SCCS/1468/12
Revision of 11 December 2012


Scientific Committee on Consumer Safety

SCCS

# OPINION ON

# Acetaldehyde

The SCCS adopted this opinion at its 16th Plenary meeting

of 18 September 2012

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged.

Revised opinions carry the date of revision.

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## 1. BACKGROUND

Acetaldehyde is classified as carcinogen category 2 (CMR substance according to Regulation 1272/2008 (CLP regulation)) under the EU chemicals legislation. The substance is not regulated in an Annex to the Cosmetics Directive.

The Scientific Committee on Cosmetic Products and non-food products intended for consumers expressed its opinion (SCCNFP/0821/04) with the following conclusions:

- Based on the information on the amount of fragrance compound present in the finished cosmetic products provided, the SCCNFP is of the opinion that acetaldehyde can be safely used as a fragrance/flavour ingredient at a maximum concentration of 0.0025% in the fragrance compound.
- SCCNFP does not recommend any further restrictions to the use of Acetaldehyde as a fragrance/flavour ingredient in cosmetic products.

This assessment was based on the presence of acetaldehyde in the fragrance compound of cosmetic products. This exposure was estimated to pose no increased life time cancer risk. However, the exposure to acetaldehyde from other uses of ethanol and/or acetaldehyde in cosmetics was not considered.

Moreover, concentration limits proposed on the basis of above opinion were considered so low that they might cause analytical problems when applied in practice.

Consequently, Industry was requested to prepare an exposure and safety assessment taking into account all possible sources of acetaldehyde exposure from cosmetic products.

This information was submitted by COLIPA in July 2011. This submission contains the requested safety assessment of acetaldehyde in all cosmetic products at concentration of 100 ppm, regardless whether deliberately added or from incidental presence.

## 2. TERMS OF REFERENCE

The SCCS is requested to answer the following questions:

1. *Is acetaldehyde safe when present up to 100 ppm in cosmetic products taking into account the new data provided?*

2. *And/or does the SCCS recommend any other concentration limit with regard to the use of Acetaldehyde as an ingredient in cosmetic products?*

3. *Does the SCCS have any further scientific concerns regarding the use of Acetaldehyde in mouth-washing products?*

# 3. OPINION

## 3.1 Chemical and Physical Specifications

### 3.1.1 Chemical identity

#### 3.1.1.1 Primary name and/or INCI name

Acetaldehyde (INCI name)

#### 3.1.1.2 Chemical names

IUPAC Name: Acetaldehyde
Synonyms: Acetic aldehyde, acetic ethanol, acetylaldehyde, ethanal, ethyl aldehyde

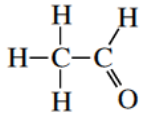
#### 3.1.1.3 Trade names and abbreviations

/

#### 3.1.1.4 CAS / EC number

CAS: 75-07-0
EC: 200-836-6

#### 3.1.1.5 Structural formula



#### 3.1.1.6 Empirical formula

Formula: $C_2H_4O$

### 3.1.2 Physical form

Colourless liquid or gas with a characteristic pungent odour

### 3.1.3 Molecular weight

Molecular weight: 44.05 g/mol

### 3.1.4 Purity, composition and substance codes

/

### 3.1.5 Impurities / accompanying contaminants

Acetic acid

### 3.1.6 Solubility

Miscible with water, benzene, diethyl ether and ethanol

### 3.1.7 Partition coefficient (Log Pow)

Log $P_{ow}$: 0.5 (measured)

Ref.: 31

### 3.1.8 Additional physical and chemical specifications

| | |
|---|---|
| Melting point: | -123 °C |
| Boiling point: | 20.1 °C |
| Flash point: | -38 °C |
| Vapour pressure: | 98 kPa at 20 °C |
| Density: | 0.78 g/cm³ |
| Viscosity: | 0.2456 mPa x sec at 15 °C |
| pKa: | 13.57 |
| Refractive index: | / |
| UV_Vis spectrum (200-800 nm): | / |
| Conversion factor: | mg/m³ = 1.80 × ppm |

### 3.1.9 Homogeneity and Stability

Pure acetaldehyde is flammable; it polymerizes violently in the presence of trace amounts of metals or acids. Acetaldehyde may undergo auto-polymerisation upon contact with air or moisture. Upon prolonged storage, it may form unstable peroxides.

Solutions of acetaldehyde in water, DMSO, 95% ethanol or acetone should be stable for 24 hours under normal laboratory conditions.

## 3.2 Function and uses

Acetaldehyde is an intermediate in the production of acetic acid, acetic anhydride, cellulose acetate, vinyl acetate resins, acetate esters, pentaerythritol, synthetic pyridine derivatives, terephthalic acid and peracetic acid. Other uses of acetaldehyde include silvering of mirrors; leather tanning; denaturant for alcohol; fuel mixtures; hardener for gelatine fibres; glue and casein products; preservative for fish and fruit; synthetic flavouring agent; paper industry; and manufacture of cosmetics, aniline dyes, plastics and synthetic rubber.

The concentration of acetaldehyde in alcoholic beverages is generally below 500 mg/l. Low levels of acetaldehyde are also reported to occur in several essential oils.

Acetaldehyde is an intermediate product in the metabolism of ethanol and sugars and also occurs as a natural metabolite in small quantities in human blood.

In cosmetic products, two possibilities of occurrence of acetaldehyde can be distinguished:

1) Acetaldehyde is used as a **fragrance/flavour ingredient** in fragrance compounds used in cosmetic products. The SCCNFP concluded in its opinion of 25th May 2004 that acetaldehyde can be safely used as a fragrance/flavour ingredient at a maximum concentration of 0.0025% (25 ppm) in the fragrance compound.

2) In addition, acetaldehyde can also be found in cosmetic products in the form of unavoidable **traces** originating mainly through:

- Plant extracts and botanical ingredients
- Ethanol.

## 3.3 Toxicological Evaluation

### 3.3.1 Acute toxicity

#### 3.3.1.1 Acute oral toxicity

In rats oral $LD_{50}$ values ranged from >600 to 1 930 mg/kg bw

In mice the oral $LD_{50}$ is reported to be 1 230 mg/kg bw

Oral $LD_{50}$ in dogs is >600 mg/kg bw

Ref.:1

#### 3.3.1.2 Acute dermal toxicity

In rabbit a dermal $LD_{50}$ of 3 540 mg/kg bw has been reported.

Ref.: 1

#### 3.3.1.3 Acute inhalation toxicity

Rats: $LC_{50}$, 20 500mg/m³ for 30 min, 13 000 mg/m³ for 4 hours
Mice: $LC_{50}$, 1 500 ppm (2 700 mg/m³) for 4 hours
Hamster: $LC_{50}$, 17 000 – 24 000 ppm (30 600 – 43 200 mg/m³) for 4 hours

Ref.: 1, 31

### 3.3.2 Irritation and corrosivity

#### 3.3.2.1 Skin irritation

On the basis of a number of short- and long-term studies, acetaldehyde liquid and vapour has been shown to be acutely irritating to the skin.

Ref.: 2, 3

In a test with rabbits carried out according to OECD Test Guideline 404, acetaldehyde was not found to be irritating to the skin. In another test not conducted in accordance with test guidelines, in the same species 500 mg acetaldehyde produced slight irritation of the skin.

In a test with human volunteers, all 13 subjects showed erythema in a non-occlusive patch test with a 10% acetaldehyde preparation (vehicle not specified, probably water).

Ref.: 3

Concentrations greater than 1% in solution are likely to be irritating to the human skin.

Ref.: 4

Comment
Acetaldehyde is a skin irritant.

#### 3.3.2.2 Mucous membrane irritation

In the rabbit eye, 40 mg acetaldehyde produced marked irritation. Long-term inhalative exposure of experimental animals to acetaldehyde vapour causes irritation of the mucous membranes of the eye, nose and upper respiratory tract.

Ref.: 3

All of the 14 humans exposed in controlled studies to acetaldehyde vapour at 135 ppm (240 $mg/m^3$) for 30 minutes reported mild irritation to the upper respiratory tract.

Ref.: 1

In another human study, inhalative exposure for 15 minutes to acetaldehyde in concentrations of ≥91 $mg/m^3$ resulted primarily in eye irritation in the majority of 24 volunteers. Sensitive persons showed eye symptoms even at concentrations as low as 45 $mg/m^3$. Irritation of the upper respiratory tract seems to be less sensitive and is not described up to 246 $mg/m^3$.

Ref.: 3

Twenty volunteers were exposed in a cross over design for 4 hours to 0 or 91 $mg/m^3$ acetaldehyde. No subjective irritative symptoms were reported (assessed by questionnaire). Before and after exposure the olfactory threshold for n-butanol and the mucociliary transport time was determined and did not show any change. Concentrations of interleukin-1ß and interleukin-8 in nasal secretion were not increased after exposure to acetaldehyde. mRNA levels of inflammatory factors (interleukin-1ß, -6 and -8, TNFα, granulocyte-macrophage colony stimulating factor, monocyte chemotactic protein 1, and cyclooxygenases 1 and 2) were determined in nasal epithelial samples and did not show any increase after exposure. The authors concluded that test persons were not adversely affected by acute exposure to 91 mg /$m^3$.

Ref.: 5

Comment
The SCCS considers that acetaldehyde is an eye and respiratory tract irritant.

### 3.3.3 Skin sensitisation

The skin sensitisation potential of acetaldehyde was tested in a modified Cumulative Contact Enhancement Tests (CCET). Fifteen female albino Dunkin-Hartley guinea pigs were tested. A 0.2 ml aliquot of acetaldehyde was applied to a 2x4 cm lint cloth and then applied to shaved skin on the upper back for 24 hours under occlusion. Induction applications (15% acetaldehyde in saline) were administered on days 0, 2, 7 and 9. The animals also received two intradermal injections of 0.1 ml FCA in the same region on day 7. Animals were challenged 14 days after the final induction at doses of 2.5%, 5.0% and 10.0% acetaldehyde in saline. At challenge, a 0.015 ml aliquot of Acetaldehyde in saline was applied to a Finn Chamber and then applied to a shaved site on the lateral back for 24 hours under occlusion. Reactions were read 48 and 72 hours after start of exposure. Acetaldehyde showed significant sensitising capacity and a clear dose-response relationship was observed. Specifically, at the 48-hour reading, challenge at 2.5% produced 4/15 sensitisation reactions; challenge at 5.0% produced 7/15 sensitisation reactions; challenge at 10.0% produced 13/15 sensitisation reactions. At the 72-hour reading, challenge at 2.5% produced 5/15 sensitisation reactions; challenge at 5.0% produced 9/15 sensitisation reactions; challenge at 10.0% produced 13/15 sensitisation reactions. The animals were rechallenged 78 days after the start of the experiment with acetaldehyde at concentrations of 0.035 and 2.5%, and no significant reactions were observed.

Ref.: 6

Comment

This was a non-guideline method and it is unknown whether the reactions observed were irritant (false positive) in nature.

In a study of intolerance reactions to air-oxidized and non-oxidized surface-active ethoxylated (fatty) alcohols, six of 528 consecutive patients tested also with a 1% preparation of acetaldehyde in water produced reactions (erythema plus oedema, papules or vesicles). In ten further patients, only erythematous reactions occurred. In the follow-up test, only one of the six patients still reacted to 1% and 0.33% acetaldehyde in water. The relevance of these reactions has not been clarified, although the authors consider the presence of slight quantities of acetaldehyde in the oxidized surfactants to be possible.

Ref.: 3

A maximization test was carried out with 2% acetaldehyde in petrolatum on 28 healthy, male and female volunteers. Application was under occlusion to the same site on the volar forearms of all subjects for five alternate-day 48-hour periods. The initial patch site was pretreated with 2% aqueous sodium lauryl sulphate (SLS) under occlusion for 24 hours. Following a 10- to 14-day rest period, challenge patches were applied under occlusion to fresh sites for 48 hours. Challenge applications were preceded by 30-minute applications of 2% aqueous SLS under occlusion on the left side of the back whereas the test materials were applied with SLS treatment on the left and petrolatum on the right. Reactions were read at patch removal and again 24 hours after patch removal. No sensitisation reactions were produced.

Ref.: 7

The skin sensitisation potential of acetaldehyde was assessed in 4 female patients with eczematous reactions to lower aliphatic alcohols. The individuals were patch tested with 2% acetaldehyde in water. The study consisted of 48-hour patch tests using A1-test units conducted on the upper backs of the patients. Reactions were read at removal and 24 and 48 hours post-removal. No evidence of skin sensitisation reactions to acetaldehyde was observed at any time interval.

Ref.: 8

Following participation in a human repeated insult patch test with ethanol, one subject became strongly sensitised and was further tested for cross-reactivity. A 0.15 ml 1% aqueous acetaldehyde was applied to a 12 mm Webril patch and reactivity to acetaldehyde was observed. In the same study the author inadvertently sensitised himself to acetaldehyde during a test to determine a non-irritant concentration of acetaldehyde. The exposure consisted of single applications of 5% and 10% acetaldehyde for a 3-hour period followed by single sequential applications of 0.5% and 1% for 24 hours, all within an 8-day period. A subsequent application of 2% acetaldehyde produced a strong allergic response and prompted a flare at the 10% application site that was made 20 days earlier.

Ref.: 9

Comment
There is limited evidence for skin sensitisation. The SCCS considers the HRIPT tests as unethical. Respiratory sensitisation has not been investigated to date.

### 3.3.4 Dermal / percutaneous absorption

No *in vitro* studies of dermal absorption have been found.

Comment
Some studies are available concerning increase in blood acetaldehyde after dermal exposure to ethanol (see Section 3.3.12 Special investigations). However, no quantitative conclusions can be drawn from these studies regarding skin absorption of acetaldehyde.

In the safety evaluation 100% skin absorption is used as no experimental data is available.

### 3.3.5 Repeated dose toxicity

#### 3.3.5.1 Repeated Dose (28 days) oral toxicity

| | |
|---|---|
| Guideline: | / |
| Species/strain: | SPF-bred (Cpb:WU) Wistar rats |
| Group size: | Control groups 20 males and 20 females, treated groups 10 males and 10 females |
| Test substance: | Acetaldehyde |
| Batch: | / |
| Purity: | 99.8% |
| Vehicle: | Water |
| Dose levels: | 0 (control), 5, 25, 125, 675 mg/kg bw/d |
| Dose volume: | / |
| Route: | Oral |
| Administration: | Drinking water |
| GLP: | / |
| Study period: | Before 1988 |

In a 4 weeks study, acetaldehyde was added to drinking water of rats (5 weeks old at start of experiment), providing daily intake levels of 0, 25, 125, or 675 mg/kg bw/d. The rats were weighed at weekly intervals and observed daily. Food and liquid intakes were measured over weekly periods. Early in week 5, the rats were killed.

There were no deaths and the rats appeared to be healthy throughout the study. The only clearly compound-related effect reported was moderate or slight focal hyperkeratosis of the forestomach in the high-dose group (8/10 males and 8/10 females). In the control group, very slight or slight focal hyperkeratosis of the forestomach was noted in 6/20 females and 4/20 males. In the high-dose group, the relative kidney weights were slightly increased in males, and urinary production was decreased. The effects and reported variations in serum biochemistry, were generally attributed to reduced water intake. Acetaldehyde exposure did not affect indices of liver function and produced no evidence of histological change in this organ.

Ref.: 10

Comment
A NOAEL of 125 mg/kg bw/d can be derived from the study.

#### 3.3.5.2 Repeated Dose (28 days) inhalation toxicity

Several studies investigating toxicity of acetaldehyde by inhalation have been published but most of them are not recent ones and did not followed standardized procedures. Two short-term studies conducted by the same research group are considered as the most reliable and informative and then are the principal studies used for risk assessment of acetaldehyde by inhalation.

In a first 28 days study, Appelman et al (1982) have exposed groups of 10 male and 10 female Wistar rats to 0, 400, 1000, 2200 or 5000 ppm acetaldehyde for 6 h/day, 5 days/week. Treatment-related changes observed at the 5000 ppm level included dyspnoea and excitation during the first 30 min of each exposure, yellow-brown fur, severe growth retardation, more neutrophils and less lymphocytes in the blood, a reduced production of urine with a high density, increased lung weights, and severe degenerative, hyperplastic and metaplastic changes of the nasal, laryngeal and tracheal epithelium. Major lesions seen at 1000 and 2200 ppm included growth retardation and an increased production of urine in males, slight to moderate degeneration with or without hyper- and metaplasia of the nasal

epithelium, and only at 2200 ppm, minimal epithelial changes in the larynx and trachea. The only change observed at the 400 ppm level that could be attributed to acetaldehyde was slight degeneration of the nasal olfactory epithelium seen as loss of microvilli and thinning and disarrangement of the layer of epithelial cells.

Ref: 40

Comment
This study does not follow OECD/GLP guidelines. No NOAEL can be derived from this study and, based on the slight degeneration of the nasal olfactory epithelium, 400 ppm could be considered as a LOAEL.

In a second 28 days study, Appelman et al (1986) have studied the effect of short-term increases and interruption in exposure on the inhalation toxicity of acetaldehyde during 28 days in male Wistar rats. Male rats were exposed to 110, 150 and 500 ppm for 6 h per day/5 days per week. One group of animals was exposed without interruption, the exposure of a second group was interrupted for 1.5 h between the first and second 3-h periods, the exposure of a third group was similarly interrupted and for six 5 min periods exposure was increased six fold. Peak exposures of up to 3000 ppm superimposed on 500 ppm acetaldehyde caused irritation and excitation, and reduced body weight gain. No such effects occurred after interrupted or uninterrupted exposure to 500 ppm acetaldehyde without peak loads. A reduced phagocytotic index of lung macrophages was found in each of the groups exposed to 500 ppm acetaldehyde, the effect being most marked in the group with superimposed peaks of 3000 ppm. Degeneration of the nasal olfactory epithelium was observed in rats uninterruptedly exposed to 500 ppm acetaldehyde. Interruption of the exposure or interruption combined with peak exposure did not visibly influence this adverse effect on the nose. No compound-related effects were seen in rats interruptedly or uninterruptedly exposed to 150 ppm acetaldehyde or interruptedly exposed to 110 ppm with peak loads of 660 ppm. As a consequence 150 ppm acetaldehyde can be considered a 'no-toxic-effect level' in male rats exposed for 6 h/day, 5 days/week, during a 4-week period.

Ref: 41

Comment
This study does not follow OECD/GLP guidelines. Based on the degeneration of the nasal olfactory epithelium observed at 500 ppm, which could be considered as a LOAEL and 150 ppm (273 mg/m³) as a NOAEL. The NOAEL, based on continuous exposure, would then be (273 x 5/7 x 6/24) 49 mg/m³.
This value will be used in the safety assessment for non-cancer effects by inhalation.

#### 3.3.5.3 Sub-chronic (90 days) oral toxicity

In a group of rats exposed to 0.05% acetaldehyde in the drinking water (estimated to be about 40 mg/kg bw for 6 months, an increase in collagen synthesis in the liver was reported. Since no other indices of toxicity were reported, the significance of this finding is unknown.

Ref.: 11

| | |
|---|---|
| Guideline: | / |
| Species/strain: | Male Wistar rats |
| Group size: | Control groups 10 males, low dose group 4 males, high dose group 10 males |
| Test substance: | Acetaldehyde |
| Batch: | / |
| Purity: | / |
| Vehicle: | Water |
| Dose levels: | 0 (control), 120, 500 mg/kg bw/d |
| Dose volume: | / |

Route: Oral
Administration: Drinking water for 11 weeks
GLP: /
Study period: Before 1996

24 male rats, 10 weeks old, where divided into 3 groups, a control group (10 males), a low dose group (4 males) and a high dose group (10 males). The low and high dose groups received drinking water containing 20 mM (corresponding to 120 mg/kg bw/day) and 120 mM acetaldehyde (corresponding to 500 mg/kg bw/day), respectively for 11 weeks. The control group received drinking water without acetaldehyde. The general health of the rats was good and no signs of illness could be observed throughout the experiment. No differences in the body weights or in the liquid intakes of the rats were detected between different groups during the study. Among the high dosed rats microvesicular fatty degeneration was found. On morphometric analysis, a significantly greater accumulation of fat could be detected both in the periportal and in the pericentral areas of the hepatic acinus in the livers of rats receiving the high dose acetaldehyde. In rats receiving a high dose of acetaldehyde, some foci of inflammatory cells were found in the liver specimens of seven out of ten rats. No inflammatory changes were found either in the rats receiving a low dose of acetaldehyde or in the controls.

Ref.: 37

Comment
Based on the accumulation of fat and inflammatory changes in the liver of the male rats receiving 500 mg/kg bw/day, a NOAEL of 120 mg/kg bw/day for acetaldehyde can be derived.

The potential toxicity of acetaldehyde administered perorally in aqueous solution to white rats and guinea pigs at dose levels of 0.5, 10, or 100 mg/kg bw/d for periods of 5-6 months was studied. In guinea pigs, indices monitored at every dose level, with the exception of the high-dose level, included peripheral blood cholinesterase and leukocyte phagocytic activity, as well as the ratio of protein fractions in blood serum. In rats, conditional reflex activity and blood pressure levels were evaluated at every dose level.

Rats in the high-dose group were reported to exhibit inhibition of reflex activity, increases in blood pressure, as well as unspecified histological variations in the internal organs. A transient disruption of the conditioned reflex activity also was reported in rats receiving 10 mg acetaldehyde/kg bw/d at the 2 and 3 month of treatment. Compound-related effects reported in guinea pigs were limited to a statistically significant reduction in eosinophil count in groups treated at 10 mg/kg bw/d. No apparent adverse effects were reported in groups of animals administered 0.5 mg/kg bw/d.

Ref.: 12

Comment
No documentation for the above study was available to SCCS for evaluation. The study is not suitable for the estimation of the NOAEL/LOAEL.

No dermal study with acetaldehyde has been found.

#### 3.3.5.4 Chronic (> 12 months) toxicity

See Section 3.3.7 Carcinogenicity

### 3.3.6 Mutagenicity / Genotoxicity

*This section is based on IARC, 1999, 2011, 2012 (Ref.: 13, 14, and 15)*

Acetaldehyde did not cause differential killing of repair-deficient *Escherichia coli* K-12 *uvrB*/*recA* cells and was not mutagenic to *Salmonella typhimurium* or *E. Coli* WP2 *uvrA* after vapour exposure, with or without metabolic activation. It induced chromosome malsegregation in *Aspergillus nidulans* and was mutagenic in *Drosophila melanogaster* after injection but not after feeding.

*In vitro* and without exogenous metabolic activation, acetaldehyde induced gene mutations in mouse lymphoma L5178T cells, sister chromatid exchanges in Chinese hamster ovary cells and aneuploidy in embryonic Chinese hamster diploid fibroblasts.

Numerous *in vitro* studies have consistently shown that acetaldehyde causes DNA–protein crosslinks, DNA strand breaks, DNA adducts, sister chromatid exchanges, chromosomal aberrations, and micronuclei in eukaryotic cells *in vitro* (ref.: 16, 17). In comparison with other assays, the Comet assay requires relatively high concentrations of acetaldehyde to show a positive result, probably reflecting the formation of crosslinks (ref.: 16). Acetaldehyde induced also DNA protein crosslinks, sister chromatid exchanges and chromosomal aberrations in rodents *in vivo* (ref.: 14)

***Table 1:*** *Genetic and related effects of acetaldehyde in vitro (See IARC (Ref 13) for references)*

| Test system | Results -S9 | Results +S9 | Dose (LEDorHID | References |
|---|---|---|---|---|
| *Escherichia coli K-12 uvrB/recA*, differential toxicity | – | – | 78200 | Hellmér & Bolcsfoldi (1992) |
| *Salmonella typhimurium* TA100, TA104, TA1535, TA98, TA97, reverse mutation | – | – | 10 mg/plate | Zeiger et al. (1992) |
| *Salmonella typhimurium* TA100, TA1535, TA1537, TA97, TA98, reverse mutation | – | – | 5–10 mg/ plate | Phillips & Jenkinson (2001) |
| *Saccaromyces cerevisiae*, (repair-deficient) strand breaks | + | NT | 39100 | Ristow et al. (1995) |
| *Aspergillus nidulans*, chromosome malsegregation | + | NT | 35500 | Crebelli et al. (1989) |
| *Vicia faba*, sister chromatid exchange | + | NT | 16000 | Zhang et al. (1991) |
| *Hordeum* species, sister chromatid exchange | + | NT | 16000 | Zhang et al. (1991) |
| Plant (other), sister chromatid exchange | + | NT | 16000 | Zhang et al. (1991) |
| *Drosophila melanogaster*, somatic mutation (and recombination) | – | NT | 120000 | Graf et al. (1994) |
| Gene mutation, mouse lymphoma L5178Y cells, Tk locus in vitro | (+) | (+) | 4200 | Wangenheim & Bolcsfoldi (1988) |
| Gene mutation, mouse lymphoma L5178Y cells, Tk locus in vitro | – | – | 35900 | Phillips & Jenkinson (2001) |
| Sister chromatid exchange, mouse embryos in vitro | + | NT | 300 | Lau et al. (1991) |
| Chromosomal aberrations, Chinese hamster lung cells in vitro | – | – | 8000 | Phillips & Jenkinson (2001) |
| Chromosomal aberrations, Chinese hamster ovary cells in vitro | – | NT | 32000 | Lin et al. (1989) |
| Chromosomal aberrations, mouse embryos in vitro | + | NT | 800 | Lau et al. (1991) |
| DNA strand breaks, human lymphocytes in vitro | + | NT | 1380 | Blasiak et al. (2000) |
| DNA strand breaks, human colonic mucosa in vitro | + | NT | 460 | Blasiak et al. (2000) |
| DNA strand breaks, human gastric mucosa in vitro | + | NT | 46000 | Blasiak et al. (2000) |
| Sister chromatid exchange, human lymphocytes in vitro | – | NT | 40000 | Zhang et al. (1991) |
| Chromosomal aberrations, human lymphocytes in vitro | – | – | 8000 | Phillips & Jenkinson (2001) |
| Chromosomal aberrations, human lymphoid cell lines in vitro | – | NT | 32000 | Hsu et al. (1991) |
| Chromosomal aberrations, human lymphoblast cell lines in vitro | – | NT | 8000 | Brown et al. (1991) |

[a]+, positive; (+), weak positive; –, negative; NT, not tested [b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/ml.

***Table 2:*** *Genetic and related effects of acetaldehyde in vivo (See IARC (Ref 13) for references)*

| Test system | Results[a] | Dose |
|---|---|---|

| (LED or HID)[b] | References | | |
|---|---|---|---|
| DNA adducts, BD6 rat tissues in vivo | – | 4300 | Izzotti et al. (1998) |
| DNA strand breaks, rat brain cells in vivo | + | 4000 | Singh et al. (1995) |
| DNA strand breaks, Wistar rat liver cells in vivo | + | 5000 | Navasumrit et al. (2000) |
| Sister chromatid exchange, mouse cells in vivo | + | 1600 | Zhang et al. (1991) |
| Sister chromatid exchange, mouse bone marrow in vivo | + | 600 | Piña Calva & Madrigal-Bujaidar (1993) |
| Micronucleus formation, B6C3F1 mouse spermatids in vivo | – | 28500 | Pylkkänen & Salonen (1987) |
| Micronucleus formation, BD6 rat bone-marrow cells and pulmonary alveolar macrophages in vivo | – | 50 g/l in drinking water | Balansky et al. (1993) |
| Micronucleus formation, CD-1 mouse polychromatic erythrocytes in *vivo* | – | 3500 | Choy et al. (1995) |
| Micronucleus formation, CD-1 mouse polychromatic erythrocytes in *vivo* | – | 2500 | Choy et al. (1996) |
| Micronucleus formation, mouse in vivo | – | 2000 | Phillips & Jenkinson (2001) |
| Chromosomal aberrations, Wistar rat bone marrow in vivo | – | 200 g/l in drinking-water | Tavares et al. (2001) |
| Aneuploidy, Chinese hamster spermatogonia in vivo | – | 6250 | Daniel & Roane (1987) |
| Aneuploidy, (C57BL x CBA) F1 Mouse oocytes in vivo | + | 4800 | O'Neill & Kaufman (1987) |
| Dominant lethal test, mice | (+) | 1260 × 3 | Rao et al. (1994) |
| Dominant lethal test, mice | + | 25000 | Berryman et al. (1992) |

[a]+, positive; (+), weak positive; –, negative; NT, not tested [b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/ml; in-vivo tests, mg/kg bw/day

***Table 3:*** *Genetic and related effects of acetaldehyde in vivo (See IARC (Ref 13) for references)*

| Test system | Results[a] | References |
|---|---|---|
| **Studies on alcoholics** | | |
| Gene mutation, human lymphocytes, HPRT locus in vivo | – | Cole & Green (1995) |
| Sister chromatid exchange, human lymphocytes in vivo | + | Butler et al. (1981) |
| Sister chromatid exchange, human lymphocytes in vivo | (+) | Seshadri et al. (1982) |
| Sister chromatid exchange, human lymphocytes in vivo | + | Kucheria et al. (1986) |
| Sister chromatid exchange, human lymphocytes in vivo | + | Rajah & Ahuja (1996) |
| Sister chromatid exchange, human lymphocytes in vivo | +c | Karaoğuz et al. (2005) |
| Micronucleus formation, human buccal mucosa cells in vivo | – | Stich & Rosin (1983) |
| Micronucleus formation, human buccal epithelium in vivo | + | Ramirez & Saldanha (2002) |
| Micronucleus formation, human lymphocytes in vivo | + c | Castelli et al. (1999) |
| Micronucleus formation, human lymphocytes in vivo | + | Maffei et al. (2000) |
| Micronucleus formation, human lymphocytes in vivo | + | Maffei et al. (2002) |
| Micronucleus formation, human lymphocytes in vivo | (+) | Ishikawa et al. (2006) |
| Chromosomal aberrations, human lymphocytes in vivo | + | De Torok (1972) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Lilly (1975) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Mitelman & Wadstein (1978) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Obe et al. (1980) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Badr & Hussain (1982) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Kucheria et al. (1986) |
| Chromosomal aberrations, human lymphocytes in vivo | – | Rajah & Ahuja (1996) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Gattás & Saldanha (1997) |
| Chromosomal aberrations, human lymphocytes in vivo | + c | Castelli et al. (1999) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Hüttner et al. (1999) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Maffei et al. (2002) |
| Chromosomal aberrations, human lymphocytes in vivo | + | Burim et al. (2004) |
| Aneuploidy, human sperm in vivo | + | Robbins et al. (1997) |

[a]+, positive; (+), weak positive; –, negative; NT, not tested bw/day [c] In these studies, people who consumed alcohol were also heavy smokers.

The most abundant DNA adduct that results from the reaction of acetaldehyde is *N*2-ethylidenedeoxyguanosine (*N*2EtidG). This adduct is too unstable to be purified and isolated, but can be converted into the stable adduct *N*2EtdG by treatment with a reducing

agent (sodium cyanoborohydride). The reduction step can also be carried out by a mixture of GSH and ascorbic acid, which may occur *in vivo.*

Ref.: 18, 19

Fang and Vaca examined the levels of the *N*2EtdG adduct in a group of Swedish alcohol abusers compared to controls. They found that chronic alcoholics had higher levels of the *N*2EtdG adduct in both lymphocytes and granulocytes compared with controls. Balbo *et al.* measured later the level of *N*2-EtdG in blood leukocyte DNA of two groups of subjects, one consisting of alcohol drinkers and abstainers and the other of heavy drinkers. A significant trend between *N*2-EtdG level and daily alcohol dose was found.

Ref.: 18, 20

In addition to the major adduct *N*2EtidG, three acetaldehyde-derived DNA adducts have been identified. These are: *N*2-(2,6-dimethyl-1,3-dioxan-4-yl) deoxyguanosine (*N*2-Dio-dG); an interstrand crosslink, and two diastereoisomers (R and S) of α-methyl-γ-hydroxy-1,*N*2-propanodeoxyguanosine (α-Me-γ-OH-PdG).

Ref.: 21

Comment
*In vitro* and without exogenous metabolic activation, acetaldehyde induced gene mutations in mouse lymphoma L5178T cells, sister chromatid exchanges in Chinese hamster ovary cells and aneuploidy in embryonic Chinese hamster diploid fibroblasts. Acetaldehyde induced also DNA protein cross links, sister chromatid exchanges and chromosomal aberrations in rodents *in vivo.* Increased frequency of acetaldehyde DNA adducts in humans has been found in relation to alcohol use.

### 3.3.7 Carcinogenicity

***Animal studies***

*Oral administration*
*Rat*

| | |
|---|---|
| Guideline: | / |
| Species/strain: | Sprague-Dawley rats |
| Group size: | Control group: 100 male and 100 female rats. Dosed groups: 50 male and 50 female rats. |
| Test substance: | Acetaldehyde |
| Batch: | / |
| Purity: | > 99.0% |
| Vehicle: | Water |
| Dose levels: | 0 (control), 50, 250, 500, 1500, and 2500 mg/l |
| Dose volume: | Drinking water supplied *ad libitum* |
| Route: | Oral |
| Administration: | Drinking water |
| Positive control: | / |
| GLP statement: | Yes |
| Study period: | Before 2002 |

Groups of 50 male and 50 female Sprague-Dawley rats, 6 weeks of age, were exposed to 0, 50, 250, 500, 1500 or 2500 mg/l acetaldehyde in the drinking-water for 104 weeks. The experiment was terminated when the last animal died at 161 weeks of age.

No significant differences in the daily consumption of beverages and feed, behaviour, body weight, or survival were observed between treated and control animals, nor were any treatment-related non-oncological pathological changes detected by gross inspection or histopathological examination.

Complete histopathology was performed on all animals. In female rats administered 0, 50, 250, 500, 1500 and 2500 mg/l acetaldehyde, respectively, the incidence of malignant mammary tumours (adenocarcinomas) was 6% (3/50), 18% (9/50), 6% (3/50), 20% (10/50) [*P* = 0.04 compared with controls; one-sided Fisher's exact test, but not significant in two-sided test], 16% (8/50) and 12% (6/50). Slight treatment-related increases were observed in the incidence of Zymbal gland carcinomas, ear duct carcinomas and oral cavity carcinomas in both sexes [not statistically significant]. Nasal cavity carcinomas (4%, 2/50) were only observed in male rats administered 2500 mg/l acetaldehyde. Sporadic incidences of lung adenomas and adenocarcinomas, forestomach acanthomas and squamous-cell carcinomas and intestinal fibromas and adenocarcinomas were observed in male and/or female rats administered acetaldehyde [no statistically significant difference]. Testicular interstitial-cell tumours were observed in all groups [not statistically significant]. The incidence of uterine adenocarcinomas was increased in rats administered 250 mg/l acetaldehyde (10% (5/50) versus 0/50 controls) [*P* = 0.03, one-sided, P = 0.056 two-sided]. The incidence of cranial osteosarcomas was increased in male rats administered 50 mg/L (10% (5/50) versus 0/50 controls) [*P* = 0.03 one-sided, P = 0.056 two-sided] and 2500 mg/L acetaldehyde (14% (7/50) versus 0/50 controls) [*P* = 0.01 two-sided ]. Lymphomas and leukaemias combined were observed in all groups; compared with the controls (12% (6/50) males and 4% (2/50) females), the incidences were increased in male rats administered 50 mg/L (28%, 14/50) [*P* = 0.04 one-sided, P = 0.08 two-sided and 1500 mg/l acetaldehyde (30%, 15/50) [*P* = 0.02 one-sited, 0.05 two-sided].

Ref.: 22

Comment

The IARC working group (ref.: 13) evaluating the study noted "that a variety of tumours were observed in male and female rats administered acetaldehyde in the drinking-water. In some instances, the incidence in the treated groups was significantly greater than that in the respective control groups; nevertheless, these increases may be due to chance because no obvious dose–response relationship was observed in any of the tissues. The Working Group expressed concerns whether the doses were accurate due to the volatility of acetaldehyde." A similar criticism has been raised by BfR-Kommission für kosmetische Mittel (ref. 39).

Despite some uncertainties in the dose-response relationship and other criticisms of the study, as stated by IARC and BfR, the SCCS calculated a T25 value from the study.

In Table 4 the frequencies of tumour sites with significant increase and the corresponding T25 are shown.

**Table 4:** Tumour types with significant increased frequencies (two-sided Fisher's exact test compared to control, P ≤ 0.05, bold) after exposure to acetaldehyde in drinking water

| ***Tumour*** | ***Control*** | ***50 mg/l*** | ***250 mg/l*** | ***500 mg/l*** | ***1500 mg/l*** | ***2500 mg/l*** | ***T25 mg/kg bw/d*** |
|---|---|---|---|---|---|---|---|
| **Male** | | *5* mg/kg bw/d* | *25 mg/kg bw/d* | *49 mg/kg bw/d* | *147 mg/kg bw/d* | *246 mg/kg bw/d* | |
| Cranial osteosarcomas | 0 (0%) | 5 (10%) | 1 (2%) | 2 (4%) | 0 (0%) | **7 (14%)** | 283 |
| Lymphomas and leukaemias | 6 (12%) | 14 (28%) | 10 (20%) | 9 (18%) | **15 (30%)** | 8 (16%) | 116 |

| ***Tumour*** | ***Control*** | ***50 mg/l*** | ***250 mg/l*** | ***500 mg/l*** | ***1500 mg/l*** | ***2500 mg/l*** | ***T25 mg/kg bw/d*** |
|---|---|---|---|---|---|---|---|
| **Female** | | *5 mg/kg bw/d* | *27 mg/kg bw/d* | *53 mg/kg bw/d* | *155 mg/kg bw/d* | *260 mg/kg bw/d* | |
| Uterine adenocarcinomas | 0 (0%) | 0 (0%) | 5 (10%)** | 1 (2%) | 2 (4%) | 1 (2%) | 44 |

* *Recalculated using data about body weight and drinking volume (average for each group up to week 104) provided by M. Soffritti (2008, personal communication).From Lachenmeier et al., 2009a; Ref.: 27)*

** P= *0.056*

T25 calculated on the basis of significant increased tumour frequencies for cranial osteosarcomas and lymphomas and leukaemias combined in male rats were 283 and 116 mg/kg bw/d respectively and 44 mg/kg bw/d for uterine adenocarcinomas in female. Lachenmeier *et al*. (2009a) (Ref.: 27) derived and used a T25 value of 127 mg/kg bw/day based on the total number of malignant tumours in male rats.

*Inhalation*

Rats

Four groups of 105 male and 105 female Cpb:WU albino Wistar rats, six weeks of age, were exposed by whole-body inhalation to concentrations of 0, 750, 1500 or 3000 (reduced progressively over a period of 11 months to 1000 ppm due to toxicity) ppm [0, 1350, 2700 or 5400–1800 mg/m$^3$] acetaldehyde vapour [purity unspecified] for 6 h per day on five days per week for a maximum of 28 months. Each group comprised five subgroups, three of which were used for interim kills at weeks 13 (5 males and 5 females), 26 (5 males and 5 females) and 52 (10 males and 10 females), respectively. One group was exposed for 12 months and killed after a recovery period of 12 months (30 males and 30 females). The remaining animals (55 males and 55 females) were killed after maximum 28 months.

Of the animals killed at these intervals, only one had a tumour of the respiratory tract: a female in the high-dose group killed in week 53, bearing a nasal squamous-cell carcinoma. At day 468, the mortality rate in the high-dose group was 50% (28/55) for males and 42% (23/55) for females.

By day 715, all high-dose rats had died and, at termination of the study at day 844, only a few animals were still alive in the mid-dose group. At the end of the study, the incidences of nasal carcinomas (carcinomas *in situ*, squamous-cell carcinomas and adenocarcinomas) were in males: 1/49 (2%), 17/52 (33%), 41/53 (77%) and 37/49 (76%) in the control, low-, mid- and high-dose groups, respectively; and in females: 0/50 (0%), 6/48 (13%), 34/53 (64%), and 43/53 (81%) in the control, low-, mid- and high-dose groups, respectively. One carcinoma *in situ* of the larynx was found in a female of the mid-dose group and one female of the low-dose group developed a poorly differentiated adenocarcinoma in the lung.

Ref.: 23

Comment

SCCS notes that the experiment lasted for 28 month and not 24 months as stipulated by modern guidelines. A T25 of 121 mg/kg bw/day based on nasal carcinomas in males was calculated in the previous SCCNFP Opinion on Acetaldehyde (ref.: 28).

Hamster

Groups of 35 male Syrian golden hamsters were exposed to 0 or 1500 ppm [2700 mg/m$^3$] acetaldehyde vapour for seven hours per day on five days per week for 52 weeks, and

received weekly intratracheal instillations of 0, 0.0625, 0.125, 0.25, 0.5 or 1 mg benzo[*a*]pyrene suspended in saline for the same period.

Groups of five animals were killed at the 52nd week and the remainder allowed surviving untreated for an additional 26 weeks.

There was no significant difference in mortality between the animals exposed to acetaldehyde and those exposed to air, except for the subgroup treated with the highest dose of benzo[*a*]pyrene, for which the mortality in the acetaldehyde-exposed animals was increased more rapidly than the mortality in the corresponding benzo[*a*]pyrene group exposed to air ($p < 0.001$ in both groups).

No tumour was found in hamsters exposed to acetaldehyde only; but 3/30, 4/30, 9/30, 25/29 and 26/28 hamsters exposed to benzo[*a*]pyrene alone developed respiratory-tract tumours and 1/28, 5/29, 8/29, 16/29 and 29/30 hamsters exposed to benzo[*a*]pyrene and acetaldehyde vapour developed the same type of tumour.

Ref.: 24

Comment
Acetaldehyde alone did not induce tumours under the experimental conditions used, nor did acetaldehyde affect the carcinogenic effect of benzo[*a*]pyrene.

Groups of 36 male and 36 female Syrian golden hamsters, six weeks of age, were exposed for seven hours per day on five days per week to room air (chamber controls) or to decreasing concentrations of acetaldehyde (distilled and analysed by gas chromatography) (initial concentration, 2500 ppm [4500 mg/m$^3$]; final concentration, 1650 ppm [2970 mg/m$^3$]) for 52 weeks. Six animals killed and examined from each group had no tumour. The remaining animals were observed until 81 weeks and killed. The incidences of respiratory-tract tumours were 0/30 (0%), 8/29 (29%), 0/28 (0%) and 5/29 (17%) in control males, exposed males, control females and exposed females, respectively ($p < 0.05$). The acetaldehyde-induced tumours were predominantly laryngeal carcinomas with a few laryngeal polyps, and nasal polyps and carcinomas.

Ref.: 25

***Human studies***

Several case-control studies have been carried out in chemical plants. In the former German Democratic Republic, nine cancer cases were found in a factory where the main process was dimerization of acetaldehyde and where the main exposures were to acetaldol (3-hydroxybutanal), Acetaldehyde, butyraldehyde, crotonaldehyde and other higher, condensed aldehydes, as well as to traces of acrolein. Of the cancer cases, five were bronchial tumours and two were carcinomas of the oral cavity. All nine patients were smokers. The relative frequencies of these tumours were reported to be higher than those expected in the German Democratic Republic. The IARC Working Group (Ref.: 13) noted the mixed exposure, the small number of cases and the poorly defined exposed population.

IARC concluded in 1999 (Ref.: 13) that "There is *inadequate evidence* in humans for the carcinogenicity of acetaldehyde.

In 2012 IARC (Ref.: 15) has concluded: "Acetaldehyde associated with the consumption of alcoholic beverages is *carcinogenic to humans (Group 1).*"

In reaching the above conclusion the IARC made the following considerations:

- Upon ingestion of alcoholic beverages, ethanol is converted into acetaldehyde, which is then oxidized to acetate.

- Ethanol and acetaldehyde are both carcinogenic in experimental animals.
- There is sufficient epidemiological evidence showing that humans who are deficient in the oxidation of acetaldehyde to acetate have a substantially increased risk for development of alcohol-related cancers, in particular of the oesophagus and the upper aero-digestive tract.

Comment
The term upper aero-digestive tract include oral cavity, pharynx, larynx and oesophagus.

On the basis of the calculated T25-values, acetaldehyde should be considered a "low potency" carcinogen (ref.: 38).

### 3.3.8 Reproductive toxicity

Several studies on the developmental effects of acetaldehyde have been conducted, primarily to investigate its role in ethanol-induced teratogenicity. In these studies, reviewed by IARC, acetaldehyde was given by amniotic or intraperitoneal injection, not by ingestion or inhalation. Dose-related embryotoxic, fetotoxic and teratogenic effects were seen in most of these studies, particularly in rats, but maternal toxicity was often not assessed adequately or reported in any of these investigations. Dose-related embryotoxic effects were observed in *in vitro* studies on rat embryos exposed to acetaldehyde. Effects on the placenta have been observed following intraperitoneal injection of acetaldehyde into pregnant rats. Foetal malformations and resorptions were found in mice and rats treated with acetaldehyde.

Ref.: 14

*Ethanol*
It is widely accepted that ethanol has profound effects on the female as well as the male reproductive system. Moreover, ethanol is a well-documented human teratogen that can cause a spectrum of physical and mental dysfunctions following prenatal exposure. Multiple terms are used to describe the continuum of effects that result from prenatal exposure to ethanol, the most commonly known of which is foetal alcohol syndrome

Ref.: 14

Comment
SCCS notes that it is not known whether acetaldehyde, the primary metabolite of ethanol, is involved in the aetiology of the human foetal alcohol syndrome.

#### 3.3.8.1 Two generation reproduction toxicity

No data submitted

#### 3.3.8.2 Teratogenicity

No data submitted

### 3.3.9 Toxicokinetics

The main part of this section is taken from IARC (2012) (Ref.: 15).

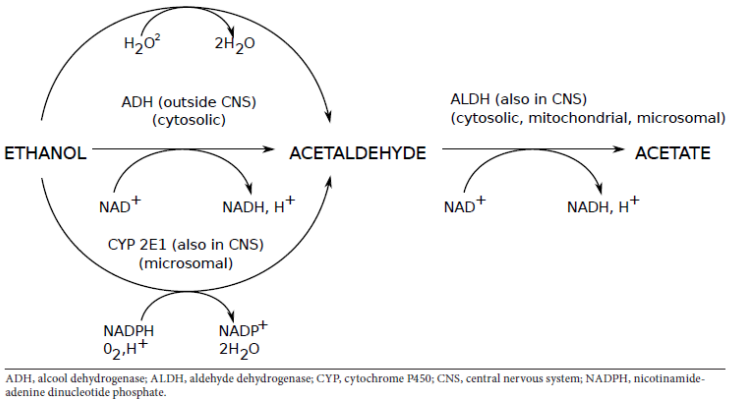



**Figure 1:** Ethanol and acetaldehyde metabolism (taken from ref. 15)

Acetaldehyde is the first metabolite in the oxidation of ethanol. Ethanol is metabolized to acetaldehyde by three major pathways (see Fig. 1) the alcohol dehydrogenase (ADH) pathway, the microsomal ethanol oxidizing cytochrome P450 (CYP) pathway, and the catalase-$H_2O_2$ system. Acetaldehyde, to which many deleterious effects of ethanol can be attributed, is oxidized to acetate primarily by acetaldehyde dehydrogenases (ALDHs).

Interindividual variations of the acetaldehyde-mediated effects will depend on the genetic polymorphisms and other factors affecting the metabolism and levels of acetaldehyde, and its effects on the target organs.

Several degradation reactions are known to form endogenous acetaldehyde in the human body. Without external alcohol ingestion, acetaldehyde concentrations are below the level of detection, except in the gastrointestinal tract.

*Aldehyde dehydrogenase pathway*

Acetaldehyde is metabolized by ALDHs, which are widely expressed in the mitochrondria (low-Km enzyme) and cytosol (high-Km enzyme) of most tissues. Oxidation of acetaldehyde is regulated by the rate of acetaldehyde formation, ALDH activity and the cytosolic and mitochondrial redox states. Ethanol consumption is not known to induce *ALDH* expression. Chronic alcohol abuse is reported to reduce the ALDH activity. The high-Km ALDH1A1 (Km = 50 μM) accounts for most of the acetaldehyde oxidizing capacity in the cytosolic compartment of the liver and other tissues. This enzyme is also abundant in the erythrocytes. The low-Km (about 5 μM) ALDH2 is located in the mitochondria and is believed to be responsible for the bulk of the oxidation of the ethanol-derived acetaldehyde. This enzyme is not significantly expressed in the erythrocytes. Of all the polymorphisms in genes encoding enzymes that metabolize alcohol and acetaldehyde, the *ALDH2*2* allele has the greatest functional impact on the human phenotype. This allele is common in East-Asian populations, about 5–10% are homozygotes and 30–40% are heterozygotes. In both groups the acetaldehyde levels are elevated, which creates several toxic effects and also euphoric reinforcing reactions. The relevance of the elevated acetaldehyde for the development of cancers is briefly mentioned in section 3.3.7. Carcinogenicity.

*Other pathways in the metabolism and reactions of acetaldehyde*

In addition to the ALDH-catalysed reactions, acetaldehyde may also be oxidized to a minor extent by CYP2E1 and by different oxidases. Due to its chemical reactivity, most, if not all, of the ethanol-derived acetaldehyde that is not further oxidized binds to a variety of constituents. These interactions vary between easily reversible and firm covalent bonds. Different kinds of Schiff's bases, which are formed by acetaldehyde and the free amino groups of amino acids, peptides and proteins, are the most common products. Some of these unstable products become stable under reducing conditions, such as during alcohol intoxication. Although only a small fraction of all acetaldehyde formed during ethanol

oxidation produces these adducts, they are important in some of the chronic toxic actions of alcohol. The acetaldehyde adducts may play a role in the carcinogenic effects of ethanol.

*Levels of acetaldehyde in tissues*
From the liver, where most of the ethanol derived acetaldehyde is formed and oxidized, the remaining acetaldehyde, free and/or loosely bound, escapes into the *vena hepatica*, reaching concentrations of approximately 70 µM (3 µg/ml) under normal conditions. Thereafter, the concentration of acetaldehyde in the blood will be diluted by the *vena cava* blood and further reduced by the circulation in the heart and the lungs before reaching peripheral tissues. Human data show that acetaldehyde levels in pulmonary arterial blood are in the range of 0–4.4 µM (0 – 0.2 µg/ml), 30 and 60 minutes after ethanol consumption. Acetaldehyde in peripheral arterial or venous blood is below the limit of detection (< 1 µM; <0.04 µg/ml), during ethanol intoxication in Caucasian male populations. However, in Caucasian women, acetaldehyde levels of 1–8 µM (0.04 – 0.3 µg/ml) have been detected during the use of oral contraceptives and during the high-estradiol phase of the normal cycle. Except for the blood and the liver, in which acetaldehyde concentration should be approximately the same as in the *vena hepatica*, little is known about acetaldehyde levels in other tissues during ethanol oxidation in humans. In Asian subjects carrying the ALDH*2 allele, blood acetaldehyde levels above 200 µM (8 µg/ml) have been reported.

Increased levels of acetaldehyde in the saliva are also reported in after alcohol intake. The acetaldehyde in the saliva is almost exclusively derived from microbiological alcohol oxidation and correlate positively with the blood alcohol concentration. Levels varying between 15 to 25 µM (0.7 – 1.1 µg/ml and 20 to 40 µM (0.9 – 1.8 µg/ml) at blood ethanol concentrations of 10 to 20 mM (0.5 – 0.9 $^{0}$/oo), respectively.

Ref.: 15

*Levels of acetaldehyde in blood*
Acetaldehyde has been measured in blood from 225 teetotallers (people that do not drink alcohol). A mean acetaldehyde level of 7.7 $\pm$ 0.7 µM (0.3 $\pm$ 0.03 µg/ml) (range 6.1 – 10.1 µM) was found. The authors reported that in an alcoholic population the mean acetaldehyde level was 25.3 + 15.6 µM (1.1 $\pm$ 0.7 µg/ml). In a subsequent study among students (645 women and 332 men), blood samples were drawn for clinical indications over a two year period. The mean blood acetaldehyde level in this group was 9.7 $\pm$ 2.1 µM (0.4 $\pm$ 0.1 µg/ml). The levels were a little higher among men than among women. The higher acetaldehyde levels among the students than the teetotallers were explained by the alcohol intake by the students.

Ref.: 44, 45

Comment
The results concerning acetaldehyde levels in blood in the two articles above (ref. 44, 45) are in contrast to that reported in the paragraph above *"Acetaldehyde in peripheral arterial or venous blood is below the limit of detection (< 1 µM; <0.04 µg/ml), during ethanol intoxication in Caucasian male populations."* (ref. 15). The text give the impression that *"free and/or loosely bound"* acetaldehyde was measured. The analytical procedure used in the two experiments cited above (ref. 44, 45) involve heating the samples to 70$^{o}$C and it is claimed that they measure whole blood-associated acetaldehyde (both free and bound acetaldehyde). Other authors claim that the heating introduces artefacts.

### 3.3.10 Photo-induced toxicity

No data submitted

### 3.3.11 Human data

No data submitted

### 3.3.12 Special investigations

*Acetaldehyde from food intake*
Acetaldehyde has been analysed in a wide variety of food matrices. The analysis was conducted using headspace gas chromatography. The samples were digested in full automation with simulated gastric fluid. 140 authentic samples were analyzed. The authors estimated that the average exposure from food (without alcoholic beverages) would be around 40 *µ*g/kg bw/day for the German population. The median intake was calculated to be 24 – 28 *µ*g/kg bw/day.

Ref.: 42

*Food flavouring substance*
The use of acetaldehyde as a flavouring substance was evaluated by JECFA in 1997. The Committee estimated that the intake of acetaldehyde at the estimated level of 11 mg per person per day in Europe (183 µg/kg bw/d) would not present safety concern.

Ref.: 32

*Tobacco products*
All tobacco products contain acetaldehyde. Cigarette mainstream smoke typically contains 800–900 µg acetaldehyde/cigarette. This implies that a smoker will inhale of the order 10 - 20 mg (170 – 330 µg/kg bw/d) acetaldehyde per day. In rooms where cigarettes are smoked the acetaldehyde level may be in the order of 200–300 µg/$m^3$ (4 µg/kg bw/d). Levels of acetaldehyde in ambient air generally average 5 µg/$m^3$.

During the last years the levels of acetaldehyde in snuff have been reduced from about 36 to 6 µg/g dry weight.

Ref.: 15

***Exposure to acetaldehyde from different uses of ethanol***

*Acetaldehyde formation by oxidation of exogenous ethanol*
The major part of the total acetaldehyde to which the body is exposed during alcohol ingestion originates from ethanol oxidation. The liver and the gut are the primary sites of acetaldehyde formation to such an extent that the rate of alcohol oxidation exceeds the rate of acetaldehyde breakdown, which consequently leads to diffusion of the surplus acetaldehyde into the bloodstream. Under normal conditions, the acetaldehyde produced at other sites is usually directly oxidized within the tissue. The exception is the aerodigestive tract, where acetaldehyde is produced at least partly by microbial alcohol oxidation. Consequently, acetaldehyde can be detected both in breath and saliva during alcohol intoxication.

As discussed above acetaldehyde levels in pulmonary arterial blood are in the range of 0–4.4 µM (0 – 0.2 µg/ml), 30 and 60 minutes after ethanol consumption. Acetaldehyde in peripheral arterial or venous blood is below the limit of detection (< 1 µM; <0.04 µg/ml), during ethanol intoxication. The acetaldehyde in the saliva correlates positively with the blood alcohol concentration. Levels varying between 15 to 25 µM (0.7 – 1.1 µg/ml and 20 to 40 µM (0.9 – 1.8 µg/ml) at blood ethanol concentrations of 10 to 20 mM (0.5 – 0.9 $^0$/oo), respectively, have been reported.

*Acetaldehyde in alcoholic beverages*
All alcoholic beverages contain acetaldehyde in variable amounts: average levels in different types vary between 60 to > 7000 µM (2.5 - > 300 µg/ml). Lachenmeier and co-workers have estimated the average exposure to acetaldehyde from its content in alcoholic beverages to 112 µg/kg bw/day. The life-time cancer risk was calculated to 7.6 x $10^{-4}$. The authors pointed out that alcohol consumption is a direct source of acetaldehyde exposure,

which in conjunction with other sources (food flavourings, tobacco) results in a magnitude of risk requiring intervention. An initial public health measure could be to reduce the acetaldehyde content in alcoholic beverages as low as technologically possible, and to restrict its use as a food flavour additive.

Ref.: 27

*Mouthwashes*

La Vecchia (2009) reviewed 10 epidemiological studies on the link between mouthwash use and oral cancer risk. Information on alcohol in the mouthwashes was only available in two of the studies. One of these showed an increased risk of oral cancer among those using alcohol containing mouthwashes while no increased risk was found among those using mouthwashes without alcohol. The other showed no increase in relation the use of alcohol containing mouthwashes.

Ref.: 29

Gandini *et al* (2012) reviewed and performed a meta-analysis of 18 epidemiological studies including the 10 studies reviewed by La Vecchia on the use of mouthwashes and cancer. The authors concluded that there was no statistically significant associations found between regular use of mouthwash and risk of oral cancer (RR=1.13 [0.95-1.35]). There was no association between reported use of mouthwash specifically containing alcohol and risk of oral cancer (RR=1.16 [0.44-3.08]).

Ref.: 43

Comment
SCCS notes that in the Abstract it is written: "*There was no association between reported use of mouthwash specifically containing alcohol and risk of oral cancer (RR=1.16 [0.44-3.08])*" while in the text of the article the same RR was given as *(RR=1.0 [0.39-2.60]).*

Ethanol is contained in a number of ready to-use mouthwashes typically between 5 and 27% vol. The acetaldehyde levels in saliva after use of alcohol-containing mouthwashes have been measured. Ready to-use mouthwashes and mouth rinses (n = 13) were rinsed in the mouth by healthy, non-smoking volunteers (n = 4) as intended by the manufacturers (20 ml for 30 sec). Saliva was collected at 0.5, 2, 5 and 10 min after mouthwash use. The acetaldehyde content in the saliva was 41 ± 15 µM, (range 9–85 µM) after 0.5 min, 52 ± 14 µM, (range 11–105 µM) after 2 min, 32 ± 7 µM, (range 9–67 µM) after 5 min and 15 ± 7 µM, (range 0–37 µM) after 10 min. The contents were significantly above endogenous levels and corresponding to concentrations normally found after alcoholic beverage consumption. A twice-daily use of alcohol-containing mouthwashes leads to a systemic acetaldehyde exposure of 0.26 µg/kg bw/d on average, which corresponds to a lifetime cancer risk of 3 x $10^{-6}$. However, the local acetaldehyde-contents in the saliva are reaching concentrations associated with DNA adduct formation and sister chromatid exchange *in vitro*, so that concerns for local carcinogenic effects in the oral cavity remain.

Ref.: 30

Comment
The results from measurements of acetaldehyde in the saliva indicate the levels are similar after ingestion of alcoholic beverages and the use of mouthwashes containing ethanol. Since most of the acetaldehyde in the saliva is formed by microbiological alcohol oxidation this may also be anticipated.

*Exposure of skin to ethanol*
Sixteen adults sprayed an aerosol containing 44% ethanol over the body for approximately 10 sec (mean amount used per treatment: 9.72 g). Blood samples were taken after a 15

min period and analysed by gas chromatography. Subsequent samples were taken 5, 10, 30 and 60 min after that. Ten of the panelists produced at least one blood sample with a detectable alcohol content (detection limit: 5 mg/l). The maximum value recorded was 13 mg/l. However, there remained some uncertainty in the analytical method, as other alcohols may co-elute. Using another gas chromatographic column (detection limit: 9 mg/l), none of the blood samples exhibited detectable levels of ethanol. The application as a spray also includes a potential pulmonary uptake. Despite the high concentration of ethanol (44%) and the high exposure to large surfaces, the maximum blood levels were only slightly elevated above physiological blood levels.

Ref.: 33

Miller and coworkers reported the blood alcohol level after using an alcohol-based instant hand sanitizer (62% (v/v) ethanol) under most extreme conditions (applying 5 ml, 25 times over the course of 2 hours). The blood alcohol level measured immediately following the final application was below the detection limit (< 5 mg/dl). In a subsequent study of 5 subjects using 5 ml of the product with a repetition of 50 times over 4 hours, the result was confirmed as all participants had blood ethanol levels less than 5 mg/dl.

Ref.: 34

Twelve volunteers applied three hand-rubs containing 95% (hand-rub A), 85% (hand-rub B) and 55% ethanol (hand-rub C; all w/w). For hygienic hand disinfection, 4 ml were applied 20 times for 30 s, with 1 minute break between applications. For surgical hand disinfection, 20 ml of each hand rub was applied to hands and arms up to the level of the elbow 10 times for 3 minutes, with a break of 5 minutes between applications. Blood concentrations of ethanol and acetaldehyde were determined immediately prior and up to 90 minutes after application using head space gas chromatography. The median of absorbed ethanol after hygienic hand disinfection was 1365 mg (A), 630 mg (B), and 358 mg (C). The proportion of absorbed ethanol was 2.3% (A), 1.1% (B), and 0.9% (C). After surgical hand disinfection, the median of absorbed ethanol was 1067 mg (A), 1542 mg (B), and 477 mg (C). The proportion of absorbed ethanol was 0.7% (A), 1.1% (B), and 0.5% (C). The highest median acetaldehyde concentration after 20 hygienic hand disinfections was 0.57 mg/l (hand-rub C, after 30 min), after 10 surgical hand disinfections 3.99 mg/l (hand-rub A, after 20 minutes). The authors concluded that the overall dermal and pulmonary absorption of ethanol was below toxic levels in humans and allows the conclusion that the use of the evaluated ethanol-based hand-rubs is safe.

Ref.: 35

*Inhalation of ethanol*

An occupational physician reported to the French Health Products Safety Agency (Afssaps) a case of adverse effect of acute pancreatitis (AP) in a teaching nurse, after multiple demonstrations with ethanol-based hand sanitizers (EBHSs) used in a classroom with defective mechanical ventilation. It was suggested by the occupational physician that the exposure to ethanol may have produced a significant blood ethanol concentration and subsequently the AP. In order to verify if the confinement situation due to defective mechanical ventilation could increase the systemic exposure to ethanol *via* inhalation route, a physiologically based pharmacokinetic (PBPK) modelling was used to predict ethanol blood levels. Under the worst case scenario, the simulation by PBPK modelling showed that the maximum blood ethanol concentration which can be predicted of 5.9 mg/l is of the same order of magnitude to endogenous ethanol concentration (mean = 1.1mg/l; median = 0.4 mg/l; range = 0–35 mg/l) in non-drinker humans. The present study does not support the likelihood that EBHS leads to an increase in systemic ethanol concentration high enough to provoke an acute pancreatitis.

Ref.: 36

General comment

The blood level of acetaldehyde depends on the rate of formation from ethanol, the intake of acetaldehyde from different sources and on the rate of oxidation of acetaldehyde to acetic acid.

All alcoholic beverages contain acetaldehyde in variable amounts. Lachenmeier and co-workers (ref. 27) have estimated the average exposure to acetaldehyde from its content in alcoholic beverages to 112 µg/kg bw/d. This may, however, be a small amount compared to that formed from alcohol by oxidation. On the other hand it will add to the amount formed by microbiological alcohol oxidation in the upper aerodigestive tract were there are considerable evidence that acetaldehyde is involved in tumour formation in humans.

JECFA estimated in 1997 the intake of acetaldehyde from food flavouring substances to 183 µg/kg bw/d, while Uebelacker and Lachenmeier estimated the mean intake of acetaldehyde in the German population from food to 40 µg/kg bw/d (median intake 24 – 28 µg/kg bw/d) in 2011 (ref. 42) after having measured acetaldehyde in 140 authentic food samples. Another important source of acetaldehyde is cigarettes. A smoker will inhale of the order 10 - 20 mg (170 – 330 µg/kg bw/d) acetaldehyde per day.

Measurements of the blood levels have given widely different results. In the recent IARC evaluation it was stated that *"Acetaldehyde in peripheral arterial or venous blood is below the limit of detection (< 1 µM; <0.04 µg/ml), during ethanol intoxication in Caucasian male populations."* (ref. 15). On the other hand the group of Halvorson (ref. 44, 45) measured mean values of 7.7 µM acetaldehyde in teetotallers, 9.7 µM in students and 25.3 µM in alcoholics. The reason for the apparent divergence is not clear. It should be noted that while in the first case it is stated that *"free and/or loosely bound"* acetaldehyde was measured while in the latter case "*whole blood-associated acetaldehyde (both free and bound acetaldehyde)"* was measured.

A blood level of 7.7 µM acetaldehyde will correspond to 0.3 µg/ml. Assuming a blood volume of 5 liter and a bodyweight of 60 kg, this will correspond to 25 µg/kg bw. The size of the intake that give rise to 0.3 µg/ml blood is not known as the "steady state" concentration will depend not only on the intake but also on how fast acetaldehyde is removed.

### 3.3.13 Safety evaluation

For some health endpoints it is not possible to establish a threshold. This is especially the case for mutagens and genotoxic carcinogens. The decision on a threshold and a non-threshold mode of action for carcinogens may not always be easy to make, especially when, although a biological threshold may be postulated, the data do not allow identification of it. If this is not clear, *the assumption of a non-threshold mode of action would be the prudent choice for risk characterisation of carcinogens* (46).

*Derivation of T25 for calculation of lifetime cancer risk*
Although, the long-term experiments with acetaldehyde and especially the oral study is criticised, a quantitative lifetime cancer risk (LCR) may be calculated in order to obtain an indication of the potential cancer risk.

In the present Opinion the same T25 = 121 mg/kg bw/d will be used as in the previous SCCNFP Opinion on Acetaldehyde (Ref.: 28). The T25 of 121 mg/kg bw/d is based on nasal carcinomas in male rats from the inhalation study of Woutersen *et al*. (1986) (Ref.: 23). These tumours occurred at the site of contact. However, since acetaldehyde is considered to be a genotoxic carcinogen it is expected that it may induce cancer by all routes of exposure and the site of tumour formation in humans may be different from that found in carcinogenicity studies with rodents. In this respect it should be noted that formaldehyde which also induced tumours primarily at the site of contact in experimental carcinogenicity studies has recently been found also induce leukaemia in humans (ref. 15). An oral study where acetaldehyde was added to the drinking water of rats was published by Soffritti *et al*.

in 2002 (ref.: 22). A T25 = 116 mg/kg bw/d based on lymphomas and leukaemias combined in male rats was calculated. Moreover, Lachenmeier *et al*. (2009a) (Ref.: 27) have derived and used a T25 = 127 mg/kg bw/d based on the total number of malignant tumours in male rats. Although, this oral study has several shortcomings, the finding that the average of the two calculated T25 values is the same ([116 + 127]/2 = 121.5) as the T25 from the inhalation study gives confidence in the T25 value used.

*Derivation of systemic exposure dose (SED).*
Dose calculations: According to the Notes of Guidance, an aggregate value of **17.4 g/day** will be used in the calculation of the MoS.

The applicant considers a concentration of acetaldehyde up to 100 ppm.

100 ppm corresponds to 1.74 mg/day.

In the absence of dermal absorption data, it is assumed that 100% is absorbed (see the previous Opinion; Ref: 28)

SED 1.74/60 0.029 mg/kg bw/d

*Calculation of lifetime cancer risk*

T25 = 121 mg/kg bw/d

$$HT_{25} = \frac{T_{25}}{(\text{body weight}_{human}/\text{body weight}_{animal})^{0.25}}$$

$$HT25 = T25 / (bw_h / bw_r)^{0.25} = 121 / (60/0.5)^{0.25} = 121/3.3 = 37 \text{ mg/kg bw/d}$$

$$\text{Lifetime cancer risk} = \frac{SED}{HT_{25} / 0.25}$$

$$= 0.029 / (37/0.25) = 2 \times 10^{-4}$$

The calculation is based on several worst case considerations. 100% skin absorption is used in the calculation. Moreover, since acetaldehyde is highly volatile, a significant fraction may evaporate and not be dermally available. Since 100% dermal absorption is used, the calculation will actually cover absorption by all routes.

The only permitted use of acetaldehyde in cosmetics is up to 25 ppm acetaldehyde in fragrance compounds. Acetaldehyde should otherwise only be found in cosmetic products in the form of unavoidable traces originating mainly through plant extracts and botanical ingredients and ethanol. The probability of cancer risk for a lifetime exposure to 100 ppm from all cosmetic products is $2 \times 10^{-4}$. It can be derived from the above calculation that a safe concentration with a LCR of $10^{-5}$ would be 5 ppm in all cosmetic products.

In the case of non-cancer effects, a NOAEL of 49 mg/m³ has been derived from a 28 day inhalation study in rats. As a worse case approach, complete evaporation of acetaldehyde present in all cosmetic products (total exposure of 1.74 mg/d) in a small room (10 m³) without ventilation would result in a concentration of 0.174 mg/m³. In this worst case scenario, the Margin of Exposure would be much higher than 100.

It should be noted that the estimated intake of acetaldehyde used as flavouring substances in food was estimated to 11 mg/person per day in Europe (183 µg/kg bw/d) by JECFA in 1993. More recently, Uebelacker and Lachenmeier estimated the mean intake of acetaldehyde in the German population from food to 40 µg/kg bw/d (median intake 24 – 28 µg/kg bw/d) (ref. 42). These values should be compared with the maximum estimated intake from cosmetics (29 µg/kg bw/d).

Based on the recent IARC evaluations (ref. 15) that "*there is sufficient epidemiological evidence that acetaldehyde has increased the risk of alcohol related cancer in particular of the oesophagus and the upper aero-digestive tract*" including oral cavity. SCCS is of the opinion that acetaldehyde should not be intentionally used in mouth-washing products.

### 3.3.14 Discussion

This evaluation considers potential exposures from acetaldehyde in cosmetic products alone. Other relevant exposures from food and alcoholic beverages and smoking, for example, have not been assessed and are likely to be considerably higher compared to cosmetic exposure.

Acetaldehyde is a naturally occurring substance, also in human metabolic pathways. It is the main metabolite of ethanol. It is metabolised to acetic acid.

*Physico-chemical properties*
Pure acetaldehyde is flammable; it polymerizes violently in the presence of trace amounts of metals or acids. Acetaldehyde may undergo auto-polymerisation upon contact with air or moisture. Upon prolonged storage, it may form unstable peroxides. Solutions of acetaldehyde in water, DMSO, 95% ethanol or acetone should be stable for 24 hours under normal laboratory conditions.

*Irritation, sensitisation*
Acetaldehyde is a skin, eye and respiratory tract irritant. There is limited evidence for skin sensitisation. Respiratory sensitisation has not been investigated to date.

*Dermal absorption*
Some studies are available concerning increase in blood acetaldehyde after dermal exposure to ethanol. However, no quantitative conclusions can be drawn from these studies regarding skin absorption of acetaldehyde. A dermal absorption of 100% is used in the risk characterization.

*General toxicity*
No toxicity studies have been performed according to present day requirements. A NOAEL of 125 mg/kg bw/d was found in a 4 week study based on relative increase in kidney weight and focal hyperkeratosis of the forestomach.

In a 28-day inhalation study with rats, a NOAEL of 49 mg/m³ based on the degeneration of the nasal olfactory epithelium was established.

*Mutagenicity*
*In vitro* and without exogenous metabolic activation, acetaldehyde induced gene mutations in mouse lymphoma L5178T cells, sister chromatid exchanges in Chinese hamster ovary cells and aneuploidy in embryonic Chinese hamster diploid fibroblasts. Increased frequency of acetaldehyde DNA adducts in humans has been found in relation to alcohol use.

*Carcinogenicity*
Acetaldehyde has been found to induce tumours in rats after oral and inhalation exposure and in hamster after inhalation exposure. IARC conclude that there is *sufficient evidence* in experimental animals for the carcinogenicity of acetaldehyde.

Acetaldehyde is a carcinogen classified as Carc Cat 2 according to Annex VI of regulation 1272/2008 (CLP). IARC concludes that "Acetaldehyde associated with the consumption of alcoholic beverages is *carcinogenic to humans (Group 1).*" In reaching this conclusion the IARC made the following considerations: Upon ingestion of alcoholic beverages, ethanol is converted into acetaldehyde, which is then oxidized to acetate. Ethanol and acetaldehyde are both carcinogenic in experimental animals. There is sufficient epidemiological evidence showing that humans who are deficient in the oxidation of acetaldehyde to acetate have a substantially increased risk for development of alcohol-related cancers, in particular of the oesophagus and the upper aero-digestive tract.

In the present Opinion the same T25 = 121 mg/kg bw/d will be used as in the previous SCCNFP Opinion on Acetaldehyde (Ref.: 28). The T25 of 121 mg/kg bw/d is based on nasal carcinomas in male rats from the inhalation study of Woutersen *et al*. (1986) (Ref.: 23). These tumours occurred at the site of contact. However, since acetaldehyde is considered to be a genotoxic carcinogen it is expected that it may induce cancer by all routes of exposure and the site of tumour formation in humans may be different from that found in carcinogenicity studies with rodents. In this respect it should be noted that formaldehyde which also induced tumours primarily at the site of contact in experimental carcinogenicity studies has recently been found also induce leukaemia in humans (ref. 15). An oral study where acetaldehyde was added to the drinking water of rats was published by Soffritti *et al*. in 2002 (ref.: 22). A T25 = 116 mg/kg be/d based on lymphomas and leukaemias combined in male rats was calculated. Moreover, Lachenmeier *et al*. (2009a) (Ref.: 27) have derived and used a T25 = 127 mg/kg bw/d based on the total number of malignant tumours in male rats. Although, this oral study has several shortcomings, the finding that the average of the two calculated T25 values is the same ([116 + 127]/2 = 121.5) as the T25 from the inhalation study gives confidence in the T25 value used. On the basis of the calculated T25-values, acetaldehyde should be considered a "low potency" carcinogen (ref.: 38).

*Reproductive toxicity*
No reproductive toxicity studies have been performed according to present day requirements. SCCS notes that it is not known whether acetaldehyde, the primary metabolite of ethanol, is involved in the aetiology of the human foetal alcohol syndrome.

*Toxicokinetics*
Acetaldehyde is the first metabolite in the oxidation of ethanol. Ethanol is metabolized to acetaldehyde by three major pathways: the alcohol dehydrogenase (ADH) pathway, the microsomal ethanol oxidizing cytochrome P450 (CYP) pathway, and the catalase-$H_2O_2$ system. Acetaldehyde, to which many deleterious effects of ethanol can be attributed, is oxidized to acetate primarily by acetaldehyde dehydrogenases (ALDHs). Inter-individual variations of the acetaldehyde-mediated effects will depend on the genetic polymorphisms and other factors affecting the metabolism and levels of acetaldehyde, and its effects on the target organs.

The major part of the total acetaldehyde to which the body is exposed during alcohol ingestion originates from ethanol oxidation. The liver and the gut are the primary sites of acetaldehyde formation to such an extent that the rate of alcohol oxidation exceeds the rate of acetaldehyde breakdown, which consequently leads to diffusion of the surplus acetaldehyde into the bloodstream. Under normal conditions, the acetaldehyde produced at other sites is usually directly oxidized within the tissue.

Acetaldehyde is metabolized by ALDHs, which are widely expressed in the mitochrondria (low-Km enzyme) and cytosol (high-Km enzyme) of most tissues. The high-Km ALDH1A1 (Km = 50 µM) accounts for most of the acetaldehyde oxidizing capacity in the cytosolic compartment of the liver and other tissues. The low-Km (about 5 µM) ALDH2 is located in the mitochondria and is believed to be responsible for the bulk of the oxidation of the ethanol-derived acetaldehyde. Of all the polymorphisms in genes encoding enzymes that metabolize alcohol and acetaldehyde, the *ALDH2*2* allele has the greatest functional impact on the human phenotype. This allele is common in East-Asian populations, about 5–10% are homozygotes and 30–40% are heterozygotes. In both groups the acetaldehyde levels are elevated, which creates several toxic effects and also euphoric reinforcing reactions.

## 4. CONCLUSION

1. *Is Acetaldehyde safe when present up to 100 ppm in cosmetic products taking into account the new data provided?*

The SCCS is of the opinion that acetaldehyde, present up to 100 ppm in cosmetic products, is not safe based on life-time cancer risk. However, the calculations are based on a number of worse case considerations which will lead to an overestimation of the risk.

Exposure from the dermal, inhalation and oral route cannot be properly assessed. In addition, there are no data available on metabolism of acetaldehyde in the skin.

2. *And/or does the SCCS recommend any other concentration limit with regard to the use of Acetaldehyde as an ingredient in cosmetic products?*

The SCCS is of the opinion that acetaldehyde should not be used as an intended ingredient in cosmetic products except used as a fragrance/flavour ingredient at a maximum concentration of 0.0025% (25 ppm) in the fragrance compound (ref previous opinion on acetaldehyde), resulting in approximately 5 ppm in the final finished product.

3. *Does the SCCS have any further scientific concerns regarding the use of Acetaldehyde in mouth-washing products?*

Based on the recent IARC evaluations (ref. 15), there is sufficient epidemiological evidence that acetaldehyde has increased the risk of alcohol related cancer in particular of the upper aero-digestive tract, assumed to be caused by the formation of acetaldehyde, SCCS is of the opinion that acetaldehyde should not be intentionally used in mouth-washing products.

## 5. MINORITY OPINION

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