Health Council of the Netherlands

Acetaldehyde

GSW/1706
124-15

Re-evaluation of the carcinogenicity and genotoxicity

2014/28

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Re-evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Acetaldehyde*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-8234/JR/cn/246-W19
Bijlagen : 1
Datum : 13 november 2014

Geachte minister,

Graag bied ik u hierbij het advies aan over de gevolgen van beroepsmatige blootstelling aan aceetaldehyde.

Dit advies is een herevaluatie van een eerder door de Gezondheidsraad uitgebracht advies voor een classificatie als kankerverwekkende stof. De raad is gevraagd om deze herevaluatie omdat de voorgestelde classificatie uit het eerdere advies afwijkt van de classificatie die op dit moment in de Europese Unie wordt gehanteerd. Tevens is de raad gevraagd de stof te classificeren voor mutageniteit. De classificaties in het voorliggende advies zijn gebaseerd op het Europese classificatiesysteem.

De conclusie van het advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad. De subcommissie heeft daarbij gebruik gemaakt van commentaren die zijn ontvangen op een openbaar concept van dit advies en van de oordelen die intern zijn ingewonnen bij de Beraadsgroep Gezondheid en omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. J.L Severens,
vicevoorzitter

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Acetaldehyde

Re-evaluation of the carcinogenicity and genotoxicity

Subcommittee on the Classification of Carcinogenic Substances of the
Dutch Expert Committee on Occupational Safety,
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2014/28, The Hague, Noverber 13, 2014

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Infrastructure & the Environment, Social Affairs & Employment, Economic Affairs, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

This report can be downloaded from www.healthcouncil.nl.

Preferred citation:
Health Council of the Netherlands. Acetaldehyde - Re-evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2014; publication no. 2014/28.

ISBN: 978-94-6281-016-7

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens het uitoefenen van hun beroep kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de Subcommissie Classificatie van carcinogene stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de Gezondheidsraad, hierna kortweg aangeduid als de commissie. Verder heeft het ministerie aan de Gezondheidsraad gevraagd om een aantal stoffen te herevalueren en daarbij ook een voorstel voor classificatie voor mutageniteit in geslachtscellen te doen. In het voorliggende advies herevalueert de commissie aceetaldehyde. Aceetaldehyde wordt vooral gebruikt als intermediair bij de synthese van diverse producten, waaronder de synthese van azijnzuur. Het wordt verder onder meer gebruikt als oplosmiddel bij de productie van diverse chemische stoffen en als conserveringsmiddel voor bijvoorbeeld vis en fruit.

De commissie concludeert dat aceetaldehyde beschouwd moet worden als kankerverwekkend voor de mens, en beveelt aan de stof in categorie 1B te classificeren.* Op basis van de beschikbare gegevens beveelt de commissie aan om aceetaldehyde te classificeren als mutageen voor geslachtscellen in categorie 1B (stof die beschouwd moet worden als een stof die erfelijke mutaties

* Zie bijlage F (carcinogeniteit) en G (mutageniteit) voor classificatiesysteem.

veroorzaakt in de geslachtscellen van mensen).* Aceetaldehyde heeft een stochastisch genotoxisch werkingsmechanisme.

* Zie Annex F (carcinogeniteit) en G (mutageniteit) voor classificatiesysteem.

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the Subcommittee on Classifying carcinogenic substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the committee. In addition, the ministry asked the Health Council to re-evaluate a series of substances, and to include in the re-evaluation a proposal for classification on germ cell mutagenicity. In this report, such a re-evaluation was made for acetaldehyde. Acetaldehyde is mainly used as intermediate, for instance in the production of acetic acid. It, furthermore, is used for instance as a solvent in the production of various chemical substances, and as a fish and fruit preservative.

The committee concludes that acetaldehyde is presumed to be carcinogenic to man, and recommends classifying the substance in category 1B.* Based on the available data, the committee furthermore recommends classifying acetaldehyde as a germ cell mutagen in category 1B (substance to be regarded as if it induces heritable mutations in the germ cells of humans).* The substance acts by a stochastic genotoxic mechanism.

* See Annex F (carcinogenicity) and G (mutagenicity) for the classification system.

Chapter 1

Scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification (see Annex A). The assessment and the proposal for a classification are expressed in the form of standard sentences (see Annex F). In addition to classifying substances on carcinogenicity, the Health Council also assesses the genotoxic properties of the substance in question.

Recently, with reference to the EU Regulation 1272/2008 on classification, labelling and packaging of substances, the ministry of Social Affairs and Employment asked the Health Council to update the evaluations and classification on carcinogenicity of a series of substances, and to propose for these substances a classification on germ cell mutagenicity as well.

In this report, such an update was performed for acetaldehyde. An earlier evaluation of this substance was published in 2012.[1] The re-evaluation now includes a proposal for classification on germ cell mutagenicity.

The Committee is aware that acetaldehyde is an intermediate substance in the metabolism of ethanol, and that it has been suggested that acetaldehyde accounts for a great part of the toxic effects of ethanol. However, the Committee

emphasizes that this report focuses on acetaldehyde alone and does not consider combined exposure with ethanol and ethanol-related adverse health effects.

1.2 Committee and procedures

The re-evaluation is performed by the Subcommittee on Classifying carcinogenic substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee. The members of the Committee are listed in Annex B. The submission letter (in English) to the Minister can be found in Annex C.

In 2014 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex D. The Committee has taken these comments into account in deciding on the final version of the report. The received comments, and the replies by the Committee, can be found on the website of the Health Council.

1.3 Data

The evaluation and recommendation of the Committee is standardly based on scientific data, which are publicly available. The starting points of the Committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the Committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of acetaldehyde, such an IARC-monograph is available, of which the summary and conclusion of IARC (1999) is inserted in Annex E.

Furthermore, relevant data from the European Chemicals Agency (ECHA) were retrieved and included in this advisory report.

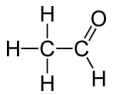
Additional data were obtained from the online databases Toxline, Medline and Chemical Abstracts, covering the period up to September 2014, using acetaldehyde and CAS no 75-07-0 as key words in combination with key words representative for carcinogenesis and mutagenesis.

Chapter 2

Identity of the substance

2.1 Name and other identifiers of the substance

Table 1 Substance identity.

EC number	:	200-836-8
EC name	:	Acetaldehyde, ethanal
CAS number (EC inventory)	:	75-07-0
CAS number	:	75-07-0
CAS name	:	Acetaldehyde
IUPAC name	:	Acetaldehyde
CLP Annex VI Index number	:	605-003-00-6
Molecular formula	:	C_2H_4O
Molecular weight range	:	44.05 g/mol
Structural formula	:	

2.2 Composition of the substance

Not applicable.

2.3 Physico-chemical properties

Table 2 Summary of physico-chemical properties.

Properties	Value	Reference	Comment
State of the substance	: Liquid at 20 °C and 101.3 kPa	IUCLID 2000	
Melting/freezing point	: -123.5 °C	SCCNFP 2004[2]	
Boiling point	: 20.4 °C	SCCNFP 2004[2]	
Relative density	: 0.78 g/cm^3 at 20 °C	IUCLID 2000	
Vapour pressure	: 98 kPa at 20 °C	SCCNFP 2004[2]	
Surface tension	: -	IUCLID 2000	
Water solubility	: Miscible at 20 °C	IUCLID 2000	
Partition coefficient n-octanol/water	: log *P*, 0.43	IARC 1999[3]	
Flash point	: -40 °C (open cup), -38 °C (closed cup)	IARC 1999[3]	
Flammability	: Extremely flammable	IUCLID 2000	
Explosive properties	: -	IUCLID 2000	
Self-ignition temperature	: -		
Oxidising properties	: -		
Granulometry	: -		
Stability in organic solvents	: - (and identity of relevant degradation products)		
Dissociation constant (pK$_a$)	: 13.6 at 25 °C	NTP 2010	
Viscosity	: 0.2456 mPa x sec at 15 °C	SCCS 2012	

2.4 International classifications

2.4.1 European Commission

Acetaldehyde is classified for carcinogenicity in Annex VI of regulation (EC) No 1272/2008 as follows: Carc 2 (suspected human carcinogen; H351: suspected of causing cancer). The substance is not classified for mutagenic activity. The classification by the European Commission dates from 1991.

2.4.2 IARC

In 1999, IARC concluded that there was inadequate evidence in humans for the carcinogenicity of acetaldehyde, and that there was sufficient evidence in experimental animals (see Annex E).[3] Therefore, IARC classified the substance in Group 2B (‘possibly carcinogenic to humans’).

In 2010, IARC evaluated the risk of cancer due to alcohol consumption, including acetaldehyde. It confirmed that there was sufficient evidence in animal experiments for the carcinogenicity of acetaldehyde.[4] Moreover, in 2012 IARC

concluded that ‘acetal-dehyde associated with alcohol consumption’ is carcinogenic to humans (Group 1).[5]

Chapter 3

Manufacture and uses

3.1 Manufacture

Not relevant for classification.

3.2 Identified uses

Acetaldehyde is an aldehyde, occurring widely in nature. For instance, it occurs naturally in coffee, bread, and ripe fruit, and is produced by plants as part of their normal metabolism. Acetaldehyde is also formed endogenously in humans in small amounts, for instance during the breakdown of ethanol in the body. It is, furthermore, present in tobacco smoke.

Acetaldehyde is produced on a large industrial scale for many purposes and uses.[6] For instance, it is used as an intermediate in the production of acetic acid; in the production of cellulose acetate, pyridine derivates, perfumes, paints (aniline dyes), plastics and synthetic rubber; in leather tanning and silvering mirrors; as a denaturant for alcohol; in fuel mixtures; as a hardener for gelatine fibres; in glue and casein products; as a preservative for fish and fruit; in the paper industry; and, as a flavouring agent.

Chapter 4

Summary of toxicokinetics

The data presented below is a summary from evaluations and reviews by others, such as IARC,[3-5] IPCS,[7] DFG,[8] and SCCNFP.[2]

4.1 Absorption, distribution and elimination

In human volunteers, a significant uptake (45-70%) by the respiratory tract of inhaled acetaldehyde was observed after a very short exposure duration of 45 to 75 seconds. In various tissues of rats, acetaldehyde was found to be increased after a single exposure by inhalation, compared to unexposed control animals. Limited data obtained from animal experiments suggest that acetaldehyde (administered by intraperitoneal injection) may be partially transferred from maternal to foetal blood. It is also found in foetal liver. In a few studies acetaldehyde was detected in the blood and brain of animals, which were given the substance by intragastric administration or intraperitoneal injections. No data are available on dermal or percutaneous absorption.

Data on elimination are very limited. In one study using dogs, a single administration of acetaldehyde via a stomach tube revealed the presence of the substance in urine in minor quantities, but in most dogs no urinary acetaldehyde could be detected at all. Most likely this is due to the rapid metabolism of the substance in the liver.

4.2 Metabolism

Quantitative data on metabolism of acetaldehyde are based on animal experiments. Acetaldehyde is rapidly oxidized into acetate by NAD+-dependent acetaldehyde dehydrogenases. These enzymes are located in the cells of most tissues, including the liver, mucosal tissue of the respiratory tract, and the testes of mice. Acetaldehyde dehydrogenases show genetic polymorphism that gives rise to differences in vulnerability in humans concerning toxicity. To a minor part, the substance is probably oxidized by cytochrome P450 2E1, and by different aldehyde oxidases. Acetate is further metabolised into carbon dioxide and water by the citric acid cycle. There is no reason to believe that metabolism of acetaldehyde in rodents is significantly different from that of humans.

In general, data indicate a highly effective metabolism, in that half-time values in the blood for acetaldehyde were found to be three minutes in rats (after repeated exposure by inhalation) and mice (single intraperitoneal injection). For humans, no reliable data on half-times are available.

Acetaldehyde is a highly reactive electrophile, which reacts with nucleophilic groups of cellular macromolecules, such as proteins and DNA, to form adducts.

Chapter 5

Genotoxicity

Numerous studies have been performed on the genotoxic properties of acetaldehyde (see Tables 3 through 11).

5.1 Non-human information

5.1.1 In vitro data

Data on in vitro mutagenicity testing are presented in Table 3.

Table 3 Summary of in vitro mutagenicity studies.

Method	Cell type	Concentration Range[a]	Results - negative + positive	Klimisch[9] score[b]	References
Micro-organisms					
Reverse mutation; multi-substance study	*S. typhimurium* TA98, TA100, TA1535, TA1537	0 - 10,000 µg/plate	- (tested in two laboratories)	2	Mortelmans et al. 1986[10]
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.005, 0.01, 0.1, 1.0, 5.0, and 10 µg/plate: + and - S9	-	2	ECHA registration data, vitro.001, study report 1979 (echa.europe.eu;)
Reverse mutation	*S. typhimurium* TA100, TA102, TA104	0.1 - 1.0 ml/chamber, vapour; - and + S9	-	2	Dillon et al. 1998[11]

Reverse mutation	*S. typhimurium* TA104	Max. non-toxic dose: 2,515 µg/ml; -S9	-	3; only one strain tested	Marnett et al. 1985[12]
Reverse mutation	*S. typhimurium* TA102	0 - 3 µg/plate; cytotoxic over 5,000 µg/plate	-	3; only one strain tested, no positive control	Chang et al. 1997[13]
Reverse mutation	*S. typhimurium* TA1535, TA1537	10 µg/plate (exact dose not given)	-	3; one dose tested only	Rosenkranz 1977[14]
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	0.5% in air (highest dose; - and + S9)	-	4; from secondary source	JETOC 1997[15]
Reverse mutation	*S. typhimurium* TA98 and TA100	No exposure concentration given; +/- S9	-	4; abstract only	Sasaki and Endo 1978[16]
Reverse mutation	*E. coli WP2 uvrA*	Six different concentrations in the range of 0.02 to 10 mM for 18 hours (-S9)	- (also alkylation rate did not increase)	2	Hemminki et al. 1980[17]
Reverse mutation	*E. coli WP2 uvrA*	0.5% in air (highest dose; - and + S9)	-	4; from secondary source	JETOC 1997[15]
Reverse mutation	*E. coli WP2 uvrA*	0.1%	+	4; abstract only; no data on controls; no data on viability	Igali and Gaszó 1980[18]
Chromosomal aberration	*Aspergillus nidulans*	Up to 300 µg/ml; -S9	+ (chromosomal malsegregation); percentage survivors decreases from 100 µg/ml onwards	3	Crebelli et al. 1989[19]
Mammalian cells					
Gene mutation	Human TK6 cells; mutants determined at the *hprt* and *tk* locus	0.001, 0.005, 0.01, 0.05, 0.25, 0.5, 1.0, 2 and 4 mM for 24 hours	- *hprt* locus; + *tk* locus (dose-dependent increase)	1	Budinsky et al. 2013[20]
Gene mutation	Human lymphocytes, *hprt* locus	0 - 2.4 mM (24 hr-treatment, 0-0.6 mM (48-hr treatment); doses selected were based on low-cytotoxicity); -S9	+ (dose-related increase in number of mutants)	2	He and Lambert 1990[21]
Gene mutation spectrum	Human lymphocytes, *hprt* locus	2.4 mM for 22 hours; cloning efficiency was 50% at 1.2 mM compared to control	+ (mutation spectrum of acetaldehyde induced mutations was different from control)	2	Noori and Hou 2001[22]
Gene mutation	Human lymphocytes from donors, *hprt* locus	1.2 to 2.4 mM for 24 hours; 0.2 to 0.6 mM for 48 hours	+ (dose-dependent increase in number of mutants); large genomic deletions; most lesions are likely point mutations	3; no positive control; no data on cytotoxicity	Lambert et al. 1994[23]

Gene mutation; multi-substance study	Mouse lymphoma L5178T cells, *tk* locus	176 - 352 µg/ml; -S9	+; growth reduces with increasing exposure	2	Wangenheim and Bolcsfoldi 1988[24]
Gene mutation	Human fibroblast cell line with shuttle vector plasmid containing *supF* suppressor tRNA gene	0, 0.25, 0.5, 1.0 and 2.0 M	+ (after replication). Mutations were specified as tandem based substitutions (GG→TT); single-strand and double strand DNA mutations increased with increasing dose	2	Matsuda et al. 1998[25]
Gene mutation (6-TG resistant mutations)	Normal human fibroblasts	Concentrations up to 10 mM for 5 hours; positive and negative control included; cell viability tests performed	+ (bell-shaped dose-response relationship); survival at 5 mM was 50%; cells treated with 8 and 10 mM showed delayed recovery of the growth rate.	2	Grafström et al. 1994[26]
Chromosome aberrations	Different DNA-repair deficient Chinese hamster ovary cells	0.3, 0.6, 1.0, 1.8, 2.5 and 3.6 mM for 2 hours; 100 metaphases scored/ group	CA: + (concentration-related increase)	2; no positive control	Mechilli et al. 2008[27]
Chromosome aberration	Primary rat skin fibroblasts	0.1 - 10 mM for 12 and 24 hours; 50 metaphases analysed/ dose	12 hours: - 24 hours: + ($p<0.05$), except lowest dose, concentration-related increase in aneuploidy	3; no positive controls; no data on cytotoxicity	Bird et al. 1982[28]
Chromosome aberration	Chinese hamster embryonic diploid fibroblasts	0, 20, 40 and 60 µg/ ml; -S9	+	3; no data on cytotoxicity; no positive control	Dulout and Furnus 1988[29]
Chromosome aberration	Human peripheral lymphocytes (from 3 healthy volunteers)	0, 0.001 and 0.002 % (v/v); 100 or 200 mitoses scored/ sample	-	3; no positive control; no data on cytotoxicity	Obe et al. 1979[30]
Chromosome aberration	Human peripheral blood lymphocytes	0.02 and 0.04 mg/mL culture medium; no positive control	+	4; abstract only	Badr and Hussain 1977[31]
Micronuclei	Human lymphoblastoid TK6 cells	0.005, 0.01, 0.05, 0.25, 0.5, 1.0, and 2 mM; plates sealed due to volatility substances	+ (dose-related increase); with increasing exposure also the number of apoptotic cells increased	1	Budinsky et al. 2013[20]

Micronuclei	Human lymphoblastoid TK6 cells	8 different concentrations tested, between 0.005 and 4 mM; negative and positive controls included; only data analysed when cytotoxicity was below 55%	+ (0.25, 0.5 and 1.0 mM)	2	ECHA registration data, vitro.002, study report 1979 (echa.europe.eu)
Micronuclei; multi-substance study	Human lympho-cytes isolated from peripheral blood from one healthy non-smoking donor	0, 0.6, 0.8 and 1.0 mM	+ (dose-related increase, $p<0.05$); - (after hybridization with a centromeric DNA probe)	2; optimal doses were assessed determining degree of decrease in bi-/ mononucleated ratio	Migliore et al. 1996[32]
Micronuclei; multi-substance study	HepG2 and Hep3B cells	0, 0.9 and 9 mM for 24 hours; per experimental point 1,500 cells evaluated.	+ (concentrations-related increase)	2; no data on cytotoxicity	Majer et al. 2004[33]
Micronuclei	MCL-5 human lymphoblastoid cell line	0 - 2 % (v/v; a range of 6 differrent concentrations) for 22 hours; > 4,000 cells per dose examined	+ (from 0.4 % onwards, $p<0.05$), dose-dependent increase -: aneuploidy	2; no positive control included	Kayani and Parry 2010[34]
Micronuclei	Primary rat skin fibroblasts	0.1 - 10 mM for 12, 24 or 48 hours; > 1,000 cells analysed/ dose	+ ($p<0.05$; except lowest dose tested)	3; no positive controls; no data on cytotoxicity	Bird et al. 1982[28]
Micronuclei	V79 Chinese hamster cells	0.5 - 10 mM (MN);	+ (dose-dependent increase)	2; No positive control	Speit et al. 2008[35]

[a] + or - S9, with or without metabolic activation system.

[b] Klimisch score is expressed in reliability levels (cited from original publication):

- Reliability 1 (reliably without restriction). For example, guideline study (OECD, etc.); comparable to guideline study; test procedure according to national standards (DIN, etc.).
- Reliability 2 (reliable with restrictions). For example, acceptable, well-documented publication/study report which meets basic scientific principles; basic data given: comparable to guidelines/standards; comparable to guideline study with acceptable restrictions.
- Reliability 3 (not reliable). For example, method not validated; documentation insufficient for assessment; does not meet important criteria of today standard methods; relevant methodological deficiencies; unsuitable test system.
- Reliability 4 (not assignable). For example, only short abstract available; only secondary literature (review, tables, books, etc.).

5.1.2 *In vivo data*

A summary on the in vivo mutagenicity of acetaldehyde is shown in Table 4.

Table 4 Summary of in vivo mutagenicity studies (animal studies).

Method	Animal	Exposure conditions	Results	Klimisch[9] score[a]	References
Somatic cell mutagencicity					
Gene mutation and micronuclei	Wildtype and knock-out mice with inactive ALDH2[b] gene; micronuclei determined in reticulocytes; mutations were determined by T-cell receptor (TCR) gene mutation assay	Inhalation, 125 and 500 ppm vapour, continuously for two weeks; negative control was inhalation of clean air	*Micronuclei*: + in knock-out mice ($p<0.05$); - in wild-type mice. *Mutation* (TCR mutant frequency): + in knock-out mice ($p<0.05$); - in wild-type mice.	2	Kunugita et al. 2008[36]
Gene mutation and micronuclei	Wildtype and knock-out mice with inactive ALDH2 gene; micronuclei determined in reticulocytes; mutations were determined by TCR gene mutation assay	Oral administration, 0 and 100 mg/kg bw, daily, once a day for two weeks; 5 - 10 animals/ group	*Micronuclei*: + in knock-out mice ($p<0.05$); - in wild-type mice. *Mutation* (TCR mutant frequency): + in knock-out mice ($p<0.05$); - in wild-type mice	2	Kunugita et al. 2008[36]
Micronuclei; multi-substance study	Male SD and F344 rats, bone marrow erythrocytes and peripheral blood erythrocytes	Highest dose tested was maximum tolerated dose; at least four animals/group	+ (250 mg/kg bw, intraperitoneal injection, both cell types)	2; only highest dose tested	Wakata et al. 1998[37]
Micronuclei	5 male CD-1 mice	0 - 400 mg/kg bw, Intraperitoneal injection, three dose levels; tests on acute toxicity performed	+ (dose-related increase)	2	Morita et al. 1997[38]
Micronuclei	Male Han rats, 5 animals/group	Single intraperitoneal injection of 125 or 250 mg/kg bw; blood samples collected after 0, 24, 48 and 72 hours	+ (at 24 and 48 hours), dose-related increase; no data at 72 hours due to toxicity	2	Hynes et al. 2002[39]
Chromosomal aberrations	Rat embryos	Single intra-amniotic injection of 7,800 mg/kg bw	+	4; original publication available in Russian only	Bariliak and Kozachuk 1983[40]
Germ cell mutagenicity					
Meiotic micronuclei in spermatids	C57BL/6J x C3H/He mouse early spermatids	125, 250, 375 and 500 mg/kg bw per day, single dose, intraperitoneal injection; 4 animals/ group	- ; survival rate was significantly decreased in highest exposure group	2	Lähdetie 1988[41]

Sex-linked recessive lethal mutations; multi-substance study	*Drosophila melanogaster*	1) Single injection of 22,500 ppm; 2) 25,000 ppm in feed; data presented on mortality and sterility	+ (injection) - (feed)	2	Woodruff et al. 1985[42]

[a] See footnote in Table 3 for explanation of the Klimisch-scores.
[b] ALDH2, aldehyde dehydrogenase 2 family (mitochondrial), converts acetaldehyde into acetate.

Germ cells

Lähdetie (1988) studied the induction of meiotic micronuclei in spermatids of mice.[41] Mice (4 animals per group) were given a single intraperitoneal injection of acetaldehyde at a concentration of 0 (control vehicle), 125, 250, 375 and 500 mg/kg bw. A group of mice served as positive control (cyclophosphamide injection). Thirteen days after treatment the mice were killed to examine the presence of meiotic micronuclei in early spermatids (1,000 spermatids scored per mouse). Compared to the vehicle control, the number of spermatids with micronuclei did not increase after acetaldehyde treatment, whereas in the positive control it did. The author reported that at a dose of 500 mg/kg bw all animals died due to acute toxicity, whereas all survived at lower doses. In a separate experiment, the author also investigated the sperm morphology in mice treated with acetaldehyde for a short period (up to 250 mg/kg bw; 5-day exposure regimen). However, acetaldehyde did not decrease sperm count, testis weight or seminal vesicle weight, nor did it induce abnormal sperm at the doses. The highest administered dose was lethal to half of the animals in the group.

The Committee noted that in a sex-linked recessive lethal mutation assay, acetaldehyde was positive after injection (Woodruff et al. 1985).[42] This shows that the substance induces mutations in germ lines of the insect.

Somatic cells

Kunugita et al. (2008) studied the induction of gene mutations and micronuclei in knock-out mice having an inactive acetaldehyde dehydrogenase (Aldh2, converts acetaldehyde into acetate) gene.[36] Both wildtype and the knockout mice inhaled acetaldehyde at concentrations of 0, 225 or 900 mg/m^3, continuously for two weeks. In addition, groups of mice (5-10 animals per group) were given acetaldehyde orally at doses of 0 or 100 mg/kg bw, once a day for two weeks. Two weeks after the last exposure, all animals were killed and the number of reticulocytes with micronuclei was determined. Also the mutations in the *TCR* gene of T-lymphocytes was measured. Irrespective the route of exposure, in

knockout mice, the number of micronuclei positive cells, and the frequency of *TCR* gene mutations in lymphocytes was statistically significantly increased compared to the respective controls. In wildtype animals, acetaldehyde did not cause any effects on these endpoints. See Table 5 for a summary of the results.

In a well-performed study, Wakata et al. (1998) showed that in bone marrow polychromatic and peripheral blood erythrocytes of SD and F344 rats, micronuclei were induced after exposure to acetaldehyde by a single intraperitoneal injection of 250 mg/kg bw.[37] Bone marrow and blood cells were harvested 24 hours after the treatment. The study included concurrent negative (solvent/vehicle) and positive (cyclophosphamide) controls.

In addition, Morita et al. (1997) reported on acetaldehyde-induced micronuclei in bone marrow polychromatic erythrocytes of male CD-1 mice.[38] Five/six mice received the substance by a single intraperitoneal injection. Dose levels were based on acute toxicity test results. Two different lots were used, because the experiment was performed in two different laboratories. Twenty four hours after injections, bone marrow cells were harvested for the micronucleus assay. In Table 6 a summary of the results is shown.

Table 5 Induction of micronuclei and TCR gene mutations in knockout mice (Kunugita et al 2008).[36]

Exposure route	Exposure level	Micronuclei in reticulocytes	Mutant frequency in T-cell receptor gene
Knock-out mice (Aldh2 -/-)			
Inhalation	0 (control)	-	-
	225 mg/m^3	+ [a]	Not determined
	900 mg/m^3	+ [b/c]	+[b]
Oral administration	0 (control)	-	-
	100 mg/kg bw	+ [b/c]	+ [b/c]
Wildtype mice (Aldh2 +/+)			
Inhalation	0 (control)	-	-
	225 mg/m^3	-	-
	900 mg/m^3	-	-
Oral administration	0 (control)	-	-
	100 mg/kg bw	-	-

[a] Compared to Aldh2 +/+ control mice (p<0.05).
[b] Compared to Aldh2 +/+ control mice (p<0.01).
[c] Compared to Aldh2 -/- control mice (p<0.05).

Table 6 Induction of micronuclei in male CD mice (Morita et al. 1997).[38]

Manufact. lot	LD_{50}	Dose	Percentage of micronuclei in bone marrow cells		
	mg/kg bw	mg/kg bw	mean	SD	*p*-value[a]
Wako	470	0	0.12	0.08	-
		95	0.22	0.15	0.132
		190	0.33	0.10	0.010
		380	0.85	0.21	0.000
Merck	338	0	0.12	0.08	-
		100	0.10	0.07	0.726
		200	0.44	0.11	0.002
		300	0.62	0.16	0.000
		400	1.10	0.25	0.000

[a] *P*-value of pairwise comparisons.

Table 7 Induction of micronuclei in blood cells of rats treated with acetaldehyde (Hynes et al. 2002).[39]

Dose (mg/kg bw)	Time (h)	Laboratory[a]	Mean RET[b] ± SD	Mean MNRET[b] per 20,000 RET ± SD	Mean MNNCE[b] ± SD
0	0	GW	1.29 ± 0.29	0.13 ± 0.06	0.01 ± 0.00
		LL	1.47	0.14	0.01
125	24	GW	0.80 ± 0.12	0.21 ± 0.07	0.01 ± 0.00
		LL	0.91	0.19	0.01
	48	GW	1.32 ± 0.21	0.30 ± 0.09	0.01 ± 0.00
		LL	1.37	0.19	0.01
	72	GW	1.82 ± 0.18	0.14 ± 0.05	0.01 ± 0.00
		LL	1.65	0.18	0.01
250	24	GW	1.00 ± 0.42	0.28 ± 0.07	0.02 ± 0.01
		LL	0.99	0.32	0.01
	48	GW	1.31 ± 0.25	0.33 ± 0.11	0.02 ± 0.01
		LL	1.14	0.39	0.01
	72	GW	1.90 ± 0.42	0.14 ± 0.05	0.01 ± 0.01
		LL	1.42	0.16	0.01

[a] GW, GlaxoWellcome; LL, Litron Laboratories.

[b] RET, reticulocytes; MNRET, micronucleated reticulocytes; MNNCE, micronucleated monochromatic erythrocytes. No data on statistical significance presented.

Hynes et al. (2002) exposed male Wistar Han rats (5 animals per group) to acetaldehyde by a single intraperitoneal injection of 125 or 250 mg/kg bw.[39] For micronuclei testing, peripheral blood cells were harvested 0, 24, 48 and 72 hours after the injection. Micronuclei were scored by flow cytometric analysis. The study included negative (vehicle) and positive (cyclophosphamide) controls. Acetaldehyde at a dose of 250 mg/kg bw induced micronuclei, with maximum increases at 48 hours (see Table 7).

5.2 Human information

Table 8 summarizes a few studies performed on humans, in which effects were related to acetaldehyde. All volunteers were alcohol abusers or smokers.

Table 8 Summary of human studies.

Method	Population	Cells	Results and remarks	Quality and/or reliability of study	References
DNA-adducts (^{32}P-postlabelling)	Alcohol abusers (n=24) and controls (n=12)	Peripheral white blood cells (granulocytes and lymphocytes)	+ in alcohol abusers compared to controls ($p<0.001$). Average adduct levels (adducts /10^7 nucleotides): - abusers: 3.4 ± 3.8 - controls: 2.1 ± 0.8	Reliability low in that subjects in the alcoholic group were heavy smokers; in control group one moderate smoker.	Fang and Vaca 1997[43]
DNA-adducts	Cancer-free male Japanese alcoholic patients with different acetaldehyde dehydrogenase (ALDH) genotypes	Peripheral white blood cells	+, adduct level was significantly higher in alcoholics with ALDH2*1*2 genotype compared to alcoholics with ALDH2*1*1 genotype.	Past exposure to ethanol; no non-alcoholic healthy controls included	Matsuda et al. 2006[44]
Acetaldehyde specific DNA-adducts (N^2-ethylidene-deoxiguanosine)	Smokers, before and after smoking cessation	Leucocytes	Decrease in number of adducts after cessation. Note: cigarette smoke contains acetalde-hyde, but also other potential carcinogens.	Reliability low, because of smoking history participants and co-exposure	Chen et al. 2007[45]

5.3 Other relevant information

In the Tables 9 and 10 data are shown on the DNA damaging and genotoxic (other than mutagenicity) properties of acetaldehyde.

Table 9 Summary of other information on DNA damage.

Method	Cell type	Concentration	Results	Klimisch[9] score[a]	References
In vivo studies					
DNA-protein crosslinks	Male Fischer-344 rats; DNA-protein cross-links studied in nasal respiratory mucosa and olfactory cells	1) Inhalation; 100, 300, 1,000 and 3,000 ppm; single 6-hour exposure 2) inhalation; 1,000 ppm; 6-hours/day, daily, 5-days samples of three rats were combined	1) + (respiratory mucosa; dose-dependent increase, $p<0.05$); - (olfactory mucosa) 2) + (respiratory mucosa); + (olfactory mucosa, $p<0.05$)	2	Lam et al. 1986[46]

In vitro tests using human cells

DNA single and double strand breaks	Human lymphocytes from two healthy donors	0, 1.56, 6.25, 25 and 100 mM for one hour; for each dose 50 cells were analysed from each subject	+ (single strand breaks at all exposures) + (double strand breaks at 100mM only) Authors reported that > 80% of cells were not viable after exposure to 100 mM for 2 hours	2; no positive control	Singh and Khan 1995[47]
Comet assay[b]	Human peripheral blood lymphocytes	3, 10, 30 and 100 mM for one hour; doses were based on cytotoxicity data	+ (dose-dependent)	2	Blasiak et al. 1999[48]
Comet assay[a]	Human lymphocytes, gastric and colonic mucosa cells	3 mM (lympho-cytes), 100 mM (gastric and colonic mucosa cells)	+ No differences were noted among the different cell types; viability was over 70% at the tested doses	2; one dose tested only	Blasiak et al. 2000[49]
Comet assay[a]	Human bronchial epithelial cells	Exposure to 3, 10, 30 and 100 mM for 1 hour in thiol free medium	+, dose-dependent effects - for single strand breaks	2	Grafström et al. 1994[26]
DNA-adducts	DNA form primary human liver cells, samples from normal liver	Incubation of cells with 5.7 mM [$^{13}C_2$]acetaldehyde; 12 liver samples analysed	+ (N^2-ethyl-deoxiguanosine adducts)	3	Wang et al. 2006[50]
Alkaline elution assay[a]	Human lymphocytes	10 - 20 mM for 4 hours	+, DNA cross-links - ,DNA strand-breaks	3; No data on cytotoxicity; no positive controls	Lambert et al. 1985[51]
Alkaline elution assay[a]; multi-substance study	Normal human bronchial epithelial cells and humane leucocytes	1 mM for 1 hour	- (without metabolic activation); at 1 mM no significant growth reduction noted	3; only one concentratio n used	Saladino et al. 1985[52]
Alkaline elution assay[a]	Human bronchial epithelial cells	10 mM for 1 hour	-	3; only one dose tested; no data on con-trols; 10 mM acetaldehyd e induced 50% cytotoxicity	Grafström et al. 1986[53]
DNA-protein crosslinks	EBV-transformed human Burkitt's lymphoma cells (EBV, Epstein Barr virus)	0.035, 0.175, 0.875, 3.5 and 17.5 mM for 2 hours; Maximum tolerated dose was 17.5 mM	+ (> 5 mM, $p<0.05$)	2	Costa et al. 1997[54]
DNA-adducts	normal epithelial cells, and SV40T antigen-immortalized human buccal epithelial cells	1-100 mM for one hour; ^{32}P-postlabeling assay	+ (N^2-ethyl-3'-dG-monophosphate adducts, dose-dependent	2	Vaca et al. 1998[55]

In vitro tests using rodent cells					
Comet assay[a]	V79 Chinese hamster cells	0.2 - 20 mM	-; authors reported more than 50% reduction of cell viability at 20 mM	2; no positive control	Speit et al. 2008[35]
Alkaline elution assay[a]	Chinese hamster ovary cells (K1 cells)	0.5, 1.5 and 4.5 mM for 90 minutes	- (strand breaks); + (crosslinks); cell viability > 80%	2; no positive control	Marinari et al. 1984[56]
Alkaline elution assay[a]; multi-substance study	Primary rat hepatocytes	0.03, 0.3 and 3 mM for 3 hours; cytotoxicity < 55%	-	3	Sina et al. 1983[57]
Other test systems					
DNA-adducts	Calf thymus DNA	1 M for 30 minutes at 37 °C; negative control included	+ (without metabolic activation)	3; only one concentration tested	Ristow and Obe 1978[58]
DNA-adducts	Calf thymus DNA	0.01-40 mM for 20 to 96 hours	+ (mainly N^2-ethylidene-deoxi-guanosine DNA-adducts, but also (< 10%) 1,N-propano-deoxi-guanosine, N^2-dimethyldioxane-deoxiguanosine, and a cross-link adduct detected).	2	Wang et al. 2000[59]
DNA-adducts	Calf thymus DNA	1.8 mM for 92 hours; ^{32}P-postlabeling assay	+ (N^2-ethyl-3'-dG-monophosphate adducts)	3	Fang and Vaca 1995[60]
DNA-adducts	Calf thymus DNA in 2'-deoxy-guanosine-3'-monophosphate	Up to 79,000 µg/ml	+	3	Fang and Vaca 1997[43]
DNA-protein crosslinks	Calf thymus DNA in 2'-deoxy-guanosine-3'-monophosphate	100, 300 and 1,000 mM for one hour	+	3	Lam et al. 1986[46]
Alkaline elution assay[a]	*Saccharomyces cerevisiae* (yeast)	0.85 M for 2 or 4 hours	+	3; no positive control; no data on statistical analysis	Ristow et al. 1995[61]
DNA repair host-mediated assay, in vivo; multi-substance study	repair-deficient *E.coli* K-12 *uvrB/recA*; tests performed in mice	Highest tested concentration 370 mM/L; - and + S9	- (- and + S9)	3; method not validated	Hellmer and Bolcsfoldi 1992[62]

[a] See footnote in Table 3 for explanation of the Klimisch-scores.
[b] Comet assay and alkaline elution assay: DNA single and double strand breaks, DNA cross-links.

Table 10 Summary of genotoxicity studies.

Method	Cell type	Concentration	Results and remarks	Klimisch[9] Score[a]	References
Mammalian cells (in vitro tests)					
Sister chromatid exchange	Different DNA-repair deficient Chinese hamster ovary cells	0.3, 0.6, 1.0, 1.8, 2.5 and 3.6 mM for 2 hours; 250 metaphases scored/ group	+	2; no positive control	Mechilli et al. 2008[27]
Sister chromatid exchange	Chinese hamster ovary cells	0, 30, 100 and 300 µM; -S9	+ (dose-dependent increase	2	Brambilla et al. 1986[63]
Sister chromatid exchange	V79 Chinese hamster cells	0.2 - 5 mM	+ (dose-dependent increase)	2; No positive control	Speit et al. 2008[35]
Sister chromatid exchange	Chinese hamster ovary cells	0, 0.8, 2, 4, 7.8, 39.4 and 78 µg/ml; + and - S9; 20 metaphases/sample scored	+, dose-related response	3; no data on cytotoxicity; no positive control	de Raat et al. 1983[64]
Sister chromatid exchange	Chinese hamster ovary cells	$0.25x10^{-3}$, $0.5x10^{-3}$, $1x10^{-3}$, and $1.5x10^{-3}$ % (v/v); - S9; 100 mitoses scored/ sample	+	3; no positive controls, no data on cytotoxicity	Obe et al. 1979[65]
Sister chromatid exchange	Human peripheral lymphocytes	0 - 1,080 µM; -S9; reduction of cell growth noted above 720 µM	+, dose-related response	2; no positive controls	Böhlke et al. 1983[66]
Sister chromatid exchange	Human peripheral lymphocytes	1 - 100 µM	+	2; no positive controls	Knadle 1985[67]
Sister chromatid exchange	Human lymphocytes and fibroblast of normal subjects	40, 400 and 800 µM;	+	3; limited information on test protocol	Véghelyi and Osztovics 1978[68]
Sister chromatid exchange	Human lymphocytes	0, 63, 125, 250 500 and 2,000 µM; -S9	+ (dose-dependent increase)	3; no positive controls; no data on cytotoxicity	Norppa et al. 1985[69]
Sister chromatid exchange	Human lymphocytes	0, 0.0005, 0.001, and 0.002 % (v/v); -S9	+, dose-related response	3; no positive controls; no data on cytotoxicity	Ristow and Obe 1978[58]
Sister chromatid exchange	Human lymphocytes	0 - 500 µM; - S9	+, dose-related response	3; no data on cytotoxicity; no positive controls	Sipi et al. 1992[70]
Sister chromatid exchange	Human peripheral lymphocytes	100 - 400 µM; - S9; exposure performed in capped bottles	+ (dose-dependent increase)	3; no positive controls; no data on cytotoxicity	Helander and Lindahl-Kiessling 1991[71]
Sister chromatid exchange	Human peripheral lymphocytes	$2x10^{-3}$ % (v/v); + or - acetaldehyde metabolizing enzyme ALDH	+	3; no positive controls, no data on cytotoxicity	Obe et al. 1986[72]
Sister chromatid exchange	Human lymphocytes	100 - 2,400 µM; - S9	+ (dose-dependent increase	3; no positive controls used, no data on cytotoxicity	He and Lambert 1985[73]

Sister chromatid exchange	Human peripheral lymphocytes	0 - 0.001% (v/v); -S9	+ (dose-dependent increase)	3; limited information on test protocol	Jansson 1982[74]
Rodents (in vivo somatic cell tests)					
Sister chromatid exchange	Bone-marrow cells of Chinese hamsters (strain not specified)	Single intra-peritoneal injection of 0.01, 0.1 and 0.5 mg/kg bw; 6-7 animals/ dose; negative and positive control included	+ at the highest exposure level only; at this level signs of intoxica-tion were noted; no signs of intoxication at 0.1 and 0.01 mg/kg bw	2	Korte et al. 1981[75]
Sister chromatid exchange	Male mouse (NIH) bone marrow cells	0.4, 4.0, 40 and 400 mg/ kg bw, single intraperitoneal injection	+ (40 and 400 mg/kg bw, $p<0.05$) Mitotic index and average generation time did not differ from control	3; number of mice per group not given; no positive control	Torres-Bezauri et al. 2002[76]
Sister chromatid exchange	Male CBA mouse	Single intraperi-toneal injection of 1 or 0.5 mL of a 10^{-4} % (v/v) solution; one animal/ dose	+	3; low number of animals in study, no positive controls	Obe et al. 1979[30]
Rodents (in vivo germ cell tests)					
Sister chromatid exchange	Mouse spermatogonial cells	Single intraperitoneal injection; 0.4, 4.0, 40 and 400 mg/kg bw; 4 - 5 animals/concentration; cells were isolated, 53 h after injection.	+ (all doses applied, $p<0.05$); no clear exposure-response relationship observed	2; authors did test for intoxication; concentrations used were considered non-toxic/-lethal	Madrigal-Bujaidar et al. 2002[77]

[a] See footnote in Table 3 for explanation of the Klimisch-scores.

Germ cells

Madrigal-Bujaidar et al. (2002) injected NIH mice (4-5 mice per group) with acetaldehyde at concentrations of 0 (vehicle control), 0.4, 4, 40 and 400 mg/kg bw (single treatment), or cyclophosphamide (positive control).[77] Fifty-three hours later, the animals were killed, and the tunica albuginea was removed from each testes to obtain spermatogonial cells in the seminiferous tubules. A statistically significant increase in the number of cells with sister chromatid exchange was reported (30 metaphases per mouse scored; see Table 11). The authors determined a LD_{50}-dose of 560 mg/kg bw.

Somatic cells

Lam et al. (1986) reported on the formation of DNA-protein crosslinks in the nose tissue of male Fischer-344 rats after inhalation exposure.[46] The animals

were exposed to acetaldehyde at concentrations of 0,180, 540, 1,800 and 5,400 mg/m^3 for a single six hours, or to 5,400 mg/m^3, 6 hours a day for 5 consecutive days. Immediately after the final exposure the animals were killed, and nasal respiratory mucosa was obtained for further examination. After a single inhalation, a dose dependent increase in DNA-protein crosslinks was observed in the respiratory mucosa, but not in the olfactory mucosa. Short-term repeated inhalation induced DNA-protein crosslinks in the respiratory and the olfactory mucosa.

In bone marrow cells of Chinese hamsters (6-7 animals per group), a single intraperitoneal injection of acetaldehyde increased the number of sister chromatid exchanges at the two highest doses applied (0.1 and 0.5 mg/kg bw; Korte et al., 1981).[75] The authors reported that exposure to concentrations of 0.6 mg/kg bw and higher was lethal.

Table 11 Sister chromatid exchanges in spermatogonial cells of mice treated with acetaldehyde (Madrigal-Bujaidar et al. 2002).[77]

Dose (mg/kg bw)	SCE/cell ± SD[a]	SCE increase
0	1.9 ± 0.16	
0.4	2.9 ± 0.33[b]	1.1
4	4.1 ± 0.34[b]	2.2
40	4.6 ± 0.51[b]	2.7
400	5.1 ± 0.8[b]	3.2
50 (cyclophosphamide)	6.0 ± 0.1[b]	4.1

[a] SCE, sister chromatid exchange.
[b] Statistically significant different compared to control, $p < 0.05$.

5.4 Summary and discussion of mutagenicity

Below, only data are summarized of reliable (with or without restrictions) experimental design (according to the Klimisch criteria (1997)).[9]

Germ cell genotoxicity

The Committee found two animal studies on germ cell genotoxicity by acetaldehyde. The first is the study by Lähdetie et al. (1988), in which a single intraperitoneal injection of acetaldehyde did not induce meiotic micronuclei in early spermatids nor sperm abnormalities.[41] The second study is published by Mardigal-Bujaidar et al. (2002), and considers the induction of sister chromatid exchanges in mouse spermatogonial cells.[77] 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.

Mutagenicity in bacteria and mammalian cells

Numerous data have been presented on the mutagenic properties of acetaldehyde in bacteria, mammalian cells (other than germ cells) and rodents (see Tables 3 and 4). Overall, negative outcomes were found in bacteria using the reverse mutation assay, whereas positive outcomes (gene mutations, chromosome aberrations) were reported in mammalian cells in vitro, and in rodents in vivo (gene mutation and micronuclei in blood cells). In part of these positive studies also a dose-related response was found. Based on these findings, the Committee concludes that acetaldehyde has mutagenic properties in at least somatic mammalian cells in vitro and in vivo.

DNA damage and cytogenicity

In addition to mutagenicity testing, various studies have been performed showing that acetaldehyde induced DNA damage (DNA-crosslinks, DNA-adducts, and DNA strand breaks) (see Table 9) in vivo and in vitro. Together with data on mutagenicity, these data indicate that acetaldehyde may damage DNA directly. Therefore, the Committee is of the opinion that acetaldehyde acts by a stochastic genotoxic mechanism. Data on human volunteers are limited, since factors like alcohol (ab)use and smoking may have influenced the outcomes (see Table 8).

Numerous data have been presented on the induction of sister chromatid exchanges by acetaldehyde using in vitro, and to a lesser extent, in vivo test systems. In most of these studies acetaldehyde scored positive, and in some of these studies also a dose-related response was found. Based on these findings, the Committee concludes that acetaldehyde induces cytogenetic effects.

5.5 Comparison with criteria

According to the criteria in Annex VI of the European regulation No. 1272/2008 (see Annex G), classification as a mutagen in category 1 is warranted when positive evidence for *in vivo heritable germ cell* mutagenicity in humans (1A) or mammals (1B) has been reported. No data have been presented on human germ cell mutagenicity, and the only animal germ cell mutagenicity study did not show

mutagenic activity (Lähdetie et al., 1988).[41] Overall, due to a lack of data the Committee concludes that there is no positive direct evidence for in vivo heritable germ cell mutagenicity of acetaldehyde.

In addition, substances may be categorized in 1B if there are

positive results from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells.

The latter may be based on a)

supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo

or b)

by demonstrating the ability of the substance or its metabolites to interact with the genetic material of germ cells

(see Annex G). Sufficient evidence has been found for in vivo mutagenicity testing in somatic cells of mammals. 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.[77] 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 (see Chapter 4). However, in another animal study no abnormal sperm cells, and no meiotic micronuclei in spermatids were observed at dose levels inducing acute toxicity (Lähdetie et al. 1988).[41]

Overall, the Committee is of the opinion that some evidence exists that acetaldehyde has potential to cause mutations in germ cells. Therefore, it recommends classifying the substance in category 1B.

5.6 Conclusions on classification and labelling

Based on the available data, the Committee recommends classifying acetaldehyde as a germ cell mutagen in category 1B (substance to be regarded as if they induce heritable mutations in the germ cells of humans).

The Committee is furthermore of the opinion that acetaldehyde acts by a stochastic genotoxic mechanism.

Chapter 6

Carcinogenicity

6.1 Non-human information

Data on animal carcinogenicity studies are summarized in Table 12.

Table 12 Summary of animal carcinogenicity studies on acetaldehyde exposure.

Species	Design	Exposure levels	Observations and remark	References
Oral administration				
Rats, Sprague Dawley	50 animals/sex/group; animals kept in observation until spontaneous death (last animal died in week 161); gross necroscopy and histopathological examinations.	0 - 50 - 250 - 500 - 1,500 - 2,500 mg acetaldehyde/ L drinking water (*ad libitum*; dose in kg/kg bw not given).	*Klimisch-score*: 2. *General*: No difference between control and exposed animals on consumption, body weight and survival. *Lesions*: Number of malignant tumour-bearing animals did not differ significantly from controls; Number of tumours per 100 animals was statistically significantly increased at 50 (females only), and at 2,500 mg/L (males – female – both sexes, $*p<0.05$): - 0 mg/L: 34% - 46% - 40% - 50 mg/L: 52% - 82%* - 67% - 2,500 mg/L: 66%*- 78%*- 72%. *Remark*: The EFSA noted that the animals may have been infected with *mycoplasma pulmonis*. Therefore, DECOS considers the study of questionable relevance.	Soffritti et al., 2002[78]

Rats, Wistar	10 male animals/ group; study duration 8 months; immuno-histochemistry and histopathological examination of the tongue, epiglottis, and forestomach; no other tissue examined.	0 or 120 mM in drinking water (*ad libitum*; dose in kg/kg bw not given).	*Klimisch-score*: 3 (only one dose used, short exposure period, limited examination of tissues). *General*: No difference between control and exposed animals on consumption, body weight and survival. *Lesions*: No cancerous or dysplastic lesions observed. Microscopic examination revealed hyperplasia in basal layers of squamous epithelia in the examined tissues of exposed animals.	Homann et al., 1997[79]
Inhalation				
Rats, Wistar	105 animals/sex/ group; six hours/day, five days/ week for 28 months; gross necroscopy and histopathological examination.	0 - 1,350 - 2,700 - 5,400 mg/m^3; due to toxicity, the highest exposure level was reduced to 1,800 mg/m^3 over a period of 11 months.	*Klimisch-score*: 2. *General*: lower survival and body weights were observed in exposed animals compared to controls. *Lesions*: exposure induced malignant tumour in the respiratory tract. See main text and Table 13. *Note*: only the respiratory tract was examined for the presence of abnormalities.	Woutersen et al., 1986[80]
Hamster, Syrian golden	36 animals/sex/group; seven hours/day, five days/week for 52 weeks, week 53-81, post-exposure period; gross necroscopy and histopathological examination; 6 animals/ sex were killed for interim examination.	4,500 mg/m^3 (week 1-9), 4,050 mg/m^3 (week 10-20), 3,600 mg/m^3 (week 21-29), 3,240 mg/m^3 (week 30-44) and 2,970 mg/m^3 (week 45-52); due to considerable growth retardation and to avoid early death, exposures were reduced gradually during experiment.	*Klimisch-score*: 2 (no standard procedure of doses applied). *General*: from week 4 onwards, exposed animals showed significant reduced body weight compared to controls; reduction diminished partly in the post-exposure period. *Lesions*: exposure induced rhinitis, hyperplasia and metaplasia in the nasal, laryngeal and tracheal epithelium. Also laryngeal and nasal carcinomas and polyps were observed; respiratory tract tumours: 0/30 - 8/29 (male, control-exposed) 0/28 - 5/29 (female, control-exposed)	Feron et al., 1982[81]
Hamster, Syrian golden	35 animals/group (males only); 7 hours/day, five days/week for 52 weeks, animals killed after 78 weeks; at week 52, 5 animals were killed for interim examination; gross necroscopy and histopathological examination.	0 or 2,700 mg/m^3	*Klimisch-score*: 2 (only one sex used, only one dose applied). *General*: in exposed animals, body weights were slightly lower than in controls. In the last part of the exposure period mortality increased more rapidly in exposed animals than in controls. *Lesions*: no substance-related tumours found. Acetaldehyde induced hyperplastic, metaplastic and inflammatory changes. *Note*: exposure level may have been too low to induce adverse health effects.	Feron et al., 1979[82]

Dermal exposure				
Rats	14 to 20 animals; subcutaneous injection.	(Total) dose not known; repeated injections.	*Klimisch-score*: 4 (data from secondary source; original study in Japanese; no abstract available)) *General*: no data. *Lesions*: spindle-cell sarcomas at site of injections (in four animals that survived the period up to 554 days).	Watanabe and Sugimoto 1956[83]
Intratracheal installation				
Hamsters, Syrian golden	35 animals/sex/group; weekly installations for 52 weeks, experiment was terminated at week 104.	0 or 2% acetaldehyde (installation volume, 0.2 mL).	*Klimisch-score*: 3 (only one dose applied; experiment not performed according to today's standard methods). *General*: no clear effects on body weight or mortality. *Lesions*: No substance-related tumours found. Hyperplastic and inflammatory changes observed in the bronchioalveolar region of exposed animals.	Feron et al., 1979[82]

6.1.1 Carcinogenicity: oral administration

Male and female Sprague-Dawley rats (50 animals/sex/group) were exposed to 0, 50, 250, 500, 1,500 and 2,500 mg/L acetaldehyde in drinking water (dose in kg bw not given), beginning at six weeks of age (Soffritti et al., 2002).[78] Animals were kept under observation until spontaneous death. In various organs and tissues neoplastic lesions were observed. However, no clear increase in number of tumour-bearing animals was found in any of the exposed groups compared to the control group. The investigators reported a significantly increased total number of tumours (per 100 animals) in groups exposed to 50 mg/L (females only), and 2,500 mg/L (males; females). The Committee noted the lack of statistical analysis, and the limited examination of non-neoplastic end-points. Furthermore, the European Food Safety Authority (EFSA) has evaluated the studies performed by the European Ramazzi Foundation of Oncology and Environmental Sciences, who performed this study, and noted that the animals used by this foundation, may have been infected with Mycoplasma pulmonis. This may have resulted in chronic inflammatory changes.[84] For these reasons, the Committee considers the findings of the study of questionable relevance.

Homann et al. (1997) have given male Wistar rats (N=10/group) either water containing acetaldehyde (120 mM) or tap water to drink for eight months.[79] Animals were then sacrificed, and of each animal tissue samples were taken from the tongue, epiglottis, and forestomach. No tumours were observed. However, in these organs, microscopic examination revealed statistically significant

hyperplasia of the basal layers of squamous epithelia in rats receiving acetaldehyde (compared to controls). Furthermore, in the three organs of the treated animals, cell proliferation was significantly increased, and the epithelia were significantly more hyperplastic, than in control animals.

6.1.2 *Carcinogenicity: inhalation*

In a carcinogenicity study by Woutersen et al. (1986), Wistar rats (105 animals/sex/group) inhaled acetaldehyde at a concentration of 0, 750, 1,500 or 3,000 ppm (0, 1,350, 2,700 or 5,400 mg/m^3) for six hours a day, five days per week for a maximum of 28 months.[80] The highest exposure level was reduced progressively over a period of eleven months to 1,000 ppm (1,800 mg/m^3) due to toxicity. The study focussed on lesions in the respiratory tract.

In general, animals exposed to acetaldehyde showed lower survival rates and body weights compared to controls. This was most pronounced in males exposed to the highest concentration of acetaldehyde. Gross examination at autopsy did not reveal acetaldehyde-related lesions, except for decolourisation of the fur and nasal swellings in all exposed groups. Microscopic examination revealed several non-neoplastic lesions in the respiratory tract of males and females, such as: hyperplasia in the respiratory nasal and olfactory epithelium; squamous metaplasia in the respiratory nasal epithelium; and, squamous metaplasia/hyperplasia in the larynx. These lesions were mainly noted in the mid and/or high exposure groups, and were statistically significantly increased compared to controls. No lesions were found in the lungs.

In the nose, also exposure-related neoplastic lesions were observed (see Table 13). It concerned squamous cell carcinoma in the respiratory epithelium of the nose, and adenocarcinomas in the olfactory epithelium. The relative lower tumour incidences in the high exposure groups were explained by the investigators by early mortality due to other causes than cancer. According to the authors, the observations support the hypothesis that nasal tumours arise from degeneration of the nasal epithelium. The same research group reported earlier on degeneration of the olfactory epithelium in rats inhaling acetaldehyde for four weeks, under comparable experimental conditions (Appelman et al., 1986).[85]

In a separate publication, the same authors reported on the interim results obtained in the first 15 month of the study (Woutersen et al. 1984).[86] In short, nasal lesion were reported in exposed animals, indicating chronic and permanent inflammation.

In a study by Feron et al. (1982), Syrian golden hamsters (n=36/sex/group) inhaled decreasing concentrations of acetaldehyde (from 2,500 ppm to 1,650

ppm (equal to 4,500 to 2,970 mg/m^3)) or clean room air, for seven hours a day, five days per week for 52 weeks.[81] The concentrations were reduced during the study because of considerable growth retardation and to avoid early death. Acetaldehyde induced rhinitis, hyperplasia and metaplasia of the nasal, laryngeal and tracheal epithelium. The exposed animals also developed laryngeal carcinomas with a few laryngeal polyps, and nasal polyps and carcinomas. The incidences of respiratory tract tumours were 0/30 (males, control), 8/29 (males, exposed), 0/28 (females, control) and 5/29 (females, exposed) (see Table 14). According to the Committee, the study by Feron et al. supports the findings of the carcinogenicity study by Woutersen et al. (1986) with rats.

Male Syrian golden hamsters (n=35/group) were exposed to 1,500 ppm (2,700 mg/m^3) acetaldehyde combined with weekly intratracheal instillations of benzo[a]pyrene (0.0625, 0.125, 0.25, 0.5 or 1 mg/kg bw) (Feron et al., 1979).[82] The exposure was for seven hours a day, five days per week for 52 weeks. No tumours were found in hamsters exposed to acetaldehyde alone, whereas in animals treated with benzo[a]pyrene alone, or with a combination of acetaldehyde and benzo[a]pyrene, a dose-related increase in respiratory-tract tumours were found.

Table 13 Respiratory tract tumour incidences in rats, which were exposed by inhalation to acetaldehyde for 28 months.[80]

Exposure level (ppm)	0	750	1,500	3,000-1,000
Male animals				
Nose:				
Papilloma	0/49	0/52	0/53	0/49
Squamous cell carcinoma	1/49	1/52	10/53[a]	15/49[b]
Carcinoma in situ	0/49	0/52	0/53	1/49
Adenocarcinoma	0/49	16/52[b]	31/53[b]	21/49[b]
Larynx: carcinoma in situ	0/50	0/50	0/51	0/47
Lungs: poorly differentiated adenocarcinoma	0/55	0/54	0/55	0/52
Female animals				
Nose:				
Papilloma	0/50	1/48	0/53	0/53
Squamous cell carcinoma	0/50	0/48	5/53	17/53[b]
Carcinoma in situ	0/50	0/48	3/53	5/53
Adenocarcinoma	0/50	6/48[a]	26/53[b]	21/53[b]
Larynx: carcinoma in situ	0/51	0/46	1/47	0/49
Lungs: poorly differentiated adenocarcinoma	0/53	1/52	0/54	0/54

[a] Fischer exact test: $p<0.05$.
[b] Fischer exact test: $p<0.001$.

Table 14 Respiratory tract tumour incidences in hamsters, which were exposed by inhalation to acetaldehyde for 52 weeks (Feron et al., 1982).[81]

	Incidence of tumours: males		Incidence of tumours: females	
	Control	Acetaldehyde	Control	Acetaldehyde
Nose				
Adenoma	0/24	1/27	0/23	0/26
Adenocarcinoma	0/24	0/27	0/23	1/26
Anaplastic carcinoma	0/24	1/27	-	-
Larynx				
Polyp/papilloma	0/20	1/23	0/22	1/20
Carcinoma in situ	0/20	3/23	0/22	0/20
Squamous cell carcinoma	0/20	2/23	0/22	1/20
Adeno-squamous cell carcinoma	-	-	0/22	2/20
Total	0/30	8/29[a]	0/28	5/29

[a] Statistical significance (Fisher's exacttest).

6.1.3 *Carcinogenicity: dermal exposure*

Watanabe et al. (1956) reported on the induction of sarcomas in rats given acetaldehyde by subcutaneous injections.[83] The Committee noted the limited study design, such as the small number of animals and the lack of a control group.

6.1.4 *Carcinogenicity: other routes of exposure*

No tumours were found in Syrian golden hamsters (n=35/sex/dose), which were given acetaldehyde by intratracheal installations, weekly or biweekly, for 52 weeks, followed by a recovery period for another 52 weeks (Feron et al., 1979).[82] Doses applied were 0.2 mL of 2% or 4% solutions. In positive controls, which were given benzo[a]pyrene and N-nitrosodiethylamine, a variety of tumours in the respiratory tract were found.

6.2 Human information

No human studies addressing the carcinogenicity of acetaldehyde alone have been retrieved from public literature.

In East-Germany, nine cancer cases were found in a factory where the main process was dimerization of acetaldehyde, and where the main exposures were to acetaldol, acetaldehyde, butyraldehyde, crotonaldehyde and other higher, condensed aldehydes, as well as to traces of acrolein.[87,88] Of these 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 observed in the population of East-Germany. A matched control group was not included. The Committee noted the combined exposure with other potential carcinogenic substances, the small number of cases, and the poorly defined exposed population.

6.3 Other relevant information

6.3.1 Alcohol consumption

Regarding the general population, some investigators suggest a role for acetaldehyde in cancer development (and other disorders) in humans after alcohol consumption, in particular in people with a genetic predisposition of one of the enzymes that are involved in ethanol metabolism.[3,4,89-95] Acetaldehyde is the major metabolite of ethanol (ethyl alcohol).[3,92,96-98] First, ethanol is oxidized by alcohol dehydrogenase (ADH) to acetaldehyde, and subsequently acetaldehyde is converted by aldehyde dehydrogenase (ALDH2) to acetate. Both enzymes show genetic polymorphisms. This means that depending on the genotype, the enzymes may lead to a faster breakdown of ethanol to acetaldehyde, and/or to a slower breakdown of acetaldehyde to acetate. Thus, people having unfavourable genotypes of these enzymes are likely to be exposed internally to higher levels of acetaldehyde after alcohol consumption than would be the case when not having one of these isoenzymes. This would increase the susceptibility to cancer development after alcohol consumption, since it is suggested that acetaldehyde possesses carcinogenic properties.

Several studies reported on the association between genetic polymorphism and ethanol-related cancer development, all suggesting a role for acetaldehyde. As a result, a few meta-analyses have been performed to get more clarity. For instance, Chang et al. (2012) performed a meta-analysis to study the association between ADH1B* and ADH1C genotypes in head and neck cancer risk.[99] The analysis included twenty-nine studies. According to the authors, having at least one of the fast alleles ADH1B*2 or ADH1C*1 reduced the risk for head and neck cancer (odds ratios: 0.50 (95% confidence interval (CI), 0.37-0.68) for ADH1B*2; 0.87 (95%CI, 0.76-0.99).

Wang et al. (2012) performed a meta-analysis to derive a more precise estimate of the relationship between ADH1C genotypes, and breast cancer risk.[100] Twelve case-control studies were included in the analysis, covering 6,159 cases and 5,732 controls (all Caucasians). The investigators did not find any significantly increased breast cancer risk that could be related to any ADH1C genotype.

Boccia et al. (2009) reported on a meta-analysis to study the relationship between ALDH2 homozygous and heterozygous genotypes, alcohol consumption, and head and neck cancer.[101] The analysis included six case-control studies, covering 945 Japanese cases and 2,917 controls. For the analysis, the investigators used a Mendelian randomization approach. The homozygous genotype ALDH2*2*2 (unable to metabolize acetaldehyde) reduced the risk of head and neck cancer, whereas the heterozygous genotype ALDH2*1*2 (partly able to metabolize acetaldehyde) did significantly increase the risk compared to the homozygous ALDH2*1*1 genotype (able to metabolize acetaldehyde). According to the authors, the reduction of cancer risk in ALDH2*2*2 was most likely explained by the fact that people having this genotype consumed markedly lower levels of alcohol compared to the other genotypes, probably due to discomfort. Therefore, the authors conclude that their study supports the hypothesis that alcohol increases head and neck cancer risk through the carcinogenic action of acetaldehyde.

The same results were obtained by Fang et al. (2011), who carried out a meta-analysis of ALDH2 genotypes and esophageal cancer development.[102] Data from sixteen studies (hospital- or population-based, one multicenter study) were analysed, covering 2,697 Asian cases and 6,344 controls. The analysis showed that the heterozygous ALDH2*1*2 genotype increased the risk of esophageal cancer, whereas the homozygous ALDH2*2*2 genotype reduced the risk.

Yokoyama and Omori (2005) reviewed a number of case-control studies (including those performed by themselves) on the relationship of genetic polymorphism of ADH1B, ADH1C and ALDH2 genotypes and esophageal, and head and neck cancer risk.[103] They found positive associations between the less-active ADH1B*1 genotype and inactive heterozygous ALDH2*1*2 genotype,

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* ADH has seven isoenzymes, which are divided into five classes. Most relevant for alcohol metabolism in the liver of adults are the class one isoenzymes ADH1B and ADH1C (formerly known as ADH2 and ADH3 isoenzymes).[99] For each isoenzyme two or three different alleles are known, leading to different genotypes and thus to functional polymorphism. The genotypes of the isoenzyme ADH1B are expressed as ADH1B*1, ADH1B*2 and ADH1B*3; those for the isoenzyme ADH1C are expressed as ADH1C*1 and ADH1C*2. The metabolic speed is highest for homozygote genotypes ADH1B*2, ADH1B*3 and ADH1C*1. ADH1B*1 and ADH1C*2 are considered slow metabolisers.

and the risk for esophageal cancer in East Asian heavy drinkers. Light-to-moderate drinkers showed a higher vulnerability. According to the authors, some studies suggest similar associations for the risk for head and neck cancer in moderate-to-heavy-drinking Japanese. Data on ADH1C genotype were controversial.

The Committee emphasizes that in none of the studies on genetic polymorphism and alcohol-related cancer risk, a direct association was found between acetaldehyde and cancer, although the indirect data are suggestive for this.

6.3.2 *Cell transformation tests*

Koivisto and Salaspuro (1998) reported on a transformation test in which human colon adenocarcinoma cell line Caco-2 were used to study changes in cell proliferation, cell differentiation, and adhesion due to exposure to acetaldehyde.[104] In the absence of cell cytotoxicity, on acute exposure (for 72 hours), acetaldehyde (0.5 or 1 mM) inhibited the cell proliferation rate, but on chronic exposure (for five weeks) it stimulated cell proliferation. Furthermore, acetaldehyde clearly disturbed the cell differentiation (concentration applied was 1 mM for 7, 14 or 21 days); and, a clear decrease of adhesion of Caco-2 cells to collagens was observed when acetaldehyde was applied to the cells at a concentration of 0.5 or 1 mM for four days. According to the authors, the increased proliferation rate, disturbed differentiation, and reduced adhesion, would *in vivo* predict more aggressive and invasive tumour behaviour.

Eker and Sanner (1986) used a rat kidney cell line in a two-stage cell transformation assay.[105] Acetaldehyde (up to 3 mM) did not affect cytotoxicity nor did it induce colony formation of the cells. When acetaldehyde treatment (3 mM) was followed by a tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), the ability of the cells to form colonies was increased.

In a poorly reported study by Abernathy et al. (1982), acetaldehyde (10-100 µl/ml (LC_{50}, 25 µg/ml)) induced cell transformation in C3H/10T½ cells, in the presence of TPA.[106] Treatment with acetaldehyde alone did induce transformed foci.

The Committee emphasizes that the value of transformation test in assessing carcinogenic potential is under debate. Therefore, it attaches little value to the outcomes of these tests.

6.4 Summary and discussion of carcinogenicity

Epidemiological studies are not available. In the literature, it is suggested that acetaldehyde may play a role in cancer development in humans after alcohol consumption, in particular in combination with a genetic predisposition for enzymes that convert ethanol in acetaldehyde, and for enzymes that convert acetaldehyde in acetate. The Committee emphasizes that in none of the studies on genetic polymorphism and alcohol-related cancer risk, a direct association was found between acetaldehyde and cancer, although the indirect data are suggestive for this.

Regarding animal carcinogenicity studies, chronic inhalation of acetaldehyde induced squamous cell carcinomas and adenocarcinomas in the nose of male and female rats. In hamsters, inhaling the substance, one study showed the presence of laryngeal and nasal tumours, whereas in another study – using a lower exposure concentration – no tumours were observed at all.

6.5 Comparison with criteria

For epidemiological data there is little or no data to support statements concerning an association between exposure to acetaldehyde and cancer. Therefore, the Committee is of the opinion that human data are insufficient to make a final conclusion on the carcinogenic potential of acetaldehyde in humans. For animal data, the Committee found sufficient evidence of carcinogenicity, since a causal relationship was established between malignant tumours in animals and chronic inhalation to acetaldehyde in two studies (Woutersen et al., 1986, Feron et al., 1982), the main route of exposure in an occupational environment.[80,81] According to the CLP classification criteria, acetaldehyde should, therefore, be classified as “presumed to have carcinogenic potential for humans”, which corresponds to classification in category 1B. Supporting evidence for its carcinogenic potential is that the substance has mutagenic properties, and acts by a stochastic genotoxic mechanism.

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. Most likely the difference in outcome is explained by differences in criteria used presently (criteria laid down in Regulation No. 1272/2008) and used in the late eighties of the twentieth century (criteria laid down in Annex VI of Directive 67/548/EEC).

6.6 Conclusions on classification and labelling

The Committee concludes that acetaldehyde is *presumed to be carcinogenic to man*, and recommends classifying the substance in category 1B*.

* See for classification system Annex F.

Chapter 7

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A	Request for advice
B	The Committee
C	The submission letter (in English)
D	Comments on the public review draft
E	IARC evaluation and conclusion
F	Classification on carcinogenicity
G	Classification on mutagenicity

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Quality of Life, Zeist; Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- J. Van Benthem
  Genetic Toxicologist, National Health Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist, SHELL International BV, The Hague
- G.J. Mulder
  Emeritus Professor of Toxicology, Leiden University, Leiden
- M.J.M. Nivard
  Molecular Biologist and Genetic Toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  Epidemiologist, Maastricht University, Maastricht
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- J.M. Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

With respect to the data presentation and interpretation, the Committee consulted an additional expert, Mr. A. Muller, Toxicologist from Bureau REACH, National Health Institute for Public health and the Environment, Bilthoven.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter (in English)

Subject : Submission of the advisory report *Acetaldehyde*
Your Reference: DGV/MBO/U-932342
Our reference : U-8234/JR/cn/246-W19
Enclosed : 1
Date : November 13, 2014

Dear State Secretary,

I hereby submit the advisory report on the effects of occupational exposure to acetaldehyde.

This advisory report is a re-evaluation of an advisory report on the classification as a carcinogenic substance that has earlier been published by the Health Council. The Council is asked for a re-evaluation because the proposed classification differs from the classification that applies in the European Union. In addition, the Council is asked to also propose a classification for mutagenicity. The classifications are based on the European classification system.

The conclusions in the advisory report were drawn by a subcommittee of the Health Council's Dutch Expert Committee on Occupational Safety (DECOS). The subcommittee has taken comments into account from a public review, and included the opinions by the Health Council's Standing Committee on Health and the Environment.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,
(signed)
Professor J.L. Severens,
Vice President

Annex **D**

Comments on the public review draft

A draft of the present report was released in 2014 for public review. The following organisations and persons have commented on the draft document:

- D. Coggon, University of Southampton, UK
- T.J. Lentz and Q. Ma, National Institute for Occupational Safety and Health (NIOSH), Cincinnati OH, USA.

Annex **E**

IARC evaluation and conclusion

Acetaldehyde (Group 2B), Volume 71 (1999) (p. 319)

Summary of Data Reported and Evaluation

Exposure data

Exposure to acetaldehyde may occur in its production, and in the production of acetic acid and various other chemical agents. It is a metabolite of sugars and ethanol in humans and has been detected in plant extracts, tobacco smoke, engine exhaust, ambient and indoor air, and in water.

Human carcinogenicity data

An increased relative frequency of bronchial and oral cavity tumours was found among nine cancer cases in one study of chemical workers exposed to various aldehydes. Oesophageal tumours have been associated with genetically determined, high metabolic levels of acetaldehyde after drinking alcohol.

Three case-control studies assessed the risk of oral, pharyngeal, laryngeal and oesophageal cancer following heavy alcohol intake, according to genetic polymorphism of enzymes involved in the metabolism of ethanol to acetaldehyde (alcohol dehydrogenase 3) and in the further metabolism of acetaldehyde (aldehyde dehydrogenase 2 and glutathione S-transferase M1).

Despite limitations in the study design and the small size of most of the studies, these studies consistently showed an increased risk of alcohol-related cancers among subjects with the genetic polymorphisms leading to higher internal doses of acetaldehyde following heavy alcohol intake as compared to subjects with other genetic polymorphisms.

Animal carcinogenicity data

Acetaldehyde was tested for carcinogenicity in rats by inhalation exposure and in hamsters by inhalation exposure and by intratracheal instillation. It produced tumours of the respiratory tract following inhalation, particularly adenocarcinomas and squamous-cell carcinomas of the nasal mucosa in rats and laryngeal carcinomas in hamsters. In hamsters, it did not cause an increased incidence of tumours following intratracheal instillation. Inhalation of acetaldehyde enhanced the incidence of respiratory-tract tumours produced by intratracheal instillation of benzo[a]pyrene.

Other relevant data

Acetaldehyde is metabolized to acetic acid. During inhalation exposure of rats, degeneration of nasal epithelium occurs and leads to hyperplasia and proliferation.

Acetaldehyde causes gene mutations in bacteria and gene mutations, sister chromatid exchanges, micronuclei and aneuploidy in cultured mammalian cells, without metabolic activation. In vivo, it causes mutations in Drosophila melanogaster but not micronuclei in mouse germ cells. It causes DNA damage in cultured mammalian cells and in mice in vivo. Acetaldehyde-DNA adducts have been found in white blood cells from human alcohol abusers.

Evaluation

There is inadequate evidence in humans for the carcinogenicity of acetaldehyde. There is sufficient evidence in experimental animals for the carcinogenicity of acetaldehyde.

Overall evaluation

Acetaldehyde is possibly carcinogenic to humans (Group 2B).

Previous evaluations: Vol. 36 (1985); Suppl. 7 (1987).

Synonyms: Acetic aldehyde; ‘Aldehyde’; Ethanal; Ethylaldehyde.

Annex **F**

Classification on carcinogenicity

The Committee expresses its conclusions in the form of standard phrases*:

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category[a]	
		67/548/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	The compound is presumed to be as carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	not applicable	not applicable
(4)	The compound is probably not carcinogenic to man.	not applicable	not applicable

[a] See Section 3.6 (Carcinogenicity) of Regulation No. 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances.

* Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.[107]

Annex **G**

Classification on mutagenicity

Source: Section 3.5 (Germ cell mutagenicity) of Regulation No. 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances.

3.5.1 Definitions and general considerations

3.5.1.1 A mutation means a permanent change in the amount or structure of the genetic material in a cell. The term 'mutation' applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term 'mutagenic' and 'mutagen' will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

3.5.1.2 The more general terms 'genotoxic' and 'genotoxicity' apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxicity test results are usually taken as indicators for mutagenic effects.

3.5.2 Classification criteria for substances

3.5.2.1 This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from

mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class.

3.5.2.2 For the purpose of classification for germ cell mutagenicity, substances are allocated to one of two categories as shown in Table 3.5.1.

3.5.2.3 Specific considerations for classification of substances as germ cell mutagens

3.5.2.3.1 To arrive at a classification, test results are considered from experiments determining mutagenic and/or genotoxic effects in germ and/or somatic cells of exposed animals. Mutagenic and/or genotoxic effects determined in in vitro tests shall also be considered.

3.5.2.3.2 The system is hazard based, classifying substances on the basis of their intrinsic ability to induce mutations in germ cells. The scheme is, therefore, not meant for the (quantitative) risk assessment of substances.

Table 3.5.1 Hazard categories for germ cell mutagens.

Categories	Criteria
CATEGORY 1:	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.
Category 1A:	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
Category 1B:	The classification in Category 1B is based on: • positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or • positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/ genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or • positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
CATEGORY 2:	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: • positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: • somatic cell mutagenicity tests in vivo, in mammals; or • other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays. *Note*: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

3.5.2.3.3 Classification for heritable effects in human germ cells is made on the basis of well conducted, sufficiently validated tests, preferably as described in Regulation (EC) No 440/2008 adopted in accordance with Article 13(3) of Regulation (EC) No 1907/2006 ('Test Method Regulation') such as those listed in the following paragraphs. Evaluation of the test results shall be done using expert judgement and all the available evidence shall be weighed in arriving at a classification.

3.5.2.3.4 In vivo heritable germ cell mutagenicity tests, such as:

- rodent dominant lethal mutation test;
- mouse heritable translocation assay.

3.5.2.3.5 In vivo somatic cell mutagenicity tests, such as:

- mammalian bone marrow chromosome aberration test;
- mouse spot test;
- mammalian erythrocyte micronucleus test.

3.5.2.3.6 Mutagenicity/genotoxicity tests in germ cells, such as:

(a) mutagenicity tests:

- mammalian spermatogonial chromosome aberration test;
- spermatid micronucleus assay;

(b) Genotoxicity tests:

- sister chromatid exchange analysis in spermatogonia;
- unscheduled DNA synthesis test (UDS) in testicular cells.

3.5.2.3.7 Genotoxicity tests in somatic cells such as:

- liver Unscheduled synthesis test (UDS) in vivo;
- mammalian bone marrow Sister Chromatid Exchanges (SCE);

3.5.2.3.8 In vitro mutagenicity tests such as:

- in vitro mammalian chromosome aberration test;
- in vitro mammalian cell gene mutation test;
- bacterial reverse mutation tests.

3.5.2.3.9 The classification of individual substances shall be based on the total weight of evidence available, using expert judgement (See 1.1.1). In those instances where a single well-conducted test is used for classification, it shall provide clear and unambiguously positive results. If new, well validated, tests arise these may also be used in the total weight of evidence to be considered. The relevance of the route of exposure used in the study of the substance compared to the route of human exposure shall also be taken into account.

3.5.3 Classification criteria for mixtures

3.5.3.1 Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.5.3.1.1 The mixture shall be classified as a mutagen when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 mutagen and is present at or above the appropriate generic concentration limit as shown in Table 3.5.2 for Category 1A, Category 1B and Category 2 respectively.

Table 3.5.2 Generic concentration limits of ingredients of a mixture classified as germ cell mutagens that trigger classification of the mixture.

Ingredient classified as:	Concentration limits triggering classification of a mixture as:		
	Category 1A mutagen	Category 1B mutagen	Category 2 mutagen
Category 1A mutagen	≥ 0,1 %	-	-
Category 1B mutagen	-	≥ 0,1 %	-
Category 2 mutagen	-	-	≥ 1,0 %

Note. The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).

3.5.3.2 Classification of mixtures when data are available for the complete mixture

3.5.3.2.1 Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients classified as germ cell mutagens. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of germ cell mutagenicity test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.5.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles

3.5.3.3.1 Where the mixture itself has not been tested to determine its germ cell mutagenicity hazard, but there are sufficient data on the individual ingredients and similar tested mixtures (subject to paragraph 3.5.3.2.1), to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.5.4 Hazard communication

3.5.4.1 Label elements shall be used in accordance with Table 3.5.3, for substances or mixtures meeting the criteria for classification in this hazard class.

Table 3.5.3 Label elements of germ cell mutagenicity.

Classification	Category 1A or Category 1B	Category 2
GHS Pictograms		
Signal word	Danger	Warning
Hazard Statement	H340: May cause genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H341: Suspected of causing genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
Precautionary Statement Prevention	P201, P202, P281	P201, P202, P281
Precautionary Statement Response	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405
Precautionary Statement Disposal	P501	P501

3.5.5 Additional classification considerations

It is increasingly accepted that the process of chemical-induced tumorigenesis in humans and animals involves genetic changes for example in proto-oncogenes and/or tumour suppresser genes of somatic cells. Therefore, the demonstration of mutagenic properties of substances in somatic and/or germ cells of mammals in vivo may have implications for the potential classification of these substances as carcinogens (see also Carcinogenicity, section 3.6, paragraph 3.6.2.2.6).

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

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