# Committee for Risk Assessment

# RAC

## 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

## Adopted

## 2 June 2022

2 June 2022

CLH-O-0000007129-71-01/F

# OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

**Chemical name:** **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**

The proposal was submitted by **Ireland** and received by RAC on **15 July 2021.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

# PROCESS FOR ADOPTION OF THE OPINION

**Ireland** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 August 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 October 2021**.

### ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

Co-Rapporteur, appointed by RAC: **Agnes Schulte**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **2 June 2022** by **consensus**.

**Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)**

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

# GROUNDS FOR ADOPTION OF THE OPINION

## 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.

## HUMAN HEALTH HAZARD EVALUATION

## 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.

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

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.**

## 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 ($2 \times 10^{-3}$ 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 (See "Additional key elements" in the BD).

## 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.

## 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.<br><br>GLP compliant.<br><br>Triplicate plates per dose, test run in duplicate.<br>Mean number of revertant colonies per dose level and strain not reported. | 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.<br>156, 313, 625, 1250, 2500 and<br>5000 µg/plate.<br>± Metabolic activation with S9 mix.<br>Preparation details not reported.<br>Vehicle control: Dimethyl sulfoxide<br><br>Positive controls: | Result: positive ± metabolic activation.<br>↑ Revertant colonies in *S. typhimurium strains* TA 100 and TA 1535 (+S9) and in *E. coli* strain WP2 uvrA (± S9)<br><br>No | Anonymous, 1995.<br><br>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 |
|---|---|---|---|---|
| No information on cytotoxicity. | | 2-aminoanthracene (+S9)<br><br>2-acetylaminofluo-rene, sodium azide, 9-aminoacridine, N-ethyl-N-nitro-N-nitrosoguanidine (-S9)<br><br>Number of replicates: 3 plates/dose and test run in duplicate.<br><br>Reliability: reliable. | information on cytotoxicity reported. | |
| Non-guideline: in vitro gene mutation study in bacteria.<br><br>Not GLP compliant.<br><br>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.*<br><br>100, 250, 500, 1000, 2000 and 5000 µg/plate.<br><br>± Metabolic activation with S9 mix. Preparation details not reported.<br><br>Vehicle control: dimethyl sulfoxide<br><br>Positive controls:<br><br>2-aminoanthracene; 2-acetylaminofluorene, 9-aminoacridine and N-ethyl-N-nitro-N-nitrosoguanidine.<br><br>Number of cells evaluated: not reported.<br><br>Number of replicates: Not reported.<br><br>Reliability: *unreliable.* | Result: positive + metabolic activation.<br><br>↑ Revertant colonies in *S. typhimurium* strains TA 100 and TA 1535 (+S9).<br><br>No cytotoxicity up to 5000 µg/plate | Anonymous, 1987. ECHA dissemination site, 2021. |
| Non-guideline: *in vitro* gene mutation in mammalian cells.<br><br>Not GLP compliant<br><br>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).<br><br>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.<br><br>± Metabolic activation with rat liver S9 (Arochlor 1254 | Result: negative ± metabolic activation.<br><br>Cytotoxicity reported at 100 × $10^{-4}$ % (-S9). No cytotoxicity data available for (+S9). | 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 |
|---|---|---|---|---|
| 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).<br><br>Vehicle control: dimethyl sulfoxide<br><br>Negative control: untreated cells.<br><br>Positive controls:<br><br>N-dimethylnitrosamine (+S9); ethylmethanesulphonate (-S9)<br><br>Exposure time: 16 hours (-S9), 5 hours (+S9).<br><br>Expression period: 7-9 days.<br><br>Number of cells evaluated:<br>200 cells/dose for frequency of mutants per $10^6$ viable cells.<br><br>Number of replicates: 2<br><br>Reliability: reliable. | | |
| Non-guideline: *in vitro* gene mutation in mammalian cells.<br><br>GLP compliant.<br><br>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 | TK 10 310 (ARALDIT CY 179) (trade name of EC No. 219-207-4) (purity: not reported). | Mouse lymphoma (L5178Y), subline TK $^{+/-}$<br><br>12.5, 25, 50, 100, 150, 200 and 250 µg/mL.<br><br>± Metabolic activation with S9 mix.<br>Preparation details not reported.<br><br>Vehicle control: dimethyl sulfoxide.<br><br>Negative control: untreated cells.<br><br>Positive controls:<br><br>N-dimethylnitrosamine (+S9); ethylmethanesulphonate (-S9)<br><br>Exposure period:<br>4 hours.<br><br>Expression period:<br>3 days.<br><br>Selection time: 14 days | Result:<br>positive ± metabolic activation.<br><br>↑ Mutant colony count at<br>≥ 150 µg/mL (+S9) and<br>≥ 100 µg/mL (-S9).<br><br>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 |
|---|---|---|---|---|
| spontaneous mutant frequency, no sizing of mutant colonies and no reporting of cytotoxicity or mutant frequency data per dose. | | for mutant selection and 11-12 for viability.<br><br>Selection agent: 5-bromodeoxyuridine.<br><br>Number of cells evaluated: $4 \times 10^5$ cells/tube for mutant selection and 200 cells/tube for viability control.<br><br>Number of replicates: not reported.<br><br>Reliability: reliable. | | |
| Non-guideline: *in vitro* sister chromatid exchange (SCE) assay in mammalian cells.<br><br>Not GLP compliant.<br><br>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.<br><br>$3.125 \times 10^{-4}$ % to 100 $\times 10^{-4}$ % by volume. Exact concentrations not reported.<br><br>No metabolic activation.<br><br>Vehicle control: dimethyl sulfoxide.<br><br>Negative control: untreated cells.<br><br>Positive control: ethylmethane sulphonate.<br><br>Medium: BrdU-containing medium.<br><br>Pre-incubation time: 20 hours.<br><br>Exposure duration: 5 hours.<br><br>Expression time: 24 hours.<br><br>Spindle inhibitor: 0.2 µg/mL colchicine or 0.1 µg/mL colcemide 1-2 hours prior to harvest.<br><br>Number of cells evaluated: Minimum of 15 cells/dose.<br><br>Number of replicates: 3<br><br>Reliability: reliable. | Result: positive – metabolic activation.<br><br>↑ SCE frequency in 3 of 6 concentrations tested (exact concentrations not reported).<br><br>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.<br><br>Not GLP compliant.<br><br>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. | Epoxy resin ERL-4221 (trade name of EC No. 219-207-4) (purity: not reported). | Hepatocytes derived from rat liver.<br><br>Six concentrations between $1.0 \times 10^{-4}$ % and $1000 \times 10^{-4}$ % by volume. Exact concentrations not reported.<br><br>Vehicle control: dimethyl sulfoxide.<br><br>Positive controls: N-dimethylnitrosamine, 4-nitroquinoline-N-oxide.<br><br>Pre-incubation period: 1 hour.<br><br>Exposure duration: 2 hours.<br><br>Number of replicates: not reported.<br><br>Reliability: unreliable. | Result: equivocal.<br><br>↑ 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 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.<br><br>GLP compliant.<br><br>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.<br><br>0, 250, 500 and 1000 mg/kg bw/day via gavage for 28 days.<br><br>Vehicle: corn oil.<br><br>Positive control: N-ethyl-N-nitrosourea.<br><br>Sampling time: 28 + 3 days. Three days after the final dose, animals were sacrificed and genomic DNA extracted.<br><br>Tissue selection: liver, forestomach, nasal tissue | Result: positive.<br><br>↑ Mutant frequency in forestomach & liver at 1000 mg/kg bw/day.<br><br>No ↑ in mutant frequency in nasal tissue or germ cells.<br><br>↑ 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).<br><br>Reliability: reliable. | | |
| OECD TG 486: unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo.*<br><br>GLP compliant.<br><br>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.<br><br>0, 500, 1000 and 2000 mg/kg bw/day via oral gavage as a single administration.<br><br>Vehicle: water.<br><br>Positive control: N-dimethylnitrosamine.<br><br>Post exposure period: 2-4 hours or 12-16 hours.<br><br>Reliability: reliable. | Result: negative.<br><br>No ↑ in mean net nuclear grain counts at any dose. | Anonymous, 1999. ECHA dissemination site, 2021. |
| OECD TG 474: mammalian erythrocyte micronucleus test.<br><br>GLP compliant.<br><br>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.<br><br>0, 500, 1000 and 2250 mg/kg bw via i.p. as a single administration.<br><br>Vehicle: peanut oil<br><br>Positive control: cyclophosphamide via i.p.<br><br>Sampling time: 24, 48 and 72 hours post treatment.<br><br>Tissue selection: bone marrow.<br><br>≥ 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.<br><br>Reliability: reliable. | Result: negative.<br><br>No ↑ in mean number of MN PCE at any sampling point.<br><br>↓ Ratio of PCE/ (NCE & PCE) in females at 500 & 2250 mg/kg bw/day at 48 hours.<br><br>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 ($15.6 \times 10^{-6}$-$78.0 \times 10^{-6}$), 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 ($0.6 \times 10^{-6}$-$99.6 \times 10^{-6}$), 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)**.

**ANNEXES:**

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).