

**Committee for Risk Assessment**  
**RAC**

Annex 3  
**Records**

of the targeted public consultation on the influence of  
acetaldehyde dehydrogenase (ALDH2) polymorphism on the  
physiological levels of acetaldehyde

**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**

The proposal for the harmonised classification and labelling (CLH) of acetaldehyde (ethanal, EC 200-836-8; CAS 75-07-0) was submitted by the Dutch competent authority and was subject to a public consultation, from 28 July until 11 September 2015. The comments received by that date are compiled in Annex 2 to the opinion.

However, during its June 2016 meeting, the Committee for Risk Assessment (RAC) asked for further information on the mode of action (MoA) of acetaldehyde - in particular, experimental and human studies that could clarify the influence of acetaldehyde dehydrogenase (ALDH2) genetic polymorphism on the physiological levels of acetaldehyde and possible health effects.

ECHA decided to organise an additional public consultation, giving parties concerned a second opportunity to provide information on the genetic polymorphism of aldehyde dehydrogenase (ALDH2). A list of additional references not included in the original CLH proposal from the Dutch competent authority and a series of selected slides summarising the weight of evidence available for acetaldehyde were submitted for PC.

A supplementary publication on developmental toxicity of salicylic acid in monkeys and a summary of the classification for all the substances used for the read-across were submitted for PC. The consultation started on 29 June 2015 and finished on 25 July 2016. The comments received are compiled in this annex.

**Substance name: acetaldehyde****CAS number: 75-07-0****EC number: 200-836-8****Dossier submitter: Netherlands****GENERAL COMMENTS**

Date	Country	Organisation	Type of Organisation	Comment number
15.07.2016	Netherlands		MemberState	1

Comment received

Information on the mode of action of acetaldehyde and in particular, experimental and human studies on the influence of ALDH2 polymorphism.

There is general consensus in the literature that acetaldehyde is the active metabolite of ethanol. In addition, all alcoholic beverages contain acetaldehyde in variable amounts: average levels in different types vary between 60 to > 7000 µM (Lachenmeier & Sohnius, 2008).

In that respect, it should be considered that in 2012 IARC classified Acetaldehyde associated with consumption of alcoholic beverages as Cat. 1 carcinogen (IARC monograph Volume 100E). One of the considerations that was noted by IARC to derive this classification is particularly relevant for the questions posed in this PC and was not included in the CLH report:

“after alcoholic beverage consumption, carriers of an inactive allele of the ALDH2 enzyme show accumulating levels of acetaldehyde in the peripheral blood, a direct consequence of their enzyme deficiency, and show increased levels of N2EtdG and methylhydroxypropano-dG adducts in lymphocyte DNA. The latter adducts have been shown to be formed from acetaldehyde; during DNA replication, these methylhydroxypropano-dG adducts cause mutations” (IARC Monograph 100E, page 471, point 8)

This conclusion was based on the combined evidence of multiple studies, including Matsuda et al. (2006). This study was already included in the CLH report with regard to the formation of acetaldehyde adducts after consumption of ethanol. With respect to ALDH2 polymorphism, it is particularly interesting to note that levels of the adducts were significantly higher in \*1/\*2 carriers (28.3 adducts in 10<sup>9</sup> bases) than in \*1/\*1 genotypes (3.9 adducts in 10<sup>9</sup> bases).

These findings are further substantiated by the publications of Ishikawa et al from 2003, 2006, and 2007. They investigated the difference in micronucleus frequency between individuals with different metabolic genotypes. Amongst others, they found higher micronucleus frequencies in non-smoking drinkers carrying an ALDH2\*2 allele.

Very closely related are the results of a study by Muto et al. (2002), who investigated the association between ALDH2 polymorphisms and the occurrence of field cancerization in patients with head and neck cancer. The results are in agreement with those of the studies included in the CLH report, that the ALDH2\*2 genotype increases the risk of multiple lesions and second esophageal carcinomas. Particularly interesting is that the levels of acetaldehyde in breath were measured after alcohol ingestion (figure 1). Participants were asked to drink 200 ml of 6% ethanol in grapefruit juice over 10 min after at least 2 h of fasting.

These results show that alcohol consumption indeed leads to increased acetaldehyde exposure and that an inactive ALDH2 allele increases both the height and the duration of the levels of acetaldehyde.

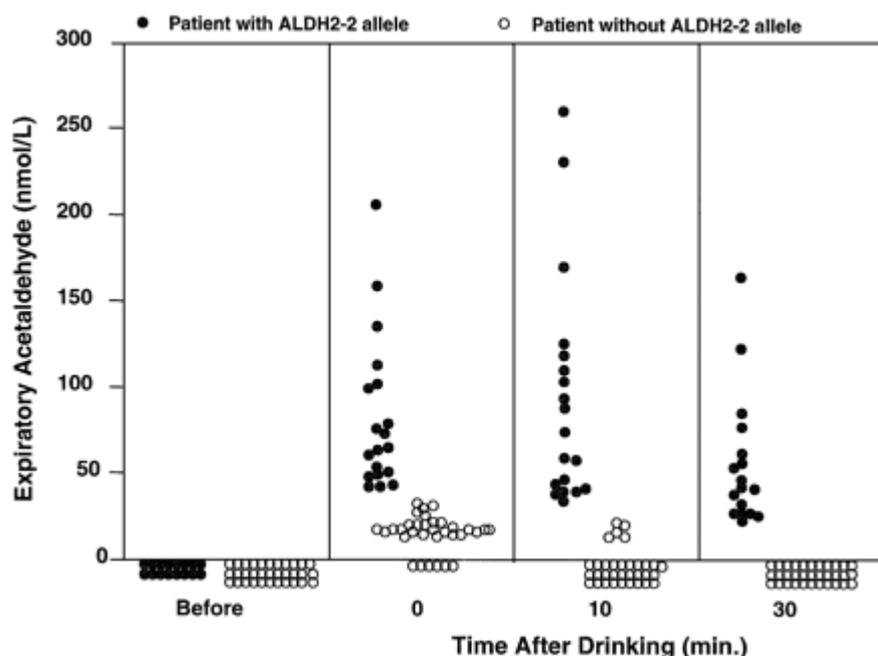


Figure 1: Acetaldehyde breath test. Acetaldehyde levels in expired breath are measured by gas chromatography. Each point represents one individual who was sampled several times: filled circles, subjects with the ALDH2-2 allele; open circles subjects lacking the ALDH2-2 allele.

Another study to ALDH2 activity and breath acetaldehyde concentrations showed that Caucasians who drank a small dose of alcohol (0.25 g/kg) after taking the alcohol-sensitizing drug calcium carbimide, which blocks the action of ALDH isozyme reached breath acetaldehyde levels between 200 and 1300 nmol/l. Without calcium carbimide, breath acetaldehyde concentration reached between 5 and 50 nmol/l in European subjects after drinking a moderate dose of ethanol (0.4–0.8 g/kg). The concentration of breath acetaldehyde in Japanese subjects after drinking alcohol reached between 200 and 500 nmol/l at the peak (Jones, 1995).

In our opinion, this information provides clear evidence that in humans with reduced ALDH activity there is a clear increase in acetaldehyde concentration and a reduction in half-life. This increased AUC of acetaldehyde results in an increase of DNA adducts and micronucleus formation in somatic cells within the systemic circulation. It is considered likely that exposure of mammals with reduced ALDH activity to acetaldehyde will also result in an increase in acetaldehyde AUC, DNA adducts and micronuclei. The increase of micronuclei in somatic cells was confirmed in knock-out mice (Kunugita, 2008).

### Carcinogenicity

In the provided slides, it was stated that there is no new information regarding carcinogenicity compared to the situation in 1991. Although we agree that there are no additional carcinogenicity studies, there is additional information that is very relevant for the assessment of the classification for carcinogenicity. As additional considerations regarding cancer, 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, namely DPX-formation at the site of contact.

Overall, the data justify classification in category 1B. Based on all available information it is considered likely that an additional carcinogenicity study in a second species will be positive and should be avoided.

#### References

IARC Monograph 100E (2012)

<http://monographs.iarc.fr/ENG/Monographs/vol100E/mono100E-11.pdf>

Ishikawa H, Ishikawa T, Yamamoto H et al. (2007). Genotoxic effects of alcohol in human peripheral lymphocytes modulated by ADH1B and ALDH2 gene polymorphisms. *Mutat Res*, 615: 134–142. doi:10.1016/j.mrfmmm.2006.11.026 PMID:17207821

Ishikawa H, Miyatsu Y, Kurihara K, Yokoyama K (2006). Gene-environmental interactions between alcohol-drinking behavior and ALDH2 and CYP2E1 polymorphisms and their impact on micronuclei frequency in human lymphocytes. *Mutat Res*, 594: 1–9. PMID:16126235

Ishikawa H, Yamamoto H, Tian Y et al. (2003). Effects of ALDH2 gene polymorphisms and alcohol-drinking behavior on micronuclei frequency in non-smokers. *Mutat Res*, 541: 71–80. PMID:14568296

Jones AW (1995). Measuring and reporting the concentration of acetaldehyde in human breath. *Alcohol Alcohol*, 30: 271–285. PMID:7545981

Kunugita N, Isse T, Oyama T, Kitagawa K, Ogawa M, Yamaguchi T, et al. (2008) Increased frequencies of micronucleated reticulocytes and T-cell receptor mutation in *Aldh2* knockout mice exposed to acetaldehyde. *J Toxicol Sci*. 33(1):31-6.

Lachenmeier DW & Sohnius EM (2008). The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: evidence from a large chemical survey. *Food Chem Toxicol*, 46: 2903–2911. doi:10.1016/j.fct.2008.05.034 PMID:18577414

Matsuda T, Yabushita H, Kanaly RA et al. (2006). Increased DNA damage in ALDH2-deficient alcoholics. *Chem Res Toxicol*, 19: 1374–1378. doi:10.1021/tx060113h PMID:17040107

Muto M, Nakane M, Hitomi Y, Yoshida S, Sasaki S, Ohtsu A, Yoshida S, Ebihara S, and Esumi H (2002) Association between aldehyde dehydrogenase gene polymorphisms and the phenomenon of field cancerization in patients with head and neck cancer. *Carcinogenesis* 23 (10): 1759-1766 doi:10.1093/carcin/23.10.1759

#### RAC's response

Thank you for your extensive comments.

RAC agrees that this data shows how individuals with differing genotype may respond to the intake of ethanol and how the metabolism of acetaldehyde in the body is important in

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this regard. However, ethanol exposition is not matter of consideration in risk assessment for acetaldehyde as pure substance. Therefore, the relevance of the data to the hazard assessment of acetaldehyde (i.e. following inhalation, oral, dermal uptake, not formation in the body systemically following ethanol uptake) is questionable.

RAC makes no comment about the necessity for further carcinogenicity testing as this is would go beyond the remit of giving an opinion on how acetaldehyde should be classified. However, there is no reason to believe that the results of such a test would lead to a significantly different hazard assessment.

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2016	France	UNDV	BehalfOfAnOrganisation	2

Comment received

Dear Sir, Dear Madam,

If the proposal to classify acetaldehyde as carcinogen 1A is accepted, it will be a huge problem for our members which are french wine distilleries. Their activities are very important for french wineries because they are environnemental tools for french wineries.

All members will not manage to comply with these new acceptable levels of acetaldehyde.

It is absolutely necessary for us to keep these current rules : 1% acetaldehyde in ethanol because it is absolutely necessary for our distilleries to keep selling ethanol on the market (industrial uses, bio-carburation).

Our position is that no change in the classification of acetaldehyde is justified

- Acetaldehyde occurs naturally in foods and beverages, especially in beer and alcoholic beverages, and it's difficult to get very low level of concentration
- Exposure to low doses is natural; small amounts are found in the beverage itself by sugar fermentation, and considerably more is produced as part of ethanol metabolism.
- The evaluation of carcinogenicity and genotoxicity for acetaldehyde are made with high exposure level that overwhelms acetaldehyde detoxification processes

The current entry in Annex VI of CLP Regulation for acetaldehyde must be Carc.2, H351.

Thank you very much in advance for the interest you could allow to our request

Yours faithfully

RAC's response

RAC notes the comments; they relate predominantly to the risk assessment of acetaldehyde and do not further influence the hazard assessment being undertaken by the committee.

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Date	Country	Organisation	Type of Organisation	Comment number
26.07.2016	n/n		BehalfOfAnOrganisation	3
Comment received				
<p>See the attached comments in response to the targeted consultation relating to acetaldehyde ALDH2 polymorphism, submitted by the Acetaldehyde Working Group.</p> <p>We are very appreciative of the interaction with ECHA and would welcome the opportunity of further discussions on these issues.</p> <p>ECHA note - The following attachment was submitted with the comment above:</p> <ul style="list-style-type: none"><li>• <i>AWG Comments on acetaldehyde polymorphism ECHA RAC</i></li><li>• <i>Teegarden 2008</i></li></ul>				
RAC's response				
<p>Thank you for your comment and the additional information on ALDH2 polymorphism and its impact on acetaldehyde concentrations in nasal tissue.</p> <p>RAC is reassured by the additional information as it further indicates that the potential carcinogenicity of acetaldehyde is not expected to be increased significantly in individuals who have a deficient ALDH2 phenotype. The existing studies in rats and hamsters are relevant for carcinogenicity hazard assessment of this substance.</p>				

**NON-CONFIDENTIAL ATTACHMENTS:**

1. *AWG Comments on acetaldehyde polymorphism ECHA RAC*, Submitted on 25/07/2016 by Regnet
2. *Teegarden 2008*, Submitted on 25/07/2016 by Regnet