Health Council of the Netherlands

# 1,4-Dioxane

Re-evaluation of the carcinogenicity and genotoxicity

2015/26

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**Gezondheidsraad**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *1,4-Dioxane*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U- 866033/DC/fs/246-W20
Bijlagen : 2
Datum : 13 november 2015

Geachte minister,

Graag bied ik u hierbij het advies *1,4-Dioxane* aan.

Dit advies is een herevaluatie van een eerder door de Gezondheidsraad uitgebracht advies voor 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.

Dit advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. De subcommissie heeft daarbij gebruik gemaakt van commentaren die zijn ontvangen op het openbare concept van dit advies. Het advies is getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

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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# 1,4-Dioxane

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. 2015/26, The Hague, November 13, 2015

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 and Sport, Infrastructure and the Environment, Social Affairs and Employment, and Economic Affairs. 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.

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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 Raad, 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 1,4-dioxaan. De stof wordt vooral gebruikt als oplosmiddel in de papier-, katoen- en textielindustrie, in koelvloeistof voor auto's, als uitgangsstof voor de synthese van andere stoffen, als schuimmiddel in de polymeerindustrie en bij de productie van cosmetische stoffen en shampoos.

De commissie concludeert dat 1,4-dioxaan 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 verder aan om 1,4-dioxaan te classificeren als mutageen voor geslachtscellen in categorie 2 (stof die reden geeft tot bezorgdheid voor de mens omdat zij mogelijk

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

erfelijke mutaties in de geslachtscellen van mensen veroorzaakt). De stof kan kanker veroorzaken via een niet-stochastisch genotoxisch werkingsmechanisme.

# 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 1,4-dioxane. 1,4-Dioxane is mainly used as solvent in the paper, cotton and textile industry; in coolant for cars, and as base component for the synthesis of other substances, such as foaming agents in the polymer industry, production of cosmetics, and shampoos.

The Committee concludes that 1,4-dioxane is presumed to be carcinogenic to man, and recommends classifying the compound in category 1B.*

Based on the available data, the Committee recommends classifying 1,4-dioxane as a germ cell mutagen in 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 substance acts via a non-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 (see Annex G), the ministry of Social Affairs and Employment asked the Health Council to update the evaluations and classifications 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 1,4-dioxane. An earlier evaluation of this substance was published in 2011.[1] The re-evaluation now includes a proposal for classification on germ cell mutagenicity.

## 1.2 Committee and procedures

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. The members of the Committee are listed in Annex B. The submission letter (in English) to the State Secretary can be found in Annex C.

In 2015 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 1,4-dioxane, such an IARC-monograph is available, of which the summary and conclusion of IARC (1999) is inserted in Annex E.

Furthermore, relevant data of 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 October 2015, using 1,4-dioxane and CAS no 123-91-1 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 | : | 204-661-8 |
| EC name | : | 1,4-dioxane |
| CAS number (EC inventory) | : | 123-91-1 |
| CAS number | : | 123-91-1 |
| CAS name | : | 1,4-dioxane |
| IUPAC name | : | 1,4-dioxane |
| CLP Annex IV Index number | : | 603-024-00-5 |
| Molecular formula | : | $C_4H_8O_2$ |
| Molecular weight range | : | 88.12 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 | : Colourless liquid | ATSDR 2012[2] | |
| Melting/freezing point | : 11.8 °C | ATSDR 2012[2] | |
| Boiling point | : 101.1 °C | ATSDR 2012[2] | |
| Relative density | : 1.0329 | ATSDR 2012[2] | |
| Vapour pressure | : 38.1 mm Hg at 25 °C | ATSDR 2012[2] | |
| Surface tension | : - | | |
| Water solubility | : Miscible | ATSDR 2012[2] | |
| Partition coefficient ($n$-$_{octanol/water}$) | : Log Kow -0.27 | ATSDR 2012[2] | |
| Flash point | : 5-18 °C | ATSDR 2012[2] | |
| Flammability | : Limits at 25 °C lower: 2.0%; upper: 22% | ATSDR 2012[2] | |
| Explosive properties | : Vapour forms explosive mixture with air over wide range | ATSDR 2012[2] | |
| Self-ignition temperature | : 180 °C | ATSDR 2012[2] | |
| Oxidising properties | : none | ECHA[3] | |
| Granulometry | : - | | |
| Stability in organic solvents | : Yes | ECHA[3] | |
| Dissociation constant (pKa) | : No dissociating properties | ECHA[3] | |
| Viscosity | : 1.27 $mm^2/s$ at 20 °C; 0.93 $mm^2/s$ at 40 °C | ECHA[3] | |

## 2.4 International classifications

### *2.4.1 European Commission*

1,4-Dioxane is classified for carcinogenicity in Annex VI of regulation (EC) No 1272/2008 of the European Parliament as follows: Carc 2 (suspected human carcinogen; H351: suspected of causing cancer). The substance is not classified for germ cell mutagenicity. The classification by the European Commission dates from January 2000.

### *2.4.2 Health Council of the Netherlands*

In 2011, the Dutch Expert Committee on Occupational Standards, a Committee of the Health Council of the Netherlands concluded that 1,4-dioxane should be regarded as carcinogenic to humans (comparable with EU category 1B) and considered the substance as a non genotoxic carcinogen.[1,4] Furthermore, the

Committee recommended an HBROEL TWA 8 hours for 1,4-dioxane of 20 $mg/m^3$ (6 ppm). This was based on the lowest observed adverse exposure limit (LOAEL) of 180 $mg/m^3$ (50 ppm) for nasal lesions in rats after lifetime exposure to 1,4-dioxane.[1]

### 2.4.3 IARC

In 1999, IARC concluded that there was inadequate evidence in humans for the carcinogenicity of 1,4-dioxane, and that there was sufficient evidence in experimental animals (see Annex E). Therefore, IARC classified the compound in Group 2B (‘possibly carcinogenic to humans’).[5]

Chapter 3

# Manufacture and uses

## 3.1 Manufacture

Not relevant for classification.

## 3.2 Identified uses

1,4-Dioxane is used as a solvent in the production of lacquers, varnishes, cleaning and detergent preparations, adhesives, cosmetics, deodorant fumigants, emulsions and polishing compositions, pulping of wood, extraction medium for animal and vegetable oils, laboratory chemical (eluent in chromatography), cassettes, plastic and rubber, and insecticides and herbicides (BASF information; HSDB 1996; Grant Chemicals 1977). Furthermore, it is used as a stabilizer for 1,1,1-trichloroethane. However, this use is diminished considerably as a result of the restriction of the use of substances depleting the ozone layer (Grant Chemicals 1977).[6]

Chapter 4

# Summary of toxicokinetics

The data presented below is a summary based on evaluations and reviews by others, such as DECOS, IARC, ATSDR, DFG, and EPA.[1,2,4,5,7,8]

## 4.1 Absorption, distribution and elimination

### *4.1.1 Absorption*

#### Inhalation and oral

Four healthy volunteers inhaled 50 ppm 1,4-dioxane (180 mg/m$^3$) for 6 hours, after which the blood and the urine was examined (Young et al., 1977).[9] The substance was rapidly and extensively absorbed as evidenced by a rapid accumulation in plasma. Limited human data are available to evaluate the oral or inhalatory absorption of 1,4-dioxane.

1,4-Dioxane was rapidly and almost completely absorbed after oral and inhalation exposure of mice (Sweeney et al., 2008).[10]

#### Dermal

Dermal absorption occurs, but it is low, probably due to evaporation of the material. In experiments with Rhesus monkeys, 2.3 and 3.4% of the dioxane, which was applied non occlusively as a methanol solution or as lotion on the

forearm skin, was excreted in the urine (Marzulli et al., 1981).[11] In vitro studies show that 12% of an applied dose passes through excised skin under occlusion, and only 0.3% when not occluded (ECETOC 1983).[12]

### 4.1.2 *Distribution*

No data are available for the distribution of 1,4-dioxane in human tissues. In addition, no data are available for the distribution of 1,4-dioxane in animals following oral or inhalation exposure. After intraperitoneal administration of $^{3}$H-labelled dioxane to rats, $^{3}$H label was found in all tissues investigated at comparable levels (Woo et al., 1977) between 1 and 16 hours after administration. Mikheleev et al., (1990) report similar findings.[13,19,20]

### 4.1.3 *Elimination and pharmacokinetics*

In humans exposed for 6 hours to 180 mg 1,4-dioxane/m$^3$ (in a chamber under dynamic airflow conditions) dioxane in plasma rapidly accumulated to nearly steady state after 4 hours of exposure. It was excreted in urine as its metabolite ß-hydroxyethoxyacetic acid (HEAA) over the next 24 hours of which approx. 50% during the first 6 hour period. In humans exposed for 6 hours to 180 mg 1,4-dioxane/m$^3$ (50 ppm) 99.3% of the absorbed dose (assuming that urinary excretion was the only excretory route) was eliminated via the urine as ß-hydroxyethoxyacetic acid (HEAA); the remainder was unchanged dioxane (Young et al., 1977).[9] After the 6 hr exposure period the plasma 1,4-dioxane concentration decreased exponentially, indicating that the elimination was not saturated. The plasma elimination T½ was 59 minutes (Young et al., 1977).[9]

Physiologically-based pharmacokinetic (PB-PK) models were developed by Reitz et al., (1990) and Leung and Paustenbach (1980), which were further improved by Sweeney et al., (2008).[10,14,15] The plasma concentrations as well as HEAA urinary excretion after exposure to dioxane by inhalation or gavage in mice and rats could reasonably well be predicted, but the human volunteer data of Young et al., (1977) did not fit adequately in the model.[9] Only the urinary excretion data of Young et al., (1978) were well predicted by the model.[16] A physiologically based pharmacokinetic modelling study indicates that 1,4-dioxane may also be excreted into human milk (Fisher et al., 1997).[17]

1,4 -Dioxane is rapidly excreted in rats via the urine. The major metabolite is 2-hydroxyethoxyacetic acid (HEAA) (Woo et al., 1977a,b).[18,19] At low pH, HEAA is rearranged (reversibly) to 1,4-dioxan-2-one.

## 4.2 Metabolism

1,4-Dioxane is metabolized by cytochrome P-450's, possibly of the 2A and 2D family (Sweeney et al., 2008).[11] Induction of the cytochrome P-450 enzymes increases the rate of HEAA formation, whereas inhibition decreases HEAA formation (Woo 1977b, Woo 1978).[19,20]

Repeated oral administration of 1,000 mg/kg of 1,4-dioxane induced dioxane metabolism in rats, but at doses of 10 mg/kg no such effect was observed (Young et al., 1978).[16]

At a single oral dose of 20 mg/kg in mice the metabolism was so rapid that 1,4-dioxane could hardly be detected in blood; saturation of metabolism seemed to occur above 200 mg/kg (Sweeney et al., 2008).[10]

In rats the capacity to metabolise 1,4-dioxane to HEAA is also limited. A single oral dose of 10 mg/kg bw was rapidly metabolised and excreted (as HEAA) via the urine, while a single oral dose of 100 1,000 mg/kg bw, saturated the metabolism, resulting in a decreased proportion of urinary excretion of HEAA, and increased excretion of 1,4-dioxane in urine and the expired air (Dietz et al., 1982, Reitz et al., 1990, Young et al., 1978).[15,16,21] Young et al., (1978) observed a statistically significant increase of $^{14}CO_2$ excretion at multiple oral doses of $^{14}C$-labelled dioxane compared to the control; it is unclear as yet how this mechanistically reflects metabolism of dioxane.[16] It has been suggested by SCOEL that at high dose another, presumably reactive metabolite of 1,4-dioxane, β-hydroxyethoxyacetaldehyde (HEA) might be responsible for toxicity: in the toxicity studies, morphological and biochemical changes were observed at exposure concentrations which lead to saturation of the metabolism.[22] SCOEL postulated, without further evidence that HEA may be assumed to be the reactive metabolite that is responsible for some of the toxicity seen with 1,4-dioxane, including carcinogenicity in experimental animals.[22]

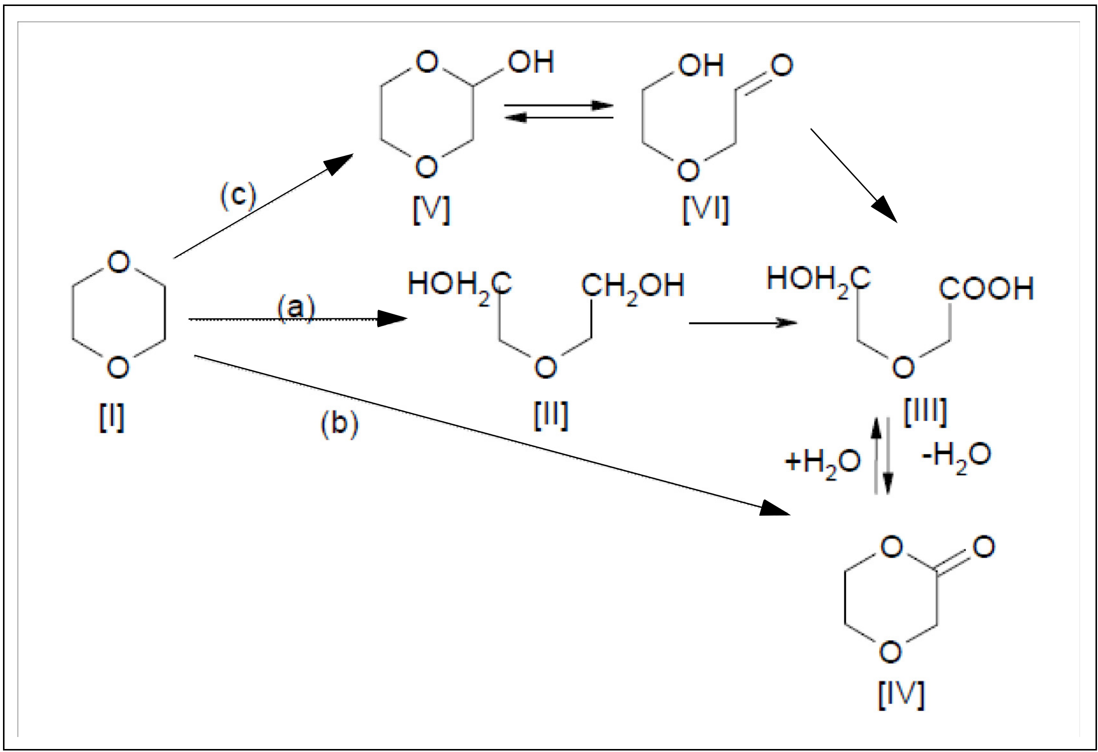


*Figure 1* Suggested metabolic pathways of 1,4-dioxane in the rat (Woo et al. 1977a in EPA 2013).[8,18] [I], 1,4-dioxane; [II], diethylene glycol; [III], β-hydroxyethoxy acetic acid (HEAA); [IV], 1,4-dioxane-2-one; [V], 1,4-dioxane-2-ol; [VI] β-hydroxyethoxy acetaldehyde (HEA). Note: Metabolite [V] is a likely intermediate in pathway b as well as pathway c. The proposed pathways are based on the metabolites identified; the enzymes responsible for each reaction have not been determined. The proposed pathways do not account for metabolite degradation to the labelled carbon dioxide identified in expired air after labelled 1,4-dioxane exposure.

Chapter 5

# Genotoxicity

## 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* | Results<br>- negative<br>+ positive | Klimisch Score** | References |
|---|---|---|---|---|---|
| *Micro-organisms* | | | | | |
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1535, TA1537<br>E. coli WP2uvrA and WP2 | 0, 156, 313, 625, 1,250, 2,500, and 5,000 µg/plate<br>+/- preincubation | - | 2 | Morita et al., 1998[23] |
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 0, 5.17, 15.5, 31.0, 62.0 and 103 mg/plate | - (highest dose bacteriostatic - S9) | 2 | Stott et al., 1981[24] |
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1535, TA1537 | 0,100, 133, 1,000, 1,333, and 10,000 µg/plate | - | 2 | Haworth et al., 1983[25] |
| Reverse mutation | *S. typhimurium* TA100, TA1535 | 0, 10, 31, 103 mg/plate preincubation | - | 3 (only two strains; methodological deficiencies) | Nestmann et al., 1984[26] |

| | | | | | |
|---|---|---|---|---|---|
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1530, TA1535, TA1537 | Dose levels not provided | - | 3 (dose levels not provided) | Khudoley et al., 1987[27] |
| Reverse mutation | *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | 4, 20, 100, 500, 2,500 µg/plate | - | 2 | Echa registration data, vitro 001 study report 1979-04-02 (echa.europe.eu)[3] |
| *Mammalian cells* | | | | | |
| Gene mutation | Mouse lymphoma L5178Y cells, tk locus | 0, 1,250, 2,500 and 5,000 µg/ml:<br>3 and 24 hr exposure | - (slight decrease in relative survival at 5,000 µg/ml +S9) | 2 | Morita and Hayashi 1998[23] |
| Gene mutation | Mouse lymphoma L5178Y cells, tk locus | 0, 312.5, 625, 1,250, 2,500, 5,000 µg/ml (-S9)<br>0, 1,000, 2,000, 3,000, 4,000, 5,000 µg/ml (+S9) | - | 2 | McGregor et al., 1991[28] |
| Gene mutation | Chinese hamster ovary, K1 cells | 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 mg/ml | - | 2 | Echa registration data, vitro 003 study report 1991-8-9 (echa.europe.eu)[3] |
| Micronucleus | Chinese hamster ovary, K1 cells | 0, 1,250, 2,500 and 5,000 µg/ml:<br>5 and 44 hr exposure (+/-S9) | - | 2 | Morita and Hayashi 1998[23] |
| Chromosome aberration | Chinese hamster ovary, K1 cells | 0, 1,250, 2,500 and 5,000 µg/ml (+/-S9) | - | 2 | Morita and Hayashi 198[23] |
| Chromosome aberration | Chinese hamster ovary cells | 1,050, 3,500, 10,520 µg/ml (+/-S9) | - | 3 (no data on purity; no data on negative control or cytotoxicity) | Galloway et al., 1987[29] |
| *Other supporting studies* | | | | | |
| Sister chromatid exchange | CHO-K1 cells | 0, 1250, 2,500 and 5,000 µg/ml (+/- S9)<br>3 and 26 hr exposure | - (dose-related cytotoxicity observed) | 2 | Morita and Hayashi 1998[23] |
| Sister chromatid exchange | CHO cells | 1,050, 3,500, 10,520 µg/ml (+/-S9); positive and negative controls included | ± (-S9 at 10,520 µg/ml);<br>- (+S9) | 3 (no data on purity, negative control or cytotoxicity) | Galloway et al., 1987[29] |

| | | | | | |
|---|---|---|---|---|---|
| UDS | Rat primary hepatocytes F344 | Incubation with 0, 0.001, 0.01, 0.1 or 1 mM; -S9 only | - (at 1mM signs of cytotoxicity) | 2 | Goldsworthy et al., 1991[30] |
| UDS | Rat primary hepatocytes | $10^{-8}$ to 1 M | - | 3 (methodological deficiencies) | Stott et al., 1981[24] |
| 'Comet assay'; DNA damage, single strand break measured by alkaline elution*** | Rat primary hepatocytes | 0.03, 0.3, 3.0, 10, 30 mM; positive and negative controls included; -S9 only | + (at cytotoxic concentrations of 0.3 and higher) | 3 (methodological deficiencies) | Sina et al., 1983[31] |
| DNA damage (Mutatox assay) | *Photobacterium phosphoreum M169* (strain sensitive to DNA damaging agents, DNA-intercalating agents, DNA-synthesis inhibitors, and direct mutagens. | Not specified; -S9 only | - | 4 (no standard test, relevance unknown; concentrations not specified) | Kwan et al., 1990 (results taken from ATSDR 2012)[2] |
| Aneuploidy | *S. cerevisiae* D61M | 1.48, 1.96, 2.44, 2.91, 3.38, 4.31, 4.75% (repeated plating after addition-nil incubation of 5 hr at 3.85 and 4.31%); positive and negative controls included | - (toxicity observed; only tested -S9) | 3 (no metabolic activation; no validated method) | Zimmerman et al., 1985[32] |

* + or - S9, with or without metabolic activation system. ** See Annex H.

## Conclusion

The in vitro studies summarised in Table 3 show no mutagenic activity of 1,4-dioxane when using bacteria or mammalian cells. Negative outcomes were also found in the unscheduled DNA synthesis and sister chromatide exchange assay.

### 5.1.2 In vivo data

Data on the in vivo mutagenicity testing are presented in Table 4.

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

| Method | Animal | Exposure conditions | Results | Klimisch score* | References |
|---|---|---|---|---|---|
| *Somatic cell mutagenicity* | | | | | |
| Micronuclei | CD-1 mice, male peripheral blood; 5/group | 0, 500, 1,000, 2,000 and 3,200 mg/kg bw (two intraperitoneal injections, 1/day); positive and negative control | - (toxicity at 3,200 mg/kg bw, 1/5 males died at this dose), cytotoxicity not tested, but IP dosing | 2 | Morita 1994[33] |
| Micronuclei | B6C3F1 mice, male bone marrow; 5/group | 0, 2,000, 3,000, 4,000 mg/kg bw (intraperitoneal injection) 0, 500, 1,000, 2,000 mg/kg bw (intraperitoneal injection, 3x); two studies in two different labs | - (decreased PCE/NCE ratio) - (500 and 1,000 mg/kg bw were positive in one trial and one laboratory only; no dose-related increase). Decreased PCE/NCE ratio | 2 | McFee et al., 1994[34] |
| Micronuclei | C57BL6 mice, male bone marrow: 10/group | 0, 900, 1,800, 3,600 mg/kg bw (oral gavage) for 24 hr, 3,600 mg/kg bw also for 48 hr sampling time | + (dose-related increase) no data on cytotoxicity | 2 | Mirkova 1994[35] |
| | C57BL6 mice, male bone marrow 4/group | 0, 900, 1,800, 3,600 mg/kg bw (oral gavage) for 24 hr, 3,600 mg/kg bw also for 48 hr sampling time | + (dose-related increase) no data on cytotoxicity | 2 | |
| | C57BL6 mice, male bone marrow 10/group | 0 and 3,600 mg/kg bw (oral gavage) for 24 hr | + (no data on cytotoxicity) | 3 (methodological deficiencies) | |
| | C57BL6 mice female bone marrow: 5/group | 0 and 5,000 mg/kg bw (oral gavage) for 24 hr or 48 hr sampling time | + (no data on cytotoxicity) | 3 (methodological deficiencies) | |
| | BALB/c mice, males bone marrow; 6/group | 0 and 5,000 mg/kg bw (oral gavage) for 24 hr | - (1/6 death occurred in 5,000 mg/kg bw after 24 hr); irrelevant exposure levels. No data on cytotoxicity | 3 (methodological deficiencies) | |

| | | | | | |
|---|---|---|---|---|---|
| Micronuclei *Follow-up study of Morita and Hayashi 1998* | CD-1 mice, male bone marrow; 5/ group | 1,500, 2,500 and 3,500 mg/kg bw (oral gavage, 5 days); 24 hr sampling time; CRESH and FISH staining used to demonstrate aneuploidy; implantation of BrdU releasing osmotic pumps used to demonstrate cell proliferation in liver and to increase sensitivity of the test | + (dose-related increase in MN frequency and decrease in PCE/NCE ratio; >90% micronuclei caused by chromosome breakage; induction of cell proliferation | 2 | Roy et al., 2005[36] |
| | CD-1 mice, male hepatocytes; 5/ group | 1,500, 2,500 and 3,500 mg/kg bw (oral gavage, 5 days) 24 hr sampling time; CRESH and FISH staining used to demonstrate aneuploidy; implantation of BrdU releasing osmotic pumps used to demonstrate cell proliferation in liver and to increase sensitivity of the test | + (from 2,500 mg/kg bw dose-related increase in MN in proliferating cells only; caused by chromosome breakage; induction of cell proliferation | 2 | |
| Micronuclei Follow-up of study Mirkova 1994 | CBA mice, male bone marrow; 4 animals | 1,800 mg/kg bw (oral, gavage); Giemsa staining** | - (decreased PCE/NCE ratio) | 2 | Tinwell and Ashby 1994[37] |
| | CBA mice, male bone marrow; 8 animals | 1,800 mg/kg bw (oral, gavage); Acridine orange staining | - | 3 (one dose only; no data cytotoxicity; acridine orange staining**) | |
| | C57BL6 mice, male bone marrow; 4 animals | 3,600 mg/kg bw (oral, gavage); acridine orange staining | - | 3 (max. dose level; no data on cytotoxicity methodological deficiencies; acridine orange staining**) | |
| Micronuclei *Follow-up of study Mirkova 1994, same dose levels* | CD-1 mice, male peripheral blood and hepatocytes; 5/group | 1,000, 2,000 and 3,000 mg/kg bw (oral gavage); partial hepatoectomy 24 hr after dosing; peripheral blood obtained from tail vein 24 hours after hepatectomy; hepatocytes analysed 5 days after hepatectomy | - (in peripheral blood) + (in hepatocytes; from 2,000 mg/kg bw; dose-related increase); intraspecies differences at 2,000, but not at 3,000 mg/kg bw; valid positive and negative controls | 3 (method not validated: partial hepatectomy to stimulate mitosis) | Morita and Hayashi 1998[23] |

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| Transgenic rodent gene mutation Analysis of GST-P positive foci and PCNA-positive cell index | *Gpt* delta transgenic male rats; 30 animals divided in four groups (number of animals per group not given) | 0, 200, 1,000 or 5,000 ppm in drinking water for up to 16 weeks; at the end of treatment all animals were killed, and livers excised for further analyses | - (0 to 1,000 ppm) + (5,000 ppm), for increased mutation frequency of *gpt* transgenes ($p<0.001$), GST-P-positive foci ($p<0.001$), and PCNA-positive cell index ($p<0.001$) | 4 (poster abstract only; no details on methods or outcomes reported) | Fukushima et al., 2009[38] |
| *Germ cell mutagenicity* | | | | | |
| Sex-linked recessive lethal mutations | *Drosophila melanogaster* | 35,000 ppm in feed for 7 days, or 50,000 ppm by injection; negative controls included | 3 (classification based on studies in mammalians; no OECD guideline anymore) | | Yoon et al., 1985[39] |
| Meiotic non-disjunction | *Drosophila melanogaster* | 1, 1.5, 2, 3 and 3.5% (feeding); negative controls included; oocytes were obtained for evaluation 24 and 48 hr after mating | + (not dose related, cytotoxic doses) | 3 (less relevant test system;_ unusual strains) | Munõz and Barnett 2002[40] |
| Dominant lethal test | Mouse, male NMRI, 20/sex | 2,550 mg/kg bw (single intraperitoneal injection) | - | 3 (no positive control; no toxicity observed in highest dose; methodological deficiencies) | BASF 1977[41] (results taken from ECHA registration data, Ex Key Genetic toxicity in vivo.001)[3] |
| *Other supporting studies* | | | | | |
| UDS | Male rat liver F344 and primary hepatocytes | 1% (1,500 mg/kg bw/day) in drinking water for 1 week (pretreatment rats) followed by hepatocyte incubation with 0, 0.001, 0.01, 0.1 or 1 mM; -S9 only | - (at 1 mM signs of cytotoxicity) | 2 | Goldsworthy et al., 1991[30] |
| UDS | Male rat liver F344; 3/group | 1,000 mg/kg bw (oral, gavage), 2 hr and 12 hr sampling time | - (cytotoxicity not observed) | 2 | |
| UDS | Male rat liver F344; 3/group | 1% (1,500 mg/kg bw/day) in drinking water for 2 weeks or 2% (3,000 mg/kg bw/day) in drinking water for 1 week | - (no increase in NG; no cytotoxicity observed) - Two-fold hepatocytes proliferation observed at 1% | 2 | |
| UDS | Male F344 rats; 3/group; nasal epithelial cells and hepatocytes examined | 1% (1,500 mg/kg bw/day) in drinking water for 8 days (pre-treatment), followed by 0, 10, 100 or 1,000 mg/kg bw (single gavage dose) | - (at highest dose signs of toxicity were observed); only morphologically normal cells were scored | 2 | |

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| UDS | SD rat liver; 4 rats/group | 1,000 mg/kg bw ($^{14}C$ oral gavage) | - | 3 (no positive control; (methodological deficiencies) | Stott et al., 1981[24] |
| UDS | SD rat liver; 6 males/group | 0, 10, 1,000 mg/kg bw/day (drinking water for 11 wks) | + (1.5 fold increase at 1,000 mg/kg, a cytotoxic concentration) | 3 (no positive control; (methodological deficiencies) | |
| 'Comet assay'; DNA damage, single strand break measured by alkaline elution assay*** | Female SD rats, 3-5/group; histopathological examination of liver | 0, 168, 840, 2,550, 4,200 mg/kg bw (oral gavage twice) for 21 and 4 h before sacrifice | + (from 2,550 mg/kg bw, dose-related increase; but irrelevant dose levels)<br><br>*Histopathology* liver: 3/5 rat of 2,550 mg/kg showed mild to minimal periportal vacuolar degenerations in liver samples in the absence of hepatic necrosis or substantial cellular toxicity. No histopathological lesions found in other dose groups. | 2 | Kitchin and Brown 1990[42] |
| Replicative DNA synthesis (marker for cell proliferation) | Male F344 rats; 4/group; hepatocytes isolated after exposure for testing | Gavage; 1,000, 1,500, 2,000 and 4,000 mg/kg bw; 24 hr and 48 hr response time; thymidine and BrdU incorporation | + (24 hr-response time: dose-related increase from 1,000 mg/kg bw, but no increase at 4,000 mg/kg bw; relationship was bell shaped; no hepatotoxicity at any dose level)<br>(48 hr-response time; no hepatocytotoxicity) | 2 | Miyagawa et al., 1999[43] |
| Replicative DNA synthesis assay | Rat hepatocytes | 0, 1,000, 2,000 mg/kg bw, oral gavage; positive and negative controls included | ± at 2,000 mg/kg bw (signs cytotoxicity at 1,000 and 2,000 mg/kg bw) | 3 (no validated test method) | Uno et al., 1994[44] |
| DNA alkylation | SD rat liver; 4-6 males/group | 1,000 mg/kg bw $^{14}C$ (gavage); DNA isolation from hepatocytes and HPLC analysis | - | 3 (positive control missing; (methodological deficiencies; limited study) | Stott et al., 1981[24] |
| RNA synthesis; inhibition of RNA polymerase A and B | Male SD rat; numbers not reported | Intravenous injection; activity measured in isolated hepatocytes; 10 and 100 mg/rat (2 and 20 mg/kg bw) | + | 3 (no positive control; no validate method) | Kurl et al., 1981[45] |

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|---|---|---|---|---|---|
| DNA repair, host mediated assay, in vivo | Repair-deficient *E coli* K-12 *uvrB/recA;* tests performed in mice | Highest tested concentration 1150 mM; + and – S9; positive and negative controls included | - | 3 (method not validated) | Hellmer and Bolcsfoldi 1992[46] |

* See Annex H. ** According to OECD guideline, the Giemsa stain is preferred for detection of micronuclei; the acridine orange stain is a DNA stain that can eliminate artefacts. *** Comet assay and alkaline elution assay: DNA single and double strand breaks, DNA cross-links.

## Conclusion

### Germ cells

No animal studies are available on the mutagenicity of 1,4-dioxane in germ cells. The outcome of a sex-linked recessive lethal mutagenicity test using *Drosophila melanogaster,* was negative (Yoon et al., 1985).[39] However, the Committee considers this test not relevant for humans.

### Somatic cells

As summarised in Table 4, a number of studies using mice have been performed on the mutagenic properties of 1,4-dioxane. The induction of micronuclei was mainly investigated in bone marrow cells, but also in peripheral blood cells and in hepatocytes. Furthermore, the Committee noted that dose levels over the limit dose of 2,000 mg/kg bw have been used. The Committee does not consider these higher dose levels relevant for evaluation of the genotoxicity.

1,4-Dioxane did not induce an increase in bone marrow cells with micronuclei in animals which were given the substance by intraperitoneal injection. In one study a decreased ratio of PCE/NCE was reported, which is an indirect measure of bone marrow toxicity (McFee et al., 1994).[34] This indicates that 1,4-dioxane at least reached the bone marrow.

In studies in which mice were given the substance orally positive results were observed in dose level above the limit dose of 2,000 mg/kg bw up to 5,000 mg 1,4-dioxane/kg bw. However, in a few studies a dose-related statistically significant increase in number of cells with micronuclei already started at doses below this limit dose. For instance, Mirkova et al., (1994) reported a statistically significant dose-related increase in bone marrow cells with micronuclei from 900 mg/kg bw/day and Roy et al., (2005) from 1,500 mg/kg bw which paralleled with a dose-related decrease in the PCE/NCE ratio, a measure for cytotoxicity in bone marrow cells and thus bioavailability in bone marrow cells.[35,36] Decreases in

bone marrow cell proliferation were also observed. Roy et al., (2005) also observed that the induced micronuclei are formed primarily from chromosomal breakage.[36]

In other studies, no induction of cells with micronuclei by 1,4-dioxane was observed below the limit dose of 2,000 mg/kg bw although in one study a decreased ratio of PCE/NCE was reported (Tinwell and Ashby 1994).[37]

Overall, the Committee noted that in the majority of the animal studies no data on cytotoxicity were reported, which makes it difficult to interpret the outcomes correctly. However, in most studies dose levels were used exceeding the limit dose, making them less relevant. Secondly, the differences in outcomes among the studies could also be partially explained by the use of a small number of animals, different dose regimen and testing methods. However, the Committee cannot ignore the dose-related positive findings of the micronuclei studies of Roy et al., (2005) and Mirkova et al., (1994) in bone marrow in which at doses below the limit dose of 2,000 mg/kg bw statistically significant increases in cells with micronuclei were found. Based on these results, the Committee considers that 1,4-dioxane may have genotoxic potential.

Other in vivo studies have also been summarised in Table 4. Kitchen and Brown 1990 found a dose-related increase in DNA single-strand breaks at 2,500 and 5,000 mg/kg bw 1,4-dioxane (oral administration by gavage) in the liver of rats.[42] At these relatively high dose levels no significant cytotoxicity was observed. In another study, 1,4-dioxane did not induce DNA-alkylation in hepatocytes of rats, which were given the substance by gavage at a concentration of 1,000 mg/kg bw (Stott et al., 1981).[24] No other reliable data on DNA damage due to exposure to 1,4-dioxane are available.

In vivo data on unscheduled DNA synthesis showed negative outcomes. Miyagawa et al., (1999) showed that cell proliferation (measured as replicative DNA synthesis) could occur without signs of hepatotoxicity.[43] In their study, rats were exposed to 1,4-dioxane to up to 4,000 mg/kg bw (single administration by gavage). Tests for cell proliferation were performed 24 or 48 hours after administration. After 24 hours a clear bell-shaped relationship was found with no significant increase in proliferation at the highest concentration tested. However, data obtained after 48 hours did not show indications of cell proliferation at any concentration level.

The majority of these studies support the conclusion that 1,4-dioxane may have genotoxic potential.

## 5.2 Human information

In Table 5 data are shown on 1,4-dioxane exposure in humans.

*Table 5* Summary of human studies.

| Method | Population | Cells | Results and remarks | Quality/reliability of study | References |
|---|---|---|---|---|---|
| Chromosomal aberrations | 6 German workers; 6-15 year exposure to unspecified airborne levels | Human peripheral lymphocytes | Negative (compared to controls) | 4 (Data from secondary sources; no study details given) | Thiess et al., 1976[47] (results taken from EU Risk Assessment Report 2002) |

## 5.3 Summary and discussion of mutagenicity

Below, only data are summarised of reliable experimental design according to the Klimisch criteria 1 and 2 (see Annex H).

### Germ cell genotoxicity

As no genotoxicity studies of 1,4-dioxane in germ cells were found, the Committee is not able to make a conclusion whether 1,4-dioxane is mutagenic in germ cells.

### Somatic cell genotoxicity

1,4-Dioxane was investigated in genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, structural and numerical chromosome aberrations. The Committee noted that in the majority of the animal studies no data on cytotoxicity were reported, which makes it difficult to interpret the outcomes. Also in most studies dose levels were used exceeding the limit dose, making them less relevant to determine the genotoxicity of 1,4-dioxane. Furthermore, the differences in outcomes among the studies could also be partially explained by the use of a small number of animals, different dose regimen and testing methods.

1,4 Dioxane did not induce gene mutations in bacteria nor in mammalian cells in vitro. Exposure to 1,4-dioxane did not result in an increase in cells with

chromosome aberrations or micronuclei. The majority of the supporting genotoxicity tests (Table 3) confirmed the negative findings in in vitro tests.

Unexpectedly, the in vivo genotoxicity studies gave contradictory results. Exposure to high doses of 1,4-dioxane, above the limit dose of 2,000 mg/kg bw, resulted in an increase of cells with micronuclei indicating to a cytotoxic rather than a genotoxic effect. Occasionally positive results were also found in micronucleus tests with doses below the limit dose of 2,000 mg/kg bw. The Committee cannot ignore these positive findings and considers that 1,4-dioxane also has a genotoxic potential. Aneuploidy was not observed. The majority of the supportive in vivo genotoxicity tests (Table 4) confirmed the in vivo results.

As the important in vitro tests are negative but part of the in vivo tests unexpectedly positive predominantly at doses above the limit dose, it can be concluded that 1,4-dioxane has to be considered as a non-stochastic genotoxic substance and that the positive results may be due to cytotoxicity and thus proliferation induction. The positive results found in the tests measuring replicative DNA synthesis as a marker for cell proliferation confirm this mode of action. Since occasionally positive results in the micronucleus tests were found at doses below the limit dose of 2,000 mg/kg bw a stochastic genotoxic mechanism as secondary mode of action cannot be excluded.

Overall, the Committee concludes that 1,4-dioxane is mutagenic in vivo in mammalian cells and acts predominantly by a non-stochastic genotoxic mechanism.

## 5.4 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 or animal germ cell mutagenicity. Overall, due to a lack of data the Committee concludes that there is no positive evidence for in vivo heritable germ cell mutagenicity of 1,4-dioxane.

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). In case of 1,4-dioxane no supporting evidence is available that suggests that the substance has potential to cause mutations in germ cells.

A substance may be classified as a germ cell mutagen in category 2 if there is positive evidence from animal studies and/or from in vitro studies obtained from: somatic cell mutagenicity tests in vivo, or other in vivo somatic cell genotoxicity tests, which are supported by positive results from in vitro mutagenicity assays. 1,4-Dioxane did not show genotoxicity in vitro. In vivo data show an increase in micronuclei formation in several studies. Therefore, the Committee concludes that 1,4-dioxane should be classified in category 2.

## 5.5 Conclusions on classification and labelling

Based on the available data, the Committee recommends classifying 1,4-dioxane as a germ cell mutagen in 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 substance may cause cancer via a non-stochastic genotoxic mechanism.

Chapter **6**

# Carcinogenicity

## 6.1 Non-human information

Data on animal carcinogenicity studies are summarized in Table 6.

*Table 6* Summary of animal carcinogenicity studies on 1,4-dioxane exposure.

| Species | Design | Exposure levels | Observations and remarks (Klimisch score)* | References |
|---|---|---|---|---|
| *Inhalation* | | | | |
| Rat F344/ DuCrj | 50 males*/group; study duration: 6 h/ day, 5 days/wk for 104 weeks; hematology, clinical biochemistry, gross necropsy and histopathological examination<br><br>*Reason for selecting male animals was the absence of mesotheliomas in females in a previous 2-year oral study with 1,4-dioxane (Kano et al., 2009)[48] | 0, 50, 250, 1,250 ppm (v/v) (calculated as 180, 900 and 4,500 mg/ $m^3$) by inhalation (whole body vaporisation technique);<br><br>Actual exposure levels were:<br>50.2 $\pm$ 1.4<br>250.9 $\pm$ 3.2<br>1,247.5 $\pm$ 18.6 ppm | *Klimisch-score*: 1<br><br>*Neoplastic lesions:* +<br>Significant induction of nasal squamous cell carcinomas, hepatocellular adenomas, peritoneal mesotheliomas and subcutis fibroma (see Table 7).<br>*General:* Decreased survival rate at 250 and 1,250 ppm towards end of 2-yr exposure period. At 1,250 ppm terminal body weights decreased, relative liver weight increased and plasma ALT, AST and gamma-GTP enzyme activities increased.<br>*Non-neoplastic lesions:* Increased incidences of nuclear enlargement in respiratory and olfactory epithelia in all exposed. Increased incidences of nuclear enlargement in liver of 1,250 ppm and in kidney of 250 and 1,250 ppm exposed groups. Statistically significant inflammation and necrosis, recurrent cell death and repair in respiratory and olfactory epithelia and atrophy in olfactory epithelium, hydropic change and sclerosis of lamina | Kasai et al., 2009[49] |

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| | | | propria and proliferation nasal gland within exposed groups.<br>At 1,250 ppm necrosis of hepatocytes and hydropic changes in renal proximal tubule were observed as well as squamous cell hyperplasia in nasal cavity and altered cell foci in liver. At 250 ppm and above squamous cell metaplasia was observed | |
| Rat Wistar | 288 rats/sex for dose group; 192 rats/sex for control; study duration 7 hr/ day, 5 days/wk, during 2 years; haematology, clinical biochemistry, Gross necropsy and histopathological examinations | 111 ppm (400 mg/ $m^3$) by inhalation (whole body) | *Klimisch-score*: 3 (methodological deficiency as no MTD was used at selecting concentration levels)<br><br>*Neoplastic lesions*: -<br>No substance-related tumours found.<br>*General*: no observable substance-related effects with respect to behaviour, growth, or mortality rate. no differences between control and exposed animals on haematology and clinical chemical, all were within the physiological limits; no substance-related gross and microscopic findings | Torkelson et al., 1974[50] |
| *Oral administration* | | | | |
| Rat F344/ DuCrj | 50 animals/sex/ group; study duration 104 weeks; haematology, clinical biochemistry, gross necropsy and histopathological examination | 0, 0.02, 0.1, 0.5% (w/w) in drinking water (*ad libitum*)<br><br>Actual dose levels: m: 0, 11, 55, 274 mg/kg bw/day; f: 0, 18, 83, 429 mg/ kg bw/day | *Klimisch-score*: 2<br><br>*Neoplastic lesions:* +<br>Significant induction of nasal squamous cell carcinomas in females and hepatocellular adenomas and carcinomas in males and females, peritoneal mesotheliomas in males, and mammary gland adenomas in females (see Table 8).<br>*General:* Significantly decreased survival rates at 0.5%; retarded growth rates and decreased terminal body weights; relative liver weights significantly increased in 0.1 and 0.5% dosed males and 0.5% dosed females; no effect on food nor water consumption | Yamazaki et al., (1994), Japan Bioassay Research Center (1998) Summarised by Kano et al., 2009[48] |
| Mouse Crj:BDF1 | 50 animals/sex/ group; study duration 104 weeks; haematology, clinical biochemistry, gross necropsy and histopathological examination | 0, 0.05, 0.2, 0.8% w/w) in drinking water (*ad libitum*).<br><br>Actual dose levels: m: 0, 49, 191, 677 mg/kg bw/day; f: 0, 66, 278, 964 mg/kg bw/day | *Klimisch-score*: 2<br><br>*Neoplastic lesions:* +<br>Significant induction of hepatocellular tumours in both sexes. Two nasal tumours in the highest dose groups for tumour incidences (see Table 9).<br>*General:* Significantly decreased survival rates at 0.2 and 0.8% females. Significantly retarded growth rates and terminal body weights in 0.2 and 0.8% males and females. Relative liver weight significantly increased in 0.8% males and females and in 0.2% males; significantly decreased food and water consumption in 0.8% males and females | Yamazaki et al., (1994), Japan Bioassay Research Center (1998), Summarised by Kano et al., 2009[48] |

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| Rat<br>Sherman | 60 animals/sex/ group;<br>study duration 716 days; haematology, gross necropsy and histopathological examination | 0, 0.01, 0.1, 1% in drinking water (*ad libitum*)<br><br>Actual dose levels<br>m: 0, 9.6, 94, 1,015 mg/kg bw/day<br>f: 0, 19, 148, 1,599 mg/kg bw/day | *Klimisch-score*: 2<br><br>*Neoplastic lesions:* +<br>Treatment related hepatocellular carcinomas and nasal squamous cell carcinomas (see Table 10).<br>*General:* Body weights were significantly lower in animals exposed to 1% than controls. water consumption was slightly less in animals exposed to 1% than controls; severe reduction in survival rate of animals exposed to 1% during first 4 months of study (p <0.05); after 4 month survival rate was the same for all groups; a significantly increased liver weight and liver/ body weight ratio in rats exposed to 1% 1,4-dioxane; gross and histopathological examination revealed variable degrees of renal tubular epithelial and hepatocellular degeneration and necrosis, accompanied by regenerative activities in liver (hepatocellular hyperplastic nodule formation) and renal tubuli in rats at 0.1 and 1.0%.<br>No difference between control and exposed animals on haematology | Kociba et al., 1974[51] |
| Rat<br>Osborne-Mendel | 35 rats/sex/group; study duration 110 weeks; gross necropsy and histopathological examination | 0, 0.5, 1% (v/v) in drinking water (*ad libitum*).<br><br>Actual dose levels<br>m: 0, 240, 530 mg/ kg bw<br>f: 0, 350, 640 mg/kg bw | *Klimisch-score*: 2<br><br>*Neoplastic lesions:* +<br>Significant induction of nasal squamous cell carcinomas in males and females and hepatocellular adenomas in females (see Table 11).<br>*General:* a significant positive dose-related trend in mortality; no clinical signs other than fluctuations in mean body weights of males probably due to mortality.<br>*Histopathology*:<br>Tubular degeneration in kidney<br>Liver cytomegaly<br>Gastric ulceration of stomach:<br>- m: 0/33, 5/28, 5/30<br>Pneumonia:<br>- m: 8/30, 15/31, 14/33<br>- f: 6/30, 5/34, 25/32 | NCI 1978[52] |
| Mouse<br>B6C3F1 | 50 mice/sex/group; study duration 90 weeks; gross necropsy and histopathological examination | 0, 0.5, 1% (v/v) in drinking water (*ad libitum*).<br><br>Actual dose levels<br>m: 0, 720, 830 mg/ kg bw/day<br>f: 0, 380, 860 mg/kg bw/day | *Klimisch-score*: 2<br><br>*Neoplastic lesions:* +<br>Significant induction of hepatocellular adenomas or carcinomas in females and males (see Table 12).<br>*General:* A significant positive dose-related trend in mortality for females.<br>Pneumonia:<br>- m: 1/49, 9/50, 17/47<br>- f: 2/50, 33/47, 32/36<br>Rhinitis:<br>- m: 0/49, 1/50, 1/49<br>- f: 0/50, 7/48, 8/39<br>No clinical signs other than altered body weights | NCI 1978[52] |

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| Rat<br>SD | 30 male/group; study duration 13 months; necropsy at 16 months; gross necropsy; histopathological examination only in nasal cavity with gross lesions | 0, 0.75, 1.0, 1.4, 1.8% drinking water (*ad libitum*). Total dose/rat based on a daily fluid intake of 36 ml: 104, 142, 191, 198, 213 and 256 gram. Using a ref. body weight of 0,523 kg chronic exposure male CD: 0, 430, 574, 803, 1,032 mg/kg bw/day) | *Klimisch-score*: 3 (only one sex; limited reporting of results, no tables and graphs, limited duration)<br><br>*Neoplastic lesions:* -<br>*Non-neoplastic lesions:*<br>Nasal cavity, squamous cell carcinomas (0, 0.75, 1.0, 1.4, 1.8%):<br>0/30, 1/30,1/30, 2/30, 2/30 | Hoch-Ligeti et al., 1970[53] |
| Rat<br>Wistar, | 26 exposed males, 9 control males; study duration 63 wk; gross necropsy and histopathological examination | 0, 1% in drinking water (*ad libitum*) (using a ref. body weight of 0,462 kg chronic exposure male Wistar: 640 mg/kg bw/day) | *Klimisch score*: 3 (rats received 1 wk terramycin prior to start test; limited number of rats; one sex; only one dose, limited duration; Control group of 9 rats).<br>*Neoplastic lesions*:<br>*(0 and 1%, respectively)*:<br>- Lymphosarcoma: 1/9, 0/26<br>- Liver tumours: 0/9, 6/26<br>- Kidney cell carcinoma: 0/9, 1/26<br>Histological changes in liver | Argus et al., 1965[54] |
| Osborne rat and B6C3F1 mice | 35/sex/group; study duration 42 weeks. Control group 34 weeks | 0,.5 and 1.0 % in drinking water<br>0.5 and 1.0% in diet | *Klimisch score*: 3 (minimal reported; purity not specified)<br><br>*Neoplastic lesions*: -<br>*General*: Mortality only in rats; increased weight gain in male rat and mice; histopathological lesions of lung and liver in rats only | King et al., 1973[55] |
| Guinea pig | 24 Guinea pigs; study duration 23 months | 0.5-2% in drinking water | Klimisch score 4<br><br>*Neoplastic lesions*: 2 gallbladder carcinomas; 3 early hepatomas; 1 kidney adenoma | Hoch-Ligeti and Argus (1970)[53] |
| *Intraperitoneal injection* | | | | |
| Mice A/J Pulmonary tumour assay | 16/sex/group; study duration 24 weeks; gross necropsy of limited organs (liver kidney, spleen intestines, stomach, thymus and salivary and endocrine glands); histopathological examination of gross lesions; lungs and livers examined on tumours | Intraperitoneal: 0, 4,800, 12,000, and 24,000 mg/kg bw<br><br>Oral: 0 and 24,000 mg/kg bw<br><br>3 applications/wk for 8 weeks, followed by 16 wks observation | *Klimisch score*: 3 (Limited gross necropsy and histopathology; short duration)<br>*Neoplastic lesions:*<br>Intraperitoneal, lung tumours<br>(0, 4,800, 12,000, 24,000, respectively):<br>- m: 1/14, 1/16, 6/16, 2/11<br>- f: 7/15, 3/16, 5/16, 3/13<br>Oral, lung tumours (0 and 24,000, respectively):<br>- m: 51/135 and 4/15<br>- f: 32/131 and 5/14 | Stoner et al., 1986[56] |

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| Mice A/J Pulmonary tumour assay | 30 males/group; study duration 16 weeks; removal of lungs and histopathological examination | 0, 400, 1,000 and 2,000 mg/kg bw; 3 applications/wk for 8 weeks, followed by 8 wks observation | *Klimisch score*: 3 (only lung tumours studied, short duration)<br><br>*Neoplastic lesions:*<br>Lung tumours in %( 0, 400, 1,000, and 2,000 respectively): 33, 17, 48, and 62 | Maronpot et al., 1986[57] |
| *Dermal administration* | | | | |
| Mice, Swiss-Webster | 30/sex/group; study duration 78 weeks. gross necropsy and histopathological examination. | 3 applications/wk of 0.2 mM 1,4-dioxane solution in acetone on shaved back for 78 wks. Acetone as negative control | *Klimisch score*: 3 (minimal reported; purity not specified)<br><br>*Neoplastic lesions*: no papilloma, one malignant lymphoma. One suspected carcinoma (f) and one subcutaneous tumour (m)<br>*General*: increase in male body weight | King et al., 1973[55] |
| Osborne rat and B6C3F1 mice | 35/sex/group; study duration 42 weeks. Control group 34 weeks | 0,.5 and 1.0 % in drinking water; 0.5 and 1.0% in diet | *Klimisch score*: 3 (limited test design no haematology clinical biochemistry; minimal reported; purity not specified)<br><br>*General*: Mortality only in rats; increased weight gain in male rat and mice.<br>Histopathologic lesions in the lung and liver in rats only. | King et al., 1973[55] |

* See Annex H.

### *6.1.1 Carcinogenicity: inhalation*

Male F344/DuCrj rats (50/group) were whole-body exposed to 0, 180, 900 and 4,500 mg 1,4-dioxane/m$^3$, for 6 hours a day, 5 days/week for 104 weeks (Kasai et al., 2009).[49] Details on tumour incidences are shown in Table 7. In summary, 1,4-dioxane induced a statistically significant increase in hepatocellular adenomas (highest exposure group only), peritoneal mesothelioma (two highest exposure groups), and in nasal squamous cell carcinoma (highest exposure group only). The investigators also reported on pre-neoplastic lesions, such as squamous cell metaplasia, characterized by replacement of transitional and respiratory epithelia by squamous epithelium with or without keratinisation occurred in rats exposed to 900 mg/m$^3$ and higher. In addition, increased incidences of nuclear enlargement in the respiratory and olfactory epithelia, and atrophy and respiratory metaplasia in the olfactory epithelium, were noted in the nasal cavity of male rats exposed at 180 mg 1,4-dioxane/m$^3$ and higher. Torkelson exposed Wistar rats to 400 mg 1,4-dioxane/m$^3$ for 7 hours a day, five days a week for a total of 2 years.[50] The substance did not induce neoplastic lesions.

*Table 7* Tumour incidences in male rats exposed to 1,4-dioxane for 2 years (Kasai et al., 2009).[49]

| Exposure level (ppm, by inhalation) | 0 | 50 | 250 | 1,250 |
|---|---|---|---|---|
| • Nose cavity: squamous cell carcinoma | 0 | 0 | 1 | 6* |
| • Liver: heptocellular adenoma | 1 | 2 | 3 | 21** |
| • Liver: hepatocellular carcinoma | 0 | 0 | 1 | 2 |
| • Kidney: renal cell carcinoma | 0 | 0 | 0 | 4 |
| • Peritoneum: mesothelioma | 2 | 4 | 14** | 41** |
| • Mammary gland: fibroadenoma | 1 | 2 | 3 | 5 |
| • Mammary gland: adenoma | 0 | 0 | 0 | 1 |
| • Zymbal gland: adenoma | 0 | 0 | 0 | 4 |
| • Subcutis: fibroma | 1 | 4 | 9** | 5 |

Fischer exact test: * $p \leq 0.05$, ** $p \leq 0.01$

### 6.1.2 Carcinogenicity: oral administration

A number of animal carcinogenicity studies have been performed in which animals received 1,4-dioxane orally in drinking water (see Table 6). Regarding the well-performed studies, all showed that 1,4-dioxane induced tumours in for instance the nasal cavity and the liver of rats and mice. Details on tumour incidences for the distinctive studies are shown in the Tables 8 to 12. In addition,

*Table 8* Tumour incidences in rats exposed to 1,4-dioxane for 2 years (Kano et al., 2009).[48]

| Exposure level (%, w/w, in drinking water) | 0 | 0.02 | 0.1 | 0.5 |
|---|---|---|---|---|
| *Male rats (mg/kg bw/day)* | 0 | 11 | 55 | 274 |
| • Nose cavity: squamous cell carcinoma | 0 | 0 | 0 | 3 |
| • Liver: hepatocellular adenoma | 3 | 4 | 7 | 32** |
| • Liver: hepatocellular carcinoma | 0 | 0 | 0 | 14** |
| • Liver: combined hepatocellular adenoma or carcinoma | 3 | 4 | 7 | 39** |
| • Peritoneum: mesothelioma | 2 | 2 | 5 | 28** |
| • Mammary gland: fibroadenoma or adenoma | 1 | 2 | 2 | 6 |
| • Subcutis: fibroma | 5 | 3 | 5 | 12 |
| *Female rats (mg/kg bw/day)* | 0 | 18 | 83 | 429 |
| • Nose cavity: squamous cell carcinoma | 0 | 0 | 0 | 7** |
| • Liver: hepatocellular adenoma | 3 | 1 | 6 | 48** |
| • Liver: hepatocellular carcinoma | 0 | 0 | 0 | 10** |
| • Liver: combined hepatocellular adenoma or carcinoma | 3 | 1 | 6 | 48** |
| • Peritoneum: mesothelioma | 1 | 0 | 0 | 0 |
| • Mammary gland: fibroadenoma or adenoma | 8 | 8 | 11 | 18* |
| • Subcutis: fibroma | 0 | 2 | 1 | 0 |

Fischer exact test: * $p \leq 0.05$, ** $p \leq 0.01$

*Table 9* Tumour incidences in mice exposed to 1,4-dioxane for 2 years (Kano et al., 2009).[48]

| Exposure level (%, w/w, in drinking water) | 0 | 0.05 | 0.2 | 0.8 |
|---|---|---|---|---|
| *Male mice (mg/kg bw/day)* | 0 | 49 | 191 | 677 |
| • Nose cavity: adenocarcinoma | 0 | 0 | 0 | 0 |
| • Nose cavity: esthesioneuroepithelioma | 0 | 0 | 0 | 1 |
| • Liver: hepatocellular adenoma | 9 | 17 | 23** | 11 |
| • Liver: hepatocellular carcinoma | 15 | 20 | 23 | 36** |
| • Liver: combined hepatocellular adenoma or carcinoma | 23 | 31 | 37** | 40** |
| *Female mice (mg/kg bw/day)* | 0 | 66 | 278 | 964 |
| • Nose cavity: adenocarcinoma | 0 | 0 | 0 | 1 |
| • Nose cavity: esthesioneuroepithelioma | - | - | - | - |
| • Liver: hepatocellular adenoma | 5 | 31** | 20** | 3 |
| • Liver: hepatocellular carcinoma | 0 | 6* | 30** | 45** |
| • Liver: combined hepatocellular adenoma or carcinoma | 5 | 35** | 41** | 46** |

Fischer exact test: * $p \leq 0.05$, ** $p \leq 0.01$.

*Table 10* Tumour incidences in male and female rats (combined) exposed to 1,4-dioxane for 2 years (Kociba et al., 1974).[51]

| Exposure level (%, in drinking water) | 0 | 0.01 | 0.1 | 1 |
|---|---|---|---|---|
| • Nose cavity: squamous cell carcinoma | 0 | 0 | 0 | 3*** |
| • Liver: hepatocellular carcinoma | 1 | 0 | 1 | 10** |
| • Liver: hepatic tumours all types | 2 | 0 | 1 | 12* |

Fisher exact probability test: *$p$=0.00022, **$p$=0.00033, ***$p$=0.05491.

*Table 11* Tumour incidences in rats exposed to 1,4-dioxane for 2 years (NCI 1978).[52]

| Exposure level (%, v/v, in drinking water) | 0 | 0.5 | 1.0 |
|---|---|---|---|
| *Male rats* | | | |
| • Nose cavity: adenocarcinoma | 0/33 | 1/35 | 3/34 |
| • Nose cavity: squamous cell carcinoma | 0/33 | 12/33 | 16/34*** |
| • Nose cavity: rhabdomyoma | 0/33 | 1/33 | 0/34 |
| • Liver: hepatocellular adenoma | 2/31 | 2/31 | 1/33 |
| • Liver: hepatocellular carcinomas | 0/31 | 1/31 | 0/33 |
| • Testis/epididymis: mesothelioma | 2/33 | 4/33 | 5/34 |
| *Female rats* | | | |
| • Nose cavity: adenocarcinoma | 0/33 | 0/35 | 1/35 |
| • Nose cavity: squamous cell carcinoma | 0/34 | 10/35*** | 8/35**** |
| • Nose cavity: rhabdomyoma | - | - | - |
| • Liver: hepatocellular adenoma | 0/31 | 10/33 | 11/32** |

Fischer exact test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p$=0.003.

*Table 12* Tumour incidences in mice exposed 1,4-dioxane for 2 years (NCI 1987).[52]

| Exposure level (%, v/v, in drinking water) | 0 | 0.5 | 1.0 |
|---|---|---|---|
| *Male mice* | | | |
| • Nose cavity: adenocarcinoma | 0/49 | 0/50 | 1/47 |
| • Liver: heptocellular adenoma or carcinoma | 8/49 | 19/50**** | 28/47*** |
| *Female mice* | | | |
| • Nose cavity: adenocarcinoma | 0/50 | 1/48 | 0/37 |
| • Liver: heptocellular adenoma or carcinoma | 0/50 | 21/48 | 35/37*** |

Fischer exact test: * $p$<0.05, ** $p$<0.01, *** $p$<0.001, **** $p$=0.014.

the tumour development was preceded by the induction of non-neoplastic lesions, which progressed to hepatocellular adenoma and carcinoma in rats and mice and to nasal squamous cell carcinoma in rats at higher dosages. Liver tumours were observed at higher tumour incidences in rats and mice from a concentration of approximately 0.05% 1,4-dioxane and higher, whereas neoplastic lesions in the nose were observed in rats at a concentration of 0.5% 1,4-dioxane and higher.

### 6.1.3 *Carcinogenicity: dermal exposure and other routes of exposure*

The Committee noted the low quality of the animal carcinogenicity studies on dermal exposure and administration of 1,4-dioxane by intraperitoneal injection. For this reason, the Committee considers these studies not relevant in assessing the carcinogenic properties of the substance.

## 6.2 Human information

*Table 13* Summary of human studies.

| Method | Population | Exposure level | Results and remarks | Quality and/or reliability of study | References |
|---|---|---|---|---|---|
| Cross sectional study; Germany | 74 workers exposed to unspecified airborne levels for 3-41 years | Concentrations up to 54 mg/m$^3$ | No evidence of liver of kidney cancer no higher cancer deaths than population at large. Two pensioned employees died and were diagnosed cancer: squamous epithelial carcinoma and myelofibrosis leukaemia | Low (secondary source, no other study details given) | Thiess et al., 1976 (source EU risk assessment report 2002)[47] |

| | | | | | |
|---|---|---|---|---|---|
| Mortality follow-up study; USA, chemical company plant | 165 employees exposed to 1,4-dioxane since 1954 | < 25 ppm (~ 90 mg/m$^3$), during 28-89 months | Manufacturing department: seven deaths, two from cancer (expected 4.9 and 0.9); processing department: five deaths of which one from cancer (expected 4.9 and 0.8) | Low | Buffler et al., 1978[58] |
| Retrospective study | 80 men | 0.18-184 mg/m$^3$ for some years | No signs of exposure related health effects | Low (secondary source, no other study details given) | Barber, 1934 (source EU risk assessment report 2002)[47] |

Data on human carcinogenicity are shown in Table 13. The Committee noted the low quality of study reporting, in that data were obtained from secondary sources, and that study details were missing. Also, the size of the cohorts, and thus the power of the studies, were low. In none of the studies evidence for carcinogenicity due to occupational exposure to 1,4-dioxane could be assessed.

## 6.3 Other relevant information

*Table 14* Initiation/promotion and cell transformation studies.

| Method | Cell type | Concentration | Results and remarks | Klimisch Score* | References |
|---|---|---|---|---|---|
| *Initiation/promotion studies* | | | | | |
| Mice, SENCAR | 25-40 females/dose; early papilloma development as potential predictor of carcinoma yields | 1,000 mg/kg bw oral, subcutaneous, or dermal for 2 wks, followed by 1 µg TPA dermal 3x/wk for 20 wks. A single dose of 1,000 mg/kg bw in a satellite group followed by acetone dermal served as negative control. TPA is a tumour promotor | - | 2 | Bull et al., 1986[59] |
| Rat SD | 8-9 male/group GGT-enzyme altered foci of hepatocytes determined 10 days after last treatment sacrifice and staining liver sections for GGT | Partial hepatectomy of rats was followed by 30 mg intraperitoneal treatment with diethynitrosamine DENA/kg (initiator). Thereafter treatment with 0, 100 and 1,000 mg 1,4-dioxane/kg bw (gavage 1/d, 5 times/wk for 7 weeks. Controls with and without DENA initiation included | + (Increase in number and total volume of foci only at toxic doses of 1,000 mg/kg bw) | 2 | Lundberg et al., 1987[60] |

| | | | | | |
|---|---|---|---|---|---|
| Mice, Swiss-Webster | 30/sex/group; study duration 78 weeks. Gross necropsy and histopathology | 50 µg DMBA (dimethyl-benzanthracene) for 1 wk, as initiator, followed by 3 applications/wk of 0.2 mM 1,4-dioxane solution on shaved back for 78 wks. Acetone was the negative control and croton oil the positive control | + *Neoplastic lesions* of skin, lung and kidney in survivors: 4 papillomas (2m, 2f); 6 suspected carcinomas (3m, 3f); 2(m) subcutaneous tumours.<br><br>Skin tumours increased sharply after 10 weeks. No skin tumours observed after dermal application in absence of DMBA initiation (Table 8).<br><br>*General*: mortality up to 25/36 after 60 weeks | 3 (limited test design no haematology clinical biochemistry; minimal reported; purity not specified) | King et al., 1973[55] |
| *Cell transformation* | | | | | |
| | Balb/3T3 cells | 0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 mg/ml; 48 hr and 13 days treatment; positive and negative controls included | + (at cytotoxic concentrations of 2 mg/ml) | 2 | Sheu et al., 1988[61] |
| | Balb/3T3 cells | + and -S9 | - (with and without S9) | 4 | Microbial Associates 1980 (source EU-risk assessment report)[47] |
| Liver pre-neoplastic marker (glutathione S-transferase, placental form); cell proliferation (PCNA-positive index); see Table 4. | *Gpt* delta transgenic rats, males; 30 animals divided in four groups (no. of animals per group not given) | 0, 200, 1,000 or 5,000 ppm in drinking water for up to 16 weeks; at the end of treatment all animals were killed, and livers excised for further analyses | - (0 to 1,000 ppm)<br>+ (5,000 ppm) for GST-P-positive foci ($p$<0.001), and PCNA-positive cell index ($p$<0.001) | 4 (poster abstract only; no details on methods or outcomes reported) | Fukushima et al., 2009[38] |

* See Annex H.

As summarized in Table 14, 1,4-dioxane was clearly positive in a liver foci assay, (Lundberg et al*.,* 1987), while a mouse skin papilloma test with a single dose of 1,4-dioxane was negative (Bull et al., 1986).[59,60] No peroxisomal proliferation activity was observed after oral dosing with 1,4-dioxane (1% and 2% in drinking water for 5 days in two studies; Goldsworthy et al*.,* 1991, see Table 4).[30]

## 6.4 Summary and discussion of carcinogenicity

Only a few epidemiological studies are available concerning the carcinogenic properties of 1,4-dioxane; they show no indications for carcinogenicity. However, as these studies have limited power, the Committee is of the opinion that the human data are insufficient for conclusions.

Two carcinogenicity studies have been conducted, in which rats were exposed by inhalation to 1,4-dioxane vapour. In a recent study (Kasai et al., 2009), male F344/DuCrj rats were exposed to 1,4-dioxane concentrations of 180, 900 and 4,500 mg/m$^3$ (50, 250 and 1,250 ppm) for 2 years, 6 h/day, 5 days/wk.[49] In this study, an increased incidence of squamous cell carcinoma in the nasal cavity and hepatocellular adenoma in the liver was observed after exposure to 4,500 mg/m$^3$. Moreover, the incidence of peritoneal mesothelioma was statistically significant increased (dose dependently) after exposure to 900 and 4,500 mg/m$^3$ as well. Non-neoplastic and pre-neoplastic changes in the nasal cavity (nuclear enlargement of the olfactory and respiratory epithelium, and atrophy and metaplasia of the olfactory epithelium) were observed at the lowest exposure level, 180 mg/m$^3$, and above. In the inhalation study of Torkelson, Wistar rats were exposed to 400 mg 1,4-dioxane/m$^3$ for 7 hours a day, five days a week for a total of 2 years (Torkelson et al., 1974).[50] The substance did not induce neoplastic lesions, probably because the exposure was too low. Moreover, the nasal cavity was not examined. Therefore, the Committee decided that this study cannot be used to indicate a lack of carcinogenic potential of 1,4-dioxane.

1,4-Dioxane has been shown to be carcinogenic in several drinking water studies in rats, mice and guinea pigs (Kano et al., 2008, 2009).[48,62] The target organs were the liver, and nasal cavities, while also peritoneal mesothelioma were induced. The relevance of the effects on the nasal cavity for humans after exposure via drinking water was questioned by Stickney et al., (2003).[63] Although the nasal lesions and nasal tumours were consistently seen after exposure to 1,4-dioxane through the drinking water, such lesions could result from water entering the nasal cavity when the animals drink from sipper bottles (Sweeney et al., 2008).[10] However, because nasal tumours were also observed after inhalatory exposure in rats, these are considered relevant for humans by the Committee.

## 6.5 Comparison with criteria

According to the criteria in Annex VI of the European regulation No. 1272/2008 substance, classification as a known or presumed human carcinogen is warranted when positive evidence for carcinogenicity is obtained in humans (category 1A), or rodents (category 1B). In humans, no evidence for carcinogenicity is found. However, the Committee is of the opinion that the studies of Kasai et al., 2009 and Kano et al., 2008, 2009 show consistent carcinogenic effects (hepatocellular adenoma, squamous cell carcinoma in the nasal cavity and peritoneal mesothelioma) after exposure to dioxane by inhalation and via drinking water respectively.[48,49,62] Because of these sound positive studies of Kasai et al., 2009 and Kano et al., 2008, 2009, the Committee recommends classifying 1,4-dioxane as a substance that is presumed to have carcinogenic potential for humans. This corresponds with a classification in category 1B.

The Committee noticed that from 2000, the European Commission classified the substance as a carcinogen in category 2 (according to the current CLP-system). The classification was based on other carcinogenicity studies as described in the present report.

## 6.6 Conclusions on classification and labelling

Based on the available data, the Committee concludes that 1,4-dioxane is presumed to be carcinogenic to man, and recommends classifying the substance for carcinogenicity in category 1B.*

* See for classification system Annex F.

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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 |
| H | Criteria for testing reliability of animal and in vitro studies |

# 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
- T.M.M. Coenen, *scientific secretary*
  Health Council of the Netherlands, The Hague

With respect to the data presentation and interpretation, the Committee consulted an additional expert, J.J.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 *1,4-Dioxane*
Your Reference: DGV/BMO/U-932542
Our reference : U- 866033/DC/fs/246-W20
Enclosed : 2
Date : November 13, 2015

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to 1,4-dioxane.

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 2015 for public review. The following organisations and persons have commented on the draft document:

- H. Stengel, BASF SE, Ludwigshafen, Germany
- T.J. Lentz, P. Joseph, National Institute for Occupational Safety and Health (NIOSH), USA.

All comments received and the response of the Committee will be publicly available (www.gezondheidsraad.nl) from the moment of presentation of the final report.

Annex **E**

# IARC evaluation and conclusion

## 1,4-dioxane (Group 2B)

VOL.: 71 (1999) (p. 589).[5]

Summary of Data Reported and Evaluation.

### Exposure data

Exposure to 1,4-dioxane may occur during its manufacture and its use as a solvent in a wide range of organic products. It has been detected in ambient air.

### Human carcinogenicity data

Deaths from cancer were not elevated in a single, small prospective study of workers exposed to low concentrations of dioxane.

### Animal carcinogenicity data

1,4-Dioxane was tested for carcinogenicity by oral administration in mice, rats and guinea-pigs. It produced an increased incidence of hepatocellular adenomas and carcinomas in mice, tumours of the nasal cavity, liver subcutaneous tissues, mammary gland and peritoneal mesotheliomas in rats and tumours of the liver

and gall-bladder in guinea-pigs. No increase in tumours was seen in rats following inhalation exposure. In the mouse-lung adenoma assay, intraperitoneal injection of 1,4-dioxane increased the incidence of lung tumours in males; no such effect was seen following oral administration. In a two-stage liver foci assay in rats, 1,4-dioxane showed promoting activity.

### Other relevant data

1,4-Dioxane is rapidly absorbed upon inhalation or after oral administration, but its penetration of skin is poor. The major metabolite is β-hydroxyethoxyacetic acid, which is rapidly excreted. In rats, the elimination of 1,4-dioxane and its metabolites is progressively delayed as doses are increased, indicating saturation of metabolism. No clinical signs or changes in mortality were found in a cohort of exposed workers. In rats, 1,4-dioxane produces degenerative and necrotic changes in liver and renal tubules. High doses can significantly increase the total hepatic cytochrome P450 content. No reproductive effects of 1,4-dioxane exposure of rats have been reported. Most of the broad of tests for genotoxic activity have produced negative results, but positive results were obtained in a cell transformation assay and conflicting results were obtained in mouse bone-marrow cell tests for micronucleus induction.

### Evaluation

There is inadequate evidence in humans for the carcinogenicity of 1,4-dioxane. There is sufficient evidence in experimental animals for the carcinogenicity of 1,4-dioxane.

### Overall evaluation

1,4-dioxane is possibly carcinogenic to humans (Group 2B).

Previous evaluations: Vol. 11 (1976); Suppl. 7 (1987).
Synonyms: ‘1,4-diethylene dioxide’.

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] | |
|---|---|---|---|
| | | (before 16 December 2008) | (as from 16 December 2008) |
| 1A | The compound is known to be carcinogenic to humans.<br>• It acts by a stochastic genotoxic mechanism.<br>• It acts by a non-stochastic genotoxic mechanism.<br>• It acts by a non-genotoxic mechanism.<br>• 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.<br>• It acts by a stochastic genotoxic mechanism.<br>• It acts by a non-stochastic genotoxic mechanism.<br>• It acts by a non-genotoxic mechanism.<br>• 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: 2010; publication no. A10/07.

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*

3.5.1.1A 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.

3.5.1.2The 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*

3.5.2.1This 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.

3.5.2.2For 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 Specific considerations for classification of substances as germ cell mutagens*

3.5.2.3.1To 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.

3.5.2.3.2The 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:<br>• positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or<br>• 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<br>• 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:<br>• positive evidence obtained from experiments in mammals and/ or in some cases from in vitro experiments, obtained from:<br>• somatic cell mutagenicity tests in vivo, in mammals; or<br>• other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.<br>*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. |
|---|---|

3.5.2.3.3Classification 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.

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

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

3.5.2.3.5In vivo somatic cell mutagenicity tests, such as:

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

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

h mutagenicity tests:
  - mammalian spermatogonial chromosome aberration test;
  - spermatid micronucleus assay;

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

3.5.2.3.7Genotoxicity tests in somatic cells such as:

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

3.5.2.3.8In vitro mutagenicity tests such as:

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

3.5.2.3.9The 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*

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

3.5.3.1.1The 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.

| | Concentration limits triggering classification of a mixture as: | | |
|---|---|---|---|
| Ingredient classified 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).

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

3.5.3.2.1Classification 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.

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

3.5.3.3.1Where 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*

3.5.4.1Label 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 tumourigenesis 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).

Annex

# H

# Criteria for testing reliability of animal and in vitro studies

To assess the reliability of animal and in vitro studies, the Committee uses the criteria set by Klimisch et al., 1997.[64] A summary of the criteria of the reliability scores is given below. Only studies with a reliability score of 1 or 2 are considered in assessing genotoxicity and carcinogenicity.

## 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.).

## 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 opinions 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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