Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

sodium 3-(allyloxy)-2-hydroxypropanesulphonate

EC Number: 258-004-5

CAS Number: 52556-42-0

Index Number: NA

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CONTENTS

1 PHYSICAL HAZARDS 3

1.1 FLAMMABLE SOLIDS 3

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 3

3 HEALTH HAZARDS 3

3.1 SERIOUS EYE DAMAGE/EYE IRRITATION 3

3.1.1 Other data 3

3.1.1.1 [Unnamed. 2012] 3

3.2 REPRODUCTIVE TOXICITY 6

3.2.1 Animal data 6

3.2.1.1 [Anonymous, 2013] 6

3.2.1.2 [Unnamed, 2017a] 16

3.2.1.3 [Unnamed, 2017b] 26

1 PHYSICAL HAZARDS

1.1 Flammable solids

Study reference:

Disseminated registration dossier

Test type

determination of the flammability of sodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS)

following EU-Method A.10 and UN N.1

Detailed study summary and results:

Material and methods

- EU-Method A.10 and UN N.1

Results

- The test item was heated with the flame of a teclu burner. The test item started to burn at contact with the flame and carbonised. After removing the flame, the test item burned only for a short time with a little flame; then the flame died. The flame only propagated for 2 cm at the surface of the test item string before it died. to verify this result, the other end of the prepared test item string was heated with the flame of the teclu burner, yielding the same result.
- Therefore, the test itemsodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS) should be considered as **not highly flammable.**

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

3 HEALTH HAZARDS

3.1 Serious eye damage/eye irritation

3.1.1 Other data

3.1.1.1 [Unnamed. 2012]

Study reference:

Anonymous. Study report. 2012.

Disseminated registration dossier

Detailed study summary and results:

Test type

OECD Guideline 437 (Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants)

GLP

Test substance

- Sodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS)
- Aqueous solution (see confidential annex for further information on composition)
- The test item is a non-surfactant liquid. It was tested directly, without dilution or preparation of a solution.
- Is the substance skin corrosive or skin irritant? No in in vitro studies; dermal irritation in an acute dermal study

Test animals

- Cattle: species fresh bovine cornea
- Fresh bovine eyes were obtained from the slaughterhouse on the day of the test. The cattle were between 12 and 60 months old. The eyes were transported to the test facility in Hank's balanced salt solution (supplemented with 0.01% streptomycin and 0.01% penicillin). Only corneas which were free from defects were used. Then the corneas were dissected and incubated in medium at 32 ± 1 °C in an incubation chamber for 1 hour.
- 3 replicates for each treatment groups (negative control, positive control and test item)

Administration/exposure

- *Duration of test/exposure period*: 10 minutes
- *Total dose: amount/concentration of test material applied to skin in mg/ml and rationale for dose level selection*
- *Post exposure observation period:* 2 hours at 32 °C.
- *Control group and treatment:* positive control (sodium hydroxide solution (10% NaOH dissolved in 0.9% sodium chloride solution) and negative control (Physiological sodium chloride solution: 0.9% NaCl) included
- The test item sodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS) was brought onto the cornea of a bovine eye which previously had been incubated with cMEM (complete minimum essential medium) without phenol red at 32±1 °C for one hour and whose opacity had been determined. The test item was incubated on the cornea for 10 minutes at 32±1 °C. After removal of the test item and two hours post-incubation, opacity and permeability values were measured.

Results and discussion

After the initial incubation, the medium was changed and the baseline opacity for each cornea was recorded. None of the corneas showed tissue damage; therefore all corneas were used.

Correction of measured absorption at 490 nm: as cuvettes with a pathlength of 0.2 cm are used in the measurement of the fluorescein-Na solution in the spectral photometer, the pathlength must be corrected to 1 cm.

Coefficient: 1/0.2 = 5: all absorptions were multiplied with this coefficient.

Calculation of IVIS: IVIS = opacity difference + (15 x corrected OD490 value)

The absorption (570 nm) and opacity values which were measured before and after exposition are given in the following table:

Absorption and Opacity Values Negative Control

Parameter	Negative control		
Absorption before exposition	0.1826	0.1843	0.1736
Absorption after exposition	0.2772	0.2472	0.1824
Opacity before exposition	1.5226	1.5286	1.4914
Opacity after exposition	1.8932	1.7669	1.5219
Opacity difference	0.3706	0.2382	0.0305

Mean opacity difference of the negative control is 0.2131

Parameter	Test Item: sodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS)			Positive control		
Absorption before exposition	0.1623	0.1618	0.2444	0.1792	0.2138	0.1321
Absorption after exposition	2.0500	2.1496	2.1244	2.2342	1.9648	2.1006
Opacity before exposition	1.4531	1.4514	1.7555	1.5108	1.6361	1.3555
Opacity after exposition	112.2018	141.1237	133.1680	171.4747	92.2147	126.0666
Opacity difference	110.7487	139.6723	131.4125	169.9639	90.5786	124.7111

For the permeability measurement, three replicates for each treatment group were measured. The optical density values at 490 nm are given in the following table:

Optical density at 490 nm

Repl.	Negative control			sodium 3-(allyloxy)-2-hydroxypropanesulphonate (HAPS)			Positive control		
Meas.	0.0101	0.0106	0.0059	0.3672	0.2928	0.2957	0.3771	0.3820	0.3852
Corr.	0.0505	0.0530	0.0295	1.8360	1.4640	1.4785	1.8855	1.9100	1.9260
Mean	0.0443			-					

*In order to correct the path length, a factor of 5 was taken into account when calculating the IVIS

Test group	IVIS	Mean IVIS	Relative standard deviation IVIS
Negative Control 0.9% NaCl	1.128	0.878	40.3%
	1.033		
	0.473		
Test Item	137.411	150.293	7.9%
	160.755		
	152.712		
Positive Control 10% NaOH	197.369	156.148	25.4%
	118.351		
	152.723		

The positive control induced a very severe irritation on the cornea, mean IVIS was 156.148.
The negative control showed no irritation, mean IVIS was 0.878.
Validity criteria are fulfilled based on the results of positive and negative controls.
A mean IVIS of 150.293 was calculated for HAPS, corresponding to an ICCVAM classification as very severely eye irritant. According to OECD Guideline no. 437 (2009), a substance that induces an IVIS ≥ 55 is defined as a corrosive or severe irritant.

3.2 Reproductive toxicity

3.2.1 Animal data

3.2.1.1 [Anonymous, 2013]

Study reference:

Anonymous. Study report. 2013

Detailed study summary and results:

Test type

OECD guideline 421; GLP compliant

Test substance

- Aqueous solution with 35.2% concentration of HAPS (see confidential annex for further information on composition)

- Aqueous solution, light yellow liquid

Test animals

- Wistar Han (IGS) rat male/female
- 12/sex/dose
- Source: Charles River, Sandhofer Weg 7, 97633 Sulzfeld, Germany
- Age at study initiation: 10 - 11 weeks
- Weight at study initiation: 274 - 309 g (males); 174 - 199 g (females)
- Fasting period before study: no
- Housing: males in groups of three, females individual
- Diet : Maintenance diet for rats and mice, No. 1324 TPF, ad libitum
- Water : sterilised community tap water, ad libitum
- Acclimation period: 6 days (satellite group) or 9-10 days (all other groups)
- ENVIRONMENTAL CONDITIONS
    - Temperature (°C): 22 +- 3 °C
    - Humidity (%): 30 - 70 %
    - Air changes (per hr): 10
    - Photoperiod (hrs dark / hrs light): 12 h/12h

Administration/exposure

- Route of administration – oral gavage
- Vehicle: water
- PREPARATION OF DOSING SOLUTIONS: The test item has been delivered as a 35.2% aqueous solution and a relative density of 1.17 g/cm^3. Dosing referred to the effective content of the active ingredient (HAPS). The raw solution also contained 3.7% NaOH increasing the pH up to 13.2, causing severe corrosive effects during application. Thus, the composition was neutralised to a pH of 7 with concentrated HCl. For preparation of the application solutions the neutralised test solution was diluted with autoclaved community tap water. The test item preparation was intended for an application volume of 4 mL per kg body weight.
- Analytical verification of doses or concentrations: no
- Duration and frequency of test/exposure period: daily
- Doses/concentration levels, rationale for dose level selection: 62.5, 250 and 1000 mg/kg bw/day based on a dose range finding study. The test item was administered in escalating doses up to 1000 mg/kg/day over a time period of 22 days which produced no acute toxic effects in the test animals.
- control animals: yes (dosed with vehicle).

Description of test design:

- *details on mating procedure:*

  - Impregnation procedure: cohoused
  - M/F ratio per cage: 1/1
  - Length of cohabitation: up to 14 days
  - Proof of pregnancy: vaginal plug / sperm in vaginal smear referred to as day 0 of pregnancy
- Males were dosed daily for 42 to 57 days, including the day before the scheduled termination of the in-life phase. This included two weeks of dosing prior to mating and continued throughout the mating period until approximately four weeks post-mating.
- Females were dosed two weeks prior to mating, covering at least two complete oestrous cycles, the variable time to conception, the duration of pregnancy and at least four days after delivery, up to and including the day before scheduled termination of the in-life phase. Therefore the duration of the study following acclimatisation depended on the female performance and was at least 47 days (to 55 days): 14 days pre-mating, up to 14 days until mating, an average of 21 days of gestation, and between 8 and 14 days of lactation. Females showing no evidence of copulation were re-mated for a second mating phase, during which dosing was continued. Those animals were dosed daily for 67 to 76 days.
- Due to several fatalities in the high dose group it became doubtful that a meaningful evaluation of the potential of the substance to affect fertility, pregnancy and maternal or suckling behaviour in at least 8 females as demanded in the guideline could be conducted. To prevent cancellation of the study, a satellite group was added with 24 animals (12 males/ 12 females). The group was treated identically to the high dose group (1000 mg/kg body weight) and supplementary included into the study on day 32. These animals were dosed for either 42 (males) or 47 (females) days. Further 24 animals (12 male/ 12 female) served as vehicle control.

- Parental animals: Observations and examinations:
  - 1. Viability / fatalities: Daily
  - 2. General clinical signs / behaviour: Daily
  - 3. Body weight: Once weekly (including once before beginning of application)
  - 4. Group/Individual food consumption: Once weekly (including once before beginning of application)
  - 5. Group/Individual water consumption: Once weekly (including once before beginning of application)
- Oestrous cyclicity (parental animals): not reported
- Sperm parameters (parental animals): not reported
- Litter observations: yes
- Post-mortem examination (parental animals):

- SACRIFICE
  - Male animals: All surviving animals
  - Maternal animals: All surviving animals
- GROSS NECROPSY
  - Full macroscopic examination was performed.
- HISTOPATHOLOGY / ORGAN WEIGHTS

- Testes and epididymis of all male adult animals were weighted. The ovaries, testes, epididymis, accessory sex organs and all other organs showing macroscopic lesions of all adult animals were preserved in the appropriate fixatives. The numbers of implantation sites per uterus horn and corporea lutea per ovary were counted and recorded. Implantation sites were counted on the day of necropsy, corporea lutea were usually counted under a microscope after at least one day of fixation in a formalin solution. Detailed histological examinations were performed in the ovaries, testes and epididymis (with special emphasis on the stage of spermatogenesis and histopathology of interstitial testicular cell structure) on the animals of the highest dose group and the control group.Post-mortem examinations (offspring): no
- Statistics
  - Spread sheet calculations were performed using Microsoft® Excel® 2011 for Mac.
  - Food- and water consumption of male animals were documented sorted by experimental groups, whereas the body weight was documented for each animal individually. Body weight, food- and water consumption, litter size and litter weight were documented for each female animal individually.
  - The arithmetic mean, standard deviation and median were calculated for all grouped numerical data originating from monitoring the body weight, food- and water consumption, organ weights (gross pathology) and litter size and weight (for details see appendix). Where appropriate, detailed column statistics were applied (minimum / maximum data, 25% quantiles, standard error, upper and lower confidence interval 95%).
  - If appropriate, the respective test item groups were compared to the vehicle group by assessing statistical significance using a two-tailed unpaired Student´s t-test. For all calculations, the significance level was set to 0.05.
- Reproductive indices: examination of reproductive organs
- Offspring viability indices: body weight and sex ratio

Results and discussion

P0 (first parental generation)

General toxicity (P0)

- Clinical signs: effects observed, treatment-related

- Mild discomfort throughout the whole application period was observed for the male animals treated with the high dose of the test item (wiping of nose and mouth through the cage bedding, salivation after application, bleeding of mucous membranes at nose and mouth, respiratory sounds). Due to the solely appearance of the symptoms in the animals treated with the high dose of the test item, it could be estimated with high probability that the test item induced slight irritating effects after application of the highest concentration. Hence, a test item related effect could not be excluded.

- Mortality: mortality observed, non-treatment related
  - 8 animals (3 males / 5 females) died in the high dose group (days 4, 5, 6, 12, 16, 18, 42, 45) and one female in the satellite group (on day 42). Five of these animals died immediately after application or half an hour after application. No mortalities occurred in other groups.
  - Death could be a result of reflux after gavage dosing leading to an accidental aspiration of dose formulation. Due to the high incidence and the exclusive occurrence within the animals treated with the high dose, a test item related effect could not be excluded. However, only one female died in the satellite group also exposed to 1000 mg/kg bw/day.
- Body weight and weight changes: effects observed, non treatment related
  - No significant differences were observed between all test item treated male animals and the vehicle control animals.
  - Body weights of all female animals were comparable during the first two exposure weeks (pre-mating phase). During post-mating, the body weight of the animals treated with the high and the medium dose slightly increased between days 0 and 7 and decreased between days 7 and 20 of gestation, indicating that pregnancies of these animals were aborted between days 7 and 14. The body weight of animals treated with the low dose slightly increased during gestation phase due to 4 animals that became pregnant after first pairing.
  - After second pairing, similar findings were observed.
  - A test item related effect after the 2 first exposure weeks could be masked by the variances in body weight resulting from the different number of females achieving pregnancy per dose group.

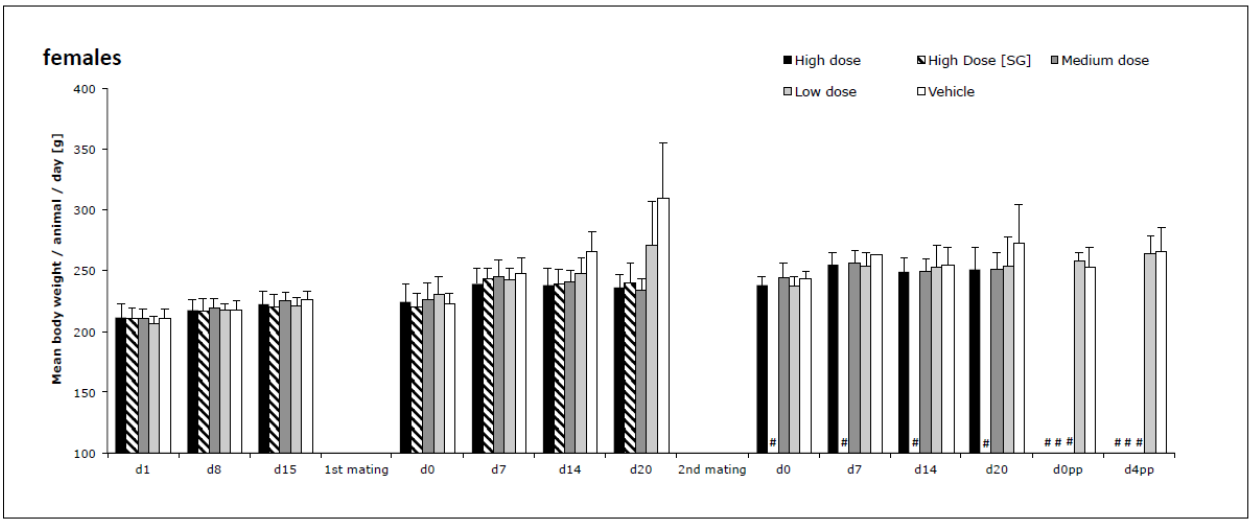

Figure 1 Mean absolute body weight [g] of female animals, sorted by groups and treatment day (high dose: n=9 [pre-mating]; n=6 [1st post-mating]; n=6 [2nd post-mating]; satellite group: n=11 [pre-mating]; n=11 [1st post-mating]; medium dose: n=12 [pre-mating]; n=12 [1st post-mating]; n=12 [2nd post-mating]; low dose: n=12 [pre-mating]; n=12 [1st post-mating]; n=8 [2nd post-mating]; vehicle control group: n=12 [pre-mating]; n=12 [1st post-mating]; n=2 [2nd post-mating];). #: no values applicable as animals did not pass this study phase. Means and standard deviations of each group are given.

-
- Food consumption: effects observed, non-treatment-related
    - No significant differences in food consumption could be observed for all test item treated animals throughout the whole in-life phase.
    - Occasionnally, female animals treated with high dose of the test item had a decreased food intake of about 10%, without clear tendency. Individual fluctuations were most likely effects of natural origin. Differences between days 20 and necropsy were most likely caused by the physiological condition of animals being in their lactation phase in contrast to those that did not achieve pregnancy.
- Water consumption: effects observed, non-treatment-related
    - An increased water intake of all animals (male (increase between 6% and 37%) and female (increase between 9% and 40%)) treated with the highest dose of the test item could be observed throughout the whole in-life phase when compared to their respective vehicle control animals. Additionally, single fluctuations were observed for the male animals of the medium dose group (increase between 8 and 29%).
- Ophthalmological findings, hematological findings, clinical biochemistry findings, urinalysis findings, immunological findings: not examined
- Behaviour (functional findings): not examined
- Organ weight findings including organ / body weight ratios: no statistically significant effects observed for mean weight and relative weight of testes and epididymis. A statistically significant increase of the mean weight of ovaries and uterus was detected for all test item dose groups compared to the vehicle control group. These differences result with high probability from the physiological changes the organs passed during pregnancy.

- Necropsy findings: no test item related prevalent findings were observed during necropsy, neither for male nor for female animals. Findings were regarded to be spontaneous in nature.
- Histopathological findings: non neoplastic: effects observed, treatment-related
    - The ovaries, testes, and epididymis from a total of 51 adult rats (high dose: 10 male/ 6 female; satellite group: 11 female; vehicle control: 12 male/ 12 female) and all other organs showing macroscopic lesions were examined histopathologically.
    - The morphology of the ovaries of the infertile treated females of the high dose group (including animals of the satellite group) was slightly different from those examined in the dams of the vehicle control group. In the main, a minimal to slight ovarian hypertrophy/hyperplasia characterised by the presence of many, partly cystic corpora lutea, several tertiary follicles and an increase in the number of interstitial cells was noted in the treated females. These findings may be due to the infertile state and estrus cycle of the ovaries. However, a relationship to the treatment with the test item could not excluded. All other microscopic findings recorded in the reproductive organs (epididymides, testes, ovaries, uterus horn and some other macroscopic alterations) of the examined animals were considered to be due to the actual estrus cycle or were spontaneous in nature. These findings were within the normal background pathology commonly seen in rats of this age. The differences noted were regarded as random events. After consultation with the Sponsor, an examination of the medium and low dose animals was not performed.

Reproductive function / performance (P0)

- Oestrous cycle: not examined
- Sperm measures: not examined
- Reproductive performance: effects observed, treatment-related: severe changes that were definitely test item related were observed in all experimental groups.
    - The test item prevented or significantly reduced the achievement of pregnancy in all tested dose levels. Offspring was only present in the low dose group (62.5 mg/kg bw/day) and in the control group (1 animal did not achieve pregnancy). At a dosage of 62.5 mg/kg, 5 of 12 animals were able to achieve pregnancy but only 2 of these animals had a normal litter size and development. One animal gave birth to 5 pups. One animal gave birth to 1 pup (runt) that could not be found on day 4 post-partum. One animal gave birth to at least 2 pups, but one was found dead the next day. There was no pregnancy at 250 and 1000 mg/kg bw/day (including the satellite group).
    - The presence of corpora lutea about 24 days after first pairing in the satellite group indicated that an implantation of the zygote took place but embryonic development did not occur or was aborted during the first days of gestation. The absence of corpora lutea about 24 days after second pairing in the high and medium dose groups indicated that, with prolonged

dosing the implantation of the zygote or the ovarian maturation were impaired by the test item. Due to the long intervals between evidence of copulation after first mating and necropsy (about 40 days or more for the animals that went into second mating), the feasibility to detect corporea lutea and implantation sites of the first pairing in these animals was very low. Likewise, the interval of 24 days between evidence of copulation and necropsy for the satellite group was too long to detect implantation sites.

- Based on the results of the study a specific physiological cause of the toxic effect could not be identified.

Table : Summary report of effects on reproduction / development, Part 1

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

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

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

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

Table : Summary report of effects on reproduction / development, Part 2

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

Overall reproductive toxicity:

LOAEL = 62.5 mg/kg bw/day (nominal) based on reproductive effects in the absence of other toxic effects, treatment related.

Table : Summary of the results

Dose Level	High dose		High dose [SG]		Medium dose		Low dose		Vehicle	
	1000 mg/kg BW		1000 mg/kg BW		250 mg/kg		62,5 mg/kg		-	
Parameter	M	F	M	F	M	F	M	F	M	F
Viability / general clinical signs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Clinical signs / behaviour	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Body weight	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Food consumption	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Water consumption	Effects	Effects	Effects	Mild effects	NAD	NAD	NAD	NAD	NAD	NAD
Necropsy	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Organ weight	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Histopathology	NAD	Effects	NAD	Effects	n.e.	n.e.	n.e.	n.e.	NAD	NAD
Reproduction / Development	NAD	Effects	NAD	Effects	NAD	Effects	NAD	Effects	NAD	NAD

M = Male animals
F = Female animals
NAD = No abnormality detected

Conclusion:

A daily oral administration of the test item Sodium 3-(allyloxy)-2- hydroxypropanesulphonate (HAPS) to male Wistar rats at dose levels of 62.5 mg, 250 mg and 1000 mg/kg body weight over a time period of 42 to 57 days did not produce any pathological evidence for toxic effects on the reproduction performance of male rats. However, effects of the spermatogenesis may not have had an adequate time to become evident (such as reduced sperm counts affecting the fertility), as chemical exposure did not cover a complete cycle of spermatogenesis in male test animals. Therefore, and due to the lack of pregnancies in the test item treated female animals, an effect of the test item on the spermatogenesis could not be excluded.

The study has shown that a daily oral administration of the test item at a dosage of 250 mg/kg body weight results in a prevention of pregnancies in female Wistar rats. At a dosage of 62.5 mg/kg, this effect was still apparent in the majority of the animals of this group. Five of 12 animals were able to achieve pregnancy but only 2 of these animals had a normal litter size and development. Hence, a daily oral administration of the test item to female Wistar rats at dose levels above 62.5 mg/kg body weight over a time period of 47 to 76 days produced severe pathological evidence for toxic effects on the reproduction performance of female rats regarding the achievement of pregnancy, litter size and survival rate of pups. It must therefore be assumed that the NOAEL regarding reproduction and development of the test item is below the herein used lowest dose of 62.5 mg/kg.

3.2.1.2 [Unnamed, 2017a]

Study reference:

Unnamed. Study report. 2017

Detailed study summary and results:

Test type

OECD guideline 414; GLP compliant

Test substance

- 38.2 wt% HAPS in mixture.
- Aqueous solution, light yellow liquid

Test animals

- Rat Wistar
- 24 females/dose
- Age of animals at arrival: 5.7-6.1 weeks
- Housing: pre-mating period: 2-3 females per cage; 2 males per cage
- during mating hours: 1 male with 1-3 females;
- during gestation: 2-3 sperm positive females per cage
- Environmental Conditions
    - Illumination: Artificial light, from 6 a.m. to 6 p.m.
    - Temperature: 22 -23 °C
    - Relative humidity: 30 - 45 %
    - Ventilation: above 10 air exchanges/hour by central air-conditioning system.
    - Environmental conditions were maintained by an air-condition system. Temperature and relative humidity were verified and recorded daily during the study

Administration/exposure

- The test item was administered at appropriate concentrations, prepared with the vehicle (dilution with distilled water). The pH value of the formulations was checked and adjusted to pH 6.0-7.0 using 85% ortho-phosphoric acid. Preparation of the test item formulations was made using a magnetic stirrer with a frequency of one to three days. The formulations were stored in a refrigerator (at 5±3 °C). According to the results of the stability measurements in course of the Method Validation the stability of the test item in water was at least 1 day at room temperature and 3 days at 5±3 °C at the concentration levels of 2.5 mg/mL and 550 mg/mL.
- Oral gavage
- Vehicle: water
- Control animals: yes, concurrent vehicle
- Analytical verification of doses or concentration: yes

- In the course of this study an HPLC-UV method has been validated for the analysis of Test Item in water. The analytical method was successfully validated, in accordance with the parameters stipulated in the study plan. The procedure was found to be suitable for the analysis.
- Selectivity no interfering peak was observed
- Repeatability (7 replicates) CV% ≤ 0.9 %
- Linear range 50 - 1500 µg/mL
- Limit of Quantification 50 µg/mL
- Recovery of Test Item from Water 94% at 2.5 mg/mL concentration level (RSD ≤ 0.4)
- 100% at 550 mg/mL concentration level (RSD ≤ 0.6)
- Stability of the test item in Water at room temperature after 1 day 101 % (ca. 2.5 mg/mL)
- 101 % (ca. 550 mg/mL)
- Stability of the test item in Water at 5 ± 3 °C after 3 days 101 % (ca. 2.5 mg/mL)
- 94 % (ca. 550 mg/mL)
- Stock solution stability at least 3 days at 5 ± 3 °C
- Stability in the autosampler at least 21 hours

Description of test design:

- Details on mating procedure: The females were paired to males in the mornings for two to four hours (one male: one to three females) until the number of sperm positive females / group achieves twenty two. Vaginal smears were prepared from each female, stained with 1 % aqueous methylene blue solution and examined for presence of sperm and for estrus cycle. The day of mating is regarded as day 0 of pregnancy (vaginal plug and/or sperm in the vaginal smear). Sperm positive females were separated and caged in groups of 1 to 3 animals, however individual caging was avoided if possible.
- Duration of treatment / exposure: From day 6 up to and including day 19 post coitum; daily
- Dose levels: 100, 300 and 1000 mg/kg bw/day (nominal)
  - The dose levels refer to the Sodium 3-allyoxy-2-hydroxy-1-propanesulfonate quantity in the dosing solutions calculated with 38.2 wt% in aqueous solution.
- Matermal examinations
  - DETAILED CLINICAL OBSERVATIONS: General clinical observations of the sperm positive females was made once a day, after treatment at approximately the same time, considering the peak period of anticipated effects after dosing. Individual observation included the check of behavior and general condition.
  - BODY WEIGHT: The body weight of the male animals was not measured. The body weight of the female rats was measured at least once in the pre-mating period, but was not statistically evaluated. Body weight of sperm positive females was measured on gestation

days 0, 3, 6, 9, 12, 15, 18 and 20 (accuracy of 1 g). Corrected body weight was calculated for the 20th day of pregnancy (body weight on day 20 minus the weight of the gravid uterus).

- FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study): The food consumption was measured between gestation days 0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18 and 18 to 20 by re-weighing the non-consumed diet.

- A Caesarean section and gross pathology were performed on gestational day 20. Organs of the dams were examined macroscopically.
- The number of implantations, early and late resorptions, live and dead foetuses in each uterine horn and the number of corpora lutea were recorded. Each foetus was weighed and examined for sex and external abnormalities. The placentas were weighed and examined externally.The body of about half of each litter was subjected to visceral examination by means of a dissecting microscope after fixation in Sanomiya mixture. The heads were examined by Wilson's free-hand razor blade method. After double staining, the skeletons were examined by means of a dissecting microscope. All abnormalities found during the foetal examinations were recorded.
- Statistical analysis was performed with SPSS PC+ software. The heterogeneity of variance between groups was checked by Bartlett's homogeneity of variance test. Where no significant heterogeneity was detected, a one-way analysis of variance was carried out. If the obtained result was positive, the histogram has been checked first. If the data were almost normal, the data were transformed with a simple function to bring the distribution to normality. Where after transforming the data (x3) no significant heterogeneity was detected, ANOVA was carried out followed by Duncan's Multiple Range test to assess the significance of inter-group differences. Where significant heterogeneity was found, the normal distribution of data was examined by Kolmogorov-Smirnov test. In case of a non-normal distribution, the non-parametric method of Kruskal-Wallis One-Way analysis of variance was used. Chi2 test was performed if feasible .
- Dams or litters were excluded from the data evaluation in cases of:
  - Sperm positive but non pregnant females or
  - dams with 3 or less implantations independent of their viability (total exclusion)
- A male/female foetus was considered as retarded in body weight, when its weight was below the average minus twofold standard deviation of the control male/female foetuses (2.85 and 2.83 g respectively).

Results and discussion

General toxicity (maternal animals):

- Clinical signs: effects observed, non treatment related
  - Alopecia of one female was observed in the 300 mg/kg bw/day group which was not attributed to the treatment.

- Mortality: no mortality observed
- Body weight and weight changes: effects observed, non treatment related
    - The mean body weight of the females was similar in all groups on each measurement day. There were no treatment related differences in the body weight gain of the animals. Between gestation days 6 and 9 there was a statistically significant ($p<0.05$) increase of body weight gain indicated in the 300 mg/kg bw/day group which was judged to be without a dose response. There were no significant differences in the mean corrected body weight and body weight gain of the animals in the different groups.
- Food consumption: effect observed, non treatment-related
    - The food consumption of the dams in the 1000 mg/kg bw/day group was slightly lower between days 6 and 9 as well as between days 9 and 12. Though a statistical significance ($p<0.01$ and $p<0.05$ respectively) considering the slight manner (-6% in both periods), these differences were considered as non-adverse. Statistically significant decrease ($p<0.01$) was also indicated in the pre-treatment period in the 100 mg/kg bw/day group group without a biological relevance
- Water consumption, ophthalmological finding, haematological findings, clinical biochemistry findings, urinalysis findings, immunological findings, neuropathological findings: not examined
- Behaviour (functional findings): no effects observed
- Organ weight findings: not examined
- Gross pathological findings: no effects observed

Maternal developmental toxicity

The number of evaluated litters was 84 (22 each in the test item treated groups and 18 in the control).

- Number of abortions: no effects observed
- Pre and post-implantation loss: effects observed, non treatment-related
    - Pre-implantation loss: mean 5.7%; 10%; 9.8%; 4.6%
    - Post-implantation loss: mean 8.4%; 11.3%; 6.4%; 8.0%
- Total litter losses by resorption: no effect observed
- Early or late resorptions: no effects observed
- Dead foetuses: no effects observed
- Changes in pregnancy duration: no effects observed

Effect levels (maternal animals): NOAEL = 1000 mg/kg bw/day (nominal)

INTRAUTERINE MORTALITY, VIABLE FETUSES, SEX DISTRIBUTION

(mean, SD)

GROUPS (mg/kg bw/day):		Control	100	300	1000	
NUMBER OF DAMS:		18	22	22	22	
Corpora Lutea	Mean:	14.1	13.4	13.6	13.6	
	SD:	1.53	1.50	1.84	1.79	NS
Preimplantation Loss %	Mean:	5.7	10.0	9.8	4.6	
	SD:	7.48	9.74	17.44	6.65	NS
Implantation	Mean:	13.3	12.1	12.3	13.0	
	SD:	1.94	2.20	2.83	1.86	NS
Early Embryonic Death %	Mean:	5.5	8.0	3.6	6.4	
	SD:	6.53	15.56	6.34	9.09	NS
Late Embryonic Death %	Mean:	2.9	2.5	2.8	1.2	
	SD:	4.37	4.86	4.83	3.10	NS
Dead Fetuses %	Mean:	0.0	0.8	0.0	0.4	
	SD:	0.00	2.57	0.00	1.94	NS
Postimplantation Loss %	Mean:	8.4	11.3	6.4	8.0	
	SD:	8.31	16.52	7.23	9.13	NS
Total Intrauterine Mortality %	Mean:	13.4	20.7	15.8	12.2	
	SD:	11.85	15.45	16.89	10.51	NS
Viable fetuses	Mean:	12.2	10.6	11.5	12.0	
	SD:	2.10	2.58	2.74	2.26	NS
Male fetuses %	Mean:	46.8	47.6	44.2	45.1	
	SD:	11.50	15.62	21.76	13.07	NS
Female fetuses %	Mean:	53.2	52.4	55.8	54.9	
	SD:	11.50	15.62	21.76	13.07	NS

REMARKS : NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
U = Mann-Whitney U - test Versus Control
DN = Duncan's multiple range test

(sum, %)

GROUPS (mg/kg bw/day): NUMBER OF DAMS:		Control 18	100 22	300 22	1000 22
Corpora Lutea	Sum:	254	294	299	299
Preimplantation Loss	Sum:	14	28	29	14
(Data compared to no. of corpora lutea)	%:	6	10	10	5
Implantation	Sum:	240	266	270	285
Early Embryonic Death	Sum:	14	23	10	18
(Data compared to no. of implantations)	%:	6	9	4	6
Late Embryonic Death	Sum:	6	7	8	3
(Data compared to no. of implantations)	%:	3	3	3	1
Dead Fetuses	Sum:	0	2	0	1
(Data compared to no. of implantations)	%:	0	1	0	0
Postimplantation Loss	Sum:	20	32	18	22
(Data compared to no. of implantations)	%:	8	12	7	8
Total Intrauterine Mortality	Sum:	34	60 *	47	36
(Data compared to no. of corpora lutea)	%:	13	20	16	12
Viable fetuses	Sum:	220	233	252	263
Male fetuses	Sum:	104	114	115	117
(Data compared to no. of viable fetuses)	%:	47	49	46	44
Female fetuses	Sum:	116	119	137	146
(Data compared to no. of viable fetuses)	%:	53	51	54	56

REMARKS:
* = $p < 0.05$; CH^2
** = $p < 0.01$; CH^2

Foetuses

The number of examined foetuses was 220, 233, 252 and 263 in the control, 100, 300 and 1000 mg/kg bw/day groups, respectively.

- Foetal body weight changes: no effects observed
    - There were no treatment related differences in the mean body weight of the foetuses (also not if combined by sex) as well as in the placental- and relative placental weight. The mean body weight of female foetuses was statistically significantly ($p<0.05$) higher in the 100 mg/kg bw/day group, however the difference was only 0.1 g and no dose response was indicated.
- Reduction in number of live offspring: no effects observed
- Changes in sex ratio: no effects observed
- Changes in litter size and weights: no effects observed
    - Body weight retardation (limit: below 2.85 g for males and below 2.83 g for females) was evaluated as an external variation. There were no significant differences in the foetal- and litter incidences.
- Changes in postnatal survival: not examined

- External malformations / variations: effects observed, non treatment-related
    - The number of litters with malformed foetuses was two in the 100 and three in the 1000 mg/kg bw/day dose group. There were no malformations found in the control and 300 mg/kg bw/day dose group
    - At external examination two foetuses were found with short tail and one of them with hypoplastic pollex (not proved at skeletal examination) in the 100 mg/kg bw/day dose group. Both of these foetuses had multiple malformed vertebrae and in addition one of them had fused ribs. In this group a third foetus had also fused ribs and multiple malformations of the thoracic vertebrae. In the high dose group two foetuses were found with bent scapula or/and ulna or/and slightly shorter femur. A third foetus in the high dose group had a bipartite thoracic vertebra with dumb-bell shaped cartilage. All of the malformations found in the low and high dose group occur sporadically also in control rat foetuses according to the Background pregnancy and foetal data of Toxi-Coop Zrt. and occurred with low incidence or without a dose response and were considered to be without a test item relationship in this study
- Skeletal malformations / variations: effects observed, non treatment-related
    - There were three malformations found each in the high and low dose group and none in the mid dose. In the high dose group one foetus was found with bent scapula (bilateral), bent ulna (unilateral) and slightly shorter femur (unilateral). Another foetus had bent scapula (bilateral). The third foetus had a bipartite thoracic vertebra with dumb-bell shaped cartilage. All of the malformations found in the high dose group occur sporadically also in control rat foetuses according to the Background pregnancy and foetal data of Toxi-Coop Zrt. and were considered to be without a test item relationship in this study. The two foetuses found with short tail at external examination in the 100 mg/kg bw/day group in a common litter were proved to have multiple malformed vertebrae and one of these foetuses had also fused ribs. One foetus had fused arches and bipartite cartilage of cervical vertebrae, fused, hemicentric, in cartilage bipartite thoracic vertebrae, hemicentric, misshapen lumbar cetnra, absence of a part of caudal vertebrae and in addition variations such as asymmetric, dumb-bell shaped thoracic centra and slightly dumb-bell shaped cartilage. The neck rib was fused with the first rib bilateral in this foetus. In the other foetus of the same litter fusion was observed of the exoccipital with the first cervical vertebra as well as bipartite cartilage or fusion of other cervical vertebrae; misshapen cartilage of thoracic centra; hemicentric thoracic and lumbar centra; fused cartilage of thoracic and lumbar vertebrae; displaced and misshapen lumbar centra; dumb-bell shaped cartilage of sacral centrum; abnormal flexion of caudal part of vertebral column; fusion of first caudal vertebrae with Sacral IV and absence of a part of caudal vertebrae. In addition vertebral variations such as dumb-bell shaped (including slightly dumb-bell shaped cartilage) and asymmetric bipartite or unossified thoracic centra

were recorded for this foetus. The third foetus in the 100 mg/kg bw/day group had fused ribs and misaligned, bipartite (including cartilage) thoracic centra; a hemicentric and displaced centrum of Th XI (the present half of centrum (left) was common with the right side of Th XII centrum). Considering the lack of dose response in case of multiple malformed vertebrae (with or without fused ribs) this malformations were not considered to be a consequence of the treatment. Moreover according to the background data of Toxi-Coop Zrt. multiple malformed vertebrae may occur incidentally in rat foetuses.

Fetal malformations					
Vertebrae					
thoracic bipartite and cartilage dumb-bell shaped	N	0	0	0	1
	%	0	0	0	1
	Litters N	0	0	0	1
	%	0	0	0	5
- multiple malformed #	N	0	1	0	0
	%	0	1	0	0
	Litters N	0	1	0	0
	%	0	5	0	0
- multiple malformed vertebrae and fused ribs *** **and** ****	N	0	2	0	0
	%	0	2	0	0
	Litters N	0	2	0	0
	%	0	9	0	0
Pectoral girdle, forelimbs					
- bent scapula	N	0	0	0	1
	%	0	0	0	1
	Litters N	0	0	0	1
	%	0	0	0	5
- bent scapula and ulna	N	0	0	0	1
	%	0	0	0	1
	Litters N	0	0	0	1
	%	0	0	0	5

- Variations: Skeletal findings such as retardation of the skull, incomplete or irregular ossification of the skull bones, unossified hyoid bone, less than 3 ossified or bipartite sternebra, wavy ribs, a presence of a neck rib, dumb-bell shaped, bipartite and/or asymmetric vertebrae, slightly dumb-bell shaped cartilage of thoracic centra, unossified thoracic centra or lumbar arches, asymmetric pelvic articulation of sacral/lumbar arches, unossified pubic or ischii as well as asymmetric ossification of metacarpal/metatarsal or if less than 3/3.5 ossified were classified as variations. There was no significant difference in the incidence of allover skeletal variations. Statistically significantly increased incidence of markedly incomplete ossification of one or more skull bones ($p<0.05$) in the 300 ($p<0.01$) and the 1000 mg/kg bw/day dose group. There was no dose response indicated and no statistical significance was indicated if the litter incidence was evaluated in the high dose group. The incidence of wavy ribs was statistically significantly higher in the low ($p<0.05$) and high dose ($p<0.01$) group. The result of evaluation was not significant in the 300 mg/kg bw/day group if the litter incidence was evaluted. The incidence of wavy ribs was within the

historical control level, hence the increase of these both variations was judged to be not adverse.

- incomplete ossification, marked (one bone or more) or not ossified	N	1	5	10 *	12 **
	%	1	4	8	9
	Litters N	1	4	7 *	6
	%	6	18	32	27
Fetal variations					
Ribs					
- wavy	N	0	5 *	4	9 **
	%	0	4	3	7
	Litters N	0	3	3	5 *
	%	0	14	14	23

- Visceral malformations / variations: effects observed, non treatment related
  - There were no malformations found.
  - Slightly dilated lateral brain ventricles or slightly dilated perimeningea space as well as pinched liver lobe were found only in the control group. Slightly dilated third brain ventricle was recorded for one foetus in the 100 mg/kg bw/day group. Hydroureter (or convoluted ureter in one foetus) as well as hydroureter with dilated renal pelvis and slightly malpositioned kidney was found with a low incidence and without a dose response. In the high dose group only hydroureter was found.

Effect levels (foetuses): NOAEL = 1000 mg/kg bw/day

PREGNANCY DATA OF FEMALES, MORTALITY, MALFORMATIONS

(sum, %)

Dose groups	Control		100mg/kg bw/day		300 mg/kg bw/day		1000 mg/kg bw/day	
Number of sperm positive females	22		24		24		24	
Number of females with no implantation but corpora lutea	0		0		0		0	
Number of females with no implantation and no corpora lutea	3		2		1		2	
Number and percent of pregnant females (females with implantation)	19	86%	22	92%	23	96%	22	92%
Number of dams with less than 3 implantations	1*		0		1		0	
Number of dams with total intrauterine death	1*		0		0		0	
Number of pregnant females died	0		0		0		0	
Number of evaluated litters	18		22		22		22	
Number and percent of evaluated litters with malformed fetuses	0	0%	2	9.1%	0	0%	3	13.6%

Remark: *= the same female

Applicant's summary:

Treatment of pregnant Hsd. Han: WIST Rats from gestational day 6 to 19 by oral administration of Sodium 3-allyloxy-2-hydroxy-1-propanesulfonate (HAPS), caused no mortality, no clinical signs and necropsy

alterations and no adverse effects on the body weight as well as food consumption of the maternal animals. The treatment of the dams did not increase the pre- and post-implantation loss and had no influence on the mean number of viable foetuses and their sex distribution. Sodium 3-allyloxy-2-hydroxy-1-propanesulfonate (HAPS) caused no foetal malformations and did not influence adversely the occurrence of variations.
Based on these observations the No Observed Adverse Effect Level (NOAEL) was determined as follows:
NOAEL maternal toxicity: 1000 mg/kg bw/day
NOAEL developmental toxicity: 1000 mg/kg bw/day

3.2.1.3 [Unnamed, 2017b]

Study reference:

Unnamed. Study report. 2017

Detailed study summary and results:

Test type

Range-finding study. OECD guideline 414; GLP compliant

Test substance

- 38.2 wt% HAPS in aqueous solution.
- aqueous solution, light yellow liquid

Test animals

- Rat Wistar
- Species / Strain: Hsd. Han: WIST Rats
- Source: TOXI-COOP ZRT. 1103 Budapest, Cserkesz u. 90.
- Hygienic level: SPF at arrival and kept in good conventional environment during the study.
- Age of females at arrival: females: 7-8 weeks
- Mating: Age of animals at mating: Females: Young adult and nulliparous females, 8-9 weeks of age at start of the mating period; Males: 25 weeks of age at start of the mating period; Body weight of females at mating: The group averages of the body weight of the females were as similar as possible on the first day of gestation; Number of animals: 50 females; 80 males
- Number of animals: 5-6 females/dose
- Acclimatisation time for females: 6 days
- Reason for Selection of Species: The rat is commonly used species for toxicological studies in accordance with international recommendations. The Wistar strain is a well-known laboratory model

with sufficient historical data. Rats are recommended as rodents for prenatal developmental toxicity studies.

- Identification of Animals: The individual identification of the adult males was performed in the pre-experimental phase by a permanent marker pen on the tail and females were identified by ear punching. Males were identified from 1 to 80 and females from 101 to 150. After randomisation of the sperm positive females, the animals' cages were marked by identity cards, with information about study number, sex, dose group, cage number, individual animal numbers, date of mating and scheduled necropsy.
- Litters: At Caesarean section, the litters were identified with the litter numbers. The flasks used for fixation and all sheets used for recording data of the foetuses were marked only with these numbers and not with the dose groups up to the end of foetal examinations (to avoid bias).
- Foetuses: In the course of the Caesarean section, after removal from the uterus the foetuses were randomly allocated and identified thereafter. For possible visceral examination the foetuses were identified by finger-cutting, for possible skeletal examination by means of water-proof plastic ribbon tied around their neck.
- Housing Conditions:
    - Only healthy animals were used for the study. Health status certified by the breeder.
    - Animal room: 16/A
    - Housing: pre-mating period: 2-3 females per cage; 2 males per cage - during mating hours: 1 male with 1- 3 females - during pregnancy: 1-3 sperm positive females per cage
    - Cage type: Type II polypropylene/polycarbonate with stainless steel covers equipped by self-feeding baskets
    - Bedding: Certified laboratory wood bedding (Lignocel Hygienic Animal Bedding produced by J. Rettenmaier & Söhne GmbH+Co.KG; D-73494 Rosenberg Holzmühle 1 Germany). The cages and bedding was changed at least twice a week.
    - Room sanitation: At the end of each working day floors were swept and then mopped with an acceptable disinfectant. Water bottles were cleaned on a rota basis as required during the course of the study.
- Environmental Conditions:
    - Illumination: Artificial light, from 6 a.m. to 6 p.m.
    - Temperature: 21 -22°C
    - Relative humidity: 30 - 42 %
    - Ventilation: above 10 air exchanges/hour by central air-conditioning system.
    - Environmental conditions were maintained by an air-condition system. Temperature and relative humidity were verified and recorded daily during the study.
    - Food and Water Supply: Animals received ssniff® SM R/M-Z+H complete diet for rats and mice produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany and tap water, as for

human consumption, ad libitum. The food was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. The supplier will provide an analytical certificate of the standard diet for the batch used.

- Water quality control analysis was performed periodically. The quality control results are available at Toxi-Coop Zrt's archives.

Administration/exposure

- The test item was administered at appropriate concentrations, prepared with the vehicle (dilution with distilled water). The pH of the formulations was checked and adjusted to pH 6.0-7.0 using 85% ortho-phosphoric acid. Preparation of the test item formulations was made daily using a magnetic stirrer.
- Vehicle: Distilled water (Humaqua); Batch number: 1511-5519; 1512-5503; Expiry date: May 19, 2016; June 03, 2016; Supplier: TEVA Storage: at room temperature
- Route of Administration and Reason for the Selection: The test item was administered orally (by gavage) from gestational day 5 to 19, daily. The route of application was selected in compliance with international guidelines.
- Analytical verification of doses or concentrations: no
- Details on mating procedure: The males were paired to females in the mornings for two to four hours (one male: one to three females) until the number of sperm positive females / group achieved at least five. Vaginal smears were prepared from each female, stained with 1 % aqueous methylene blue solution and examined for presence of sperm and for estrus cycle. The day of mating was regarded as day 0 of pregnancy (vaginal plug and/or sperm in the vaginal smear). Sperm positive females were separated and caged in groups of 1 to 3 animals.
- Dose levels: 0, 10, 37.5, 125, 500 mg/kg bw/day - The dose levels refer to the Sodium 3-allyoxy-2-hydroxy-1-propanesulfonate quantity in the dosing solutions calculated with 38.2 wt% in aqueous solution.
- Control group: yes, concurrent vehicle
- Maternal examinations:
    - Clinical Observations: General clinical observations of the sperm positive females was made once a day, after treatment at approximately the same time, considering the peak period of anticipated effects after dosing.
    - Body Weight: The body weight of the male animals was not measured. The body weight of the female rats was measured at least once in the pre-mating period, but was not statistically evaluated. Body weight of sperm positive females was measured on gestation days 0, 3, 5, 8, 11, 14, 17 and 20 (accuracy of 1 g) and evaluated. Corrected body weight was calculated for the 20th day of pregnancy (body weight on day 20 minus the weight of the gravid uterus).

- Food Consumption: The food consumption was measured between gestation days 0 to 3, 3 to 5, 5 to 8, 8 to 11, 11 to 14, 14 to 17 and 17 to 20 by re-weighing the non-consumed diet (accuracy: 1 g).
- Mortality: Observations for signs of morbidity and for mortality were made twice daily, at the beginning and before the end of the working period.
- Necropsy: All sperm positive females were sacrificed by decapitation under deep Isofluran anaesthesia on day 20 of gestation. The abdomen was opened, the uterus with cervix and left ovary was removed and weighed. The right ovary was placed into a Petri dish after removal. After removing the uterus gross pathology of dams' viscera was performed.
- The number of corpora lutea in each ovary and implantation sites in each uterine horn, live foetuses, early and late embryonic death and foetal death were counted. Animals, in which unambiguous implantation sites, but not foetuses have been found, were considered as pregnant. Uteri that appeared non-gravid were further examined to confirm the non-pregnant status.
- Foetuses were removed from the opened uterus and sunk in a Petri-dish filled up with water. Spontaneous movement of foetuses was observed as a viability assessment. Euthanasia of the foetuses was performed by hypothermia.

- Foetal examinations
  - The foetuses were washed with tap water and randomly laid on a filter paper with written ordinal numbering. Bleeding from the umbilical cord after it is cut was observed as an indication of viability before euthanasia. Each live foetus and its placenta was weighed individually (foetuses accuracy 0.01 g, placentas accuracy 0.001 g), and subjected to external examination. The gender of the foetuses was determined according to the anogenital distance. All abnormalities found during the external examination were recorded. The foetuses were individually identified and about the half of each litter was fixed for visceral and the other half for skeletal examination. The body of those subjected for visceral examination were macerated in Sanomiya mixture and thereafter fixed in isopropanol. After fixation the bodies were micro dissected by means of a dissecting microscope and the heads of the animals were examined by Wilson's free-hand blade method. The abdominal region of those subjected to possible skeletal examination was opened, the viscera were removed and the cadaver was fixed in alcohol. The skeletons were stained by KOH-Alizarin red-S method and examined by means of a dissecting microscope. All changes were recorded. Soft tissues were discarded after examination. Fixed or stained foetuses are preserved until the issue of the final report, thereafter discarded.

Results and discussion

General toxicity (maternal animals):

- Clinical signs: no effects observed
- Mortality: no mortality observed (none of the females died before scheduled necropsy)
- Body weight and weight changes: effect observed, non treatment related
- Food consumption: no effects observed
- Water consumption, ophthalmological findings, haematological findings, clinical biochemistry findings, urinalysis finding, immunological findings, organ weight findings, neuropathological findings: not examined
- Behaviour (functional findings): no effects observed
- Gross pathological findings: no effects observed

Maternal developmental toxicity:

- Number of abortions: no effects observed
- Pre and post-implantation loss: no effects observed
- Total litter losses by resorption: no effects observed
- Early or late resorption: no effects observed
- Dead foetuses: no effects observed
- Changes in pregnancy duration: no effects observed
- Changes in number of pregnant: no effects observed

Effect levels: NOAEL = 500 mg/kg bw/day (no effect)

Foetuses

All females were pregnant except one in the control and one in the 37.5 mg/kg bw/day group, thus the number of evaluated litters was 4, 5, 4, 6 and 6 in the control, 10, 37.5, 125 and 500 mg/kg bw/day groups.

- Foetal body weight changes: no effects observed. There was no dose related reduction indicated in the foetal weight. Moreover the body weight of the foetuses was higher in the test item treated groups than in the control. There were no foetuses under the body weight retardation limit (below 1.24 g for males and 1.48 g for females) and there were no other variations found.
- Reduction in number of live offspring: no effects observed
- Changes in sex ratio: no effects observed
- Changes in litter size and weights: no effects observed
- External malformations: no effects observed
- Skeletal malformations: effects observed, non treatment related: There were two malformations found at skeletal examination in the 37.5 mg/kg bw/day group. One foetus was observed with a split

and misaligned sternum and another foetus with bent ulna. Considering the low incidence and the lack of dose response these malformations were not attributed to the treatment

- Visceral malformations: no effects observed

Effect levels: NOAEL developmental toxicity: 500 mg/kg bw/day (no effect)