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 (penta-PSCA)

EC Number: -
CAS Number: -

CLH-O-0000006923-68-01/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

(Penta-PSCA)

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 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 6-[C12-18-ALKYL-(BRANCHED, UNSATURATED)-2,5-DIOXOPYRROLIDIN-1-YL]HEXANOIC ACID

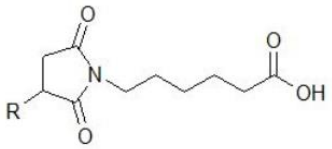
CONTENTS

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 6-[C12-18-ALKYL-(BRANCHED, UNSATURATED)-2,5-DIOXOPYRROLIDIN-1-YL]HEXANOIC ACID

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
Other names (usual name, trade name, abbreviation)	Penta-PSCA
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	$C_{22}H_{37}NO_4$ - $C_{28}H_{49}NO_4$
Structural formula	 R=C12-18-alkenyl-(evan and odd, branched, unsaturated); mainly C15
SMILES notation (if available)	-
Molecular weight or molecular weight range	>= 379.0 — <= 463.0
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

The substance in a UVCB substance.

Table 2: Constituents (non-confidential information) (Source: ECHA dissemination site)

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)
6-(3-C12-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoic acid	conf *	-	-

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 6-[C12-18-ALKYL-(BRANCHED, UNSATURATED)-2,5-DIOXOPYRROLIDIN-1-YL]HEXANOIC ACID

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)**
6-(3-C13-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoic acid	conf	-	-
6-(3-C14-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoic acid	conf	-	-
6-(3-C15-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoic acid	conf	-	-
6-(3-C16-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoic acid	conf	-	-
6-(3-C17-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoic acid	conf	-	-
6-(3-C18-alkenyl(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl)hexanoic acid	conf	-	-
Pentapropene	conf	-	-
Water EC 231-791-2	conf	-	-
Unknown impurities	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	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	-	-	Repr. 1B	H360FD	GHS08 Dgr	H360FD	-	Repr. 1B, H360FD ≥ 0.03%	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid	-	-	Repr. 1B	H360FD	GHS08 Dgr	H360FD	-	Repr. 1B, H360FD ≥ 0.03%	-

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	*data conclusive but not sufficient for classification*	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.

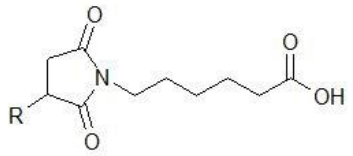
The substance has not been notified in the C&L inventory.

RAC general comment

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

Read across assessment

An analogue read-across approach between penta-(polypropenylsuccinimido)-caproic acid (PSCA), penta-PSCA (see Figure below) and penta-PSCA Na-TEA has been proposed by the Dossier Submitter (DS) based on similarities in structure, ions released in biological media and expected toxicological similarity. All substances (source and target) 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. Penta PSCA is a UVCB substance.



R=C12-18-alkenyl-(evan and odd, branched, unsaturated); mainly C15

Figure: chemical structure of penta-PSCA

Three Member States Competent Authorities (MSCA) agreed with the read-across approach during the general consultation whereas two other MSCAs requested additional justifications. One commenting MSCA did not support the proposed read across for local toxicity due to uncertainties in the composition of the test substance. The DS argued that read across could be applied and clarified that additional information is available in a confidential annex to the CLH report. One MSCA noted that tetra-PSCA and penta-PSCA

belong to a homologous series of (Polypropenylsuccinimido)-caproic acid (PSCA) and can thus be considered to belong to a "chain length category". Thus the substances have a high structural similarity.

RAC agrees with the grouping approach and read across proposed by the DS for STOT RE and reproductive toxicity. The assessment of reproductive toxicity and STOT RE of penta-PSCA was based on studies performed with penta-PSCA Na-TEA. In addition, for the assessment of STOT RE, a 28-day study in rats with exposure to tetra-PSCA was included. For local effects RAC considers that the classification should be based on data on penta-PSCA.

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 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 Na-TEA. 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

The substance had no active registration at the time of dossier compilation. However, the following former uses are indicated at ECHA dissemination site:

	Use(s)	Technical function
Manufacture	Manufacturing and Filling of Penta-PSCA	-
Formulation	Formulation of products containing Penta-PSCA 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 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 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].

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	-8 ± 3°C	REACH registration	OECD 102
Boiling point	347 ± 30°C (decomposes on boiling).	REACH registration	OECD 103
Relative density	1.01	REACH registration	OECD 109
Vapour pressure	1.0 Pa	REACH registration	calculated
Surface tension	37.2 ± 0.1 mN/m (90%, 25°C)	REACH registration	OECD 115
Water solubility	0.23 ± 0.11 g/L (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 (20°C)	REACH registration	calculated
Flash point	113±2°C	REACH registration	EU A.9
Flammability	-	-	-
Explosive properties	-	-	-
Self-ignition temperature	290 °C	REACH registration	Method A.15
Oxidising properties	-	-	-
Granulometry	-	-	-
Stability in organic solvents and identity of relevant degradation products	-	-	-
Dissociation constant	pKa1 (COO- + H+ ⇌	REACH registration	-

Property	Value	Reference	Comment (e.g. measured or estimated)
	COOH) : 4.74 ± 0.10 pKa2 (R3NH+ ⇌ R3N + H+) : -1.60 ± 0.40		
Viscosity	16200 ± 400 mPa.s (dynamic, 20°C) / 2050 ± 50 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)

There are no specific toxicokinetics data available.

The substance 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. In the registration dossier an assessment based on available toxicity data has been made.

A repeated dose study with the read-across 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

Not assessed

10.2 Acute toxicity - dermal route

Not assessed

10.3 Acute toxicity - inhalation route

Not assessed

10.4 Skin corrosion/irritation

Table 6: Summary table of animal studies on skin corrosion/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 404 GLP	Rabbit, New Zealand White n=3	Penta-PSCA	0.5 ml (no vehicle) 4h semiocclusive	Erythema score, mean (24, 48, 72h) = 1.1 (max. 2) Full reversibility within 7 days Oedema score mean (24, 48, 72h) = 0	Anonymous, 1993a

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

The skin irritation potential of the registration substance was investigated according to the OECD Guideline 404. 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.

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

For Penta-PSCA the mean score for oedema was 0 and for erythema was 1.1, which was fully reversible.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the available data no classification is indicated for Penta-PSCA.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

For the evaluation of skin corrosion/irritation the DS included one study performed according to OECD TG 404 and GLP where 3 female New Zealand White rabbits were exposed to 0.5 mL penta-PSCA 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 was 1.1 (all animals; max. 2) while the mean score for oedema was 0. The effect was fully reversible within 7 days. On this basis the DS did not propose any classification for skin corrosion/irritation.

Comments received during consultation

Two commenting MSCAs supported the proposal by the DS for 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 scoring value is between 2,3 and 4 for erythema/eschar or for oedema in at least 2 of 3 tested animals. The study summarised by the DS showed a mean score for erythema of 1.1 (all animals; max. 2) while the mean score for oedema was 0. The effect was fully reversible within 7 days.

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

10.5 Serious eye damage/eye irritation

Table 7: 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	Penta-PSCA	0.1 ml to the conjunctival sac	cornea opacity mean score (24, 48, 72h) = 0.3 max. score = 1 iris score mean score (24, 48, 72h) = 0.4	Anonymous, 1993b

				max. score = 1 conjunctivae score mean score (24, 48, 72h) = 1.3 max. score = 3 chemosis score mean score (24, 48, 72h) = 0.6 max. score = 3 All effects were fully reversible within 7 days.	

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

Penta-PSCA was investigated for its eye irritation potential according to the Guideline OECD 405. 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 (see also Table 7). No individual animal data are available.

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

Application of Penta-PSCA to rabbit eyes resulted in mean scores (24, 48, 72h) of 1.3 for conjunctival redness, 0.6 for chemosis, 0.4 for iris and 0.3 for cornea. The adverse effects were full reversibility.

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

Based on mean scores (24, 48, 72h) of 1.3 for conjunctival redness, 0.6 for chemosis, 0.4 for iris and 0.3 for cornea and full reversibility within 7 days no classification for eye irritation is indicated.

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 (Anonymous, 1993b). The animals were observed for 7 days to assess reversibility. Conjunctival redness, chemosis, discharge, corneal effects and lesion were observed in all animals. However, all the observed effects were fully reversible within 7 days. The scores are presented in the table below.

Table: *Scoring from OECD TG 405*

	Mean score (24, 48, 72 h)	**Max score**
Corneal opacity	0.3	1
Iris score	0.4	1
Conjunctivae score	1.3	3
Chemosis score	0.6	3

On this basis the DS proposed no classification for serious eye damage/eye irritation.

Comments received during consultation

Two commenting MSCAs supported the proposal by the DS for no classification for serious eye damage/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 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 in at least 2 out of 3 animals and which is fully reversible within 21 days.

The study presented by the DS showed a mean score $\leqslant$ 1 for corneal opacity and iritis, and a mean score $\leqslant$ 2 for conjunctival redness and oedema (chemosis) for the three animals tested. The observed effects were fully reversible within 7 days.

Despite the absence of individual animal data, RAC concurs with the opinion by the DS that **no classification for serious eye damage/eye irritation is warranted for penta-PSCA**.

10.6 Respiratory sensitisation

Not assessed

10.7 Skin sensitisation

Not assessed

10.8 Germ cell mutagenicity

Not assessed

10.9 Carcinogenicity

Not assessed

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

No data are available on the substance Penta-PSCA. The structurally similar substance 6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts (Penta-PSCA Na-TEA) is used as a source substance for read-across to the target substance Penta-PSCA. Both substances (source and target substance, respectively) belong to the group of 2,5 dioxopyrrolidin hexanoates. Based on the available date reproductive toxicity does not seem to be related to TEA. A justification for read-across is given in Annex I.

Table 8: 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% mean body weight gain (at 0, 100, 300 and	Anonymous 2013a

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		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 9. 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 10. 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 9: 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 10); 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 10). 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 10. 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 10: 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 bw/day	17.9 g (-31.4%)		13.5 g (-30.8%)	19.0 g (-27.2%)	15.9 g (-47.5%)
Body weight gain (mean differences) [%]	Control	+ 11%	+ 6%	+ 9%	+ 57%	+ 5%
	40mg/kg bw/day	+ 8%	+ 8%	+ 6%	+ 55%	+ 6%
	200mg/kg	+ 7%	+ 7%	+ 6%	+ 49%	+ 4%

	bw/day					
	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 11. 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 11: 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 bw	56	214	356	75.7	13(+2)	66.3	70.4
	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 12). 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 13). 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 14 and Table 15.

Table 12: 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 13: 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 14: 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 15: 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 16). 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 17).

Table 16: 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 their litters during lactation	0	0	1 (female no 73)	0
Number of females which reared their pubs until day 4 post partum	11	10	8	0

Table 17: 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 18 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 18).

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 18).

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 17).

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 18: 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 litter check	Litters affects	-	**4***	**4***	2
	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

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 read across 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

No data are available on the substance Penta-PSCA. The structurally similar substance Penta-PSCA Na-TEA is used as a source substance for read-across to the target substance Penta-PSCA. Both substances (source and target substance, respectively) belong to the group of 2,5 dioxopyrrolidin hexanoates. Based on the available date reproductive toxicity does not seem to be related to TEA. A justification for read-across is given in Annex I.

Table 19: 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)	$NOAEL_{parental\ toxicity}$ = 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	Anonymous, 2012c

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	Dose Volume: 10 mL/kg body weight m: 4 weeks f: ~7 weeks	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)	
OECD 414 (Prenatal Developmental Toxicity Study) Rat, Wistar n = 5/sex/dose	Penta-PSCA Na-TEA. Oral, gavage 0, 8, 40, 200 mg/kg bw/day Vehicle: water day 6 post-coitum – day 20 post coitum	$NOAEL_{parental\ toxicity}$ = 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 (skeletal, visceral malformations) ≥8 mg/kg bw: small spleen ≥40 mg/kg bw: supernumerary ribs 200 mg/kg bw: skeletal abnormalities and variations	Anonymous, 2013b

For the read-across 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 17 and Table 18):

- 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 21. No apparent maternal toxicity was seen.

Table 20: 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 21: 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 22. 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 23.

Table 22: 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 23: 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 24):

- 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 24).
- 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 24).

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 24: 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

The read-across substance 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 the read-across substance 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 no data on reproductive toxicity are available. However, one OECD 414 and one OECD 422 study with the read-across substance Penta-PSCA Na-TEA 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 II).

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 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 $\leq$ 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 $\geq$ 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 source substance 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 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:
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. The potency of the source substance is a borderline case between medium and high potency. Considering the severitiy of effects and the nature of the test substance Penta-PSCA Na-TEA (UVCB, including 55% Penta-PSCA) and based on the ECHA guidance (2017) a specific concentration limit of 0.03% is proposed for Penta-PSCA.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

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

In the OECD TG 422 study penta-PSCA Na-TEA induced adverse effects on fertility in the absence of marked parental toxicity. The fertility parameters that were affected included the birth index and the pup viability index. These parameters were already significantly altered at 40 mg/kg bw/d. In addition, increased pre-implantation loss, reduced litter size and reduced fertility index were reported in a dose dependant manner. Further, in the high dose group a high incidence of post-implantation loss was reported, and all pregnant

females had a total litter loss. These effects were not considered as secondary non-specific consequences of parental toxicity.

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

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

Developmental toxicity

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

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

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

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

Adverse effects on or via lactation

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

Setting of specific concentration limits (SCLs)

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

The DS considered that post-implantation losses reported in the OECD TG 422 screening study and small spleen reported in the OECD TG 414 study were the leading effects for reproductive toxicity following exposure to penta-PSCA Na-TEA. The resulting ED_{10} values for penta-PSCA Na-TEA for these effects are summarised in the table below.

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

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

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

***Table:** Potency boundaries for SCL setting*

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

Based on the potency boundaries and the calculated ED_{10} values, a medium potency was assumed for penta-PSCA Na-TEA by the DS. In addition, the CLP Guidance (ECHA, 2017, version 5, 2017, point 3.7.2.6.5 and point 3.7.2.6.5) states 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, were assessed by the DS and are summarised below.

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

In addition, according to the DS, it had to be considered that the studies were conducted with the salt penta-PSCA Na-TEA and the reprotoxic effects may be due to the dissolving product penta-PSCA and also considered relevant for 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 as a medium potency toxicant. As the ED_{10} is closed to the high potency group and the reported developmental toxicity effects are severe with a LOAEL at 8 mg/kg bw/d a shift into the high potency group can be considered. No additional modifying factors are considered to influence the potency.

Conclusion on concentration limit

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

Relevant studies with TEA

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

In a reproduction/developmental screening test (OECD TG 421), Wistar rats were exposed via oral gavage to 0, 100, 300 or 1000 mg/kg bw/d TEA. Males were exposed for 2 weeks premating, 2 weeks mating and 1 week post-mating, females for 2 weeks premating, max 2 weeks mating, gestation and up to lactation day 4. General toxicity: Transient salivation was reported in most high dose animals and one low dose animal for a few minutes immediately after exposure, however, was likely to be induced by the unpleasant taste of TEA or by local irritation of the upper digestive tract, and not considered to be a sign of systemic toxicity. A slightly lower body weight gain in the 1000 mg/kg bw/d females during gestation was likely caused by an increase in post-implantation loss rather than a systemic toxic effect of the TEA. Reproductive toxicity: In the low- and mid-dose group no adverse effects on reproductive performance or fertility were reported. In the high dose group, a lower mean number of implantation sites (about 20% below control levels), increased post-implantation loss (19.4%* [*$p \leqslant 0.05$] vs. 3.7% in control) and a lower average litter size (about 33% below control) was reported. No further details were available. No test substance-related adverse findings were reported in F1 pups.

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

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

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

Comments received during consultation

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

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

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

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

Based on the results from the OECD TG 422 DRF study with exposure to 0, 100, 300 and 1000 mg/kg bw/d of penta-PSCA Na-TEA (purity 90%) showing a decreased fertility index and birth index in the high dose group in the presence of decreased body weight gain and reduced food consumption, the doses for the main OECD 422 study were 0, 40, 200 and 1000 mg/kg bw/d (purity > 90%).

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

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

Table: *Reproductive parameters*

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

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

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

Table: *Breeding parameters*

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

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

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

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

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

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

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

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

Summary

In the OECD TG 422 study, penta-PSCA Na-TEA induced adverse effects on fertility. The fertility parameters affected included a decrease in the birth index and the gestation

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

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

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

Developmental toxicity

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

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

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

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

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

*corrected for gravid uterus weight

All female rats in the study were pregnant. No differences were reported for control and

exposed rats regarding the number of corpora lutea, implantation sites and number of live foetuses, see results in the table below. During necropsy enlarged placentas were found in one control dam and in one high dosed dam and were therefore not considered as treatment related.

Table: *Reproduction data*

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

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

Table: *Foetal toxicity*

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

*Small spleen: approx. 75% of expected size

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

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

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

Skeletal abnormalities were reported in all examined foetuses (n=28) at 200 mg/kg bw/d, comprising thin skull zygomatic jugal arch, abnormal curvature of pectoral girdle clavicle. Additional findings were absent humerus deltoid tuberosity in forelimb in 24 foetuses, short mid region of rib cage in 17 foetuses, abnormal curvature of hyoid body in 14 foetuses and abnormal spacing of zygomatic arch structures in 6 foetuses.

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

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

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

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

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

Summary

In the OECD TG 422 screening study penta-PSCA Na-TEA induced adverse effects on development including a statistically significant increase in post-implantation loss at all dose levels as well as pups mortality on PND0-4 at 40 and 200 mg/kg bw/d and no live pups at 1000 mg/kg bw/d. These effects were not considered to be a secondary non-specific consequence of parental toxicity. In the 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). These effects were seen at doses that were not associated with maternal toxicity.

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

RAC is of the opinion that based on the clear evidence of adverse effects reported on the developing foetuses following exposure to penta-PSCA Na-TEA and a read across to penta-PSCA the **classification of penta-PSCA 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)

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

RAC supports the DS assessment for the setting of concentration limits from the OECD TG 422 screening reproductive toxicity study and the developmental toxicity study in rats following exposure to penta-PSCA Na-TEA. During the general consultation, it was noted that separated SCL for developmental toxicity and effects on fertility and sexual function should be set according to the CLP Guidance (paragraph 3.7.2.6.6.1). Therefore, RAC has assessed the data to set separate SCL for effects on sexual function and fertility and for

developmental toxicity.

Concentration limit for effects on fertility and sexual function

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

Concentration limit for developmental effects

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

As regards 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 with an LOAEL at 8 mg/kg bw/d and ED_{10} of 7.8 mg/kg bw/d). Other adverse effects (increased number of supernumerary ribs and skeletal abnormalities) were reported following exposure from 40 mg/kg bw/d of penta-PSCA Na-TEA.

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

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 read-across substance Tetra-PSCA and in a combined repeated dose toxicity study with Repro/Dev Toxicity screening with the read-across substance Penta-PSCA Na-TEA. For read-across justification seen Annex I.

One 28-day repeated dose toxicity study with the read-across substance Tetra-PSCA (source substance) is available. In addition the structurally similar substance 6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid, sodium and tris(2-hydroxyethyl)ammonium salts (Penta-PSCA Na-TEA) is used as a source substance for read-across. Both substances (source and target substance, respectively) belong to the group of 2,5 dioxopyrrolidin hexanoates. Based on the available repeated dose toxicity data for TEA effects observed in the studies with the source substance Penta-PSCA Na-TEA do not seem to be related to the dissolving product TEA. A justification for read-across is given in Annex I.

Table 25: 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 1000 mg/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 read-across-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 read-across-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 source 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 26). 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 27 and Table 28). Most of the effects were reversible within the observation period of 14 days. A LOAEL of 200 mg/kg bw can be derived.

Table 26: 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 27: 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 28: **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 ≤ 10	C ≤ 30	C ≤ 16
Category 2	10 < C ≤100	30 < C ≤ 300	16 < C ≤ 163

For the 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 source 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 29: 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 ≤ 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 addition studies with the source substance Penta-PSCa Na-TEA are presented. 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 ≤ 300 mg/kg bw (males, 28d) and outside the GV of 16 < C ≤ 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 ≤ 300 mg/kg bw (males, 28d) or 16 < C ≤ 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 28-day study rats were exposed to 0, 8, 40, 200 and 1000 mg/kg bw/d tetra-PSCA. The main target organ following exposure to tetra-PSCA was the liver in both sexes and the kidney and forestomach in male rats. The effects observed at 200 mg/kg bw/d were not considered by the DS sufficiently severe (moderate and low incidence of eosinophilic bodies in the kidneys of males, increased relative liver weight in females (+10%), no significant changes in haematology and clinical chemistry) to warrant a classification for STOT RE 2.

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

Relevant studies with TEA

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

1000 mg/kg bw/d.

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

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

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

Comments received during consultation

Comments were received from two MSCAs noting that repeated dose toxicity was assessed based on a 28-day study with the substance tetra-PSCA and two studies (OECD TG 422 and an associated range finding study) with the substance penta-PSCA Na-TEA. Both MSCAs supported no classification for STOT RE since the observed effects below the Guidance Value for classification were insufficient for a classification. One MSCAs noted that the data available for a classification for STOT RE was limited (OECD screening study and a 28-day study on structurally similar substances).

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 (purity 90%, 3/sex/dose). Male rats were dosed 14 days during pre-mating and 14 days during mating (total 28 days). Females were dosed during pre-mating, mating and 14 days during gestation (total 42 days). During the treatment bedding in mouth was noted in all dose groups (m, f) in a dose dependent manner. Further, salivation was noted in the high dose group, and these findings were considered to be treatment related.

Differences in mean food consumption of males at 100, 300 and 1000 mg/kg bw/d compared to the control animals were, respectively: -8%, -8% and -29% during the pre-mating period and 0%, -9% and -17% after the mating period. Differences in mean food consumption of females at the dose levels of 100, 300 and 1000 mg/kg bw/d compared to the control animals were, respectively: -6%, -12% and -29% during the pre-mating period and -5%, -5% and -19% during the gestation period. Differences in mean body weight gain of males at the dose levels of 100, 300 and 1000 mg/kg bw/d were, respectively: +14%, +13% and +4% during the pre-mating period, +4%, +2% and +2% during the mating period and +9%, +7% and +8% after the mating period. Differences in mean body weight gain of females at the dose levels of 100, 300 and 1000 mg/kg bw/d were, respectively: +9%, +9% and +4% during the pre-mating period and +25%, +29% and +20% during the gestation period.

No macroscopic findings were noted in males and females. Clinical chemistry investigations showed statistically significant lower relative haematocrit value (0.4 compared to 0.44 in control) and lower albumin concentration (45.72 g/L compared to 53.99 g/L in control) in females in the high dose group. No further test item-related changes in haematology or clinical biochemistry parameters were noted in males or females at any dose level. No organ parameters were examined. A LOAEL of 1000 mg/kg bw/d can be derived based on statistically significant reduction in food consumption, reduction of body weight and body weight gain at 1000 mg/kg bw/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 (purity > 90%) by oral gavage (Anonymous, 2012c). The male rats were exposed for 28 days in total and the female rats for 14 days prior to mating, through the mating and gestation periods until the F1 generation reached day 4 post-partum (in total for approx. 42 days).

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

Table: *Parental toxicity from OECD TG 422*.

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

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

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 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 by 9% in males and 11% in females. Histopathological examinations showed swelling of hepatocytes in males and females at 1000 mg/kg bw/d as well as granulation tissue accompanied by calcification. In addition, effects on forestomach (mucosa degeneration) and kidney (eosinophilic bodies) in males as well as haematological and clinical alteration were observed in males and females. Most of the effects were reversible within the recovery period. A LOAEL of 200 mg/kg bw/d was derived.

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

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

In 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 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 tetra-PSCA and the read across to penta-PSCA, RAC is of the opinion that **no classification for STOT RE is warranted for penta-PSCA** according to the CLP criteria.

10.13 Aspiration hazard

Not assessed

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed

12 ADDITIONAL LABELLING

Not relevant

13 REFERENCES

Anonymous, 1993a. Acute Dermal Irritation / Corrosion (Penta-PSCA) https://echa.europa.eu/de/registration-dossier/-/registered-dossier/21896/7/4/2

Anonymous, 1993b. Acute Eye Irritation / Corrosion (Penta-PSCA) https://echa.europa.eu/de/registration-dossier/-/registered-dossier/21896/7/4/3

Anonymous (1995) Repeated Dose 28-Day Oral Toxicity in Rodents (Tetra-PSCA). ECHA dissemination site https://echa.europa.eu/de/registration-dossier/-/registered-dossier/21896/7/6/2/?documentUUID=ff136799-07b9-4824-a236-830aae880aed

Anonymous (2012c). Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test (Penta-PSCA Na-TEA). ECHA dissemination site https://echa.europa.eu/de/registration-dossier/-/registered-dossier/21896/7/6/2

Anonymous (2013a). (Pentapropylensuccinimido)-hexanoic acid, sodium and triethanolamine salts: Dose Range-Finding Study for a Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test in the Han Wistar Rat, study D28366

Anonymous (2013b). Prenatal Developmental Toxicity Study (Penta-PSCA Na-TEA). ECHA dissemination site https://echa.europa.eu/de/registration-dossier/-/registered-dossier/19944/7/9/3

ECHA (2008). Guidance on information requirements and chemicals safety assessment Chapter R.6 – QSARs and grouping of chemicals. https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

ECHA (2017a). Guidance on Application of CLP Criteria, July 2017, https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5

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ECHA (2017c). Read-Across Assessment Framework (RAAF). https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

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 Na-TEA and Tetra-PSCA as source substances has been applied to the endpoints toxicity to reproduction and repeated dose toxicity. 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, sodium and tris(2-hydroxyethyl)ammonium salts (Penta-PSCA Na-TEA) and 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 6-[C12-18-alkyl-(branched, unsaturated)-2,5-dioxopyrrolidin-1-yl]hexanoic acid (Penta-PSCA). All three substances (source and target substance) belong to the group of 2,5 dioxo-pyrrolidin hexanoates.

The source 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 30: Endpoints and used studies

Endpoint	Source Substance	Study type and reference
Reproductive toxicity	Penta-PSCA Na-TEA (Emulsogen 3971, purity 90%)	Anonymous, 2012c OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test Reliability: Score 1 (GLP)
	Penta-PSCA Na-TEA (Emulsogen 3971, purity 90%)	Anonymous, 2013a Dose Range finding study for OECD 422 Reliability: Score 1

	Penta-PSCA Na-TEA [no further information]	Anonymous, 2013b OECD 414 (Prenatal Developmental Toxicity Study) Reliability: Score 2 (reduced number of animals, 5 instead of 20; Non-GLP)
Repeated dose toxicity	Penta-PSCA NaTEA (Emulsogen 3971, purity 90%))	Anonymous, 2012c OECD Guideline 422 Reliability: Score 1 (GLP)
	Penta-PSCA NaTEA (Emulsogen 3971, purity 90%)	Anonymous, 2013a Dose Range finding study for OECD 422 Reliability: Score 1
	Tetra-PSCA	Anonymous, 1995 OECD 407 Reliability: Score 2

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

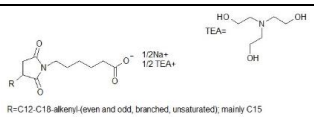
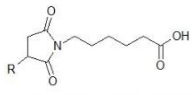
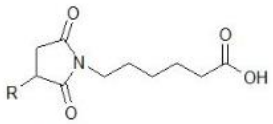
According to the ECHA (2008) Guidance on QSARs and grouping of chemicals, the used data needs to be assessed for its adequacy. Therefore, the available experimental data have been evaluated for adequacy and reliability.

The combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test is according OECD 422 guideline and GLP. It is, like the dose range finding study, well designed and documented (full report available). Therefore Klimisch score 1 applies. 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. GLP is not documented but full study report is available. Therefore it is classified with Klimisch score 2. The OECD 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 31: Substance identities

	Source substance	Source substance	Target 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

Source 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 source substance Penta-PSCA Na TEA 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 Tetra-PSCA:

For the OECD 407 study with the source substance Tetra-PSCA no information on purity is available.

Target substance Penta-PSCA:

Information on the composition of Penta-PSCA is given in the confidential Annex II. No influence from impurities on the classification can be assumed.

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 32).

Penta-PSCA and Tetra-PSCA are 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 Tetra-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 32: 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 chemical	Target substance	Source chemical
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 33.

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 33: 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 chemical	Target chemical	Source 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)

The toxicological profile of TEA (CAS 102-71-6, EC 203-049-8), an ion that is systemically available when Penta-PSCA Na-TEA (source 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 34: 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	Eye Irrit 2, H319 [751 notifiers] or Eye Dam 1, H318 [51 notifiers]

	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"*	
Resp. Irritation	Info from inhal. repeated dose study: larynx irritation with a LOAEL 0.02 ng/L; Reddish crusts on nasal edges	STOT SE 3, H335 [35 notifier]
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	STOT RE 2, H373 [11 notifier]

	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 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.

Reproductive Toxicity:

Table 35: 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 Rat, Wistar, strain Crl:WI(Han) 10/sex/dose	TEA, 99.5% Oral, gavage 0, 100, 300, 1000 mg/kg bw/day 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.	NOAEL $_{\text{maternal toxicity}}$ > 1000mg/kg bw/day NOAEL $_{\text{developmental tox}}$ = 300mg/kg bw/day 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	Anonymous, 2010

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 36: 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	TEA (88.5%) (impurities: MEA<0.12; DEA 6.0; TEA-1EO 5.3) Oral (feed) 91 days, continuous exposure	NOAEL = 1000 mg/kg bw/day significant differences in body weight gain and feed efficiency in females of the mid-dose group.	Anonymous, 1989

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
n = 20/sex/group	0, 250, 500, 1000 mg/kg bw/day	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.	

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 the source 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	> 3209 mg/kg bw
		NOAEL devtox	300 mg/kg bw	963 mg/kg bw
	OECD 408, TEA 88.5%	NOAEL	1000 mg/kg bw	2855 mg/kg bw

Penta-PSCA NaTEA	OECD 422, TEA 31%	NOAELmat.tox	40 mg/kg bw	40 mg/kg bw
		LOAEL devtox/fert	40 mg/kg bw	40 mg/kg bw
	Dose rang finding study, TEA 31%	NOAELmat.tox	300 mg/kg bw	300 mg/kg bw
		NOAEL devtox/fert	300 mg/kg bw	300 mg/kg bw
	OECD 414, TEA ?	NOAELmat.tox	40 mg/kg bw	40 mg/kg bw
		LOAEL devtox	8 mg/kg bw	8 mg/kg bw

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 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 – 6. Conclusion

An analogue read-across approach between Penta-PSCA Na-TEA (source chemical), Tetra-PSCA (Source Chemical) and Penta-PSCA (target chemical) 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 (source chemical), Tetra-PSCA (source chemical) and TEA (ion) indicate that there is no need for classification for repeated dose toxicity for none of these compounds as well as for the target substance Penta-PSCA.

Reproductive toxicity

For reproductive toxicity an OECD 422 study carried out with Penta-PSCA Na-TEA (source chemical) 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 (source 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 Tetra-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 repeated dose toxicity and reproductive 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)

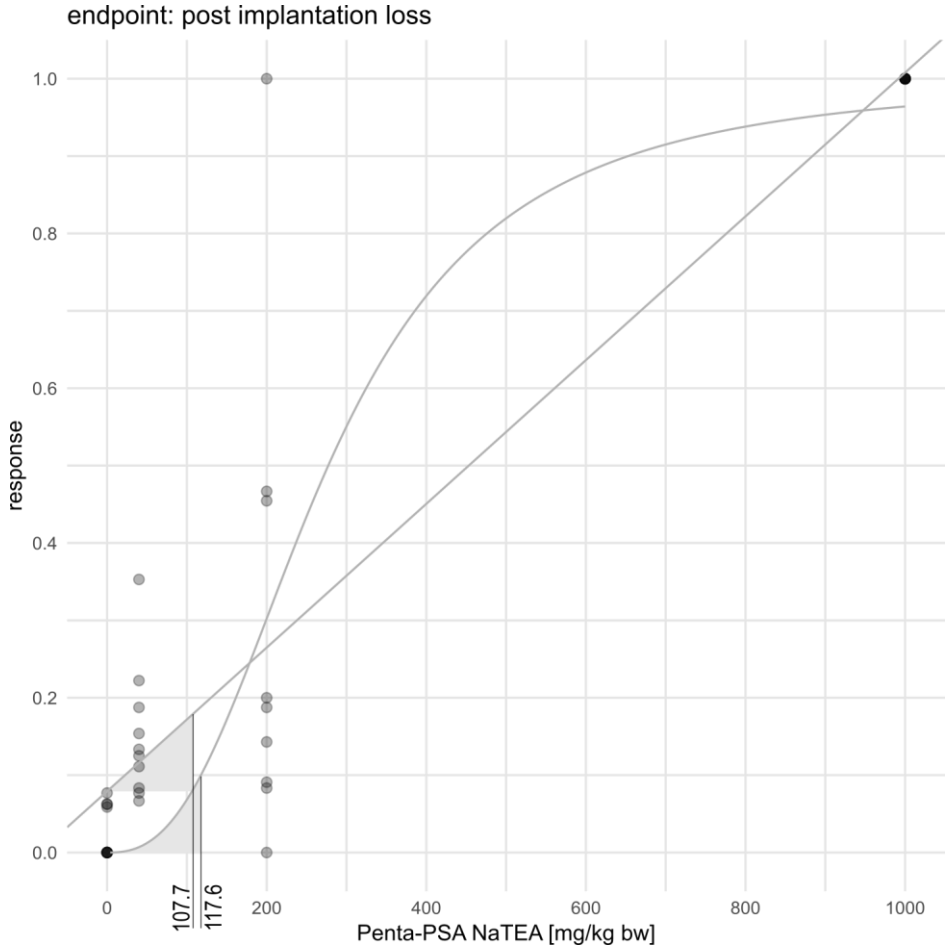


Figure 1: Linear and sigmoidal response fitting yield similar ED_{10}

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)

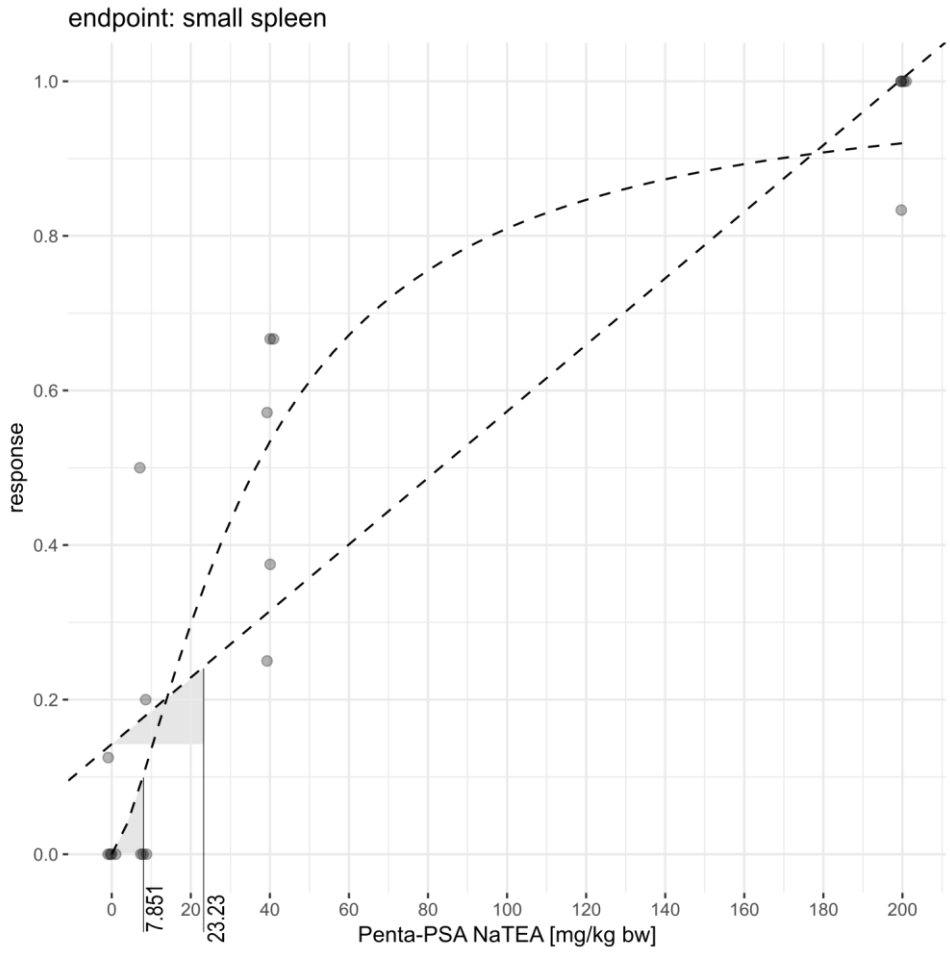


Figure 2: Linear and sigmoidal response fitting