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: 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo [4.1.0]heptane-3-carboxylate

EC Number: 219-207-4

CAS Number: 2386-87-0

Index Number: -

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Version number: 2.0 **Date: July 2021**

CONTENTS

1 PHYSICAL HAZARDS

Not evaluated as part of this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not available.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

Not evaluated as part of this dossier.

3.2 Acute toxicity - dermal route

Not evaluated as part of this dossier.

3.3 Acute toxicity - inhalation route

Not evaluated as part of this dossier.

3.4 Skin corrosion/irritation

Not evaluated as part of this dossier.

3.5 Serious eye damage/eye irritation

Not evaluated as part of this dossier.

3.6 Respiratory sensitisation

No data available.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 Guinea pig maximisation test

Study reference:

Anonymous, 1991a. Guinea pig maximization test of ERL-4221. (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

Similar to OECD 406: Skin sensitisation. The study pre-dated the adoption of the OECD test guideline. Deviations from the test guideline included incomplete reporting of the results of the range finding studies, no rationale for the selection of the intradermal induction dose, no individual animal data reported and no

data supporting the statement that the periodic testing of the positive control resulted in 100 % positive reactions. GLP compliant. Unpublished report.

- *Year of study:* 1991.

Test substance

- *Name:* ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* Not reported.
- *Impurities*: Not reported.
- *Batch number:* Not reported.

Test animals

- *Species/strain/sex:* Guinea pig, Hartley albino, male and female.
- *No. of animals per sex per dose:* 10/sex in the treatment group and 5/sex in the vehicle control group.
- *Age and weight at the study initiation:*
    - *Age:* 5-6 weeks old
    - *Weight:* 338 -390 g (males) and 300 -381 g (females)

Administration/exposure

- *Vehicle control:* Propylene glycol.
- *Positive control:* Dinitrochlorobenzene. No concurrent positive control group was included in the study. The study summary indicates that the laboratory performed periodic (approximately every 4 to 6 months) studies with the positive control to demonstrate the sensitivity of the test system.
- *Range finding study:*
    - 2 animals were administered intradermal injections of 0.1 ml of 5 % test material in propylene glycol. Observations were made at 24 and 48 hours post injection for signs of necrosis or ulceration. The study summary notes that only “local necrosis”, described as no extensive necrosis or ulceration, was observed. No further details are reported. This dose was selected for intradermal induction in the main study.
    - 6 animals were administered 0.1 ml of 10 %, 25 %, 50 % and 100 % test material on 2 x 2 cm filter papers on four different shaved sites on the dorsal and lateral areas of each animal. The sites were covered with plastic. After 24 hours the patches were removed and the skin was assessed for signs of erythema, oedema and eschar formation at 24 and 48 hours after patch removal. No results are reported however 100 % was selected for topical applications in the main study.
- *Induction:*
    - Prior to the start of the study, the hair on the shoulder region of each animal was clipped short.
    - Day 0 – Test animals received three pairs of intradermal injections:
        - 0.1 ml FCA (Freunds Complete Adjuvant)/water emulsion

        - 0.1 ml of 5 % test material in propylene glycol
        - 0.1 ml of 5 % test material in FCA/water emulsion
    - Day 6 – Test areas were pre-treated with 10 % sodium lauryl sulphate.
    - Day 7 –Test animals received a topical application of 0.2 ml of 100 % test material on 2 x 4 cm filter paper which was secured to the test site with an occlusive dressing. The length of the treatment was not stated.
    - Animals in the vehicle control group were treated in the same way as those in the test group except they received propylene glycol or 70 % ethanol instead of the test material.
- *Challenge*
    - Day 21 – The hair on a 5 x 5 cm area on the flank of each animal was removed by clipping. Test and vehicle control animals received a topical application of 0.1 ml of 100 % test material on 2 x 2 cm filter paper for 24 hours.
- *Assessment:* Dermal assessment of all animals performed 24 and 48 hours after removal of the challenge patches.
- *Grading system used:* A four point scale (0.5, 1, 2 or 3) was used to grade skin reactions.
- *Conclude whether the test substance is positive, negative or equivocal:*
    - *Score of 0:* Negative
    - *Score of 0.5:* Equivocal
    - *Score of 1 or above:* Positive
- *Statistical methods:* None applied.

Results and discussion

One male in the treatment group died on day 11. The animal had discoloured lungs, yellow liver colouring and the abdominal cavity was filled with fluid. The cause of death was not established. No other clinical signs were reported.

At 24 hours, positive reactions were observed in the test group in 12/19 animals: 11/19 with score of 1 and 1/19 with a score of 2. At 48 hours, 8/19 animals in this group had positive reactions (all with a score of 1). The sensitisation rate was 63 % at 24 hours and 42 % at 48 hours. No positive reactions were observed in the vehicle control group (0/10) at either time point. The study summary does not include individual animal data.

Table A1: Summary of the skin sensitisation reactions in the guinea pig maximisation test with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 1991a. ECHA dissemination site, 2021)

Group	No. of animals	Time (hours)	Dermal scores							
			0	0.5	1	2	3	Ed*	N*	E*
Test material	19	24	0	7	11	1	0	5	0	0
	19	48	4	7	8	0	0	0	0	0
Vehicle control	10	24	0	0	0	0	0	0	0	0
	10	48	0	0	0	0	0	0	0	0

* These abbreviations are not defined in the study summary however the dossier submitter considers they could refer to oedema, necrosis and eschar.

The study summary states that historical control data of the test laboratory demonstrated that the positive control was valid as it resulted in positive reactions in 100 % of treated animals. However, no data relating to the positive control is included in the study summary.

Under the conditions of the study, the test material, ERL-4221, is considered to be a skin sensitiser.

3.8 Germ cell mutagenicity

3.8.1 *In vitro* data

3.8.1.1 Bacterial reverse mutation test - 1

Study reference:

Anonymous, 1995. Bacterial reverse mutation study of UVR-6110 (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

OECD 471: Bacterial reverse mutation test. The study summary deviated from the test guideline in that the mean number of revertant colonies per dose level and strain were not reported. GLP-compliant. Unpublished study.

- *Year of study:* 1995.
- *Number of replicates:* Three plates per dose and the test was run in duplicate.
- *Positive controls:*
    - *+S9: mix:* All strains: 2-aminoanthracene.
    - *–S9 mix:* TA 100: 2-acetylaminofluorene.

      TA 1535: sodium azide.

      TA 1537: 9-aminoacridine.

      WP2 uvrA: N-ethyl-N-nitro-N-nitrosoguanidine.
- *Vehicle control:* Dimethyl sulfoxide.

- *Criteria for evaluating results:* The test was considered positive if the mean number of revertants was more than double that observed in the negative control and when the increase was dose dependent and significant.

Test substance

- *Name:* 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate. Identical to substance identified in CLH dossier.
- *Degree of purity:* Not reported.
- *Impurities*: Not reported.
- *Batch number:* Not reported.

Administration/exposure

- *Strain or cell type or cell line: S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA.
- *Type and composition of metabolic activation system:* S9 mix. Preparation method not reported.
- *Test concentrations:* 156, 313, 625, 1250, 2500 and 5000 µg/plate
- *Method:* Pre-incubation method.
- *Statistical methods:* No information reported.

Results and discussion

No information on cytotoxicity is reported. An increase in revertant colonies was reported for *S. typhimurium* strains TA 100 and TA 1535 in the presence of metabolic activation and *E. coli* WP2 uvrA in the presence and absence of metabolic activation. No increase in revertant colonies was reported for *S. typhimurium* strains TA 100 and TA 1535 in the absence of metabolic activation and in *S. typhimurium* strains TA 98, TA1537 and *E coli* strain WP2 uvrA in the presence and absence of metabolic activation. The mean number of revertant colonies per dose level and strain were not reported in the study summary.

Under the conditions of the study, the test material was considered positive in the presence of metabolic activation in *S. typhimurium* strains TA 100 and TA 1535 and in *E coli* strain WP2 uvrA in the presence and absence of metabolic activation.

3.8.1.2 Bacterial reverse mutation test - 2

Study reference:

Anonymous, 1987. AMES-8706-017 (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

Non-guideline study. The study pre-dated the adoption of the OECD 471 (bacterial reverse mutation test) but the method employed was reported to be similar, with the following deviations: no information on which positive controls were used for which strains or on number of replicates per dose, limited reporting of the method, and the mean number of revertant colonies per dose level and strain were not reported. The study summary notes that the study report is in Japanese. Not GLP compliant. Unpublished study.

- *Year of study:* 1987

- *Number of replicates:* Not reported.
- *Positive controls:* 2-acetylaminofluorene, 9-aminoacridine, N-ethyl-N-nitro-N-nitrosoguanidine and 2-aminoanthracene. No information on which positive controls were used with which strains.
- *Vehicle control:* Dimethyl sulfoxide.
- *Criteria for evaluating results:* Not reported.

Test substance

- *Name:* Celloxide 2021P (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3- carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* Not reported.
- *Impurities*: Not reported.
- *Batch number:* CELPS-HF-2.

Administration/exposure

- *Strain or cell type or cell line, target gene: S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E coli* strain WP2 uvrA.
- *Type and composition of metabolic activation system:* S9 mix. Preparation method not reported.
- *Test concentrations:* 100, 250, 500, 1000, 2000 and 5000 µg/plate.
- *Method:* Pre-incubation method.
- *Statistical methods:* No information reported.

Results and discussion

The study summary reports that no cytotoxicity was reported up to the limit concentration (no further details reported). An increase in revertant colonies was reported for *S. typhimurium* strains TA 100 and TA 1535 in the presence of metabolic activation. No increase in revertant colonies was reported for *S. typhimurium* strains TA 100 and TA 1535 in the absence of metabolic activation and in *S. typhimurium* strains TA 98, TA1537 and *E coli* strain WP2 uvrA in the presence or absence of metabolic activation. The mean number of revertant colonies per dose level and strain were not reported in the study summary.

Under the conditions of the study, the test material was considered positive in the presence of metabolic activation in *S. typhimurium* strains TA 100 and TA 1535.

The dossier submitter notes that the registration dossier has assigned a Klimisch score of 4 and indicates that the study report is in Japanese. The dossier submitter notes that the study summary provides limited details which makes assessment of the reliability of the study difficult.

3.8.1.3 *In vitro* mammalian gene mutation test - 1

Study reference:

Anonymous, 1980. Bakelite cycloaliphatic epoxy resin ERL-4221: *in vitro* mutagenesis studies, (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

Non-guideline study. The study pre-dated the adoption of OECD 476 (*in vitro* mammalian gene mutation test) but the method employed was similar with the following deviations: limited reporting of the method, no information was reported on culture/cell density, a longer expression time was used, no information on whether the reported mutant frequency was corrected for cloning efficiency and no reporting of cytotoxicity or mutant frequency data per dose. Not GLP compliant. Unpublished study.

- *Year of study:* 1980
- *Number of replicates:* Two.
- *Positive and negative control groups:*
    - *Positive controls:* N-dimethylnitrosamine (+S9); ethylmethanesulphonate (-S9).
    - *Negative control:* Untreated cells.
    - *Vehicle control:* Dimethyl sulfoxide
- *Criteria for evaluating results:* The test was considered positive or negative depending on the level of statistical significance compared with the concurrent control and whether there was evidence of a dose response following treatment.

Test substance

- *Name:* Epoxy resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* Not reported.
- *Impurities*: Not reported.
- *Batch number:* 42-136.

Administration/exposure

- *Strain or cell type or cell line, target gene:* Chinese hamster ovary cells, *HGRPT* gene.
- *Type and composition of metabolic activation system:* Liver S9 mix prepared from livers of male Sprague Dawley rats treated with Arochlor 1254.
- *Test concentrations:* Five concentrations between 6.25 x 10^{-4} % and 100 x 10^{-4} % (-S9) and 12.5 x 10^{-4} % and 200 x 10^{-4} % (+S9). Exact concentrations not reported.
- *Method:*
    - *Exposure time:* 16 hours (-S9) and 5 hours (+S9).
    - *Expression time:* 7 – 9 days.
    - *Number of cells evaluated:* 100 cells/dish (200/dose) were evaluated for frequency of mutants per 10^6 viable cells.
    - *Cytotoxicity:* Assessed by the percentage of cells surviving treatment, frequency of mutant colonies and number of mutants per 10^6 viable cells.
- *Statistical methods:* Data was transformed using Box-Cox Transformation and was then analysed using Student's T-test.

Results and discussion

Cytotoxicity was reported at 100 x 10^{-4} % in the absence of metabolic activation. No data on cytotoxicity in cells treated in the presence of metabolic activation was obtained due to equipment malfunction. No dose related increase in mutation frequency was reported with or without metabolic activation. The mutation frequency per dose level were not reported in the study summary.

Under the conditions of the study, the test material was considered negative in the presence and absence of metabolic activation.

3.8.1.4 *In vitro* mammalian gene mutation test - 2

Study reference:

Anonymous, 1984. L5178Y/TK +/- mouse lymphoma mutagenicity test – *in vitro*, (Unpublished Report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

Non-guideline study. The study pre-dated the adoption of the OECD 490 (*in vitro* mammalian gene mutation test using the thymidine kinase gene) but the method employed was similar with the following deviations: limited reporting of the method, different positive controls were used, no information was reported on the acceptable spontaneous mutant frequency, a different selective agent was used, no sizing of mutant colonies was mentioned and there was no reporting of cytotoxicity or mutant frequency data per dose. GLP compliant. Unpublished study.

- *Year of study:* 1984
- *Number of replicates:* Not reported.
- *Positive and negative control groups:*
    - *Positive controls:* N-dimethylnitrosamine (+S9); ethylmethanesulphonate (-S9).
    - *Negative control:* Untreated cells.
    - *Vehicle control:* Dimethyl sulfoxide.
- *Criteria for evaluating results:* The test was considered positive if the mutant colony count at any test concentration was a factor of 2.5 or more greater than that in the solvent control.

Test substance

- *Name:* TK 10 310 (ARALDIT CY 179) (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* Not reported.
- *Impurities*: Not reported.
- *Batch number:* 608632.

Administration/exposure

- *Strain or cell type or cell line, target gene:* Mouse lymphoma (L5178Y), subline TK $^{+/-}$. Prior to the study, cells from growing stock cultures were cleansed of spontaneous TK$^{-/-}$ mutants by exposing to

a combination of thymidine, hypoxanthine, methotrexate and glycine for 24 hours. Following this cells were incubated with thymidine, hypoxanthine and glycine for a further 3 days before use.

- *Type and composition of metabolic activation system:* S9 mix derived from rat liver. Preparation method not reported.
- *Test concentrations:* 12.5, 25, 50, 100, 150, 200 and 250 µg/ml. The highest dose of 250 µg/ml was selected due to the solubility limit of the test material.
- *Method:*
  - *Exposure duration:* 4 hours.
  - *Expression time:* 3 days.
  - *Selection time and agent:* 14 days for mutant selection and 11-12 days for viability. The selection agent was 5-bromodeoxyuridine.
  - *Number of cells evaluated:* 4 x 10^5 cells per tube for mutant selection and 200 cells per tube for viability control.
  - *Cytotoxicity*: Assessed by cloning efficiency, relative total growth and mutant frequency.
- *Statistical methods:* Not reported.

Results and discussion

No cytotoxicity was reported up to the highest dose tested. An increase in the mutant colony count was reported at 150 µg/ml and above in the presence of metabolic activation and at 100 µg/ml and above in the absence of metabolic activation. The absolute values were not reported other than for the highest concentration, the solvent control and the positive control. In the presence of metabolic activation, the mutant frequency at 250 µg/ml was 16.9 x 10^{-6} compared with 4.6 x 10^{-6} in the solvent control. In the absence of metabolic activation, the mutant frequency at 250 µg/ml was 557.7 x 10^{-6} compared with 2.9 x 10^{-6} in the solvent control. No information was provided on colony sizing.

Table A2: Summary of the results in the *in vitro* mammalian gene mutation test with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 1984. ECHA dissemination site, 2021)

	- S9			+ S9		
	250 µg/ml	**Solvent control**	**Positive control**	**250 µg/ml**	**Solvent control**	**Positive control**
Mutant frequency	557.7 x 10^{-6}	2.9 x 10^{-6}	274.6 x 10^{-6}	16.9 x 10^{-6}	4.6 x 10^{-6}	Not reported

Under the conditions of the study, the test material was considered positive in the presence and absence of metabolic activation.

3.8.1.5 *In vitro* sister chromatid exchange assay in mammalian cells

Study reference:

Anonymous, 1980. Bakelite cycloaliphatic epoxy resin ERL-4221: *in vitro* mutagenesis studies, (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

Non-guideline study. The endpoint study record was entitled "*in vitro* DNA damage and/or repair" however the study was an *in vitro* sister chromatid exchange (SCE) assay in mammalian cells. The study pre-dated the adoption of the now deleted OECD 479 (*in vitro* sister chromatid exchange assay in mammalian cells) but the method employed was similar, with the following deviations: limited reporting of the method, a lower number of cells per concentration were assessed for SCE, the test was only performed without metabolic activation and there was no reporting of cytotoxicity or mutant frequency data per dose. Not GLP compliant. Unpublished study.

- *Year of study:* 1980
- *Number of replicates:* Triplicate although no assessment of SCE was undertaken in two replicates due to excessive toxicity.
- *Positive and negative control groups:*
    - *Positive control:* Ethylmethanesulphonate.
    - *Negative control:* Untreated cells.
    - *Vehicle control:* Dimethyl sulfoxide.
- *Criteria for evaluating results:* The test was considered positive if there was a dose dependent increase in SCE.

Test substance

- *Name:* Epoxy resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* Not reported.
- *Impurities*: Not reported.
- *Batch number:* 42-136.

Administration/exposure

- *Strain or cell type or cell line, target gene:* Chinese hamster ovary cells.
- *Type and composition of metabolic activation system:* None.
- *Test concentrations:* 3.125×10^{-4} % to 100×10^{-4} % by volume. Exact concentrations not reported.
- *Method:*
    - *Medium:* BrdU-containing medium.
    - *Pre-incubation time:* 20 hours.
    - *Exposure duration:* 5 hours.
    - *Expression time:* 24 hours.
    - *Spindle inhibitor:* 0.2 µg/ml colchicine or 0.1 µg/ml colcemide added 1 to 2 hours before harvesting cells.

- *Stain:* Hoechst 33258 dye.
- *Number of cells evaluated:* Minimum of 15 cells per dose level.

- *Statistical methods:* The study summary indicates that the original data were analysed by an appropriate parametric test but no further details are provided.

Results and discussion

The study summary states that excessive cytotoxicity was observed in the first two replicates, reported as a decrease in the number of mitotic cells and chromosome preparations that were not suitable for scoring (no details on the number of mitotic cells observed or the concentrations tested). For this reason, the results from only one replicate are reported.

A statistically significant increase in the SCE frequency was observed in three of the six concentrations tested (exact concentrations not reported). Under the conditions of the study, the test material was considered positive in the absence of metabolic activation.

3.8.1.6 *In vitro* unscheduled DNA synthesis (UDS) in mammalian cells

Study reference:

Anonymous, 1980. Bakelite cycloaliphatic epoxy resin ERL-4221: *in vitro* mutagenesis studies, (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

Non-guideline study: *in vitro* unscheduled DNA synthesis (UDS) in mammalian cells. The study pre-dated the adoption of the now OECD 482 (DNA damage an repair/unscheduled DNA synthesis in mammalian cells *in vitro*) but the method employed was similar with the following deviations: the number of replicates were not reported, limited reporting of the method, the number of cells per culture assessed was not reported, and there was no reporting of cytotoxicity or mutant frequency data per dose. Not GLP compliant. Unpublished study.

- *Year of study:* 1980.
- *Number of replicates:* Not reported.
- *Positive and negative control groups:*
  - *Positive controls:* N-dimethylnitrosamine (indirect acting mutagens – it is noted this is not an appropriate positive control for this test system) and 4-nitroquinoline-N-oxide (direct acting mutagens).
  - *Negative control:* None specified.
  - *Vehicle control:* Dimethyl sulfoxide.
- *Criteria for evaluating results:* The test was considered positive if there was a dose dependent, statistically significant increase in the UDS activity measured as average DPM per 10^6 viable hepatocytes.

Test substance

- *Name:* Epoxy resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.

- *Degree of purity:* Not reported.
- *Impurities*: Not reported.
- *Batch number:* 42-136.

Administration/exposure

- *Strain or cell type or cell line, target gene:* Hepatocytes derived from rat liver.
- *Type and composition of metabolic activation system:* None. The study was conducted using primary hepatocytes.
- *Test concentrations:* Six concentration between 1.0 x 10^{-4} % and 1000 x 10^{-4} % by volume. Exact concentrations not reported.
- *Method:*
    - *Medium:* ^{3}H-thymidine and hydroxyurea.
    - *Preincubation period:* 1 hour.
    - *Exposure duration:* 2 hours.
    - *Cytotoxicity*: Assessed by mitotic index, cloning efficiency and relative total growth, nuclear bound radiolabel.
- *Statistical methods:* The study summary indicates that the original data were analysed by an appropriate parametric test but no further details are provided.

Results and discussion

The study summary reports that only one concentration (the exact concentration is not reported) induced a statistically significant increase in ^{3}H-thymidine incorporation. A gradual decrease in the amount of radioactivity incorporation over the range of tested concentrations was reported, which was concluded in the study summary as evidence of cytotoxicity. Absolute values relating to cytotoxicity were not reported. The study summary reports that two of the six tested concentrations (the exact concentrations are not reported) induced a statistically significant increase in UDS. The study summary also reports that the three lowest concentrations (the exact concentrations are not reported) produced "highly numerically elevated levels of UDS activity" but which were not statistically significant. The study summary concludes that result to be equivocal.

The dossier submitter notes that the study summary includes limited details which makes evaluation of the reported equivocal result difficult.

3.8.2 Animal data

3.8.2.1 Transgenic rodent somatic and germ cell gene mutation assay

Study reference:

Anonymous, 2016. Gene mutation assay of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate in Muta mouse. (Unpublished report).

Detailed study summary and results:

Test type

OECD 488: transgenic rodent somatic and germ cell gene mutation (TGR) assay. A sampling time of "28 + 3 days" was used, which is acceptable for the assessment of somatic tissues but not optimal for the assessment of germ cells. GLP compliant.

Test substance

- *Name:* 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate. Identical to substance identified in CLH dossier.
- *Degree of purity:* 96 %
- *Impurities:* Not reported.
- *Batch number:* CELP-FB-008.

Test animals

- *Species/strain/sex:* mouse (transgenic), CD2-LacZ80/HazfBR (MutaMouse), male.
- *No. of animals per sex per dose:* 5 males/group.
- *Age and weight at the study initiation:* 9 weeks old and 24.4 – 28.1 g.

Administration/exposure

- *Doses/concentration levels:* 0, 250, 500 and 1000 mg/kg bw/day. Doses were selected based on the results of a range finding study where no signs of toxicity were reported up to 1000 mg/kg bw/day.
- *Vehicle:* Corn oil.
- *Route of administration:* Oral gavage.
- *Duration of treatment:* Animals were treated for 28 consecutive days.
- *Positive control groups and treatment:* 100 mg/kg bw/day N-ethyl-N-nitrosourea administered via i.p. for 2 days.
- *Historical control data*: Mutant frequency in liver, stomach and testis from negative control animals in TGR assays (lacZ assay) with male MutaMouse conducted in the test laboratory are reported.
- *Tissue selection and justification*:
    - Liver: main site of metabolism.
    - Forestomach: site of first contact following oral administration.
    - Nasal tissue: target organ in the 90-day oral repeated dose toxicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3- carboxylate.
    - Germ cells: collected in case future assessment of germ cell mutagenicity was required.
- *Method:* Three days after the final dose, animals were sacrificed. Liver, forestomach, nasal cavity, seminiferous tubules and vas deferens/cauda epididymis were removed, prepared and stored in an ultra low temperature freezer (-80 ^{0}C). Germ cells (spermatozoa, spermatid and spermatocytes) were collected from seminiferous tubules and vas deferens/cauda epididymis. For the liver, forestomach, and germ cell sample, genomic DNA was extracted from each animal. Due to the small amount of nasal tissue extracted from each animal, the nasal tissue was pooled per treatment group and the genomic DNA was extracted from the pooled sample. The genomic DNA was then packaged using Transpack packaging extract. The packaged DNA samples were then incubated with an *Escherichia coli C (lacZ^{-}, gal E^{-})* solution and the number of plaques formed were counted.

- *Assessment:* The total number of plaques were counted. The number of mutant plaques and the mutant frequency was calculated.
- *Statistical methods:* Bartlett's test for homogeneity of variance, Dunnett's multiple comparison test, Steel's test, Student's t-test, Aspin-Welch's t-test and Cochran-Armitage trend test.

Results and discussion

No clinical signs of toxicity and no effect on body weights were reported in any of the treatment groups. A slight increase in absolute and relative liver weight was observed at 1000 mg/kg bw/day when compared with the control. No other organ weights were reported.

Table A3: Body weight and liver weight data in male mice in the TGR assay with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 2016)

Dose group (mg/kg bw/day)	Mean terminal body weight (g)	Mean liver weight (g)	Mean liver weight per body weight (%)
0	27.3 ± 1.3	1.15 ± 0.07	4.21 ± 0.2
250	27.9 ± 1.1	1.17 ± 0.05	4.21 ± 0.2
500	27.4 ± 0.9	1.21 ± 0.04	4.43 ± 0.15
1000	27.1 ± 2.6	1.25 ± 0.13	4.6 ± 0.29
Positive control	26.0 ± 1.1	1.22 ± 0.08	4.68 ± 0.19

A statistically significant increase in mutant frequency was observed in the forestomach and liver at 1000 mg/kg bw/day when compared to the concurrent negative control. The mean mutant frequencies (x 10^{-6}) in the forestomach were reported to be 49.1 ± 11.7, 52.2 ± 15.4, 54.9 ± 5 and 78.5 ± 10.7 at 0, 250, 500 and 1000 mg/kg bw/day, respectively. The study report noted that although the increase in mutant frequencies observed in the forestomach at 1000 mg/kg bw/day (78.5 ± 10.7 x 10^{-6}) was only marginally outside the acceptable range of the test laboratory (15.6 x 10^{-6} – 78.0 x 10^{-6}), the increase was considered to be biologically relevant and therefore the study authors concluded that under the conditions of the study, the test material induced gene mutations in the forestomach. The mean mutant frequencies (x 10^{-6}) in the liver were reported to be 48.2 ± 14.1, 62 ± 12.5, 61.2 ± 13.8 and 78.2 ± 18.1 at 0, 250, 500 and 1000 mg/kg bw/day, respectively. The study report noted that as the increase in the mutant frequency in the liver at 1000 mg/kg bw/day group (78.2 ± 18.1 x 10^{-6}) was within the acceptable ranges of the test laboratory for this tissue (0.6 x 10^{-6} – 99.6 x 10^{-6}), it was considered marginal and not biologically significant. No increase in mutant frequency was observed in nasal tissue or germ cells at any dose. The positive control substance elicited a statistically significant increase in mutant frequency in the four tissue samples when compared with the concurrent negative control.

Table A4: Mutant frequencies in male mice in the TGR assay with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 2016)

	Mean mutant frequency (x 10^{-6})			
Dose group (mg/kg bw/day)	**Liver**	**Forestomach**	**Nasal tissue**	**Germ cells**
0	48.2 ± 14.1	49.1 ± 11.7	53.7	32.6 ± 5.8
250	62.0 ± 12.5	52.2 ± 15.4	40.6*	33.7 ± 5.5
500	61.2 ± 13.8	54.9 ± 5.0	50.2	40.3 ± 17.8
1000	78.2 ± 18.1*	78.5 ± 10.7*	54.3	42.4 ± 8.8
Positive control	143.8 ± 21.7*	624.7 ± 96.1	215.7*	82.1 ± 26.4*

***** P ≤ 0.05

Table A5: Historical negative control data for the TGR assay (*lacZ* assay) (Anonymous, 2016)

		Mutant frequency (x 10^{-6})		
Organ	**n**	**Mean**	**Range**	**Acceptable range#**
Liver	137	50.1 ± 16.5	16.6 – 95.0	0.6 – 99.6
Stomach	43	46.8 ± 10.4	31.1 – 84.7	15.6 – 78.0
Testis	10	46.6 ± 27.7	12.2 – 83.5	-
Nasal tissue	-	-	-	-

Acceptable range reported as mean ± 3 SD

The dossier submitter agrees that the increase in the mutant frequency in the forestomach at 1000 mg/kg bw/day is biologically and statistically significant. Therefore, the dossier submitter considers that under the conditions of the study, the increase in mutant frequency in the forestomach, as the site of first contact following oral administration, indicates that the test material is a direct acting mutagen.

3.8.2.2 Unscheduled DNA synthesis test with mammalian liver cells *in vivo*

Study reference:

Anonymous, 1999. Unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo*. (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

OECD 486: unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo*. The study deviated from the test guideline in that the number of slides evaluated per animal, the number of cells scored for each animal and individual and group data was not reported. GLP compliant study.

Test substance

- *Name:* Union Carbide Cycloaliphatic Epoxy Resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* 89 %.

- *Impurities:* Not reported.
- *Batch number:* TF3-24462.

Test animals

- *Species/strain/sex:* Rat, Sprague Dawley, male.
- *No. of animals per sex per dose:* 10 males/dose.
- *Age and weight at the study initiation:* 8 weeks old and 257.4 g – 280.6 g.

Administration/exposure

- *Doses/concentration levels:* 0, 500, 1000 and 2000 mg/kg bw/day. The study summary indicates that the test doses were corrected for the purity of the test material (89 %) but no further details are reported. Doses were selected based on the results of a range finding study in which mortality was observed at ≥4000 mg/kg bw and clinical signs of toxicity including lethargy, piloerection, diarrhoea, tremors and crusty eyes were observed at doses of between 500 mg/kg bw to 5000 mg/kg bw.
- *Vehicle:* Water.
- *Route of administration:* Oral gavage.
- *Duration of treatment:* Single dose.
- *Post exposure period:* 2 to 4 hours or 12 to 16 hours.
- *Positive control group:* 35 mg/kg bw N-dimethylnitrosamine.
- *Historical control data*: None reported.
- *Method:* 3 days after administration of the test material, animals were sacrificed and livers removed. Hepatocytes were isolated and plated. 90 to 180 minute after plating, hepatocytes were washed once with 10 µCi ^{3}H-thymidine. After 4 hours, ^{3}H-thymidine was remove and the cultures washed 3 times in a medium containing cold thymidine and then incubated for 17-20 hours in a cold thymidine medium. Cells were then fixed and at least 3 slides per animal were dipped in NTB-2 emulsion and stored for 5-12 days in light boxes. Slides were then developed and stained.
- *Criteria for scoring and number of cells analysed per animal:* Not reported.
- *Assessment:* An increase in mean net nuclear grain count at least 5 counts over the negative control was considered significant. The test was considered positive if there was a dose-related significant increase in mean net nuclear counts in at least one dose over the negative control or a significant increase in mean net nuclear counts in two successive doses without a dose response.
- *Statistical methods:* Not reported.

Results and discussion

No clinical signs of toxicity other than 1 animal in the high dose group exhibiting diarrhoea at sacrifice.

No increase in mean net nuclear grain counts were reported at any dose. In hepatocytes isolated 2 to 4 hours post exposure, the mean net nuclear grain counts were 0.2, 0.1, -0.2 and -0.3 for the 0, 500, 1000 and 2000 mg/kg bw groups, respectively. The mean net nuclear grain count for the positive control was 17.6. In hepatocytes isolated 12 to 16 hours post exposure, the mean net nuclear grain counts were -0.2, -0.4, -0.2 and 0.4 for the 0, 500, 1000 and 2000 mg/kg bw groups, respectively. The mean net nuclear grain count for the

positive control was 10.5. Under the conditions of the study, the test material did not induce a significant increase in UDS *in vivo*.

3.8.2.3 Mammalian erythrocyte micronucleus test

Study reference:

Anonymous, 1991b. Micronucleus assay in mouse bone marrow erythrocytes: ERL-4221 (Unpublished report).

Detailed study summary and results:

Test type

OECD 474: mammalian erythrocyte micronucleus test. The study deviated from the test guideline in that 1000 rather than 4000 polychromatic erythrocytes per animal were scored. GLP compliant study.

Test substance

- *Name*: ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* Not reported.
- *Impurities:* Not reported.
- *Batch number:* TF3-01564.

Test animals

- *Species/strain/sex:* Mouse, Swiss Albino Crl:CD-1 (ICR)BR.
- *No. of animals per sex per dose:* 5/sex/dose/sampling point.
- *Age and weight at the study initiation:* 63 days old and 19.6 g– 35.2 g (females) and 27.0 g – 37.6 g (males).

Administration/exposure

- *Doses/concentration levels:* 0, 500, 1000 and 2250 mg/kg bw. Doses were selected based on the results of a range finding tests where mice were administered single intraperitoneal injections of 500 – 4000 mg/kg bw of the test material. Mortality and clinical signs of toxicity was observed at ≥ 2500mg/kg bw.
- *Vehicle:* Peanut oil
- *Route of administration:* Intraperitoneal injection
- *Duration of treatment:* Single administration.
- *Positive control group:* Cyclophosphamide administered via intraperitoneal injection.
- *Historical control data*: None reported.
- *Sampling regime*: Animals were sacrificed 24, 48 and 72 hours post treatment. Bone marrow was collected from the femur, stained and fixed.
- *Criteria for scoring and number of cells analysed per animal:* ≥ 1000 erythrocytes were counted. 1000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei (MN). The number of normochromatic erythrocytes (NCE) was also counted.

- *Statistical methods:* One-tailed Fisher exact tests, binomial approximation tests, one-tailed Cochran-Armitage.

Results and discussion

Clinical signs of toxicity including decreased motor activity, collapse, weakness, ataxia and laboured breathing were observed at 2250 mg/kg bw.

A significant decrease in the ratios of PCE/(NCE & PCE) was reported in females at 500 and 2250 mg/kg bw groups at 48 hours, which the study report concludes as evidence of cytotoxicity (values not reported).

No increase in the mean number of MN PCE was observed at any dose or sampling time. The positive control elicited statistically significant increase in MN PCE.

Table A6: Results from the mammalian erythrocyte micronucleus test with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (Anonymous, 1991b)

Dose (mg/kg bw)	Sex	Mean number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes		
		24 hour	48 hour	72 hour
0	M	1.2	0.0	0.4
	F	0.4	0.2	0.2
500	M	0.8	1.0	0.8
	F	1.8	0.2	1.2
1000	M	1.0	1.4	0.8
	F	0.6	0.6	0.6
2250	M	2.0	0.6	0.6
	F	0.8	0.2	1.4
Cyclophosphamide (25 mg/kg bw)	M	9.8*	Not tested	Not tested
	F	11.0*	Not tested	Not tested
Cyclophosphamide (50 mg/kg bw)	M	14.2*	Not tested	Not tested
	F	16.2*	Not tested	Not tested

* $p < 0.01$

The study report notes that when the absolute number of MN PCE from the 5 animals per dose group were pooled (i.e. 5000 PCE), there was a statistically significant increase in MN PCE in males at 1000 mg/kg bw/day at 48 hours only. However, as there was no dose response, this increase was not considered biologically significant.

Under the conditions of the study, the test material did not induce a significant increase in the frequency of MN-PCE.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Dermal carcinogenicity study

Study reference:

Anonymous, 1964. Results of long term test for mouse skin carcinogenicity of four residues from the sevin process and of six epoxides, (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

Non-guideline dermal carcinogenicity study. 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate, acetone or the positive control, 3-methylcholanthrene, were applied dermally to groups of 40 male mice by brushing three times per week for 26 months. Animals were examined for the development of papillomas or carcinomas. Limited details provided in the study summary. Not GLP compliant. Unpublished study.

Test substance

- *Name:* EP-4221 (reported to be 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* 100 %
- *Impurities:* Not reported.
- *Batch number:* Not reported.

Test animals

- *Species/strain/sex:* Mouse, C3H/Anf, male.
- *No. of animals per sex per dose:* 40 mice per group.
- *Age and weight at the study initiation:* 3 months old. Weight not reported.

Administration/exposure

- *Route of administration:* Dermal. Animals were painted with a “brushful” (reported to be approximately 0.1 – 0.2 g) of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate to a clipped intrascapular region approximately 1 cm in diameter, with a single brush stroke against the direction of the hair.
- *Duration of test/exposure period:* Up to 29 months.
- *Doses/concentration levels, rationale for dose level selection:* One treatment group of 4000 – 8000 mg/kg bw undiluted 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (reported to be based on 0.1 – 0.2 g applied to a mouse of 25 g body weight). Rationale for dose selection not reported.
- *Frequency of treatment;* 3 times per week.
- *Control group and treatment:*

  - Positive control: Dermal application of 0.2 % 3-methylcholanthrene (3-MC) in acetone 3 times per week.
  - Negative control: Acetone

- *Historical control data:* None reported.
- *Post exposure observation period:* None.
- *Test substance formulation preparation:* 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was applied undiluted.
- *Satellite groups:* No.
- *Area covered:* Not reported. The application site was reported to be 1 cm in diameter.
- *Occlusion :* Not reported.
- *Total volume applied:* 0.1 - 0.2 g per application.
- *Removal of test substance (e.g. water or solvent):* Not reported.
- *Statistical methods:* Not reported.

Results and discussion

- *Mortality and time to death:* The mortality in the test group was 4/40, 5/40 and 23/40 at 12, 18 and 24 months, respectively. The mortality in the negative control was higher: 8/40, 14/40 and 36/40 at 12, 18 and 24 months, respectively.

Table A7: Mortality observed in a dermal carcinogenicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 1964. ECHA dissemination site, 2021)

	Mortality		
Group	**12 Months**	**18 months**	**24 Months**
Test	4/40	5/40	23/40
Negative control	8/40	14/40	36/40
Positive control	38/40	40/40	40/40

- *Clinical signs:* Not examined
- *Body weight gain:* Not examined
- *Food/water consumption:* Not examined
- *Ophthalmoscopic examination:* Not examined
- *Clinical chemistry:* Not examined
- *Haematology:* Not examined
- *Urinalysis:* Not examined
- *Organ weights:* Not examined
- *Necropsy findings: nature and severity:* No non-neoplastic findings reported.

- *Histopathological findings: nature and severity:* No non-neoplastic findings reported.
- *Tumour incidence data by sex, dose and tumour type:* 1/40 animals in the test group developed a skin tumour at 23 months, which was characterised as a papilloma. 2/40 animals in the negative control group developed skin tumours at 23 months, which were characterised as papillomas. In the positive control group, 39/40 animals developed skin tumours from 3 months and the vast majority were characterised as carcinomas.

Table A8: Tumour incidence observed in a dermal carcinogenicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 1964. ECHA dissemination site, 2021)

Group	Appearance of first tumour	Number of mice with papillomas (tumours)	Number of mice with carcinomas	Tumour index	Cancer index
Test	23 months	1/40	0/40	6.7	0.0
Negative control	23 months	2/40	0/40	40.0	0.0
Positive control	3 months	39/40	37/40	100.0	94.9

- *Local or multi-site responses:* Local
- *Progression of lesions to malignancy:* Not applicable.
- *Mode of action (genotoxic, non-genotoxic):* Not applicable.
- *Tumour latency:* Not applicable.

Under the conditions of the study, 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate did not induce the formation of skin tumours in male mice.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Prenatal developmental toxicity study

Study reference:

Anonymous, 2007. A prenatal developmental toxicity study of cycloaliphatic epoxy resin ERL-4221 in rats (Unpublished report).

Detailed study summary and results:

The study summary is included as supporting information for the assessment of specific target organ toxicity – repeated exposure.

Test type

OECD 414: Prenatal developmental toxicity study. GLP compliant.

Test substance

- *Name:* Cycloaliphatic Epoxy Resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* 92.3 %
- *Impurities:* Not reported.
- *Batch number:* 87068.

Test animals

- *Species/strain/sex:* Rat, Crl:CD(SD)IGS BR, female (same strain of males used for mating).
- *No. of animals per sex per dose:* 25/female/dose.
- *Age and weight at the study initiation:* 84 days old and 223 - 307g (female).

Administration/exposure

- *Route of administration:* Oral gavage.
- *Duration and frequency of test/exposure period:* Females were treated daily from gestation day (GD) 6 to 19.
- *Doses/concentration levels:* 0, 5, 25, 125 and 500 mg/kg bw/day.
- *Control group and treatment:* Corn oil via oral gavage.
- *Vehicle:* Corn oil.

Description of test design:

- *Details on mating procedure:* Animals were mated 1 male to 1 female. The length of cohabitation was not reported. Day 0 of pregnancy was determined by the presence of a copulatory plug and/or the presence of sperm in the vaginal smear.
- *Assessment:*
    - Females were sacrificed on GD 20. The number of corpora lutea, resorptions (early and late), implantation sites and foetuses were recorded. The placentae were also examined. Uterus, liver and kidney weights were also recorded.
    - Foetuses: Viable foetuses were sexed, weighed and euthanised. An external examination was performed, including examination of the eyes, palate and external orifices. For late resorptions, crown-rump measurements and degrees of autolysis were recorded if present. Visceral and skeletal examinations were performed.

Results and discussion

All females survived to scheduled sacrifice. At 500 mg/kg bw/day, a statistically significant decrease in mean body weight gain was observed during GD 6-20 (94 g compared with 115 g in the control group). At 125 mg/kg bw/day, maternal body weight gain was reduced at time points during the gestation period but there was no statistically significant effect for the whole gestation period (GD 6-20). A decrease in food consumption was observed at ≥ 125 mg/kg bw/day, which reached statistical significance at various time points.

Absolute mean kidney weights were statistically significantly increased at ≥ 125 mg/kg bw/day. The mean kidney weights were 2.09 g, 2.18 g, 2.15 g, 2.29 g and 2.34 g, at 0, 5, 25, 125 and 500 mg/kg bw/day, respectively. There was a non-statistically significant increase in absolute mean liver weights at ≥ 125 mg/kg

bw/day. The reported weights were 17.46 g, 17.60 g, 17.47 g, 18.52 g and 18.41 g at 0, 5, 25, 125 and 500 mg/kg bw/day, respectively. No effect on uterus weights was observed.

There was no effect on the number of corpora lutea, implantation sites, viable foetuses, sex ratio or resorptions (both early and late). Pre- and post-implantation losses in the treatment group were comparable to the control group. At 500 mg/kg bw/day, foetal body weight was statistically significantly reduced (3.3 g compared with 3.6 g in the control). No skeletal or visceral malformations associated with treatment were observed.

At 500 mg/kg bw/day, mean foetal body weigh was significantly decreased. The mean foetal body weights were reported as 3.6 g, 3.6g, 3.5 g, 3.6 g and 3.3 g at 0, 5, 25, 125 and 500 mg/kg bw/day, respectively. At the same dose, the mean litter incidence of the ossified cervical centrum number 1 was statistically significantly decreased (11.7 %) when compared with the control (25.7 %). The study report notes that the incidence in the high dose group was within the historical control range of the test laboratory (6.58 % - 27.6 %). There was also a non-statistically significant increase in the mean litter incidence of unossified sternebrae numbers 5 and/or 6 (26.4 % compared with 7.6 % in the control) and unossified sternebrae numbers 1, 2, 3 and/or 4 (1.6 % compared with 0.3 % in the control). The study report notes that the incidence of these two variations at 500 mg/kg bw/day was outside the historical control range of the test laboratory (2.13 % – 21.4 % for unossified sternebrae numbers 5 and/or 6 and 0.0 % - 1.0 % for unossified sternebrae numbers 1, 2, 3 and/or 4). The study authors considered that the skeletal variations observed at 500 mg/kg bw/day were indicative of developmental delay.

Table A9: Litter incidence of skeletal variations observed in a prenatal developmental toxicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 2007)

	Dose (mg/kg bw/day)				
	0	**5**	**25**	**125**	**500**
Ossified cervical centrum #1	25.7 ± 22.2	15.9 ± 15.3	20.6 ± 21.2	23.9 ± 21.3	11.7 ± 18.5 *
Unossified sternebra(e) #5 and/or #6	7.6 ± 10.4	5.7 ± 6.3	12.3 ± 16.5	8.8 ± 11.2	26.4 ± 27.5
Unossified sternebra(e) #1, #2, #3 and/or #4	0.3 ± 1.4	0.6 ± 1.8	0.9 ± 3.3	1.1 ± 4.3	1.6 ± 3.6

*$p < 0.01$

3.11 Specific target organ toxicity – single exposure

Not evaluated as part of this dossier.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Repeated dose 90-day oral toxicity study

Study reference:

Anonymous, 2001. A 90-day oral (gavage) toxicity study of cycloaliphatic epoxy resin ERL-4221 in rats. (Unpublished report).

Detailed study summary and results:

Test type

OECD 408: repeated dose 90-day oral toxicity study in rodents. The study deviated from the test guideline in that a functional observation battery was not performed and thyroid hormone levels were not measured. GLP compliant study.

Test substance

- *Name*: Cycloaliphatic Epoxy Resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity*: 87 %.
- *Impurities*: Not reported.
- *Batch number*: 87068.

Test animals

- *Species/strain/sex*: Rat, Crl:CD(SD)IGS BR, male and female.
- *No. of animals per sex per dose*: 25/sex in the 0 and 500 mg/kg bw/day groups and 20/sex in the 5 and 50 mg/kg bw/day groups.
- *Age and weight at the study initiation:* 6 weeks old and 124 g – 179 g (females) and 170 g – 224 g (males).

Administration/exposure

- *Route of administration*: Oral gavage.
- *Duration and frequency of test/exposure period:* Daily administration for 91 - 92 days.
- *Doses/concentration levels, rationale for dose level selection:* 0, 5, 50 and 500 mg/kg bw/day. The dose levels were selected based on the results of a range finding study where 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was administered by oral gavage once daily for 14 days to groups of 20 (10/sex/dose) rats at dose levels of 0, 100, 500, 750 and 1000 mg/kg bw/day. Fine periportal hepatocellular vacuolation was observed in both sexes at ≥ 100 mg/kg bw/day (see summary 3.12.1.2).
- *Post exposure observation period*: 5/sex in the 5 mg/kg bw/day and 50 mg/kg bw/day groups and 10/sex in the 0 and 500 mg/kg bw/day groups were subject to a 28-day recovery period.
- *Vehicle:* Corn oil.
- *Control group and treatment:* Corn oil via oral gavage.
- *Test substance formulation preparation:* Test formulations were prepared daily to ensure homogenous suspensions. Individual animal dosages were adjusted based on body weight data.
- *Actual dose (mg/kg bw/day):* Doses were corrected for the epoxy equivalent weight (EEW) of 92.5 %, which the study report states is a measure of the total epoxide activity of the sample. Doses based on EEW were 0, 50, 50, 500 mg/kg bw/day. Actual administered doses were 0, 5.4, 54, 540 mg/kg bw/day.

- *Stability and homogeneity of the preparation:* The formulations were analysed and found to be homogenous and stable after 3 days at room temperature.
- *Statistical methods*: The majority of parameters were analysed using ANOVA followed by Dunnett's test. The percentages of motile spermatozoa and the sperm with normal morphology were analysed using Kruskal-Wallis non-parametric ANOVA test followed by the Mann-Whitney U-test.

Results and discussion

- *Body weight and body weight changes:*
  - Males: A non-statistically significant decrease in body weight was observed at 500 mg/kg bw/day throughout the study, which did not return to control levels by the end of the recovery period. At week 13, the body weight at 500 mg/kg bw/day was 546 ± 53 g compared with 585 ± 56 g in the control group. At week 17, the body weight at 500 mg/kg bw/day was 575 ± 68 g compared with 617 ± 73 g in the control group.
  - Females: No effect on body weight was observed in females at any dose during the main study or at the end of the recovery period.

Table A10: Body weight data in the repeated dose 90-day oral toxicity study in rodents with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 2001)

	Mean body weight (g)							
	Males				**Females**			
Dose (mg/kg bw/day)	**0**	**5**	**50**	**500**	**0**	**5**	**50**	**500**
Week 0	201 ± 12	197 ± 11	199 ± 12	197 ± 14	151 ± 11	144 ± 12	151 ± 11	149 ± 13
Week 4	400 ± 24	396 ± 30	395 ± 35	386 ± 33	236 ± 17	229 ± 18	239 ± 21	229 ± 18
Week 8	510 ± 40	505 ± 50	500 ± 47	480 ± 47	275 ± 21	268 ± 20	277 ± 25	265 ± 24
Week 13	585 ± 56	586 ± 70	574 ± 64	546 ± 53	293 ± 24	287 ± 21	296 ± 28	285 ± 23
Week 17	617 ± 73	616 ± 73	594 ± 81	575 ± 68	311 ± 19	288 ± 26	318 ± 31	320 ± 33

- *Food/water consumption:* No effects on food consumption. Water consumption not reported.
- *Clinical signs:* All animals survived to scheduled sacrifice. Clinical signs observed in males and females in the 500 mg/kg bw/day group post dosing included salivation, and yellow material on the urogenital area, hind limbs and ventral neck/trunk.
- *Sensory activity, grip strength and motor activity:* Not examined.
- *Ophthalmologic findings:* No treatment related ocular lesions.
- *Haematological findings:* In females at ≥ 50 mg/kg bw/day, there was a statistically significant decrease in neutrophil count and a statistically significant increase in lymphocyte counts at week 13. Both parameters had returned to control levels at the end of the recovery period (week 17).
- *Clinical biochemistry findings:* Blood urea nitrogen (BUN) levels were statistically significantly increased in males and females at ≥ 50 mg/kg bw/day at weeks 5 and 13 when compared with the

control. There was a statistically significant increase in mean phosphorus levels in males at 500 mg/kg bw/day at week 5 and in both males and females at ≥ 50 mg/kg bw/day at week 13 when compared with the control. There was a trend to increased potassium levels with increasing dose, which was statistically significant in females at 500 mg/kg bw/day at week 13.

Creatine kinase levels were statistically significantly decreased in males and females at 500 mg/kg bw/day at week 5 when compared with the control. At week 13, creatine kinase levels were statistically significantly decreased in males at ≥ 5 mg/kg bw/day and females at 500 mg/kg bw/day. Cholesterol levels were statistically significantly decreased in males at 500 mg/kg bw/day at week 5 and 13. In females, cholesterol levels were statistically significantly decreased at 50 mg/kg bw/day at week 13, with a non statistically significant decrease at 500 mg/kg bw/day.

At 500 mg/kg bw/day, direct bilirubin levels were statistically significantly increased in males in week 5 and in males and females in week 13. There was no effect on indirect bilirubin levels. Sorbitol dehydrogenase levels were statistically significantly increased in males and females at 500 mg/kg bw/day at weeks 5 and 13.

At the end of the recovery period at week 17, there was no significant difference in any of the parameters in any of the treatment groups when compared with the control.

Table A11: Clinical chemistry findings at week 13 in the repeated dose 90-day oral toxicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 2001)

Parameter	Week	Dose (mg/ kg bw/day)							
		Males				Females			
		0	5	50	500	0	5	50	500
Urea nitrogen (mg/dL)	5	13.5 ± 2.8	13.6 ± 1.2	23.2 ± 2.1**	25.6 ± 3.9**	16.3 ± 2.1	17.0 ± 3.0	23.5 ± 3.0**	20.8 ± 3.1**
	13	12.9 ± 2.4	13.6 ± 2.0	22.7 ± 2.1**	22.4 ± 3.6**	14.1 ± 2.5	13.8 ± 2.8	18.7 ± 3.1**	17.1 ± 4.3**
	17	17.8 ± 4.3	17.7 ± 3.5	15.2 ± 1.4	16.7 ± 1.4	17.0 ± 2.8	18.2 ± 2.0	19.0 ± 3.0	16.2 ± 2.8
Phosphorus (mg/dL)	5	8.4 ± 0.6	8.6 ± 0.3	8.8 ± 0.5	9.2 ± 0.7*	7.6 ± 0.6	7.9 ± 0.6	8.0 ± 0.7	8.4 ± 0.7
	13	6.2 ± 0.6	6.4 ± 0.6	6.9 ± 0.6 **	7.4 ± 0.5 **	6.0 ± 0.9	6.4 ± 0.6	6.8 ± 0.5**	6.9 ± 0.6 **
	17	6.3 ± 1.7	7.1 ± 0.3	6.4 ± 0.6	6.7 ± 0.8	5.7 ± 0.5	5.6 ± 0.6	5.8 ± 0.6	5.9 ± 0.6
Creatine kinase (U/L)	5	379 ± 155.9	368 ± 145.1	375 ± 102.5	192 ± 77.3 **	567 ± 387.8	658 ± 449.0	465 ± 201.4	135 ± 67.8 *
	13	243 ± 127.2	161 ± 56.6 *	157 ± 57.5 **	67 ± 27.7**	273 ± 170.8	316 ± 193.2	259 ± 158.2	106 ± 56.7 *
	17	251 ± 126.1	362 ± 199.6	218 ± 118.9	494 ± 329.7	324 ± 169.1	345 ± 227.5	337 ± 94.4	425 ± 311.9
Cholesterol (mg/dL)	5	60 ± 7.8	65 ± 12.5	48 ± 12.4	36 ± 13.6**	66 ± 10.5	72 ± 12.8	63 ± 18.2	51 ± 12.0
	13	69 ±	75 ±	56 ±	45 ±	67 ±	74 ±	52 ±	56 ±

Parameter	Week	Dose (mg/ kg bw/day)							
		Males				Females			
		0	5	50	500	0	5	50	500
		11.4	17.8	15.7	16.3**	13.5	16.7	16.0*	18.8
	17	88 ± 15.9	88 ± 25.1	90 ± 10.4	80 ± 22.4	90 ± 31.9	89 ± 7.4	76 ± 7.3	74 ± 23.1
Potassium (mEq/L)	5	5.37 ± 0.5	5.64 ± 0.4	5.58 ± 0.6	5.68 ± 0.4	5.37 ± 0.5	5.59 ± 0.5	5.56 ± 0.3	5.63 ± 0.4
	13	5.09 ± 0.3	5.21 ± 0.3	5.16 ± 0.4	5.32 ± 0.5	4.93 ± 0.6	5.27 ± 0.5	5.32 ± 0.5	5.54 ± 0.6 **
	17	5.52 ± 0.5	5.65 ± 1.0	5.24 ± 0.3	5.79 ± 0.4	5.14 ± 0.3	6.22 ± 1.6*	4.5 ± 0.3	5.16± 0.6
Direct bilirubin (mg/dL)	5	0.04 ± 0.0	0.04 ± 0.0	0.05 ± 0.0	0.08 ± 0.0 **	0.06 ± 0.0	0.04 ± 0.0	0.04 ± 0.0	0.07 ± 0.0
	13	0.04 ± 0.0	0.04 ± 0.0	0.05 ± 0.0	0.06 ± 0.0**	0.04 ± 0.0	0.05 ± 0.0	0.05 ± 0.0	0.08 ± 0.0**
	17	0.00 ± 0.0	0.01 ± 0.0	0.00 ± 0.0	0.02 ± 0.0	0.01 ± 0.0	0.03 ± 0.0	0.01 ± 0.0	0.00 ± 0.0
Sorbitol dehydrogenase (U/L)	5	16.7 ± 4.7	26.8 ± 17.3	22.3 ± 5.9	43.4 ± 20.7**	12.5 ± 2.9	14.1 ± 4.8	22.2 ± 11.1	30.4 ± 18.5**
	13	17.5 ± 4.4	19.6 ± 4.1	20.8 ± 4.2	33.4 ± 13.1**	17.6 ± 5.4	17.9 ± 5.8	21.9 ± 7.9	27.5 ± 8.2**
	17	20.7 ± 5.5	17.4 ± 6.5	26.4 ± 8.0	30.2 ± 28.6	31.8 ± 29.9	18.3 ± 3.3	22.2 ± 8.8	15.3 ± 7.3

* p< 0.05 ** p < 0.01

- *Urinalysis:* At 500 mg/kg bw/day, there was a statistically significant decrease in urine pH in males and females at weeks 5 and males at week 13 when compared with the controls. At this dose, urine creatine levels were also statistically significantly decreased in males at weeks 5 and 13. At the end of the recovery period at week 17, there was no significant difference in any of the parameters in any of the treatment groups when compared with the control.

Table A12: Urinalysis findings at week 13 in the repeated dose 90-day oral toxicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 2001)

Parameter	Week	Dose (mg/kg bw/day)							
		Males				Females			
		0	5	50	500	0	5	50	500
Urine pH	5	7.3 ± 1.1	7.9 ± 0.7	6.8 ± 1.0	6.0 ± 0.7**	7.2 ± 1.1	6.5 ± 1.2	6.7 ± 1.1	5.8 ± 0.4*
	13	6.3 ± 1.0	6.2 ± 0.7	6.3 ± 0.7	5.6 ± 0.5*	6.4 ± 1.2	6.0 ± 0.7	6.0 ± 0.6	5.6 ± 0.8
	17	7.9 ± 1.2	6.9 ± 1.0	7.0 ± 1.0	6.8 ± 1.2	6.9 ± 0.8	7.4 ± 1.5	6.4 ± 0.2	6.3 ± 0.9
Urine creatinine (mg/dL)	5	140.7 ± 64.1	90.5 ± 40.0*	131.4 ± 35.6	85.6 ± 18.0*	72.3 ± 32.7	97.8 ± 30.5	84.3 ± 63.4	64.3 ± 16.8
	13	267.8 ± 115.9	226.2 ± 99.8	249.1 ± 89.0	135.9 ± 38.2**	101.7 ± 75.8	123.1 ± 74.4	107.2 ± 59.3	107.8 ± 44.0
	17	146.1 ± 83.4	187.2 ± 59.1	124.0 ± 92.6	141.1 ± 81.1	101.2 ± 46.5	84.7 ± 27.4	105.5 ± 17.2	81.9 ± 35.6

* p< 0.05 ** p < 0.01

- *Oestrus cycle:* No effect on oestrus cycle.
- *Spermatogenic evaluations:* No effect on mean testicular and epididymal sperm count, sperm production rate, sperm motility or sperm morphology was observed at any dose.
- *Gross pathology findings:* 3/15 males in the 500 mg/kg bw/day group had pale livers at 13 weeks.
- *Organ weights:* Absolute liver weights were statistically significantly increased in females at ≥ 50 mg/kg bw/day (9.48 g at 50 mg/kg bw/day and 9.98 g at 500 mg/kg bw/day compared with 8.3g in the control) and in males at 500 mg/kg bw/day (19.64 g compared with 16.36 g in the control). Relative liver weights were statistically significantly increased in females and males at ≥ 50 mg/kg bw/day.

  Absolute kidney weights were statistically significantly increased in females at 500 mg/kg bw/day (2.14 g compared with 1.89 g in the control) and there was a non-statistically significant increase in males at the same dose (4.57 g compared with 3.96 g in the control). Relative kidney weights were statistically significantly increased in females and males at 500 mg/kg bw/day. At the end of the recovery period at week 17, there was no significant difference in absolute or relative weights of either organ.

Table A13: Organ weights in the repeated dose 90-day oral toxicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 2001)

Organ weights	Dose (mg/kg bw/day)							
	Males				Females			
	0	5	50	500	0	5	50	500
Week 13								
Absolute kidney (g)	3.96 ± 0.4	3.82 ± 0.3	3.91 ± 0.5	4.57 ± 0.6**	1.89 ± 0.2	1.96 ± 0.1	1.97 ± 0.2	2.14 ± 0.1**
Relative kidney (g/100 g)	0.714 ± 0.1	0.690 ± 0.1	0.721 ± 0.1	0.875 ± 0.1**	0.665 ± 0.1	0.705 ± 0.1	0.700 ± 0.1	0.817 ± 0.1 **
Absolute liver (g)	16.36 ± 1.9	16.38 ± 2.7	18.09 ± 2.3	19.64 ± 2.9 **	8.30 ± 0.7	8.21 ± 1.7	9.48 ± 0.9**	9.98 ± 1.0**
Relative liver (g/100 g)	2.932 ± 0.2	2.928 ± 0.2	3.318 ± 0.2**	3.751 ± 0.3**	2.923 ± 0.2	2.937 ± 0.6	3.375 ± 0.3**	3.809 ± 0.3**
Week 17								
Absolute kidney (g)	3.94 ± 0.6	3.96 ± 0.5	4.03 ± 0.6	4.2 ± 0.4	2.02 ± 0.1	1.77 ± 0.2	2.07 ± 0.2	2.16 ± 0.2
Relative kidney (g/100 g)	0.677 ± 0.1	0.670 ± 0.0	0.708 ± 0.0	0.766 ± 0.1	0.696 ± 0.0	0.655 ± 0.0	0.696 ± 0.1	0.719 ± 0.0
Absolute liver (g)	16.18 ± 2.0	16.82 ± 2.5	15.76 ± 3.1	16.52 ± 2.4	8.93 ± 0.7	7.69 ± 1.0	9.25 ± 1.4	9.26 ± 1.4
Relative liver (g/100 g)	2.75 ± 0.1	2.836 ± 0.1	2.757 ± 0.3	2.997 ± 0.4	3.074 ± 0.2	2.842 ± 0.2	3.097 ± 0.2	3.066 ± 0.2

* p< 0.05 ** p < 0.01

- *Histopathology findings:* An increased incidence of periportal hepatocellular vacuolation was observed in males and females at ≥ 50 mg/kg bw/day. The incidence was reported as 4/15, 5/15, 15/15 and 15/15 in males and 2/15, 2/15, 12/15 and 15/15 in females in the 0, 5, 50 and 500 mg/kg bw/day groups, respectively. The severity was reported to be minimal at 0, 5 and 50 mg/kg bw/day and mild at 500 mg/kg bw/day. At the end of the recovery period, the incidence in the treatment groups was comparable to the control.

  Degeneration of the olfactory epithelium of the nasal tissue was observed in males and females at ≥ 50 mg/kg bw/day. The study report states that the degeneration was characterised by the loss of sustentacular cells, vacuolation and desquamation of neuroepithelial cells, which resulted in decreased height of the olfactory epithelium. The incidence was reported as 0/15, 0/15, 2/15 and 12/15 in males and 0/15, 0/15, 3/15 and 13/15 in females for the 0, 5, 50 and 500 mg/kg bw/day treatment groups, respectively. No effect on basal cells, the underlying structures or connective tissue was reported.

Table A14: Incidence of degeneration of olfactory epithelium at week 13 in the repeated dose 90-day oral toxicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3- carboxylate (Anonymous, 2001)

Olfactory epithelium degeneration	**Dose (mg/kg bw/day)**							
	Males				**Females**			
	0	**5**	**50**	**500**	**0**	**5**	**50**	**500**
Number of animals examined	15	15	15	15	15	15	15	15
Cross section of nasal cavity level 1	0	0	0	1	0	0	0	0
Mild	-	-	-	1	-	-	-	-
Cross section of nasal cavity level 2	0	0	2	12	0	0	0	10
Minimal	-	-	-	1	-	-	-	-
Mild	-	-	2	4	-	-	-	2
Moderate	-	-	-	3	-	-	-	8
Severe	-	-	-	4	-	-	-	-
Cross section of nasal cavity level 3	0	0	2	11	0	0	3	13
Minimal	-	-	-	-	-	-	3	-
Mild	-	-	2	2	-	-	-	2
Moderate	-	-	-	7	-	-	-	11
Severe	-	-	-	2	-	-	-	-
Cross section of nasal cavity level 4	0	0	2	11	0	0	1	11
Minimal	-	-	1	-	-	-	1	-
Mild	-	-	1	6	-	-	-	5
Moderate	-	-	-	4	-	-	-	6
Severe	-	-	-	1	-	-	-	-

At the end of the recovery period, olfactory epithelium degeneration was observed in both sexes at ≥ 50 mg/kg bw/day but at a lower incidence: 0/10, 0/5, 2/5 and 7/10 in males and 0/10, 0/5, 3/5 and 9/10 in females in the 0, 5, 50 and 500 mg/kg be/day groups, respectively. Evidence of regenerative changes was reported: basal cell proliferation and regeneration of sustentacular and neuroepithelial cells. Foci of replacement of olfactory epithelium by ciliated columnar epithelium was observed in 6/10 males and 9/10 females at 500 mg/kg bw/day and the study director considered this change to be part of the repair process, suggesting that local damage to basal cells prevented repair to olfactory epithelium.

Table A15: Incidence of degeneration of olfactory epithelium at the end of the recovery period (week 17) in the repeated dose 90-day oral toxicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (Anonymous, 2001)

Olfactory epithelium degeneration	Dose (mg/kg bw/day)							
	Males				Females			
	0	5	50	500	0	5	50	500
Number of animals examined	10	5	5	10	10	5	5	10
Cross section of nasal cavity level 1	0	0	0	0	0	0	0	0
Cross section of nasal cavity level 2	0	0	0	7	0	0	0	3
Minimal	-	-	-	1	-	-	-	-
Mild	-	-	-	6	-	-	-	3
Cross section of nasal cavity level 3	0	0	2	6	0	0	3	7
Minimal	-	-	1	-	-	-	2	-
Mild	-	-	1	6	-	-	1	7
Cross section of nasal cavity level 4	0	0	0	6	0	0	1	5
Minimal	-	-	-	-	-	-	1	-
Mild	-	-	-	6	-	-	-	5

A NOAEL of 5 mg/kg bw/day is identified based on effects observed in the nasal cavity (olfactory epithelial degeneration) and in the liver (increase in absolute liver weight and increased incidence of periportal hepatocellular vacuolation) at 50 and 500 mg/kg bw/day.

3.12.1.2 Repeated dose 14-day oral toxicity study

Study reference:

Anonymous, 2000. A 14-day oral (gavage) range-finding study in rats of cycloaliphatic epoxy resin ERL-4221, (Unpublished report). ECHA Dissemination site, 2021.

Detailed study summary and results:

Test type

The study was performed as a range finding study for the 90-day repeated dose toxicity study (Anonymous 2001, as described in section 3.12.1.1). The study duration was 14 days. No haematological or clinical chemistry analysis, or urinalysis was performed. The study summary does not report incidences of effects observed per dose group. GLP compliant. Unpublished study.

Test substance

- *Name:* Cycloaliphatic Epoxy Resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate). Identical to substance identified in CLH dossier.
- *Degree of purity:* 83.1 %

- *Impurities:* Not reported.
- *Batch number:* 87068

Test animals

- *Species/strain/sex:* Rat, Crl:CD(SD)IGS BR, male and female.
- *No. of animals per sex per dose:* 10/sex/dose.
- *Age and weight at the study initiation:* 44 days old and 197 g – 202 g (females) and 286 g - 291 g (males).

Administration/exposure

- *Route of administration:* Oral gavage.
- *Duration and frequency of test/exposure period:* Daily administration for 14 days.
- *Doses/concentration levels:* 0, 100, 500, 750 and 1000 mg/kg bw/day.
- *Post exposure observation period:* None.
- *Vehicle:* Corn oil.
- *Control group and treatment:* Corn oil via oral gavage.
- *Test substance formulation preparation:* Test formulations were prepared daily to ensure homogenous suspensions.
- *Statistical methods*: ANOVA followed by Dunnett's test.

Results and discussion

- *Body weight and body weight changes:*
    - Males: The study summary reports a dose related decrease in mean body weight (not statistically significant) at ≥ 500 mg/kg bw/day and a statistically significant decrease in body weight gain at ≥ 750 mg/kg bw/day. No body weight data reported.
    - Females: The study summary reports that a slight decrease in mean body weight (6 %, not statistically significant) was observed at 1000 mg/kg bw/day. Body weight gain was reported to be statistically significantly reduced at this dose. No body weight data reported.
- *Food/water consumption:* A decrease in food consumption was reported in the first week of the study in males at ≥ 750 mg/kg bw/day. Water consumption not reported.
- *Clinical signs:* All animals survived to scheduled sacrifice. Salivation was observed in both sexes at ≥ 500 mg/kg bw/day, yellow material in the urogenital region in females at ≥ 750 mg/kg bw/day.
- *Sensory activity, grip strength and motor activity assessments:* Not examined.
- *Ophthalmologic findings:* Not examined.
- *Haematological findings:* Not examined.
- *Clinical biochemistry findings:* Not examined.
- *Urinalysis:* Not examined.
- *Gross pathology findings:* 1/10 males at 500 and 1000 mg/kg bw/day had small testes and epididymis.

- *Organ weights*: In males, absolute liver weights were reported to be increased at ≥ 100 mg/kg bw/day, without an indication of statistically significance, and relative (to body weight) liver weights were statistically significantly increased at ≥ 100 mg/kg bw/day. In females, absolute and relative (to body and brain weights) liver weights were reported to be statistically significantly increased at ≥ 500 mg/kg bw/day. The study summary reports that there were statistically significant changes in absolute or relative weights of spleen, heart, kidneys and thymus of males or females but no information is provided on the doses where these effects were observed. Organ weight values were not reported.
- *Histopathology findings:* Fine periportal hepatocellular vacuolation was observed in males and females at ≥ 100 mg/kg bw/day. The study summary notes that the severity of the lesion appeared to be qualitatively and/or quantitatively greater at ≥ 500 mg/kg bw/day, without providing details of the exact incidences. 1/10 males at 500 and 1000 mg/kg bw/day with small testes and epididymis had mild to moderate seminiferous tubule degeneration of the testes and lumeal cellular debris and hypospermia of the epididymis.

4 ENVIRONMENTAL HAZARDS

Not evaluated as part of this dossier.

5 REFERENCES

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