Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

1,4-dioxane

EC Number: 204-661-8
CAS Number: 123-91-1

CLH-O-0000001412-86-264/F

Adopted

15 March 2019

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: 1,4-dioxane
EC number: 204-661-8
CAS number: 123-91-1
Dossier submitter: Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
23.05.2018	Germany		MemberState	1
Comment received				
The German CA supports the NL initiative for identifying 1,4-dioxane as germ cell mutagen, Category 2 and increasing the classification for carcinogenicity from Category 2 to 1B. More recent information on toxicokinetic behaviour in humans can be found in Göen et al., Metabolism and toxicokinetics of 1,4-dioxane in humans after inhalational exposure at rest and under physical stress. Arch. Toxicol. (2016) 90:1315-1324 Furthermore the dossier submitter states in the CLH report that a change in the existing entry for 1,4-dioxane in Annex VI to the CLP Regulation is justified "due to new data". In this context, we would have appreciated if this "new data" had been specifically marked as such in the dossier and how this new data specifically changes the toxicological assessment from 1999 when the substance was discussed in the TC C&L.				
Dossier Submitter's Response				
Thank you for your support and pointing us towards the recent work on toxicokinetics of 1,4-dioxane in humans. It is noted that the study of Göen et al. (2016) confirms the rapid and almost complete metabolism of 1,4-dioxane to HEAA, reaching a steady state within 3-4h in the blood. In addition, exercise or work elevates the internal blood concentration of 1,4-dioxane likely by increased inhalation uptake. Overall the results seem to be in agreement with the studies by Young and coworkers (1977). The elimination half-life of HEAA was found to be 3.4 hours, only slightly higher than the 2.7 hours found by Young et al. (1977). Göen et al. (2016), noted that despite of the fast elimination kinetics, considerable amounts of HEAA were still detected after 16 h (post-exposure or 24h since the start of exposure) in the urine. The levels were rather low compared to the maximum elimination levels of HEAA indicating near complete elimination and limited accumulation may occur. These results are again in agreement with those of Young et al. (1977).				

Current classification as Carc. 2 (H351) dates from August 2001 (2001/59/EC (28th ATP)) and was based on the data presented in the RAR (Dossier Submitter NL). At that time, the following was considered:
"1,4-Dioxane can be considered as a carcinogen for test animals. In drinking water studies with rats and mice, liver and kidney damage and liver adenomas and carcinomas were induced. In rats also nasal adenomas and carcinomas were observed, accompagnied by non-neoplastic lesions in the nasal cavity. These lesions were also observed in mice, but in mice 1,4-dioxane induced no increased incidence of nasal tumours. The liver, kidney and nasal damage were still seen at concentrations of 0.02%, 0.1% and 0.1%, respectively, in drinking water, while at 0.01% (equivalent to 10 mg/kg bw/day) no effects were seen. The liver tumours were seen at 1,4-dioxane drinking water concentrations of ≥0.05% for mice and of ≥0.1% for rats. The nasal tumours in rats were observed at 1,4-dioxane drinking water concentrations of ≥0.5%.
No nasal and liver tumours, as observed after oral administration in drinking water, were seen after inhalatory exposure to 1,4-dioxane.
Some indication for liver tumours were also obtained in guinea-pigs, but no information on non-neoplastic lesions was provided.
For both liver and nasal tumours, cytotoxic effects and organ damage are considered to be involved, which are subject to nonlinear kinetics, implicating a threshold.
Initiator/promotor assays also demonstrated that 1,4-dioxane has no initiator activity but may act as a tumour promotor. Furthermore for what their scientific value might be, in several epidemiological studies no indications of association between (low-level) exposure to 1,4-dioxane and genotoxicity or carcinogenicity in humans have been obtained.

Although 1,4-dioxane has been demonstrated to be carcinogenic in rat and mouse and some indications have been obtained that the substance is also carcinogenic in guinea pigs, the absence of genotoxicity and the involvement of a threshold mechanism of action (i.e. specific organ toxicity) justify down-grading of the classification of 1,4-dioxane from Carc. cat 2 to Carc. cat 3."

Current proposal for an update of the carcinogenicity classification is based on new data and include the repeated dose and carcinogenicity studies in mice and rats by Kano et al. and Kasai et al. from 2008/2009. These studies confirm that there is positive evidence for carcinogenicity in at least two species (rat and mouse). Further, the mutagenicity study by Roy Thilagar and Eastmond (2005) provide, together with Mirkova (1994) and Morita and Hayashi (1998), some evidence for mutagenicity of 1,4-dioxane (which is used as basis for the proposed classification as Muta. 2). A genotoxic mode of action can therefore not be excluded. Additionally, it is noted that there is a change in the classification criteria for carcinogenicity (CLP vs. DSD). Previously, under the DSD-regulation, a non-genotoxic chemical would in general not be classified as carc. 2 (similar to the current 1B under the CLP-regulation). Current CLP-criteria do not exclude considering non-genotoxic chemicals as presumed human carcinogens. Based on all of this, a classification in category 1B (Carc. 1B; H350) is proposed.

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	Belgium	International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.)	Industry or trade association	2

Comment received
please see attachment ECHA note – An attachment was submitted with the comment above. Refer to public attachment A.I.S.E comments to proposal for harmonized classification and labelling 1,4-dioxane.pdf
Dossier Submitter's Response
Thank you for your comments. *Use of substance in Industry:* Although not important for the proposed classification, thank you for pointing out that 1,4-dioxane is not used as solvent for the production of cleaning products or cosmetics but is rather a by-product in these formulations. See comments nr 4 and 9 for our response with respect to issues raised for carcinogenicity and genotoxicity, respectively.
RAC's response
Noted.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	United States	American Chemistry Council	Industry or trade association	3
Comment received				
ACC does not support revision of the carcinogen classification of 1,4-dioxane. ECHA note – An attachment was submitted with the comment above. Refer to public attachment ACC comment on CLH proposal for 1,4-dioxane.pdf				
Dossier Submitter's Response				
Thank you for your comments. Although not important for the proposed classification, thank you for pointing out that 1,4-dioxane is not used as solvent for the production of cleaning products or cosmetics but is rather a by-product. *Carcinogenicity* In the comments, ACC has put some focus on the study by Dourson et al. (2017) that suggest a non-genotoxic (regenerative hyperplasia) mode of action. This study was not assessed in the CLH proposal as it was drafted before this study was published. However, as mentioned in response to comment no. 4, the mode of action proposed may be plausible but also non-genotoxic carcinogenicity is relevant for classification. Since there is also information suggesting 1,4 dioxane is genotoxic at higher dose levels, and the toxicity of metabolites cannot be excluded, no definite conclusions can be made about the mode of action underlying carcinogenicity. ACC further mentions that the only target organ seen at the mid-dose is the liver in the mouse strain which is more susceptible to such tumours. This is not considered true since also peritoneal mesotheliomas were observed (in rats) at the mid-dose level (upon				

inhalation). Regardless of the high background incidence in mice, liver tumours can still be relevant if a clear effect and dose response is seen (which is the case here).

As mentioned in the response to comment number 4, cytotoxicity with subsequent regenerative cell proliferation with a theoretical threshold would still be considered relevant for humans and in the opinion of the dossier submitter should not lead to a downgrade of the classification.

Please see further our response to comment number 4 as the comments provided followed similar arguments and set-up.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	Belgium	International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.)	Industry or trade association	4

Comment received

please see attachment

ECHA note – An attachment was submitted with the comment above. Refer to public attachment A.I.S.E comments to proposal for harmonized classification and labelling 1,4-dioxane.pdf

Dossier Submitter's Response

Thank you for your comments.

Carcinogenicity, see also the response to comment number 1:
Current proposal for an update of the carcinogenicity classification is based on new data and include the repeated dose and carcinogenicity studies in mice and rats by Kano et al. and Kasai et al. from 2008/2009. These studies confirm that there is positive evidence for carcinogenicity in at least two species (rat and mouse). Further, the mutagenicity study by Roy Thilagar and Eastmond (2005) provide, together with Mirkova (1994) and Morita and Hayashi (1998), some evidence for mutagenicity of 1,4-dioxane (which is used as basis for the proposed classification as Muta. 2). A genotoxic mode of action can therefore not be excluded. Additionally, it is noted that there is a change in the classification criteria for carcinogenicity (CLP vs. DSD). Previously, under the DSD-regulation, a non-genotoxic chemical would in general not be classified as carc. 2 (similar to the current 1B under the CLP-regulation). Current CLP-criteria do not exclude considering non-genotoxic chemicals as presumed human carcinogens. Based on all of this, a classification in category 1B (Carc. 1B; H350) is proposed.

Most of the arguments provided are based on the exposure levels being higher than the level where metabolism is saturated and as a consequence, the carcinogenic effects observed are irrelevant for humans because they are not exposed to such high levels. However, we do not agree with these arguments because the classification of the substance is based on the hazard of the chemical, not possible relevance of exposure. Further, the dose levels causing clear carcinogenic effects may indeed be at a level where metabolism is saturated. However this is also considered irrelevant because classification considers the hazard at reasonable high dose levels (generally below the limit dose).

Further, it is unkown if one of the metabolites can contribute to the carcinogenic potential although most experts and data suggest the parent is responsible.

A.I.S.E. mentions that Torkelson did not observe any tumours in a high number of rats (thus considered to be a sensitive assay, though only one concentration group was included), but this is explained by the much lower concentration levels compared to those of Kasai et al. (2009).
Further, A.I.S.E. mentions that at the mid-dose of 250 ppm (Kasai et al. (2009)) only the incidence of peritoneal mesotheliomas was significantly increased and these are common in F344 rats and in humans only associated with asbestos exposure. However, it is noted that there is a signifant increase in tumor incidence and a clear dose-response for mesothelioma. Therefore, this effect is considered to be treatment-related and not attributable to spontaneous occurrence and variability in F344 rats.
In conclusion, the dossier submitter is of the opinion that classification as Carc. 1B is warranted based on the intrinsic hazard of the chemical (clear evidence of carcinogenicity, observed in several species at reasonable dose levels) regardless of the mode of action being threshold, non-threshold or dependent on metabolism.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
23.05.2018	Germany		MemberState	5

Comment received

We agree with the dossier submitter's proposal to classify 1,4-dioxane as carcinogenic in category 1B. The new available data confirm that the substance has the potential to induce tumours in rats and mice. In this context, the induction of mesothelioma in male F344 rats not only after oral exposure but also after inhalation is specifically noteworthy. However, already at the time of the TC C&L it had been acknowledged that 1,4-dioxane is carcinogenic in two species. Still, specifically based on assumptions on the mode of action, it was agreed to classify the substance only in category 2. Hence, we would have appreciated if reflections on the potential mode of action for tumorigenesis had been included in the CLH report. (Quote from the European Union Risk Assessment Report on 1,4-Dioxane (page 83):
"Despite the fact that the substance is a carcinogen in two species (rats and mice), with some indication for a third species (guinea pigs), the current classification as category 3 carcinogen (R40) is agreed with because the substance is a low potent carcinogen and the available data indicate a non-genotoxic mechanism. For both liver and nasal tumours, cytotoxic effects and organ damage are considered to be involved, which are subject to non-linear kinetics, implicating a threshold.")

Several long-term studies in rats and mice with 1,4-dioxane are available. One reliable inhalation study in male rats shows a significant induction of nasal squamous cell carcinomas, hepatocellular adenomas, peritoneal mesotheliomas and subcutis fibroma. All available oral studies are based on addition of 1,4-dioxane to drinking water of rats or mice of both genders. Neoplastic effects attributable to treatment included nasal squamous cell carcinomas, hepatocellular adenomas and carcinomas, peritoneal mesotheliomas, and mammary gland adenomas. Tumours at high doses are preceded by non-neoplastic lesions at lower doses.
It has been questioned, whether the observed squamous cell carcinoma in the nasal cavity in the drinking water studies are of human relevance because they could result from water entering the nasal cavity when the animals drink and therefore present an exposure that is unlikely to happen in humans. There are no repeated-dose studies

available with dosing via other routes such as oral gavage or dermal. Therefore, the incidence of nose cavity tumours after oral administration by non-local exposure, which would be human relevant, cannot be excluded. Inhalation represents a relevant route for human exposure; human relevance is given from the incidence of squamous cell carcinoma in the nasal cavity in the inhalation study in rats of both genders.
However, the mechanism of the development of nose cavity tumours by oral or inhalation route could be different: In 13-week dose range finding studies, possibly pre-neoplastic enlarged nuclei in the respiratory epithelium and olfactory epithelium were evident for both inhalation route (only performed in rats (Kasai 2008)) and oral administration with drinking water (mice/rats (Kano 2008)). The authors of these studies report differences between oral and inhalation route: With oral administration they observe a "wide distribution of the enlarged nuclei over the entire upper respiratory tract including nasal cavity and trachea, in the absence of localization of the enlarged nuclei in the anterior and dorsal part of the nasal epithelia that the inhaled air first contacts." They argue for the possibility of conveyance of 1,4-dioxane or its toxic metabolites after gastro-intestinal absorption via the blood stream to epithelial tissues and by exhalation through the nasal cavity from the alveolar space. This is supported by nuclear enlargement and degeneration of bronchial epithelium with similar incidences as nuclear enlargements of epithelia in the nasal cavity. This is in contrast to the inhalation study, where "the incidences and severities of nuclear enlargement in the 1,4-dioxane-exposed males and females tended to decrease along the passage of inspiratory airflow through the upper and lower respiratory tracts. […] This difference in the route of exposure can be accounted for in terms of a first-pass effect such that the inhaled 1,4-dioxane comes into first contact with the anterior portion of the respiratory epithelium, while the orally administered 1,4-dioxane is conveyed to the respiratory epithelial cells through the nasal blood flow after its first entrance in the gastrointestinal system including the liver." The DS should consider placing a part of the short summary for or at least a reference to Section 10.12.1 (Short Summary and overall relevance of the provided information on repeated dose toxicity and carcinogenicity) also in the Carcinogenicity section.
Conclusively, there is sufficient evidence for carcinogenicity from incidence of tumours in rats and mice of both sexes, which makes 1,4-dioxane a presumed human carcinogen.
The T25 value calculations appear plausible and deviating from the GCL seems not to be indicated.
However, the DS should discuss the potential mode of action, especially as the present dossier identifies 1,4-dioxane as a mutagenic substance, thereby classifying it as a genotoxic carcinogen. It should be evaluated, if a practical threshold for carcinogenicity can be derived, as toxicokinetic data suggest a saturation of the metabolism at high doses. Moreover, gene mutations have not been observed and genotoxicity is observed only in vivo and at high doses.

Dossier Submitter's Response

Thank you for your support and comments.
It is acknowledged there was doubt about the mode of action resulting in carcinogenicity and it seems to be a threshold mode of action rather than non-threshold. See also our response to comment 1:
Current proposal for an update of the carcinogenicity classification is based on new data and include the repeated dose and carcinogenicity studies in mice and rats by Kano et al. and Kasai et al. from 2008/2009. These studies confirm that there is positive evidence for carcinogenicity in at least two species (rat and mouse). Further, the mutagenicity study by Roy Thilagar and Eastmond (2005) provide, together with Mirkova (1994) and Morita and Hayashi (1998), some evidence for mutagenicity of 1,4-dioxane (which is used as basis for the proposed classification as Muta. 2). A genotoxic mode of action can therefore not be excluded. Additionally, it is noted that there is a change in the classification criteria for carcinogenicity (CLP vs. DSD). Previously, under the DSD-regulation, a non-genotoxic chemical would in general not be classified as carc. 2 (similar to the current 1B under the

CLP-regulation). Current CLP-criteria do not exclude considering non-genotoxic chemicals as presumed human carcinogens. Based on all of this, a classification in category 1B (Carc. 1B; H350) is proposed.

Thank you for the comments on the differences between the occurrence of epithelial carcinomas in the respiratory tract via the oral and inhalatory route (and the acknowledgement they should be regarded as relevant for humans). Unfortunately we cannot alter the CLH report at this stage of the CLH-process anymore to include more details in the short summary on carcinogenicity or a reference to the data on toxicity after repeated exposure.

A practical threshold based on saturated metabolism would require knowledge on which entity, the parent or the metabolite, causes the carcinogenic effects and if this is caused via a threshold or non-threshold mode of action. It is possible to speculate that the parent compound is likely responsible since most of the effects are observed at first pass organs and because of the difference in nasal tumour distribution throughout the respiratory tract between the two exposure routes. However, it is also reported that one of the metabolites of 1,4 dioxane, 1,4-dioxane-2-one is more toxic (ATSDR 2012). Overall there is uncertainty on the mode of action also considering that a genotoxic mode of action cannot be excluded, and the metabolite might also contribute towards the carcinogenic potential.

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	Sweden		MemberState	6
Comment received				
The Swedish Chemicals Agency agrees with the proposal to classify 1,4-dioxane as Carc. 1B based on sufficient evidence in animals (clear evidence of tumors in multiple species in multiple organs).				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	Germany	BASF SE	Please select organisation type..	7
Comment received				
Please find our comments attached. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments_BASF SE_CLH_123-91-1_20180606 (002).pdf				
Dossier Submitter's Response				
Thank you for your comments. See also our response to comments 3 and 4 and our general response: Current proposal for an update of the carcinogenicity classification is based on new data and include the repeated dose and carcinogenicity studies in mice and rats by Kano et al. and Kasai et al. from 2008/2009. These studies confirm that there is positive evidence for				

carcinogenicity in at least two species (rat and mouse). Further, the mutagenicity study by Roy Thilagar and Eastmond (2005) provide, together with Mirkova (1994) and Morita and Hayashi (1998), some evidence for mutagenicity of 1,4-dioxane (which is used as basis for the proposed classification as Muta. 2). A genotoxic mode of action can therefore not be excluded. Additionally, it is noted that there is a change in the classification criteria for carcinogenicity (CLP vs. DSD). Previously, under the DSD-regulation, a non-genotoxic chemical would in general not be classified as carc. 2 (similar to the current 1B under the CLP-regulation). Current CLP-criteria do not exclude considering non-genotoxic chemicals as presumed human carcinogens. Based on all of this, a classification in category 1B (Carc. 1B; H350) is proposed.

Please view our response to comment number 3 and 4.

RAC's response

Noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	United States	American Chemistry Council	Industry or trade association	8

Comment received

ACC does not support the classification of 1,4-dioxane as a mutagen.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ACC comment on CLH proposal for 1,4-dioxane.pdf

Dossier Submitter's Response

Please view our response to comment number 9.

In addition, we would like to note that in the comments, it is stated that the positive results in the study by Roy Thilagar and Eastmond (2005) were observed in the presence of strong tissue toxicity. However, even though the PCE/PNC ratio declined with increasing dose, the authors determined that the increase in micronuclei were primarily a result of chromosomal breakage and there was no mentioning of "strong toxicity/cytotoxicity". Therefore the results are considered relevant for classification. Roy Thilagar and Eastmond (2005) also noted that reasons for the discrepant micronucleus assay results among various investigators was unclear, but could be related to the inherent variability present when detecting moderate to weak responses using small numbers of animals, as well as differences in strain, dosing regimen, or scoring criteria.

RAC's response

RAC assessed the data for mutagenicity and concluded as follows: 1,4-dioxane did not induce genotoxicity *in vitro* showing that 1,4-dioxane has no direct mutagenic potential. Results from *in vivo* studies showed an increase in micronuclei (MN) formation in several studies in bone marrow cells and hepatocytes, but not in peripheral blood cells. However, the results from the studies were inconsistent. In the majority of the studies in bone marrow cells an induction of MN was reported above the limit dose of 2000 mg/kg bw according to the OECD TG 474, and in the hepatocytes an induction of MN was only reported at or above the limit dose of 2000 mg/kg bw. It should also be mentioned that in most of the *in vivo* studies no data on cytotoxicity were reported, which limits the interpretation of the results. RAC therefore considers that a classification of 1,4-dioxane as Muta. 2 as proposed by the DS is not justified based on the CLP criteria, leading to no classification for mutagenicity.

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	Belgium	International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.)	Industry or trade association	9

Comment received

please see attachment

ECHA note – An attachment was submitted with the comment above. Refer to public attachment A.I.S.E comments to proposal for harmonized classification and labelling 1,4-dioxane.pdf

Dossier Submitter's Response

Thank you for your comments.

Mutagenicity:
We agree that the *in vitro* tests were negative. Regarding the *in vivo* tests A.I.S.E. mentions the results are mostly inconsistent and depend on the mouse strain used while the positive results were found in non-OECD guideline studies. We also agree that local organ toxicity, such as inflammation, can lead to the increased formation of micronuclei. However we do not agree that the positive micronuclei results in the liver or bone marrow in the study of Roy Thilager and Eastmond (2015) can be attributed to local organ damage as there was no sign of inflammation or high general toxicity. Further, this positive study was assigned a klimisch score of 2 which is considered reliable regardless even though it did not follow an OECD guideline. OECD guideline studies are considered reliable but non-guideline studies should not be disregarded, especially when the studies are given a klimisch score of 2.
The liver is indeed not a standard organ for measurement of genotoxicity. In this case it is deemed relevant since carcinogenic effects after oral administration are predominantly found in the liver. It is acknowledged that the dose levels were much higher than the levels that were able to cause carcinogenic effects. However the carcinogenicity studies were also longer (lifetime). Further, the effects followed a dose dependent manner starting below the usual limit dose levels.
We do recognize that many experts regard 1,4 dioxane as a non-genotoxic carcinogen. Some of the mentioned work was not available when this CLH proposal was drafted (e.g. Dourson., 2017). The analysis by Dourson et al. seems plausible but as indicated a possible genotoxic mode of action cannot be excluded.
Overall, in many opinions 1,4-dioxane is regarded as a non-genotoxic carcinogen. However the new mutagenicity study by Roy Thilagar and Eastmond (2005) provide, together with Mirkova (1994) and Morita and Hayashi (1998), some evidence for mutagenicity of 1,4-dioxane. This is used as basis for the proposed classification as Muta. 2. Since the genotoxicity observed (in somatic cells only) starts at doses below the limit dose and clearer effects are observed at higher doses, this fulfills the criteria for muta. 2. Therefore the dossier submitter is of the opinion that classification as muta. 2 is appropriate regardless of the possible mode of action associated with its carcinogenicity.

RAC's response

See RAC's response to comments No. 8.

Date	Country	Organisation	Type of Organisation	Comment number
23.05.2018	Germany		MemberState	10
Comment received				
We agree with the dossier submitter's proposal to classify 1,4-dioxane as mutagenic in category 2 based on new available data. The study results by Roy et al. 2005 confirm the findings by Mirkova 1994: 1,4-dioxane has the potential to induce micronuclei in bone marrow erythrocytes. Together with the study by Morita and Hayashi 1998, the Roy study further shows that 1,4-dioxane has the potential to induce micronuclei in liver cells. In this context, however, we would have appreciated if the findings from Roy et al. 2005 had been presented in the report in more detail by providing numerical information for each dose group. In vitro studies on mutagenicity were almost exclusively negative, but two positive studies are reported in which either cytotoxicity was present (comet assay in rat hepatocytes positive above cytotoxic conc.) or no data on cytotoxicity is available (Sister Chromatid Exchange at 10 520 ug/ml). Positive results from in vivo micronuclei studies were obtained only at high doses, at or close to the limit dose of 2000 mg/kg (OECD TG 474). There are no positive studies available with less than 1000 mg/kg bw and it should be noted that none of the positive studies has been performed under GLP or relevant OECD test guidelines. Most studies did not report on cytotoxicity, but cytotoxicity should be considered more carefully in evaluating the relevance of study results for a classification of 1,4-dioxane as a germ cell mutagen. However, at least in Roy, Thilagar and Eastmond (2005), a dose-related increase in micronuclei in CD-1 mice bone-marrow and hepatocytes with additional evidence for chromosome breakage with no overt cytotoxicity (hepatocyte proliferation or PCE/NCE ratio in bone marrow reduced by less than 50 % at highest dose) is reported. Conclusively, and supported by other in vivo studies at similar doses in which no cytotoxicity data were reported, this indicates sufficient evidence for mutagenicity to classify 1,4-dioxane as a Category 2 germ cell mutagen.				
Dossier Submitter's Response				
Thank you for your support and shared considerations.				
RAC's response				
See RAC's response to comments No. 8.				

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	Sweden		MemberState	11
Comment received				
The Swedish Chemicals Agency agrees with the proposal to classify 1,4-dioxane as Muta. 2 based on positive evidence in vivo. Cytotoxic mechanisms seem to be involved, however genotoxic effects cannot be excluded since mutagenicity was also observed at doses below the limit dose (including dose-related increases).				
Dossier Submitter's Response				
Thank you for your support and considerations.				
RAC's response				
See RAC's response to comments No. 8.				

Date	Country	Organisation	Type of Organisation	Comment number
08.06.2018	Germany	BASF SE	Please select organisation type..	12
Comment received				
Please find our comments attached. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments_BASF SE_CLH_123-91-1_20180606 (002).pdf				
Dossier Submitter's Response				
Thank you for your comments. See also our response to comment numbers 8 and 9.				
RAC's response				
See RAC's response to comments No. 8.				

PUBLIC ATTACHMENTS
1. A.I.S.E comments to proposal for harmonized classification and labelling 1,4-dioxane.pdf [Please refer to comment No. 2, 4, 9]
2. Comments_BASF SE_CLH_123-91-1_20180606 (002).pdf [Please refer to comment No. 7, 12]
3. ACC comment on CLH proposal for 1,4-dioxane.pdf [Please refer to comment No. 3, 8]