores (24, 48, 72h) of 2, 1, 1.75 (Anonymous, 1967) and 1.08, 1, 1.08 (Anonymous, 1966) for conjunctiva redness, corneal opacity and chemosis respectively. It is further noted that Triethanolamine as well as triethanolammonium compounds are (self-)classified as Eye Irrit. 2. Penta-PSCA Na-TEA dissolves in biological fluids. The DS is of the opinion that the concept of generic concentrations limits of ingredients of a mixture for their effects on eyes can be applied (CLP regulation, Annex I, part 3, Table 3.3.3) for the classification of penta-PSCA Na-TEA. Based on the structure and the molecular weight of penta-PSCA Na-TEA (ranging from 465 to 549) the fraction of TEA will be 13-16% and a classification of the mixture is indicated.

On this basis the DS proposed a classification of penta-PSCA Na-TEA as Eye Irrit. 2, H319.

Comments received during consultation

One commenting MSCA did not support the proposed read across for local toxicity and would take into account only the one study on the substance itself. The MSCA was of the opinion that no classification due to lack of data should be considered. Since the substance dissolves in biological fluids, the DS argued the concept of generic concentration limits for ingredients in a mixture should be considered and that a classification as Eye Irrit. 2 could be justified on this basis.

A second MSCA supported the proposed classification for serious eye damage/irritation as

Eye. Irrit. 2, H319.

Assessment and comparison with the classification criteria

Individual animal scores were not available for all studies in the background document. Therefore, for these studies only a qualitative comparison with the CLP criteria could be achieved. 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 is reported and which is fully reversible within 21 days.

In a study on serious eye damage/eye irritation according to OECD TG 405 and GLP, three New Zealand White rabbits were exposed to 0,1 mL 40% penta-PSCA Na-TEA (other components in the tested mixture included teak oil, oleyl alcohol, polypropylene and water) in the conjunctival sac on one eye of each test animal (Anonymous, 1993c). The observed mean scores (24, 48, 72h) were 0.4, 0.4, 2 and 0.9 for cornea opacity, iris, conjunctivae redness and chemosis respectively. Individual animal data for conjunctivae score are 1.0, 2.7 and 2.3. The conjunctiva score observed in this study fulfils the criteria for a classification as Eye Irrit. 2. It is however noted that there are uncertainties related to the composition of the mixture tested, and how the other substances in the tested mixture would contribute to the eye irritation observed in this study.

A study with penta-PSCA showed mean scores (24, 48, 72h) of 0.3, 0.4, 1.3 and 0.6 for corneal opacity, iris, conjunctival redness and chemosis respectively (Anonymous, 1993d), and hence penta-PSCA does not fulfil the criteria for classification for serious eye damage/irritation.

The DS also included several studies with TEA. Two studies gave clear positive results with mean scores (24, 48, 72h) of 2, 1, 1.75 (Anonymous, 1967) and 1.08, 1, 1.08 (Anonymous, 1966) for conjunctiva redness, corneal opacity and chemosis respectively. It is also noted that TEA (triethanolamine) as well as triethanolammonium compounds are (self-)classified as Eye Irrit. 2.

It is noted that penta-PSCA Na-TEA dissociate in biological fluids or water to sodium ion, triethanolammonium ion and penta-PSCA. Therefore, the concept of generic concentrations limits of ingredients of a mixture for their effects on eyes can be applied (CLP regulation, Annex I, part 3, Table 3.3.3). Based on the structure and the molecular weight of penta-PSCA Na-TEA, the fraction of TEA will be 13-16% and a classification of penta-PSCA Na-TEA is indicated.

Overall, taking into account one study with penta-PSCA Na-TEA (40%) and two studies with TEA which fulfils the criteria for a classification as Eye Irrit. 2 H319, RAC is of the opinion that **classification as Eye Irrit. 2, H319 for penta-PSCA Na-TEA is warranted**.

10.6 Respiratory sensitisation

No assessed

10.7 Skin sensitisation

No assessed

10.8 Germ cell mutagenicity

No assessed

10.9 Carcinogenicity

Not assessed

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

The evaluation of this endpoint is based on an OECD 422 screening study with the substance Penta-PSCA Na-TEA. Based on the available date reproductive toxicity does not seem to be related to TEA. For further details see Annex I.

Table 11: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Dose Range finding study for OECD 422 Rat, RccHanTM: WIST(SPF) N= 3/sex/group	Test substance: Penta-PSCA Na-TEA (Emulsogen 3971, purity 90%) Oral (Gavage) Dose volume: 10ml/kg Vehicle: water Dose levels: 0, 100, 300, 1000 mg/kg/day M: 4 weeks f: 6 weeks (sacrif on day 14 of gestation)	$NOAEL_{parental\ toxicity}$ = 300 mg/kg bw/day 1000 mg/kg bw: 2/3 females not pregnant, salivation (2m, 2f), food consumption↓, bw↓, bw gain↓ $NOAEL_{fertility}$ = 300 mg/kg bw Fertility index: 100%, 100%, 100% and 33.3% Conception rate: 100%, 100%, 100% and 33.3% mean food consumption (compared to control at 100, 300 and 1000mg/kg bw): m: pre-pairing -8%, -8%, -29% m: after pairing period ±0%, -9% ,-17% f: pre-pairing -6%, -12% and -29% f: during gestation -5%, -5%, -19%	Anonymous 2013a

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		mean body weight gain (at 0, 100, 300 and 1000 mg/kg bw) m: pre-pairing +14%, +14%, +13% , +4% m. pairing +4%, +4%, +2%, +2% m: after pairing +8%, +9%, +7% ,+8%. f: pre-pairing +9%, +9%, +9%, +4% f: during gestation +25%, +25%, +29%, +20% Corrected body weight gains females: 3.1%, -3.6%, -3.6%, -4.6%	
OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) GLP Rat, RccHanTM: WIST(SPF) n=11/sex/dose	Test substance: Penta-PSCA Na-TEA (Emulsogen 3971, purity >90%) Oral (Gavage) Vehicle: water Dose levels: 0 mg/kg/day (Group 1, control group) 40 mg/kg/day (Group 2) 200 mg/kg/day (Group 3) 1000 mg/kg/day (Group 4) Dose Volume: 10 ml/kg body weight m: 4 weeks f: ~7 weeks	$NOAEL_{parental\ toxicity}$ = 40 mg/kg bw/day (reduced food consumption, salivation) $LOAEL_{fertility}$ = 40 mg/kg bw/day $LOAEL_{developmental\ toxicity}$ = 40 mg/kg bw/day • reduced fertility index (100%, 90.9%, 90.9%, 72.7%) • reduced gestation index (100%, 100%, 90%, 0%*) • increased pre-implantation loss in mid and high dosed animals • post-implantation loss at all dose levels (mean incidence per dam: 0.4, 2.0*, 3.8* and 8.5* at dose levels of 0, 40, 200 and 1000 mg/kg bw/day) • reduction of litter size at all dose levels (mean number of living pups per dam 11.9, 10.7, 8.8 and 0* respectively) • reduction in birth index (96.7%, 84.3%*, 68.8%*, 0.0%*) • postnatal loss (days 0-4) in mid and low dose groups • viability index: 99.2%, 91.6%* and 69.3%* at dose levels of 0, 40 and 200 mg/kg bw/day, respectively $LOAEL_{F1\text{-}generation}$ = 40 mg/kg bw/day (mortality)	Anonymous, 2012c

The dose levels for the OECD 422 study were selected based on a previous dose range-finding toxicity study (Anonymous, 2013a) carried out with Han Wistar rats, using dose levels of 0, 100, 300 and 1000 mg/kg/day (n=3/sex/dose). Males were dosed 14 days during pre-pairing and 14 days during pairing. Females were dosed during pre-pairing, pairing and 14 days during gestation. For evaluation of fertility, the number and distribution of implantation sites, live or dead embryos, and early and late embryonic deaths in each uterine horn were recorded. Also the number of corpora lutea in each ovary was recorded. Two of three females at the dose level of 1000 mg/kg bw/day were not pregnant. Consequently, fertility indexes (number of females achieving pregnancy as a percentage of females paired) and conception rates (number of females achieving pregnancy as a percentage of females mated) were 100%, 100%, 100% and 33.3% at the dose levels of 0, 100, 300 and 1000 mg/kg bw/day, respectively. All pregnant females had living fetuses at Caesarean section on day 14 post coitum. Reproduction data are presented in Table 12. During the treatment bedding in mouth was noted in all dose groups with a dose-dependent frequency. Salivation was noted at the dose level of 1000 mg/kg bw/day. These findings were considered to be test item-related. Differences in mean food consumption (food consumtion was not recorded during pairing) and mean body weight gain of males and females are presented in Table 13**Error! Reference source not found.**. Corrected body weight gains for females were -3.1%, -3.6%, -3.6% and -4.6% at the dose levels of 0, 100, 300 and 1000 mg/kg bw/day respectively. No macroscopical findings were noted in males and females at any dose level Clinical laboratory investigations showed lower relative hematocrit value and lower albumin concentration in females at the high dose level. No further test item-related changes in hematology or clinical biochemistry parameters were noted in males or females at any dose level. . Based on these results, dose levels of 0, 40, 200 and 1000 mg/kg bw were considered to be suitable for the subsequent combined repeated dose toxicity study with reproduction /developmental toxicity screening.

Table 12: Reproduction data (Anonymous, 2013a)

		0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Number of dams		3	3	3	1
Corpora lutea	Total	37	37	43	12
	mean	12.33	12.33	14.33	12.00
	Std.Dev	5.51	1.53	1.53	0
Pre-implantation loss	Total	5	3	6	0
	% of corp. lutea	13.51	8.11	13.95	0.00
	mean	1.67	1.00	2.00	0.00
	Std.Dev	1.15	1.00	0.00	0.00
	No of dams affected	3	2	3	-
Implantation sites	Litters affected	32	34	37	12
	% of corp. lutea	86.49	91.89	86.05	100.00
	mean	10.67	11.33	12.33	12.00

	Std.Dev	6.66	1.15	1.53	0.00
Post implantation loss	Total	2	1	4	0
	% of implant, sites	6.25	2.94	10.81	0.00
	mean	0.67	0.33	1.33	0.00
	Std.Dev	1.15	0.58	0.58	0.00
	No of dams affected	1	1	3	-
Implantation site scars	Total	0	0	0	0
Early resorption	Total	2	1	2	0
Late resorptionss	Total	0	0	2	0
Total embryos	Total	30	33	33	12
	% of implant sites	93.75	97.06	89.19	100.00
	mean	10.00	11.00	11.00	12.00
	Std.Dev	7.81	1.00	2.00	0.00

In the main OECD 422 study (Anonymous, 2012c) rats were dosed daily via gavage with 0, 40, 200 or 1000 mg/kg bw (further named as group 1, 2, 3 and 4). The dose volume was 10 ml/kg bw. The dose formulations were prepared weekly and they were stable for at least 8 days. The test substance Penta-PSCA Na-TEA was administered to male rats for 28 days and to female rats for 14 days prior to pairing, through the pairing and gestation periods until the F1 generation reached day 4 post partum (in total approx. 7 weeks).

During the pairing period, females were housed with sexually mature males (1:1) until evidence of copulation was observed. The females were removed and housed individually if the daily vaginal smear was sperm positive, or a copulation plug was observed. The day on which a positive mating was determined (copulation plug or sperm) was designated day 0 post coitum. If a female did not mate during the 14-day pairing period, a second pairing of this female with a male in the same group, which had already mated successfully, was considered. All dams were allowed to give birth and rear their litters (F1 pups) up to day 4 post partum. Day 0 was designated as the day on which a female had delivered all her pups.

Viability, clinical signs, food consumption and body weights were investigated. Clinical observation was done weekly. FOB assessment was done in 5 animals/sex. Blood samples were investigated from 5 males from each group. Blood samples from 5 lactating females from each group were obtained on day 5 post partum. Estrous cyclicity and sperm parameters were not examined. The litters were examined for litter size, live births, still births and any gross anomalies. The sex ratio of the pups was recorded. Pups were weighted individually (without identification) on days 0 (if possible), 1 and 4 post partum.

Males were sacrificed after treatment of at least 28 days, when no longer needed for the assessment of reproductive effects. Pups were sacrificed on day 4 post partum. Dams were sacrificed on day 5

post partum. The number of implantation sites and corpora lutea was recorded for all dams with litters. The uteri of non-pregnant females were placed in a solution of ammonium sulfide to visualize possible hemorrhagic areas of implantation sites. Testes and epididymides from all parental males were weighted. Adrenal glands (weighted as pairs), brain, heart, kidneys (weighted as pairs), liver, thymus and spleen were weighted from 5 males and 5 females per group.

For males tissue preservations of prostate, seminal vesicles with coagulating gland, testes and epididymides were made. For females ovaries were preserved. From 5 animals per sex and group (plus animals which died spontaneously or had to be terminated in extremis) the following tissues were preserved: gross lesions, brain, spinal cord, small and large intestine, stomach, liver, kidneys, adrenals, spleen, heart, thymus, thyroids (parathyroids if possible), trachea and lungs, uterus, urinary bladder, lymph nodes, peripheral nerve, bone marrow. First control and high-dose groups were examined. When test item-related morphologic changes were detected in organs of any high-dose animal, those same organs from the mid- and low-dose group were examined to establish a no-effect level, if possible. Pups were subjected to a detailed macroscopic examination.

Parental toxicity:

All animals survived until the scheduled necropsy.

Salivation and bedding in mouth is documented for all animals in group 3 and 4 and several in group 2. Decreased activity was seen in all males and some females in group 4 and four females in group 3. Ruffled fur and yellow discoloured faeces were observed at 1000 mg/kg bw/day in all animals.

Locomotor activity was reduced at dose levels of 1000 and 200 mg/kg bw/day. In males, reduction of locomotor activity was statistically significant; mean beam counts per minute were 425 and 273 in group 4 and 3, respectively. In the control group 1294 counts per minute were recorded. In females, differences to the control values were not statistically significant; mean beam counts per minute were 577 and 763 in group 4 and 3, respectively, and 974 counts per minute in the control group. At the dose level of 40 mg/kg bw/day, locomotor activities of males and females were similar to the respective control values.

Food consumption in males was reduced in all dose levels (see Table 13); while this reduction was statistically significant in the high dose group during the entire pre-pairing period in the mid dose group statistical significance was reached on days 1-4 and 11-14 of the pre-pairing period.

Female food consumption was also reduced in all dose groups (see Table 13). For group 4 the reduction in food consumption was statistically significant during the entire pre-pairing, gestation and lactation periods. For group 3 reduction in food consumption was statistically significant on days 1 - 8 and 11 - 14 of the pre-pairing period and entire gestation and lactation periods. For group 2 reduction in food consumption was statistically significant on days 0 - 14 of the gestation period.

An overview on body weight gain is compiled in Table 13. A description on body weight development is given below:

1000 mg/kg bw/day: For males and females body weights were statistically significantly reduced starting from day 4 of the pre-pairing period until the completion of the study. Slight decrease of body weights (by 2%) was noted at this dose level between day 1 and 4 of the pre-pairing period. Afterwards, body weight gain was stable in males and slightly increased in females although until the completion of the pre-pairing period remained lower than the control values. During the pairing period in males body weight gain increased and was higher than the control values. In females during the gestation and lactation periods body weight gain remained lower than the control values. Reduction in female body weight gain

was statistically significant starting from day 3 until the completion of the pre-pairing period and during the entire gestation period. Reduction in male body weight gain was statistically significant starting from day 2 until the completion of the pre-pairing period, increase in body weight gain was statistically significant on day 3 and from day 5 until the completion of the pairing period.

200 mg/kg bw/day: Male body weights were statistically significantly reduced starting from day 12 of the pre-pairing period until day 9 of the pairing period. Slight decrease of body weights (by 1%) was noted at this dose level on day 2 of the pre-pairing period. Afterwards, body weight gain increased although until the completion of the pre-pairing period remained lower than the control values. Reduction in body weight gain was statistically significant starting from day 11 until the completion of the pre-pairing period. During pairing period, body weight gain was similar to the control values.

In females body weights were similar or slightly, not statistically significantly lower than the respective control values during the most of the study period. Statistically significantly lower body weights were noted on days 4 and 5 of the lactation period. Slight decrease of body weights (by 1%) was noted at this dose level on day 2 of the pre-pairing period. Afterwards, body weight gain increased slightly but until the completion of the gestation period remained lower than the control values. During lactation period, body weight gain was similar to the control values. Reduction in body weight gain was statistically significant on days 2, 11 and 14 of the pre-pairing period and on days 5, 11, 13 and 14 of the gestation period.

40 mg/kg bw/day: In males body weights and body weight gain were considered not to be affected by the treatment. On day 12 of the pre-pairing period, body weight gain was statistically significantly lower when compared to the control value. Because during the remaining study period body weight gain and body weights were similar to the respective control values, the isolated difference in body weight gain on day 12 of the pre-pairing period was considered to be incidental.

In females no significant differences in body weights were noted at this dose level. A slight decrease of body weights (by 1%) was noted on day 2 of the pre-pairing period. Body weight gain was occasionally lower if compared to the control values. On day 14 of the pre-pairing period and day 3 of the gestation period body weight gain was statistically significantly lower than the control values. During the remaining study period body weight gain was similar or slightly lower than the respective control values.

Table 13: Food consumption and body weight gain (Anonymous, 2012c).

	dose	males		females		
period		pre-pairing	pairing	pre-pairing	gestation	lactation
Food consumption [g/animal/day]	Control	26.1		19.5 g	26.1 g	30.3 g
	40mg/kg bw/day	25.4 g (-2.7%)		18.8 g (-3.6%)	23.7 g (-9.2%)	27.6 g (-8.9%)
	200mg/kg bw/day	23.4 g (-10.3%)		17.2 g (-11.8%)	22.4 g (-14.2%)	20.0 g (-34.0%)
	1000 mg/kg	17.9 g		13.5 g	19.0 g	15.9 g

	bw/day	(-31.4%)		(-30.8%)	(-27.2%)	(-47.5%)
Body weight gain (mean differences) [%]	Control	+ 11%	+ 6%	+ 9%	+ 57%	+ 5%
	40mg/kg bw/day	+ 8%	+ 8%	+ 6%	+ 55%	+ 6%
	200mg/kg bw/day	+ 7%	+ 7%	+ 6%	+ 49%	+ 4%
	1000mg/kg bw/day	- 1%	+ 9%	+ 2%	+ 33%	± 0%

To assess maternal effects of the test substance in this study (ECHA, 2017) the mean maternal body weight change (difference between the initial and terminal body weight minus the sum of the weights of the foetuses) was calculated. The animal individual animals data are presented in Table 14. The mean body weight changes were 78.8g, 70.4g, 63.2g and 62.6g for the groups of 0, 40, 200 and 1000 mg/kg bw, respectively, indicating maternal toxicity at high doses. It has to be considered that the body weight of dead pups at first litter check is not documented in the study; this is also highlighted in the table.

Table 14: Maternal body weight change from start of study to end of gestation (Anonymous, 2012c).

Dose group	Animal No	Initial body weight (starting 14 days prior to pairing) [g]	body weight end of gestation [g]	Total weight of pups [g] at day 1	Number of pups on day 1 (+ Number of pups dead at first litter check) *caution: weight of dead pubs not given in the report and not included in this analysis*	Body weight gain (calculation: initial bw - bw end of gestation – pup weight) [g]	Mean body weight gain/group [g]
0 mg/kg bw	45	218	381	88.7	13	74.3	78.8
	46	229	378	72.2	12	76.8	
	47	213	397	82.3	15	101.7	
	48	219	385	93.2	15	72.8	
	49	224	385	85	16	76	
	50	229	394	71.4	12	93.6	
	51	212	340	59.7	9	68.3	
	52	227	392	79.5	12	85.5	
	53	241	380	59	8	80	
	54	221	378	96.5	15	60.5	
	55	223	329	28.7	4	77.3	
40 mg/kg	56	214	356	75.7	13(+2)	66.3	70.4

bw	57	230	348	49,6	7	68.4	
	58	234	385	74.2	11(+1)	76.8	
	59	233	287	Not pregnant	-		
	60	223	359	71.6	12	64.4	
	61	224	378	55.3	8	98.7	
	62	226	355	67*	11(+1)	62	
	63	229	376	88.2	14	58.8	
	64	224	355	76.6	11	54.4	
	65	221	390	85.3	13(+1)	83.7	
	66	232	352	49.4	7	70.6	
200 mg/kg bw	67	221	358	51.4*	11	85.6	63.2
	68	226	274 #	Dead at first litter check	13	48	
	69	225	335	52.5	11(+1)	57.5	
	70	217	264	Not pregnant	-	47	
	71	242	347	48.9	7	56.1	
	72	240	412	85.1	13	86.9	
	73	225	341	31.6	6(+5)	84.4	
	74	232	346	74.5	13	39.5	
	75	226	356	45.7	8(+1)	84.3	
	76	213	332	47.8	8(+5)	71.2	
	77	227	344	59.6	10	57.4	
1000 mg/kg bw	78	216	287	No pubs at first litter check	-	71	62.6
	79	231	342	Not mating	-	111	
	80	218	314	-	0	96	
	81	225	257	Not pregnant	-	32	
	82	221	243	Not pregnant	-	22	
	83	223	275	-	(+6)	52	
	84	232	284	-	0	52	
	85	225	243	No pubs at first litter check	-	18	
	86	231	368[1]	-	0	137	
	87	213	299	-	(+1)	86	
	88	218	230	Not pregnant		12	

* Pub weight at day 0, # bw after delivery, [1] bw at begin on lactation day 1(292g) indicate delivery

Reduction of body temperature was statistically significant at the high dose level (males: mean body temperature 37.6°C compared to 38.3°C in control; females: 37.7°C compared to 38.7°C).

Hematology showed a higher platelet count (1318 x 10^9/L compared to 1004 x 10^9/L in the control group) and statistically significantly higher relative prothrombin time (0.90 compared to 0.82 in the control) in males at 1000 mg/kg bw/day. No effects on hematology parameters, which were considered to be test item-related were noted in females at any dose level.

Clinical biochemistry showed a slight but dose dependent change of potassium concentration in males across all dose groups and therefore this effect was considered to be possibly test item-related. At 1000 mg/kg bw/day the mean concentration was 4.60 mmol/ versus 4.00 mmol/L in the control group, however, this value was within the historical control data (HCD). In females, at the dose level of 1000 mg/kg bw/day, increased concentration of total protein (72.11 g/ versus 65.35 g/L in the control group) and increased concentration of albumin (47.27 g/L versus 41.98 g/L in the control group) were noted and considered test substance related. Higher concentrations of cholesterol and lower concentrations of phosphorus were also found in females. Isolated findings in group 3 males (but not in group 4) were bilirubin↓, phosphorus ↑, protein↓, albumin↓. In group 2 females a higher activity of ALAT was noted but without dose response.

At 1000 mg/kg bw/day in females a statistically significant different liver to body weight ratio was observed but no statistically significant higher absolute liver weight was recorded (for details see Table 15). In females higher brain weight to body weight ratio at 1000 mg/kg and lower heart weight to brain weight ratio at 200 and 1000 mg/kg bw/day were considered to be the result of lower body weights (Table 16). Males at the high dose showed a statistically significant increase in absolute and relative liver and spleen weights. Mean liver weight was 13.39 g versus 9.83 g in the control group. Mean spleen weight was 0.86 g versus 0.70 g in the control group. Further, at the dose level of 1000 mg/kg bw/day, statistically significantly reduced absolute testis and epididymides weights as well as statistically significantly reduced epididymides weights relative to brain weights were noted. Mean testis weights (left/right) were 1.88/1.84 g versus 2.07/2.04 g in the control group. Mean epididymides weights were 0.596/0.595 g versus 0.700/0.706 g in the control group. The higher kidney/body weight ratio in males at the high dose was noted in the absence of significant changes of absolute kidney weights or kidney weights to brain weights ratio and was therefore considered to be secondary to reduced body weights. For further details see Table 17 and Table 18.

Table 15: Organ weight and organ/body weight ratios (%), females [mean organ weight (g), SD, organ/body weight ratio (%), SD, n number of animals] **(Anonymous, 2012c).**

Organ		0 mg/kg bw/day	40 mg/kg bw/day	200 mg/kg bw/day	1000 mg/kg bw/day
Body weight	Mean bodyweight [g]	259.8g	253.7g	245.3g	**231.1g ****
	Standarddeviation	12.6	13.5	14.3	22.8
	organ/body weight ration [%]	-	-	-	-
	Standarddeviation	-	-	-	-
	N	11	10	10	8
Brain	Mean organ weight [g]	1.93g	1.93g	1.98g	1.88g
	Standarddeviation	0.10	0.03	0.09	0.06

	organ/body weight ration [%]	0.73 %	0.78 %	0.80 %	**0.86 %****
	Standarddeviation	0.03	0.04	0.04	0.08
	N	5	5	5	5
Heart	Mean organ weight [g]	0.88g	0.78g	0.77g	**0.71g ****
	Standarddeviation	0.07	0.02	0.08	0.09
	organ/body weight ration [%]	0.33 %	0.31 %	0.31 %	0.32 %
	Standarddeviation	0.02	0.02	0.02	0.05
	N	5	5	5	5
Liver	Mean organ weight [g]	8.88g	8.37g	9.59g	9.70g
	Standarddeviation	1.73	0.64	1.13	0.88
	organ/body weight ration [%]	3.37 %	3.36 %	3.87 %	**4.42 %****
	Standarddeviation	0.62	0.31	0.29	0.45
	N	5	5	5	5
Thymus	Mean organ weight [g]	0.207g	0.163g	0.162g	0.175g
	Standarddeviation	0.063	0.053	0.070	0.064
	organ/body weight ration [%]	0.078 %	0.064 %	0.065 %	0.079 %
	Standarddeviation	0.023	0.018	0.026	0.027
	N	5	5	5	5
Kidney	Mean organ weight [g]	1.62g	1.56g	1.65g	1.47g
	Standarddeviation	0.15	0.13	0.21	0.09
	organ/body weight ration [%]	0.62 %	0.63 %	0.67 %	0.67 %
	Standarddeviation	0.04	0.07	0.08	0.08
	N	5		5	5
Adrenals	Mean organ weight [g]	0.085g	0.082g	0.091g	0.089g
	Standarddeviation	0.012	0.010	0.021	0.007
	organ/body weight ration [%]	0.032 %	0.033 %	0.036 %	0.041 %
	Standarddeviation	0.004	0.004	0.007	0.007
	N	5	5	5	5
Spleen	Mean organ weight [g]	0.75g	0.75g	0.82g	0.54g
	Standarddeviation	0.18	0.07	0.15	0.07
	organ/body weight ration [%]	0.28 %	0.30 %	0.33 %	0.25 %
	Standarddeviation	0.06	0.02	0.06	0.04

	N	5	5	5	5

Table 16: Organ/brain weight ratios (%) for organs with significant changes, females [organ/brain weight ration (%), SD, n] **(Anonymous, 2012c).**

Organ		0 mg/kg bw/day	40 mg/kg bw/day	200 mg/kg bw/day	1000 mg/kg bw/day
Heart	organ/brain weight ration [%]	45.60 %	40.64 %	38.71 %	**37.89 %****
	Standarddeviation	1.61	1.51	3.61	5.80
	N	5	5	5	5
Spleen	organ/brain weight ration [%]	38.47 %	39.10 %	41.35 %	**28.97 %***
	Standarddeviation	7.18	4.22	7.35	3.07
	N	5	5	5	5

Table 17: Organ weight and organ/body weight ratios (%), males [mean organ weight (g), SD, organ/body weight ratio (%), SD, n number of animals] (Anonymous, 2012c).

Organ		0 mg/kg bw/day	40 mg/kg bw/day	200 mg/kg bw/day	1000 mg/kg bw/day
Body weight	Mean body weight [g]	379.3g	380.9g	366.7g	**336.2g****
	Standarddeviation	12.9	13.8	13.4	17.8
	organ/body weight ration [%]	-	-	-	-
	Standarddeviation	-	-	-	-
	N	11	11	11	11
Brain	Mean organ weight [g]	2.08g	2.06g	2.01g	2.06g
	Standarddeviation	0.07	0.07	0.07	0.11
	organ/body weight ration [%]	0.55 %	0.54 %	0.55 %	**0.60 %***
	Standarddeviation	0.04	0.04	0.03	0.02
	N	5	5	5	5
Heart	Mean organ weight [g]	0.98g	1.02g	1.10g	0.90g
	Standarddeviation	0.08	0.03	0.10	0.08
	organ/body weight ration [%]	0.26 %	0.27 %	0.28 %	0.26 %
	Standarddeviation	0.02	0.01	0.03	0.02
	N	5	5	5	5

Liver	Mean organ weight [g]	9.83	10.03	9.97	**13.39****
	Standarddeviation	0.41	0.92	1.08	0.64
	organ/body weight ration [%]	2.59 %	2.62 %	2.73 %	**3.91 %****
	Standarddeviation	0.06	0.16	0.28	0.15
	N	5	5	5	5
Thymus	Mean organ weight [g]	0.335g	0.376g	0.302g	0.283g
	Standarddeviation	0.056	0.087	0.034	0.045
	organ/body weight ration [%]	0.088 %	0.099 %	0.083 %	0.083 %
	Standarddeviation	0.013	0.022	0.010	0.013
	N	5	5	5	5
Kidney	Mean organ weight [g]	2.21g	2.37g	2.25g	2.35g
	Standarddeviation	0.04	0.20	0.21	0.17
	organ/body weight ration [%]	0.58 %	0.62 %	0.62 %	**0.69** % *
	Standarddeviation	0.03	0.04	0.06	0.06
	N	5	5	5	5
Adrenals	Mean organ weight [g]	0.090g	0.091g	0.082g	0.096g
	Standarddeviation	0.008	0.014	0.009	0.013
	organ/body weight ration [%]	0.024 %	0.024 %	0.023 %	0.027 %
	Standarddeviation	0.002	0.003	0.002	0.004
	N	5	5	5	5
Spleen	Mean organ weight [g]	0.70g	0.81g	0.79g	**0.86g ***
	Standarddeviation	0.07	0.06	0.10	0.12
	organ/body weight ration [%]	0.18 %	0.21 %	0.22 %	**0.25** % **
	Standarddeviation	0.02	0.01	0.03	0.03
	N	5	5	5	5
Testis (L)	Mean organ weight [g]	2.07g	20.3g	1.99g	**1.88g***
	Standarddeviation	0.14	0.12	0.18	0.18
	organ/body weight ration [%]	0.55 %	0.53 %	0.54 %	0.56 %
	Standarddeviation	0.04	0.04	0.06	0.05
	N	11	11	11	11
Testis (R)	Mean organ weight [g]	2.04g	2.03g	1.91g	**1.84g ****
	Standarddeviation	0.14	0.14	0.13	0.17

	organ/body weight ration [%]	0.54 %	0.53 %	0.52 %	0.55 %
	Standarddeviation	0.04	0.04	0.04	0.05
	N	11	11	11	11
Epididymidis (R)	Mean organ weight [g]	0.706g	0.719g	0.662g	**0.595g ****
	Standarddeviation	0.063	0.056	0.055	0.030
	organ/body weight ration [%]	0.186 %	0.189 %	0.181 %	0.177 %
	Standarddeviation	0.015	0.014	0.017	0.008
	N	11	11	11	11
Epididymidis (L)	Mean organ weight [g]	0.700g	0.696g	0.677g	**0.596g ****
	Standarddeviation	0.085	0.072	0.070	0.034
	organ/body weight ration [%]	0.185 %	0.183 %	0.185 %	0.177 %
	Standarddeviation	0.022	0.017	0.022	0.007
	N	11	11	11	11

Table 18: Organ/brain weight ratios (%) for organs with significant changes, males [organ/brain weight ration (%), SD, n]

Organ		0 mg/kg bw/day	40 mg/kg bw/day	200 mg/kg bw/day	1000 mg/kg bw/day
Liver	organ/brain weight ration [%]	473.85 %	486.91 %	495.15 %	**652.05 %****
	Standarddeviation	34.68	43.33	52.13	31.49
	N	5	5	5	5
Spleen	organ/brain weight ration [%]	33.69 %	39.49 %	39.17 %	**41.82 %***
	Standarddeviation	4.20	3.64	4.27	6.15
	N	5	5	5	5
Epididymidis (R)	organ/brain weight ration [%]	34.461 %	35.390 %	32.762 %	**28.927% ***
	Standarddeviation	3.548	3.801	3.181	1.451
	N	5	5	5	5
Epididymidis (L)	organ/brain weight ration [%]	35.485 %	34.592 %	32.796 %	**28.775 %***
	Standarddeviation	5.391	4.095	2.984	1.156
	N	5	5	5	5

No test item related findings were noted during necropsy of males and females at any dose level.

Histopathology showed no differences in the completeness of stages or cell populations of the testes between controls and high-dose animals. Further histological findings were:

- Liver: Central to diffuse hepatocellular hypertrophy was recorded at minimal severity in both males and females at the dose level of 1000 mg/kg bw/day.
- Kidneys: Incidence and severity of hyaline droplets in the epithelium were increased in males at the dose level of 1000 mg/kg bw/day.
- Stomach: Squamous hyperplasia of the forestomach was recorded at minimal to slight severity in animals at the dose level of 1000 mg/kg bw/day, and few females at the dose levels of 200 and 40 mg/kg bw/day.
- Thyroid Gland: Follicular cell hypertrophy was recorded at minimal severity in both males and females at the dose level of 1000 mg/kg bw/day.
- Other Findings: No test item-related histological findings were recorded in ovaries of females which did not give birth or in the reproductive organs of infertile males.

Reproduction and breading

Percentage of mating was 100% in all groups (copulation plug or sperm). With the exception of one female at the high dose level (no. 79) mating of all females was recorded during the first pairing period. After 14 days of unsuccessful pairing of female no. 79 with male no. 35, a second pairing of this female with male no. 39 was commenced. Mating of this female was confirmed on day two of the pairing with male no. 39. However, no pregnancy was documented.

Mean (median) precoital times calculated for the first pairing period were 2.4 (3), 2.3 (3), 2.8 (3) and 3.8 (4) days in order of ascending dose levels.

One female at the dose level of 40 mg/kg bw/day, one female at the dose level of 200 mg/kg bw/day, and three females at the dose level of 1000 mg/kg bw/day were not pregnant (see Table 19). Consequently, fertility indexes (numbers of females pregnant as percentages of females paired) were 100%, 90.9%, 90.9% and 72.7% at the dose levels of 0, 40, 200 and 1000 mg/kg bw/day, respectively (see Table 20).

Table 19: Parental breeding performance (Anonymous, 2012c)

	0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1000 mg/kg/day
Female numbers	45-55	56-66	67-77	78-88
Number of females paired	11	11	11	11
Number of females mated	11	11	11	11
Number of females not pregnant	0	1 (female no 59)	1 (female no 70)	3 (female no 81, 82, 88)
Number of females which lost their litters before first litter check	0	0	1 (female no 68)	8 (female no 78, 79, 80, 83, 84, 85, 86, 87)
Number of females which lost	0	0	1	0

their litters during lactation			(female no 73)	
Number of females which reared their pubs until day 4 post partum	11	10	8	0

Table 20: Reproductive indices (Anonymous, 2012c)

	0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1000 mg/kg/day
Percentage mating (%)	100.0	100.0	100.0	100.0
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**$^{\#\#}$	-

Fischer´s Exact test, signif. at 5% ($^{\#}$), 1% ($^{\#\#}$)

No effects on corpora lutea count were observed at any dose level. Mean numbers of corpora lutea per dam were 11.3, 11.1, 9.8 and 12.1 in order of ascending dose levels. See also Table 21 for further details.

No effects on duration of gestation were observed at any dose level. Mean duration of gestation was 21.6, 21.9, 22.1 and 22.4 days, in order of ascending dose level.

At the dose level of 1000 mg/kg bw/day, a lower number of implantations was noted. Mean number of implantations per dam was 8.5 at this dose level, compared to 12.2 in the control group. This difference was not statistically significant but below the historical control range (containing values from 11.4 to 13.7) (see Table 21).

Treatment with the test item caused an increase in post-implantation loss at all dose levels with a total post implantation loss in six litters at the high dose level. Mean incidence of post implantation loss per dam was 0.4, 2.0, 3.8 and 8.5 at the dose levels of 0, 40, 200 and 1000 mg/kg bw/day, respectively. The differences to the control value were statistically significant in all dose groups (Table 21).

Treatment with the test item caused reduction of litter size at first litter check in all dose groups. At the dose level of 1000 mg/kg bw/day, no living pups were found at first litter check. In two litters (no. 83 and 87) dead pups were found at first litter check. In remaining litters, beginning of delivery was noticed; first delivered pups were noted or supposed in the cage, but no living pups were found in the cages during the first litter check. It should be considered that at least some of the females at the high dose level delivered their pups but cannibalized them shortly thereafter. At the dose level of 200 mg/kg bw/day 24 pups (from 4 litters) were found dead at first litter check whereas mean number of living pups per dam was 8.8. At the dose level of 40 mg/kg bw/day 5 pups (from 4 litters) were found dead at first litter check whereas mean number of living pups per dam was 10.7. In the control group, no dead pups at first litter check were noted; mean number of living pups per dam was 11.9. Differences at the mid- and low-dose levels were not statistically significant. However, lower litter size resulted from test item-related increase of post-implantation loss and values at these dose levels were beyond the HCD (containing values of mean number of living pups per dam from 10.3 to 13.2).

In all dose groups, statistically significant reduction in birth index (number of pups born alive as a percentage of implantation sites) was noted. Mean birth index was 96.7%, 84.3%, 68.8% and 0.0% at the dose levels of 0, 40, 200 and 1000 mg/kg bw/day, respectively (Table 20).

Increased postnatal loss was noted in all dose groups. At the dose level of 200 mg/kg bw/day, 27 pups (from 5 litters) were lost during lactation. At the dose level of 40 mg/kg bw/day, 9 pups (from 1 litter) were lost during lactation. In the control group, one pup was lost during lactation. Consequently, statistically significant reduction in viability index (number of pups on day 4 post partum as a percentage of pups born alive) was noted in mid- and low-dose groups. Mean viability index was 99.2%, 91.6% and 69.3% at the dose levels of 0, 40 and 200 mg/kg bw/day, respectively.

Table 21: Breeding data (Anonymous, 2012c).

		0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1000 mg/kg/day
Number of litters		11	10	10	8
Duration of gestation	mean	21.6	21.9	22.1	22.4
	Std.Dev	0.5	0.3	0.6	0.7
	N	11	10	10	8
Corpora lutea	Total	124	111	88	97
	mean	11.3	11.1	9.8	12.1
	Std.Dev	2.7	3.9	2.5	5.2
	N	11	10	9	8
Implantations	Total	134	127	109	68
	mean	12.2	12.7	12.1	8.5[1]
	Std.Dev	4.0	3.2	2.8	3.7
	N	11	10	9	8
Post implantation loss	Litters affected	4	**10****	8	**8****
	Total	4	**20****	**34****	**68***
	mean	0.4	**2.0**[##]**	**3.8**[#]**	**8.5**[##]**
	Std.Dev	0.5	1.6	4.1	3.7
	N	10	10	9	8
Living pups at first check	Total	131	107	88	**0**[##]**
	mean	11.9	10.7	8.8	**0**
	Std.Dev	3.6	2.5	4.0	0.0
	N	11	10	10	8
Dead pups at first	Litters	-	**4***	**4***	2

litter check	affects				
	Total	0	5	24	7
	mean	0.0	0.5	2.4	0.9
	Std.Dev	0.0	0.7	4.2	2.1
	N	11	10	10	8
Living pups on day 4 post partum	Total	130	98	61	0
	mean	11.8	9.8	**6.1**[#]	**0.0**[##]
	Std.Dev	3.5	3.2	4.1	0.0
	N	11	10	10	8
Postnatal loss days 0-4 post partum	Litters affects	1	1	5	-
	Total	1	**9****	**27****	0
	mean	0.1	0.9	2.7	0.0
	Std.Dev	0.3	2.8	3.6	0.0
	N	11	10	10	8

Steel test, significant at 5% (#), 1% (##); Fischer´s Exact test, signif. at 5% (*), 1% (**)

[1] not statistically significant but below the historical control range (containing values from 11.4 to 13.7).

F1-generation:

During first litter check, no milk in the stomach was noted in one pup at the dose level of 1000 mg/kg bw/day, 15 pups (from 2 litters) at the dose level of 200 mg/kg bw/day and 3 pups (from 2 litters) at the dose level of 40 mg/kg bw/day. All these pups were dead at first litter check. At the low-dose level 2 further pups (from one litter) had no milk in the stomach on day 2 post partum. Several dead pups at the first litter check were found partially cannibalized (6 pubs from 2 litters in group 4, 4 pubs from 2 litters in group 3).

Pups sex ratio was not affected by exposure to the test item at any dose level. At first litter check, percentages of male pups were 53%, 44% and 51% at the dose levels of 0, 40 and 200 mg/kg bw/day.

Mean body weights of pups on day 1 post partum were: 6.4 g, 6.6 g and 5.9 g and mean body weight gains during lactation were +47.5%, +48.5%, and +40.2%, at the dose levels of 0, 40, and 200 mg/kg/day, respectively (not statistically significant). No data on body weight for high dose pups as they were found dead.

No findings during necropsy of pups at any dose level are documented.

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

The substance Penta-PSCA Na-TEA, tested in a OECD 422 study, showed signs of toxicity in parent animals at 200 and 1000 mg/kg bw/day.

At 1000 mg/kg bw/day toxic effects in the parents have been observed, e.g. significantly reduced body weight (m, f), significantly reduced food consumption (m, f), significantly reduced body temperature (m, f), reduced locomotor activity (m, f), significantly increased liver weight (m) and liver hypertrophy (m, f), reduced testis and epididymidis 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). These effects are considered as adverse but not marked systemic effects and thus negative impact on fertility parameters are relevant for classification purposes (ECHA, 2017a).

At 200 mg/kg bw/day minor parental toxicity like reduced locomotor activity (m, f), reduced food consumption (m, f) and reduced body weight (m) are documented. For parental toxicity a NOAEL of 40 mg/kg bw/day (reduced body weight, reduced food consumption, salivation) can be set.

A dose dependent decrease in birth index (C: 96.7%, 40 mg/kg bw/day: 84.3%, 200 mg/kg bw/day: 68.8%, 1000 mg/kg bw/day: 0) and viability index (C: 99.2%, 40 mg/kg bw/day: 91.6%, 200 mg/kg bw/day 69.3%, 1000 mg/kg bw/day: 0) and fertility index (C: 100.0%, 40 mg/kg bw/day: 90.9%, 200 mg/kg bw/day: 90.9% and 1000 mg/kg bw/day 72.7%) was observed. In all dose groups the reduction in birth index (number of pups born alive as a percentage of implantation sites) is statistically significant. The viability index (number of pups on day 4 post partum as a percentage of pups born alive) was also statistically reduced in mid- and low-dose groups. Other important fertility parameters (ECHA, 2017b) like post-implantation loss, reduced litter size and postnatal loss were already increased at 40 mg/kg bw/day indicating that substance specific adverse effects on fertility already occur below paternal LOAEL of 200 mg/kg bw/day. In the highest dose all pregnant females lost their litters before first litter check.

For effects on fertility a LOAEL of 40 mg/kg bw/day (increased pre-implantation loss, post-implantation loss, reduced litter size, reduced fertility index, reduced gestation index, reduced conception rate) can be derived. For toxicity in F1-generation a LOAEL of 40 mg/kg bw/day can be derived based on the mortality seen in all dose levels.

The study demonstrates adverse effects on fertility for the substance Penta-PSCA Na-TEA.

A previous dose range finding study resulted in significant reduced food consumption and reduced body weight and body weight gain at 1000 mg/ kg bw in males and females. 2/3 animals were not pregnant at this dose level. No effects on reproduction parameter were seen.

10.10.3 Comparison with the CLP criteria

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 when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

- The classification of a substance in Category 1A is largely based on evidence from humans.

- The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. 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.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

In a Reproduction/Developmental Toxicity Screening Test the substance Penta-PSCA Na-TEA caused marked adverse effects on fertility. Fertility parameters (birth index, viability index and post-implantation loss) were already significantly altered at a dose level of 40 mg/kg bw/day. In addition increased pre-implantation loss, reduced litter size and reduced fertility index have been seen in a dose dependant manner. At the highest dose level (1000 mg/kg bw/day) substance administration results in high post implantation loss and all pregnant females lost their litter before first litter check. For effects on fertility a LOAEL of 40 mg/kg bw/day (increased pre-implantation loss, post-implantation loss, reduced litter size, reduced fertility index, reduced gestation index, reduced conception rate) can be derived

The following weighting parameters have to be considered:

- High incidence (viability index and birth index reduced up to 100%, implantation loss 100% at highest dose) (concern ↑)
- The generic nature of the maternal toxicity (loss of appetite, reduction in body weight, and at higher levels moderate toxicity such as reduced body temperature (m, f), reduced locomotor activity (m, f) makes it very difficult to suggest a causal relationship between reproductive and parental toxicity (concern ↑)
- In a 28-day repeated dose toxicity study, at the dose level of 1000 mg/kg bw/day, statistically significantly reduced absolute testis and epididymides weights as well as statistically significantly reduced epididymides weights relative to brain weights were noted (concern↑). These changes were however not accompanied by histopathological changes (concern ↓).
- Species differences in sensitivity is unknown and only rats have been tested (concern ↑)
- Toxicokinetics/toxicodynamics data are not available. A direct effect of the substance and/or its main metabolites cannot be ruled out (concern ↑).
- Modes of Action (including ED properties) are unknown (concern↑).

Aspects of developmental toxicity of the substance will be discussed in the following Chapter.

10.10.4 Adverse effects on development

For the evaluation of developmental toxicity two studies with the substance Penta-PSCA Na-TEA are available.

Table 22: 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 Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) GLP Rat, Han Wistar (m, f) n=11/sex/dose	Test substance: Penta-PSCA Na-TEA (Emulsogen 3971, >90%) Oral, gavage Vehicle: water Dose levels: 0 mg/kg/day (Group 1, control group) 40 mg/kg/day (Group 2) 200 mg/kg/day (Group 3) 1000 mg/kg/day (Group 4) Dose Volume: 10 mL/kg body weight m: 4 weeks f: ~7 weeks	$NOAEL_{parental\ toxicity}$ = 40 mg/kg bw/day (reduced food consumption, salivation) $LOAEL_{fertility}$ = 40 mg/kg bw/day $LOAEL_{developmental\ toxicity}$ = 40 mg/kg bw/day • reduced fertility index (100%, 90.9%, 90.9%, 72.7%) • reduced gestation index (100%, 100%, 90%, 0%*) • increased pre-implantation loss in mid and high dosed animals • post-implantation loss at all dose levels (mean incidence per dam: 0.4, 2.0*, 3.8* and 8.5* at dose levels of 0, 40, 200 and 1000 mg/kg bw/day) • reduction of litter size at all dose levels (mean number of living pups per dam 11.9, 10.7, 8.8 and 0* respectively) • reduction in birth index (96.7%, 84.3%*, 68.8%*, 0.0%*) • postnatal loss (days 0-4) in mid and low dose groups • viability index: 99.2%, 91.6%* and 69.3%* at dose levels of 0, 40 and 200 mg/kg bw, respectively $LOAEL_{F1\text{-}generation}$ = 40 mg/kg bw/day (mortality)	Anonymous, 2012c
OECD 414 (Prenatal Developmental Toxicity Study) Rat, Wistar	Penta-PSCA Na-TEA. Oral, gavage 0, 8, 40, 200 mg/kg bw/day Vehicle: water day 6 post-coitum – day 20 post	$NOAEL_{parental\ toxicity}$ = 40 mg/kg bw/day (reduced food consumption and body weight gain) $NOAEL_{fertility}$ = 200 mg/kg bw $LOAEL_{developmental\ toxicity}$ = 8 mg/kg bw/day	Anonymous, 2013b

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
n = 5/sex/dose	coitum	(skeletal, visceral malformations) ≥8 mg/kg bw: small spleen ≥40 mg/kg bw: supernumerary ribs 200 mg/kg bw: skeletal abnormalities and variations	

For the substance Penta-PSCA Na-TEA a OECD 422 Screening Test is available investigating concentrations of 0, 40, 200, 1000 mg/kg bw/day. The study (Anonymous, 2012c) has been described in detail in Chapter 10.10.1.

Parental toxicity (reduced food consumption, salivation, reduced locomotor activity, reduced body weights in males) has been seen at 200 mg/kg bw/day and above, a NOAEL of 40 mg/kg bw/day has been derived.

For developmental toxicity a LOAEL of 40 mg/kg bw/day can be derived based on the following main findings (see also Table 20 and Table 21):

- Postimplantation loss was statistically significant increased at 40, 200 and 1000 mg/kg bw/day.
- A reduction of litter size was seen at all dose levels. All pregnant high dose females lost their litter before first litter check.
- The birth index was statistically significant reduced in all dose groups (96.7%, 84.3%*, 68.8%*, 0.0%*)
- postnatal loss (days 0-4) was significant at 40 and 200 mg/kg bw/day

Based on the results of the Screening test a Prenatal Developmental Toxicity Study with a reduced number of animals (5/sex/dose) was performed in order to investigate if the effects found in the OECD 422 study originated from a fertility impairment or fetotoxicity.

Female Wistar rats were exposed to concentrations of 0, 8, 40, 200 mg/kg bw/day from day 6 post coitum till day 20 post coitum via gavage once a day. Caesarean section and necropsy were done on day 21 post coitum.

All females survived till scheduled necropsy. At 200 mg/kg bw/day the food consumption and the body weight gain of the dams were slightly reduced. Mean differences are presented in the table below. Individual animal data can be seen in Table 24. No apparent maternal toxicity was seen.

Table 23: Food consumption and body weight gain of female rats (Anonymous, 2013b).

	Food consumption, difference to control	Body weight gain during treatment	Corrected body weight gain (corrected for

			gravid uterus weight)
Control	0.0%	+55%	+17.9%
8mg/kg bw/day	+3.3%	+51%	+12.6%
40mg/kg bw/day	-3.7%	+53%	+13.1%
200 mg/kg bw/day	-6.6%	+52%	+14.4%

Table 24: Corrected body weight gain - individual data (Anonymous, 2013b).

Dose group	Female #	Body weight (day 6) [g]	Body weight (day 21) [g]	Uterus weight [g]	Corrected weight gain [g]	Corrected weight gain [%]
0 mg/kg bw	1	240.7	375.2	100.2	34.4	14.3
	2	227.5	347.7	65.2	55.0	24.2
	3	228.1	344.1	85.5	30.6	13.4
	4	224.5	362.5	107.3	30.8	13.7
	5	233.3	354.0	65.0	55.7	23.9
8 mg/kg bw	6	242.4	366.8	104.2	20.2	8.3
	7	247.1	383.1	112.0	24.0	9.7
	8	252.2	389.9	105.2	32.6	12.9
	9	235.0	365.1	78.1	52.1	22.2
	10	241.8	332.0	66.2	24.0	9.9
40 mg/kg bw	11	229.1	364.1	94.0	41.0	17.9
	12	227.4	349.0	104.5	17.0	7.5
	13	245.7	371.8	100.1	26.0	10.6
	14	233.1	364.0	82.7	48.2	20.7
	15	221.4	318.7	78.2	19.2	8.6
200 mg/kg bw	16	231.5	335.8	80.2	24.1	10.4
	17	226.7	364.0	94.1	43.2	19.1
	18	239.5	360.1	82.5	38.1	15.9
	19	223.7	333.5	78.9	30.9	13.8
	20	236.5	362.3	95.7	30.1	12.7

All females were pregnant. The ovaries and uterine content were examined after termination. Examinations included gravid uterus weight, number of corpora lutea, implantations, early resorptions and late resorptions. No differences were found for control and treated animals upon

investigation on number of corpora lutea, implantation sites and number of live fetuses. Data (per dam) are presented in Table 25. During necropsy enlarged placentas were found in one control female and in one high dosed female and therefore considered not test-substance related. A summary of reproduction data are documented in Table 26.

Table 25: Reproduction data per dam (Anonymous, 2013b).

Dose group	Female #	Corpora lutea	Implantations	Embryonic deaths (total)	Fetuses live, total	Fetuses dead, total	Fetuses malfomated, total
0 mg/kg bw	1	15	15	0	15	0	0
	2	14	14	4	10	0	0
	3	14	13	1	12	0	0
	4	17	16	1	15	0	0
	5	17	14	5	9	0	0
8 mg/kg bw	6	17	16	0	16	0	0
	7	18	17	0	17	0	0
	8	17	15	0	15	0	0
	9	13	12	1	11	0	0
	10	14	14	4	10	0	0
40 mg/kg bw	11	15	15	1	14	0	0
	12	17	16	1	15	0	0
	13	17	15	0	15	0	0
	14	14	12	0	12	0	0
	15	12	12	0	12	0	0
200 mg/kg bw	16	13	13	1	12	0	2
	17	21	14	1	13	0	0
	18	12	12	1	11	0	0
	19	15	12	1	11	0	0
	20	15	13	0	13	0	0

Table 26: Summary of reproduction data (Anonymous, 2013b).

	0 mg/kg bw	8 mg/kg bw	40 mg/kg bw	200 mg/kg bw
Corpora lutea	77	79	75	76
Mean	15.4	15.8	15.0	15.2
StDev.	1.5	2.2	2.1	3.5
Pre-Implantation loss	5	5	5	12

Mean	1.0	1.0	6.7	2.4
StDev.	1.2	0.7	1.0	2.9
No. of dams affected	3	4	1.0	3
Implantation sites	72	74	70	64
Mean	14.4	14.8	14.0	12.8
StDev.	1.1	1.9	1.9	0.8
Post-implantation loss	11	5	2	4
% of impl. sites	15.3	6.8	**2.9 ##**	6.3
Mean	2.2	1.0	0.4	0.8
StDev.	2.2	1.7	0.5	0.4
No. of dams affected	4	2	2	4
Embryonic resorptions	10	4	2	4
% of impl. sites	13.9	5.4	**2.9 #**	6.3
Mean	2.0	0.8	0.4	0.8
StDev.	1.9	1.3	0.5	0.4
No. of dams affected	4	2	2	4
Fetal resorptions	1	1	0	0
% of impl. sites	1.4	1.4		
Mean	0.2	0.2		
StDev.	0.4	0.4		
No. of dams affected	1	1		
Fetuses, total	61	69	68	60
% of impl. sites	84.7	93.2	**97.1 ##**	93.8
Mean	12.2	13.8	13.6	12.0
StDev.	2.8	3.1	1.5	1.0

#/## Fisher´s Exact Test significant at level 5% (#) of 1% (##)

Fetal body weights were not effects at any dose level. No statistically significant differences in the sex ratio of the foetuses were noted in any group. Fetal evaluation included external examinations of all pups per litter as well as soft tissue examinations, skeletal examinations and head examinations for half per litter (total numbers see Table 27):

- In the external examination two fetuses from one litter at 200 mg/kg bw/day exhibited abnormalities. One had no lower jaw, small mouth opening and possibly a cleft palate and the other a cleft palate.

- Visceral abnormalities were seen in all fetuses of 200 mg/kg bw/day. They had small spleen and seven foetuses had incomplete fusion of nasal septum to palate. Small spleen was found down to the dose level of 8 mg/kg bw/day (see Table 27).
- Skeletal abnormalities were found in all examined foetuses (n=28) at dose of 200 mg/kg bw/day, comprising thin skull zygomatic jugal arch, abnormal curvature of pectoral girdle clavicle. Additional findings were absent humerus deltoid tuberosity in forelimb in 24 fetuses, short mid region of rib cage in 17 fetuses, abnormal curvature of hyoid body in 14 fetuses and abnormal spacing of zygomatic arch structures in 6 fetuses.
- Variations noted at 200mg/kg bw: increased ossification/thick tympanic ring in 28 fetuses, fusion of zygomatic arch in 22 fetuses, increased ossification of scapula in pectoral gridle in 13 fetuses and slight curved or slightly bent forelimb radius in 10 fetuses.
- Increased number of supernumerary ribs was found dose dependent at 200 and 40 mg/kg bw/day (see Table 27).

Clear toxic effects (LOAEL = 8 mg/kg bw) were found for foetuses at doses that were not associated with an apparent maternal toxicity (NOAEL = 40 mg/kg bw).

Table 27: Fetotoxicity in a Prenatal Developmental Toxicity Study **(Anonymous, 2013b)**

	Dose level Foetuses examined	Control n=32	8 mg/kg bw/day n=36	40 mg/kg bw/day n=35	200 mg/kg bw/day n=32
Small Spleen	Spleen small or small severe (total)	1 (3%)	4 (11%)	17 (49%)	32 (100%)
	Small (ca. 75% of expected size)	1 (3%)	4 (11%)	17 (49%)	32 (100%)
	Small severe (ca. 50% of expected size)	0 (-)	0 (-)	1 (3%)	27 (84%)
		Control n=29	8 mg/kg bw/day n=33	40 mg/kg bw/day n=33	200 mg/kg bw/day n=28
Supernumerary ribs	Left	7 (24%)	8 (24%)	26 (79%)	24 (82%)
	Right	8 (28%)	5 (15%)	24 (73%)	25 (89%)

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

Penta-PSCA Na-TEA was investigated for its developmental toxicity in a OECD 414 study and in a OECD 422 screening study in Wistar rats.

In a combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (Anonymous, 2012c) rats were exposed to concentrations of 0, 40, 200, 1000 mg/kg

bw/day prior to pairing, through the pairing and gestation periods until the F1 generation reached day 4 post partum. Minor parental toxicity has been seen at 200 mg/kg bw/day and above, a NOAEL of 40 mg/kg bw/day has been derived. For developmental toxicity a LOAEL of 40 mg/kg bw/day can be derived based on postimplantations loss, reduction of litter size, reduced birth index and postnatal loss.

In the prenatal developmental toxicity study (Anonymous, 2013b) the NOAEL for maternal toxicity was 40 mg/kg bw/day based on reduced food consumption and body weight gain seen at 200 mg/kg bw/day. Intrauterine exposed foetuses showed an increased frequency of a small spleen seen in a dose dependant manner down to the dose level of 8 mg/kg bw/day. At 40 and 200 mg/kg bw/day an increased number of supernumerary ribs (rudimentary) was found. In addition skeletal abnormalities were found in all fetuses at a dose of 200 mg/kg bw/day, comprising thin skull zygomatic jugal arch, abnormal curvature of pectoral girdle clavicle, absent humerus deltoid tuberosity in forelimb, short mid region of rib cage, abnormal curvature of hyoid body and abnormal spacing of zygomatic arch structures. Based on these findings a LOEAL of 8 mg/kg bw/day can be defined for developmental toxicity of Penta-PSCA Na-TEA.

Fetotoxicity was found at doses that were not associated with apparent maternal toxicities. The studies demonstrate adverse effects on the developmental of offsprings for Penta-PSCA Na-TEA.

10.10.6 Comparison with the CLP criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). Adverse effects on development

- The classification of a substance in Category 1A is largely based on evidence from humans.
- The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. 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.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

In the Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test (OECD 422) the application of read-across substance Penta-PSCA Na-TEA shows developmental toxic effects with a $LOAEL_{developmental\ toxicity}$ = 40 mg/kg bw/day.

The observation was further substantiated in a modified Prenatal Developmental Toxicity Study (OECD 414). Fetotoxic effects such as cleft palate formation, visceral abnormalities (small spleen)

and skeletal abnormalities were observed. A LOAEL for developmental toxicity effects of 8 mg/kg bw/day can be set. The effects were seen at doses that were not associated with an apparent maternal toxicity. The NOAEL for maternal toxicity is 40 mg/kg bw/day.

The following weighting parameters have to be considered:

- High incidence, severity (up to 100% incidence in small spleen possibly related to immunotoxicity) and rare findings (e.g cleft palate)(concern ↑);

- The generic nature of the maternal toxicity (loss of appetite, reduction in body weight, and at higher levels moderate toxicity such as reduced body temperature (m, f), reduced locomotor activity (m, f)) makes it very difficult to suggest there is a causal relationship between reproductive and parental toxicity (concern↑).

- Species difference in sensitivity is unknown as only rats have been tested. Relevance to humans cannot be excluded (concern ↑).

- Toxicokinetics/toxicodynamics data are not available. A direct effect of the substance and its main metabolites on the developing organism cannot be ruled out (concern ↑).

- Modes of Action (including ED properties) are unknown (concern↑)..

10.10.7 Adverse effects on or via lactation

No data available

10.10.8 Conclusion on classification and labelling for reproductive toxicity

For the substance Penta-PSCA Na-TEA one OECD 414 and one OECD 422 study are available.

Fertility parameters (birth index, viability index and post-implantation loss) were already significantly altered at a dose level of 40 mg/kg bw/day. In addition increased pre-implantation loss, reduced litter size and reduced fertility index have been seen in a dose dependant manner. At the highest dose level (1000 mg/kg bw/day) substance administration results in high post implantation loss and all pregnant females lost their litter before first litter check. Based on the OECD 422 study a LOAEL of 40 mg/kg bw/day for developmental toxicity and fertility can be derived.

In order to determine if the effects seen in the OECD 422 study originate from fertility impairment or from fetotoxicity and to see if substance application affects further developmental toxicity parameters a OECD 422 was carried out. In this study a LOAEL of 8 mg/kg bw/day has been derived based on visceral abnormalities (small spleen at 8 mg/kg bw) and skeletal abnormalities (supernumerary ribs at 40 and 200 mg/kg bw/day, several abnormalities and variations in skeleton at 200 mg/kg bw/day).

Reproductive toxicity of Penta-PSCA Na-TEA was found at doses that were not associated with apparent maternal toxicity (seen at 200 mg/kg bw/day and above). Fertility effects and developmental toxicity were seen at lower concentrations (40 mg/kg bw/day and 8 mg/kg bw/day) then paternal toxicity effects.

The chemical moiety responsible for the reprotoxic effects (fertility and developmental toxicity) is assumed to be the 2,5 dioxopyrrolidin hexanoate. TEA showed no reproductive toxicity in an OECD 421 screening test (see also read-across justification Annex I).

Based on read-across the substance 6-(C10-13-alkenyl-(even and odd, branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoic acid has to be classified for its adverse effects on fertility and development as Repro 1B, H360FD.

Reproductive toxicity was only tested for exposure via oral route. In the absents of valid data by other routes the hazard statement can not be specified by indicating a specific route of exposure (ECHA, 2017).

Concentration limits:

According to the CLP guidance (ECHA, 2017), concentration limits for adverse effects should be based on the lowest ED_{10} effective dose with a 10% effect level above the background. Post-implantation loss and small spleen, were the leading effects for reproductive toxicity. The resulting ED_{10} values for the testing with Penta-PSCA Na-TEA are:

	Statistic modelling	
Effects	Linear response	Sigmoidal response
Post-implantation loss	107.7 mg/kg bw	117.6 mg/kg bw
Small spleen	23.2 mg/kg bw	7.8 mg/kg bw

Details on statistic analysis are given in Annex III.

The lowest ED_{10} value of all the key studies for effects warranting classification determines the overall ED_{10} of the substance (ECHA, 2017). For a preliminary potency evaluation the following boundaries according CLP guidance apply:

Potency group	Boundaries
High potency group	ED10 value ≤ 4 mg/kg bw/day
Medium potency group	4 mg/kg bw/day < ED10 value < 400 mg/kg bw/day
Low potency group	ED10 value ≥ 400 mg/kg bw/day.

Based on the potency boundaries and the calculated ED_{10} values a medium potency can be assumed for the substance. Following modifying factors have to be considered (ECHA, 2017):

- Type and severity of the effect: The type of effects observed in reproductive toxicity studies following exposure to Penta-PSCA Na-TEA (source substance) included beside others post-implantation loss and small spleen at low doses and these were considered to be severe. As the lowest ED_{10} is close to the boundary of a higher potency group a change of the potency group has to be considered
- Data availability: The data available for Penta-PSCA Na-TEA (OECD 422 and OECD 414 study, full reports available) were considered adequate considering the REACH requirements. However only LOAELs could be derived based on the available data. The Prenatal Developmental Toxicity Study was done according OECD 414 but with a reduced number of animals. This reduced design was chosen as the study was designed to clarify whether the effects found in the OECD 422 originated from the fertility impairment or fetotoxicity.

- Dose-response relationship: The lowest ED_{10} (7.8 mg/kg bw, small spleen) of the source substance Penta-PSCa Na-TEA is similar to the LOAEL of 8 mg/kg bw.
- Mode or mechanism of action: No information is available.
- Toxicokinetics: No information is available.
- Bio-accumulation of substance: The source and the target substance are not considered to be bioaccumulating based on registration data.

In addition it has 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. 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, the substance is considered 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 a shift into the high potency group can be considered. No additional modifying factor applies.

Conclusion on concentration limit:
The potency of the source substance is a borderline case between medium and high potency. Small spleen was the most sensitive adverse effects seen down to the dose level of 8 mg/kg bw/day with an EC_{10} of 7.8 mg/kg bw. All other adverse effects in foetuses (increased number of supernumerary ribs, skeletal abnormalities) were found at exposure to 40 mg/kg Penta-PSCA Na-TEA and/or above. For the UVBC Penta-PSCA Na-TEA (UVCB, including 55% Penta-PSCA) therefore the generic concentration limits apply.

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 and 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 the low dose of 40 mg/kg bw/d. In addition, increased pre-implantation loss and reduced litter size and reduced fertility index were reported in a dose dependant manner. Further, in the high dose group (1000 mg/kg bw/d) a high incidence of post-implantation loss was reported, and all pregnant females lost their litter before the first litter check (day not available). These effects were not considered as secondary non-specific consequences of parental toxicity.

Based on the adverse effects reported on sexual function and fertility the DS proposal was to classify penta-PSCA Na-TEA 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 study (combined repeated dose toxicity study with the screening test) 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 (OECD TG 414) in Wistar rats (5/females/group) with exposure to 0, 8, 40 and 200 mg/kg bw/d penta-PSCA Na-TEA from gestation day 6-20 (Anonymous, 2013b). The 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 foetotoxicity. 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 penta-PSCA Na-TEA 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)

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: *ED10 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 ED10 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 severe effects observed in the reproductive toxicity studies following exposure to penta-PSCA Na-TEA are post-implantation loss and small spleen. As the lowest ED_{10} for these effects 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. The PNDT study was done 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 close to the effective dose of 8 mg/kg bw/d.
- *Mode or mechanism of action*: No information was available.
- *Toxicokinetic:* No information was available.
- *Bioaccumulation:* penta-PSCA Na-TEA was not considered to be bioaccumulating based on the REACH registration data.

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 factor applies.

Conclusion on concentration limit

The potency of penta-PSCA Na-TEA was a borderline case between medium and high potency. Small spleen was the most sensitive adverse effects seen down to the dose level of 8 mg/kg bw/d with an ED_{10} of 7.8 mg/kg bw/d. All other adverse effects in the foetuses (increased number of supernumerary ribs, skeletal abnormalities) were found at exposure to 40 mg/kg bw/d penta-PSCA Na-TEA and/or higher, therefore the DS considered that the generic concentration limits should apply.

In absence of data, the DS did not propose a classification for effects on or during lactation.

Comments received during consultation

Comments were received from three MSCAs, with one MSCA submitting two comments. All comments supported a classification as Repr. 1B; H360FD, no classification for lactation and that the GCL should be applied. As regards the assessment of a concentration limit one MSCA noted that in addition to the ED_{10} values calculated by the DS for effects on development a 10% decrease in the fertility was reported in the OECD TG 422 from 40 mg/kg bw/d supporting a GCL for effects on fertility and sexual function.

Assessment and comparison with the classification criteria

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, 2013a) with an associated OECD TG 422 DRF study (Anonymous, 2012c) 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 dose-range finding study with exposure to 0, 100, 300 and 1000 mg/kg bw/d of penta-PSCA Na-TEA (90% purity) showing a decreased fertility index and conception rate in the high dose group in the presence of decreased body weight gain and reduced food consumption, the doses for the main OECD TG 422 study were 0, 40, 200 and 1000 mg/kg bw/d (> 90% purity).

In the OECD TG 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 losses 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 (days 0-4) 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	4/0.4	20**/2.0##	34**/3.8#	68*/8.5##

(total/mean)				
Living pups at first check (total/mean)	131/11.9	107/10.7	88/8.8	0##/0
Dead pups at first litter check (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##
Pup mortality 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/d)	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.

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 viability index. These parameters were already statistically significantly altered at 40 mg/kg bw/d. In addition, a reduced litter size and reduced fertility index were reported in a dose dependent manner. Further, in the high dose group all dams experienced total litter loss. RAC considers that the effects on reproduction reported are considered not to be a secondary non-specific consequence of parental toxicity.

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

Developmental toxicity

For the assessment of developmental toxicity following exposure to penta-PSCA Na-TEA the DS included 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 (> 90% purity). The second study was 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. 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 before first litter check. The birth index was statistically significant reduced in all dose groups (96.7%, 84.3%*, 68.8%*, 0.0%*), while pup mortality on PND0-4 was increased 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 (OECD TG 414 in rats), 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*
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 cleft palate and the second foetuses had 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/da (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.

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 postnatal mortality (days 0-4) at 40 and 200 mg/kg bw/d and complete litter loss at 1000 mg/kg bw/d. These effects were not considered to be a secondary non-specific consequence of parental toxicity. In the developmental toxicity study (OECD 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.

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

Adverse effects on lactation

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

Setting of specific concentration limits (SCLs)

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. RAC notes that according to the CLP Guidance (paragraph 3.7.2.6.6.1) separate SCL should be set for effects on sexual function and fertility and developmental toxicity. Overall, RAC did not propose a SCL.

Concentration limit for effects on sexual function and fertility

The most sensitive effects on fertility and sexual function reported in the OECD TG 422 study with penta-PSCA Na-TEA was 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. RAC is however 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 the modifying factors is not relevant for setting a SCL for effects on sexual function and fertility. Due to the ED_{10} value obtained (well within the medium potency values), it is not relevant to modify the potency group (CLP Guidance, ECHA 2017, Version 5 point 3.7.2.6.5). RAC concludes that based on the ED_{10} at 40 mg/kg bw/d the GCL should be applied for penta-PSCA Na-TEA for sexual function and fertility.

Concentration limit for developmental toxicity

Post-implantation loss and small spleen were considered as the main effects for reproductive 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 to 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. However, as the other adverse effects in the foetuses in the developmental toxicity study and on fertility in the OECD TG 422 were reported from doses of 40 mg/kg bw/d, RAC concludes that the GCL should be applied for penta-PSCA Na-TEA.

10.11 Specific target organ toxicity-single exposure

Not assessed

10.12 Specific target organ toxicity-repeated exposure

Repeated dose toxicity has been investigated in a 28-day study with the source substance Tetra-PSCA and in a combined repeated dose toxicity study with Repro/Dev Toxicity screening with the substance Penta-PSCA Na-TEA. TEA is not used as read-across substance as available data on repeated dose toxicity show that the effects seems to be mainly related to the 2,5 dioxopyrrolidin hexanoat and not to TEA. For details see Annex I.

Table 28: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference

Dose Range finding study for OECD 422 Rat, RccHanTM: WIST(SPF) N= 3/sex/group	Test substance: Penta-PSCA Na-TEA (Emulsogen 3971, purity 90%) Oral (Gavage) Dose volume: 10ml/kg Vehicle: water Dose levels: 0, 100, 300, 1000 mg/kg/day m. 28 days f: 42 days (sacrif on day 14 of getation)	$NOAEL_{parental\ toxicity}$ = 300 mg/kg bw/day $LOAEL_{parental\ toxicity}$ (m, f) = 1000 mg/kg bw/day 1000 mg/kg bw: 2/3 females not pregnant, salivation (2m, 2f), food consumption↓, bw↓, bw gain↓ mean food consumption (compared to control at 100, 300 and 1000mg/kg bw): m: pre-pairing -8%, -8%, -29% m: after pairing period ±0%, -9% ,-17% f: pre-pairing -6%, -12% and -29% f: during gestation -5%, -5%, -19% mean body weight gain (at 0, 100, 300 and 1000 mg/kg bw) m: pre-pairing +14%, +14%, +13% , +4% m. pairing +4%, +4%, +2%, +2% m: after pairing +8%, +9%, +7% ,+8%. f: pre-pairing +9%, +9%, +9%, +4% f: during gestation +25%, +25%, +29%, +20% Corrected body weight gains females: 3.1%, -3.6%, -3.6%, -4.6% *Guidance values (28 days, rat):* *STOT RE 2: 30 < C ≤ 300 mg/kg bw/day* *STOT RE 1: C ≤ 30 mg/kg bw/day* *Guidance values (42 days, rat):* *STOT RE 2: 21 < C ≤ 214 mg/kg bw/day* *STOT RE 1: C ≤ 21 mg/kg bw/day*	Anonymous 2013a

OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) GLP Rat, Han Wistar (m, f) n= 11/sex/dose	Test substance: Penta-PSCA Na-TEA (Emulsogen 3971, purity >90%) Oral (Gavage) Vehicle: water Dose levels: 0 mg/kg/day (Group 1, control group) 40 mg/kg/day (Group 2) 200 mg/kg/day (Group 3) 1000 mg/kg/day (Group 4) Dose Volume: 10 mL/kg body weight m: 4 weeks f: ~7 weeks	$LOAEL_{parental\ toxicity}$ (m, f) = 200 mg/kg bw/day 1000mg/kg bw: - Salivation, bedding in mouth, ruffled fur, reduction in locomotor activity - reduced food consumption (m:-31.4%, f: pre-pairing -30.8%, gestation -27.2%, lactation -47.5%) - reduction in body weight gain (m: pre-pairing -1%, pairing +9%, f: pre-pairng +2%, gestation + 33%, lactation 0%) 200 mg/kg bw - Salivation, bedding in mouth, reduction in locomotor activity - reduced food consumption m: -10.3%, f: pre-pairing -11.8%, gestation -14.2%, lactation -34.0%) - reduction in body weight gain (m: pre-pairing +7%, pairing +7%, f: pre-pairng +6%, gestation + 49%, lactation 4%) 40 mg/kg bw: - Salivation, bedding in mouth $LOAEL_{F1\text{-}generation}$ = 40 mg/kg bw/day (mortality) *Guidance values (28 days, rat):* *STOT RE 2: 30 < C ≤ 300 mg/kg bw/day* *STOT RE 1: C ≤ 30 mg/kg bw/day* *Guidance values (49 days, rat):* *STOT RE 2: 16 < C ≤ 163 mg/kg bw/day* *STOT RE 1: C ≤ 16 mg/kg bw/day*	Anonymous, 2012c

OECD 407 GLP Rat, Crj: CD(SD) (m, f) n=6/sex/dose	Test substance: Tetra-PSCA Oral, gavage 8, 40, 200, 1000 mg/kg bw/day Vehicle: carboxymethyl cellulose) 28d	NOAEL (m, f) = 40 mg/kg bw LOAEL (m, f) = 200 mg/kg bw/day <u>1000mg/kg bw:</u> Salivation (m, f) Spontaneous movement ↓, hunchback posture (m,f) Respiratory rate ↓ (m,f) Depilation in the lower neck region (m, f) soft stool, reddish tears, reddish tear traces and ptosis (m) relative kidney weight ↑ (m), surface spotting relative liver weight ↑ (m: +24%, f: +35%) swelling of hepatocytes (m, f) moderate/severe eosinophilic bodies in kidney ↑ (m) granulation tissue accompanied by calcification (m) mucosal degeneration, forestomach (m) <u>200mg/kg bw:</u> Salivation (m, f) Spontaneous movement ↓ (m) Respiratory rate ↓ (m) Relative liver weight ↑ (f,: +10%) relative kidney weight ↑ (f) slight eosinophilic bodies in kidney ↑ (m) <u>after recovery:</u> relative liver weight ↑ at former 1000 mg/kg bw group (m: +9%, f: +11%) relative kidneys ↑ at former 1000 mg/kg bw group (m) relative kidneys ↑ at former 200 mg/kg bw group (f) eosinophilic bodies in the kidney ↑ (m) in the 1000 mg/kg bw group necrosis of the mucosa of the glandular stomach in males in the 200 mg/kg bw *Guidance values (28 days, rat):* *STOT RE 2: 30 < C ≤ 300 mg/kg bw/day* *STOT RE 1: C ≤ 30 mg/kg bw/day*	Anonymous, 1995

In a dose range-finding toxicity study (Anonymous, 2013a) carried out with Han Wistar rats, using the substance Penta-PSCA Na TEA in dose levels of 0, 100, 300 and 1000 mg/kg/day (n=3/sex/dose), males were dosed 14 days during pre-pairing and 14 days during pairing (in total 28 days). Females were dosed during pre-pairing, pairing and 14 days during gestation (in total 42 days). During the treatment bedding in mouth was noted in all dose groups (m, f) with a dose-dependent frequency. Salivation was noted at the dose level of 1000 mg/kg bw/day. These findings were considered to be test item-related. Differences in mean food consumption (food consumtion was not recorded during pairing) of males at the dose levels of 100, 300 and 1000 mg/kg bw/day were, respectively: -8%, -8% and -29% during the pre-pairing period and ±0%, -9% and -17% during the after pairing period. Differences in mean food consumption of females at the dose levels of 100, 300 and 1000 mg/kg bw/day were, respectively: -6%, -12% and -29% during the pre-pairing 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/day were, respectively: +14%, +14%, +13% and +4% during the pre-pairing period, +4%, +4%, +2% and +2% during the paring period and +8%, +9%, +7% and +8% during the after pairing period. Differences in mean body weight gain of females at the dose levels of 100, 300 and 1000 mg/kg bw/day were, respectively: +9%, +9%, +9% and +4% during the pre-pairing period and +25%, +25%, +29% and +20% during the gestation period. Corrected body weight gains were -3.1%, -3.6%, -3.6% and -4.6% at the dose levels of 0, 100, 300 and 1000 mg/kg bw/day respectively. No macroscopical findings were noted in males and females at any dose level Clinical laboratory investigations showed statistically significant lower relative hematocrit 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 at the high dose level. No further test item-related changes in hematology or clinical biochemistry parameters were noted in males or females at any dose level. No organ data were examined. A LOAEL of 1000 mg/kg bw 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 laboratory in females.

For the OECD 422 study with the substance Penta-PSCA Na-TEA (Anonymous, 2012c) the test substance was orally administered (gavage) in concentrations of 0, 40, 200 or 1000 mg/kg bw to male rats for 28 days in total and to female rats for 14 days prior to pairing, through the pairing and gestation periods until the F1 generation reached day 4 post partum (in total approx. 7 weeks). The observed results are presented in detail in Chapter 10.10. For parental toxicity a NOAEL of 40 mg/kg bw/day (reduced body weight, reduced food consumption, salivation) can be set as parental toxicity has been seen at 200 mg/kg bw/day and above. At 200 mg/kg bw/day effects like reduced locomotor activity (m, f), reduced food consumption (m, f) and reduced body weight (m) are documented. At 1000 mg/kg bw/day significantly reduced body weight (m, f), significantly reduced food consumption (m, f), significantly reduced body temperature (m, f), reduced locomotor activity (m, f), significantly increased liver weight (m) and liver hypertrophy (m, f), reduced testis and epididymidis 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 described. The higher kidney weight to body weight ratio in males at the high dose and the higher brain weight to body weight ratio in high dosed females were considered to be the result of lower body weights.

The read-across substance Tetra-PSCA was investigated for its repeated dose toxicity according to the OECD Guideline 407 (Anonymous, 1995). Tetra-PSCA in carboxymethyl cellulose (vehicle) was administered once daily for 28 days via gavage in doses of 0, 8, 40, 200 and 1000 mg/kg

bw/day. In addition the study included recovery groups for doses of 200 and 1000 mg/kg bw/day. Observations and examinations included clinical observation, body weight and food consumption, haematology, clinical chemistry, urinalysis, gross pathology and histopathology. Organ weights were determined for brain, liver, spleen, kidney, adrenals, testes (or ovaries). Histopathology included the examination of the following tissues:

Concentration	Tissue
control group 1000 mg/kg bw/day	liver, spleen, kidneys, heart, stomach, intestines, testes, adrenals
200 mg/kg bw/day	liver, kidneys (males only), stomach (males only), testes
40 mg/kg bw/day	liver (females only), kidneys (males only)
8 mg/kg bw/day	kidneys (males only)
Control group - recovery 1000 mg/kg bw/day - recovery	liver, kidneys (males only), stomach (males only), testes
200 mg/kg bw/day- recovery	liver, kidneys (males only), testes

No deaths occurred. No effects due to administration of the test substance were seen on body weight and food consumption during the period of administration or on the urinalyses at the time administration was concluded. Clinical signs observed were salivation in males and females at 200 mg/kg bw/day and higher, decreased spontaneous movement and decreased respiratory rate in males at 200 mg/kg bw/day. At 1000 mg/kg bw/day in females and males 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 observed. Soft stool, reddish tears, reddish tear traces and ptosis were documented for males in the 1000 mg/kg group. No clinical effects were seen at the end of the recovery period. Relative kidney weight was increased in females at 200 mg/kg bw/day and males at 1000 mg/kg bw/day. Male and female liver weight was increased in the 1000 mg/kg bw/day group 24% and 35% respectively. After recovery liver weight in the high dosed group was increased 9% in males and 11% in females (Table 29). Swelling of hepatocytes in males and females at 1000 mg/kg bw/day was seen in histopathology 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 (see Table 30 and Table 31). Most of the effects were reversible within the observation period of 14 days. A LOAEL of 200 mg/kg bw can be derived.

Table 29: relative liver weights (Anonymous, 1995).

sex	control	200 mg/kg bw/day	1000 mg/kg bw/day	recovery Control	recovery 200 mg/kg bw/day	recovery 1000 mg/kg bw/day
Males	3.05 ± 0.22	3.15 ± 0.14	**3.81** ± 0.07**	2.71 ±0.05	2.74 ± 0.09	**2.98** ± 0.09**
Females	3.14 ± 0.18	**3.47* ± 0.17**	**4.25** ± 0.13**	2.77 ± 0.12	3.05 ± 0.28	**3.08* ± 0.16**

*: significantly different from vehicle control at $p < 0.05$; ** at $p < 0.01$ (Bartlett's test)

Table 30: Histopathological findings (Anonymous, 1995).

effect	sex	severity	control	200 mg/kg bw/day	1000 mg/kg bw/day	recovery Control	recovery 200 mg/kg bw/day	recovery 1000 mg/kg bw/day
LIVER								
Swelling of hepatocytes	males		0	0	3	0	0	0
	Females		0	0	4	0	0	0
KIDNEY								
Eosinophilic bodies	males	++	0	3	1	0	0	1
		+++	0	0	3	0	0	0
		++++	0	0	2	0	0	0
	females	++	0	-	0	0	-	-
		+++	0	-	0	0	-	-
		++++	0	-	0	0	-	-
FORESTOMACH								
Mucosa degeneration	males	+	0	0	4	0	-	0
		++	0	0	1	0	-	0
	females	+	0	-	0	-	-	-
		++	0	-	0	-	-	-

+, very slight; ++, slight; +++, moderate; ++++ severe

Table 31: **Haematology and clinical chemistry (mean ± SD) (Anonymous, 1995).**

effect	sex	control	200 mg/kg bw/day	1000 mg/kg bw/day	recovery Control	recovery 200 mg/kg bw/day	recovery 1000 mg/kg bw/day
HAEMATOLOGY							
RBC [x10^4/mm^3]	males	786 ± 14	751 ± 32	**716** ± 38**	829 ± 47	809 ±019	781 ± 42
	females	738 ± 2	731 ± 36	707 ± 33	794 ± 24	772 ± 21	**743** ± 23**
WBC [x10^2/mm^3]	males	74 ± 12	**108** ± 13**	**107** ± 19**	117 ± 13	93 ± 20	112 ± 17
	females	71 ± 18	77 ± 10	82 ± 21	75 ± 30	57 ± 8	73 ±11
Hb [g/dt]	males	15.5 ± 0.06	15.0 ± 0.4	**14.1** ± 0.4**	15.6 ± 0.05	15.2 ± 0.7	15.3 ± 0.3
	females	15.1 ± 0.6	14.8 ± 0.6	**13.9** ± 0.5**	15.6 ± 0.4	15.1 ± 0.3	**15.0** ± 0.5**
Ht [%]	males	44.5 ± 1.4	44.0 ± 1.0	**41.2** ± 1.0**	45.0 ± 2.0	43.7 ± 1.6	44.1 ± 1.4
	females	42.1 ± 1.4	41.0 ± 2.5	**38.9** ± 1.5**	43.3 ± 0.8	41.9 ± 0.8	42.0 ± 1.5
Platelet [x10^4/mm^3]	males	121.6 ± 10.9	130.2 ± 15.6	136.4 ± 16.5	115.5 ± 9.9	116.7 ± 10.4	110.6 ± 11.2
	females	129.1 ± 11.6	132.5 ± 16.7	120.2 ± 6.3	120.6 ± 9.4	124.4 ± 11.6	**135.7** ± 6.1**
PT [sec]	males	16.6 ± 3.9	14.9 ± 2.1	17.8 ± 0.9	14.1 ± 1.5	14.5 ± 2.1	15.7 ± 2.5
	females	12.0 ± 0.7	11.5 ± 0.5	11.7 ± 0.9	11.3 ± 0.5	11.0 ± 0.2	10.9 ± 0.3
APTT [sec]	males	28.9 ± 3.1	30.7 ± 2.9	32.7 ± 3.3	27.2 ± 3.8	24.1 ± 3.4	26.4 ± 1.9
	females	20.7 ± 0.8	22.1 ± 2.1	**25.9** ± 3.6**	21.8 ± 2.3	20.2 ± 3.9	20.2 ± 1.7
CLINICAL CHEMISTRY							
ALP [lU/l]	males	512 ± 67	490 ± 57	476 ± 67	373 ± 43	336 ± 35	342 ± 40
	females	310 ± 34	265 ± 54	249 ± 31	200 ± 28	196 ± 42	171 ± 27
Glucose [mg/dl]	males	133.1 ± 13.4	**111.8** ± 13.8**	**108.3** ± 12.6**	150.8 ± 15.6	132.6 ± 16.2	129.7 ± 17.6
	females	116.5 ± 11.1	121.7 ± 14.0	103.8 ± 22.5	124.4 ± 12.8	135.9 ± 16.5	127.6 ± 10.4
TG [mg/dl]	males	51 ± 15	66 ± 20	58 ± 11	63 ± 11	61 ± 13	46 ± 16

	females	29 ± 4	31 ± 7	**41* ± 10**	35 ± 11	33 ± 16	31 ± 6
Creatinine [mg/dl]	males	0.45 ± 0.07	0.39 ± 0.03	0.40 ± 0.05	0.54 ± 0.03	**0.47** 0.02**	**0.47** ± 0.04**
	females	0.46 ± 0.03	0.43 ± 0.04	**0.39* ± 0.04**	0.50 ± 0.04	0.49 ± 0.05	0.49 ± 0.04
T-Bil [mg/dl]	males	0.18 ± 0.03	0.22 ± 0.04	**0.25** ± 0.04**	0.15 ± 0.02	**0.18* 0.01**	**0.19*0.02**
	females	0.18 ± 0.01	0.20 ± 0.02	0.21 ± 0.02	0.17 ± 0.03	0.19 ± 0.04	0.17 ± 0.02
Cl [mEq/l]	males	106.2 ± 1.5	106.4 ± 1.3	106.0 ± 0.9	106.7 ± 2.0	107.2 ± 1.2	106.8 ± 1.9
	females	109.3 ± 1.0	**106.7** ± 1.3**	**106.8** ± 1.1**	108.7 ± 1.5	108.4 ± 1.7	108.9 ± 2.6

*: significantly different from vehicle control at $p < 0.05$; ** at $p < 0.01$ (Bartlett's test)

10.12.1 Comparison with the CLP criteria

A substance is classified with STOT RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity to humans or be harmful to human health following repeated exposure by the oral, dermal or inhalation routes. This can be on the basis of human data or evidence from studies in animals that cause such effects at or below given Guidance Values. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included under this classification.

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: • reliable and good quality evidence from human cases or epidemiological studies; or • observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of- evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided in the CLP regulation in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2

The guidance values for classification as STOT RE (oral exposure) are as follows (CLP-guidance document 3.9.2.2, Haber's rule):

Rat [mg/kg bw]	90 day	28d	49d
Category 1	$C \leq 10$	$C \leq 30$	$C \leq 16$
Category 2	$10 < C \leq 100$	$30 < C \leq 300$	$16 < C \leq 163$

For the available 28day study with the source substance Tetra-PSCA a NOAEL of 40 mg/kg and a LOAEL of 200 mg/kg bw/day (m, f) can be derived. The main target organ of the substance is the liver in both sexes and the kidney and forestomach in male rats. Slight adverse effects like salivation (m, f), decreased spontaneous movement (m), decreased respiratory rate (m), relative kidney weight increased (f + 10.5%) and slight increase of eosinophilic bodies in male kidneys are documented for concentrations of 200 mg/kg bw/day. At 1000 mg/kg bw liver weight increase in m + 24%, f + 35% as well as swelling of hepatocytes and granulation tissue were observed.

Studies with the substance Penta-PSCA Na-TEA with exposure durations from 28 (m) to 49 (f) days resulted in LOAEL of 200 mg/kg bw (OECD 422) or 1000 mg/kg bw (range finding sudy). The dose range finding study showed significant reductions in food consumption and body weight gain in males and females at 1000 mg/kg bw. The OECD 422 study showed parental toxicity (reduced food consumption, salivation) at 200 mg/kg bw and effects on target organs (liver, kidney, forestomach, thyroid gland) at 1000 mg/kg bw.

The effect levels (m, f) of all available studies are presented in the table below.

Table 32: Effects levels from repeated dose toxicity studies

Study Test substance	Sex	Duration of exposure	NOAEL	LOAEL
OECD 407 Tetra-PSCA	m	28 d	40 mg/kg bw/day	200 mg/kg bw/day
	f	28 d	40 mg/kg bw/day	200 mg/kg bw/day
Range-finding study Penta-PSCA Na-TEA	m	28 d	300 mg/kg bw/day	1000 mg/kg bw/day
	f	42 d	300 mg/kg bw/day	1000 mg/kg bw/day
OECD 422 Penta-PSCA Na-TEA	m	28 d	40 mg/kg bw/day	200 mg/kg bw/day
	f	49 d	40 mg/kg bw/day	200 mg/kg bw/day

10.12.2 Conclusion on classification and labelling for STOT RE

In the 28-day study in rats, toxicology effects caused by the source substance Tetra-PSCA have been observed from a dose level of 200 mg/kg bw, i.e. within the GV of $30 < C \leq 300$ mg/kg bw (28d, m/f) for STOT RE 2. However the effects observed at this dose level are not considered sufficiently severe (moderate and low incidence eosinophilic bodies in the kidneys of males, increased rel. liver weight in females +10%, no significant changes in haematology and clinical chemistry) to warrant a classification for STOT RE 2.

In the OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test), toxicology effects caused by Penta-PSCA Na-TEA have been observed from a dose level of 200 mg/kg bw, i.e. within the GV of $30 < C \leq 300$ mg/kg bw (males, 28d) and outside the GV of $16 < C \leq 163$ mg/kg bw (females, 49d) for STOT RE 2. Effects seen in males are 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.

In a previous dose-range finding study with a limited number of animals exposed to Penta-PSCA Na-TEA significant reductions in food consumption and body weight gain were seen in males and females at 1000 mg/kg bw outside the GV of $30 < C \leq 300$ mg/kg bw (males, 28d) or $16 < C \leq 163$ mg/kg bw (females, 42d) for STOT RE2.

Based on the available data no classification for STOT RE is proposed.

No data on exposure via dermal and inhalation route is available. No conclusion on classification for these route can be made.

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 combined repeated dose toxicity study with the reproduction/developmental toxicity screening study (OECD TG 422) (Anonymous, 2012c) and an associated dose range finding (DRF) study to the OECD TG 422 (Anonymous, 2013a). Both studies were performed in Han Wistar rats with exposure to penta-PSCA Na-TEA. The DS also included a 28-day study (OECD TG 407) in CD(SD) rats with exposure to tetra-PSCA in a read across assessment (Anonymous, 1995).

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 DRF study (3/sex/group) to 0, 100, 300 and 1000 mg/kg bw/d penta-PSCA Na-TEA (28 days (m) and 42 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.

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 were 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 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.

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

In a 90-day oral repeated dose toxicity study, Cox CD rats (20/sex/group) were exposed

to 0, 250, 500 and 1000 mg/kg bw/d TEA in feed (Anonymous, 1989). Results from the study included a statistically significant differences in body weight gain and feed efficiency in female rats in the mid-dose group. No changes in organ weight to body weight ratios were reported. Pathology and histopathology showed no treatment related effects. Further, no changes were reported in haematology. It was concluded from the study that 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 and 0.5 mg TEA/L (aerosol) showed only local irritating effects in the submucosa of the larynx region of the rats. No systemic effects were reported.

In a dermal 90-day study, Fisher rats (Anonymous, 1987) were exposed 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 exposure related microscopic lesions were seen in examined organs. Haematological changes reported in the high dose groups of both sexes could be related to the inflammatory response resulting from dermal irritation. At 2000 mg/kg bw/d the final body weight was statistically significantly reduced in male and female rats accompanied by a depression in body weight gain.

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.

Comments received during consultation

Comments were received from two MSCAs. Both MSCAs supported no classification for STOT RE since the observed effects below the Guidance Value for classification were insufficient for a classification. One MSCA noted that the data available for a classification for STOT RE was limited.

Assessment and comparison with the classification criteria

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 (90% purity, 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 0, 100, 300 and 1000 mg/kg bw/d

were, respectively: +14%, +14%, +13% and +4% during the pre-mating period, +4%, +4%, +2% and +2% during the paring period and +8%, +9%, +7% and +8% after the mating period. Differences in mean body weight gain of females at the dose levels of 0, 100, 300 and 1000 mg/kg bw/d were, respectively: +9%, +9%, +9% and +4% during the pre-mating period and +25%, +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 was derived based on statistically significant reduction in food consumption, reduction of body weight and body weight gain at 1000 mg/kg bw/d 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 (> 90% purity) 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.

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 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 (percentage not reported) 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.

In summary RAC supports the assessment of the repeated dose toxicity studies performed by the DS. In the OECD TG 422, the relevant effects induced following exposure to penta-PSCA Na-TEA were observed from a dose of 200 mg/kg bw/d (within the GV of $30 < C \leqslant 300$ mg/kg bw/d (males, 28d) and just at the GV of $20 < C \leqslant 200$ mg/kg bw/d (females, 42d) for a classification as 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 the 28-day study in rats with tetra-PSCA, effects were reported from 200 mg/kg bw/d (within the GV of $30 < C \leqslant 300$ mg/kg bw/d 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 conclusion: Based on the data available for penta-PSCA Na-TEA and the read across to tetra-PSCA, RAC is of the opinion that **no classification for STOT RE is warranted** according to the CLP criteria.

10.13 Aspiration hazard

No assessed

11 EVALUATION OF ENVIRONMENTAL HAZARDS

No assessed

12 ADDITIONAL LABELLING

Not relevant

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ANNEX I: STRUCTURAL ANALOGUE READ-ACROSS JUSTIFICATION FOR THE ENDPOINTS TOXICITY TO REPRODUCTION AND REPEATED DOSE TOXICITY

I – 1. Hypothesis for the analogue approach

In the following section the read-across has been described according to the Read-Across Guidance (ECHA, 2017c) as well as ECHA guidance R.6 (2008).

In the present CLH-Dossier read-across using Penta-PSCA as source substances has been applied to the endpoints Skin corrosion/irritation and eye irritation. Basis for the analogue approach is the similarity in structure, identic ions in biological media and similar toxicity.

6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (Penta-PSCA) as well as 6-[(C10-C13)-alkyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid Tetra-PSCA are used as source substances for read-across to the target substance Penta-PSCA Na-TEA. All substances (source and target substances) belong to the group of 2,5 dioxo-pyrrolidin hexanoates.

The target substance Penta-PSCA Na-TEA is the salt of the corresponding acid Penta-PSCA. Penta-PSCA Na-TEA is dissolved in a biological fluid and an immediate dissociation in sodium ion, triethanolammonium ion and Penta-PSCA can be assumed.

Tetra-PSCA and Penta-PSCA belong to a homologous series of (Polypropenylsuccinimido)-caproic acid and can thus be considered as to belong to a "chain length category". The substances have a high structural similarity. They differ only in the number of C-atoms of the alkyl side chain (branched, unsaturated) at position 3 of the ring structure.

Endpoints for which the read-across applies are documented in the table below.

Table 33: Endpoints and used studies

Endpoint	Source Substance	Study type and reference
Skin corrosion/irritation	Penta-PSCA (no information on purity)	Anonymous, 1993b OECD Guideline 404 Reliability: Score 2 (GLP)
Eye Irritation	Penta-PSCA (no information on purity)	Anonymous, 1993d OECD Guideline 405 Reliability: Score 2 (GLP)
Repeated dose toxicity	Tetra-PSCA	Anonymous, 1995 OECD 407

		Reliability: Score 2

Reliability and adequacy of the source studies used for read-across

According to the ECHA (2008) Guidance on QSARs and grouping of chemicals, the used data needs to be assessed for its adequacy. Therefore, the available experimental data have been evaluated for adequacy and reliability.

The available skin and eye irritation studies with the source substance Penta-PSCA are according to OECD guidelines 404 and 405 and GLP. However the reporting is limited (ECHA dissemination site) and no information on purity of the substance is available. Therefore Klimisch score 2 applies.

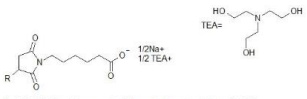
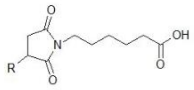
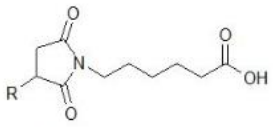
The OECD 407 study with Tetra-PSCA is a well documented GLP study. As no information on the test substance (purity) is supplied Klimisch score 2 has been applied.

I – 2. Identity and characterisation of the source substance and target substance

The identity of the source and target substances is compiled in the following table:

Table 34: Substance identities

	Target substance	Source substance (dissolving product)	Source substance (dissolving product)
Public name:	6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts	6-[(C10-C13)-alkyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid	6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid
EC number:	-	-	-
CAS number:	-	2156592-54-8	-
Molecular formula:	$C_{22}H_{36}NO_4.1/2Na.1/2C_6H_{16}NO_3$ - $C_{28}H_{48}NO_4.1/2Na.1/2C_6H_{16}NO_3$	$C_{19}H_{31}NO_4$ - $C_{23}H_{39}NO_4$	$C_{22}H_{37}NO_4$ - $C_{28}H_{49}NO_4$
Molecular weight range [g/mol]:	~531	conf	>= 379.0 — <= 463.0
Synonyms:	Penta-PSCA Na-TEA	Tetra-PSCA	Penta-PSCA

Chemical structure	 R=C12-C18-alkenyl-(even and odd, branched, unsaturated); mainly C15	 R=C10-13-alkenyl-(even and odd,branched, unsaturated); mainly C12	 R=C12-18-alkenyl-(evan and odd, branched, unsaturated); mainly C15
Purity	~90%	-	-

I – 3. Purity and Impuritites

Target substance Penta-PSCA Na-TEA:

Based on the structure and the molecular weight of Penta-PSCA Na-TEA (ranging from 465 to 549) the theoretic fraction of TEA will be 13-16%.

OECD 422 study as well as the dose range finding study with the Penta-PSCA Na TEA have been conducted with the same batch. A certificate of analysis for this batch gave the following result on the composition of the UVCB:

Pentapropylenesuccinimido-capronate	55.0%
Sodium	2.9%
Triethanolamine	31.2%
Water	9.2%
Olefins	1.7%

For the OECD 414 study with the target substance no information on detailed composition is available. However, as the study has been conducted by the same laboratory in the same time period sponsored by the same industry, the same characteristics can be assumed.

Source substance Penta-PSCA

For OECD 404 and 405 studies no informations on purity are available.

Source substance Tetra-PSCA

For OECD 407 informations on purity are available.

I – 4. Analogue approach justification

Phys-chem properties

The substances are low molecular weight compounds with a shared 2,5-dioxopyrrolidin-1-ylhexanoic acid. These substances have similar water solubility and partition coefficient octanol/water (Kow) (see Table 35).

Penta-PSCA is considered to be a weak acid and not to be dissociated under acidic and neutral conditions in aqueous media. Penta-PSCA Na-TEA as salt of Penta-PSCA is expected to behave similarily as it is present as non-dissociated acid under acidic and neutral conditions in aqueous media. Therefore, the same species (dissociated and non-dissociated ions) are expected under similar (eco-)toxicologically relevant conditions at same pH in aqueous media.

The substances are surfactants. The surface tension is 38.2 mN/m for Tetra-PSCA, 37.2 mN/m (at 90% saturation concentration and 20°C) for Penta-PSCA and 31.6 mN/m (at 1g/l and 20°C) for Penta-PSCA Na-TEA.

Based on these physico-chemical properties and resulting behaviour of the analogues, it is justified that Penta-PSCA and Penta-PSCA Na-TEA are appropriate reference materials for read-across.

Toxicity

Based on the available limited data the acute toxicity of all three substances seems to be low.

Penta-PSCA Na-TEA dissolves in biological fluids and dissociates in sodium ion, triethanolammonium ion and Penta-PSCA. The acids have low to no irritating properties. TEA shows some irritating potential and is (self-)classified as irritating (Eye Irrit 2, H319; Skin Irrit 2, H315).

A subacute toxicity study with Tetra-PSCA showed that the main target organs of the substance are the liver in both sexes and the kidney and forestomach in male rats. Minor adverse effects (salivation (m, f), decreased spontaneous movement (m), decreased respiratory rate (m), rel. kidney weight increased (f)) are documented for concentrations of 200 mg/kg bw/day and above. A NOAEL of 40 mg/kg bw/day has be derived. A combined repeated dose toxicity study with reproduction/developmental screening with the substance Penta-PCSA Na-TEA also gave a NOAEL (maternal toxicity) of 40 mg/kg bw/day, supporting the read across hypothesis. While at 200 mg/kg bw/day effects like reduced locomotor activity (m, f), reduced food consumption (m, f) and reduced body weight (m) are documented at 1000 mg/kg bw/day toxic effects on the liver, the kidney, the forestomach as well as on testis and epididymidis weights are described.

Triethanolamine (TEA), a dissolving product in concentrations about 31%, which is not common to source and target substance, does not influence the anticipated (sub)chronic toxicity as shown by the data on repeated dose toxicity and reproduction toxicity (see Chapter I-5.2.2). The corrected NOAELs/LOAELs for maternal toxicity are a factor of 10-80 higher than the derived values for the source substance. For developmental toxicity a factor of 20-120 applies indicating that the observed effects were due to the dissolving product Penta-PSCA and not due to TEA.

I – 5. Data Matrix of selected physicochemical and toxicological information

Table 35: Phys-chem properties of 2,5 dioxo-pyrrolidin hexanoates

	6-[(C10-C13)-alkyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (Tetra-PSCA)	**6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (Penta-PSCA)**	**6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts (Penta-PSCA NaTEA)**
Read-across	Source substance	Source substance	Target chemical

	(dissolving product)	(dissolving product)	
State of the substance at 20°C and 101.3 kPa	liquid	liquid	liquid
Melting point	-2 °C	-8 ± 3°C.	4 ± 3 °C.
Boiling point	412 ± 10°C	347 ± 30°C (decomposes on boiling).	112 ± 13 °C at 101.9 kPa
Relative density	1.037	1.01.	1.07
Vapour pressure	1.6 Pa (calculated)	1.0 Pa (calculated)	10^{-8}Pa (calculated)
Dissociation constant pKa:	The pKa values of Tetra-PSCA at 25 °C are: pKa1 (COO- + H+ ⇌ COOH) : 4.74 ± 0.10 pKa2 (R3NH+ ⇌ R3N + H+) : -1.60 ± 0.40	pKa1 (COO- + H+ ⇌ COOH) : 4.74 ± 0.10 pKa2 (R3NH+ ⇌ R3N + H+) : -1.60 ± 0.40	4.74 ± 0.2 at 25°C (read-across)
Water solubility	0.19 ± 0.08 g/L at 20°C (critical micelle concentration)	0.23 ± 0.11 g/L (20°C) (critical micelle concentration)	0.077 ± 0.039 g/L at 20°

I -5.2 Toxicological data

I -5.2.1Toxicological profiles of 2,5 dioxo-pyrrolidin hexanoates

Information on toxicological endpoints of all three representatives of 2,5 dioxo-pyrrolidin hexanoates is compiled in Table 36.

REACH registrants for 2,5 dioxo-pyrrolidin heaxnoates use read-across for several toxicological endpoints including acute toxicity, chronic toxicity and reproductive toxicity. Therefore only limited data are available.

Table 36: Toxicological data of 2,5 dioxo-pyrrolidin hexanoates

SUBSTANCE	**6-[(C10-C13)-alkyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid** **(Tetra-PSCA)**	**6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid** **(Penta-PSCA)**	**6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts** **(Penta-PSCA NaTEA)**
Read-across	Source substance	Source substance	Target chemical
Acute Tox Oral	no data	>2000mg/kg bw	>800 mg/kg bw
Acute Tox Dermal	>2000mg/kg bw	no data	no data

Acute Tox Inhalation	no data	no data	no data
Skin irritation	Not irritating	Not irritating	Not irritating
Eye irritation	Irritating	Not irritating	Irritating
Subacute toxicity study (oral)	NOAEL = 40 mg/kg bw/day (salivation (m, f), decreased spontaneous movement (m), decreased respiratory rate (m), rel. kidney weight increased (f))	no data	no data
Combined repeated dose toxicity study (OECD 422) (oral)	no data	no data	$NOAEL_{parental\ toxicity}$ = 40 mg/kg bw/day (reduced food consumption, salivation) $LOAEL_{F1\text{-}generation}$ = 40 mg/kg bw/day (mortality) $LOAEL_{fertility}$ = 40 mg/kg bw/day $LOAEL_{developmental\ toxicity}$ = 40 mg/kg bw/day (reduced fertility index, reduced gestation index, increased pre-implantation loss, post-implantation loss, reduction of litter size, reduction in birth index, postnatal loss (days 0-4), reduced viability index)
Reproductive toxicity (OECD 414 PNDT Study) (oral)	no data	no data	$NOAEL_{parental\ toxicity}$ = 40 mg/kg bw/day (reduced food consumption and body weight gain) $LOAEL_{developmental\ toxicity}$ = 8 mg/kg bw/day (external, skeletal, visceral malformations)
Mutagenicity	not mutagenic	no data	no data
Carcinogenicity	no data	no data	no data

I -5.2.2 Toxicological profile of 2,2',2''-nitrilotriethanol (TEA)

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON FOR 6-[C12-18-ALKYL-(BRANCHED, UNSATURATED)-2,5-DIOXOPYRROLIDIN-1-YL]HEXANOIC ACID, SODIUM AND TRIS(2-HYDROXYETHYL)AMMONIUM SALTS

The toxicological profile of TEA (CAS 102-71-6, EC 203-049-8), an ion that is systemically available when Penta-PSCA Na-TEA (target chemical) dissolves in biological fluids, is presented in the table below. Information on the toxicological profile of TEA is provided to clarify that the toxicological adversity of the concerned endpoints is related to 2,5 dioxo-pyrrolidin hexanoates but not to TEA.

Table 37: Toxicological profile of TEA

2,2',2''-nitrilotriethanol (TEA, triethanolamine)	**Info from registration dossiers and Substance evaluation report (2015)**	**Self-Classification (C&L-inventory)** **[number of notifiers, in total 3904]**
Acute Tox, oral	not acutely toxic	Acut Tox 4, H302 [52 notifiers]
Acute Tox, dermal	not acutely toxic	Acut Tox 4, H312 [1 notifier]
Acute Tox, inhalation	not acutely toxic	Acut Tox 4, H332 [1 notifier]
Skin irritation	Negativ animal studies *SEV: "five studies with dermal application of TEA resulted in indications of only very slight irritation."*	Skin Irrit 2, H315 [219 notifier] or Skin Corr 1C, H314 [1 notifier]
Eye irritation	Negative studies and two animal study with positive effects (mean scores 24-48h, 4 animals): Study [1]: redness 1.08 cornea opacity 1 chemosis 1.08 Study [2]: redness 2 cornea opacity 1 chemosis 1.75 *SEV: "Available animal data demonstrated that TEA is a slight eye irritant, but not classifiable"*	Eye Irrit 2, H319 [751 notifiers] or Eye Dam 1, H318 [51 notifiers]
Resp. Irritation	Info from inhal. repeated dose study: larynx irritation with a LOAEL 0.02 ng/L; Reddish crusts	STOT SE 3, H335 [35 notifier]

	on nasal edges	
Skin Sensitization	GPMT negative, some human evidence *SEV: "...based on human data, including in a highly exposed population, and animal data, TEA has a low potential to induce skin sensitisation and does not meet the criteria for classification."*	Skin Sens 1, H317 [41 notifiers]
Respiratory Sensitisation	2 case reports *SEV: "considering the very high tonnages of TEA used in a wide variety of applications and over a long period of time and the absence of other reports, the eMSCA concludes that TEA is not a respiratory sensitiser"*	Resp Sens 1, H334 [1 notifier]
Repeated dose toxicity	<u>Dermal:</u> [1] at 250 mg/kg bw/day and above skin lesions: minimal to mild epidermal thickening (acanthosis), to chronic active inflammation, erosion, and ulceration. NOAEL (local effects) = 125 mg/kg bw/day [2] 250 mg/kg bw/day: acanthosis; 2000mg/kg bw/day: inflammation at the site of application. LOAEL (local effects) = 250 mg/kg bw/day (skin lesions) <u>Oral:</u> no effects NOAEL = 1000 mg/kg bw/day <u>Inhal:</u> focal inflammatory changes in the submucosa of the larynx, no systemic effect	STOT RE 2, H373 [11 notifier]

	LOAEC (local effects) = 0.02 mg/litre (equivalent to 23 mg/kg bw/day) NOAEC (systemic effects) = 0.5 mg/litre (equivalent to 575 mg/kg bw/day)	
Reproductive toxicity	OECD 421 Screening test: At 1000 mg/kg bw/day: - Lower mean number of implantation sites (about 20% below control) - Increased postimplantation loss (19.4%* [*=p≤0.05] vs. 3.7% in control) - Lower average litter size (about 33% below control). NOAEL (for developmental toxicity) (F1): 300 mg/kg bw/day NOAEL (for reproductive performance and fertility) (P): > 1000 mg/kg bw/day (male/female) NOAEL (for systemic toxicity) (P): > 1000 mg/kg bw/day *SEV: "Reproductive toxicity was not an initial concern for TEA and was not identified as an additional concern."* DevTox: read-across zu MEA (monoethanolamine): *SEV: "No evidence of an adverse effect on development"*	-

Mutagenicity	negative	-
Carcinogenicity	equivocal evidence of carcinogenic (renal tubule cell adenoma in male rats, liver hemangiosarcoma in male mice, hepatocellular adenoma in female mice) *SEV: "Not carcinogenic in rats and mice"* <u>Local effects (dermal application):</u> <u>Rat:</u> 125 mg/kg bw/day and above: Local effects (acanthosis and inflammation and ulceration, female rats had epidermal erosion) <u>Mice:</u> 100 mg/kg bw/day and above: skin irritation with visible crusts. epidermal hyperplasia, supparative inflammation, ulceration and dermal chronic inflammation	-

TEA shows some irritating potential in two positive eye irritation tests and in dermal repeated dose studies as well as in the carcinogenicity study with dermal application.

The most relevant endpoints to elucidate the read-across between Penta-PSCA Na-TEA and Tetra PSCA are repeated dose toxicity and reproductive toxicity. Therefore the relevant studies are described in more detail.

<u>Reproductive Toxicity:</u>

Table 38: Reproductive/developmental toxicity screening testing of TEA (source: ECHA dissemination site)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD 421 (OECD TG 421, Reproduction/Developmental Toxicity Screening Test) GLP	TEA, 99.5% Oral, gavage 0, 100, 300, 1000 mg/kg bw/day	NOAEL $_{\text{maternal toxicity}}$ > 1000mg/kg bw/day NOAEL $_{\text{developmental tox}}$ = 300mg/kg bw/day	Anonymous, 2010

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Rat, Wistar, strain Crl:WI(Han) 10/sex/dose	Exposure males: premating period of 2 weeks and a mating period (max. 2 weeks) + approximately 1 week post-mating Exposure females: premating period of 2 weeks and a mating period (max. 2 weeks) and the entire gestation period as well as 4 days of lactation in females.	1000 mg/kg bw/day • lower mean number of implantation sites (about 20% below control) • Increased post-implantation loss (19.4%* [*=p≤0.05] vs. 3.7% in control) • Lower average litter size (about 33% below control). 300mg/kg bw/day No test substance related adverse effects 100mg/kg bw/day No test substance related adverse effects	

In the OECD 421 study with TEA rats were exposed via gavage to concentrations of 0, 100, 300 or 1000 mg/kg bw/day in water (vehicle). The animals were exposed during the premating period of 2 weeks and a mating period (max. 2 weeks) in both sexes, approximately 1 week post-mating in males, and the entire gestation period as well as 4 days of lactation in females.

Body weight and food consumption were determined once a week. For the males, mating and fertility indices (male mating index and male fertility index) were calculated for F1 litters. The parturition and lactation behaviour of the dams was generally evaluated. The status (sex, liveborn or stillborn) and number of all delivered pups were determined as soon as possible on the day of birth. At the same time, the pups were also examined for macroscopically evident changes. The number of live pups/litter was calculated on the day after birth, and on lactation day 4. The live pups were examined daily for clinical symptoms. The pups were weighed on the day after birth (PND 1) and on PND 4.

Parental animals were sacrificed, necropsied and assessed by gross pathology. Special attention was given to the reproductive organs. The following organs or tissues of parental animals were assessed: all gross lesions, adrenal glands, pituitary gland, testis, epididymides, prostate gland, seminal vesicles, coagulation glands, ovaries, uterus, oviducts, vagina. The uteri of all cohabited female F0 parental animals have been examined for the presence and number of implantation sites. All pups with scheduled sacrifice on PND 4 were examined externally and eviscerated; their organs were assessed macroscopically.

Male mating index, male fertility index, female mating index, female fertility index, gestation index and live birth index were investigated but not documented in the registration.

Most high-dose animals and one low-dose animal showed transient salivation for a few minutes immediately after each treatment. This was likely to be induced by the unpleasant taste of the test substance or by local irritation of the upper digestive tract. It is not considered to be a sign of systemic toxicity. The slightly lower body weight gain of the 1000 mg/kg females during gestation was likely caused by the increased post-implantation loss rather than a systemic toxic effect of the test compound.

No systemic effects were observed up to the highest dose. No test substance related adverse effects on reproductive performance or fertility were documented for the low and the mid dose. High dosed animals showed a lower mean number of implantation sites (about 20% below control), increased post-implantation loss (19.4%* [*=p≤0.05] vs. 3.7% in control) and a lower average litter size (about 33% below control). No further details are available. No test substance-related adverse findings were observed in F1 pups.

No further details on body weights, organ weights, histopathology, litter observations or reproductive indices are available.

Based on the effects seen for maternal systemic toxicity a NOAEL of > 1000 mg/kg bw/day can be derived. For developmental toxicity a NOAEL of 300 mg/kg bw/day can be set.

Repeated dose toxicity:

There is one oral subchronic toxicity study (90 days) available which is presented in detail below. The available studies via the inhalation and the dermal route are mentioned for completeness but not in detail.

Table 39: Repeated dose toxicity (oral) of TEA (source: ECHA dissemination site)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
OECD Guideline 408 Non-GLP Rat, Cox CD n = 20/sex/group	TEA (88.5%) (impurities: MEA<0.12; DEA 6.0; TEA-1EO 5.3) Oral (feed) 91 days, continuous exposure 0, 250, 500, 1000 mg/kg bw/day	NOAEL = 1000 mg/kg bw/day significant differences in body weight gain and feed efficiency in females of the mid-dose group. no significant differences in organ to body weight ratios. Histopathology: Tissue alterations, mild and not considered significant no gross or histopathologic indications of a treatment-related effect.	Anonymous, 1989

For this repeated dose study (Anonymous, 1989) male and female rats (20/sex/dose) were exposed to concentrations of 0, 250, 500 or 1000 mg TEA/kg bw per day via food. This continuous exposure over 91 days resulted in significant differences in body weight gain and feed efficiency in female rats of the mid-dose group but no significant differences in organ weight to body weight ratios. Pathology and histopathology showed no treatment related effects. Also hematology showed no

adverse effects. No dose-response-related systemic effects of TEA up to concentrations of 1000 mg/kg bw/day are documented. A NOAEL of 1000 mg/kg bw can be set.

The available 28-day inhalation study (Gamer, 2008) with test concentrations of 0.02, 0.1, 0.5 mg TEA/l (aerosol) showed only local irritating effects in the submucosa of the larynx region of rats but no systemic effects. The NOAEC was 0.5 mg/L air.

In a dermal 90-day study (Anonymous, 1987) Fischer rats were exposed to 0, 125, 250, 500, 1000 or 2000 mg TEA/kg bw/day (vehicle acetone). The main compound-related effects observed were inflammation of the skin and acanthosis, which were seen in 2000, 1000, 500, and 250 mg/kg male rats and in 2000, 1000, and 500 mg/kg female rats. Non-compound related microscopic lesions were seen in examined organs. Haematological changes in high dose rats of both sexes can be attributed to an inflammatory response resulting from dermal irritation. At 2000 mg/kg bw/day the final body weight was decreased significantly in males and females accompanied by a depression in weight gain.

Conclusion:

The available tests were conducted with TEA of 99.5% or 88.5% purity. When substance Penta-PSCA Na-TEA is dissolved in a biological fluid an immediate dissociation in sodium ion, triethanolammonium ion and Penta-PSCA can be assumed. Based on a certificate of analysis (for the OECD 422 study) TEA comprises 31% of Penta-PSCA Na-TEA. To compare the observed effect levels a correction has to be made:

compound	**Study type, TEA content**	**Effect level**	**Derived**	**Corrected (31% TEA)**
TEA	OECD 421, TEA 99.5%	NOAELmat.tox	> 1000 mg/kg bw/day	> 3209 mg/kg bw/day
		NOAEL devtox	300 mg/kg bw/day	963 mg/kg bw/day
	OECD 408, TEA 88.5%	NOAEL	1000 mg/kg bw/day	2855 mg/kg bw/day
Penta-PSCA NaTEA	OECD 422, TEA 31%	NOAELmat.tox	40 mg/kg bw/day	40 mg/kg bw/day
		LOAEL devtox/fert	40 mg/kg bw/day	40 mg/kg bw/day
	Dose rang finding study, TEA 31%	NOAELmat.tox	300 mg/kg bw/day	300 mg/kg bw/day
		NOAEL devtox/fert	300 mg/kg bw/day	300 mg/kg bw/day
	OECD 414, TEA ?	NOAELmat.tox	40 mg/kg bw/day	40 mg/kg bw/day
		LOAEL devtox	8 mg/kg bw/day	8 mg/kg bw/day

TEA is not common to source and target substance, however is does not influence the anticipated (sub)chronic toxicity as shown by the data on repeated dose toxicity and reproduction toxicity The corrected NOAELs/LOAELs for maternal toxicity are a factor of 10-80 higher than the derived

values for Penta-PSCA Na-TEA. For developmental toxicity a factor of 20-120 applies indicating that the observed effects were due to the dissolving product Penta-PSCA and not due to TEA.

I – 6. Conclusion

An analogue read-across approach between Penta-PSCA Na-TEA (target chemical), Penta-PSCA (source chemical) and Tetra-PSCA has been applied based on similarity in structure, similar ions in biological media and similar sub-acute toxicity.

Repeated dose toxicity

The oral repeated dose toxicity studies with Penta-PSCA Na-TEA (target chemical), Tetra-PSCA and TEA (ion) indicate that there is no need for classification for repeated dose toxicity for none of these compounds..

Reproductive toxicity

For reproductive toxicity an OECD 422 study carried out with Penta-PSCA Na-TEA was analyzed. In this study fertility parameters (birth index, viability index and post-implantation loss) were already significantly altered at a dose level of 40 mg/kg bw/day. In addition increased pre-implantation loss, reduced litter size and reduced fertility index have been seen in a dose-dependent manner. At the highest dose level (1000 mg/kg bw/day) substance administration resulted in high post implantation loss and all pregnant females lost their litter before first litter check.

In an OECD 414 study developmental toxicity of Penta-PSCA Na-TEA was seen at 8 mg/kg bw (small spleen). At 40 and 200 mg/kg bw/day an increased number of supernumerary ribs (rudimentary) was found. In addition skeletal abnormalities were found in all fetuses at a dose of 200 mg/kg bw/day. The NOAEL for maternal toxicity was 40 mg/kg bw.

In the OECD 421 study carried out with TEA some reproductive toxicity parameters were altered only at the highest dose tested (1000 mg/kg bw/day) without maternal toxicity. The substance was subject in 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).

The pronounced effects seen already at low doses with Penta-PSCA Na-TEA (target chemical) demonstrate that the effects can be attributed to Penta-PSCA, which is systemically available when dissolves in biological fluids and not to the presence of TEA ion. This is a further prove that read-across between Penta-PSCA Na-TEA and Petra-PSCA can be accepted for the reproductive toxicity endpoint.

No information on a possible mode of action is available, neither for the source nor for the target substance.

Based on thorough analysis of all available information a read-across approach for the endpoints Irritation and repeated dose toxicity is considered appropriate.

References:

Anonymous (1987). Subchronic Dermal Toxicity of TEA: 90-Day Study with rats and mice (TEA) https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15134/7/6/4

Anonymous (1989). Repeated Dose 90-Day Oral Toxicity of TEA in Rodents (TEA) https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15134/7/6/2

Anonymous (2010). Reproduction / Developmental Toxicity Screening Test) study with TEA https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15134/7/9/2/?documentUUID=b2cb4178-158b-45c5-910e-37c691d6a37a

ECHA (2017). Guidance on the application of the CLP criteria. https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5

Gamer AO et al (2008). The inhalation toxicity of di- and triethanolamine upon repeated exposure. Food and Chemical Toxicology, 46(6), 2173-2183 (source: ECHA dissemination site)

UK (2014). Substance evaluation report for 2,2',2"-NITRILOTRIETHANOL (TEA) https://echa.europa.eu/documents/10162/63d1a4e9-f3e3-45f7-8546-3042f2293dd2

ANNEX II – CONFIDENTIAL INFORMATION

ANNEX III – ED10 CALCULATION

III – 1. Summary

ED_{10} for post implantation loss were about five times those associated with small spleen in fetuses.

Linear and sigmoidal fitting of response-dose relationships yielded similar results for post implantation loss (ED_{10} = 107.7 and 117.6 mg/kg bw, resp.) but not for small spleen (23.23 and 7.851 mg/kg bw, resp)

Note that the dose-response relationships were not sigmoidal for both endpoints.

Linear dose-response functions were fit with function lm of package stats, Hill-type sigmoidal response with functions curveFit (equation="Hill", response="quantal") and tuneFit from package mixtox using R statistical software (version 3.5.1).

III – 2. Endpoint: Post-implantation loss

dose expressed as: exposure; unit: mg / kg body weight

response expressed as:

count of living pubs at first litter check / count of implantation sites; unit-less

(0 = no loss, 1 = total loss)

dose-response fitted as:

a) linear: $y = k_0 + k_1 x$ (k_0: intercept, k_1: slope)

model terms:

	Estimate	Std. Error	t	value	Pr(>|t|)
(k0)	7.91E-02	3.22E-02	2.454	0.0193	*
k1	9.29E-04	6.78E-05	13.709	1.23E-15	***

Residual standard error: 0.1598 on 35 degrees of freedom
Multiple R-squared: 0.843, Adjusted R-squared: 0.8385
F-statistic: 187.9 on 1 and 35 DF, p-value: 1.23e-15

b) Hill sigmoidal: y = 1/(1 + (Alpha/x)^Beta)

model terms:

	Estimate	Std. Error	t	value	Pr(>|t|)
Alpha	277.168	45.344	6.113	5.49E-07	***
Beta	2.564	1.095	2.341	0.025	*

ED10 predicted:

107.7 (linear response; a)

117.6 (sigmoidal response; b)

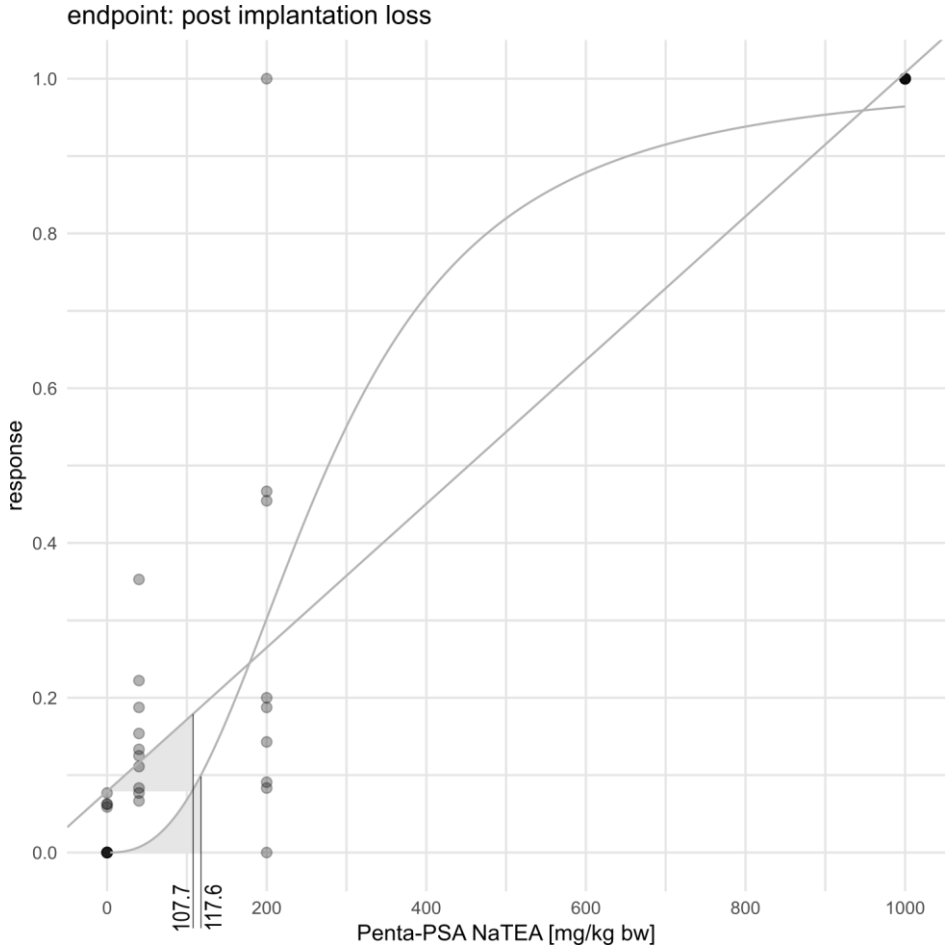


Figure 1: Linear and sigmoidal response fitting yield similar ED_{10}

III – 3. Endpoint: Small Spleen

dose expressed as: exposure; unit: mg / kg body weight

response expressed as: fetuses with small spleen / fetuses examined; unit-less

(half the fetuses of each litter were examined)

dose-response fitted as:

a) linear: $y = k_0 + k_1 x$ (k_0: intercept, k_1: slope)

model terms:

	Estimate	Std. Error	t	value	Pr(>|t|)
(k0)	1.43E-01	5.27E-02	2.70E+00	1.45E-02	*
k1	4.30E-03	5.16E-04	8.34E+00	1.36E-07	***

Residual standard error: 0.1872 on 18 degrees of freedom
Multiple R-squared: 0.7943, Adjusted R-squared: 0.7828
F-statistic: 69.49 on 1 and 18 DF, p-value: 1.357e-07

b) Hill sigmoidal: y = 1/(1 + (Alpha/x)^Beta)

model terms:

	Estimate	Std. Error	t value	Pr(>|t|)	
Alpha	36.4022	6.0839	5.983	1.17E-05	***
Beta	1.4323	0.3487	4.108	0.00066	***

ED10 predicted:

23.23 (linear response; a)

7.851 (sigmoidal response; b)

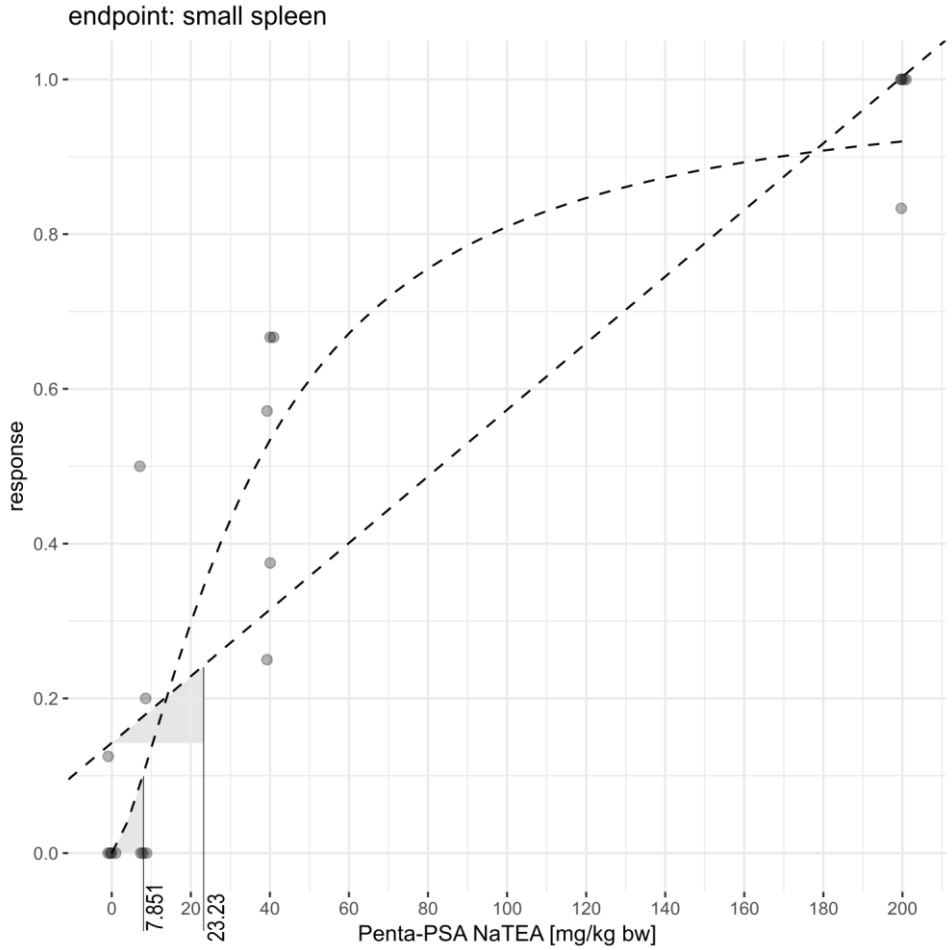


Figure 2: Linear and sigmoidal response fitting