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:

Reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis((2,3-epoxypropoxy)methyl)butane and 1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane

EC Number: -

CAS Number: -

Index Number: 603-RST-VW-Y

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CONTENTS

1 INFORMATION ON IDENTITY OF THE SUBSTANCE AND SUBSTANCE COMPOSITION

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	'no optical activity' (information provided in the context of analytical information)

1.2 Information on substance composition

The constituents of the reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis ((2,3-epoxypropoxy)methyl) butane and 1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane are **given in Table 2.**

Purity: for details see confidential annex

Table 2: Constituents

Constituent (name and numerical identifier)	Typical concentration [% (w/w)] (only for legal entity)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current Classification (* according to CLH in Annex VI Table 3.1 (CLP))
1-(2,3-epoxypropoxy)-2,2-bis[(2,3-epoxypropoxy)methyl]butane **CAS number: 3454-29-3** **EC number: 222-384-0**	for details see confidential annex	for details see confidential annex	No harmonised classification available, not listed in ECHA C&L (2019)
1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxy butane **CAS number: 18425-64-4** **EC number: -**	for details see confidential annex	for details see confidential annex	No harmonised classification available, not listed in ECHA C&L (2019)

Table 3: Impurities

For details see confidential annex.

2 PHYSICAL HAZARDS

Evaluation not performed for this substance.

3 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Evaluation not performed for this substance.

4 HEALTH HAZARDS

Acute toxicity

4.1 Acute toxicity - oral route

Evaluation not performed for this substance.

4.2 Acute toxicity - dermal route

Evaluation not performed for this substance.

4.3 Acute toxicity - inhalation route

Evaluation not performed for this substance.

4.4 Skin corrosion/irritation

Evaluation not performed for this substance.

4.5 Serious eye damage/eye irritation

Evaluation not performed for this substance.

4.6 Respiratory sensitisation

Evaluation not performed for this substance.

4.7 Skin sensitisation

Evaluation not performed for this substance.

4.8 Germ cell mutagenicity

4.8.1 In vitro data

4.8.1.1 Bacterial Reverse Mutation Assay

Study reference:

N.N., study report, 2014

Detailed study summary and results:

Test type

An in vitro bacterial reverse mutation assay according to OECD TG 471 was performed. GLP compliance is given (incl. certificate).

A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to substance identified in the CLH dossier: Reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis((2,3-epoxypropoxy)methyl)butane and 1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane; named in the ESR as 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, oligomeric reaction product with (chloromethyl)oxirane
- Test Substance Description: Clear colorless liquid
- Degree of purity: 100% (provided by sponsor)
- Impurities: not specified
- Batch number: available in confidential study report
- Expiration date of Lot/Batch: 31/10/15
- Test Substance Molecular Weight: 246.3 – 302.37 g/mol (Molecular weight of 302.37 was used for calculation of dose level)
- Storage conditions of test material: at room temperature, protected from light

Administration/exposure

- Strains: S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2
- Type and composition of metabolic activation system:
  - species and cell type: rat liver S9
  - quantity: 0.5 mL
  - induced or not induced: Induced
  - chemicals used for induction: Aroclor 1254
  - co-factors used: beta-nicotinamide-adenine dinucleotide phosphate, glucose-6-phospate, potassium chloride, magnesium chloride, phosphate buffer

- Test concentrations:
  - Initial toxicity-mutation assay (plate incorporation):
    - eight concentrations (1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate) tested in overnight cultures of strains TA98, TA100, TA1535, TA1537 and WP2 uvrA on

selective minimal agar in the presence and absence of Aroclor-induced rat liver S9. ; the maximal dose tested (5000 µg/plate) was achieved with a concentration of 100 mg/mL and a 50 µL plating aliquot.

- Number of replicates: 2

- Confirmatory mutagenicity assay (plate incorporation):
  - five concentrations (50, 150, 500, 1500 and 5000 µg/plate) tested in overnight cultures of strains TA98, TA100, TA1535, TA1537 and WP2 uvrA on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9.
  - Number of replicates: 3

- Vehicle:
  - Identification: Dimethyl sulfoxide (DMSO)
  - Justification of Choice: DMSO was selected as the solvent of choice based on the solubility of the test substance and compatibility with the target cells.
  - At approximately 500 mg/mL, the test substance formed a clear solution in DMSO, the maximum concentration tested in the solubility test conducted at BioReliance.
  - Statistical methods:
    - A statistical evaluation was not provided,
    - Data were collected and analysed including the following systems: Sorcerer Colony Counter and Ames Study Manager (Perceptive Instruments), LIMS System (BioReliance), Excel 2007 (Microsoft Corporation), BRIQS (BioReliance) and Kaye Lab Watch Monitoring System (Kaye GE).

Results and discussion

- Justification should be given for choice of tested dose levels: based on initial toxicitiy-mutation test (50, 150, 500, 1500 and 5000 µg/plate), tested up to maximum concentration according to guideline
- Cytotoxic concentrations with and without metabolic activation: no cytotoxicity nor precipitates, but tested up to recommended limit concentrations
- Genotoxic effects with and without metabolic activation: positive mutagenic responses with tester strains TA100 and TA1535 in the presence and absence of Aroclor-induced rat liver S9 and tester strain WP2 uvrA in the presence of Aroclor-induced rat liver S9.
- Concurrent negative (solvent/vehicle) and positive control data:
  - Untreated negative controls: no
  - True negative controls: no
  - Negative solvent/vehicle controls: yes, valid

  - Positive controls: yes (9-aminoacridine, 2-nitrofluorene, sodium azide, methylmethanesulfonate, other: 2-aminoanthracene), valid

- Test specific confounding factors: no cytotoxicity nor precipitates, but tested up to recommended limit concentrations
- Statistical results:
  - Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 uvrA were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 2.0-times the mean vehicle control value.
- Provide information that may be needed to adequately assess data for reliability
  - mean number of revertant colonies per plate and standard deviation: numerical values are not provided
  - Evaluation criteria:
    - Positive mutagenic responses in the initial toxicity-mutation test (ranging from 4.3- to 68.6-fold maximum increases) were observed with tester strains TA100 and TA1535 in the presence and absence of S9 activation and tester strain WP2 uvrA in the presence of S9 activation.
    - Positive mutagenic responses in the mutagenicity assay (ranging from 4.2- to 93.9-fold maximum increases) were observed with tester strains TA100 and TA1535 in the presence and absence of S9 activation and tester strain WP2 uvrA in the presence of S9 activation.

Table 4: Results of initial toxicity-mutation assay without metabolic activation

Metabolic Activation	**Test Article**	**Dose Level (µL/plate)**	**Initial Toxicity-Mutation Assay (#1) Revertant Colony Counts (Mean ±SD)**				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	DMSO	50 µL/plate	17 ± 8	98 ± 7	6 ± 1	3 ± 3	17 ± 4
	TK 30174	1.5	22 ± 4	117 ± 11	5 ± 4	6 ± 1	26 ± 13
		5.0	20 ± 8	137 ± 13	11 ± 1	6 ± 8	24 ± 0
		15	21 ± 2	130 ± 8	12 ± 0	9 ± 1	26 ± 8
		50	21 ± 4	114 ± 3	16 ± 2	10 ± 4	17 ± 8
		150	18 ± 6	168 ± 4	13 ± 2	7 ± 2	23 ± 0
		500	14 ± 4	337 ± 18	26 ± 2	4 ± 2	28 ± 5
		1500	17 ± 4	586 ± 11	80 ± 18	5 ± 4	32 ± 11
		5000	35 ± 4	1011 ± 120	152 ± 0	6 ± 0	44 ± 13
	2NF	1.0	196 ± 3				
	SA	1.0		632 ± 9	633 ± 91		

	9AAD	75				610 ± 173	
	MMS	1000					455 ± 27

Key to Positive Controls

SA sodium azide

9AAD 9-Aminoacridine

2NF 2-nitrofluorene

MMS methyl methanesulfonate

Table 5: Results of initial toxicity-mutation assay with metabolic activation

Metabolic Activation	**Test Article**	**Dose Level (µL/plate)**	**Initial Toxicity-Mutation Assay (#1) Revertant Colony Counts (Mean ±SD)**				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
With Activation	DMSO	50 µL/plate	39 ± 4	126 ± 5	14 ± 5	10 ± 6	16 ± 4
	TK 30174	1.5	40 ± 10	122 ± 11	11 ± 1	8 ± 3	26 ± 0
		5.0	40 ± 6	148 ± 1	18 ± 1	11 ± 1	22 ± 5
		15	38 ± 1	199 ± 6	32 ± 9	9 ± 3	27 ± 3
		50	38 ± 13	205 ± 15	61 ± 10	6 ± 1	28 ± 3
		150	38 ± 1	262 ± 34	103 ± 4	8 ± 4	24 ± 8
		500	32 ± 10	528 ± 101	274 ± 2	8 ± 4	36 ± 12
		1500	32 ± 4	760 ± 26	460 ± 17	7 ± 1	43 ± 1
		5000	50 ± 9	1392 ± 216	961 ± 100	8 ± 1	68 ± 1
	2AA	1.0	461 ± 85		72 ± 9		
	2AA	2.0		427 ± 153		37 ± 1	
	2AA	15					279 ± 4

Key to Positive Controls

2AA: 2-aminoanthracene

Table 6: Results of confirmatory-mutation assay with and without metabolic activation

Metabolic Activation	**Test Article**	**Dose Level (µg/plate)**	**Confirmatory-Mutation Assay (#2) Revertant Colony Counts (Mean ±SD)**				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	DMSO	50 µL/plate	14 ± 2	108 ± 13	9 ± 1	7 ± 3	21 ± 2
	TK 30174	50	18 ± 8	127 ± 12	20 ± 13	7 ± 1	25 ± 7
		150	12 ± 3	137 ± 13	17 ± 1	5 ± 3	22 ± 1
		500	10 ± 0	243 ± 6	33 ± 8	8 ± 2	23 ± 5
		1500	17 ± 6	365 ± 49	84 ± 13	5 ± 4	30 ± 4
		5000	18 ± 4	850 ± 6	148 ± 30	9 ± 3	40 ± 13
	2NF	1.0	230 ± 29				
	SA	1.0		652 ± 122	664 ± 57		
	9AAD	75				413 ± 186	
	MMS	1000					303 ± 12
	DMSO	50 µL/plate	27 ± 3	95 ± 15	10 ± 6	7 ± 3	18 ± 5
		50	22 ± 5	150 ± 17	64 ± 10	6 ± 5	23 ± 8

With Activation	TK 30174	150	27 ± 3	233 ± 41	154 ± 16	6 ± 1	28 ± 3
		500	25 ± 3	474 ± 13	349 ± 13	8 ± 2	34 ± 9
		1500	30 ± 7	594 ± 15	648 ± 124	6 ± 5	41 ± 7
		5000	23 ± 6	1138 ± 189	939 ± 233	7 ± 1	75 ± 13
	2AA	1.0	560 ± 38		121 ± 17		
	2AA	2.0		664 ± 170		54 ± 11	
	2AA	15					287 ± 55

Key to Positive Controls

SA	Sodium azide
9AAD	9-Aminoacridine
2NF	2-nitrofluorene
MMS	methyl methanesulfonate
2AA	2-aminoanthracen

4.8.1.2 In Vitro Mammalian Chromosome Aberration Assay

Study reference:

N.N., study report, 2014.

Detailed study summary and results:

Test type

An in vitro mammalian chromosome aberration test according to OECD TG 473 was performed. GLP compliance is given (incl. certificate).

A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to substance identified in the CLH dossier: Reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis((2,3-epoxypropoxy)methyl)butane and 1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane; named in the ESR as 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, oligomeric reaction product with (chloromethyl)oxirane
- Test material form: Clear colorless liquid
- Degree of purity: 100% (provided by Sponsor)
- Impurities: not specified
- Batch No.: available in confidential study report
- Expiration date of Lot/Batch: 31/10/15
- Test Substance Molecular Weight: 246.3 – 302.37 g/mol (Molecular weight of 302.37 was used for calculation of dose level)
- Storage condition of test material: Room temperature, protected from light

Administration/exposure

- Strain or cell type or cell line, target gene: Chinese hamster ovary (CHO-K1) cells (repository number CCL 61)
- Type and composition of metabolic activation system:
  - species and cell type: rat liver, S9 mix
  - induced or not induced: Induced
  - chemicals used for induction: Aroclor 1254
  - co-factors used: beta-nicotinamide-adenine dinucleotide phosphate, glucose-6-phospate, potassium chloride, magnesium chloride, phosphate buffer
- Test concentrations, and reasoning for selection of doses:
  - prior to chromosome aberration assay, a preliminary toxicity assay was conducted (levels tested ranged from 0.302 to 3020 µg/mL (10 mM))
  - based on the toxicity finding test, the doses for the chromosome aberration assay ranged
    - between 15 and 150 µg/mL for the non-activated 4-hour exposure group,
    - from 50 to 350 µg/mL for the S9-activated 4-hour exposure group, and
    - from 5 to 50 µg/mL for the non-activated 20-hour continuous exposure group
- Vehicle:
  - Identification: Dimethyl sulfoxide (DMSO)
  - Justification of choice of the vehicle: DMSO was used based on the solubility of the test substance and compatibility with the target cells
- Duration: exposure duration was 4 and 20 hours in the non-activated system and 4 hours in the S9-activated test system; harvest 20 hours after treatment initiation
- Statistical methods: a Fisher's exact test was applied to pairwise compare the percentage of aberrant cells of each treatment group with that of the vehicle control group. To measure the dose-responsiveness, the Cochran-Armitage test was applied

Results and discussion

- Justification should be given for choice of tested dose levels:
  - a preliminary toxicity assay was conducted with concentrations between 0.302 to 3020 µg/mL. No further details provided.
- Cytotoxic concentrations with and without metabolic activation: for details see Table 7
  - cytotoxicity detected for the lowest applied concentrations in the experiments without metabolic activation (5 µg/mL at 20h continous incubation and 15 µg/mL at 4h incubation with 16 hours recovery) and above 50 µg/mL with metabolic activation
- Genotoxic effects for details see Table 7

  - positive for structural aberrations in treatment groups with or without metabolic activation
  - negative for numerical (polyploid or endoreduplicated) aberrations (neither significant nor dose-dependent ($p > 0.05$; Fisher's Exact and Cochran-Armitage tests)) in treatment groups with or without metabolic activation

- Concurrent negative (solvent/vehicle) and positive control data:
  - Untreated negative controls: no
  - True negative controls: no
  - Negative solvent/vehicle controls: yes, valid
  - positive controls: yes (cyclophosphamide and mitomycin C), valid
- Test-specific confounding factors:
  - Effects of pH: not provided
  - Effects of osmolality: not provided
  - Water solubility: not provided
  - Precipitation: not provided
- Statistical results:
  - statistically significant effect with a dose-dependent behaviour ($p > 0.05$ (probably typo: expected information: $p < 0.05$)); Fisher's Exact and Cochran-Armitage tests) for structural aberrations in treatment groups with or without metabolic activation
  - no statistically significant differences ($p > 0.05$; Fisher's Exact and Cochran-Armitage tests) for numerical (polyploid or endoreduplicated) aberrations between treatment groups and vehicle controls with or without metabolic activation
- Provide information that may be needed to adequately assess data for reliability:
  - precipitation concentration: not provided
  - mitotic index: not provided
  - Evaluation criteria:
    - The frequency of cells with structural chromosome aberrations in the vehicle control must be within the historical control range.
    - The percentage of cells with aberrations must be statistically increased ($p \leq 0.05$, Fisher's exact test) in the positive control condition relative to the vehicle control

Table 7: Numerical results of the chromosomal aberration assay

Metabolic Activation	Test Substance	Concentration µg/mL	Cytotoxicity a (% of Control)	Aberrant Cells		Aberrations per Cell b, d Mean ± SD f	Total Polyploid Cells (Mean %) e
				Structural (Mean %) b	Numerical (Mean %) c		
20-hr Continuous	DMSO	NA	NA	0.0	0.5	0.000 ± 0.000	0.5
Treatment	TK30174	5	29	14.0**	1.0	0.160 ± 0.420	1.0

Without	TK30174	10	44	20.0**	0.5	0.240 ± 0.534	0.5
Activation	TK30174	25	61	43.0**	1.0	0.680 ± 0.931	1.0
	MMC	0.1	34	21.0**	0.0	0.230 ± 0.468	0.0
4-hr Treatment	DMSO	NA	NA	1.0	0.5	0.010 ± 0.100	0.5
With 16 hr Recovery	TK30174	15	32	4.0*	1.0	0.045 ± 0.231	0.5
Without	TK30174	30	44	34.0**	0.0	0.410 ± 0.637	0.0
Activation	TK30174	50	53	48.0**	0.5	0.840 ± 1.361	0.5
	MMC	0.2	43	21.0**	0.5	0.240 ± 0.495	0.5
4-hr Treatment	DMSO	NA	NA	1.5	1.5	0.015 ± 0.122	1.0
With 16 hr Recovery	TK30174	50	14	2.0	1.5	0.020 ± 0.140	0.5
With	TK30174	100	46	16.0**	0.0	0.210 ± 0.624	0.0
Activation	TK30174	275	55	18.0**	2.0	0.240 ± 0.571	2.0
	CP	5	58	26.0**	0.0	0.360 ± 0.704	0.0

DMSO: Dimethyl sulfoxide; MMC: Mitomycin C; CP: Cyclophosphamide; NA: Not Applicable; Fisher's Exact Test: * $p < 0.05$; ** $p < 0.01$.

a. Based on cell growth inhibition relative to solvent control.
b. Does not include cells with only gaps.
c. Includes polyploid and endoreduplicated cells.
d. Severely damaged cells counted as 10 aberrations.
e. Does not include endoreduplicated cell.
f. SD = Standard Deviation.

4.8.1.3 *In Vitro* Mammalian Cell Forward Gene Mutation

Study reference:

N.N., study report, 2014

Detailed study summary and results:

An in vitro mammalian cell gene test according to OECD TG 476 was performed. GLP compliance is given (incl. certificate).

A reliability of 1 is given for this study in the registration dossier.

Deviations are stated: In the definitive mutagenicity assay, there was no dose level with S9 that produced 10 to 20% relative survival but the results of the initial trial were clearly positive.

Test substance

- Test material used in the study is equivalent to substance identified in the CLH dossier: Reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis((2,3-epoxypropoxy)methyl)butane and 1-(2,3-

epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane; named in the ESR as 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, oligomeric reaction product with (chloromethyl)oxirane

- Test material form: Clear colorless liquid
- Degree of purity: 100% (provided by Sponsor)
- Impurities: not specified
- Test substance Batch No.: available in confidential study report
- Expiration date of Lot/Batch: 31/10/15
- Test Substance Molecular Weight: 246.3 – 302.37 g/mol (Molecular weight of 302.37 was used for calculation of dose level)
- Storage condition of test material: Room temperature, protected from light

Administration/exposure

- Strain or cell type or cell line, target gene: Chinese hamster Ovary (CHO-K1-BH4 from A.W. Hsie, Oak Ridge National Laboratories (Oak Ridge, TN)), hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus (hprt)
- Type and composition of metabolic activation system:
  - species and cell type: rat, liver S9-mix
  - induced or not induced: induced
  - chemicals used for induction: Aroclor 1254
  - co-factors used: beta-nicotinamide-adenine dinucleotide phosphate, glucose-6-phospate, calcium chloride, potassium chloride, magnesium chloride, phosphate buffer
- Test concentrations, and reasoning for selection of doses:
  - preliminary toxicity assay: concentrations of 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500 and 5000 µg/mL with and without S9; no visible precipitate observed; the test substance did not have an adverse impact on the pH or osmolality of the cultures; relative survival was 42.76% and 19.35% at concentrations of 313 µg/mL with S9 and 39.1 µg/mL without S9, respectively (relative survival at other higher concentrations was 0%, or was not determined due to excessive toxicity)
  - definitive mutagenicity assay:
    - Treatment:
      - Eight concentrations (50.0, 100, 200, 300, 350, 400, 450, 500 µg/mL) treated with test substance and with S9 for the 5-hour exposure group
      - Eight concentrations (5.00, 10.0, 20.0, 25.0, 30.0, 35.0, 40.0, 50.0 µg/mL) treated with test substance without S9 for the 5-hour exposure group
    - Mutant selection for concentrations of

- 100, 200, 300, 350 and 400 µg/mL with S9 and
- 5.00, 10.0, 20.0, 30.0 and 40.0 µg/mL without S9

- Evaluated concentrations
  - 100, 200, 300, 350 µg/mL with S9 and
  - 5.00, 10.0, 20.0, 30.0 and 40.0 µg/mL without S9
  - The selection of concentrations is based on the cytotoxicity

- Vehicle:
  - Identification: Dimethyl sulfoxide (DMSO)
  - No visible precipitate was observed at the beginning or end of treatment
- Experimental conditions:
  - Evaluation of cytotoxicity:
    - Replicate cultures from each treatment condition were subcultured independently in complete medium
    - Number of replication: triplicate cultures
    - 200 cells/60-mm dish
    - After 7 days' incubation at standard conditions, colonies were fixed, stained and counted.
  - Expression of mutant phenotype
    - Replicates from each treatment condition were subcultured independently in complete medium
    - maximal 106 cells/T-75 cm2 flask
    - Subculturing for the 7-day expression period, at 2- to 3-day intervals
  - Selection of the TG-resistant phenotype
    - cells from each treatment condition plated into five dishes at 2 x 105 cells/100-mm dish in hypoxanthine-free complete medium containing 10 µM TG
    - At the end of the expression period, selection for mutant phenotype
  - Cloning efficiency at the time of selection
    - 200 cells/60-mm dish plated in hypoxanthine-free complete medium without TG
    - Number of replication: triplicate cultures
    - After 7 days of incubation colonies were fixed, stained and counted for both cloning efficiency at selection and mutant selection.
- Selection agent: hypoxanthine-free complete medium containing 10 µM TG
- Number of replication: duplicate cultures
- Determination of cytotoxicity: Cytotoxicity was expressed relative to the solvent-treated control cultures

- Statistical methods: The statistical analyses used the method of Snee and Irr (1981), with significance established at the 0.05 level

Results and discussion

- Justification should be given for choice of tested dose levels:
  - based on preliminary toxicity assay concentrations of 50.0, 100, 200, 300, 350, 400, 450 and 500 µg/mL with S9, and 5.00, 10.0, 20.0, 25.0, 30.0, 35.0, 40.0 and 50.0 µg/mL without S9 were used; no precipitation observed at the beginning or end of treatment
- Cytotoxic concentrations with and without metabolic activation:
  - cultures treated at concentrations of 400 and 450 µg/mL with S9 were excluded from evaluation due to excessive toxicity. The average relative survival at concentrations of 350 µg/mL with S9 were 25.72 %. This result is a deviation from the guideline but is not regarded as relevant for the validity of the study (see Table 8 and Table 9).
  - The average relative survival of cultures treated with concentrations of 40.0 µg/mL without S9 was 15.88 % (see Table 8 and Table 9).
- Genotoxic effects with and without metabolic activation: Significant increases in mutant frequency, as compared to the concurrent vehicle controls, were observed at the highest acceptable dose level with and without S9 ($p < 0.05$). These increases were dose-dependent, and they also exceeded the 95% confidence limit for the historical vehicle control data (see Table 8 and Table 9).
- Concurrent negative (solvent/vehicle) and positive control data:
  - Untreated negative controls: no
  - Negative solvent/vehicle controls: yes, valid
  - True negative controls: no
- Positive control: yes (benzo(a)pyrene, ethylmethanesulphonate), valid
- Indicate test-specific confounding factors:
  - Effects of pH: the test substance did not have an adverse impact on the pH
  - Effects of osmolality: the test substance did not have an adverse impact on the osmolality
  - Water solubility: highest soluble or workable stock concentration was 50 mg/mL for aqueous solvents
  - Precipitation: no precipitation at the beginning or at the end of treatment
- Statistical results:
  - Significant increases in mutant frequency, as compared to the concurrent vehicle controls, at highest acceptable dose level with and without S9 ($p < 0.05$).
  - Increases dose-dependent
  - Increases exceeded the 95% confidence limit for the historical vehicle control data.

- Provide information that may be needed to adequately assess data for reliability
  - frequency of mutations: see Table 8 and Table 9
  - mean number of revertant colonies per plate: numerical values provided in confidential study report
  - evaluation criteria:
    - average absolute cloning efficiency of the vehicle control must be >50% (at initial survival and selection)
    - spontaneous mutant frequency in the vehicle control must fall within the range of 0-25 mutants per 106 clonable cells
    - The positive controls must induce a mutant frequency at least three times that of the solvent control and must exceed 40 mutants per 106 clonable cells
    - At least four analyzable concentrations with mutant frequency data required and their mutant frequencies reported.
    - The highest test substance concentration must produce 80 to 90% toxicity (OECD, 1997) unless limited by solubility of the maximum required concentration

Table 8: Results of the in vitro Mammalian Cell Forward Gene Mutation (CHO/HPRT) Assay (without metabolic activation)

Definitive Mutagenicity Assay					
Metabolic Activation and Exposure Time	**Test or Control Substance**	**Concentration (µg/mL)**	**Mutant Frequency (x10^6)**	**Cloning Efficiency (%; Initial Survival)**	**Relative Survival (%)**
Without Activation 5 hours (±0.5 hours)	DMSO	10.0[a]	7.92	57.50	84.25
		10.0[a]	0.00	79.00	115.75
	TK30174	5.00	2.49	73.50	107.69
		5.00	13.27	66.00	96.70
		10.0	1.49	69.50	101.83
		10.0	5.44	65.75	96.34
		20.0	5.13	61.17	89.62
		20.0	5.00	51.17	74.97
		25.0	nd	48.50	71.06
		25.0	nd	41.33	60.56
		30.0	19.17	38.33	56.17
		30.0	3.30	32.67	47.86
		35.0	nd	20.00	29.30
		35.0	nd	16.67	24.42
		40.0	22.57*	9.33	13.68
		40.0	20.62*	12.33	18.07
		50.0	nd	1.67	2.44
		50.0	nd	2.17	3.17
	EMS	0.200[a]	260.14**	56.33	82.54
		0.200[a]	248.06**	58.67	85.96

[a] µL/mL

nd – Not determined; discarded prior to selection because a sufficient number of higher concentrations was available, or due to excessive toxicity

*, ** Significant increase (p< 0.05 or 0.01, respectively)

Table 9: Results of the in vitro Mammalian Cell Forward Gene Mutation (CHO/HPRT) Assay (with metabolic activation)

Definitive Mutagenicity Assay					
Metabolic Activation and Exposure Time	**Test or Control Substance**	**Concentration (µg/mL)**	**Mutant Frequency (x10^6)**	**Cloning Efficiency (%; Initial Survival)**	**Relative Survival (%)**
With Activation 5 hours (±0.5 hours)	DMSO	10.0[a]	1.22	85.00	91.23
		10.0[a]	2.52	101.33	108.77
	TK30174	50.0	nd	77.00	82.65
		50.0	nd	84.33	90.52
		100	6.74	120.17	128.98
		100	4.71	91.33	98.03
		200	0.00	77.00	82.65
		200	22.32	77.00	82.65
		300	18.06	42.67	45.80
		300	10.80	39.33	42.22
		350	29.27*	19.67	21.11
		350	25.35*	28.25	30.32
		400	0.00[b]	6.33	6.80
		400	0.00[b]	6.67	7.16
		450	nd	1.50	1.61
		450	nd	2.50	2.68
		500	nd	0.33	0.36
		500	nd	1.67	1.79
	B(a)P	4.00	224.57**	28.00	30.05
		4.00	231.15**	31.67	33.99

[a] µL/mL

[b] Excluded from evaluation of mutagenicity due to excessive toxicity

nd – Not determined; discarded prior to selection because a sufficient number of higher concentrations was available, or due to excessive toxicity

*, ** Significant increase (p< 0.05 or 0.01, respectively)

4.8.2 Animal data

4.8.2.1 *In vivo* Mammalian Alkaline Comet Assay

Study reference:

N.N. study report, 2017

Detailed study summary and results:

Test type

In vivo Mammalian Alkaline Comet Assay according to OECD TG 489 (Guideline adopted 26 September 2014) with minor deviations: In the Dose Range Finding Assay, the interval between first and second dosing

was ca. 24-25 hours instead of 21 hours; during electrophoresis the buffer temperature at the end of electrophoresis was 11.2°C, which is outside the protocol range of 2 to 10°C; both deviations were not regarded to impact the study validity. GLP compliance given (incl. certificate).
A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier: Reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis((2,3-epoxypropoxy)methyl)butane and 1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane; named in the ESR as 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, oligomeric reaction product with (chloromethyl)oxirane also named TK30174
- Test material form: Liquid
- Degree of purity: 100% (provided by Sponsor)
- Impurities: not specified
- Batch number: available in confidential study report

Test animals

- Species: Sprague-Dawley rat (Hsd:SD), male and female (DRF); male (definite study)
- No. of animals per sex per dose:
  - Dose Range Finding: 3 animals/sex exposed to 2000, 1000 and 500 mg/kg/day
  - Comet Assay: 6 animals exposed to 0, 500, 1000 and 2000 mg/kg/day
- Age at time of study initiation:
  - Dose Range Finding:
    - Male and female: 6 weeks; days of acclimation: 6
  - Comet Assay: Male: 6 weeks; days of acclimation: 7
- Weight at study initiation:
  - Dose Range Finding:
    - Male: 167.2 - 181.4 g
    - Female: 133.7 - 151.7 g
  - Definitive Test: Male: 169.6 - 187.5 g

Administration/exposure

- Route of administration – oral (gavage)
- All dose formulations were administered at a volume of 10 mL/kg/day using disposable polypropylene syringes with gastric intubation tubes (needles)

- Doses/concentration levels: 500, 1000 and 2000 mg/kg bw/day in Dose Range Finder and Comet Assay; 2000 mg/kg bw/day highest guideline recommended dose for this assay, estimated to be the maximum tolerated dose
- Rationale for dose level selection: not provided
- Analytical verification of day 1 samples
- Duration and frequency of test/exposure period: Two dose administrations (Days 1 and 2); second dose ~ 21 hours after the first dose
- Control group and treatment:
  - Negative control: yes, concurrent vehicle
  - Positive control:
    - ethyl methanesulfonate (EMS); neat EMS prepared in 0.9% saline;
    - dosing concentration: 20 mg/mL
    - dose level: 200 mg/kg/day
    - dose volume: 10 mL/kg/day
    - 3 animals used; animals dosed approximately 3 to 4 hours prior to euthanasia on day 2
- Vehicle: Polyethyene glycol 400 (PEG 400); 10 mL/kg/d, no justification for vehicle selection
- Methods of slides preparation:
  - Organ preparation:
    - animals euthanized by CO_2 asphyxiation 3 to 4 hours after the last dose at day 2
    - animals dissected; liver, stomach and duodenum collected
    - section of liver, stomach and duodenum were placed in formalin for possible histopathology analysis
    - for preparation of cell suspensions and Comet slides: another section of liver, glandular stomach and duodenum were placed in chilled mincing solution (Hank's balanced salt solution with EDTA and DMSO)
  - Preparation of cell suspensions and Comet Slides:
    - Cell suspensions of each organ were prepared; aliquots of suspensions were applied to microscope slides; cells were lysed and DNA unwinded via addition of alkaline buffer; electrophoresis was conducted 30 minutes at 0.7 V/cm protected from light; staining with DNA stain (Sybr-gold)
- Number of cells analysed per animal:
  - 3 slides/wells per organ/animal

- 50 randomly selected, non-overlapping cells per slide/well scored (= 150 cells evaluated per animal) with the fully validated automated scoring system Comet Assay IV from Perceptive Instruments Ltd. (UK)

- Evaluation creteria:
  - Comet Tail Migration; defined as the distance from the perimeter of the Comet head to the last visible point in the tail
  - % Tail DNA (also known as % tail intensity or % DNA in tail); defined as the percentage of DNA fragments present in the tail.
  - Tail Moment (also known as Olive Tail moment); defined as the product of the amount of DNA in the tail and the tail length [(% Tail DNA x Tail Length)/ 100; Olive et al. 1990)].
  - Indications for cytotoxicity:
    - "Clouds", also known as "hedgehogs", are a morphological indication of highly damaged cells often associated with severe genotoxicity, necrosis or apoptosis. A "cloud" is produced when almost the entire cell DNA is in the tail of the comet and the head is reduced in size, almost nonexistent (Collins et. al., 2004). "Clouds" with visible gaps between the nuclei and the comet tail were excluded from comet image analysis.
    - Scanning of 150 cells per animal for rough estimate of the percentage of "clouds" (percentage of "clouds" was calculated by adding the total number of clouds for all slides scored, dividing by the total number of cells scored and multiplying by 100).

- Statistical methods:
  - Median value of 150 counts of % Tail DNA, Tail moment and Tail migration for each animal in each treatment group for each organ.
  - Mean and standard deviation of the median values only for % Tail DNA.
  - Statistical analysis was performed only for % Tail DNA.
  - Statistics for quantification of test substance effects on DNA damage:
    1. Levene's test (significant level of $p \leq 0.05$) for group variances for % tail DNA
    2. If differences and variations between groups were not significant, a parametric one-way ANOVA followed by a Dunnett's post-hoc test was performed (significant level of $p < 0.05$).
    3. If Levene's test indicated heterogeneous group variances ($p \leq 0.05$), the suitability of a transformation of the original data was evaluated (e.g. using logarithm transformed values of the original data) in an attempt to meet the normality criteria.
    4. Afterwards, parametric tests as described above. If parametric tests were not acceptable, non-parametric statistical methods, (Kruskal Wallis and/or Mann Whitney test) may have been used in evaluation of data.

- Statistics for dose responsiveness in test substance trated groups: linear regression analysis ($p \leq 0.01$).
- Comparison of positive control group and concurrent vehicle control group: pair-wise comparison (Student's T-test, $p \leq 0.05$); if needed, non-parametric statistical methods (Kruskal Wallis and/or Mann Whitney test) were used in evaluation of data.

Results and discussion

- Toxicitiy: no effects
- Genotoxic effects: positive
  - Liver samples: positive; dose responsive, statistically significant increase in % tail DNA in the 1000 and 2000 mg/kg/day dose; the 2000 mg/kg/day increase was outside current historical control and 95% confidence range
  - Duodenal samples: positive; dose responsive, statistically significant increase in % tail DNA in the 2000 mg/kg/day dose
  - Stomach samples: negative; no increases in % tail DNA in the stomach samples
  - The vehicle and positive control % tail DNA values were within expected ranges for all three organs. All valid assay criteria were met.
- Vehicle control: yes; Polyethylene glycol (PEG 400); valid
- Positive control: yes; ethyl methane sulfonate (EMS); valid
- Statistical results:

Treatment (10 mL/kg/day)	**Dose (mg/kg/day)**	**N° of males**	**% Tail DNA (a) in Liver Cells (Mean ± SD)**	**% Tail DNA (a) in Duodenal Cells (Mean ± SD)**	**% Tail DNA (a) in Stomach Cells (Mean ± SD)**
Vehicle (b)	0	5	0.58 ± 0.31	6.95 ± 4.04	15.63 ± 4.53
Test substance	500	6	0.54 ± 0.31 @	7.84 ± 3.61@	17.91 ± 4.70
Test substance	1000	6	2.75± 1.47 @#	8.52 ± 4.5@	18.55 ± 5.36
Test substance	2000	6	7.55 ± 6.44 @#	20.62 ± 8.61@#	21.54 ± 6.75
Positive Control (c)	200 mg/kg	3	17.62 ± 3.09*	16.78 ± 2.81*	35.31 ± 5.21 §

(a) Mean of 3,5 or 6 animal means of medians

(b) Polyethylene glycol 400 (PEG 400)

(c) Ethyl methanesulfonate (EMS), was only administered once on Study day 2 at 3 to 4 hours prior to organ collection SD = Standard Deviation

* $p \leq 0.05$ (Student's t-test); Statistically significant increase relative to the vehicle control

@ $p \leq 0.01$ (regression analysis): Statistically significant relative to the vehicle control.

$p \leq 0.05$ (ANOVA, Dunnett's post hoc); Statistically significant increase relative to the vehicle control.

§ $p \leq 0.05$ (Mann-Whitney test); Statistically significant increase relative to the vehicle control.

- 'Clouds':
  - In test substance groups ≤ 2.8%
  - In vehicle control group = 4.0%
- Levene's test for group variances for mean of medians of the % Tail DNA in the vehicle and test substance groups: significant difference in the group variance ($p < 0.05$); therefore, the suitability of a transformation of the original data was evaluated (e.g. using logarithm transformed values of the original data) in an attempt to meet the normality criteria. Afterwards, the parametric approach, ANOVA followed by Dunnett's post-hoc analysis, was used in the statistical analysis of data.
- Statistics for DNA damage: Statistically significant responses in the % Tail DNA (DNA damage) was observed in the 1000 and 2000 mg/kg/day test substance groups relative to the concurrent vehicle control group (ANOVA followed by Dunnett's post-hoc analysis, $p < 0.05$)
- Regression analysis: dose-dependent increase in the % Tail DNA across three test substance doses ($p < 0.01$)
- The positive control, EMS, induced a statistically significant increase in the % Tail DNA in liver cells as compared to the vehicle control groups (Student's t-test, $p \leq 0.05$)

- Additional information that may be needed to adequately assess data for reliability and use:
  - Clinical signs:
    - in dose range finder:
      - At 500 mg/kg/day: Piloerection in males and females
      - At 1000 mg/kg/day: Piloerection in males and females, rusty nose in males
      - At 2000 mg/kg /day: Piloerection in males and females
    - Comet assay:
      - PEG 400 (vehicle): Normal
      - At 500 mg/kg/day: Normal
      - At 1000 mg/kg/day: Piloerection, Diarrhea
      - At 2000 mg/kg/day: Lethargy, Piloerection, Crusty nose, Diarrhea
      - Positive Control: EMS (200 mg/kg): Normal
  - Mortality:
    - No mortality or significant clinical observations during the dose range finding assay, the maximum tolerated dose for the definitive comet assay was set at 2000 mg/kg

- The high dose for the Comet assay was the limit dose of 2000 mg/kg/day as determined in the DRF assay
- Mortality in the vehicle control (PEG 400): 1/6 male died
- No mortality at any test article dose levels or in the positive control group during the course of the definitive assay
- No mortality or significant differences in clinical observations between the sexes; therefore only males were used in the definitive assay

– Body weight:

- No significant bodyweight loss observed in dose range finder
- Appreciable reductions in mean group body weights in the high test substance treated group during the course of the study

– Dosing formulation analysis:

- Dosing formulations were analyzed by the analytical laboratory at BioReliance using the validated method AD90MA.GTCHEM.BTL
- Actual mean concentrations of the analyzed formulation samples between 98.2 and 134.0% of their respective target concentrations with S/L ratios of > 0.925. The 200 mg/mL formulation above the acceptable range for concentration (85.0 to 115.0% of target) but met the S/L ratio of > 0.925. This indicates that the formulations were accurately prepared, except the above.
- No test substance detected in the vehicle control sample
- Additionally, TK30174 in PEG 400, at concentration of 49.1 mg/mL, was stable at room temperature for at least 5.17 hours. Also, TK30174 in PEG 400, at concentration of 199 mg/mL, was stable at room temperature for at least 4.10 hours. TK30174 in PEG 400, at a concentration of 196 mg/mL, was stable at room temperature for at least 5.04 hours.

– Histopathology Analysis

- Based on the histopathological evaluation of liver and duodenum, no evidence of test article induced toxicity

4.8.3 Human data

No studies available.

4.8.4 Other data

No studies available.

4.9 Carcinogenicity

No studies available.

4.9.1 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

No studies available.

4.9.2 Other data (e.g. studies on mechanism of action)

No studies available.

4.10 Reproductive toxicity

4.10.1 Animal data

4.10.1.1 Combined Repeat Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test

Study reference:

N.N., study report, 2015

Detailed study summary and results:

Test type

A combined repeated dose toxicity study with the reproduction/developmental toxicity screening test according to OECD TG 422 was performed. A derivation was that some procedures were performed on day 25 or 26 post coitum rather than post partum, because the group failed to achieve pregnancy. GLP compliance is given (incl. certificate).

A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to substance identified in the CLH dossier: Reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis((2,3-epoxypropoxy)methyl)butane and 1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane; named in the ESR as 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, oligomeric reaction product with (chloromethyl)oxirane
- Concentration of constituents: 58% C15H26O6 and 25% C12H22O5
- Impurities: not specified
- Batch number: available in confidential study report

- Physical state/appearance: clear colourless liquid
- Storage conditions: ambient 10 to 30 °C in the dark

Test animals

- Rat/Wistar/male and female
- No. of animals per sex per dose: 12 per sex and dose (in total 48 males and 49 females)
- Age at the study initiation: approx. 12 weeks old
- Weight at the study initiation: males: 296 - 346 g; females: 196 - 229 g
- Acclimatisation for 6 days, during this period rats were housed in groups of three

Administration/exposure

- Route of administration – oral (gavage), by using a stainless steel cannula attached to a disposable plastic syringe
- Duration and frequency of test/exposure period:
  - up to 56 days, daily during two week pre-pairing phase, pairing, (and for females) during gestation and early lactation
  - on day 43 or day 44 surviving adult males were terminated
  - on day 5 post partum termination of all females and offspring
  - on or after day 25 post coitum all females (non-pregnant) of the highest dose group were terminated
- Doses/concentration levels, rationale for dose level selection:
  - 0, 30, 100, 300 mg/kg bw/day, actual ingested
  - volume of test and control item administered to each animal was based on the most recent scheduled body weight and was adjusted at weekly intervals
  - random allocation of animals to treatment groups was done by using a stratified body weight randomization procedure and the group mean body weights were then determined to ensure similarity between the treatment groups
- Control group and treatment: yes, concurrent vehicle
- Historical control data: yes
- Vehicle:
  - polyethylene glycol 400, 4 mL/kg for control animals
  - analytical verification confirmed the accurate use of the test item and PEG 400 as vehicle
  - justification of choice of vehicle is not provided
- Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:

- test item was prepared at the appropriate concentrations in polyethylene glycol 400
- test item formulations were stable for at least seventeen days in the dark at approximately 4°C, thus formulations were prepared weekly and stored at approx. 4 °C in the dark
- analytical verification of doses or concentrations: samples of test item formulations were taken and analysed by high performance liquid chromatography with UV detection (HPLC / UV) for concentration of 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, oligomeric reaction product with (chloromethyl) oxirane (CAS: 30499-70-8); results indicate that the prepared formulations were within 94% to 107% of the nominal concentration confirming the accuracy and suitability of the formulation procedure

Description of test design:

- Details on mating procedure:
  - M/F ratios per cage: 1:1
  - length of cohabitation: started on day 15 (day 16 for male 25 and female 37) for a maximum of 14 days
  - proof of pregnancy: presence of sperm within the vaginal smear and/or vaginal plug in situ designated as day 0 of post coitum, then males were returned to their original cages and females were transferred to individual cages
- Premating exposure period for males and females: two weeks
- Dosing schedules and pre and post dosing observation periods for P, F1:
  - twelve animals per dose and sex were treated daily throughout the study (except for females during parturition where applicable) and the first day of dosing was designated as day 1 of the study
  - prior to the start of treatment and once weekly thereafter, all animals were observed for signs of functional/behavioural toxicity
  - on day 15 (day 16 for male 25 and female 37), animals were paired on a male:female ratio of 1:1 within each dose group for a maximum of fourteen days
  - following evidence of mating (designated as day 0 post coitum) males were returned to their original cages and females were housed individually
  - on completion of the pairing phase (during Week 6), five selected males per dose group were evaluated for functional/sensory responses to various stimuli
  - pregnant females were allowed to give birth and maintain their offspring until day 5 post partum; litter size, offspring weight and sex, surface righting and clinical signs were also recorded during this period

- at day 4 post partum, five selected females per dose group were evaluated for functional/sensory responses to various stimuli; as no littering females were available at 300 mg/kg bw/day, females at this dosage were assessed on day 25 post coitum
- blood samples were taken from five males from each dose group for haematological and blood chemical assessments on day 42; male dose groups were killed and examined macroscopically on day 43 or day 44
- blood samples were taken from five randomly selected females from each dose group for haematological and blood chemical assessment on day 4 post partum; as no littering females were available at 300 mg/kg bw/day, females at this dosage were assessed on day 25 post coitum; at day 5 post partum, all females and surviving offspring were killed and examined macroscopically; females at 300 mg/kg bw/day were terminated on day 26 post coitum

- Standardization of litters: no
- Parameters assessed for P:
  - Clinical observations
    - all animals were examined for overt signs of toxicity, ill-health and behavioural change immediately before dosing, soon after dosing, and approx. one hour after dosing; all observations were recorded
  - Functional observations
    - all animals were observed for signs of functional/behavioural toxicity prior to the start of treatment and at weekly intervals thereafter; functional performance tests were also performed on five selected males and females from each dose level prior to termination
  - Behavioural assessments
    - detailed individual clinical observations were performed for each animal using a purpose built arena and this test was developed from the methods used by Irwin (1968) and Moser et al (1988) the following parameters were assessed: gait, hyper/hypothermia, tremors, skin colour, twitches, respiration, convulsions, palpebral closure, bizarre/abnormal/stereotypic behaviour, urination, salivation, defecation, piloerection, transfer arousal, exophthalmia, tail elevation, and lachrymation
  - Functional performance tests
    - Motor activity: purpose-built 44 infrared beam automated activity monitors were used to assess motor activity; random allocation of animals to the activity monitors; tests were performed at approx. the same time (at least two hours after dosing) on each occasion under similar laboratory conditions; evaluation period was thirty minutes for each animal; percentage of time each animal was active and mobile was

recorded for the overall thirty minute period and also during the final 20% of the period

- Fore limb/Hind limb grip strength: for the measurement an automated meter was used; each animal was allowed to grip the proximal metal bar of the meter with its forepaws and then was pulled by the base of the tail until its grip was broken; afterwards each animal was drawn along the trough of the meter by the tail until its hind paws gripped the distal metal bar and then was pulled by the base of the tail until its grip was broken; the force required to break the grip for each animal was recorded; three consecutive trials were performed for each animal;
- Sensory reactivity: each animal was individually assessed for sensory reactivity to auditory, visual and proprioceptive stimuli. The following parameters were assessed: grasp response, touch escape, vocalization, pupil reflex, toe pinch, blink reflex, tail pinch, startle reflex, and finger approach

– Body weight
  - individual body weights were recorded on day 1 (prior to dosing) and then weekly for males until termination and weekly for females until pairing; during pairing period females were weighed daily until mating was confirmed and afterwards body weights were recorded for females on days 0, 7, 14 and 20 post coitum, on days 1 and 4 post partum and at terminal kill

– Food consumption
  - during the pre-pairing period, weekly food consumption was recorded for each cage of adults; this was continued for males after the mating phase; for females showing evidence of mating, food consumption was recorded for the periods covering post coitum days 0-7, 7-14 and 14-20; for females with live litters, food consumption was recorded on days 1 and 4 post partum
  - one female (37R) at 30 mg/kg bw/day was killed for animal welfare considerations on day 2 after showing a dark, swollen right eye (not treatment-related); the replacement female was placed in the same cage as the original female; as the cage always contained the correct number of animals it was considered acceptable to continue with the food consumption for this cage and use it in the assessment of effects during the first week of the study although the initial food intake would have included a contribution from the excluded animal
  - food efficiency (the ratio of body weight change/dietary intake) was calculated retrospectively for males throughout the study period (with the exception of the mating phase) and for females during the pre-pairing phase; during gestation and

lactation food efficiency could not be accurately calculated for females due to offspring growth and milk production

- Water consumption
  - water intake was observed daily by visual inspection of water bottles for any overt changes
- Reproductive performance
  - mating
    - each morning cage tray-liners were checked for the presence of ejected copulation plugs and each female was examined for the presence of a copulation plug in the vagina; vaginal smear was prepared for each female and the stage of oestrus or the presence of sperm was recorded; presence of sperm within the vaginal smear and/or vaginal plug in situ was taken as positive evidence of mating (day 0 of gestation) and the males were subsequently returned to their original holding cages (unless required for additional pairing); mated females were housed individually during the period of gestation and lactation
- Reproductive indices
  - pre-coital interval and gestation length were calculated
  - fertility indices (mating index, pregnancy index) and parturition index were determined
- Pregnancy and parturition
  - each pregnant female was observed at least three times a day (early morning, mid-day and as late as possible during the normal working day) around the period of expected parturition; observations were carried out at approx. 0830 and as late as possible at weekends and public holidays (no further details provided) and for each female was recorded the date of pairing, date of mating, date and time of observed start of parturition, and date and time of observed completion of parturition
- Haematology
  - following parameters were measured on blood collected into tubes containing potassium EDTA anti-coagulant: haemoglobin (Hb), erythrocyte count (RBC), haematocrit (Hct), erythrocyte indices (mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC)) Total leukocyte count (WBC), differential leukocyte count (neutrophils (Neut), lymphocytes (Lymph), monocytes (Mono), eosinophils (Eos), basophils (Bas), platelet count (PLT), reticulocyte count (Retic), methylene blue stained slides were prepared but reticulocytes were not assessed)

- prothrombin time (CT) was assessed by 'Innovin' and activated partial thromboplastin time (APTT) was assessed by 'Actin FS' using samples collected into sodium citrate solution (0.11 mol/L)

– Blood chemistry
  - following parameters were measured on plasma from blood collected into tubes containing lithium heparin anti-coagulant: urea, calcium (Ca^{2+}), glucose, inorganic phosphorus (P), total protein (Tot. Prot.), aspartate aminotransferase (ASAT), albumin, alanine aminotransferase (ALAT), albumin/globulin (A/G) ratio (by calculation), alkaline phosphatase (AP), sodium (Na^{+}), creatinine (Creat), potassium (K^{+}), total cholesterol (Chol), chloride (Cl^{-}), total bilirubin (Bili), bile acids

– Necropsy
  - sacrifice by intravenous overdose of suitable barbiturate agent followed by exsanguination of adult males on day 43 or day 44 and of adult females on day 5 post partum and day 25 post coitum for females at 300 mg/kg bw/day; surviving offspring were terminated via intracardiac overdose of suitable barbiturate agent
  - uterus was examined for signs of implantation and number of uterine implantations in each horn was recorded; this procedure was enhanced (as necessary) by staining the uteri with a 0.5% ammonium polysulphide solution and where possible the corpora lutea were also counted
  - all adult animals (including those dying during the study) were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded

– Organ weights
  - following organs were dissected free from fat and weighed before fixation from five selected males and five selected females from each dose group: adrenals, prostate, brain, seminal vesicles, epididymides, spleen, heart, testes, kidneys, thymus, liver, thyroid (weighed post-fixation with parathyroid), ovaries, uterus (weighed with cervix), pituitary (post fixation)
  - tissues were weighed from all remaining animals: prostate, seminal vesicles, epididymides, testes, ovaries, uterus (weighed with cervix), pituitary (post fixation)
  - as all females were non-pregnant at 300 mg/kg bw/day, normal range data based on 90-d toxicity studies are also present for female (non-pregnant) animals

– Histopathology
  - from five selected males and five selected females from each dose group samples of the following tissues were removed and preserved in buffered 10% formalin, except where stated: adrenals, muscle (skeletal), aorta (thoracic), ovaries, bone and bone marrow (femur including stifle joint), pancreas, bone and bone marrow (sternum),

pituitary, brain (including cerebrum, cerebellum and pons), prostate, caecum rectum, coagulating gland, salivary glands (submaxillary), colon, sciatic nerve, duodenum, seminal vesicles, epididymides•, skin (hind limb), oesophagus, spinal cord (cervical, mid-thoracic and lumbar), eyes*, gross lesions, spleen, heart, stomach, ileum (including peyer's patches), thyroid/parathyroid, jejunum, trachea, kidneys, testes•, liver, thymus, lungs (with bronchi)#, urinary bladder, lymph nodes (mandibular and mesenteric), uterus/cervix, mammary gland, vagina

* = eyes fixed in Davidson's fluid

• = preserved in Modified Davidsons fluid

= lungs were inflated to approximately normal inspiratory volume with buffered 10% formalin before immersion in fixative

- the following tissues were preserved from all remaining animals: coagulating gland, epididymides•, gross lesions, mammary gland, ovaries, pituitary, prostate, seminal vesicles, testes•, uterus and cervix, vagina

  • = preserved in Modified Davidsons fluid

- tissues were dispatched to the Test Site (Propath UK Ltd, Willow Court, Netherwood Road, Rotherwas, Hereford, HR2 6JU) for processing (Principal Investigator: N Fower); tissues from five selected control and 300 mg/kg bw/day dose group animals, were prepared as paraffin blocks, sectioned at a nominal thickness of 5 μm and stained with haematoxylin and eosin for subsequent microscopic examination; tissues shown in below from the remaining control and 300 mg/kg bw/day animals and animals which did not achieve a pregnancy were also processed; female 37R was excluded from histopathological processing and investigation: ovaries, pituitary, prostate, coagulating gland, seminal vesicles, epididymides, gross lesions, testes, uterus/cervix, vagina, mammary gland
- sections of testes from all control and 300 mg/kg bw/day males were also stained with Periodic Acid-Schiff (PAS) stain and examined
- detailed qualitative examination of the testes was undertaken, taking into account the tubular stages of the spermatogenic cycle; examination was conducted in order to identify treatment-related effects such as missing germ cell layers or types, retained spermatids, multinucleated or apoptotic germ cells and sloughing of spermatogenic cells into the lumen and any cell-or stage-specificity of testicular findings was noted
- microscopic examination was conducted by the Study Pathologist (Jeffrey Wilson at Propath GmbH, Muttenzerstrasse 30, 4133 Pratteln, Switzerland); a peer review of findings was conducted by the test facility

- Oestrous cycle length and pattern: no data
- Sperm examination: no data

- Parameters assessed for F1:
  - on completion of parturition (day 0 post partum) number of live and dead offspring was recorded; offspring were individually identified within each litter by tattoo on day 1 post partum
  - Litter data: for each litter the following was recorded:
    - number of offspring born
    - number of offspring alive recorded daily and reported on days 1 and 4 post partum
    - sex of offspring on days 1 and 4 post partum
    - clinical condition of offspring from birth to day 5 post partum
    - individual offspring weights on days 1 and 4 post partum (litter weights were calculated retrospectively from this data)
  - Physical development
    - all live offspring were assessed for surface righting reflex on day 1 post partum
  - Laboratory investigations
    - haematological and blood chemical investigations were performed on five males and five females selected from each test and control group prior to termination (day 42 for males and day 4 post partum for females, day 25 post coitum for females receiving 300 mg/kg bw/day); animals were not fasted prior to sampling; blood samples were obtained from the lateral tail vein; if necessary repeat samples were taken by cardiac puncture at termination
  - Necropsy:
    - all offspring (including those dying during the study) were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded
  - Litter indices determined:
    - implantation losses (pre- and post-implantation loss), live birth and viability indices (live birth index, viability index) and sex ratio

Results and discussion

- Actual dose received: 0, 30, 100, 300 mg/kg bw/day, actual ingested for both sexes
- Statistical treatment of results:
  - where considered appropriate, quantitative data was subjected to statistical analysis to detect the significance of intergroup differences from control; statistical significance was achieved at $p<0.05$
  - statistical analysis was performed for the following parameters: grip strength, motor activity, body weight during gestation and lactation, body weight change, food consumption during

gestation and lactation, pre-coital interval, gestation length, litter size, litter weight, sex ratio, corpora lutea, implantation sites, implantation losses, viability indices, offspring body weight, offspring body weight change, offspring surface righting, hematology, blood chemistry, absolute organ weights, body weight-relative organ weights

- data were analysed using the decision tree from the Provantis™ Tables and Statistics Module as detailed as follows:
  - where appropriate, data transformations were performed using the most suitable method. The homogeneity of variance from mean values was analyzed using Bartlett's test. Intergroup variances were assessed using suitable ANOVA, or if required, ANCOVA with appropriate covariates. Any transformed data were analyzed to find the lowest treatment level that showed a significant effect using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response was found but the data shows non-homogeneity of means, the data were analyzed by a stepwise Dunnett's (parametric) or Steel (non-parametric) test to determine significant difference from the control group. Where the data were unsuitable for these analyses, pair-wise tests was performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric).
  - data not analyzed by the Provantis™ data capture system were assessed separately using the R Environment for Statistical Computing. Initially, the distribution of the data was assessed by the Shapiro-Wilk normality test, followed by assessment of the homogeneity of the data using Bartlett's test. Where considered appropriate, parametric analysis of the data was applied incorporating analysis of variance (ANOVA), which if significant, was followed by pair-wise comparisons using Dunnett's test. Where parametric analysis of the data was considered to be unsuitable, non-parametric analysis of the data was performed incorporating the Kruskal-Wallis test which if significant was followed by the Mann-Whitney "U" test.
  - Due to the preponderance of non-normally distributed data, reproductive parameters (implantation losses, offspring sex ratio and offspring surface righting) were analyzed using non-parametric analyses.
- probability values (p) are presented as follows: $p<0.01$ **, $p<0.05$ *, $p>0.05$ (not significant)

- Mortality
  - no unscheduled or treatment-related deaths were observed
  - one female (37R) at 30 mg/kg bw/day was killed for animal welfare considerations on day 2 after showing a dark, swollen eye; this death was clearly unrelated to treatment and in view

of its the close proximity to the start of the stud, this female was replaced and excluded from the assessment of toxicity for this study

- Clinical observations
  - only increased post-dosing salivation for all males and to a lesser extent the majority of females at 300 mg/kg bw/day was noted;
  - at 100 mg/kg bw/day one male showed incidences of piloerection on two separate occasions during the study, no similar observations were made in animals of the other dose groups
  - for both sexes at 30 mg/kg bw/day or for females at 100 mg/kg bw/day no clinical signs were observed
- Functional observations
  - no abnormal observations were apparent during behavioural assessments and there was no consistent pattern in intergroup differences for behavioural assessment scores that indicated any effect of treatment
- Functional performance tests
  - no changes in functional performance that were considered to be treatment-related
  - for females at 30 mg/kg bw/day lower mean values for the last 20% mobile activity attained statistical significance when compared to controls; no similar effects observed at higher dosages
  - sensory reactivity assessments: no treatment-related intergroup differences were noted in sensory reactivity scores for either sex at 30, 100 or 300 mg/kg bw/day
- Body weight
  - body weight gain of males was unaffected by treatment throughout the study at 30, 100 and 300 mg/kg bw/day
  - at 300 mg/kg bw/day, body weight gain of males was slightly lower than control during the final week of the study (9.2, 3.2, 6.7, 2.0 g in the control, low, mid and high dose group, respectively)with differences attaining statistical significance; group mean body weights are probably influenced by the laboratory investigations being performed during this period; body weight gains prior to this period, were essentially similar to control
  - body weight gain of females was considered to be unaffected by treatment during the two week pre-pairing period at 30, 100 and 300 mg/kg bw/day; body weight gain of females during pregnancy and lactation was considered to be unaffected by treatment at 30 and 100 mg/kg bw/day
  - body weight gain during pregnancy and lactation could not be assessed at 300 mg/kg bw/day as no females at this dosage achieved pregnancy
- Food consumption

- at 300 mg/kg bw/day food consumption for males was lower than control during the first week of treatment; afterwards food consumption was similar to control and was unaffected by treatment
- no obvious effect on food consumption was seen for males at 30 and 100 mg/kg bw/day
- food consumption of females was considered to be unaffected by treatment during the two week pre-pairing period at any dose level; food consumption of females during pregnancy and lactation was considered to be unaffected by treatment at 30 and 100 mg/kg bw/day
- food consumption during pregnancy and lactation could not be assessed at 300 mg/kg bw/day as no females at this dosage achieved pregnancy
- food conversion efficiency for both sexes during the pre-pairing phase and males during the post-mating phase of the study was considered to be unaffected at any dose level

- Water consumption
  - visual assessment of water consumption during the study did not indicate any obvious effect of treatment on water intake for either sex at 30, 100 or 300 mg/kg bw/day
- Reproductive performance, see Table 10
  - mating and fertility
    - no treatment-related effects on mating performance as assessed by pre-coital interval, with all treated animals mating within the first four days of pairing (i.e. at the first oestrus opportunity); however at 300 mg/kg bw/day none of the matings resulted in a pregnancy
- Gestation length, see Table 10
  - a tendency for gestation length to be longer than in control for females receiving 30 or 100 mg/kg bw/day with differences attaining statistical significance; however all gestation lengths were within the normally expected range of the historical control of this laboratory
- Laboratory investigations
  - females at 300 mg/kg bw/day were non-pregnant and thus were in a different physiological state to the remaining females on the study
- Haematology
  - intergroup differences for a number of haematology parameters for females at 30 and 100 mg/kg bw/day and both sexes at 300 mg/kg bw/day attained statistical significance when compared with control but none of these findings were supported by any histopathological change and, at the levels observed, these were considered not to indicate any adverse effect of treatment

- in males at 300 mg/kg bw/day higher erythrocyte counts and haemoglobin levels attained statistical significance when compared with control but all individual values for these treated animals were within the historical control range
- for females at 300 mg/kg bw/day higher erythrocyte count, haemoglobin and haematocrit level, lower mean cell haemoglobin and mean cell volume attained statistical significance when compared with control; except for one value for erythrocyte count and haemoglobin, values for these treated animals were within the historical control range; for control females, one value for erythrocyte count, mean cell haemoglobin and mean cell volume were outside this historical range
- for females at 30 and 100 mg/kg bw/day a statistically significantly lower mean cell volume compared with control was observed but all individual values were within the historical control range
- for females at all dosages, total leukocyte count and the number of neutrophils were statistically significantly lower than control; all values for these treated animals were within the historical control range, while one control value for total leukocyte count and two control values for neutrophils were outside this historical range
- at 300 mg/kg bw/day, higher numbers of lymphocytes attained statistical significance when compared with control but only one value for these treated animals exceeded the historical control range
- for females at 100 and 300 mg/kg bw/day lower platelet counts attained statistical significance compared to control; all individual values for these treated animals were within the historical control range but one control value exceeded this historical range
- for females at 30 mg/kg bw/day, there was a statistically significant increase in prothrombin time compared to control; there was no similar increase at higher dosages and all individual values for these treated animals were within the historical control range

- Blood chemistry
  - intergroup differences for a number of blood chemistry parameters for females at 30 and 100 mg/kg bw/day and both sexes at 300 mg/kg bw/day attained statistical significance when compared with control but none of these findings were supported by any histopathological change
  - for males at 300 mg/kg bw/day, higher creatinine levels attained statistical significance compared to control; this was in principle due to one treated animal with a particularly high recorded creatinine level; all other values were within the historical control range
  - for females at all dosages, albumin/globulin ratio was statistically significantly higher compared to control but no dose-response relationship was observed; furthermore no

accompanying statistically significant changes in levels of total protein or albumin were noted and all values for albumin/globulin ratio were within the historical control range

- for females at 300 mg/kg bw/day, higher glucose, calcium and inorganic phosphorus levels attained statistical significance compared to control; two glucose values and inorganic phosphorus values exceeded the historical control; all other values were within this historical range
- for females at 300 mg/kg bw/day, lower alanine aminotransferase and alkaline phosphatase levels attained statistical significance compared to control; all values were within the historical control and a decrease in these parameters is unlikely to indicate an adverse effect of treatment

- Necropsy
  - at 30, 100 or 300 mg/kg bw/day no treatment-related effects (neither the type, incidence or distribution of macroscopic findings) were observed
- Organ weights
  - at 300 mg/kg bw/day, there was a decrease in absolute and body weight relative liver weights for females compared with control, with differences attaining statistical significance; these differences were considered to reflect the difference in pregnancy state between the pregnant controls and the non-pregnant treated females; body weight relative values are considered to be the best indicator of toxicological effect for this organ and all body weight values for these treated animals were within the historical range based on 90-d toxicity studies where females are of similar age and the same pregnancy status
  - at 300 mg/kg bw/day, there was an increase in absolute and body weight relative thymus weights for females compared with control, with differences attaining statistical significance; the registrant regarded these differences as probably influenced by the difference in pregnancy state between the pregnant controls and the non-pregnant treated females; all individual thymus weights were within the historical range based on 90-d toxicity studies
  - at 300 mg/kg bw/day, statistically significantly lower absolute and body weight-relative heart weights, compared to control, were observed but all values for treated animals were within the historical control range
- Histopathology
  - microscopic examination of tissues for both sexes at 300 mg/kg bw/day did not reveal any microscopic alterations indicative of toxicity, thus examination was not extended to include animals in the low and intermediate groups
  - no evidence of histopathological changes that could give an explanation for the failure of females to achieved pregnancy at this dosage was noted

  - morphology of the reproductive organs was normal in the animals which failed to produce offspring and testes morphology did not reveal any disturbances of spermatogenic staging

- Effect levels (given in dissemination database[1]):
  - NOAEL (male/female) = 100 mg/kg bw/day (actual dose received), based on a clear effect on reproduction leading to no pregnant animals being available for assessment
  - NOAEL (systemic toxicity) = 300 mg/kg bw/day (actual dose received)

For F1 and F2 pups/litters (per dose):

- At 300 mg/kg bw/day all females failed to achieve pregnancy and therefore it is not possible to assess litter responses at this dosage
- Offspring litter size, sex ratio and viability: see
- Table 11 and Table 12
  - no adverse effect of treatment on the corpora lutea count, pre-implantation loss, numbers of implantations, post-implantation loss, litter size at birth/day 1 and subsequent offspring survival to day 4 of age at 30 or 100 mg/kg bw/day was seen
  - sex ratio for the offspring was similar in all groups and did not indicate any selective effect of maternal treatment on survival for either sex
- Offspring growth and development, see
- Table 11
  - at 30 or 100 mg/kg bw/day no adverse effect on offspring body weight and litter weights at day 1 and body weight gain to 4 post partum was observed
  - at 100 mg/kg bw/day, offspring body weights were slightly higher compared to control on day 1 of age, with differences for males attaining statistical significance; these differences were considered to reflect the slightly longer gestation period observed for parent females at this dosage rather than any treatment related effect on pre-natal/early post-natal growth
  - offspring performance during assessment of surface righting appeared to be unaffected by maternal treatment at 30 or 100 mg/kg bw/day
- Clinical signs
  - Clinical signs findings apparent for offspring on the study were typical for the age observed; neither incidence nor distribution of these observations indicated any adverse effect of maternal treatment on offspring development at 30 or 100 mg/kg bw/day
- Necropsy

[1] ECHA Dissemination: Information on Chemicals - Registered Substances. European Chemicals Agency. Online: https://echa.europa.eu/registration-dossier/-/registered-dossier/21171/1 (last modified 3 October July 2019)

- necropsy findings apparent for offspring were typical for the age observed and the low incidence and distribution of these observations did not indicate any effect of maternal treatment at 30 or 100 mg/kg bw/day

- Effect levels (given in dissemination database[2]):
  - NOAEL (F1, male/female) = 100 mg/kg bw/d (nominal), based on viability, clinical signs, mortality, gross pathology, offspring litter size / sex ratio / offspring growth and development / necropsy)

[2] ECHA Dissemination: Information on Chemicals - Registered Substances. European Chemicals Agency. Online: https://echa.europa.eu/registration-dossier/-/registered-dossier/21171/1 (last modified 3 October 2019)

Table 10: Summary of incidence of mating performance, fertility and gestation lengths

Group	Dose (mg/kg bw/day)	No. of males	Number of females			Pre-coital interval (days)					Mating Index (%)	Pregnancy Index (%)	Gestation length (days)				Females with live offspring	Parturition index (%)
		paired	paired	mated	pregnant	1	2	3	4	13			22	22 ½	23	23 ½		
1	0	12	12	12	12	4	3	0	4	1	100	100	4	8	0	0	12	100
2	30	12	12	12	12	0	7	1	4	0	100	100	0	8	4	0**	12	100
3	100	12	12	12	12	3	4	1	4	0	100	100	0	7	1	4**	12	100
4	300	12	12	12	0	5	3	1	3	0	100	0						

** Significantly different from control group p<0.01

Table 11: Summary of group mean litter size and litter weight

Group	Dose (mg/kg bw/day)		Number of corpora lutea	Number of implantation sites	Total number of offspring	Number of live offspring		Litter weight (g)		Offspring weight (g)				Offspring weight change (g)	
					born	Day 1	Day 4	Day 1	Day 4	Day 1		Day 4		Day 1-4	
										Males	Females	Males	Females	Males	Females
1	0	Mean	14.5	14.3	13.7	13.6	13.4	78.39	109.18	5.89	5.64	8.27	8.07	2.38	2.44
		S.D.	2.5	2.5	2.5	2.5	2.5	14.53	18.48	0.36	0.28	0.59	0.52	0.37	0.41
		N	12	12	12	12	12	12	12	12	12	12	12	12	12
2	30	Mean	14.3	13.9	13.3	13.2	12.4	78.72	103.96	6.14	5.85	8.69	8.21	2.55	2.36
		S.D.	1.4	1.7	1.8	1.8	2.0	9.48	12.20	0.36	0.32	0.82	0.55	0.61	0.44
		N	12	12	12	12	12	12	12	12	12	12	12	12	12
3	100	Mean	12.8	12.4	11.5	11.4	11.3	69.51	96.82	6.32*	5.99	9.00	8.74	2.68	2.75
		S.D.	3.2	3.3	2.7	2.7	2.7	15.46	17.99	0.49	0.51	1.19	1.23	0.77	0.83
		N	12	12	12	12	12	12	12	12	12	12	12	12	12

* Significantly different from control group p<0.05

Table 12: Summary of group mean implantation loss and survival indices

Group	Dose (mg/kg bw/day)		Pre-implantation (%)	Post-implantation (%)	Live birth index (%)	Viability index (%)
1	0	Mean	1.2	4.6	99.5	98.7
		S.D.	2.9	5.4	1.8	3.0
		N	12	12	12	12
2	30	Mean	3.0	4.3	99.8	94.7
		S.D.	6.8	3.9	4.1	10.9
		N	12	12	12	12
3	100	Mean	3.1	6.5	99.2	98.7
		S.D.	7.2	5.3	2.9	3.0
		N	12	12	12	12

4.10.1.2 Oral (Gavage) Reproduction Study in the Rat

Study reference:

N.N., study report, 2016

Detailed study summary and results:

Test type

This study was performed to further investigate observed fertility effects, which were previously seen in a OECD 422 study (Harlan Laboratories Ltd. Study Number 41402232) with the test item at a dosage of 300 mg/kg bw/day. Males were dosed for 38 consecutive days and females dosed for at least 4 weeks (including a two week pre-pairing phase, pairing and then to day 13 of gestation).

GLP compliance is given (incl. certificate).

A reliability of 1 is given for this study in the registration dossier.

Test substance

- Test material used in the study is equivalent to substance identified in the CLH dossier: Reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis((2,3-epoxypropoxy)methyl)butane and 1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane; named in the ESR as 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, oligomeric reaction product with (chloromethyl)oxirane
- Concentration of constituents: 58% $C_{15}H_{26}O_6$ and 25% $C_{12}H_{22}O_5$
- Impurities: not specified
- Batch number: available in confidential study report
- Test material form: Clear colorless liquid

Test animals

- Rat/Wistar/male and female

- No. of animals per sex per dose: 12 per sex and dose (in total 24 males and 24 females)
- Age at the study initiation: approx. 12 weeks old
- Weight at the study initiation: males: 314 - 349 g; females: 185 - 208 g
- Acclimatisation for 12 days

Administration/exposure

- Route of administration – oral (gavage), by using a stainless steel cannula attached to a disposable plastic syringe
- Duration and frequency of test/exposure period:
  - 38 consecutive days for males
  - daily, at least 4 weeks for females (including a two week pre-pairing phase, pairing and then to day 13 of gestation)
  - on day 39 males were terminated
  - on day 14 of gestation/ post coitum mated females were terminated
- Doses/concentration levels, rationale for dose level selection:
  - 0, 300 mg/kg bw/day, actual ingested, concentration: 0 and 75 mg/mL
  - volume of test and control item administered to each animal was based on the most recent scheduled body weight and was adjusted at weekly
  - random allocation of animals to treatment groups was done by using a stratified body weight randomization procedure and the group mean body weights were then determined to ensure similarity between the treatment groups
- Control group and treatment: yes, concurrent vehicle
- Historical control data: yes
- Vehicle:
  - polyethylene glycol 400, 4 mL/kg
  - analytical verification confirmed the accurate use of the test item
  - justification of choice of vehicle is not provided
- Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:
  - test item was prepared as a solution at the appropriate concentrations in polyethylene glycol 400
  - test item formulations were stable for at least seventeen days in the dark at approx. 4°C, thus formulations were prepared weekly and stored at approx. 4 °C in the dark
  - analytical verification of doses or concentrations: samples of test item formulations were taken and analysed for concentration of test item; results indicate that the prepared

formulations were within 96% to 100% of the nominal concentration confirming the accuracy and suitability of the formulation procedure

Description of test design:

- Details on mating procedure:
  - M/F ratios per cage: 1:1; control males were paired with females receiving 300 mg/kg bw/day and 300 mg/kg bw/day males were paired with control females
  - length of cohabitation: started on day 15 for a maximum of 14 days
  - proof of pregnancy: presence of sperm within the vaginal smear and/or vaginal plug in situ designated as day 0 of post coitum, then males were returned to their original cages and females were transferred to individual cages
- Premating exposure period for males and females: two weeks
- Dosing schedules and pre and post dosing observation periods for P, F1:
  - twelve animals per dose and sex were treated daily at the appropriate dose level (0 (Control) and 300 mg/kg bw/day) throughout the study and the first day of dosing was designated as day 1 of the study
  - on day 15 animals were paired on a male:female ratio of 1:1 within each dose group for a maximum of fourteen days; thereby were control males paired with females receiving 300 mg/kg bw/day and 300 mg/kg bw/day males were paired with control females
  - following evidence of mating (designated as Day 0 post coitum) males were returned to their original cages and females were housed individually
  - pregnant females were maintained until day 14 of gestation/post coitum; a nonmated female was killed fourteen days after the end of the pairing period
  - on day 39 male dose groups were killed and examined macroscopically
  - on day 14 of gestation/post coitum females were killed and examined macroscopically
- Standardization of litters: no
- Parameters assessed for P:
  - Clinical observations
    - all animals were examined for overt signs of toxicity, ill-health and behavioural change immediately before dosing, soon after dosing, and approx. one hour after dosing; all observations were recorded
  - Body weight
    - individual body weights were recorded on day 1 (prior to dosing) and then weekly for males until termination and weekly for females until pairing; during pairing

period females were weighed daily until mating was confirmed and afterwards body weights were recorded for females on days 0, 7, 14 post coitum

- Food consumption
    - during the pre-pairing period, weekly food consumption was recorded for each cage of adults; this was continued for males after the mating phase; for females showing evidence of mating food consumption was recorded for the periods covering post coitum days 0-7 and 7-14
- Water consumption
    - water intake was observed daily by visual inspection of water bottles for any overt changes
- Reproductive performance
    - mating
        - each morning cage tray-liners were checked for the presence of ejected copulation plugs and each female was examined for the presence of a copulation plug in the vagina; vaginal smear was prepared for each female and the stage of oestrus or the presence of sperm was recorded; presence of sperm within the vaginal smear and/or vaginal plug in situ was taken as positive evidence of mating (day 0 of gestation) and the males were subsequently returned to their original holding cages; mated females were housed individually during the period of gestation and lactation
- Reproductive indices
    - pre-coital interval and gestation length were calculated
    - fertility indices (mating index, pregnancy index) and parturition index were determined
- Pregnancy and parturition
    - for each female was recorded the date of pairing and date of mating
- Litter data:
    - terminal investigations, but comparative assessment of litter responses was not possible as all control females (paired with treated males) failed to achieve pregnancy
- Organ weights:
    - from males left epididymis and testis were removed, dissected free from fat and weighed
- Necropsy:

- sacrifice by intravenous overdose of suitable barbiturate agent followed by exsanguination of adult males on day 39
- uterus was examined for signs of implantation and number of uterine implantations in each horn was recorded; this procedure was enhanced (as necessary) by staining the uteri with a 0.5% ammonium polysulphide solution and corpora lutea were also counted
- all adult animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded

- Oestrous cycle length and pattern: no data
- Sperm examination: at necropsy of males the following evaluations were performed:
  - left testis and epididymis were removed, dissected from connective tissue and weighed separately
  - for the testis, the tunica albuginea was removed and the testicular tissue stored frozen at approx. -20ºC
  - for the epididymis the distal region was incised and a sample of the luminal fluid collected and transferred to a buffer solution for analysis of sperm motility
  - approx. 200 individual sperm were assessed (where possible) using an automated semen analyser and the number of motile, progressively motile and non-motile sperm determined; characteristics of motile sperm were also identified by using the computer assisted sperm analyser (Hamilton-Thorne TOX IVOS system)
  - cauda epididymis was separated from the body of the epididymis, weighed and afterwards frozen at approx. -20ºC
  - later frozen testicular and epididymal tissues were thawed and homogenised in an appropriate saline/detergent for determining the numbers of homogenisation resistant spermatids for each tissue using Hamilton-Thorne TOX IVOS system
  - morphological assessment was also performed on a sample of sperm that has been preserved in fixative, transferred to a glass slide and then stained with eosin stain, for determining the number with apparent structural anomalies
- Parameters assessed for F1:
  - only litter data available for treated females, thus a comparative assessment of litter responses was not possible as all control females (paired with treated males) failed to achieve pregnancy
  - post exposure observation: none
  - Litter indices determined:

- implantation losses (pre- and post-implantation loss), live birth and viability indices (live birth index, viability index) ans sex ratio

Results and discussion

- Actual dose received: 0, 300 mg/kg bw/day, actual ingested for both sexes
- Statistical treatment of results:
  - where considered appropriate, quantitative data was subjected to statistical analysis to detect the significance of intergroup differences from control; statistical significance was achieved at a level of $p<0.05$. Statistical analysis was performed on the following parameters: body weight, body weight change, pre-coital interval, absolute organ weights, body weight-relative organ weights, sperm motility, sperm counts and sperm morphology (% normal, % abnormal)
  - data were analysed using the decision tree from the Provantis™ Tables and Statistics Module as detailed as follows:
    - where appropriate, data transformations were performed using the most suitable method. The homogeneity of variance from mean values was analysed using Bartlett's test. Intergroup variances were assessed using suitable ANOVA, or if required, ANCOVA with appropriate covariates. Any transformed data were analyzed to find the lowest treatment level that showed a significant effect using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response was found but the data shows nonhomogeneity of means, the data were analyzed by a stepwise Dunnett's (parametric) or Steel (non-parametric) test to determine significant difference from the control group. Where the data were unsuitable for these analyses, pair-wise tests was performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric).
    - data not analysed by the Provantis™ data capture system were assessed separately using the R Environment for Statistical Computing. Initially, the distribution of the data was assessed by the Shapiro-Wilk normality test, followed by assessment of the homogeneity of the data using Bartlett's test. Where considered appropriate, parametric analysis of the data was applied incorporating analysis of variance (ANOVA), which if significant, was followed by pair-wise comparisons using Dunnett's test. Where parametric analysis of the data was considered to be unsuitable, non-parametric analysis of the data was performed incorporating the Kruskal- Wallis test which if significant was followed by the Mann-Whitney "U" test.

- probability values (p) are presented as follows: p>0.001 ***, p<0.01 **, p<0.05 *, p>0.05 (not significant)
- as all control females were non-pregnant, body weight, body weight gains and food consumption during gestation were compared against values from non-pregnant females and litter data (including pre- and post-implantation loss) could not be analysed statistically

For P:

- Mortality: no effects observed
  - no unscheduled deaths occurred
- Clinical observations: effects observed, treatment-related
  - only increased post-dosing salivation for both sexes, with the majority of treated animals being affected was observed;
- Body weight: effects observed, treatment-related
  - during the first two weeks of treatment body weight gain of treated males was lower compared to control, with differences attaining statistical significance; afterwards body weight gain was essentially similar to control throughout the remainder of the study, however overall body weight gain was still lower compared to control at termination
  - body weight gain of treated females appeared unaffected by treatment during the two week pre-pairing period; meaningful assessment of body weight after mating was precluded as all control females (paired with treated males) failed to achieve pregnancy
  - body weight gains of females were similar to control during the first week of pairing but were statistically significantly higher than control during the second week, when body weight gain would have been increasingly influenced by weight gain due to pregnancy
- Food consumption: effects observed, treatment-related
  - for treated males food consumption was lower than control during the two week pre-pairing period, but was similar to control during the post-pairing phase of the study
  - for treated females food consumption was unaffected by treatment during the two week pre-pairing period; a meaningful assessment of food consumption after mating was precluded as all control females (paired with treated males) failed to achieve pregnancy; food consumption was similar to control during the first week of pairing but appeared higher than control during the second week, when food intake would have been increasing influenced by weight gain due to pregnancy
  - for treated males food conversion efficiency was lower than control during the first week and to a lesser extent, during the second week of treatment; during the post-pairing period food conversion efficiency of treated males was similar to control of the study
  - for treated females food conversion efficiency was unaffected by treatment during the two week pre-pairing period

- Water consumption: no effects observed
  - visual assessment of water consumption during the study did not indicate any obvious effect of treatment on water intake for either untreated or treated animals
- Reproductive performance: effects observed, treatment-related, see Table 13
  - Mating and fertility: effects observed, treatment-related
    - no treatment-related effects on mating performance as assessed by pre-coital interval; all treated males (paired with control females) and the majority of treated females (paired with control males) mated within the first four days of pairing (i.e. at the first oestrus opportunity)
    - one treated female failed to mate but this was considered to reflect poor fertility of the control male (indicated by male reproductive organ weight and sperm analysis).
    - evidence of mating (sperm in vaginal smear, numbers of copulation plugs) was similar in both groups, however while two mated treated females (paired with control males) failed to achieve pregnancy, all mated control females (paired with treated males) failed to achieve pregnancy
- Necropsy: no effects observed
  - no treatment-related effects (neither the type, incidence or distribution of macroscopic findings) were observed in either sex
  - one control male showed small and flaccid testes and small epididymides and failed to mate with its treated female partner
- Organ weights: effects observed, treatment-related
  - for treated males, absolute and body weight relative left epididymal weights were statistically significantly lower than control
  - observed difference from control would have been greater if the control male observed to have atypically small reproductive organs had been excluded
- Sperm analysis: effects observed, treatment-related
  - for treated males, mean homogenisation resistant spermatid count from the cauda epididymis was statistically significantly lower than control; no similar decrease was observed for mean homogenisation resistant spermatid count from the testis of treated males
  - examination of sperm concentration and motility at necropsy and sperm morphology did not indicate any obvious effect of treatment
  - at necropsy no relevant findings on epididymis and testis observed in treated males
- Effect levels (given in dissemination database[3]):

[3] ECHA Dissemination: Information on Chemicals - Registered Substances. European Chemicals Agency. Online: https://echa.europa.eu/registration-dossier/-/registered-dossier/21171/1 (last modified 3 October 2019)

- LOAEL (male) = 300 mg/kg bw/day (actual dose received), based on a clear effect on male fertility

For F1:

- comparative assessment of litter responses was not possible as all control females (paired with treated males) failed to achieve pregnancy, see Table 14
- litter data available for treated females did not indicate any obvious adverse effect of treatment
- no further investigations were performed on F1

Table 13: Summary of incidence of mating performance, fertility and gestation lengths

Group	Dose (mg/kg bw/day)	No. of males	Number of females			Pre-coital interval (days)					Mating Index (%)	Pregnancy Index (%)	Females with live young at Day 14 of gestation
		paired	paired	mated	pregnant	1	2	3	4	13			
1	0	12+	12	12	0	5	3	2	2	0	100	0	0
2	300	12x	12	11	9	3	1	4	2	1	92	82	9

+ = Group 2 males; x = Group 1 males

Table 14: Summary of group mean litter data

Group	Dose of dams (mg/kg bw/day)		Number of corpora lutea	Number of implants	Number of embryonic/foetal deaths			Implantation loss %		Number of live implants
					Early	Late	Total	Pre	Post	Total
1	0	Mean								
		S.D.								
		N								
2	300	Mean	13.4	13.2	0.0	0.1	0.1	1.6	100.0	13.1
		S.D.	2.0	2.0	0.0	0.3	0.3	3.3	0.0	1.9
		N	9	9	9	9	9	9	9	9

4.10.1.3 Prenatal Developmental Toxicity Study

Study reference:

N.N., study report, 2018

Detailed study summary and results:

Test type

A prenantal developmental toxicity study according to OECD TG 414 was performed. GLP compliance is given (incl. certificate).

A reliability of 1 is given for this study in the registration dossier. According to the authors of this document the study is considered to have a lower reliability (RL 3) as the highest dose tested was too low to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) as requested by the guideline.

Test substance

- Test material used in the study is equivalent to substance identified in the CLH dossier: Reaction mass of 1-(2,3-epoxypropoxy)-2,2-bis ((2,3-epoxypropoxy)methyl) butane and 1-(2,3-epoxypropoxy)-2-((2,3-epoxypropoxy)methyl)-2-hydroxymethyl butane
- CAS number: 30499-70-8
- Concentration of constituents: 58% $C_{15}H_{26}O_6$ and 25% $C_{12}H_{22}O_5$
- Impurities: not specified
- Batch number: available in confidential study report
- Physical state/appearance: clear colourless liquid
- Storage conditions: ambient +15 °C to +25 °C in the dark

Test animals

- Species/strain/sex: rat/Wistar HanTac: WH/female
- No. of animals per sex per dose: 24 mated females per dose
- Age at the study initiation: 12 - 15 weeks old
- Weight at the study initiation:
  - in each group the weight variation did not exceeded ± 20% of the mean body weight
  - mean body weights of groups: G1 (control): 204.090 ± 15.529, G2: 201.808 ± 16.874, G3: 201.734 ± 16.276, G4: 201.699 ± 14.109

Administration/exposure

- Route of administration – oral (gavage), by using disposable plastic syringe attached with a metal feeding cannula
- Duration and frequency of test/exposure period: from gestation day (GD) 5 to GD 19 of presumed gestation, once daily at approx. the same time each day (varying by ± 3 hours)
- Doses/concentration levels, rationale for dose level selection:
  - 0, 30, 90, or 180 mg/kg bw/d, nominal
  - dose selection was based on available literature in consultation with the sponsor (no further information provided)
  - test item was administered at an equivolume of 5 mL/kg bw
  - actual volume was calculated for individual animals using the most recent body weight
  - random allocation of pregnant rats to treatment groups was done by body weight stratification method using Provantis™ software and the group mean body weights were then determined to ensure similarity between the treatment groups
- Control group and treatment: yes, concurrent vehicle
- Historical control data: yes
- Vehicle:
  - polyethylene glycol 400 (PEG-400), administration of a constant dose volume of 5 mL/kg bw for all females of all groups
  - analytical verification confirmed the accurate use of the test item and PEG-400 as vehicle
  - justification of choice of vehicle: same vehicle as in existing toxicity studies
- Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:
  - Preparation of formulation: test item was weighed into a pre-calibrated beaker (separately for each dose level), PEG-400 was added and mixed to obtain a homogeneous suspension, more PEG-400 was added up to the mark for obtaining final concentrations, a magnetic stirrer was used for mixing the suspension during sampling/gavage administration
  - analytical verification of doses or concentrations: prepared formulations were sampled in duplicates (one for analysis, one as back up) at the prior to the initiation (08 June 2018) and a day before the termination (25 June 2018) of batch one of rats; taken samples (one replicate sample was drawn from top, middle and bottom layers for each dose formulation of each set (for control only middle layer)) were analysed by using a validated analytical method
  - all tested formulations were within the acceptable limits (mean result for all layers were within ± 15% of the claimed concentration and the relative standard deviation (RSD) of assay of top, middle and bottom layer (total 3 samples) was less than or equal to 10%)

- under Study No. G16353 stability and homogeneity of the test item in the vehicle was established at concentrations of 1 and 100 mg/ml; test item was stable and homogenous in the vehicle for 4 days when stored at room temperature

Description of test design:

- Details on mating procedure:
  - M/F ratios per cage: 1:1
  - length of cohabitation: not specified
  - proof of pregnancy: presence of sperm within the vaginal smear and/or vaginal plug was observed in the morning then female was considered to be mated (day 0 of post coitum), afterwards males were returned to their original cages and females were transferred to individual cages
  - females were cohabited in batches of required numbers and this procedure was continued until sufficient numbers of Day 0 pregnant rats were available for the study
- Premating exposure period for males and females (P): none
- Dosing schedules: administered from GD 5 to GD 19 of presumed gestation, once daily at approx. the same time each day
- Standardization of litters: no
- Parameters assessed for P:
  - cage side observations: yes, daily
  - detailed clinical observations: yes, twice daily on treatment days, and once on non-treatment days
  - body weight: yes, on gestation days 0, 3, 5, 8, 11, 14, 17 and 20
  - food consumption: controlled and recorded
  - post-mortem examinations: yes,
    - on GD 20 all rats were sacrified under isoflurane anaesthesia and subjected to post-mortem examination
    - prior to post-mortem examination a generation of random numbers was performed for coding in order to avoid bias during caesarean section and subsequent foetal evaluations (animal code was written on tail by striking out the permanent accession number)
    - external observation and examination of thoracic and abdominal cavity
  - ovaries and uterine examinations of all animals:
    - gravid uterus along with the cervix was excised, weighed and immediately examined,

- following data were collected: pregnancy status, gravid uterine weight (from all rats subjected to caesarean section), number of corpora lutea, number of implantation sites, number of early and late resorptions, gross evaluation of placenta
- non-gravid uteri were subjected to 10% ammonium sulphide staining to observe implantation sites (identified as pregnant animals) or to confirm the non-pregnant status

- Parameters assessed for F1:
  - foetuses were delivered by hysterectomy on GD 20
  - body weight: yes
  - sex: yes, from the ano-genital distance
  - litter data: total number of foetuses, number of alive and dead foetuses
  - external examinations: all foetuses per litter
  - visceral examinations: half of the foetuses from each litter of all groups were prepared for fresh tissue visceral organ evaluation, foetuses were decapitated and heads were stored in 70% alcohol for sectioning using modified Wilsons Razor blade sectioning technique
  - skeletal examinations: half of the foetuses from each litter of all groups were prepared for skeletal evaluation by wet skinning followed by evisceration and staining; fixed in 70% ethyl alcohol, eviscerated and dehydrated in 95% ethyl alcohol; macerated in 1.5% KOH and stained with saturated, aqueous Alizarin red S in Mall's solution; excess stain was removed in Mall's solution and foetuses were cleared by passing through grades of glycerol with thymol crystals
  - foetal heads subjected for sectioning were pooled dam wise and stored in formalin

Results and discussion

- Actual dose received by dose level: 0, 30, 90, or 180 mg/kg bw/d, nominal
- Statistical treatment of results:
  - analysis of variance (ANOVA) after testing for homogeneity for intra group variance using Levene's test was performed for data on maternal body weight, body weight change in interval, gravid uterine weight, body weight change corrected to gravid uterine weight, and maternal food consumption
  - in case of intra group variances being heterogeneous, data was transformed and afterwards analysed by ANOVA
  - if group differences were significant a Dunnett's pairwise comparison of treated group means with control group mean was performed

- analysis of covariance (ANCOVA) by taking litter size as covariate for group into account was used for analysis of foetal weight (male and female)
- analysis of group comparison by using Kruskal-Wallis test was performed for number of corpora lutea, number of implantations, early and late resorptions, pre-implantation and post-implantation loss, external, visceral and skeletal observations for variations
- if group differences were significant Mann-Whitney pairwise comparison of treated groups with control group was conducted
- incidence of dams with and without resorptions was tested using Cochran Armitage trend test followed by Fisher's exact test for group association

For P: see Table 15, Table 16, and Table 17

- Mortality: no effects observed
- Clinical signs: no effects observed
- Body weight and weight changes: no effects observed (see Table 16)
- Food consumption and compound intake: no effects observed
- Haematological findings: no effects observed
- Organ weight findings: no effects observed
- Number of abortions: no effects observed (see Table 17)
- Pre- and post-implantation loss: no effects observed (see Table 17)
- Total litter losses by resorption: no effects observed
- Early or late resorptions: no effects observed (see Table 17)
- Dead foetuses: no effects observed (see Table 17)
- Pregnancy duration: no effects observed
- Number of pregnant: no effects observed (see Table 17)
- Effect levels (given in dissemination database[4]):
  - Maternal NOAEL: 180 mg/kg bw/day based on no observed effects at highest dose tested

For F1: see Table 15 and Table 18

- Viability: no effects observed (see Table 18)
- Sex ratio: no effects observed (see Table 18)
- Litter size and weights: effects observed, non-treatment-related: at 90 mg/kg bw/d one small foetus was observed in a litter with 12 foetuses, which was incidental and considered as non-treatment related because it also occurred in historical controls (see Table 18)

[4] ECHA Dissemination: Information on Chemicals - Registered Substances. European Chemicals Agency. Online: https://echa.europa.eu/registration-dossier/-/registered-dossier/21171/1 (last modified 3 October 2019)

- Postnatal survival: no effects observed
- External malformations: no effects observed
- Visceral malformations: no effects observed
- Skeletal malformations: no effects observed
- Effect levels (given in dissemination database[5]):
  - Developmental NOAEL: 180 mg/kg bw/day based on no observed effects on litter parameters at highest dose tested

Table 15: Summarised details of the experiment

Group No. **Dose**	**G1** **0** **mg/kg/day**	**G2** **30** **mg/kg/day**	**G3** **90** **mg/kg/day**	**G4** **180** **mg/kg/day**
Total No. of rats found sperm positive / group	24	24	24	24
Duration of treatment	GD 5 to 19 (total 15 days)			
Caesarean section (day of presumed gestation)	24	24	24	24
Number of rats sacrificed at caesarean section	2	3	2	3
Number. of rats non-pregnant at caesarean section	22	21	22	21
Number of rats pregnant at caesarean section	22	21	22	21
Number of litters examined	261	249	238	255
Total number of fetuses	0	0	0	0
Total number of dead fetuses	261	249	238	255
Number of fetuses evaluated a.External examination	126	121	113	123
b.Visceral examination	135	128	125	132
c.Skeletal examination	24	24	24	24

Table 16: Summary of maternal body weights (g) of pregnant rats

Day(s) Relative to Mating		**G1** **0** **mg/kg/day**	**G2** **30** **mg/kg/day**	**G3** **90** **mg/kg/day**	**G4** **180** **mg/kg/day**
0	Mean SD N %Diff	204.19 16.25 22 -	202.37 16.75 21 -0.89	202.81 16.39 22 -0.68	204.04 13.36 21 -0.08
3	Mean SD N %Diff	215.31 17.89 22 -	215.48 19.93 21 0.08	214.23 18.18 22 -0.50	215.04 14.94 21 -0.12

[5] ECHA Dissemination: Information on Chemicals - Registered Substances. European Chemicals Agency. Online: https://echa.europa.eu/registration-dossier/-/registered-dossier/21171/1 (last modified 3 October 2019)

5	Mean	222.31	221.48	221.81	221.27
	SD	17.79	20.42	19.40	14.60
	N	22	21	22	21
	%Diff	-	-0.37	-0.22	-0.47
8	Mean	232.65	232.48	230.83	230.84
	SD	17.75	20.92	19.33	15.62
	N	22	21	22	21
	%Diff	-	-0.07	-0.78	-0.77
11	Mean	245.20	245.29	242.95	243.11
	SD	18.25	22.51	20.33	15.31
	N	22	21	22	21
	%Diff	-	0.04	-0.92	-0.85
14	Mean	255.73	256.16	251.29	252.97
	SD	17.24	21.85	20.07	15.73
	N	22	21	22	21
	%Diff	-	0.17	-1.74	-1.08
17	Mean	278.41	280.81	274.86	276.06
	SD	18.00	22.50	24.20	17.57
	N	22	21	22	21
	%Diff	-	0.86	-1.27	-0.84
20	Mean	305.82	307.58	301.11	301.65
	SD	18.54	23.06	27.88	18.19
	N	22	21	22	21
	%Diff	-	0.58	-1.54	-1.36

Table 17: Summary of maternal data

Group No. **Parameters Dose (mg/kg/day)** **No. of pregnant rats**		**G1** **0** **22**	**G2** **30** **21**	**G3** **90** **22**	**G4** **180** **21**
Gravid Uterine Weight (g)	Mean	65.239	64.778	60.219	64.809
	SD	8.969	6.946	15.653	7.054
No. of Corpora lutea	Mean	13.64	13.90	13.27	13.71
	SD	2.19	1.79	1.67	1.85
No. of Implantations	Mean	12.45	12.52	11.59	12.71
	SD	2.06	1.78	2.84	1.65
No. of Early Resorptions	Mean	0.50	0.62	0.68	0.57
	SD	0.86	0.80	0.99	0.75
No. of Late Resorptions	Mean	0.09	0.05	0.09	0.00
	SD	0.29	0.22	0.29	0.00
No. of Pre-implantation Loss	Mean	1.18	1.38	1.68	1.00
	SD	1.50	1.63	1.81	1.18
No. of Post-implantation Loss	Mean	0.59	0.67	0.77	0.57
	SD	1.05	0.86	1.15	0.75
Dams with any Resorption	Total No.	6	10	9	10
Early Resorptions (% litter)	Mean	3.66	4.78	5.62	4.37
	SD	6.28	5.71	8.14	5.56
Late Resorptions (% litter)	Mean	0.65	0.34	0.92	0.00
	SD	2.12	1.56	3.06	0.00

Pre-implantation Loss (% litter)	Mean	8.15	9.46	13.92	6.86
	SD	10.67	10.33	19.25	8.15
Post-implantation Loss (% litter)	Mean	4.31	5.12	6.53	4.37
	SD	7.66	6.06	9.83	5.56
Implantation Index (% litter)	Mean	91.85	90.54	86.08	93.14
	SD	10.67	10.33	19.25	8.15

Table 18: Summary of litter data

Group No. **Parameters Dose (mg/kg/day)** **No. of pregnant rats**		**G1** **0** **22**	**G2** **30** **21**	**G3** **90** **22**	**G4** **180** **21**
No. of litters[$]		22	21	22	21
Total No. of fetuses		261	249	238	255
Mean litter size		11.9	11.9	10.8	12.1
Dead fetuses	Total No.	0	0	0	0
	%	0	0	0	0
Total live fetuses a. Number		261	249	238	255
b.Weight (g)	Mean	4	4	4	4
	SD	0.202	0.337	0.199	0.248
Live male fetuses a. Number		119	136	128	137
b.Weight (g)	Mean	4	4	4	4
	SD	0.237	0.297	0.178	0.264
Live female fetuses a. Number		142	113	110	118
b.Weight (g)	Mean	4	4	4	3
	SD	0.206	0.401	0.222	0.270
Sex Ratio - Male: Female		1:1.19	1:0.83	1:0.86	1:0.86
(Percentage of number of males)		(46)	(55)	(54)	(54)

[$]: litter refers to pregnant rat with fetuses

4.10.2 Human data

No studies available.

4.10.3 Other data (e.g. studies on mechanism of action)

No studies available.

4.11 Specific target organ toxicity – single exposure

Evaluation not performed for this substance.

4.12 Specific target organ toxicity – repeated exposure

Not evaluated in this dossier. However, study summaries are provided to support the evaluation of the endpoint reproductive toxicity and carcinogenicity.

4.12.1 Animal data

4.12.1.1 Repeated dose oral toxicity study

Study reference:

N.N., study report, 2019

Detailed study summary and results:

Test type

In a repeated-dose toxicity study (according to OECD TG 408), rats were exposed to doses of the test substance (0, 30, 90, or 270 mg/kg bw/d) by gavage for 90 days and effects on clinical signs, functional observations, neurology examinations, clinical chemistry, hormone analysis, hematology and histopathology were evaluated. The reversibility of any effects during a subsequent 28 days recovery period was also assessed. GLP compliance is given (incl. certificate).

A reliability of 1 is given for this study in the registration dossier. According to the authors of this document the study is considered to have a lower reliability (RL 3), as the highest dose tested was too low to induce toxicity (”the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering”) as requested by the guideline.

Test substance

- Test material used in the study is equivalent to substance identified in the CLH dossier: Araldite DY-T/CH, IUPAC : 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, oligomeric reaction product with (chloromethyl)oxirane
- CAS number: 30499-70-8
- Concentration of constituents: 58% C15H26O6 and 25% C12H22O5
- Impurities: not specified
- Batch number: available in confidential study report
- Physical state/appearance: clear colourless liquid
- Storage conditions: ambient +15 °C to +25 °C in the dark

Test animals

- Species/strain/sex: rat/Wistar HanTac: WH/male and female

- No. of animals: 10 per sex per dose (main group), 5 per sex per dose (recovery group)
- Age at the study initiation: 5-6 weeks old
- Weight at the study initiation: 96.61 - 148.49 g (males), 92.60 - 118.62 g (females)

Administration/exposure

- Route of administration – oral (gavage)
- Duration of test/exposure period: for 90 consecutive days; vehicle or dose formulations were not administered to recovery groups for 28 days following the 90-day treatment period
- Doses/concentration levels, rationale for dose level selection:
  - 0, 30, 90, or 270 mg/kg bw/d (groups G1, G2, G3, and G4)
  - vehicle control recovery group (G1R) and high dose recovery group (G4R)
  - dose selection was based on available literature in consultation with the sponsor (no further information provided)
  - test item was administered at an equivolume of 5 mL/kg bw
  - actual volume was calculated for individual animals on the first day of treatment period and adjusted using the most recent body weight during the treatment period
- Frequency of treatment: once daily at approx. the same time (± 3 hours) each day
- Control group and treatment: yes, concurrent treatment
- Historical control data: not provided
- Post exposure observation period: 28 days recovery period
- Vehicle:
  - polyethylene glycol 400 (PEG-400), administration of a constant dose volume of 5 mL/kg bw
  - analytical verification confirmed the accurate use of the test item and PEG-400 as vehicle
  - justification of choice of vehicle: same vehicle as in existing toxicity studies
- Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:
  - Preparation of formulation: test item was weighed into a pre-calibrated beaker, PEG-400 added up to the pre-mark and mixed to obtain a homogeneous suspension, more PEG-400 was added up to the mark thus final concentrations were 6, 18 and 54 mg/mL for treatment groups (G2 - G4/G4R), respectively, a magnetic stirrer was used for mixing the suspension
  - analytical verification of doses or concentrations: prepared formulations and vehicle control were sampled on test day 1 and during 2nd (day 37) and 3rd (day 65) month of the treatment in order to analyse for homogeneity and active ingredient (a.i.) concentration

- all tested formulations were within the acceptable limits (± 15% of variations from the theoretical concentrations and the relative standard deviation (RSD) of assay from six replicates at each dose level was less than or equal to 10%)
- under Study No. G16353 stability and homogeneity of the test item in the vehicle was established at concentrations of 1 and 100 mg/ml; test item was stable and homogenous in the vehicle for 4 days when stored at room temperature

- statistical methods:
  - for parameters body weight and organ weights; laboratory investigations - haematology and clinical chemistry Provantis™ was used for data capturing and analysis by using built-in statistical tests
  - neurological observations and hormones were statistically analysed by using validated package in Excel and also using licensed copies of SYSTAT Statistical package ver.12.0, normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were first tested within the group and afterwards a one-factor ANOVA modeling was performed by treatment groups, if data was non-optimal (non-normal or heteroschedastic) a transformation was performed before ANOVA
  - comparison of means of treatment and control groups was analysed by a Dunnett's test, if the 'F' test was found significant
  - all analyses and comparisons were evaluated at the 5% ($p<0.05$) level

Results and discussion

- Mortality: no effects observed
- Clinical signs: no effects observed
- Body weight gain: effects observed, treatment-related
  - dose-dependent reduction of body weight in males, particularly at the end of the 90 d treatment period without a statistically signifcance at any time point (see Table 19); a recovery of the observed effect was noted during the recovery period, only on one day a statistically significant difference between G4R and control group was seen (see Table 20)
  - dose-dependent reduction of body weight gain in males, particularly at the end of the 90 d treatment period, statistically significant during days 43-50 and 50-57 of the treatment period (see Table 23), absolute body weight gains were significantly lower in males during days 1-8 and significantly higher during days 90-97 during the treatment period, absolute and total percent body weight gain during days 90-118 during the recovery period indicating the recovery of effects observed during treatment period (see Table 24)
  - no treatment-related effects on body weight of females in G1, G2, G3, and G4 were observed during the treatment period Table 21, however in the recovery group body weights

of G4R females were reduced when compared to controls, reaching statistical significance on three days during the treatment, control and treated animals have regained alignment, but G4R has not quite caught up (see Table 22)

- body weight gain in females was higher in treatment groups G2, G3, and G4 than in G1 (control) during the treatment period (see Table 25), in the recovery group was the body weight gain of G4R females below the body weight gain of G1R females, but not statistically significant throughout the entire period (see Table 26)

- Food consumption and compound intake: effects observed, non-treatment-related
  - food consumption was not affected by treatment at any tested dose in either sex
  - food consumption was significantly lower during days 43-50 at 270 mg/kg/d high dose males, during days 71-78 and 78-85 at 270 mg/kg/d high dose recovery males during treatment period, in the registration dossier these changes were associated with apparent decrease in body weights
  - mean food consumption was higher during days 22-29, 29-36, 36-43, 43-50, 50-57, 57-64, 64-71, 71-78, 78-85 and 85-90 at 90 mg/kg/day in females, in the registration dossier these changes were not considered toxicologically relevant as mean body weights were not affected by treatment
- Ophthalmoscopic examination: no effects observed
- Neurological findings: no effects observed
  - open field and sensory observations: no treatment-related effects observed
  - motor activity: in high dose females the stereotypic time at interval 3 was significantly higher, but not statistically significant from vehicle control thus in the registration dossier the finding was not considered to be toxicologically significant
  - landing hind limb footsplay: in high dose recovery males a significantly lower higher hindlimb foot splay was observed, which was not considered to be toxicologically relevant in the registration dossier because no changes were observed in the home cage or open field observations, motor activity, and clinical signs during daily clinical observation
  - grip strength: in high dose recovery males a significantly higher hindlimb grip strength was observed, which was not considered to be toxicologically relevant in the registration dossier because no changes were observed in the home cage or open field observations, motor activity, and clinical signs during daily clinical observation
- Clinical chemistry: no effects observed
- Haematology: no effects observed
- Thyroid hormone: no effects observed
- Urinalysis: no effects observed
- Organ weights: no effects observed

- absolute/relative organ weights and ratios were not different from the vehicle control for all examined organs, except for thymus
- at 90 mg/kg bw/d and 270 mg/kg bw/d a treatment-related decrease in absolute (statistically significant decrease 17% and 22% in males of mid and high dose, respectively, no clear dose-response relationship observed in females) and relative weight of thymus (not statistically significant in males, no clear dose-response relationship observed in females) was observed, but was not considered to be relevant in the registration dossier because no significant microscopic findings were seen

- Necropsy findings: no effects observed
- Histopathological findings: no effects observed, including a qualitative assessment of stages of spermatogenesis
- Oestrous cycle length: no effects observed, stage of oestrus cycle was examined and recorded before necropsy for main and recovery groups
- Effect levels (given in dissemination database[6]):
  - NOAEL: 270 mg/kg bw/day based on no observed effects on rats at highest dose tested

Table 19: Summary of body weights (g) - Males

Day(s) Relative to Start Date		G1 0 mg/kg/day	G2 30 mg/kg/day	G3 90 mg/kg/day	G4 270 mg/kg/day
1	Mean	119.62	119.62	122.61	118.68
	SD	14.52	16.58	16.28	13.99
	N	10	10	10	10
8	Mean	156.20	157.46	161.20	156.20
	SD	14.12	19.12	19.52	17.63
	N	10	10	10	10
15	Mean	191.60	192.48	195.49	189.38
	SD	14.41	20.94	21.67	20.23
	N	10	10	10	10
22	Mean	228.64	224.07	227.70	221.61
	SD	13.32	20.55	25.13	23.77
	N	10	10	10	10
29	Mean	259.12	252.61	252.96	249.23
	SD	14.74	21.38	29.24	25.82
	N	10	10	10	10
36	Mean	281.90	277.92	274.37	270.01
	SD	16.01	23.12	33.17	28.09
	N	10	10	10	10
43	Mean	303.34	298.17	297.85	288.18
	SD	18.39	24.28	34.34	29.03
	N	10	10	10	10
50	Mean	322.67	317.12	316.37	302.30
	SD	22.25	27.24	35.25	30.36

[6] ECHA Dissemination: Information on Chemicals - Registered Substances. European Chemicals Agency. Online: https://echa.europa.eu/registration-dossier/-/registered-dossier/21171/1 (last modified 3 October 2019)

	N	10	10	10	10
57	Mean SD N	339.25 22.84 10	333.62 25.79 10	326.57 36.96 10	311.77 31.68 10
64	Mean SD N	351.90 23.60 10	346.19 25.37 10	342.06 39.04 10	323.36 32.96 10
71	Mean SD N	367.56 27.07 10	362.55 27.67 10	355.61 37.13 10	337.72 34.80 10
78	Mean SD N	375.32 29.23 10	371.85 29.43 10	367.53 37.79 10	345.89 35.72 10
85	Mean SD N	379.12 30.11 10	377.73 31.72 10	371.91 42.34 10	349.61 36.59 10
90	Mean SD N	385.02 31.25 10	382.20 32.67 10	375.67 42.14 10	352.89 34.86 10

Table 20: Summary of body weights (g) – Males recovery group

Day(s) Relative to Start Date		**G1R 0 mg/kg/day**	**G4R 270 mg/kg/day**
1	Mean SD N	123.26 18.99 5	121.74 15.00 5
8	Mean SD N	164.67 17.91 5	158.60 13.23 5
15	Mean SD N	200.77 20.38 5	193.69 12.10 5
22	Mean SD N	234.09 14.33 5	222.48 11.20 5
29	Mean SD N	260.18 10.96 5	249.34 13.44 5
36	Mean SD N	281.86 9.06 5	265.80 15.20 5
43	Mean SD N	302.10 9.11 5	287.38 14.18 5
50	Mean SD N	319.77 10.01 5	306.06 13.89 5
57	Mean SD N	330.51 11.73 5	314.93 14.73 5
64	Mean SD N	341.14 9.47 5	323.62 * 11.97 5
71	Mean SD	353.64 10.42 5	338.31 15.81 5

	N		
78	Mean SD N	360.60 9.85 5	344.13 14.17 5
85	Mean SD N	363.36 9.24 5	348.08 16.49 5
90	Mean SD N	368.16 11.51 5	351.53 18.16 5
97	Mean SD N	371.86 12.18 5	360.60 16.62 5
104	Mean SD N	374.94 12.83 5	370.05 17.34 5
111	Mean SD N	380.62 17.05 5	376.81 19.00 5
118	Mean SD N	381.25 16.36 5	382.60 19.83 5

* = p < 0.05, T-Test: [G1RvsG4R]

Table 21: Summary of body weights (g) - Females

Day(s) Relative to Start Date		**G1 0 mg/kg/day**	**G2 30 mg/kg/day**	**G3 90 mg/kg/day**	**G4 270 mg/kg/day**
1	Mean SD N	104.84 8.17 10	104.02 7.83 10	103.16 8.03 10	103.07 6.81 10
8	Mean SD N	127.28 8.10 10	125.28 8.43 10	124.89 7.33 10	125.68 6.38 10
15	Mean SD N	144.58 10.32 10	143.73 11.04 10	145.05 8.18 10	145.51 7.43 10
22	Mean SD N	161.50 9.62 10	162.43 10.84 10	163.00 7.55 10	163.21 7.27 10
29	Mean SD N	171.50 10.95 10	175.32 11.14 10	176.94 8.70 10	176.36 9.05 10
36	Mean SD N	179.51 11.15 10	186.65 13.34 10	189.92 10.16 10	187.99 9.21 10
43	Mean SD N	187.09 10.02 10	193.00 13.68 10	196.51 12.74 10	193.42 8.25 10
50	Mean SD N	194.78 10.74 10	200.81 16.05 10	203.20 11.20 10	199.98 9.68 10
57	Mean SD N	200.64 11.86 10	207.79 16.64 10	209.90 12.84 10	206.32 9.06 10
64	Mean	204.54	211.69	212.88	209.72

	SD N	12.76 10	16.05 10	14.03 10	8.40 10
71	Mean SD N	210.46 12.48 10	214.46 16.23 10	219.22 14.89 10	215.17 8.10 10
78	Mean SD N	214.93 12.37 10	219.25 18.53 10	222.01 14.38 10	216.28 8.59 10
85	Mean SD N	215.26 12.62 10	221.18 16.45 10	226.98 14.74 10	219.31 10.05 10
90	Mean SD N	213.70 12.78 10	221.57 17.63 10	227.06 14.23 10	219.97 9.74 10

Table 22: Summary of body weights (g) – Females recovery group

Day(s) Relative to Start Date		**G1R 0 mg/kg/day**	**G4R 270 mg/kg/day**
1	Mean SD N	104.64 8.46 5	103.04 8.04 5
8	Mean SD N	132.86 9.38 5	125.92 9.12 5
15	Mean SD N	150.31 11.77 5	144.22 8.16 5
22	Mean SD N	170.11 8.33 5	159.37 11.00 5
29	Mean SD N	184.31 8.74 5	173.00 11.68 5
36	Mean SD N	195.14 8.77 5	180.93 14.28 5
43	Mean SD N	201.76 11.85 5	185.59 12.33 5
50	Mean SD N	208.84 8.45 5	191.87 15.63 5
57	Mean SD N	216.70 9.67 5	198.49 16.82 5
64	Mean SD N	222.15 10.75 5	202.67 16.16 5
71	Mean SD N	227.83 9.45 5	204.84 * 16.03 5
78	Mean SD N	232.63 8.89 5	209.69 * 18.33 5
85	Mean SD	233.74 11.72	210.98 19.40

	N	5	5
90	Mean SD N	233.67 12.14 5	210.39 * 17.98 5
97	Mean SD N	234.42 11.14 5	212.57 19.86 5
104	Mean SD N	237.66 12.42 5	218.09 18.33 5
111	Mean SD N	240.46 14.24 5	224.98 20.79 5
118	Mean SD N	239.66 11.73 5	225.79 23.05 5

* = p < 0.05, T-Test: [G1RvsG4R]

Table 23: Summary of body weight gains (g) - Males

Day(s) Relative to Start Date			**G1 0 mg/kg/day**	**G2 30 mg/kg/day**	**G3 90 mg/kg/day**	**G4 270 mg/kg/day**
Absolute Weight Gain (g)	1-8 [a]	Mean	36.58	37.85	38.60	37.53
		SD	5.91	5.80	4.78	4.34
		N	10	10	10	10
	8-15 [a]	Mean	35.41	35.02	34.28	33.17
		SD	7.49	3.81	4.44	3.35
		N	10	10	10	10
	15-22 [a]	Mean	37.03	31.59	32.21	32.24
		SD	5.68	3.66	5.86	6.02
		N	10	10	10	10
	22-29 [a]	Mean	30.49	28.54	25.27	27.61
		SD	5.60	4.66	6.82	3.64
		N	10	10	10	10
	29-36 [a]	Mean	22.77	25.31	21.41	20.79
		SD	4.68	5.85	7.62	4.97
		N	10	10	10	10
	36-43 [a]	Mean	21.45	20.25	23.48	18.17
		SD	5.52	3.40	4.50	3.51
		N	10	10	10	10
	43-50 [a]	Mean	19.33	18.95	18.52	14.12 *
		SD	5.65	6.61	2.06	2.21
		N	10	10	10	10
	50-57 [a]	Mean	16.58	16.50	10.19 *	9.48 *
		SD	2.59	5.48	9.15	4.36

		N	10	10	10	10
	57-64 [a]	Mean	12.64	12.57	15.50	11.58
		SD	5.69	3.49	7.87	3.93
		N	10	10	10	10
	64-71 [a]	Mean	15.67	16.36	13.54	14.37
		SD	5.28	4.65	4.70	3.00
		N	10	10	10	10
	71-78 [a]	Mean	7.76	9.29	11.92 *	8.17
		SD	4.35	2.72	2.47	3.38
		N	10	10	10	10
	78-85 [a]	Mean	3.80	5.88	4.38	3.72
		SD	3.14	4.54	5.98	4.10
		N	10	10	10	10
	85-90 [a]	Mean	5.90	4.47	3.76	3.28
		SD	3.47	3.73	5.04	4.37
		N	10	10	10	10
	1-90 [a]	Mean	265.40	262.58	253.07	234.21
		SD	38.05	33.50	33.76	28.16
		N	10	10	10	10
Total Weight Gain (%)	1-90 [a1]	Mean	227.15	224.46	208.76	199.13
		SD	56.32	49.93	34.17	29.78
		N	10	10	10	10

[a] Anova & Dunnett; [a1] Anova & Dunnett(Log); * = $p < 0.05$

Table 24: Summary of body weight gains (g) – Males recovery

Day(s) Relative to Start Date			**G1 0 mg/kg/day**	**G4R 270 mg/kg/day**
Absolute Weight Gain (g)	1-8 [p]	Mean	41.41	36.86 *
		SD	2.34	3.11
		N	5	5
	8-15 [p]	Mean	36.10	35.09
		SD	5.68	3.94
		N	5	5
	15-22 [p]	Mean	33.32	28.79
		SD	6.84	5.15
		N	5	5
	22-29 [p]	Mean	26.08	26.86
		SD	4.50	3.80

		N	5	5
	29-36 [p]	Mean	21.68	16.46
		SD	5.48	2.13
		N	5	5
	36-43 [p]	Mean	20.24	21.58
		SD	1.71	3.30
		N	5	5
	43-50 [p]	Mean	17.67	18.68
		SD	4.97	3.26
		N	5	5
	50-57 [p]	Mean	10.74	8.86
		SD	2.69	3.76
		N	5	5
	57-64 [p]	Mean	10.63	8.70
		SD	2.74	3.45
		N	5	5
	64-71 [p]	Mean	12.50	14.69
		SD	2.53	5.24
		N	5	5
	71-78 [p]	Mean	6.96	5.82
		SD	4.79	3.26
		N	5	5
	78-85 [p]	Mean	2.76	3.95
		SD	2.38	3.95
		N	5	5
	85-90 [p]	Mean	4.80	3.45
		SD	3.03	3.10
		N	5	5
	1-90 [p]	Mean	244.90	229.79
		SD	16.64	16.99
		N	5	5
	90-97 [p]	Mean	3.70	9.07 *
		SD	1.31	1.96
		N	5	5
	97-104 [p]	Mean	3.07	9.45
		SD	5.05	4.87
		N	5	5
	104-111 [p]	Mean	5.68	6.76
		SD	5.50	4.93

		N	5	5
	111-118 [p]	Mean	0.63	5.78
		SD	4.12	2.91
		N	5	5
	90-118 [p]	Mean	13.09	31.07 *
		SD	6.83	10.67
		N	5	5
Total Weight Gain (%)	1-90 [a]	Mean	203.91	191.66
		SD	43.19	33.17
		N	5	5
Total Weight Gain (%)	90-118 [a]	Mean	10.22	26.13 *
		SD	3.92	10.50
		N	5	5

[p] = T-Test: [G1RvsG4R]; [a] = Anova & Dunnett; * = $p < 0.05$

Table 25: Summary of body weight gains (g) - Females

Day(s) Relative to Start Date			**G1 0 mg/kg/day**	**G2 30 mg/kg/day**	**G3 90 mg/kg/day**	**G4 270 mg/kg/day**
Absolute Weight Gain (g)	1-8 [a]	Mean	22.44	21.27	21.74	22.60
		SD	6.37	3.83	4.36	5.90
		N	10	10	10	10
	8-15 [a]	Mean	17.30	18.44	20.15	19.83
		SD	4.38	3.95	4.08	3.35
		N	10	10	10	10
	15-22 [a]	Mean	16.92	18.70	17.95	17.70
		SD	3.55	6.64	4.20	4.81
		N	10	10	10	10
	22-29 [a]	Mean	10.00	12.89	13.94	13.15
		SD	3.39	4.30	3.41	9.63
		N	10	10	10	10
	29-36 [a]	Mean	8.01	11.34	12.98	11.63
		SD	5.18	3.84	3.37	9.24
		N	10	10	10	10
	36-43 [a]	Mean	7.58	6.35	6.59	5.42
		SD	4.11	3.02	5.04	1.76
		N	10	10	10	10
	43-50 [a]	Mean	7.69	7.81	6.69	6.56
		SD	6.46	6.06	5.40	5.55

		N	10	10	10	10
	50-57 [a]	Mean	5.86	6.98	6.70	6.34
		SD	2.87	4.76	3.99	5.03
		N	10	10	10	10
	57-64 [a]	Mean	3.90	3.90	2.97	3.40
		SD	2.49	2.52	3.56	4.20
		N	10	10	10	10
	64-71 [a]	Mean	5.93	2.77	6.34	5.45
		SD	5.68	4.63	4.57	3.35
		N	10	10	10	10
	71-78 [a]	Mean	4.47	4.79	2.79	1.11
		SD	2.70	3.31	4.19	3.71
		N	10	10	10	10
	78-85 [a]	Mean	0.33	1.93	4.97	3.03
		SD	2.77	4.65	3.43	5.06
		N	10	10	10	10
	85-90 [a]	Mean	-1.56	0.39	0.08	0.66
		SD	1.94	2.73	3.90	4.96
		N	10	10	10	10
	1-90 [a1]	Mean	108.86	117.55	123.91	116.90
		SD	11.74	18.01	16.67	11.61
		N	10	10	10	10
Total Weight Gain (%)	1-90 [a1]	Mean	104.54	113.86	121.33	114.24
		SD	15.36	20.16	22.57	17.05
		N	10	10	10	10

[a] = Anova & Dunnett; [a1] = Anova & Dunnett(Log)

Table 26: Summary of body weight gains (g) – Females recovery

Day(s) Relative to Start Date			**G1 0 mg/kg/day**	**G4R 270 mg/kg/day**
Absolute Weight Gain (g)	1-8 [p]	Mean	28.22	22.88
		SD	5.07	4.73
		N	5	5
	8-15 [p]	Mean	17.45	18.30
		SD	4.10	5.17
		N	5	5
	15-22 [p]	Mean	19.80	15.15
		SD	3.88	6.22
		N	5	5

	22-29 [p]	Mean	14.20	13.63
		SD	4.68	1.64
		N	5	5
	29-36 [p]	Mean	10.83	7.93
		SD	2.93	4.02
		N	5	5
	36-43 [p]	Mean	6.62	4.66
		SD	4.04	4.19
		N	5	5
	43-50 [p]	Mean	7.08	6.28
		SD	3.58	3.81
		N	5	5
	50-57 [p]	Mean	7.86	6.62
		SD	4.34	1.94
		N	5	5
	57-64 [p]	Mean	5.45	4.18
		SD	4.52	2.64
		N	5	5
	64-71 [p]	Mean	5.68	2.17 *
		SD	2.84	1.19
		N	5	5
	71-78 [p]	Mean	4.80	4.85
		SD	2.98	5.19
		N	5	5
	78-85 [p]	Mean	1.11	1.29
		SD	3.67	2.81
		N	5	5
	85-90 [p]	Mean	-0.07	-0.59
		SD	3.86	2.48
		N	5	5
	1-90 [p]	Mean	129.04	107.35 *
		SD	7.32	16.65
		N	5	5
	90-97 [p]	Mean	0.74	2.19
		SD	3.43	2.06
		N	5	5
	97-104 [p]	Mean	3.25	5.51
		SD	2.84	10.68
		N	5	5

	104-111 [p]	Mean	2.80	6.90
		SD	2.85	6.33
		N	5	5
	111-118 [p]	Mean	--0.80	0.81
		SD	3.84	5.82
		N	5	5
	90-118 [p]	Mean	5.99	15.41
		SD	2.67	20.92
		N	5	5
Total Weight Gain (%)	1-90 [a]	Mean	123.88	104.74
		SD	10.91	17.56
		N	5	5
Total Weight Gain (%)	90-118 [a1]	Mean	5.82	15.92
		SD	2.84	22.58
		N	5	5

[p] = T-Test: [G1RvsG4R]; [p1] = Wilcoxon(Rank): [G1RvsG4R]; [p2] = T-Test(Log): [G1RvsG4R]; [a] = Anova & Dunnett; [a1] - Anova & Dunnett(Log); * = $p < 0.05$

4.13 Aspiration hazard

Evaluation not performed for this substance.

5 ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance.

6 REFERENCES

ECHA C&L Inventory (2019): Information on Chemicals - Classification & Labelling Inventory, European Chemicals Agency. Online: http://echa.europa.eu/information-on-chemicals/cl-inventory, Disclaimer: http://echa.europa.eu/web/guest/legal-notice