Comments on the

"CLH report - Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Acetaldehyde

EC Number: 200-836-8

CAS Number: 75-07-0

Index Number: 605-003-00-6

Version number: 2.0 Date: June 2015"

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)

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: "T*he 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

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.

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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.

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[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.