Committee for Risk Assessment

RAC

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

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

CLH-O-0000007129-71-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

2 June 2022

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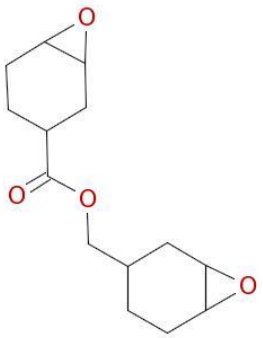
Version number: 2.0 **Date: July 2021**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate.
Other names (usual name, trade name, abbreviation)	7-oxabicyclo 4.1.0 heptane-3-carboxylic acid, 7-oxabicyclo 4.1.0 hept-3-ylmethyl ester.
ISO common name (if available and appropriate)	Not applicable.
EC number (if available and appropriate)	219-207-4
EC name (if available and appropriate)	7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate.
CAS number (if available)	2386-87-0
Other identity code (if available)	Not applicable.
Molecular formula	C14H20O4
Structural formula	
SMILES notation (if available)	O=C(OCC1CCC2OC2C1)C3CCC4OC4C3
Molecular weight or molecular weight range	252.306
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable.
Degree of purity (%) (if relevant for the entry in Annex VI)	Not applicable.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate.	Mono-constituent substance.	None.	Skin Sens. 1B; H317.

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling

No impurities relevant for classification.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

No additives relevant for classification.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	TBD	7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate	219-207-4	2386-87-0	Skin Sens. 1 Muta. 2 STOT RE 2	H317 H341 H373 (nasal cavity)	GHS07 GHS08 Wng	H317 H341 H373 (nasal cavity)	-	-	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate	219-207-4	2386-87-0	Skin Sens. 1 Muta. 2 STOT RE 2	H317 H341 H373 (nasal cavity)	GHS07 GHS08 Wng	H317 H341 H373 (nasal cavity)	-	-	-

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier.	No.
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier.	No.
Oxidising gases	Hazard class not assessed in this dossier.	No.
Gases under pressure	Hazard class not assessed in this dossier.	No.
Flammable liquids	Hazard class not assessed in this dossier.	No.
Flammable solids	Hazard class not assessed in this dossier.	No.
Self-reactive substances	Hazard class not assessed in this dossier.	No.
Pyrophoric liquids	Hazard class not assessed in this dossier.	No.
Pyrophoric solids	Hazard class not assessed in this dossier.	No.
Self-heating substances	Hazard class not assessed in this dossier.	No.
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier.	No.
Oxidising liquids	Hazard class not assessed in this dossier.	No.
Oxidising solids	Hazard class not assessed in this dossier.	No.
Organic peroxides	Hazard class not assessed in this dossier.	No.
Corrosive to metals	Hazard class not assessed in this dossier.	No.
Acute toxicity via oral route	Hazard class not assessed in this dossier.	No.
Acute toxicity via dermal route	Hazard class not assessed in this dossier.	No.
Acute toxicity via inhalation route	Hazard class not assessed in this dossier.	No.
Skin corrosion/irritation	Hazard class not assessed in this dossier.	No.
Serious eye damage/eye irritation	Hazard class not assessed in this dossier.	No.
Respiratory sensitisation	Hazard class not assessed in this dossier.	No.
Skin sensitisation	Harmonised classification proposed.	Yes.
Germ cell mutagenicity	Harmonised classification proposed.	Yes.
Carcinogenicity	Hazard class not assessed in this dossier.	No.
Reproductive toxicity	Hazard class not assessed in this dossier.	No.
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier.	No.
Specific target organ toxicity-repeated exposure	Harmonised classification proposed.	Yes.
Aspiration hazard	Hazard class not assessed in this dossier.	No.
Hazardous to the aquatic environment	Hazard class not assessed in this dossier.	No.
Hazardous to the ozone layer	Hazard class not assessed in this dossier.	No.

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification and labelling for 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate and it was not previously discussed by the Technical Committee for Classification and Labelling under Directive 67/548/EEC.

RAC general comment
The substance subject to the classification proposal is 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo [4.1.0]heptane-3-carboxylate, hereafter referred to by its EC number, EC No. 219-207-4. Ireland concluded in its Substance Evaluation Conclusion and Evaluation Report[1] (2018) for this substance that harmonised classifications for germ cell mutation, STOT RE and skin sensitisation are warranted. Herein these hazard classes are addressed for classification purposes.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was included on the CoRAP for evaluation by Ireland in 2013 to clarify concerns including skin sensitisation, mutagenicity and developmental toxicity. During the evaluation, an additional concern was identified relating to effects in the nasal tissue observed in an oral repeated dose toxicity study. The conclusion by the evaluating MSCA was that harmonised classification and labelling for germ cell mutagenicity, specific target organ toxicity – repeated exposure (STOT RE) and skin sensitisation is warranted[2].

There is no requirement for justification that action is needed at Community level

In accordance with Article 36(1) of CLP, justification for action is not required for substances, which fulfil the classification criteria for carcinogenicity, germ cell mutagenicity or reproductive toxicity. The dossier submitter proposes classification as a category 2 germ cell mutagen and therefore no justification for this hazard class is required.

Justification that action is needed at Community level is required

In accordance with Article 36(3) of CLP, justification for action is required for hazard classes other than those referred to in Article 36(1). The REACH registrant has self-classified 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate as a skin sensitiser category 1B. They have not self classified the substance for germ cell mutagenicity or STOT RE. The dossier submitter considers that the data presented in this dossier supports classification as category 2 germ cell mutagen and category 2 specific

[1] https://echa.europa.eu/documents/10162/b8804bf1-592d-b25e-9f72-89f6f9dedc6b

[2] The substance evaluation conclusion and evaluation report for 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate can be found at: https://echa.europa.eu/documents/10162/b8804bf1-592d-b25e-9f72-89f6f9dedc6b

target organ toxicant following repeated exposure. In addition, the dossier submitter considers that the available data on skin sensitisation does not allow sub-categorisation and thus considers that classification as category 1 skin sensitiser is more appropriate. Thus, in addition to category 2 germ cell mutagen, harmonised classification for the hazard classes skin sensitisation and STOT RE is also proposed due to the disagreement by the dossier submitter with the current self-classification.

5 IDENTIFIED USES

7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate is a cycloaliphatic liquid epoxy resin used in a number of industrial sectors including inks and coatings, electricity and electronics. It also used in the manufacture of polymers.

6 DATA SOURCES

Data for 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate are taken from:

- Publically disseminated REACH registration dossier (ECHA dissemination site, 2021.).
- Unpublished study reports provided by the registrants for the repeated dose toxicity, mutagenicity and reproductive toxicity endpoints.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid.	ECHA dissemination site, 2021	Measured.
Melting/freezing point	No true melting point.	ECHA dissemination site, 2021	Measured. Softening to viscous liquid at -35 to -30°C.
Boiling point	None.	ECHA dissemination site, 2021	Measured. Decomposes on heating without boiling.
Relative density	1.172	ECHA dissemination site, 2021	Measured at 20.1 ^{0}C.
Vapour pressure	2 x 10^{-3} Pa	ECHA dissemination site, 2021	Measured at 25 ^{0}C.
Surface tension	61 mN/m	ECHA dissemination site, 2021	Measured at 20 ^{0}C.
Water solubility	13850 mg/L	ECHA dissemination site, 2021	Measured at 20.2 ^{0}C and pH 7.
Partition coefficient n-octanol/water	Log Pow 1.34	ECHA dissemination site, 2021	Measured at 20 ^{0}C and pH 7.
Flash point	202 ^{0}C	ECHA dissemination site, 2021	Measured at 19.6 ^{0}C and 52.2 % humidity.
Flammability	Non-flammable	ECHA dissemination site, 2021	Estimated based on flash point data.
Explosive properties	Not explosive.	ECHA dissemination site, 2021	Calculated.
Self-ignition temperature	375 ^{0}C.	ECHA dissemination	Measured at 1013 hPa.

Property	Value	Reference	Comment (e.g. measured or estimated)
		site, 2021	
Oxidising properties	Not oxidising.	ECHA dissemination site, 2021	Calculated.
Granulometry	Not applicable.		
Stability in organic solvents and identity of relevant degradation products	No data.		
Dissociation constant	Not applicable.		
Viscosity	241 mPa/s.	ECHA dissemination site, 2021.	Measured at 20 ^{0}C.

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated as part of this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

No data available.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not evaluated as part of this dossier.

10.2 Acute toxicity - dermal route

Not evaluated as part of this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated as part of this dossier.

10.4 Skin corrosion/irritation

Not evaluated as part of this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated as part of this dossier.

10.6 Respiratory sensitisation

No data available.

10.7 Skin sensitisation

Table 8: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Similar to OECD 406: skin sensitisation (guinea pig maximisation test) GLP compliant. 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. No positive control data reported.	Guinea pig, Hartley albino. 10/sex in treatment group and 5/sex in vehicle control group.	ERL-4221 (trade name of 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: not reported). Vehicle: propylene glycol Positive control: dinitrochlorobenzene (not concurrent).	Range finding studies: 2 animals administered 5 % test material in propylene glycol via intradermal injection. Observations made 24 and 48 hours post injection. 6 animals administered 10 %, 25 %, 50 % and 100 % test material to sites on dorsal and lateral areas for 24 hours. Observations made 24 and 48 hours post patch removal. Main study: Induction: 3 pairs of injections of: - FCA/water emulsion - 5 % test material in propylene glycol - 5 % test material in FCA/water emulsion Topical application of 100 % test material on day 7. Duration of topical induction not specified. Challenge: Topical application of 100 % test material for 24 hours on day 21. Dermal assessments 24 and 48 hours post challenge patch removal.	Range finding studies: 5 % intradermal dose resulted in “local necrosis”. No results reported but 100 % selected for main study. Main study: Result: positive 1/10 males died on day 11. Cause of death was not established. At 24 hours 12/19 animals had positive skin reactions. At 48 hours, 8/19 animals had positive skin reactions. No positive skin reactions observed in the vehicle control group at 24 or 48 hour assessments. Positive skin reactions reported in 100 % of animals in the positive control.	Anonymous, 1991a. ECHA dissemination site, 2021.

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

A guinea pig maximisation test conducted with 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate is reported in the REACH registration dossier for the substance. The study is similar to OECD 406 although it was conducted before the adoption of the test guideline, and it is GLP compliant. The dose levels for the main study were selected based on the results of two range finding studies. In the first, 5 % test material in propylene glycol was administered intradermally to two guinea pigs and observations made at 24 and 48 hours post administration. The study summary reports that “local necrosis”, described as no extensive necrosis or ulceration, was observed. No further details are reported. In

the second, 10 %, 25 %, 50 % and 100 % test material were applied to patches on different sites on six guinea pigs and observations made 24 hours after removal of the patches. No results are reported, but 100 % test material was used in the main study for topical application.

In the main study, Hartley albino guinea pigs, 10/sex, received three pairs of intradermal injections on day 0: Freunds Complete Adjuvant (FCA)/water emulsion, 5 % test material in propylene glycol and 5 % test material in FCA/water emulsion. On day 7, the same animals received a topical application of 100 % test material using an occlusive dressing. Animals in the vehicle control (5/sex) were treated in the same way as the test group except that they received propylene glycol or 70 % ethanol instead of the test material. On day 21, the animals in the test and vehicle control groups received a topical application of 100 % test material for 24 hours. Dermal assessments were made 24 and 48 hours later. 1/10 males in the test group died on day 11. The study summary reports that the animal had discoloured lungs, yellow liver colouring and an abdominal cavity filled with blood. The cause of death was not established. No other clinical signs were reported for animals in the test group.

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. In the test group, 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.

Table 9: 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.

Under the conditions of the study, the test material is considered to be a skin sensitiser. Based on the results of this study, the REACH registration dossier for 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate applies a self-classification of skin sensitiser category 1B.

No human data on the skin sensitising effects of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate are available. The dossier submitter notes that the available guinea pig maximisation study summary has some limitations, in particular that there was no rationale provided for the selection of the intradermal induction dose, no individual animal data and no data supporting the statement that the periodic testing of the positive control resulted in 100 % positive reactions. Despite these limitations, a significant increase in positive reactions were observed in the test group. The dossier submitter considers that based on the results of this study, 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate is a skin sensitiser.

Further details on the above study is provided in Annex I to this report

10.7.2 Comparison with the CLP criteria

According to Annex I to the CLP Regulation, substances may be classified as skin sensitisers category 1:

(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

(b) if there are positive results from an appropriate animal test"

No human data on the skin sensitising effects of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate are available. A positive guinea pig maximisation test with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate is available. Based on the results of this study, classification as skin sensitiser category 1 is warranted.

Section 3.4.2.2.1.1 of Annex I to the CLP Regulation states that where data are not sufficient for sub-categorisation, skin sensitisers should be classified in Category 1. An assessment of the need for subcategorization is outlined below.

According to Annex I to the CLP Regulation, substances may be classified as skin sensitisers category 1A where *"…a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans"*. According to Table 3.4.3 of Annex I to the CLP Regulation, for a guinea pig maximisation test this corresponds to a positivity rate of ≥ 30 % at ≤ 0.1 % intradermal induction dose or ≥ 60 % at > 0.1 % to ≤ 1 % intradermal induction dose. According to Annex I to the CLP Regulation, substances may be classified as skin sensitisers category 1B where "…*a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans."* According to Table 3.4.4 of Annex I to the CLP Regulation, for a guinea pig maximisation test this corresponds to a positivity rate of ≥ 30 % to < 60 % at > 0.1 % to ≤ 1% intradermal induction dose or ≥ 30 % at > 1 % intradermal induction dose.

In the available guinea pig maximisation test with 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate, a positive rate of 63 % (at the 24 hour assessment) was observed at an intradermal dose of 5 %. These results are within the range for classification in category 1B. However, the dossier submitter notes that the study summary did not include a rationale for selecting the intradermal dose of 5 % in the range finding study or in the main guinea pig maximisation test. Therefore, it cannot be excluded that a lower (i.e. ≤ 1 %) intradermal dose would have led to positive skin reactions supporting classification as a skin sensitiser category 1A.

ECHA's Guidance on the application of the CLP criteria (version 5.0, July 2017)[3] (CLP Guidance), states, *"when category 1A cannot be excluded, category 1 should be applied instead of category 1B."* Based on the available data, sub-categorisation is not appropriate and classification as skin sensitiser category 1 is warranted.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the available data, classification of 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate as a skin sensitiser category 1 (without sub-categorisation) is warranted. Based on the available data, the assignment of a specific concentration limit is not warranted.

[3] https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

To assess the skin sensitising property of EC No. 219-207-4, the Dossier Submitter (DS) presented the results of the Guinea Pig Maximisation Test (GPMT) (Anonymous, 1991a) summarised in the REACH registration dossier (ECHA, 2021). The study was performed similarly to OECD TG 406 (adopted in 1992) but with some deviations, such as: 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. The study is considered reliable with restrictions.

In the range finding study:

- 2 animals were administered intradermal injections of 0.1 mL of 5 % EC No. 219-207-4 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 of 0.1 mL of 5 % test material was selected for intradermal induction in the main study.

- 6 animals were administered topically 0.1 mL of 10 %, 25 %, 50 % and 100 % EC No. 219-207-4 on 2 × 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. The skin was assessed for signs of erythema, oedema and eschar formation at 24 and 48 hours after patch removal. While no results are reported, the 100 % concentration was selected for topical applications in the main study.

There was no concurrent positive control group in the study. The study summary indicates that the laboratory performed periodic (approximately every 4 to 6 months) studies with dinitrochlorobenzene as the positive control resulting in positive reactions in 100 % of treated animals, which demonstrates the sensitivity of the test system.

In the main study, 10 Hartley guinea pigs of each sex were used in the treatment group and 5 guinea pigs/sex in the vehicle control group (propylene glycol or 70 % ethanol).

For the induction on day 0 the treated animals received three pairs of intradermal injections (0.1 mL each):

- FCA (Freund's Complete Adjuvant)/water emulsion
- 5 % test material in propylene glycol
- 5 % test material in FCA/water emulsion

Test areas were pre-treated on day 6 with 10 % sodium lauryl sulphate. On day 7 exposed animals received a topical application of 0.2 mL of 100 % test material on 2 × 4 cm filter paper which was secured to the test site with an occlusive dressing. The length of the treatment was not stated. The 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.

It was noted that 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.

For the challenge on day 21 the hair was clipped on a 5 × 5 cm area on the flank of each animal. Test and vehicle control animals received a topical application of 0.1 mL of 100 % test material on 2 × 2 cm filter paper for 24 hours. Dermal assessment of all animals was performed 24 and 48 hours after removal of the challenge patches.

At 24 hours after removal of challenge patches positive reactions were observed in the test group in 12/19 animals: 11/19 with score of 1 and 1/19 with score of 2. At 48 hours, 8/19 animals had positive reactions (all with 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.

Based on results of this study DS concluded that EC No. 219-207-4 warrants classification as Skin Sens. 1 without sub-categorisation.

Comments received during consultation

Two MSCAs noted that as no lower doses were tested in the GMPT study, the 1A sub-categorisation cannot be excluded. However, both agreed to the classification as skin sensitiser category 1 (H317) without sub-categorisation.

Assessment and comparison with the classification criteria

To assess the skin sensitisation potential of EC No. 219-207-4, no human data are available, but only 1 animal study, an acceptable GPMT, where EC No. 219-207-4 was given intradermally at 5 % concentration and induced positive skin response in 63 % (24 hours) and 42 % (48 hours) of the animals after removal of challenge patches. This response meets the classification criteria for skin sensitisation sub-category 1B, since the observed response was ≥ 30 % and the intradermal induction dose was > 1 %. However, according to the CLP Regulation (point 3.4.2.2.1.1) skin sensitisers will be classified in category 1 where data are not sufficient for sub-categorisation.

In order to classify a substance into sub-category 1A in the GPMT:

- at least 30 % of animals should be sensitised after intradermal induction dose of ≤ 0.1 % or
- at least 60 % of animals should be sensitised after intradermal induction dose of > 0.1 % to ≤ 1 %.

The intradermal induction doses of ≤ 1 % were not used in the study. Consequently, the study cannot be used to demonstrate whether or not the criteria for category 1A have been met. Therefore, sub-category 1A cannot be excluded.

In the opinion of RAC, since EC No. 219-207-4 meets the classification criteria for skin sensitisation, but no conclusion on sub-categorisation can be drawn, the substance **warrants classification as Skin Sens. 1; H317 (May cause an allergic skin reaction) without sub-categorisation.**

10.8 Germ cell mutagenicity

Table 10: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OECD 471: bacterial reverse mutation test. GLP compliant. Triplicate plates per dose, test run in duplicate. Mean number of revertant colonies per dose level and strain not reported. No information on cytotoxicity.	7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (purity: not reported).	*S. typhimurium strains* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA. 156, 313, 625, 1250, 2500 and 5000 µg/ plate. ± Metabolic activation with S9 mix. Preparation details not reported. Vehicle control: Dimethyl sulfoxide Positive controls: 2-aminoanthracene (+S9) 2-acetylaminofluorene, sodium azide, 9-aminoacridine, N-ethyl-N-nitro-N-nitrosoguanidine (-S9) Number of replicates: 3 plates/dose and test run in duplicate. Reliability: reliable.	Result: positive ± metabolic activation. ↑ Revertant colonies in *S. typhimurium strains* TA 100 and TA 1535 (+S9) & *E. coli* strain WP2 uvrA (± S9) No information on cytotoxicity reported.	Anonymous, 1995. ECHA dissemination site, 2021.
Non-guideline: *in vitro* gene mutation study in bacteria. Not GLP compliant. Study pre-dated the adoption of OECD 471 (bacterial reverse mutation test) but the method employed was reported to be similar, with the following deviations: positive controls per strain, the number of replicates per dose and the mean number of revertant colonies per dose level/strain were	Celloxide 2021P (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: not reported).	*S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E coli* strain WP2 uvrA. 100, 250, 500, 1000, 2000 and 5000 µg/plate. ± Metabolic activation with S9 mix. Preparation details not reported. Vehicle control: dimethyl sulfoxide Positive controls: 2-aminoanthracene; 2-acetylaminofluorene, 9-aminoacridine and N-ethyl-N-nitro-N-nitrosoguanidine. Number of cells evaluated: not reported. Number of replicates: Not	Result: positive + metabolic activation. ↑ Revertant colonies in *S. typhimurium* strains TA 100 and TA 1535 (+S9). No cytotoxicity up to 5000 µg/plate.	Anonymous, 1987. ECHA dissemination site, 2021.

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
not reported.		reported. Reliability: unreliable.		
Non-guideline: *in vitro* gene mutation in mammalian cells. Not GLP compliant Study pre-dated 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 on culture/cell density, a longer expression time used, no information on whether the reported mutant frequency was corrected for cloning efficiency and no reporting of cytotoxicity or mutant frequency per dose.	Epoxy resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: not reported).	Chinese hamster ovary cells (*HGRPT* gene). 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. ± Metabolic activation with rat liver S9 (Arochlor 1254 induced). Vehicle control: dimethyl sulfoxide Negative control: untreated cells. Positive controls: N-dimethylnitrosamine (+S9); ethylmethanesulphonate (-S9) Exposure time: 16 hours (-S9), 5 hours (+S9). Expression period: 7-9 days. Number of cells evaluated: 200 cells/dose for frequency of mutants per 10^6 viable cells. Number of replicates: 2 Reliability: reliable.	Result: negative ± metabolic activation. Cytotoxicity reported at 100 x 10^{-4} % (-S9). No cytotoxicity data available for (+S9).	Anonymous, 1980. ECHA dissemination site, 2021.
Non-guideline: *in vitro* gene mutation in mammalian cells. GLP compliant. Study pre-dated the adoption of OECD 490 (*in vitro* mammalian gene mutation test using the thymidine kinase gene) but the method employed was similar with the following	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) (purity: not reported).	Mouse lymphoma (L5178Y), subline TK $^{+/-}$ 12.5, 25, 50, 100, 150, 200 and 250 µg/ml. ± Metabolic activation with S9 mix. Preparation details not reported. Vehicle control: dimethyl sulfoxide. Negative control: untreated cells. Positive controls: N-dimethylnitrosamine	Result: positive ± metabolic activation. ↑ Mutant colony count at ≥ 150 µg/ml (+S9) and ≥ 100 µg/ml (-S9). No cytotoxicity reported up to 250 µg/ml.	Anonymous, 1984. ECHA dissemination site, 2021.

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
deviations: limited reporting of the method, different positive controls and selective agent used to those recommended in OECD 490, no information reported on the acceptable spontaneous mutant frequency, no sizing of mutant colonies and no reporting of cytotoxicity or mutant frequency data per dose.		(+S9); ethylmethanesulphonate (-S9) Exposure period: 4 hours. Expression period: 3 days. Selection time: 14 days for mutant selection and 11-12 for viability. Selection agent: 5-bromodeoxyuridine. Number of cells evaluated: 4 x 10^5 cells/tube for mutant selection and 200 cells/tube for viability control. Number of replicates: not reported. Reliability: reliable.		
Non-guideline: *in vitro* sister chromatid exchange (SCE) assay in mammalian cells. Not GLP compliant. 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/ concentration assessed, the test was performed without metabolic activation and there was no reporting of cytotoxicity or mutant frequency data per dose.	Epoxy resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: not reported).	Chinese hamster ovary cells. 3.125 x 10^{-4} % to 100 x 10^{-4} % by volume. Exact concentrations not reported. No metabolic activation. Vehicle control: dimethyl sulfoxide. Negative control: untreated cells. Positive control: ethylmethanesulphonate. 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 1- 2 hours prior to harvest. Number of cells evaluated: Minimum of 15 cells/dose. Number of replicates: 3 Reliability: reliable.	Result: positive – metabolic activation. ↑ SCE frequency in 3 of 6 concentrations tested (exact concentrations not reported). Excessive toxicity reported in first two replicates, reported as ↓ in the number of mitotic cells and chromosome preparations not suitable for scoring. Based on this, SCE scoring from only one replicate reported.	Anonymous, 1980. ECHA dissemination site, 2021.

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Non-guideline: *in vitro* unscheduled DNA synthesis in mammalian cells. Not GLP compliant. Study pre-dated the adoption of OECD 482 (DNA damage and repair/unscheduled DNA synthesis in mammalian cells *in vitro*) but the method employed was similar with the following deviations: the number of replicates and the number of cells per culture assessed were not reported, no reporting of cytotoxicity or mutant frequency data per dose and limited reporting of the method.	Epoxy resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: not reported).	Hepatocytes derived from rat liver. Six concentrations between 1.0 x 10^{-4} % and 1000 x 10^{-4} % by volume. Exact concentrations not reported. Vehicle control: dimethyl sulfoxide. Positive controls: N-dimethylnitrosamine, 4-nitroquinoline-N-oxide. Pre-incubation period: 1 hour. Exposure duration: 2 hours. Number of replicates: not reported. Reliability: unreliable.	Result: equivocal. ↑ UDS in 2 of 6 concentrations (exact concentrations not reported). 3 lowest concentrations also reported to have ↑ levels of UDS activity (exact concentrations not reported).	Anonymous, 1980. ECHA dissemination site, 2021.

Table 11: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
OECD 488: transgenic rodent somatic and germ cell gene mutation (TGR) assay. GLP compliant. Sampling time of "28 +3 days" not optimal for germ cell mutagenicity assessment.	7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (purity: 96 %).	5 male CD2-LacZ80/HazfBR (MutaMouse)/group. 0, 250, 500 and 1000 mg/kg bw/day via gavage for 28 days. Vehicle: corn oil. Positive control: N-ethyl-N-nitrosourea. Sampling time: "28 + 3 days". Three days after the final dose, animals were sacrificed and genomic DNA extracted. Tissue selection: liver, forestomach, nasal tissue &	Result: positive. ↑ Mutant frequency in forestomach & liver at 1000 mg/kg bw/day. No ↑ in mutant frequency in nasal tissue or germ cells. ↑ Absolute & relative liver weight at 1000 mg/kg bw/day.	Anonymous, 2016.

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		germ cells (spermatozoa, spermatid and spermatocytes from seminiferous tubules and vas deferens/caudia epididymis). Reliability: reliable.		
OECD 486: unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo.* GLP compliant. Number of slides evaluated per animal, the number of cells score per animal, and individual and group data not reported.	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) (purity: 89 %).	10 male Sprague Dawley rats/ group. 0, 500, 1000 and 2000 mg/kg bw via oral gavage as a single administration. Vehicle: water. Positive control: N-dimethylnitrosamine. Post exposure period: 2 – 4 hours or 12 – 16 hours. Reliability: reliable.	Result: negative. No ↑ in mean net nuclear grain counts at any dose.	Anonymous, 1999. ECHA dissemination site, 2021.
OECD 474: mammalian erythrocyte micronucleus test. GLP compliant. Study did not meet currently guideline requirements requiring at least 4000 polychromatic erythrocytes (PCEs) per animal.	ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: not reported).	5/sex Swiss Albino Crl:CD-1 (ICR)BR mice/sampling point. 0, 500, 1000 and 2250 mg/kg bw via i.p. as a single administration. Vehicle: peanut oil Positive control: cyclophosphamide via i.p. Sampling time: 24, 48 and 72 hours post treatment. Tissue selection: bone marrow. ≥ 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. Reliability: reliable.	Result: negative. No ↑ in mean number of MN PCE at any sampling point. ↓ Ratio of PCE/ (NCE & PCE) in females at 500 & 2250 mg/kg bw at 48 hours. Clinical signs of toxicity included ↓ motor activity, collapse, weakness, ataxia and laboured breathing at 2250 mg/kg bw.	Anonymous, 1991b.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

***In vitro* studies**

In a bacterial reverse mutation test with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate, an increase in revertant colonies was observed in *S. typhimurium* strains TA 100 and TA 1535 in the presence of metabolic activation and in *E. coli* strain WP2 uvrA in the presence and absence of

metabolic activation. In a second bacterial reverse mutation study, an increase in revertant colonies was reported for *S. typhimurium* strains TA 100 and TA 1535 in the presence of metabolic activation.

Two *in vitro* gene mutation studies with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate are available. 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was not mutagenic in Chinese hamster ovary cells at the *HGRPT* locus either with or without metabolic activation, but was mutagenic in mouse lymphoma (L5178Y) $TK^{+/-}$ cells in the presence and absence of metabolic activation. In a sister chromatic exchange assay in mammalian cells with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate, an increase in sister chromatid exchanges was observed in the presence of metabolic activation. An *in vitro* unscheduled DNA synthesis assay in mammalian cells with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was reported to be equivocal.

The dossier submitter notes that the *in vitro* study summaries reported in the REACH registration dossier for 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate provide limited details. However, the dossier submitter considers that the available *in vitro* data indicates a concern for gene mutation. In addition, the positive results in *E. coli* strain WP2 uvrA and in mouse lymphoma (L5178Y) $TK^{+/-}$ cells in the absence of metabolic activation indicate a concern for a direct action of the substance as a mutagen at the sites of first contact.

***In vivo* studies**

In a transgenic rodent somatic and germ cell mutation (TGR) assay conducted in accordance with OECD 488, 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was administered to groups of 5 male transgenic mice (MutaMouse) at 0, 250, 500 and 1000 mg/kg bw/day via oral gavage for 28 days. Three days after the final dose, animals were sacrificed and genomic DNA extracted from the liver, forestomach, nasal cavity and germ cells. No clinical signs of toxicity or effect on body weight was observed at any dose. A slight increase in absolute (1.25 g) and relative (4.6 %) liver weight was observed at 1000 mg/kg bw/day when compared with the control (1.15 g and 4.21 %, respectively).

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 notes 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. 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 by the study authors to be 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 12: 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)

Dose group	Mean mutant frequency (x 10^{-6})

(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*

***** $P \leq 0.05$

The negative historical control data for the test laboratory is presented in table 14 below. The acceptable range was based on pooled control data per tissue from studies conducted from 1998 to 2015. The dossier submitter notes time range of this data is large (17 years). No laboratory historical control data was available for the nasal tissue.

Table 13: Historical negative control data for the TGR assay (*lacZ* assay) (Anonymous, 2016)

Organ	n	Mutant frequency (x 10^{-6})		
		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 considers the increase in mean mutant frequency in the forestomach, a site of first contact, at 1000 mg/kg bw/day to be statistically and biologically significant, and indicative of a direct acting mutagen. With respect to the liver, the dossier submitter agrees with the conclusion of the study author that as the increase in the mean mutant frequency observed at 1000 mg/kg bw/day is marginal and within the acceptable limits of the test laboratory, it is not considered to be biologically relevant. With respect to the nasal tissue, the dossier submitter notes that due to the small amount of tissue available, samples per dose group were pooled and thus individual animal data was not reported. No increase in mutant frequency was observed in any of the test material pooled samples but a statistically significant increase in mutant frequency was observed in the pooled sample of the positive control. Therefore, while the sample preparation for this tissue was not optimal, the dossier submitter considers that the increase in mutant frequency in the positive control supports the validity of the negative response in this tissue in the 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate treated groups.

With respect to germ cells, the dossier submitter agrees with the study author that no increase in mutant frequency was observed. It is noted that the sampling of germ cells following "28 + 3 day" sampling regime, as used in this study, results in a mixed population of spermatogonia, spermatocytes and spermatids at different stages of development and thus does not provide complete coverage of germ cell development. In accordance with paragraph 35 of OECD 488, a negative result in germ cells after a "28 + 3 day" sampling regime is not sufficient to negate the possibility that a test substance is a germ cell mutagen. In addition, the dossier submitter notes that the result for the positive control is within the laboratory historical control range for this tissue, supporting the view that the assessment of germ cells with this sampling regime is not very sensitive. Therefore, the dossier submitter considers that based on this study no conclusion can be drawn

regarding the potential for 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3- carboxylate to act as a germ cell mutagen.

In an unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo* conducted in accordance with OECD 486 but with deviations, 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was administered as a single dose to 10 males at 0, 500, 1000 and 2000 mg/kg bw via oral gavage. Liver cells were sampled 2 to 4 hours and 12 to 16 hours following exposure. 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, compared with 17.6 in the positive control. 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, compared with 10.5 in the positive control.

In a mammalian erythrocyte micronucleus test conducted in accordance with OECD 474, a single dose of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was administered to 5 male and 5 female mice per sampling point via intraperitoneal injection at 0, 500, 1000 and 2250 mg/kg bw. The study deviated from the current version of the test guideline in that 1000 rather than 4000 polychromatic erythrocytes per animal were scored. 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 (polychromatic erythrocyte) / (normochromatic and polychromatic erythrocytes) was reported in females in the 500 and 2250 mg/kg bw groups at 48 hours, which the study authors conclude as evidence of cytotoxicity (values not reported). No increase in the mean number of micronucleated polychromatic erythrocytes was observed at any dose or sampling time.

Table 14: 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

* $p < 0.01$

Overall, the dossier submitter considers that the statistical and biologically significant increase in mutant frequency observed in the forestomach in the TGR assay indicates that 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate induces gene mutation at sites of first contact. The negative results in the *in vivo* UDS and mammalian erythrocyte micronucleus studies do not negate this concern since neither test is designed to investigate site of first contact tissues.

Further details on the above studies are provided in Annex I to this report.

10.8.2 Comparison with the CLP criteria

According to Annex I to the CLP Regulation, substances may be classified as category 1A germ cell mutagens *"if they induce heritable mutations in the germ cells of humans*" and that classification is based on positive evidence from human epidemiological studies. No epidemiological data are available to demonstrate heritable gene mutations in humans. Therefore, classification in category 1A is not warranted.

According to Annex I to the CLP Regulation, substances may be classified as category 1B germ cell mutagens if there are:

- *"positive results from in vivo heritable germ cell mutagenicity tests in mammals or*
- *positive results from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells…or*
- *positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny…"*

In the TGR assay with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate, no increase in the mutant frequency in germ cells was observed. Therefore, the first criterion above is not met. However, it is noted the "28 + 3-day" sampling regime used in the study results in a mixed population of cells at different stages of development and thus does not provide complete coverage of germ cell development. In accordance with paragraph 35 of OECD 488, a negative result in germ cells after a "28 + 3-day" sampling regime is not sufficient to negate the possibility that a test substance is a germ cell mutagen.

An increase in mutant frequency was observed in the forestomach (site of first contact following oral administration) in the TGR assay, indicating 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate induces gene mutations at the site of first contact. No biologically significant increase in mutant frequency was observed in the other somatic tissues investigated (liver and nasal tissue). In addition, no increase in the mean number of micronucleated polychromatic erythrocytes was observed in the *in vivo* erythrocyte micronucleus test and no increase in mean net nuclear grain counts was observed in the *in vivo* UDS test, both conducted with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate. In a 90-day oral repeated dose toxicity study with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3- carboxylate (see section 10.12), there was no indication that the test substance reached the male reproductive organs: no effect on mean testicular and epididymal sperm count, sperm production rate, sperm motility or sperm morphology was observed. Therefore, the second criterion above is not met.

No data is available demonstrating that 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate would show mutagenic effects in the germ cells of humans and so the third criterion above is not met. Therefore, classification in category 1B is not warranted.

According to Annex I to the CLP Regulation, substances may be classified as category 2 germ cell mutagens if positive evidence are obtained from *"somatic cell mutagenicity tests in vivo… or other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays."*

In the available TGR assay, a statistically and biologically significant increase in mutant frequency was observed in the forestomach, which is a site of first contact following oral administration. No increase in mutant frequency was observed in the other somatic tissues investigated (liver and nasal tissue). The concern for gene mutation at sites of first contact is supported by the positive results *in vitro* in *E. coli* strain WP2 uvrA and in mouse lymphoma (L5178Y) $TK^{+/-}$ cells in the absence of metabolic activation. Therefore, classification in category 2 is warranted.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the available data, classification of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate as a category 2 germ cell mutagen is warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

For assessment of the mutagenicity potential of the EC No. 219-207-4 the DS provided six *in vitro* genotoxicity studies:

- two *in vitro* bacterial reverse mutation tests,
- two *in vitro* gene mutation tests in mammalian cells,
- one *in vitro* sister chromatid exchange assay in mammalian cells,
- one *in vitro* unscheduled DNA synthesis (UDS) in mammalian cells, and

three *in vivo* genotoxicity studies:

- one *in vivo* transgenic rodent somatic and germ cell gene mutation assay,
- one *in vivo* unscheduled DNA synthesis test with mammalian liver cells,
- one *in vivo* micronucleus assay in mouse bone marrow erythrocytes.

Most of these studies were presented based on their summaries obtainable in the REACH registration dossier for the substance (ECHA, 2021).

Table 1*: Summary table of mutagenicity/genotoxicity tests in vitro*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
OECD TG 471: bacterial reverse mutation test. GLP compliant. Triplicate plates per dose, test run in duplicate. Mean number of revertant colonies per dose level and	EC No. 219-207-4 (purity: not reported).	*S. typhimurium strains* TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA. 156, 313, 625, 1250, 2500 and 5000 µg/plate. ± Metabolic activation with S9 mix. Preparation details not reported. Vehicle control:	Result: positive ± metabolic activation. ↑ Revertant colonies in *S. typhimurium strains* TA 100 and TA 1535 (+S9) and in *E. coli* strain WP2 uvrA (±	Anonymous, 1995. ECHA dissemination site, 2021.

strain not reported. No information on cytotoxicity.		Dimethyl sulfoxide Positive controls: 2-aminoanthracene (+S9) 2-acetylaminofluo-rene, sodium azide, 9-aminoacridine, N-ethyl-N-nitro-N-nitrosoguanidine (-S9) Number of replicates: 3 plates/dose and test run in duplicate. Reliability: reliable.	S9) No information on cytotoxicity reported.	
Non-guideline: in vitro gene mutation study in bacteria. Not GLP compliant. Study pre-dated the adoption of OECD TG 471 (bacterial reverse mutation test) but the method employed was reported to be similar, with the following deviations: positive controls per strain, the number of replicates per dose and the mean number of revertant colonies per dose level/strain were not reported.	Celloxide 2021P (trade name of EC No. 219-207-4) (purity: not reported).	*S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 and E coli strain WP2 uvrA.* 100, 250, 500, 1000, 2000 and 5000 µg/plate. ± Metabolic activation with S9 mix. Preparation details not reported. Vehicle control: dimethyl sulfoxide Positive controls: 2-aminoanthracene; 2-acetylaminofluorene, 9-aminoacridine and N-ethyl-N-nitro-N-nitrosoguanidine. Number of cells evaluated: not reported. Number of replicates: Not reported. Reliability: *unreliable.*	Result: positive + metabolic activation. ↑ Revertant colonies in *S. typhimurium* strains TA 100 and TA 1535 (+S9). No cytotoxicity up to 5000 µg/plate	Anonymous, 1987. ECHA dissemination site, 2021.
Non-guideline: *in vitro* gene mutation in mammalian cells. Not GLP compliant Study pre-dated adoption of OECD TG 476 (*in vitro* mammalian gene mutation test) but the method employed was	Epoxy resin ERL-4221 (trade name of EC No. 219-207-4) (purity: not reported).	Chinese hamster ovary cells (*HGRPT* gene). Five concentrations between 6.25×10^{-4} % and 100×10^{-4} % (-S9) and 12.5×10^{-4} % and 200×10^{-4} % (+S9). Exact concentrations not reported. ± Metabolic activation with rat liver S9 (Arochlor 1254	Result: negative ± metabolic activation. Cytotoxicity reported at 100×10^{-4} % (-S9). No cytotoxicity data available for (+S9).	Anonymous, 1980. ECHA dissemination site, 2021

similar with the following deviations: limited reporting of the method, no information on culture/cell density, a longer expression time used, no information on whether the reported mutant frequency was corrected for cloning efficiency and no reporting of cytotoxicity or mutant frequency per dose.		induced). Vehicle control: dimethyl sulfoxide Negative control: untreated cells. Positive controls: N-dimethylnitrosamine (+S9); ethylmethanesulphonate (-S9) Exposure time: 16 hours (-S9), 5 hours (+S9). Expression period: 7-9 days. Number of cells evaluated: 200 cells/dose for frequency of mutants per 10^6 viable cells. Number of replicates: 2 Reliability: reliable.		
Non-guideline: *in vitro* gene mutation in mammalian cells. GLP compliant. Study pre-dated the adoption of OECD TG 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 and selective agent used to those recommended in OECD TG 490, no information reported on the acceptable spontaneous mutant frequency, no sizing of	TK 10 310 (ARALDIT CY 179) (trade name of EC No. 219-207-4) (purity: not reported).	Mouse lymphoma (L5178Y), subline TK $^{+/-}$ 12.5, 25, 50, 100, 150, 200 and 250 µg/mL. ± Metabolic activation with S9 mix. Preparation details not reported. Vehicle control: dimethyl sulfoxide. Negative control: untreated cells. Positive controls: N-dimethylnitrosamine (+S9); ethylmethanesulphonate (-S9) Exposure period: 4 hours. Expression period: 3 days. Selection time: 14 days for mutant selection and 11-12 for viability.	Result: positive ± metabolic activation. ↑ Mutant colony count at ≥ 150 µg/mL (+S9) and ≥ 100 µg/mL (-S9). No cytotoxicity reported up to 250 µg/mL.	Anonymous, 1984. ECHA dissemination site, 2021.

mutant colonies and no reporting of cytotoxicity or mutant frequency data per dose.		Selection agent: 5-bromodeoxyuridine. Number of cells evaluated: 4×10^5 cells/tube for mutant selection and 200 cells/tube for viability control. Number of replicates: not reported. Reliability: reliable.		
Non-guideline: *in vitro* sister chromatid exchange (SCE) assay in mammalian cells. Not GLP compliant. Study pre-dated the adoption of the withdrawn OECD TG 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/ concentration assessed, the test was performed without metabolic activation and there was no reporting of cytotoxicity or mutant frequency data per dose.	Epoxy resin ERL-4221 (trade name of EC No. 219-207-4) (purity: not reported).	Chinese hamster ovary cells. 3.125×10^{-4} % to 100 $\times 10^{-4}$ % by volume. Exact concentrations not reported. No metabolic activation. Vehicle control: dimethyl sulfoxide. Negative control: untreated cells. Positive control: ethylmethane sulphonate. 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 1-2 hours prior to harvest. Number of cells evaluated: Minimum of 15 cells/dose. Number of replicates: 3 Reliability: reliable.	Result: positive – metabolic activation. ↑ SCE frequency in 3 of 6 concentrations tested (exact concentrations not reported). Excessive toxicity reported in first two replicates, reported as ↓ in the number of mitotic cells and chromosome preparations not suitable for scoring. Based on this, SCE scoring from only one replicate reported.	Anonymous, 1980. ECHA dissemination site, 2021.
Non-guideline: *in vitro* unscheduled DNA synthesis in mammalian cells.	Epoxy resin ERL-4221 (trade name of EC No. 219-207-4) (purity: not reported).	Hepatocytes derived from rat liver. Six concentrations between 1.0×10^{-4} % and 1000×10^{-4} % by	Result: equivocal. ↑ UDS in 2 of 6 concentrations	Anonymous, 1980. ECHA dissemination site, 2021.

Not GLP compliant. Study pre-dated the adoption of OECD TG 482 (DNA damage and repair/unscheduled DNA synthesis in mammalian cells *in vitro*) but the method employed was similar with the following deviations: the number of replicates and the number of cells per culture assessed were not reported, no reporting of cytotoxicity or mutant frequency data per dose and limited reporting of the method.		volume. Exact concentrations not reported. Vehicle control: dimethyl sulfoxide. Positive controls: N-dimethylnitrosamine, 4-nitroquinoline-N-oxide. Pre-incubation period: 1 hour. Exposure duration: 2 hours. Number of replicates: not reported. Reliability: unreliable.	(exact concentrations not reported). 3 lowest concentrations also reported to have ↑ levels of UDS activity (exact concentrations not reported).	

Table 2: *Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo (↑: increase, ↓: decrease):*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
OECD TG 488: transgenic rodent somatic and germ cell gene mutation (TGR) assay. GLP compliant. Sampling time of "28 +3 days" not optimal for germ cell mutagenicity assessment.	EC No. 219-207-4 (purity: 96 %).	5 male CD2-LacZ80/HazfBR (MutaMouse)/group. 0, 250, 500 and 1000 mg/kg bw/day via gavage for 28 days. Vehicle: corn oil. Positive control: N-ethyl-N-nitrosourea. Sampling time: 28 + 3 days. Three days after the final dose, animals were sacrificed and genomic DNA extracted. Tissue selection: liver, forestomach, nasal tissue & germ cells (spermatozoa, spermatid and spermatocytes from seminiferous tubules and vas deferens/caudia	Result: positive. ↑ Mutant frequency in forestomach & liver at 1000 mg/kg bw/day. No ↑ in mutant frequency in nasal tissue or germ cells. ↑ Absolute & relative liver weight at 1000 mg/kg bw/day.	Anonymous, 2016.

		epididymis). Reliability: reliable.		
OECD TG 486: unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo.* GLP compliant. Number of slides evaluated per animal, the number of cells score per animal, and individual and group data not reported.	Union Carbide Cycloaliphatic Epoxy Resin ERL-4221 (trade name of EC No. 219-207-4) (purity: 89 %).	10 male Sprague Dawley rats/ group. 0, 500, 1000 and 2000 mg/kg bw/day via oral gavage as a single administration. Vehicle: water. Positive control: N-dimethylnitrosamine. Post exposure period: 2-4 hours or 12-16 hours. Reliability: reliable.	Result: negative. No ↑ in mean net nuclear grain counts at any dose.	Anonymous, 1999. ECHA dissemination site, 2021.
OECD TG 474: mammalian erythrocyte micronucleus test. GLP compliant. Study did not meet currently guideline requirements requiring at least 4000 polychromatic erythrocytes (PCEs) per animal.	ERL-4221 (trade name of EC No. 219-207-4) (purity: not reported).	5/sex Swiss Albino Crl:CD-1 (ICR)BR mice/sampling point. 0, 500, 1000 and 2250 mg/kg bw via i.p. as a single administration. Vehicle: peanut oil Positive control: cyclophosphamide via i.p. Sampling time: 24, 48 and 72 hours post treatment. Tissue selection: bone marrow. ≥ 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. Reliability: reliable.	Result: negative. No ↑ in mean number of MN PCE at any sampling point. ↓ Ratio of PCE/ (NCE & PCE) in females at 500 & 2250 mg/kg bw/day at 48 hours. Clinical signs of toxicity included ↓ motor activity, collapse, weakness, ataxia and laboured breathing at 2250 mg/kg bw/day.	Anonymous, 1991b.

In vitro studies

DS noted in the summary of the in vitro studies that in a bacterial reverse mutation test with EC No. 219-207-4, an increase in revertant colonies was observed in S. typhimurium

strains TA 100 and TA 1535 in the presence of metabolic activation and in E. coli strain WP2 uvrA in the presence and absence of metabolic activation. In a second bacterial reverse mutation study, an increase in revertant colonies was reported for S. typhimurium strains TA 100 and TA 1535 in the presence of metabolic activation.

EC No. 219-207-4 was not mutagenic in Chinese hamster ovary cells at the *HGRPT* locus, with or without metabolic activation, but was mutagenic in mouse lymphoma (L5178Y) TK+/- cells in the presence and absence of metabolic activation.

In a sister chromatic exchange assay in mammalian cells with EC No. 219-207-4, an increase in sister chromatid exchanges was observed in the presence of metabolic activation. An *in vitro* unscheduled DNA synthesis assay in mammalian cells with EC No. 219-207-4 was reported to be equivocal.

In vivo studies

In a transgenic rodent somatic and germ cell mutation (TGR) assay conducted in accordance with OECD TG 488, EC No. 219-207-4 has induced a statistically significant increase in mutant frequency in the forestomach and liver at 1000 mg/kg bw/day when compared to the concurrent negative control. The mean mutant frequencies ($\times 10^{-6}$) 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 notes that although the increase in mutant frequencies observed in the forestomach at 1000 mg/kg bw/day ($78.5 \pm 10.7 \times 10^{-6}$) was only marginally outside the historical control range considered acceptable (historical mean +/- 3sd) of the test laboratory (15.6×10^{-6}-78.0×10^{-6}), the increase was considered to be biologically relevant. 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 ($\times 10^{-6}$) 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 ($78.2 \pm 18.1 \times 10^{-6}$) was within the historical control range considered acceptable (historical mean +/- 3sd) of the test laboratory for this tissue (0.6×10^{-6}-99.6×10^{-6}), it was considered by the study authors to be 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.

The DS considers the increase in mean mutant frequency in the forestomach, a site of first contact, at 1000 mg/kg bw/day to be statistically and biologically significant, and indicative of a direct acting mutagen. The DS agrees with the conclusion of the study author that the increase in the mean mutant frequency observed at 1000 mg/kg bw/day in the liver is marginal and not considered to be biologically relevant. The DS notes that due to the small amount of nasal tissue available, samples per dose group were pooled and thus individual animal data was not reported. No increase in mutant frequency was observed in any of the pooled samples, but a statistically significant increase in mutant frequency was observed in the pooled sample of the positive control. Although the sample preparation for this tissue was not optimal, the DS considers that the increase in mutant frequency in the positive control supports the validity of the negative response in nasal tissue in the EC No. 219-207-4 treated groups.

The DS agrees with the study author that no increase in mutant frequency was observed

in germ cells. It is noted that the sampling of germ cells following "28 + 3 day" sampling regime, as used in this study, results in a mixed population of spermatogonia, spermatocytes and spermatids at different stages of development and thus does not provide complete coverage of germ cell development. In accordance with paragraph 35 of OECD TG 488, a negative result in germ cells after a "28 + 3 day" sampling regime is not sufficient to negate the possibility that a test substance is a germ cell mutagen. In addition, the DS notes that the result for the positive control is within the laboratory historical control range and that, based on this study, no conclusion can be drawn regarding the potential for EC No. 219-207-4 to act as a germ cell mutagen.

In an unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo* conducted in accordance with OECD TG 486 but with deviations, EC No. 219-207-4 was administered as a single dose to 10 males at 0, 500, 1000 and 2000 mg/kg bw via oral gavage. Liver cells were sampled 2 to 4 hours and 12 to 16 hours following exposure. 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, compared with 17.6 in the positive control. 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, compared with 10.5 in the positive control.

In a mammalian erythrocyte micronucleus test conducted in accordance with OECD TG 474, a single dose of EC No. 219-207-4 was administered to 5 male and 5 female mice per sampling point via intraperitoneal injection at 0, 500, 1000 and 2250 mg/kg bw. The study deviated from the current version of the test guideline in that 1000 rather than 4000 polychromatic erythrocytes per animal were scored. 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 (polychromatic erythrocyte) / (normochromatic and polychromatic erythrocytes) was reported in females in the 500 and 2250 mg/kg bw groups at 48 hours, which the study authors conclude as evidence of cytotoxicity (values not reported). No increase in the mean number of micronucleated polychromatic erythrocytes was observed at any dose or sampling time.

Conclusion

Overall, the DS considers that the statistical and biologically significant increase in mutant frequency observed in the forestomach in the TGR assay indicates that EC No. 219-207-4 induces gene mutation at sites of first contact. The negative results in the *in vivo* UDS and mammalian erythrocyte micronucleus studies do not negate this concern since neither test is designed to investigate site of first contact tissues. Moreover, as the UDS test is useful only for some classes of substances, a negative result in a UDS assay alone is not a proof that a substance does not induce gene mutations. The mammalian *in vivo* micronucleus test identifies substances that cause cytogenetic damage (e.g. chromosome aberrations) but not gene mutations.

Based on the available data the DS concluded that classification of EC No. 219-207-4 as a category 2 germ cell mutagen (Muta. 2) is warranted.

Comments received during consultation

Two MSCAs supported the proposal to classify EC No. 219-207-4 as a category 2 germ

cell mutagen based on the positive data showing a mutagenic action at the first site of contact (forestomach).

Assessment and comparison with the classification criteria

The germ cell mutagenicity potential of EC No. 219-207-4 has been assessed in relevant *in vitro* and *in vivo* tests.

Analysis of the available data indicated that there are neither evidence from human epidemiological studies nor positive results from the *in vivo* heritable germ cell mutagenicity tests in mammals, therefore classification of EC No. 219-207-4 as Muta. 1A or Muta. 1B is not justified.

However, the positive evidence of gene mutagenicity was obtained in a transgenic rodent somatic and germ cell mutation (TGR) assay, in which EC No. 219-207-4 has induced a statistically significant increase in mutant frequency in the forestomach, thus at the site of first contact (Anonymous, 2016.). The negative results in the other *in vivo* tests such as an unscheduled DNA synthesis (UDS) test with mammalian liver cells (Anonymous, 1999. ECHA dissemination site, 2021) and mammalian erythrocyte micronucleus assay (Anonymous, 1991b) do not deny ability of EC No. 219-207-4 to induce gene mutation at the site of first contact since these assays are not designed to investigate gene mutations, particularly gene mutation at the site of first contact. Therefore, the criterion of germ cell mutagenicity category 2, that is a positive evidence obtained from experiments in mammals, has been met. This *in vivo* evidence is further supported by induction of gene mutations in bacterial reverse mutation tests and an *in vitro* assay in mouse lymphoma (L5178Y) TK+/- cells in absence of metabolic activation.

In the opinion of RAC the classification criteria of germ cell mutagenicity category 2 are met for EC No. 219-207-4 based on positive evidence from a somatic cell mutagenicity test in mammals supported by positive evidence from *in vitro* mutagenicity assays. Therefore, the substance EC No. 219-207-4 **warrants classification as Muta. 2; H341 (Suspected of causing genetic defects)**.

10.9 Carcinogenicity

No classification proposed.

The carcinogenicity study reported below is provided only as supporting information for the assessment of STOT RE.

Table 15: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Non-guideline: dermal carcinogenicity study. Test materials applied dermally to groups of 40 male C3H/Anf mice/group by brushing 3 times per week up for to 29 months. Animals were examined for the development of skin papillomas or carcinomas. Limited details provided in the study summary. Not GLP compliant.	EP-4221 (7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: 100 %). One treatment group of 4000 – 8000 mg kg/bw (undiluted). Exact dose not reported. Applied 3 times per week up for to 29 months. Positive control: dermal application of 3-methylcholanthrene. Vehicle control: acetone. Reliability: unreliable.	Result: negative Incidence of skin papillomas was 1/40 in the test group, 2/40 in the vehicle control group and 39/40 in the positive control group.	Anonymous, 1964. ECHA dissemination site, 2021.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

In a non-guideline dermal carcinogenicity study, 0.1 – 0.2 g (corresponding to 4000 – 8000 mg/kg bw) of undiluted 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was applied dermally to 40 male C3H/Anf mice three times per week for up to 29 months. The negative (acetone) and positive (3-mehtylcholanthrene) control groups were treated in the same way. Animals were examined for the development of dermal papillomas or carcinomas. There was no increase in the mortality rate in the 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate group when compared with the negative control. One animal in the 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate group developed a skin tumour at 23 months, which was characterised as a papilloma. Two 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. No further details are reported.

Table 16: 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	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

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.

10.9.2 Comparison with the CLP criteria

No classification proposed.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification proposed.

10.10 Reproductive toxicity

No classification proposed.

The pre-natal developmental toxicity study reported below is provided only as supporting information for the assessment of STOT RE.

10.10.1 Adverse effects on sexual function and fertility

No data available.

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

Not applicable.

10.10.3 Comparison with the CLP criteria

Not applicable.

10.10.4 Adverse effects on development

Table 17: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 414: prenatal developmental toxicity study. GLP compliant. Rat, Crl:CD(SD)IGS BR, male/female. 25/female dose.	Cycloaliphatic Epoxy Resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: 92 %) Vehicle: Corn oil. 0, 5, 25, 125 and 500 mg/kg bw/day administered daily from gestation day 6 to 19. Reliability: reliable	Maternal effects: ↓ Body weight at 500 mg/kg bw/day. ↓ Food consumption at ≥ 125 mg/kg bw/day. ↑ Kidney weight at ≥ 125 mg/kg bw/day. Reproductive parameters: No effect on the number of corpora lutea, implantation sites, viable foetuses, sex ratio, resorptions (both early and late) or pre-/post implantation loss. Foetal effects: ↓ Foetal body weight at 500 mg/kg bw/day. ↑ Skeletal variations at 500 mg/kg bw/day.	Anonymous, 2007.

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

In a prenatal developmental toxicity study conducted in accordance with OECD 414, 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was administered to female rats via oral gavage from gestation day (GD) 6 to 19 at 0, 5, 25, 125 and 500 mg/kg bw/day. All females survived to scheduled sacrifice. A significant decrease in mean body weight gain was observed between GD 6-20 at 500 mg/kg bw/day (94 g) compared with the control (115 g). 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 reported 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 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.

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. 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 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 at 500 mg/kg bw/day was within the historical control range of the test laboratory (6.58 % - 27.6 %). There was also a non-statisitically 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.

10.10.6 Comparison with the CLP criteria

No classification proposed.

10.10.7 Conclusion on classification and labelling for reproductive toxicity

No classification proposed.

10.11 Specific target organ toxicity-single exposure

Not evaluated as part of this dossier.

10.12 Specific target organ toxicity-repeated exposure

Table 18: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
OECD 408: repeated dose 90-day oral toxicity study in rodents. GLP compliant. Rat, Crl:CD(SD)IGS BR, male/female. 25/sex at 0 and 500 mg/kg bw/day; 20/sex at 5 and 50 mg/kg bw/day. No functional observation battery performed. Thyroid hormone levels not measured.	Cycloaliphatic Epoxy Resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: 87 %). 0, 5, 50 and 500 mg/kg bw/day. Daily administration via oral gavage for 91-92 days. Vehicle: Corn oil. 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. Reliability: reliable.	Week 13: ↓ Body weight in males at 500 mg/kg bw/day. ↓ Neutrophil count & ↑ lymphocyte count in females at ≥ 50 mg/kg bw/day. ↑ Blood urea nitrogen, mean phosphorus & sorbitol dehydrogenase in males & females at ≥ 50 mg/kg bw/day; ↑ potassium in females at 500 mg/kg bw/day, ↓ creatine kinase in males at ≥ 50 mg/kg bw/day & females at 500 mg/kg bw/day, ↓ cholesterol in males at 500 mg/kg bw/day & females at ≥ 50 mg/kg bw/day, ↓ direct bilirubin in males & females at 500 mg/kg bw/day. ↓ Urine pH & urine creatine in males at 500 mg/kg bw/day. ↑ Absolute liver weight in females at ≥ 50 mg/kg bw/day & males at 500 mg/kg bw/day. ↑ relative liver weight in males & females at ≥ 50 mg/kg bw/day; ↑ Absolute & relative kidney weight in males & females at 500 mg/kg bw/day. Degeneration of olfactory epithelium of nasal tissue in males & females at ≥ 50 mg/kg bw/day. Pale liver observed at 500 mg/kg bw/day. Periportal hepatocellular vacuolation in males &	Anonymous, 2001.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
		females at ≥ 50 mg/kg bw/day. Week 17 (end of recovery period): ↓ Body weight in males at 500 mg/kg bw/day. Degeneration of olfactory epithelium of nasal tissue in males & females at ≥ 50 mg/kg bw/day. NOAEL: 5 mg/kg bw/day.	
Repeated dose 14-day oral toxicity study. Range finding study for the OECD 408 study (Anonymous, 2001). GLP compliant. Rat, Crl:CD(SD)IGS BR, male/female. 10/sex/dose. No haematological, clinical chemistry or urine analysis was performed. Incidences of effects observed per dose group not reported.	Cycloaliphatic Epoxy Resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: 83 %). Vehicle: Corn oil. 0, 100, 500, 750 and 1000 mg/kg bw/day. Daily administration via oral gavage for 14 days. Reliability: reliable.	↓ Body weight in males at ≥ 500 mg/kg bw/day & females at 1000 mg/kg bw/day. ↓ Body weight gain in males at ≥ 750 mg/kg bw/day & females at 1000 mg/kg bw/day. ↓ Food consumption in males in week 1 at ≥ 750 mg/kg bw/day. 1/10 males at both 500 and 1000 mg/kg bw/day had small testes and epididymis. ↑ Absolute liver weight in males at ≥ 100 mg/kg bw/day and in females at ≥ 500 mg/kg bw/day. ↑ Relative liver weight in males at ≥ 100 mg/kg bw/day and in females at ≥ 500 mg/kg bw/day. Significant “changes” in absolute or relative weights of spleen, heart, kidneys and thymus noted in the study summary but no information on doses or weights reported. Periportal hepatocellular vacuolation in males and females at ≥ 100 mg/kg bw/day. LOAEL: 100 mg/kg bw/day.	Anonymous, 2000. ECHA dissemination site, 2021.
Non-guideline: dermal carcinogenicity study. Not GLP compliant. Mouse, C3H/Anf, male.40 male/group. Test materials applied dermally by brushing 3 times per week for 26 months. Animals were examined for the development of skin papillomas or carcinomas. Limited details provided in the study summary.	EP-4221 (reported to be 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: 100 %). Single dose between 4000 – 8000 mg kg/bw (undiluted). Dermal administration 3 times per week for 26 months. Positive control: dermal application of 3-methylcholanthrene. Vehicle control: acetone. Reliability: unreliable.	Result: negative Incidence of skin papillomas was 1/40 in the test group, 2/40 in the vehicle control group and 39/40 in the positive control group. Body weights & organ weights not reported. No non-neoplastic histopathological evaluation performed.	Anonymous, 1964. ECHA dissemination site, 2021.
OECD 414: prenatal developmental toxicity study	Cycloaliphatic Epoxy Resin ERL-4221 (trade name of 7-oxabicyclo[4.1.0]hept-3-	Maternal effects: ↓ Body weight at 500 mg/kg bw/day.	Anonymous, 2007.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
GLP compliant. Rat, Crl:CD(SD)IGS BR, male/female. 25/female dose. Kidney and liver weights recorded.	ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate) (purity: 92 %). Vehicle: corn oil. 5, 25, 125 and 500 mg/kg bw/day administered daily from gestation day 6 to 19. Reliability: reliable.	↓ Food consumption at ≥ 125 mg/kg bw/day ↑ Kidney weight at ≥ 125 mg/kg bw/day. No histopathological examination of liver or kidney performed. NOAEL maternal toxicity: 25 mg/kg bw/day. Reproductive parameters: No effect on the number of corpora lutea, implantation sites, viable foetuses, sex ratio, resorptions (both early and late) or pre-/post implantation loss. Foetal effects: ↓ Foetal body weight at 500 mg/kg bw/day. ↑ Skeletal variations at 500 mg/kg bw/day.	

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In a 90-day oral repeated toxicity study conducted in accordance with OECD 408, 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was administered via oral gavage to male and female rats at 0, 5, 50 and 500 mg/kg bw/day. At the end of the 90-day dosing period, 10/sex in the 0 and 500 mg/kg bw/day groups and 5/sex in the 5 and 50 mg/kg bw/day groups were subject to a 28-day recovery period.

All animals survived to scheduled sacrifice. Clinical signs of toxicity observed at 500 mg/kg bw/day included salivation, and yellow material on the urogenital area, hind limbs, neck and trunk. A non-statistically significant decrease in body weight was reported in males at 500 mg/kg bw/day during the treatment period, which remained lower than the control group at the end of the recovery period. At week 13, the mean body weight in males at 500 mg/kg bw/day was 546 ± 53 g compared with 585 ± 56 g in the control group. At the end of the recovery period (week 17), the mean body weight in males at 500 mg/kg bw/day was 575 ± 68 g compared with 617 ± 73 g in the control group. There was no effect on body weight in females.

At the end of the 13-week treatment period, a number of clinical chemistry and urinalysis parameters were statistically significantly altered (see table 19 below). At the end of the recovery period, no significant difference in any of the clinical chemistry or urinalysis parameters was observed.

Table 19: Clinical chemistry and 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
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 ± 11.4	75 ± 17.8	56 ± 15.7	45 ± 16.3**	67 ± 13.5	74 ± 16.7	52 ± 16.0*	56 ± 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**

Parameter	**Week**	**Dose (mg/kg bw/day)**							
		Males				**Females**			
		0	**5**	**50**	**500**	**0**	**5**	**50**	**500**
	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
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

* $p < 0.05$ ** $p < 0.01$

Absolute liver weights were statistically significantly increased in females at ≥ 50 mg/kg bw/day and males at 500 mg/kg bw/day. The absolute liver weights were 8.3 g, 8.21 g, 9.48 g and 9.98 g in females and 16.36 g, 16.38 g, 18.09 g and 19.64 g in males at 0, 5, 50 and 500 mg/kg bw/day, respectively. 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 and there was a non-statistically significant increase in males at the same dose. The absolute kidney weights were 1.89 g, 1.96 g, 1.97 g and 2.14 g in females and 3.96 g, 3.82 g, 3.91 g and 4.57 g in males at 0, 5, 50 and 500 mg/kg bw/day, respectively. 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 20: 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

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 at 0, 5, 50 and 500 mg/kg bw/day, 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 that in the control group.

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 at 0, 5, 50 and 500 mg/kg bw/day, respectively. No effect on basal cells, the underlying structures or connective tissue was reported.

Table 21: 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 than that observed at week 13. The incidence was 0/10, 0/5, 2/5 and 9/10 in males and 0/10, 0/5, 3/5 and 7/10 in females at 0, 5, 50 and 500 mg/kg bw/day, respectively. The study report notes that there was some evidence of regenerative changes: basal cell proliferation and regeneration of sustentacular and neuroepithelial cells was reported. 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 report considered this change to be part of the repair process, suggesting that local damage to basal cells prevented repair to olfactory epithelium.

Table 22: 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. The dossier submitter notes that no difficulties with administration of the dose via gavage cannula were reported. In addition, no clinical signs after dosing were reported which would indicate reflux of the test material. The dossier submitter notes that, given the low vapour pressure of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (-2 x 10^{-3} Pa) and the choice of vehicle (corn oil) in the study, it is considered unlikely that animals at 50 or 500 mg/kg bw/day were exposed to toxic or caustic vapours from the oral preparation. Therefore, while effects on nasal tissue following oral administration are relatively rare, the dossier submitter considers that based on the available information the effects observed on nasal tissue, which were not fully reversible after a 4 week recovery period, were treatment related.

In a 14-day oral repeated toxicity study conducted as a range finding study for the 90-day repeated dose toxicity study (Anonymous 2001, discussed above), 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was administered via oral gavage to male and female rats at 0, 100, 500, 750 and 1000 mg/kg bw/day for 14 days. All animals survived to scheduled sacrifice. A non-statistically significant decrease in body weight was observed in males at ≥ 500 mg/kg bw/day and in females at 1000 mg/kg bw/day. Organ weights were not reported however; the study summary reports that absolute liver weights were increased in males at ≥ 100 mg/kg bw/day and females at ≥ 500 mg/kg bw/day, and relative liver weights were increased in males at ≥ 100 mg/kg bw/day and in females at ≥ 500 mg/kg bw/day.

Fine periportal hepatocellular vacuolation was observed in both sexes 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 were reported to have small testes and epididymis. The same animals had mild to moderate seminiferous tubule degeneration of the testes and lumenal cellular debris and hypospermia of the epididymis. There was no indication in the study summary as to whether the nasal tissue was examined.

In a non-guideline dermal carcinogenicity study, 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was applied undiluted to the skin of male mice at a single dose of between 4000 and 8000 mg/kg bw/day for 26 months. No organ weights or non-neoplastic histopathological examinations were performed. No increase in dermal tumour incidence was observed.

In a prenatal developmental toxicity study conducted in accordance with OECD 414, 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate was administered to female rats via oral gavage from gestation day (GD) 6 to 19 at 0, 5, 25, 125 and 500 mg/kg bw/day. All females survived to scheduled sacrifice. A significant decrease in mean body weight gain was observed between GD 6-20 at 500 mg/kg bw/day (94 g) compared with the control (115 g). Absolute kidney weights were statistically significantly decreased at ≥ 125 mg/kg bw/day. The reported 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. No effect on liver weight was reported. No histopathological examination of kidney or liver were performed.

Due to the limited histopathological assessment performed in both the carcinogenicity study and the prenatal developmental toxicity study, these studies are not considered further in the weight of evidence assessment for classification for specific target organ toxicant following repeated exposure.

Table 23: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg bw/day)	Length of exposure	Extrapolated guidance value for STOT RE 2	Classification supported by the study
OECD 408: repeated dose 90-day oral toxicity study in rodents (Anonymous, 2001).	Nasal cavity effects (epithelial degeneration) observed at ≥ 50 mg/kg bw/day. Liver effects (increase in absolute liver weight and increased incidence of periportal hepatocellular vacuolation) observed at ≥50 mg/kg bw/day.	90-days	10 < C ≤ 100 mg/kg bw/day	STOT RE 2
14-day repeated dose toxicity study (Anonymous, 2000. ECHA dissemination site, 2021.)	Liver effects (fine periportal hepatocellular vacuolation) at ≥ 100 mg/kg bw/day.	14 days	60 < C ≤ 600 mg/kg bw/day	STOT RE 2

10.12.2 Comparison with the CLP criteria

According to Annex I to the CLP Regulation, substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement, including the use of the recommended guidance values (as outlined in 3.9.2.9 of the CLP Regulation) and assigned to one of two categories:

Category 1:

"Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in category 1 for target organ toxicity (repeat exposure) on the basis of:

- reliable and good quality evidence from human cases or epidemiological studies; or

- observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/ concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of evidence evaluation."

The guidance value for STOT RE category 1 is C ≤ 10 mg/kg bw/day for an oral 90-day repeated dose toxicity study in rats.

Category 2:

"Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2 (see 3.9.2.6)."

The guidance value for STOT RE category 2 is 10 < C ≤ 100 mg/kg bw/day for an oral 90-day repeated dose toxicity study in rats.

No human repeated dose toxicity data are available for oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3- carboxylate.

<u>Nasal cavity effects</u>

In the available 90-day oral repeated toxicity study in rats with 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3- carboxylate, degeneration of the olfactory epithelium of the nasal tissue was observed in both sexes at ≥ 50 mg/kg bw/day. 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. These effects were not fully reversible after a 4 week recovery period. According to 3.9.2.7.3 (b) of Annex I of the CLP Regulation, *"effects on special senses (e.g. sight, hearing and smell)"* are considered to be indications of functional impairment and should be taken into consideration in the classification process. There is no data to indicate that this effect on the olfactory epithelium is not relevant for humans and therefore it should be considered for classification purposes.

The observed effect on the nasal tissue in the 90-day repeated dose toxicity study occurred at a dose (50 mg/kg bw/day) within the guidance value range for classification as STOT RE category 2 (10 < C ≤ 100 mg/kg bw/day). Therefore, classification in category 2 is warranted.

<u>Liver effects</u>

An increase in liver weight was observed in females at ≥ 50 mg/kg bw/day and males at 500 mg/kg bw/day. This was accompanied by alterations in clinical chemistry parameters at 500 mg/kg bw/day (decreased cholesterol and increase in direct bilirubin and sorbitol dehydrogenase) and an increased incidence of periportal hepatocellular vacuolation at ≥ 50 mg/kg bw/day. The effects observed in the liver were reversible

after a 4 week recovery period. Supporting evidence is provided from a 14-day repeated dose toxicity study were fine periportal hepatocellular vacuolation was observed at ≥ 100 mg/kg bw/day. According to 3.9.2.8.1 of Annex I of the CLP Regulation, classification is not justified for "*changes in organ weight with no evidence of organ dysfunction"* and *"adaptive responses that are not considered toxicologically relevant"*. Although some alterations of clinical chemistry parameters were observed, they did not indicate functional impairment of the liver. The only histopathological finding in the liver was hepatocellular vacuolation which may be an adaptive response rather than a significant toxic response. Therefore, although the observed effect on the liver in the 90-day repeated dose toxicity study occurred at a dose (50 mg/kg bw/day) within the guidance value range for classification as STOT RE category 2 ($10 < C \leq 100$ mg/kg bw/day), the dossier submitter considers that this effect does not support classification for specific target organ toxicity following repeated exposure.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the available data, classification of 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3- carboxylate as STOT RE category 2 is warranted. The hazard statement (H373) should specify the "nasal cavity" as the organ effected. As data is available from only one route of exposure (oral), it is proposed not to state the route in the hazard statement. Based on the available data, the assignment of a specific concentration limit is not warranted.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposal presented data from a 90-day oral study according to OECD TG 408 and additional data from a 14-day oral range finding study (both in Crl:CD(SD)IGS BR rats) among other studies which were not considered further for the STOT assessment (for an overview, see table 18 of the CLH report).

90-day oral study

In the 90-day oral study (Anonymous, 2001) male and female rats received via oral gavage doses of 0, 5, 50 and 500 mg/kg bw/day (15/sex/group). At the end of the 90-day dosing period, 10/sex in the 0 and 500 mg/kg bw/day groups and 5/sex in the 5 and 50 mg/kg bw/day groups had a 28-day recovery period.

Without any mortality clinical signs of toxicity were observed at 500 mg/kg bw/day (including salivation and yellow material on the urogenital area, hind limbs, neck and trunk). Males at 500 mg/kg bw/day had a non-significant decrease in body weight which remained lower in the recovery period. At the end of the 13-week treatment period, a number of clinical chemistry and urinalysis parameters were statistically significantly altered (see table 19 in the CLH report). At the end of the recovery period, no significant difference in any of the clinical chemistry or urinalysis parameters was observed.

Liver weights were significantly increased in females (absolute and relative at ≥ 50 mg/kg bw/day) and in males (absolute at 500 mg/kg bw/day, relative at ≥ 50 mg/kg bw/day). Kidney weights were significantly increased in females (absolute and relative at 500 mg/kg bw/day) and in males (absolute at 500 mg/kg bw/day (nonsignificant),

relative at 500 mg/kg bw/day).

A dose-dependent increased incidence of minimal to mild 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 at 0, 5, 50 and 500 mg/kg bw/day, respectively. At the end of the recovery period, the incidence in the treatment groups was comparable to that in the control group.

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 at 0, 5, 50 and 500 mg/kg bw/day, respectively. No effect on basal cells, the underlying structures or connective tissue was reported.

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 than that observed at week 13. The incidence was 0/10, 0/5, 2/5 and 9/10 in males and 0/10, 0/5, 3/5 and 7/10 in females at 0, 5, 50 and 500 mg/kg bw/day, respectively. The study report notes that there was some evidence of regenerative changes: basal cell proliferation and regeneration of sustentacular and neuroepithelial cells was reported. 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 report considered this change to be part of the repair process, suggesting that local damage to basal cells prevented repair to olfactory epithelium.

14-day oral study

All rats of the dose-ranging 14-day oral study receiving doses of 0, 100, 500, 750 and 1000 mg/kg bw/day survived and showed a non-significant decrease in body weight in males at ≥ 500 mg/kg bw/day and in females at 1000 mg/kg bw/day. Organ weights were not reported, however, the study summary reports that absolute liver weights were increased in males at ≥ 100 mg/kg bw/day and females at ≥ 500 mg/kg bw/day, and relative liver weights were increased in males at ≥ 100 mg/kg bw/day and in females at ≥ 500 mg/kg bw/day.

Fine periportal hepatocellular vacuolation was observed in both sexes 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 exact incidences. 1/10 males at 500 and 1000 mg/kg bw/day were reported to have small testes and epididymis. The same animals had mild to moderate seminiferous tubule degeneration of the testes and lumenal cellular debris and hypospermia of the epididymis.

Summary of the DS's conclusion

The DS noted that no difficulties with administration of the dose via gavage cannula were reported. In addition, no clinical signs after dosing were reported which would indicate reflux of the test material. Given the low vapour pressure of EC No. 219-207-4 (2×10^{-3} Pa) and the choice of vehicle (corn oil), it is considered unlikely that animals at 50 or 500 mg/kg bw/day in the 90-day study were exposed to toxic or caustic vapours from

the oral preparation. Therefore, while effects on nasal tissue following oral administration are relatively rare, the DS considers that based on the available information the effects observed on nasal tissue, which were not fully reversible after a 4-week recovery period, were treatment related.

The DS concluded that the effects on the nasal tissue reported as degeneration of the olfactory epithelium of the nasal tissue in both sexes at ≥ 50 mg/kg bw/day are relevant effects for classification. 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. These effects were not fully reversible after a 4-week recovery period. According to 3.9.2.7.3 (b) of Annex I of the CLP Regulation, "effects on special senses (e.g. sight, hearing and smell)" are considered to be indications of functional impairment and should be taken into consideration in the classification process. There is no data to indicate that this effect on the olfactory epithelium is not relevant for humans and therefore it should be considered for classification purposes.

The observed effects on the liver (weight increase, periportal hepatocellular vacuolation) in the 90-day repeated dose toxicity study observed at a dose (50 mg/kg bw/day) which is within the guidance value range for classification as STOT RE category 2 were not considered as supportive for classification.

Comments received during consultation

Supporting comments on STOT RE category 2 for degeneration of olfactory epithelium were received from two MSCAs. In addition, one MSCA noted that the hepatocellular vacuolation observed in the two lowest dose groups (5 and 50 mg/kg bw/day) is of minimal severity and thus not relevant for classification as STOT RE.

Additional key elements

Degeneration of the olfactory epithelium is rarely seen as a target organ in oral toxicity studies. The DS confirmed that no data are available to indicate that it may be a consequence of incorrect dosing, direct cytotoxicity due to vapour inhalation or reflux.

Severity grades of olfactory degeneration are reported as mild to severe at 500 mg/kg bw/day and minimal to mild at 50 mg/kg bw/day. The effects are mainly located at cross section levels 2 to 4 of the nasal cavity (corresponding to the main levels covered by olfactory epithelium).

Although at 50 mg/kg bw/day high severity grades did not appear, the degeneration of olfactory epithelium at any grade deserves consideration for classification purposes. This is because the effects were not reversible (the lesion was seen in several rats at 50 mg/kg bw/day at the end of recovery) and the olfactory epithelium is a special sense organ responsible for smelling and contributing to tasting. Although the surface area of the olfactory epithelium is larger and its relative proportion is higher in rats than in humans, the principal structures and functions are identical for rats and humans.

In general, regeneration capacity following degeneration of the olfactory neuroepithelium is limited or lacking. In the available 90-day study, cell loss of sustentacular cells and neuroepithelial cells were observed resulting in a reduction in epithelial height (indicative of atrophy of the upper layers of sensory cells). Foci of replacement of olfactory

epithelium by ciliated columnar epithelium (which in healthy mammals only occur in the respiratory epithelium) was observed in 6/10 males and 9/10 females at 500 mg/kg bw/day (no data on lower doses available to the rapporteurs). The study report considered this change to be part of the repair process, suggesting that local damage to basal cells prevented repair to olfactory epithelium. This replacement is not equivalent to a regeneration to the neuroepithelium, and the replaced tissues are considered not to have the (full) functionality of the smelling sense.

The substance is assumed to exert its toxicity to the olfactory epithelium via systemic effects as such or via its metabolite(s).

No toxicokinetic data are available for EC No. 219-207-4. Based on the physico-chemical data (molecular weight 252 g/mol, water solubility 13 850 mg/L, Log P_{ow} 1.34 (20 °C) and the effects seen at several target organs, systemic bioavailability by the oral and dermal route is likely.

The registration of a substance with a similar structure (bis[(3,4-epoxycyclohexyl)methyl] adipate[4]) refers to the 90-day oral study of EC No. 219-207-4 as a source substance to fill the REACH data requirements. The registrants reflected on the unusual findings:

"The nasal epithelial changes were effectively the critical data on which the NOAEL derivation was based. The cause was not established, the persistence and limited recovery can be gauged by the incidence of degenerative and regenerative change present at termination following the recovery phase. It has been assumed that the nasal change is a systemic effect and extended in a dose related manner to affect the high dose group moderately and the intermediate group minimally and was not present in any of the low dose group animals."

The registrants noted that critical effects are a consequence of systemic availability, but inappropriately referred to the NOAEL of 5 mg/kg bw/day as a reason why the substance does not require classification.

The Substance Evaluation Report[5] indicated that "no mechanism of action was identified in this case, the evaluating MSCA notes that the target tissue, sustentacular cells, contain high levels of metabolising enzymes including cytochrome P450 and flavin mono-oxygenases (Harkema *et al*., 2006). Other chemicals causing degeneration and/or atrophy of the olfactory epithelium following administration by routes other than inhalation include methacrylonitrile, benzyl acetate, dipropylene glycol, o-nitrotoluene, cyclohexanone oxime, butanal oxime and methyl ethyl ketoxime. Metabolic predilection could explain regional distribution of lesions in the nasal cavity following non-inhalation exposure. For example, nasal cell cytochrome P-450 2E1 is thought to play a role in the metabolism of methacrylonitrile resulting in degeneration of nasal epithelial cells (Sells *et*

[4] https://echa.europa.eu/registration-dossier/-/registered-dossier/24900/1/1

[5] The Substance Evaluation Conclusion and Evaluation Report for 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate can be found at https://echa.europa.eu/documents/10162/b8804bf1-592d-b25e-9f72-89f6f9dedc6b

al., 2007)."

Assessment and comparison with the classification criteria

RAC agrees with the DS that the observed degeneration of the olfactory epithelium of the nasal tissue observed at ≥ 50 mg/kg bw/d in an appropriate 90-day oral study is an adverse effect which should be considered for classification as STOT RE.

The degeneration of the olfactory epithelium of the nasal tissue was characterised by the loss of sustentacular cells, vacuolation and desquamation of neuroepithelial cells, which resulted in decreased height of the olfactory epithelium.

The incidences of olfactory degeneration 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 at 0, 5, 50 and 500 mg/kg bw/day, respectively indicated a dose-response related effect at doses of ≥ 50 mg/kg bw/day.

Although the declining incidence rates at the end of recovery period indicate tendency for recovery, the effect is considered as non-reversible due to the remaining incidences of 0/10, 0/5, 2/5 and 9/10 in males and 0/10, 0/5, 3/5 and 7/10 in females at 0, 5, 50 and 500 mg/kg bw/day, respectively.

Adverse effects at 50 mg/kg bw/day are within the guidance values for STOT RE 2 (10 < C ≤ 100 mg/kg bw/day, for 90-day oral study) as given in Annex I to CLP (table 3.9.3).

As no relevant effect was observed in the 5 mg/kg bw/day dose which would be within the guidance value for STOT RE 1 (C ≤ 10 mg/kg bw/day, table 3.9.2, Annex I to CLP), RAC agrees that STOT RE 1 is not appropriate.

There are no data on effects of the nasal tissues from the other oral rat studies (14-day range finding, prenatal developmental (OECD TG 414) study) or the non-guideline dermal carcinogenicity study in mice. As histopathological examinations are lacking or incomplete and do not indicate whether the nasal tissues were examined, no information on the presence or absence of effects on the nasal tissues is available in the other studies.

An increased incidence of periportal hepatocellular vacuolation was observed in almost all males and females at ≥ 50 mg/kg bw/day. 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 that in the control group. Hepatocellular vacuolation was assumed by the DS to be adaptive. RAC considers that hepatocellular vacuolation, if of significant severity, could also be of degenerative nature. In this case and in support of the DS's view the minor severity grades at the two low doses in the 90-day study and at 100 mg/kg bw/day in the 14-day study (which are in the range of the guidance values) do not justify consideration for classification for liver effects. Significantly increased liver weights (absolute increases in females at ≥ 50 mg/kg bw/day and males at 500 mg/kg bw/day; relative increases in females and males at ≥ 50 mg/kg bw/day) as such do not correspond to the criteria for classification.

Other effects observed in the 90-day study on clinical chemistry and urinalysis parameters and kidney weights (and which indicated renal toxicity/dysfunction) occurred only at the dose of 500 mg/kg bw/day and were reversible at the end of recovery. Since these effects are related to dose levels far above the guidance values, they are not relevant for classification.

Information from a valid 90-day oral study is available and there is no evidence demonstrating a lack of relevance for humans, hence, the degeneration of the olfactory epithelium is considered as relevant for classification purposes.

In conclusion, the non-reversible degeneration of the olfactory epithelium in the 90-day study meets the classification criteria for STOT RE 2. In agreement with the DS's proposal and the provisions of table 3.9.5 of Annex I to CLP the hazard statement should specify "nasal cavity" as the organ effected. As data is available from only one route of exposure (oral), it is proposed not to state the route in the hazard statement. **STOT RE 2; H373 (May cause damage to the nasal cavity through prolonged or repeated exposure) is warranted**.

Due to the fact that the target organ toxicity was not observed at doses clearly below the guidance values (in accordance to 3.9.2.6 of the CLP guidance), RAC agrees with the DS's proposal not to propose a specific concentration limit.

10.13 Aspiration hazard

Not evaluated as part of this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated as part of this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated as part of this dossier.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

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.

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

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

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

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

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

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

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

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

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

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

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).

ECHA (2021). 7-oxabicyclo[4.1.0]heptan-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate REACH Registration dossier. (Accessed 2021). https://echa.europa.eu/registration-dossier/-/registered-dossier/13863/1

15 ANNEX I

Detailed study summaries for studies referenced in this report.

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: -

Contact details for dossier submitter: Health & Safety Authority,
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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 *

Parameter	Week	Dose (mg/ kg bw/day)							
		Males				Females			
		0	5	50	500	0	5	50	500
	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 ± 11.4	75 ± 17.8	56 ± 15.7	45 ± 16.3**	67 ± 13.5	74 ± 16.7	52 ± 16.0*	56 ± 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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