Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

acetaldehyde; ethanal

EC Number: 200-836-8
CAS Number: 75-07-0

CLH-O-0000001412-86-120/F

Adopted
16 September 2016

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.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: acetaldehyde; ethanal
CAS number: 75-07-0
EC number: 200-836-8
Dossier submitter: The Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.09.2015	Belgium	European Flavour Association		1

Comment received

Issue: new classification proposal for acetaldehyde (ethanal) as: **Muta. 1B** & **Carc. 1 B** – on behalf of the global Flavour & Fragrance industry EFFA wishes to submit comments and has major reservations regarding this proposed classification.

Current classification: according to the Annex VI of the CLP Regulation the **current classification for carcinogenicity** of acetaldehyde is: **Carc. 2**.
This harmonised classification is a straight translation (without any modification) from the previous "DSD-classification" (cfr Dangerous Substance Directive 67/548/EEC) as "**Carc. Cat. 3**" (with the "translation" from DSD to CLP category 3 became new category 2).
Currently there is **no classification for mutagenicity**.

Industry proposal:
- On **carcinogenicity**: go back to the current classification for carcinogenicity: **Carc. 2**
- On **mutagenicity**: propose new classification (based on new scientific evidence) for mutagenicity: **Muta. 2**

Rationale (see in detail explained in the attached pdf document):
- On carcinogenicity:
  - The CLH report covers mainly additional information on mutagenicity (see next point) rather than carcinogenicity.
  - There is limited new data present regarding carcinogenicity compared to the studies available for the harmonized classification in 1993, namely two studies which are both being questioned for their reliability (page 41 of the CLH-dossier)
  - In addition, CLH report confirms that no human studies addressing the carcinogenicity of acetaldehyde on its own have been retrieved from public literature, hence acknowledging that the human data on carcinogenicity are **not sufficient to derive a classification** (page 49).

- On mutagenicity:
  - Most additional information in the CLH report is related to *in vitro* mutagenicity studies in mammalian cells, including human cells, and *in vivo* animal mutagenicity studies.
  - Only two studies related to animal germ cell mutagenicity are listed in the CLH report: the first one was already available when the initial harmonized classification was established in 1993; the second one, although more recent, uses a route of administration (intra-peritoneal injection) which is not an appropriate route of exposure and does not reflect normal intake in relation to humans. Hence we question the conclusions of this study and the biological relevance (see more details in the attaché pdf document).
  - Taken into account this new scientific information (and bearing in mind the questionable biological relevance), Industry though agrees for an **alternative classification as: Muta. 2.**

Conclusion/summary:
Industry recommends to maintain the current classification for acetaldehyde with regard to carcinogenicity, i.e. Category 2, bearing the hazard statement H351 (suspected of causing cancer). On the other hand, we acknowledge that new data on mutagenicity may warrant a classification for this hazard class with Category 2, H341 (suspected of causing genetic defects). However, we disagree with the authors' conclusion that a classification for germ cell mutagenicity as Category 1B is justified.

ECHA note: The following attachment was submitted with the comment above:
- *EFFA - Response to the ECHA Public Consultation on the CLH report "Proposal for Harmonized Classification and Labelling of Acetaldehyde" based on regulation (EC) 1272/2008 (CLP Regulation), Annex VI, Part 2.*

Dossier Submitter's Response

Thank you for your comment.
Regarding the comments on carcinogenicity, the limited number of new studies does not necessarily prohibit an adaptation of the classification. Moreover, the new information on mutagenicity has at least supportive relevance for carcinogenicity, as it strengthens the mechanistic plausibility. The comment that human data is insufficient for classification is correct, however this excludes classification as Category 1A carcinogen, while the proposal is for classification as Cat 1B. In addition the classification criteria for carcinogenicity changed in the transition form DSD to CLP. Whereas the criteria in the DSD focussed mainly on carcinogens without a treshold for Cat 1B (Cat 2 under DSD), CLP focusses less on non-treshold carcinogens and includes mutagenicity in the specific considerations. Also, classification of acetaldehyde as a category 1B carcinogen would be in line with the structurally similar substance formaldehyde for which RAC concluded classification in Category 1B.
Regarding mutagenicity, we agree that the direct evidence for in vivo heritable germ cell mutagenicity is too weak for classification as 1B by itself. However, as also stated in the CLH report, substances may be categorized in 1B if there are "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". The latter may be based on a) "supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo", or b) "by demonstrating the ability of the substance or its metabolites to interact with the genetic material of germ cells". Sufficient evidence has been found for in vivo mutagenicity testing in somatic cells of mammals. The intraperitoneal study does indicate that the ability of acetaldehyde to interact with genetic material is not limited to the site of entry. Additionally, toxicokinetic studies showed that acetaldehyde was distributed in the blood, liver, kidney, spleen, heart, myocardium and skeletal muscle of rats after oral and inhalation exposure.
Thus, we remain of the opinion that acetaldehyde has the potential to cause mutations in germ cells.

RAC's response
Thank you for the comments. Your position has been noted. RAC is aware that the biological relevance of SCEs has been called into question, and will take this into account in the opinion. The relevance of studies conducted via the i.p. route will also be considered.

Date	Country	Organisation	Type of Organisation	Comment number
28.08.2015	Germany		MemberState	2
Comment received				
The German CA supports the proposal to classify acetaldehyde as carcinogen Cat. 1B. However the German CA considers that a classification as Category 1B mutagen is not justified. Due to the positive results from in vitro tests as well as from soma cell assays in vivo a classification as Category 2 mutagen is proposed. See specific comments below.				
Dossier Submitter's Response				
Thank you for your comment. See the response below.				
RAC's response				
Thank you for the comments. Your position has been noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.09.2015	Sweden		MemberState	3
Comment received				
The Swedish CA supports classification of acetaldehyde (Cas No 75-07-0) in Carc. 1B and Muta. 1B as specified in the proposal. SE agrees with the rationale for classification into the proposed hazard classes and differentiations.				
Dossier Submitter's Response				
Thank you for your comment and your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.09.2015	France		MemberState	4
Comment received				
FR-CA agrees with the classification proposal of Muta 1B and Carc 1B.				
Dossier Submitter's Response				
Thank you for your comment and your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.09.2015	Belgium	INTERNATIONAL FRAGRANCE ASSOCIATION	Industry or trade association	5
Comment received				
For the reasons indicated on the attached document, we do not believe there is sufficient evidence to support the proposed re-classification of acetaldehyde as a Category 1B carcinogen, and therefore suggest that the current classification Category 2 carcinogen be maintained. Furthermore, while there may be evidence to support a Muta. 2 classification				

to address mutagenicity in somatic cells, we are convinced that the available data on the mutagenicity in germ cells is not sufficient to support a Muta. 1B classification.

ECHA note: The following attachment was submitted with the comment above:
- *IFRA Response to the ECHA Public Consultation on the CLH report "Proposal for Harmonized Classification and Labelling of Acetaldehyde" based on regulation (EC) 1272/2008 (CLP Regulation), Annex VI, Part 2.*

Dossier Submitter's Response

Thank you for your comment. Please see the response to comment 1.

RAC's response

Thank you for the comments. Your position has been noted. RAC is aware that the biological relevance of SCEs has been called into question, and will take this into account in the opinion. The relevance of studies conducted via the i.p. route will also be considered.

Date	Country	Organisation	Type of Organisation	Comment number
26.08.2015	United Kingdom		Individual	6

Comment received

Comments on proposal for the harmonised classification and labelling of Acetaldehyde (EC 200-836-8, CAS 75-07-0)

We would like to submit comments on the proposal for the harmonised classification and labelling for acetaldehyde that has been submitted by the Netherlands to add the classifications cat 1B H350 and muta 1B H340 to this substance. The proposal document seems to be a thorough review of the available data and our comments are restricted to the scope and reliability of data that should be included and the weighting of that data in coming to a conclusion on an appropriate decision. We request that the RAC take these comments into account in their deliberations.

<u>Manufacture and uses</u>
We would like to make one minor comment regarding the list of identified uses.

To my knowledge, acetaldehyde has never been used as a denaturant for alcohol and is certainly not used now. It may be present as a very minor impurity in some naturally derived denaturants, such as wood alcohol, but it has no intended use as a denaturant.

The identified uses also correctly acknowledge that acetaldehyde occurs widely as a trace component in foodstuffs and is also formed endogenously in humans. Humans have evolved multiple detoxification mechanisms and are capable of breaking it down very quickly once formed. This is important since it means that whilst it may be theoretically possible that the substance may be able to reach more distant organs such as the testes and ovaries, there is no experimental evidence to support this.

ECHA note: An attachment was submitted with the comment above. As it contains the same content as the comment, it is not provided as a separate attachment.

Dossier Submitter's Response

Thank you for your comment.
While the extent in which acetaldehyde is currently used as alcohol denaturant is unclear, it has been listed as such by various groups, including the SCCNFP (in opinion

SCCNFP/0821/04) and the US Governmental Publishing Office (http://www.ecfr.gov/cgi-bin/text-idx?rgn=div5;node=27%3A1.0.1.1.17#se27.1.21_193).

For a response on the second comment on the ability of acetaldehyde to reach the germ cells, please see the response to comment 1.

RAC's response

Thank you for the comments. Your position has been noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.09.2015	United States	Acetaldehyde Working Group	Industry or trade association	7

Comment received

ACETALDEHYDE WORKING GROUP
1250 Connecticut Ave. NW Suite 700
Washington, DC 20036
202.419.1500

11 September 2015

European Chemicals Agency
Committee for Risk Assessment (RAC)
Helsinki, Finland

Re: Comments on the CLH Report on Acetaldehyde; Proposal for Harmonised Classification and Labeling (June 2015)

Dear Committee for Risk Assessment (RAC):

On behalf of the Acetaldehyde Working Group[1] (AWG), I am pleased to submit the following comments in response to the; Proposal for Harmonised Classification and Labeling (June 2015) on acetaldehyde prepared by RIVM, The Netherlands. AWG is a not-for-profit association, located in Washington DC, whose mission is to address human health and relevant risk assessment/regulatory issues of interest to the membership.

AWG's technical experts (both from within the membership and consultants) have been involved in the technical review of acetaldehyde and in the sponsorship/conduct of studies for several decades. In fact, several of the publications cited in the proposal were authored and/or sponsored by AWG representatives. As explained in the attached comments, AWG does not believe that the proposed classification changes for acetaldehyde are supported by the available data nor is it consistent with the applicable ECHA guidance.

The Netherlands Technical Support document, on which the RIVM proposal is based[2], presumes that acetaldehyde *"acts by a stochastic genotoxic mechanism"* (see Executive Summary (p. 11). A critical underlying premise to this thesis is that this type of mutagen produces a molecular interaction (i.e., DNA adduct) over background, irrespective of dose, and that this interaction leads to a finite probability of subsequent mutations over background. As further explained in the attached comments, available studies, some recent and not considered in the RIVM proposal, demonstrate that this stochastic mechanism is not the mode of action for acetaldehyde. In particular, these studies show that:

- While acetaldehyde is capable of interacting with DNA to form DNA adducts, available data document that exogenous exposure to acetaldehyde does not produce DNA adducts above background levels. A 2013 publication by Moeller et al. (2013)[3] demonstrated that exposure of human TK6 lymphoblastoid cells to acetaldehyde does not produce adducts above background level until an exposure concentration of 50 µM is exceeded.

- These chromosome level mutations should not be considered stochastic events induced by acetaldehyde; rather, there are a wide range of low-dose concentrations that do not induce mutations above background (Moeller et al., 2013

- Acetaldehyde is a ubiquitous compound that is endogenously produced by plants, animals and humans and as such, there is generally a continual source of endogenous and exogenous exposure to acetaldehyde.

One of the most comprehensive analyses of food sources not discussed in the RIVM proposal, was published in 2011 by researchers from the German Laboratory - *Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Weissenburger Straße 3, 76187 Karlsruhe, German (*Uebelacker and Lachenmeier, 2011). Their results document the large contribution of acetaldehyde from natural levels found in food and beverages. In fact, it is intriguing to note that the consistent and distinctive flavor profile of some beers are based on the presence of select constituents including acetaldehyde. Homeostatic mechanisms have evolved to keep intra-cellular concentrations within acceptable physiological ranges. These homeostatic mechanisms can only be overwhelmed by massive exposures that do not occur under actual industrial or consumer use scenarios.

AWG believes that the data are overwhelming that acetaldehyde is not a stochastic genotoxic substance and thus recommends that ECHA and the Member State Competent Authorities (MSCA) on the Risk Assessment Committee (RAC) reconsider this proposal. We maintain that a rational classification approach to an endogenous, ubiquitous compound like acetaldehyde can be supported by employing the recent June 2015 Guidance[4] on the Application of the CLP Criteria to the key inhalation studies on acetaldehyde by Woutersen and Feron. These studies showed that the tumors were preceded by chronic inflammation and cell regeneration/hyperplasia at the portal of entry. The guidance combined with the data on acetaldehyde supports the conclusion that no change in the classification of acetaldehyde is justified.

AWG appreciates the opportunity to provide these comments. Please do not hesitate to contact me if we can provide any further clarification/information. We are continuing to study and sponsor research on acetaldehyde and would be pleased to provide any supplemental information to ECHA, MSCA's or RAC representatives.
Sincerely,
Robert J. Fensterheim, MPH
Executive Director
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[1] AWG members include companies/organizations that either: a) produce acetaldehyde as a commercial product, b) manufacture other products that may be metabolized to acetaldehyde; or, c) organizations that do not manufacture, process or use acetaldehyde, but due to the nature of their process, emit acetaldehyde as a byproduct.
[2] Health Council of the Netherlands ACETALDEHYDE: Re-evaluation of Carcinogenicity and Genotoxicity (Pub. No. 2014/28, December 2014)

[3] Moeller, B. C., Recio, L., Green, A., et al. (2013) Biomarkers of exposure and effect in human lymphoblastoid TK6 cells following [$^{13}C_2$] acetaldehyde exposure. Toxicol. Sci. 133(1): 1-12.
[4] Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures (Version 4.1).

ECHA note: The following attachments were provided with the comment above:

- *ACETALDEHYDE WORKING GROUP. Comments on the CLH Report on Acetaldehyde; Proposal for Harmonised, Classification and Labeling (June 2015). (Submitted 11 September 2015)*
- *Research Article. Quantitative Determination of Acetaldehyde in Foods Using Automated Digestion with Simulated Gastric Fluid Followed by Headspace Gas Chromatography. Hindawi Publishing Corporation. Journal of Automated Methods and Management in Chemistry. Volume 2011, Article ID 907317, 13 pages*
- *Biomarkers of Exposure and Effect in Human Lymphoblastoid TK6 Cells Following [$^{13}C_2$]-Acetaldehyde Exposure. Toxicological sciences 133(1), 1–12 2013*

Dossier Submitter's Response

Thank you for your comment.
In response to your comments we would like to make the following remarks:

- In the study by Moeller et al (2013), a linear increase in exogenous adducts was shown in figure 3 of the article, irrespective of the formation of endogenous adducts. This shows that the proposed homeostatic mechanism to keep intracellular concentrations within acceptable physiological ranges has limitations.
- As can be seen in the CLH dossier, there is a large number of in vitro and in vivo studies that show acetaldehyde can induce gene mutations (positive for gene mutations, chromosome aberrations and micronuclei).
- While the background levels of acetaldehyde are indeed important to consider in the risk assessment, classification is hazard based. In addition, RAC classified formaldehyde, which has comparable carcinogenic properties and is also produced endogenously and present in food products, as a carcinogen in category 1B. This supports that the source of exposure is less relevant.
- Considering the risks associated with excessive alcohol abuse, the presence of acetaldehyde in certain beers cannot be considered evidence for its safety.

The key inhalation study by Woutersen and Feron considered carcinogenicity and contains insufficient information on mutagenic endpoints to be used to assess the mutagenic potential of acetaldehyde.

RAC's Response

Thank you for the comments and the additional information; the assessment of RAC will take into consideration the report provided during the public consultation. RAC agrees with the DS comments relating to hazard assessment. Background levels of acetaldehyde are not taken into account in the opinion. RAC is aware that the biological relevance of SCEs has been called into question; this is reflected in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
08.09.2015	Germany	Company-Manufacturer	Celanese	8

Comment received

General Comments
1. Comments on Identified Uses
The identified uses given in the CLH report (p. 13, section 2.2 Identified uses) seem to be quoted

from The Merck Index. (11th ed., 1989). The Merck Index was published by the United States pharmaceutical company Merck & Co., and information collected on acetaldehyde uses in 1989 does not necessarily reflect the European use pattern of acetaldehyde in 2015.
As can be seen from the existing REACH registrations, the most relevant use of acetaldehyde is as a chemical intermediate.

2. Endogenous in the human body and present in Food and beverages
New data are available on the quantitative determination of acetaldehyde in foods by headspace gas chromatography (Uebelacker and Lachenmeier, 2011 [A1]). The authors summarize "140 authentic samples were analyzed. The acetaldehyde content in apples was 0.97 +/- 0.80 mg/kg, orange juice contained 3.86 +/- 2.88 mg/kg. The highest concentration was determined in a yoghurt (17 mg/kg)."
The CLH report (p. 13, section 2.2 Identified uses) states that acetaldehyde is "occurring widely in nature" and is "formed endogenously in humans in small amounts". No further assessment of the implications of these statements for the effect of acetaldehyde exposure in humans is provided.
According to Regulation (EC) No 1272/2008 (CLP Regulation) Annex I Classification And Labelling Requirements For Hazardous Substances And Mixtures, No. 3.5.2.3.9. it is required that "The classification of individual substances shall be based on the total weight of evidence available, using expert judgement (See 1.1.1)."
It has to be taken into consideration that acetaldehyde occurs naturally in mammalians cells and is part of the physiological cellular metabolism. The ubiquitous and naturally occurring, endogenous nature of acetaldehyde needs to be considered as part of the total weight of evidence.

Note: These comments are submitted via the ECHA web form. Since it is expected that the formatting might not be transmittable via the web form, the complete set of comments will also be submitted as a public attachment.

References
[A1] "Quantitative Determination of Acetaldehyde in Foods Using Automated Digestion with Simulated Gastric Fluid Followed by Headspace Gas Chromatography" by Michael Uebelacker and Dirk W. Lachenmeier (2011) (Journal of Automated Methods and Management in Chemistry, Volume 2011, Article ID 907317)

ECHA note: The following attachment was submitted with the comment above:
Celanese Comments on Acetaldehyde CLH report 2015-09-08*.*

Dossier Submitter's Response

Thank you for your comments.

1. Noted. However, it should be mentioned that there is also a full registration for acetaldehyde.
2. While the background levels of acetaldehyde are indeed important to consider in the risk assessment, classification is hazard based. In addition, RAC classified formaldehyde, which is also produced endogenously and present in food products, as a carcinogen in category 1B. Formaldehyde has a very similar mode of action as acetaldehyde, as this is also a reactive, mutagenic compound that induces squamous cell carcinoma and adenocarcinoma in the nose after inhalation.
   See also the response to Comment 7

RAC's response

The ubiquitous, endogenous nature of acetaldehyde is acknowledged and will be taken into account by the committee.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
28.08.2015	Germany		MemberState	9
Comment received				

The German CA supports the proposal to classify acetaldehyde as carcinogen Cat. 1B.

Some comments for consideration:

4.10.1 Non-human information
(1) Carcinogenicity study in rats (Wouterson studies)
It is recommended to add the tumour data on unscheduled deaths after 15 months (Wouterson et al. 1984) and some information on the interim sacrifices of the carcinogenicity study. Otherwise it remains open why 105 rats/sex/group were tested and tumour data were only available for 55 of them.

(2) Study Woutersen et al. 1986: It is recommended to discuss the effects of excessive toxicity, caused by doses exceeding the maximal tolerated dose (MTB) as given in the Guidance on the Application of the CLP criteria (Version 4 – June 2015) chapter 3.6.2.3.3 j. On p. 216 Fig. 1 in the study of Woutersen et al. 1986 growth curves of rats exposed to acetaldehyde vapour for up to 28 month are given. Although doses in the top dose group where reduced over time the differences in body weights, between control group, top dose group and partly the mid dose group (male) as well as between control group and partly mid- and top-dose group (female), exceed the value of approximately 10% reduction in body weight gain clearly.

(3) It is further recommended to discuss the distinctively lower survival rate of the top-dose group more specifically in view on the tumour incidence.

Summary and discussion of carcinogenicity
(4) The Woutersen data did not allocate the tumours to the nasal levels or to a specific tissue type. Based on the preneoplastic findings the main sites of tumour origin are assumed to be the respiratory and the olfactory epithelium.

(5) Some information should be given on the assumed mode of carcinogenic action. Acetaldehyde is assumed to induce tumours via a local genotoxic activity as indicated from mutagenic properties in somatic cells and the production of DNA protein cross links in cells at the sites of exposure. The intracellular acidification was only minor in studies on vinyl acetate; its role is also questioned for acetaldehyde (as mentioned in 4.9.4 of the CLH report).

(6) The differences in enzyme activities of aldehyde dehydrogenases in different regions of the respiratory tract could be mentioned to explain the mode of action and the preferred sites of tumour development in the respiratory tract. See also the RAR on vinyl acetate http://echa.europa.eu/documents/10162/23433313-22b7-4e0a-a9d4-b469a451c1cf, p. 182 'Aldehyde dehydrogenase (ALDH) is the key enzyme for the elimination of acetaldehyde. Its activity is more than 2fold higher in the respiratory epithelium than in the olfactory epithelium. At high concentrations of intracellular acetaldehyde ALDH activity will not be sufficient to oxidise all acetaldehyde to acetic acid and acetaldehyde may accumulate. Saturation of metabolism of acetaldehyde by ALDH indicating limited enzyme capacity is suggested to occur at acetaldehyde concentrations of 300 ppm (Stanek and Morris, 1999).'

(7) With regards to the human relevance of animal data, the ubiquitous occurrence of acetaldehyde dehydrogenases in organs/tissues (including the upper gastrointestinal tract, see also p. 185 of the vinylacetate RAR) should be mentioned.

4.10.2 Human information
(8) Please clarify which cancer types were included in the head and neck cancer statistics (tumours of the lip, oral cavity, oropharynx, hypopharynx, larynx, nasopharynx, nasal cavity and paranasal sinuses, salivary glands, thyroid glands?)

Dossier Submitter's Response

Thank you for your comment and your support.
As the CLH proposal cannot be updated at this stage the requested additional information and statements are provided below.

(1) Included below are Tables I from Woutersen et al 1984 and 1987, which contain the tumour incidences in the rats that died in respectively the first 15 months and that were included in the recovery group.

Table I: Type and incidence of nasal tumours in rats that died or were killed in extremis during the first 15 months of exposure to acetaldehyde vapour (Woutersen et al. 1984)

Type of tumours	Incidence of tumours							
	Males dose group				Females dose group			
	Control	Low	Mid	Top	Control	Low	Mid	Top
No. of animals examined	4	1	6	32	3	1	2	31
No. of animals with nasal tumours	1	1	4	7	0	0	1	12
Squamous cell carcinoma in situ	0	0	0	0	0	0	0	2
Squamous cell carcinoma	1	0	1	4	0	0	0	8
Adenocarcinoma of olfactory epithelium	0	1	3	5	0	0	1	4

Table I: Incidence of nasal tumours in rats exposed to 0, 750, 1500 or 3000/1500 ppm acetaldehyde vapour for various periods of time. Results of animals exposed to acetaldehyde for 52 weeks followed by a recovery period of at most 26 weeks (Woutersen & Feron 1987)

	Males conc. group				Females conc group			
	Control	Low	Mid	Top	Control	Low	Mid	Top
Initial No. of rats	30	30	30	30	30	30	30	30
Effective No. of rats	29	29	30	24	28	30	29	24
No. of intercurrent deaths	0	3	7	18	1	3	5	12
No. of nasal tumour bearing animals	0	1	5	12	0	0	4	8
Adenocarcinoma	0	1	4	4	0	0	4	6
Squamous cell carcinoma	0	0	1	10	0	0	0	4

(2) and (3) The following statement was made in Woutersen et al (1986) regarding the pathology of the top-concentration rats:

Microscopic examination. The cause of early death or moribund condition of top-concentration rats was nearly always partial or complete occlusion of the nose by excessive amounts of keratin and inflammatory exudate. Several male and female top-concentration rats that died or were killed in moribund condition showed acute bronchopneumonia occasionally accompanied by tracheitis. In nearly all the affected animals, feed particles were found in the tracheo-bronchial tree. Moreover, the nose of each of these rats was partially or completely occluded by a nasal tumour or a plug of keratin with or without inflammatory exudate. One might speculate that rats being obligatory nose breathers easily inhale feed particles in attempts to breath through the mouth.

Under gross examinations, it was stated that nasal swellings were observed in all test groups and one control animal.
Based on the pathological findings and the tumour incidences reported in the tables above, it appears that the lower survival of the top-dose animals was caused by occlusion of the nose. This was either caused by tumour formation, excessive amounts of keratin, or a combination of both. The high incidence of non-neoplastic swelling in the top-dose group and resulting mortality has probably contributed to the lower incidence of adenocarcinoma compared to the mid-dose group. It should also be mentioned that the olfactory epithelium in the nose, where the adenocarcinoma originated, was damaged at all exposure levels. In contrast, the respiratory epithelium, where the squamous cell carcinoma originated, was much less effected at the mid-dose and unaffected at the low-dose. These findings indicate that the olfactory epithelium is more susceptible to the cytotoxic activity of acetaldehyde and/or more heavily exposed to acetaldehyde than the respiratory epithelium. This indicates that excessive toxicity is the reason the incidence of adenocarcinoma was highest in the mid-dose group after 28 months (but in the top-dose at earlier time points), while the top-dose showed the most squamous cell carcinoma.

(4) The assumed tumour sites are correct. More specifically, the squamous cell carcinoma originated from the respiratory epithelium and the adenocarcinoma from the olfactory epithelium.

(5) and (6) We agree with the remarks on the mode of action and in particular the notion that the availability of ALDH influences the carcinogenic potential of acetaldehyde in different tissues.

(7) We would like to emphasize that ALDH is also associated with a large degree of genetic polymorphism, which creates interindividual differences in cancer risk, as discussed in paragraph 4.10.3 of the CLH report.

(8)Regarding the question on paragraph 4.10.2, no clear differentiation was made in the studies of the different types of head and nech cancers. However, the common definition of head and neck cancer includes cancers of the larynx, throat, lips, mouth, nose, and salivary glands.

RAC's response

Thank you for the comments. Your position and the clarification provided by the DS have been noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.09.2015	Sweden		MemberState	10
Comment received				
We agree with the arguments for recommending classification of acetaldehyde in Carc. 1B.				
Dossier Submitter's Response				
Thank you for your comment and your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.09.2015	France		MemberState	11
Comment received				
Conclusion on classification and labelling, p. 49 As tumours were only observed after chronic inhalation exposure, it would be considered relevant to state the route of exposure in the hazard statement: "H350: May cause cancer by Inhalation"				
Dossier Submitter's Response				
Thank you for your comment. Due to the limitations in the oral studies, it is not possible to either confirm or dismiss the carcinogenic potential of acetaldehyde after oral exposure. As there is no reason to assume that the genotoxic activity of acetaldehyde is limited to the respiratory tract and toxicokinetic studies show systemic availability after oral exposure, we consider classification for all exposure routes appropriate.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
26.08.2015	United Kingdom		Individual	12
Comment received				
ANIMAL DATA There are no reliable studies by the oral or dermal routes of exposure for this end point. There are two reliable studies by the inhalation route, one each in hamsters and rats. All date from the 1970's/1980's and therefore none are likely to meet current protocols. In the Woutersen (1986) rat study, the top dose clearly exceeded the MTD and had to be reduced (eventually to below that of the mid dose exposure group). Results from this dose group therefore need to be interpreted with caution. The Feron (1982) study exposed hamsters to a single exposure concentration, which too exceeded the MTD as the dose had to be reduced during the study due to severe toxicity. Since this was the only dose group used, this lessens the reliability of the study, the results of which should be interpreted with caution. In the Feron study, no individual tumour reached statistical significance. Statistical				

significance was only reached (males only) when all tumours were combined. The only biologically significant tumour findings were in the larynx, according to the data presented in the proposal. In Woutersen, the only statistical significance, and most relevant, findings in both males and females were nasal adenocarcinomas (seen at all dose levels. Squamous cell carcinoma of the nose reached statistical significance in males only at the mid dose level.) Findings at the higher dose level should not be taken into account since this clearly exceeded the MTD, although they change the findings little.

HUMAN DATA

I believe this should be disregarded completely as either unreliable or irrelevant. The East German epidemiology data is clearly unreliable due to confounding with uncontrolled exposures such as smoking and a lack of control data.

As already stated in the previous section on mutagenicity, the regulation of alcoholic beverages is clearly outside of scope of the CLP regulation 1272/2008 and therefore data relating to the consumption and use of this product is not appropriate to consider. Such exposures in both dose and pattern are completely different and usually involve co-exposure to other compounds or other confounding lifestyle or dietary issues. It should also be emphasised that the database on alcohol and health effects is huge and cannot be covered in a single page! I therefore strongly urge the RAC to disregard the contents of section 4.10.2 of the proposal document.

COMPARISON AGAINST CLASSIFICATION CRITERIA

The proposal document makes a brief comparison against the classification criteria. In my view there is no relevant human data to consider. This leaves evidence from two animal studies using acetaldehyde, one in rats and one in hamsters. I believe that the hamster study warrants low weighting in any consideration due to the fact is used a single exposure well in excess of the MTD and significant findings only in males when all tumour incidence was combined, and even then only in 8/29 animals. Indeed, it is debatable as to whether this study would be more appropriately rated as unreliable, Klimisch 3. The results can only at best be regarded as equivocal. This just leaves the findings in rats to consider. Again, disregarding the top dose group findings as unreliable due to the MTD being exceeded, the only truly significant findings are the adenocarcinomas in both sexes, which reached incident rates of ~50% in the mid dose groups. Squamous cell carcinomas were just significant in the mid dose male group.

Taking into account the following:
• There is only one study with reliable results, and,
• The rat study shows effects in the nose and not the larynx and the hamster study shows the reverse, and,
• Due to differences in rat versus human nasal physiology and the fact that local concentrations in nasal tissues of rats for a given concentration are likely to exceed those of humans, couple with the proximity of the test doses in animals to the MTD.

I do not believe there is sufficient evidence to warrant a classification as category 1B. A classification as category 2 seems more appropriate, the classification that currently applies. I would also like to make the observation that none of the data being considered is new and must have been taken account when the current classification for carcinogenicity was agreed. My understanding is that the RAC does not normally revisit past decisions unless new information is available. If a different classification is agreed to the harmonised classification that currently prevails it would indicate that the Classification and Labelling Working group (under directive 67/548) mistakenly

interpreted the same data and previously made an incorrect decision.

ECHA note: An attachment was submitted with the comment above. As it contains the same content as the comment, it is not provided as a separate attachment.

Dossier Submitter's Response

Thank you for your comment.
We disagree that the findings in the high dose levels in the studies by Woutersen and Feron should be dismissed entirely due to excessive toxicity, especially as they confirm the tumour incidences at lower doses. However, the high toxicity at these doses should indeed be taken into account in the interpretation of the results.
The paragraph on alcohol consumption did not have the purpose to give a complete evaluation of the health influence of alcohol. Nor does it prove a direct association between acetaldehyde exposure and cancer, as explained at the end of this paragraph. However, alcohol consumption is wide-spread and a well-known source of acetaldehyde exposure. As such, these studies on genetic polymorphism and alcohol-related cancer risk do give supportive evidence as well as relevant mechanistic insight.

Possible reasons for the differences between hamsters and rats include differences in susceptibility of the epithelium to acetaldehyde, a difference in impact of acetaldehyde on the epithelium, a difference in breathing pattern, or a combination of these factors.
For an evaluation of the intra-species differences, we would like to refer to the classification of formaldehyde, which has a very similar mode of action as acetaldehyde. Formaldehyde induced squamous cell carcinomas in the nose in rats studies as well and also in that case, only one hamster study was available, which indicated a lower sensitivity of hamsters compared to rats. However, in addition to the rodent studies, also studies with monkeys were available for formaldehyde, which confirmed the findings in rats. It was concluded in the RAC opinion that 'The observed toxicity and carcinogenicity of formaldehyde in the respiratory tract of rats is therefore considered highly relevant for primates and humans.'

We do not agree that changing the existing classification would mean that the classification and labelling working group mistakenly interpreted the same data and previously made an incorrect decision. Although no new carcinogenicity data have become available there is new information on mutagenicity which is also relevant for the mechanism for carcinogenicity. The proposed classification is further supported by the RAC classification of formaldehyde. In addition, the classification criteria for carcinogencity changed when DSD (67/548) was replaced by CLP as GHS introduced the IARC criteria.

RAC's response

Thank you for the comments. Your position has been noted.

Date	Country	Organisation	Type of Organisation	Comment number
08.09.2015	Germany	Company-Manufacturer	Celanese	13

Comment received

Specific comments
Carcinogenicity
The CLH report acknowledges that no new data on carcinogenicity is available:
"The classification by the European Commission dates from 1991. The existing classification with Carc. Cat 2 is based on the same carcinogenicity studies as in this proposal." (p. 8, section 2.1 History of the previous classification and labelling)
The Health Council of the Netherlands' acetaldehyde evaluation [C1], which provides the basis for

the CLH report, equally notes that no new hazard data in either laboratory animals or humans was assessed: "The Committee noticed that in 1991, the European Commission classified the substance as a carcinogen in category 2 (according to the current CLP-system). The classification was based on the same carcinogenicity studies as described in the present report." (Health Council of the Netherlands, 2014, p. 48)

According to Regulation (EC) No 1272/2008 (CLP Regulation) Annex I: 3.6.2.2.6. "Some important factors which may be taken into consideration, when assessing the overall level of concern [for Carcinogenicity] are: […]
(j) the possibility of a confounding effect of excessive toxicity at test doses;
(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity."

These points are addressed in a 2011 assessment of acetaldehyde effects at EU level which concluded "Acetaldehyde is a physiological intermediate with low background concentrations. Its adverse effects (genotoxicity and mutagenicity) are limited to non-physiologically high concentrations." (Opinion of the EU's Committee for Risk Assessment (RAC) [C2])
This review supports the existing, more appropriate category 2 cancer classification for acetaldehyde, and the opinion of the EU's Committee for Risk Assessment (RAC) needs to be considered since it discusses acetaldehyde effects in detail.

Detailed comments on the mutagenicity data which constitute the basis for acetaldehyde's proposed re-classification on carcinogenicity are submitted in that section. In summary:
• The 2011 assessment of acetaldehyde effects at EU level mentioned above needs to be considered which concluded "Acetaldehyde is a physiological intermediate with low background concentrations. Its adverse effects (genotoxicity and mutagenicity) are limited to non-physiologically high concentrations." (Opinion of the EU's Committee for Risk Assessment (RAC)) [C2]
• Weight of Evidence needs to be considered: The CLH report (p. 13, section 2.2 Identified uses) states that acetaldehyde is "occurring widely in nature" and is "formed endogenously in humans in small amounts". No further assessment of the implications of these statements for the effect of acetaldehyde exposure in humans is provided.
According to Regulation (EC) No 1272/2008 (CLP Regulation) Annex I Classification And Labelling Requirements For Hazardous Substances And Mixtures, No. 3.5.2.3.9. it is required that "The classification of individual substances shall be based on the total weight of evidence available, using expert judgement (See 1.1.1)."
It has to be taken into consideration that acetaldehyde occurs naturally in mammalians cells and is part of the physiological cellular metabolism. The ubiquitous and naturally occurring, endogenous nature of acetaldehyde needs to be considered as part of the total weight of evidence.

• A report of the germ cell genotoxic effects of acetaldehyde, i.e. SCE frequencies in the male germ cells of mice apparently seems to constitute a relevant part of the new information that underlies the proposal for reclassification. The new information on acetaldehyde mutagenicity, namely Madrigal-Bujaidar et al. (2002) [C3], is summarized differently in two sections of the CLH report regarding the dose response in the SCE assay.
This ambiguous assessment of a relevant publication within the same document clearly compromises the basis of the CLH report to classify acetaldehyde for mutagenicity.
- "Madrigal-Bujaidar et al. (2002) […] a clear dose response relationship was reported…" (CLH report p. 36, section Germ cells)
- "The second study is published by Mardigal-Bujaidar et al. (2002) […] Although no clear dose-response relationship could be assessed, …" (CLH report p. 38, section Germ cell genotoxicity)
The Health Council of the Netherlands' acetaldehyde evaluation [C1], which provides the basis for the CLH report, as well as the authors Madrigal-Bujaidar et al. themselves did not identify a clear dose-response relationship for sister chromatid exchanges (SCE) in mouse spermatogonial cells.

• No Known Route Of Exposure For Portal Of Entry Carcinogen To Reach Target Tissue
The ubiquitous and endogenous nature of acetaldehyde needs to be considered when assessing effects on germ cells. The current CLH proposal justifies the use of the SCE endpoint as an indicator that exogenously administered acetaldehyde reaches the testes and is able to reach the germ cells and argues that the criterion laid out in Regulation (EC) No 1272/2008 (CLP Regulation)

Annex I, 3.5.2.2. for classification of a substance as Category 1B "It is possible to derive this supporting evidence […] by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells;" is fulfilled.
A qualitative assertion that acetaldehyde could reach germ cells is insufficient to justify the use of this data for classification purposes, since acetaldehyde, as a product of normal cellular metabolism, is present in cells continuously.

Please note that a detailed assessment of acetaldehyde carcinogenicity data will be submitted by the Acetaldehyde Working Group, a not-for-profit association located in Washington, DC.

Note: These comments are submitted via the ECHA web form. Since it is expected that the formatting might not be transmittable via the web form, the complete set of comments will also be submitted as a public attachment.

References
[C1] Reference (1) in the CLH report is cited as "Netherlands HCot. Acetaldehyde - Re-evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2014 2014/28 Contract No.: 978-94-6281-016-7." Final publication URL: http://www.gr.nl/sites/default/files/201428_ acetaldehyde.pdf
[C2] Committee for Risk Assessment (RAC) Opinion proposing harmonised classification and labelling at Community level of vinyl acetate (ECHA/RAC/DOC No CLH-O-0000001742-77-01/F; Adopted 10 June 2011; http://echa.europa.eu/documents/10162/0314bc3c-eb3c-442d-be4d-91a2c8d79311
[C3] Madrigal-Bujaidar E, Velazquez-Guadarrama N, Morales-Ramirez P, Mendiola MT. Effect of disulfiram on the genotoxic potential of acetaldehyde in mouse spermatogonial cells. Teratog Carcinog Mutagen. 2002;22(2):83-91.

ECHA note: The following attachment was submitted with the comment above:
Celanese Comments on Acetaldehyde CLH report 2015-09-08*.*

Dossier Submitter's Response

Thank you for your comment.
Although no new carcinogenicity data have become available there is new information on mutagenicity, which is also relevant for carcinogenicity as it provides mechanistic insight and supportive evidence.
In addition, the classification criteria for carcinogenicity changed when DSD (67/548/EEC) was replaced by CLP. Under the GHS criteria, there is a stronger incentive to classify a substance in Category 1B when there are positive results for two species, as is the case for acetaldehyde (rats and hamsters). DSD Annex VI part 4.2.1.2 contained a list of arguments which especially in combination would in most cases lead to category 3 (= current category 2) including lack of genotoxicity in short-term tests *in vivo* and *in vitro*. Seen the current absence of a classification for germ cell mutagenicity, not sufficient data was considered present at that time. This has changed in the interval, as shown by the large number of genotoxicity studies published in the last 25 years. The proposed classification is further supported by the RAC classification of formaldehyde, which is also an endogenous substance and has a very similar mode of action.

The RAC opinion cited in the comment (reference C2) discussed the classification of vinyl acetate, of which acetaldehyde is a metabolite. In the context of the RAC opinion 'non-physiologically high concentrations' should be interpreted as higher than naturally occurs in cells without external exposure. Vinyl acetate was mainly classified as Cat 2 carcinogen rather than 1B, because it is an indirect carcinogen, which has to be metabolised to acetaldehyde:

"Most significantly, the extensive mechanistic data suggests that vinyl acetate is carcinogenic by a secondary mechanism with a practical threshold. After inhalation and

oral exposure, vinyl acetate is rapidly and effectively hydrolysed to acetic acid and acetaldehyde, the latter is then converted to acetic acid by aldehyde dehydrogenase. The similarities of toxic and carcinogenic effects of vinyl acetate to those of acetaldehyde indicated that acetaldehyde is the critical metabolite that is responsible for the carcinogenic activity of vinyl acetate." (RAC 2011, vinyl acetate)

The carcinogenic and mutagenic potential of acetaldehyde is not questioned in this opinion, nor its relevance from a hazard perspective.
In that respect, it is interesting to note that vinyl acetate was tested at lower concentrations in an inhalation study than acetaldehyde:
"In rats, vinyl acetate induced an increased number of nasal tumours (mainly papillomas and squamous cell carcinomas) in various regions of the nasal mucosa after long-term inhalation. The total incidence was significantly increased at a concentration of 600 ppm, but a single papilloma already developed at 200 ppm. No significant tumour response was seen in mice after long-term inhalation of vinyl acetate vapour." (RAC 2011, vinyl acetate)

An oral cancer study with vinyl acetate in F344 rats and BDF1 mice (Umeda et al., 2004) demonstrated significantly increased rates of squamous cell tumours in the oral cavity (rats and mice), oesophagus and fore stomach (mice) after a 2-year administration of 10000 ppm vinyl acetate with the drinking water (equivalent mean doses in rats were 442 mg/kg bw/d for males, 575 mg/kg bw/d for females, in mice 989 mg/kg bw/d for males, 1418 mg/kg bw/d for females). Maximum increase in tumour incidences was found in the oral cavity in both species. Squamous cell carcinomas were already observed at a dose of 400 ppm in female rats (31 mg/kg bw/d). Incidences of tumours in the oral cavity and stomach were also reported in several other studies, but these were described as less reliable due to methodological limitations.

Although this is of course no direct evidence for the carcinogenicity of acetaldehyde; as the active metabolite of vinyl acetate, it is reasonable to assume that acetaldehyde has similar carcinogenic properties, as also claimed in the RAC opinion of vinyl acetate.

Concerning the comment on the study by Madrigal-Bujaidar et al (2002), this study emphasis was on germ-cell mutagenicity. The results of this study will be further discussed under comment 17. For the discussion on carcinogenicity, it is not the primary concern whether a substance is a germ-cell mutagen or a somatic mutagen, as also a somatic mutagen may induce tumors. As can be seen in the CLH proposal, there is a large body of evidence that shows acetaldehyde induces mutations. This is very relevant for the interpretation of the carcinogenicity studies, as it substantiates the hypothesis that acetaldehyde induces tumors in the nose not only by irritation, but also through direct DNA damage.

Regarding the potential of acetaldehyde to reach the germ cells see our answer to question 17.

RAC's response

Thank you for these additional points; they will be considered carefully by the committee. Please refer to the Opinion Document for the detailed review of the carcinogenicity and mutagenicity endpoints.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
28.08.2015	Germany		MemberState	14

Comment received

The German CA considers that a classification as Category 1B mutagen is not to be justified. Due to the positive results from in vitro tests as well as from soma cell assays in vivo a classification as Category 2 mutagen is proposed.

Justification
1. Statement on the available germ cells tests
The germ cell tests do not provide a justification for a classification as Category 1B mutagen. Although acetaldehyde induces 'indicator effects' (SCE's) in spermatogonia no mutagenic effects (here: clastogenic effects) were observed in spermatids of mice after i.p. injection.

Available germ cell tests
- Negative mutagenicity test (micronuclei in spermatids; Lähdati 1988)
- Positive genotoxicity test (indicator test; ‚sister chromatid exchanges' (SCE's) in spermatogonia; Madrigal-Bujaidar et al. 2002).

Comparability of both germ cell tests is given with regard to the test performance:
- Test animals: mice
- Doses: similar doses were tested
- Exposure: single i.p. injection
- Toxicity: lethality and acute toxicity at similar doses.

Reachability of the germ cells:
- The positive effect in the genotoxicity test indicates that acetaldehyde is available to reach the germ cells (after i.p. administration), and to interact with the genetic material.
- Due to the comparable test performance it is assumed that acetaldehyde also reaches the germ cells in the mutagenicity test.

Statement to the route of exposure:
- The reference on the 'non-physiological route of exposure' (i.p. injection) by the dossier submitter is correct. But in the Guidance to Regulation (EC) No 1272/2008 it is noted that in an in vivo test using intraperitoneal administration the result gives information on a possible intrinsic mutagenic property (chapter 3.5.2.4). Accordingly the results of the germ cell tests are relevant under consideration of the specific 'non-physiological route of exposure'.

Relevance of the results: Mutagenicity test vs. indicator test
- No heritable changes in the genetic material of tested cells were detected in the mutagenicity test.
- In contrast to the mutagenicity test an indicator test (here: SCE test) detected no mutations as genetic endpoint but endpoints that are related more or less closely to the formation of mutations. Accordingly, the induction of indicator effects such as SCE's does not necessarily leads to mutagenic effects. (e.g. due to repair mechanisms). [See also the publication from Madrigal-Bujaidar et al. 2002 (p. 89: ‚Thus at present there is no clear evidence that the observed damage by Ace could produce abnormal zygotes.']

2. Acetaldehyde dehydrogenase (ALDH2)
In the opinion of the German CA the general reference of the dossier submitter that it cannot be excluded that acetaldehyde may reach germ cells, especially in human with mutated form of ALDH2 (p. 40, second paragraph) is alone not sufficient for a proposal to classify acetaldehyde as Category 1B mutagen.

The dossier submitter points out that ALDH2 is the most important enzyme for aldehyde oxidation and thus an important factor for the distribution of acetaldehyde in the body. It is also known that ALDH2 enzyme with its high degree of polymorphism in humans influences the occurrence of DNA adducts in white blood cells due to exposure to alcohol (ab)use and smoking.

3. Classification criteria
Not all criteria for classification of acetaldehyde as Category 1B mutagen are met.

The hazard classification as Category 1B for germ cell mutagens according Annex VI of CLP Regulation (EC) No 1272/2008 is based also on 'positive result(s) 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'. The latter criterion may be based on (a) 'supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo', or (b) 'by demonstrating the ability of the substance or its metabolites to interact with the genetic material of germ cells'.

Whether these criteria are adequately met, is judged by the German CA as follows:
- Positive results from in vivo somatic cell mutagenicity tests in mammals are available?: YES

- Evidence that the substance has potential to cause mutations to germ cells?: There is no supporting evidence from the available germ cell tests.

(a) The germ cell tests allow conclusions about whether acetaldehyde has potential to cause genotoxic effects/mutations to germ cells (under consideration of the defined test conditions such as e.g. the route of exposure). Although an indicator test showed an increased induction of SCE's in spermatogonia of mice at a mutagenicity test no increased induction of micronuclei was determined at the analysis of spermatids of mice.

(b) For both tests can be assumed that acetaldehyde is able to reach the germ cells and to interact with the genetic material.

Overall conclusion
The German CA evaluates the following justification of the dossier submitter for a classification as Category 1B mutagen as not sufficient:
- Due to the uncertainties at the germ cell tests and the non-physiological route of exposure 'it cannot be concluded that acetaldehyde is genotoxic in germ cells on these studies alone'. (p. 39, first paragraph),
- '… it cannot be excluded that acetaldehyde may reach germ cells, especially in humans with a mutated form of ALDH2.' (p. 40, second paragraph),
- 'Overall, it is considered that some evidence exists that acetaldehyde has potential to cause mutation in germ cells.' (p. 40, third paragraph).

Due to the positive results from in vitro tests as well as from soma cell assays in vivo a classification as Category 2 mutagen is proposed.

Dossier Submitter's Response
Thank you for your comment. As noted in the comment, it has been shown that acetaldehyde can reach the germ cells. The result of the SCE test indicates that it has the ability to interact with the genetic material of the germ cells, which is supported by somatic in vivo and in vitro studies. The increase of acetaldehyde in several tissues after inhalation exposure shows that acetaldehyde can reach the systemic circulation and therefore supports the relevance of the results of the IP studies. We remain of the opinion that the negative outcome of the micronucleus assay does not disprove this, as this difference in result may be due to a lower sensitivity of the micronucleus assay, as also stated by Sweden in comment 13.
RAC's response
Thank you for the comments and analysis; your conclusion has been noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.09.2015	Sweden		MemberState	15
Comment received				
We agree that the available data demonstrate that acetaldehyde is mutagenic in somatic cells in vivo. Furthermore, there is data suggesting that acetaldehyde is genotoxic in germ cells, since sister-chromatid exchanges were induced in spermatogonial cells in mice after intraperitoneal injection. According to the criteria, positive evidence from somatic cell mutagenicity test in vivo in combination with supporting evidence that the substance has the potential to cause mutation in germ cells, which could be derived either from mutagenicity/genotoxicity tests in germ cells or by demonstrating the ability of the substance to interact with the genetic material of germ cells, would lead to classification in Category 1B. We agree that the study on sister-chromatid exchanges indicated that acetaldehyde has the ability to reach the germ cells and to interact with the genetic material of germ cells. The fact that a study on meiotic micronuclei in early spermatids and sperm abnormalities in mice was negative does not change our view that acetaldehyde has the ability to interact with the genetic material of germ cells, since the negative result might be related to a lower sensitivity of this study compared to the sensitivity of the study on sister-chromatid exchanges. In conclusion, we support the recommendation to classify acetaldehyde in Muta. 1B.				
Dossier Submitter's Response				
Thank your for your comment and your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
26.08.2015	United Kingdom		Individual	16
Comment received				
There is a significant amount of data available for acetaldehyde, both in vitro and in vivo. For the purposes of chemical regulation, the generation of in vitro data is normally regarded as a screening process. The assays are by their nature conservative so that negative results can be regarded as definitive in concluding no mutagenic potential for a substance. Positive results would normally then result in proceeding to in vivo testing in whole animal systems and results from these studies would then be used in any decision making process. When there is good reliable in vivo available, only this should be used as				

a basis for classification decisions – in vitro data should be considered for information only. In addition, higher weighting should be given to those studies that follow or are similar to standard protocols (information not provided in the proposal document) and those that use physiologically relevant routes of exposure (in this case oral and inhalation.) Other 'artificial' routes such as intraperitoneal (ip), need to be interpreted with caution because they bypass important detoxification mechanisms.

Taking these factors into account would suggest that of the somatic cell studies, the Kunugita (2008) study should be given the highest weighting. This produced negative results by the inhalation (up to 500ppm) and oral (100mg/kg) routes in wild type animals. (The knockout mouse results again should be disregarded for classification purposes; this provides useful information on mode of action but is clearly not a 'natural' situation and is not part of standard testing protocol procedures.) The remaining 3 reliable studies are all by the ip route, which will clearly bypass mechanisms for detoxification. Whilst these show positive effects, the data suggests a maximum tolerated dose (MTD) of ~250mg/kg by this route, and all studies included doses at or above this level (one only at this level). There must remain some doubt as to how relevant these results are to a hypothesis that acetaldehyde is mutagenic by relevant route of exposure.

GERM CELL MUTAGENICITY

The proposal document highlights that there are two animal studies that examined the effects of acetaldehyde on germ cells in vivo (Lahdetie, 1998 and Mardigal-Bujaidar, 2002 – both in mice). Both are by the more 'severe' and non-physiological route of dosing, namely ip. The former study showed no effects up to doses well above the MTD seen in other studies. The latter study showed some effects but no clear dose response relationship. A clear dose response relationship would affirm that the observed effects are treatment related and not just random effects, thereby adding reliability to the study. I agree that on the basis of these studies, genotoxicity in germ cells cannot be concluded but it must be emphasised that these are the definitive studies and end points when deciding on a mutagenicity classification and must be given heavy weighting in any decision making process.

HUMAN INFORMATION

The inclusion of this data is puzzling. The regulation of tobacco and alcoholic beverages is clearly outside of scope of the CLP regulation 1272/2008 and therefore data relating to the consumption and use of these products is not appropriate to consider. Resultant exposures in both dose and pattern are completely different and usually involve co-exposure to other compounds or other confounding lifestyle issues. This, coupled with the fact that the studies that are included appear to be of low reliability would lead us to conclude all as irrelevant. I therefore strongly urge the RAC to disregard the contents of section 4.9.2 of the proposal document.

COMPARISON OF DATA AGAINST CLASSIFICATION CRITERIA

The proposal document rightly compares the data against the classification criteria. There is data available in animal germ cells and I agree with the conclusion that there is no direct evidence for in vivo heritable germ cell mutagenicity of acetaldehyde, because the data that is available is either inconclusive or negative. The proposal then goes on to consider the somatic cell in vivo data and in vitro data and uses the evidence of positive effects here to conclude that a category 1B classification is appropriate. However, the regulation and guidance does not provide advice on how to weight contradictory evidence

and how to weight studies by routes that are physiologically relevant (inhalation, oral) versus those that are not (ip) when they too provide contradictory evidence. In this case, the 'gold standard' data should be studies that examine effects on germ cells. These are negative or ambiguous and should be given heavy weighting in any weight of evidence approach. For the somatic cell in vivo data, again heavier weighting should be given to the data generated by physiologically relevant routes, and these too are negative. These observations should be heavily weighted against the somatic cell positive data generated using ip dosing studies (at or around the MTD), which bypass detoxification mechanisms. Disregarding the irrelevant human data also, I conclude that at most this substance should only be classified as a category 2 mutagen. Considering the conflicting data appropriately weighted for relevance to the end point and route of exposure, I do not believe there is an adequate case for classification as category 1B.

ECHA note: An attachment was submitted with the comment above. As it contains the same content as the comment, it is not provided as a separate attachment.

Dossier Submitter's Response

Thank you for your comment.
While it is true that in vivo studies carry more weight, in vitro studies can give important mechanistic data. As such it is valid to used them as supportive evidence, especially when there is limited in vivo data and/or a strong agreement between in vitro studies as is the case for acetaldehyde.

Studies using a physiological route of exposure are indeed preferable, but as also stated in comment 12, an in vivo study using intraperitoneal administration gives information on a possible intrinsic mutagenic property. Accordingly the results of the germ cell tests are relevant under consideration of the specific 'non-physiological route of exposure'.

A similar argument applies to the use of knock-out animals. Although the results of wild-type animals carry more weight, knock-out animals can be used to demonstrate the mutagenic potential of a substance. An exemple of a substance that has been classified for mutagenicity on studies in knock-out animals is leucomalachite green.

For the response on the comments on human information, please see the respons to comment 11.

We agree that for the comparison of the data against the classification criteria a weight of evidence is needed because of the presence of contradictory data. The available in vitro data in mammalian cells are clearly positive showing that acetaldehyde can damage DNA directly and induce mutations in vitro. Contradictory in vivo data on somatic cells are available for acetaldehyde. In vivo studies showed an increase in DNA-protein cross links in respiratory tissues after inhalation exposure confirming similar results shown in vitro. The oral and inhalation studies with wild-type and ALDH2 knock-out mice by Kunugita et al (2008) show an increase in micronuclei in the knock-out mice but not in the wild-type mice. The most likely explanation for these results is that the formation of adducts and mutations depends on the dose level reaching the tested cell population. The detection of adducts and mutations also depends on the detection limit of the applied test. The differences between the positive ip micronuclei tests and the negative oral and inhalation study can also be best explained by the differences in local exposure levels. As classification of substances for mutagenicity is based on the available evidence and not dependent on the dose level required to induce such effects, the negative results obtained with the oral and inhalation study are considered not to be more important than the ip studies. Therefore, based on the results of the in vitro tests, the formation of DNA-protein cross links in vivo and the in vivo formation of micronuclei, acetaldehyde is considered

mutagenic in somatic cells in vivo. A weight of evidence evaluation is also required regarding the mutagenicity towards germ cells. No direct evidence is available showing mutagenicity in germ cells or the offspring. The only test that measured micronuclei in germ cells was negative. However, the SCE test that determines genotoxicity of the germ cells and therefore the ability to reach and interact with the germ cells was positive. Both studies are IP studies. However, the different results may be due to differences in sensitivity between these two types of effects with SCE tests possibly being more sensitive. The relevance of the IP route for systemic exposure is confirmed by the available ADME information showing increases in systemic acetaldehyde levels after inhalation exposure. The combination of the ability to interact with the germ cells plus the positive results from the mutagenicity tests in somatic cell justifies classification in category 1B.

RAC's response

Thank you for the comments; your conclusion has been noted. RAC agrees that the information in Section 4.9.2 (human information) from smokers and alcohol abusers is not relevant for classification, therefore this information is not taken into account in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
08.09.2015	Germany	Company-Manufacturer	Celanese	17

Comment received

Specific comments
Mutagenicity

1. No Known Route Of Exposure For Portal Of Entry Carcinogen To Reach Target Tissue
The current CLH proposal justifies the use of the SCE endpoint as an indicator that exogenously administered acetaldehyde reaches the testes and is able to reach the germ cells:
"There is no direct evidence that acetaldehyde reaches the germ cells or the testes and ovaries after exposure via physiological routes of exposure. However, as acetaldehyde reaches the systemic circulation and several organs it is considered likely that acetaldehyde will also reach the testes and ovaries." (p.15, section 4.1 Toxicokinetics (absorption, metabolism, distribution and elimination, Conclusion)
"Regarding the second part of the criterion, there is limited evidence that acetaldehyde is genotoxic (sister chromatid exchanges) in germ cells of mice (Madrigal-Bujaidar et al. 2002), when the substance was given by intraperitoneal injection.(5) These findings indicate that acetaldehyde is able to reach the germ cells, and interacts with the genetic material, which would be in line with the findings on absorption and distribution kinetics." (p.39, section 4.9.5 Comparison with criteria)
The CLH report thus argues that the criterion laid out in Regulation (EC) No 1272/2008 (CLP Regulation) Annex I, 3.5.2.2. for classification of a substance as Category 1B "It is possible to derive this supporting evidence […] by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells;" is fulfilled.

A qualitative assertion that acetaldehyde could reach germ cells is insufficient to justify the use of this data for classification purposes, since acetaldehyde, as a product of normal cellular metabolism, is present in cells continuously.
Intra-cellular acetaldehyde concentrations are kept at physiological concentrations through the enzymatic action of aldehyde dehydrogenase (ALDH). However, when exposures to acetaldehyde are high, the physiological concentrations may be exceeded and adverse effects produced. Therefore, an additional mutational load resulting from exogenous acetaldehyde could only be manifested when physiological concentrations are exceeded.

2. Additional new data are available
A 2011 assessment of acetaldehyde effects at EU level concluded "Acetaldehyde is a physiological

intermediate with low background concentrations. Its adverse effects (genotoxicity and mutagenicity) are limited to non-physiologically high concentrations." (Opinion of the EU's Committee for Risk Assessment (RAC) [B1])
This opinion of the EU's Committee for Risk Assessment (RAC) needs to be considered since it discusses acetaldehyde effects in detail.

3. Weight of Evidence to be considered in classification decisions
The CLH report (p. 13, section 2.2 Identified uses) states that acetaldehyde is "occurring widely in nature" and is "formed endogenously in humans in small amounts". No further assessment of the implications of these statements for the effect of acetaldehyde exposure in humans is provided.
According to Regulation (EC) No 1272/2008 (CLP Regulation) Annex I Classification And Labelling Requirements For Hazardous Substances And Mixtures, No. 3.5.2.3.9. it is required that "The classification of individual substances shall be based on the total weight of evidence available, using expert judgement (See 1.1.1)."
It has to be taken into consideration that acetaldehyde occurs naturally in mammalians cells and is part of normal cellular metabolism. The ubiquitous and naturally occurring, endogenous nature of acetaldehyde needs to be considered as part of the total weight of evidence.

4. Ambiguous assessment of relevant data in the CLH report
A report of the germ cell genotoxic effects of acetaldehyde, i.e. SCE frequencies in the male germ cells of mice apparently seems to constitute a relevant part of the new information that underlies the proposal for reclassification. The new information on acetaldehyde mutagenicity, namely Madrigal-Bujaidar et al. (2002) [B2], is summarized differently in two sections of the CLH report regarding the dose response in the SCE assay:
• "Madrigal-Bujaidar et al. (2002) […] a clear dose response relationship was reported…" (CLH report p. 36, section Germ cells)
• "The second study is published by Mardigal-Bujaidar et al. (2002) […] Although no clear dose-response relationship could be assessed, …" (CLH report p. 38, section Germ cell genotoxicity)
This seemingly contradictory assessment of a relevant publication within the same document clearly compromises the basis of the CLH report to classify acetaldehyde for mutagenicity.
Moreover, the CLH report states " This proposal for changing the harmonised classification is based on the report of the Health Council of the Netherlands.(1)" [B3] (page 8, section 2.1 History of the previous classification and labelling).
The Health Council of the Netherlands' acetaldehyde evaluation, which provides the basis for the CLH report, as well as the authors Madrigal-Bujaidar et al. themselves did not identify a clear dose-response relationship for sister chromatid exchanges (SCE) in mouse spermatogonial cells.
The report issued by the Health Council of the Netherlands states in section 5.4 Summary and discussion of mutagenicity:
"The second study is published by Mardigal-Bujaidar et al. (2002), and considers the induction of sister chromatid exchanges in mouse spermatogonial cells. Although no clear dose-response relationship could be assessed, the authors reported that acetaldehyde induced sister chromatid exchanges (see Table 11). However, based on this endpoint alone, the Committee cannot conclude that acetaldehyde is genotoxic in germ cells." (emphasis added)
The publication Madrigal-Bujaidar et al. (2002) states in the results section: "The SCE induction by Ace [acetaldehyde] in spermatogonial cells of mice is shown in Table I. It was observed that all four doses tested produced a positive response. The lowest tested dose (0.4 mg/kg) increased 1.1 SCE with respect to the basal value, and with the high dose (400.0 mg/kg), the increase was 3.2 SCEs, which is more than three times the control level; however, a dose-dependent effect was not found." (emphasis added).

Although the CLH report presented contradictory statements, it incorrectly reached the conclusion that " a clear dose response relationship was reported".

The authors Madrigal-Bujaidar et al. (2002) conclude in the discussion of their results: "The present results raise the question of whether the observed damage could be maintained during the development of spermatozoa and even be passed on to the zygote. […] Thus, at present there is no clear evidence that the observed damage by Ace [acetaldehyde] could produce abnormal zygotes."
The authors' own assessment of their experimental data should be taken into detailed consideration during a valid review and re-evaluation of acetaldehyde data.

5. Relevant Route of Exposure
Although mutations have been induced in mammals following acetaldehyde exposure, all positive studies have employed the non-physiological i.p. route of administration. When laboratory animals are exposed via relevant physiological routes, there are no meaningful positive responses. The i.p. route, bypassing site-of-contact detoxification mechanisms, is not a realistic route of exposure for humans.

Please note that a detailed assessment of acetaldehyde mutagenicity data will be submitted by the Acetaldehyde Working Group, a not-for-profit association located in Washington, DC.

Note: These comments are submitted via the ECHA web form. Since it is expected that the formatting might not be transmittable via the web form, the complete set of comments will also be submitted as a public attachment.

References
[B1] Committee for Risk Assessment (RAC) Opinion proposing harmonised classification and labelling at Community level of vinyl acetate (ECHA/RAC/DOC No CLH-O-0000001742-77-01/F; Adopted 10 June 2011; http://echa.europa.eu/documents/10162/0314bc3c-eb3c-442d-be4d-91a2c8d79311
[B2] Madrigal-Bujaidar E, Velazquez-Guadarrama N, Morales-Ramirez P, Mendiola MT. Effect of disulfiram on the genotoxic potential of acetaldehyde in mouse spermatogonial cells. Teratog Carcinog Mutagen. 2002;22(2):83-91
[B3] Reference (1) in the CLH report is cited as "Netherlands HCot. Acetaldehyde - Re-evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2014 2014/28 Contract No.: 978-94-6281-016-7." Final publication URL: http://www.gr.nl/sites/default/files/201428_ acetaldehyde.pdf

ECHA note: The following attachment was submitted with the comment above:
Celanese Comments on Acetaldehyde CLH report 2015-09-08*.*

Dossier Submitter's Response

Thank you for your comments.

1. In the *in vitro* study by Moeller et al (2013), a linear increase in exogenous adducts was shown in figure 3 of the article, irrespective of the formation of endogenous adducts. This shows that the proposed homeostatic mechanism to keep intracellular concentrations within acceptable physiological ranges has limitations. This is especially true for people with less- or inactive forms of ALDH, which are widespread through the population.
2. The RAC opinion cited in the comment (reference B1) discussed the classification of vinyl acetate, of which acetaldehyde is a metabolite. In the context of the RAC opinion 'non-physiologically high concentrations' should be interpreted as higher than naturally occurs in cells without external exposure. In addition, in our opinion the formation of DNA adducts from endogenously formed substances like acetaldehyde could contribute to the formation of tumours in control animals. The carcinogenic and mutagenic potential of acetaldehyde is not questioned in this opinion, nor its relevance from a hazard perspective.
3. While the background levels of acetaldehyde are indeed important to consider in the risk assessment, classification is hazard based. In addition, RAC classified formaldehyde, which has comparable carcinogenic properties and is also produced endogenously and present in food products, as a carcinogen in category 1B. This supports that the source of exposure is less relevant.
4. We agree that the description of the results of the study by Madrigal-Bujaidar in the CLH report is not consistent regarding the dose-response relation. However, as also stated in the comment, all doses induced a significant increase in SCEs with a

magnitude that increased with increasing dose. As the authors of the study noted, the lack of proportionality of the effect with the dose might be due to an increase in apoptosis at the high dose(s). This is not at odds with the conclusion that the results show that acetaldehyde can interact with the genetic material of the spermatogonial cells.

Under the CLP regulation, one condition that may lead to classification as 1B mutagen is:
'positive result(s) 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. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells'

While the study by Madrigal-Bujaidar indeed does not prove that the damage is passed on to the zygotes, this information is not obligatory for classification as 1B mutagen.

5. Studies using a physiological route of exposure are indeed preferable, but an in vivo study using intraperitoneal administration gives information on possible intrinsic mutagenic properties. Accordingly, the results of the germ cell tests are relevant under consideration of the specific 'non-physiological route of exposure'.

RAC's response

Thank you for these helpful comments and the additional insight relating to the possible germ cell mutagenicity of acetaldehyde. RAC's detailed, critical evaluation of the available data (especiaaly of the key *in vivo* tests) will be provided in the Opinion Document.

NON-CONFIDENTIAL ATTACHMENTS

1. IFRA Response to the ECHA Public Consultation on the CLH report "Proposal for Harmonized Classification and Labelling of Acetaldehyde" based on regulation (EC) 1272/2008 (CLP Regulation), Annex VI, Part 2. Submitted on 11.09.2015 by the International Fragrance Association. *[Please refer to comment 5]*
2. EFFA - Response to the ECHA Public Consultation on the CLH report "Proposal for Harmonized Classification and Labelling of Acetaldehyde" based on regulation (EC) 1272/2008 (CLP Regulation), Annex VI, Part 2. Submitted on 11.09.2015 by the European Flavour Association. *[Please refer to comment 1]*
3. ACETALDEHYDE WORKING GROUP. Comments on the CLH Report on Acetaldehyde; Proposal for Harmonised, Classification and Labeling (June 2015). (Submitted 11 September 2015). Submitted on 11.09.2015 by the Acetaldehyde Working Group. *[Please refer to comment 7]*
4. Celanese Comments on Acetaldehyde CLH report 2015-09-08. Submitted on 11.09.2015 by Celanese. *[Please refer to comment 8, 13, 17]*

JOURNAL ARTICLES

5. Research Article. Quantitative Determination of Acetaldehyde in Foods Using Automated Digestion with Simulated Gastric Fluid Followed by Headspace Gas Chromatography. Hindawi Publishing Corporation. Journal of Automated Methods and

Management in Chemistry. Volume 2011, Article ID 907317, 13 pages. Submitted on 11.09.2015 by the Acetaldehyde Working Group. *[Please refer to comment 7]*

6. Biomarkers of Exposure and Effect in Human Lymphoblastoid TK6 Cells Following [$^{13}C_2$]-Acetaldehyde Exposure. Toxicological sciences 133(1), 1–12 2013. Submitted on 11.09.2015 by the Acetaldehyde Working Group. *[Please refer to comment 7]*