CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,4-dioxane

EC Number: **204-661-8**

CAS Number: **123-91-1**

Index Number: **603-024-00-5**

Contact details for dossier submitter:

RIVM, The Netherlands

National Institute for Public Health and the Environment

Centre for Safety of Substances and Products

Bilthoven, The Netherlands

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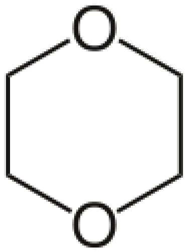
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1,4-dioxane
Other names (usual name, trade name, abbreviation)	1,4-dioxacyclohexane; diethylene dioxide; diethylene ether; diethylene-1,4-dioxide; dioxane; dioxyethylene ether; glycolethylene ether; NE 220; p-dioxane; tetrahydro-1,4-dioxane; tetrahydro-p-dioxane
EC number (if available and appropriate)	204-661-8
EC name (if available and appropriate)	1,4-dioxane
CAS number (if available)	123-91-1
Molecular formula	$C_4H_8O_2$
Structural formula	
Molecular weight or molecular weight range	88.12 g/mol
Degree of purity (%) (if relevant for the entry in Annex VI)	≥99.8 % (w/w)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	References
1,4-dioxane EC no.: 204-661-8	>99% w/w	Flam. Liq. 2 Eye Irrit. 2 STOT SE 3 Carc. 2		(EU 2002)

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling	References

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling	References
Water (CAS-No.7732-18-5)	≤0.1% w/w			No	(EU 2002)
2-methyl-1,3-dioxolane (CAS-No.497-26-7)	≤0.1% w/w		Flam. Liq. 2 Eye Irrit. 2	No	(EU 2002)
2-ethyl-1,3-dioxolane (CAS-No.2568-96-9)	≤0.03% w/w			No	(EU 2002)
hydrogen peroxide (CAS-No.7722-84-1)	≤0.001% w/w	Ox. Liq. 1 Acute Tox. 4 Skin Corr. 1A Note B		No	(EU 2002)
non volatile components	≤0.03% w/w			No	(EU 2002)

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	References
In stabilised dioxane 2,6-di-tert-butyl-p-cresol is found.				Aquatic Chronic 1 Aquatic Acute 1	(EU 2002)

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-024-00-5	1,4-dioxane	204-661-8	123-91-1	Flam. Liq. 2 Eye Irrit. 2 STOT SE 3 Carc. 2	H225 H319 H335 H351	GHS07 GHS02 GHS08 Dgr	H225 H319 H335 H351	EUH019 EUH066		Note D
Dossier submitters proposal					Carc. 1B Muta. 2	H350 H341	GHS08 Dgr	H350 H341			
Resulting Annex VI entry if agreed by RAC and COM	603-024-00-5	1,4-dioxane	204-661-8	123-91-1	Flam. Liq. 2 Eye Irrit. 2 STOT SE 3 Carc. 1B Muta. 2	H225 H319 H335 H350 H341	GHS07 GHS02 GHS08 Dgr	H225 H319 H335 H350 H341	EUH019 EUH066		Note D

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity		Yes
Carcinogenicity		Yes
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

1,4-Dioxane is classified for carcinogenicity in Annex VI of regulation (EC) No 1272/2008 as follows: Carc 2 (suspected human carcinogen; H351: suspected of causing cancer). This substance is not classified for germ cell mutagenicity. The classification by the European Commission dates from August 2001 (2001/59/EC (28th ATP)) and was based on a proposal by The Netherlands using the data in the Risk Assessment Report. In 1999, the International Agency for Research on Cancer (IARC) concluded that there was inadequate evidence in humans to conclude on the carcinogenicity of 1,4-dioxane, and that there was sufficient evidence in experimental animals. Therefore, IARC classified the compound in Group 2B (possibly carcinogenic to humans) (IARC 1999). This proposal for changing the harmonised classification of 1,4-dioxane is based on an update report of the Health Council of the Netherlands.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL.

Justification that action is needed at Community level.

Change in existing entry due to new data.

Further detail on need of action at Community level

More information on the mutagenic and carcinogenic properties of 1,4-dioxane has become available in recent years, which warrants a more severe classification for carcinogenicity compared to the current harmonised classification.

The Health Council of the Netherlands published an evaluation of this substance in 2011 and concluded that 1,4-dioxane should be regarded as carcinogenic to humans (comparable with CLP category 1B), and considered the substance as non-genotoxic carcinogen (HCN 1987, 2011).

In 2015, the Health Council performed a re-evaluation of the mutagenic and carcinogenic properties of 1,4-dioxane, which included more recent studies. In this re-evaluation, additional studies where provided that confirmed the carcinogenic properties of 1,4-dioxane. This re-evaluation by the Health Council forms the basis for the current proposal for an update of the harmonised classification of 1,4-dioxane from Cat. 2 to Cat. 1B (H350) for carcinogenicity and inclusion of a classification as Muta 2 (H341) for germ cell mutagenicity.

5 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. 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 (Chemicals 1977).

6 DATA SOURCES

This CLH report is based on a recent report of the Health Council of the Netherlands, "1,4-Dioxane - Re-evaluation of the carcinogenicity and genotoxicity", No. 2015/26, The Hague, November 13, 2015. Starting point of their report were the monographs of the International Agency for Research on Cancer (IARC) and the registration dossier at the European Chemicals Agency (ECHA).

Other sources as cited in the text and tables are mentioned in the reference list (13. References).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Colourless liquid	(ATSDR 2012)	
Melting/freezing point	11.8°C	(ATSDR 2012)	
	12°C	(EU 2002)	

Property	Value	Reference	Comment (e.g. measured or estimated)
Boiling point	100.1°C	(ATSDR 2012)	
	101°C	(EU 2002)	
Relative density	1.0329	(ATSDR 2012)	
	1.034	(EU 2002)	
Vapour pressure	38.1 mm Hg at 25°C	(ATSDR 2012)	
	40 hPa at 20°C	(EU 2002)	
Surface tension	-	(ATSDR 2012)	
	33.2 mN/m	(EU 2002)	
Water solubility	Miscible	(ATSDR 2012)	
	Miscible in all mixtures	(EU 2002)	
Partition coefficient n-octanol/water	Log K_{OW} -0.27	(ATSDR 2012; EU 2002)	
Flash point	5-18°C	(ATSDR 2012)	
	11°C	(EU 2002)	
Flammability	Limits at 25 °C lower: 2.0%; upper: 22%	(ATSDR 2012)	
	Highly Flammable (R11 and R19)	(EU 2002)	
Explosive properties	Vapour forms explosive mixture with air over wide range	(ATSDR 2012)	
	Not explosive		
Self-ignition temperature	180°C	(ATSDR 2012)	
Oxidising properties	None	(ECHA 2015; EU 2002)	
Granulometry	-	(ECHA 2015)	
Stability in organic solvents and identity of relevant degradation products	Yes	(ECHA 2015)	
Dissociation constant	No dissociating properties	(ECHA 2015)	
Viscosity	1.27 mm^2/s at 20°C; 0.93 mm^2/s at 40°C	(ECHA 2015)	

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of non-human toxicokinetic studies

Method	**Results**	**Observations and remarks (Klimisch score)***	**Reference**
Rat (Sprague-Dawley) male (n=4) Inhalation: vapour Exposure regime: once for 6 hr Doses/conc.: 50 ppm Equivalent or similar to OECD Guideline 417 (Toxicokinetics) ^{14}C-1,4 dioxane Purity > 99%	Metabolites identified: yes Details on metabolites: The amounts of 1,4-dioxane and β-hydroxyethoxyacetic acid (HEAA) in urine during exposure (0-6h) were 5.1 and 7613 µg, respectively, and afterwards (6-48 h) 1.7 and 13659 µg, respectively. Hence, more than 99.9% of the total urinary excretion of the inhaled 1,4-dioxane was HEAA.	2	(Young, Braun, and Gehring 1978)
Rat (Sprague-Dawley) male (n=2-3) Oral: gavage Exposure regime: single and repeated (17 daily doses) dosing Doses/conc.: single dosing: 10, 100 or 1000 mg/kg, no of animals 3/dose group Repeated dosing: 10 and 1000 mg/kg, no of animals, 2/dose group Equivalent or similar to OECD Guideline 417 (Toxicokinetics) ^{14}C-1,4 dioxane Purity > 99%	Metabolites identified: yes Details on metabolites: β-hydroxyethoxyacetic acid (HEAA; urine) CO_2 (expired air)	2	(Young, Braun, and Gehring 1978)
Four healthy volunteers Inhalation: vapour Exposure regime: once for 6 hr Single Dose/conc.: 50 ppm 1,4 dioxane Purity > 99% non-guideline study	Metabolites identified: yes Details on metabolites: β - hydroxyethoxyacetic acid (HEAA) Rapid uptake and elimination of parent and metabolite. Vapour caused eye irritation in 2/4 subjects	2	(Young *et al.* 1977)
Monkey, 3-6 animals/group (Pitman-Moore Rhesus monkeys) male/female Coverage (dermal absorption study): open Exposure regime: 24 hr Doses/conc.: dose: 4 mg/cm^2; area: 3-15 cm2. 1,4-dioxane with unknown purity. Monkeys were used in groups of between three and six. Site tested for skin penetration was the ventral forearm. Test substance (4 µg/sq . cm) was applied in methanol or a skin lotion . The skin-contact area ranged from 3 to 15 sq. cm^2. In each experiment, a radiotagged (^{14}C) chemical was applied to the uncovered skin of a restrained, clipped animal and was removed after 24 hr by washing with soap and water. Urine was collected over a 5-day period and was analysed for the radiolabel. A metabolism cage	Absorption: One and five minutes after treatment with 1,4-dioxane in skin lotion 36% and 15% of the applied dose, respectively, were still detectable on the skin. Total recovery: Within 24 hours after treatment 2.3% and 3.4% of the applied radioactivity were excreted via the urine. Percutaneous absorption rate: > 2.3- < 3.4 % at 24 (based on urine excretion only (e.g. does not include % in skin))	2	(Marzulli, Anjo, and Maibach 1981)

Method	Results	Observations and remarks (Klimisch score)*	Reference
was used for collecting animal urine.			

*(Klimisch, Andreae, and Tillmann 1997)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Absorption

Inhalation and oral

Four healthy volunteers inhaled 50 ppm 1,4-dioxane (180 mg/m^3) for 6 hours. Samples of blood and the urine were collected during and after exposure at 8h intervals and examined for the presence of 1,4 dioxane, as well as the metabolite β-hydroxyethoxyacetic acid (HEAA) (Young et al. 1977). 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).

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 1,4-dioxane, which was applied non occlusively as a methanol solution or as lotion on the forearm skin, was excreted in the urine (Marzulli, Anjo, and Maibach 1981). *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).

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 1,4-dioxane to rats, ^{3}H label was found in all tissues investigated at comparable levels between 1 and 16 hours after administration (Woo, Arcos, and Argus 1977; Woo, Argus, and Arcos 1978). Mikheev *et al.* report similar findings (Mikheev, Gorlinskaya, and Solovyova 1990).

Elimination and pharmacokinetics

In humans exposed for 6 hours to 180 mg 1,4-dioxane/m^3 (in a chamber under dynamic airflow conditions) 1,4-dioxane in plasma rapidly accumulated to nearly steady state after 4 hours of exposure (figure 1), (Young et al. 1977). After 5 hours of exposure, the metabolite was measured for the first time (it is not clear if they attempted earlier detection or if earlier samples did not yield detectable levels of HEAA). 1,4 Dioxanewas excreted in urine as the metabolite 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 HEAA; the remainder was unchanged 1,4-dioxane (Young *et al*. 1977). After the 6 hr exposure period the plasma 1,4-dioxane concentration decreased exponentially, indicating that the elimination was not saturated. The plasma elimination $T_{½}$ was 59 minutes (Young *et al*. 1977).

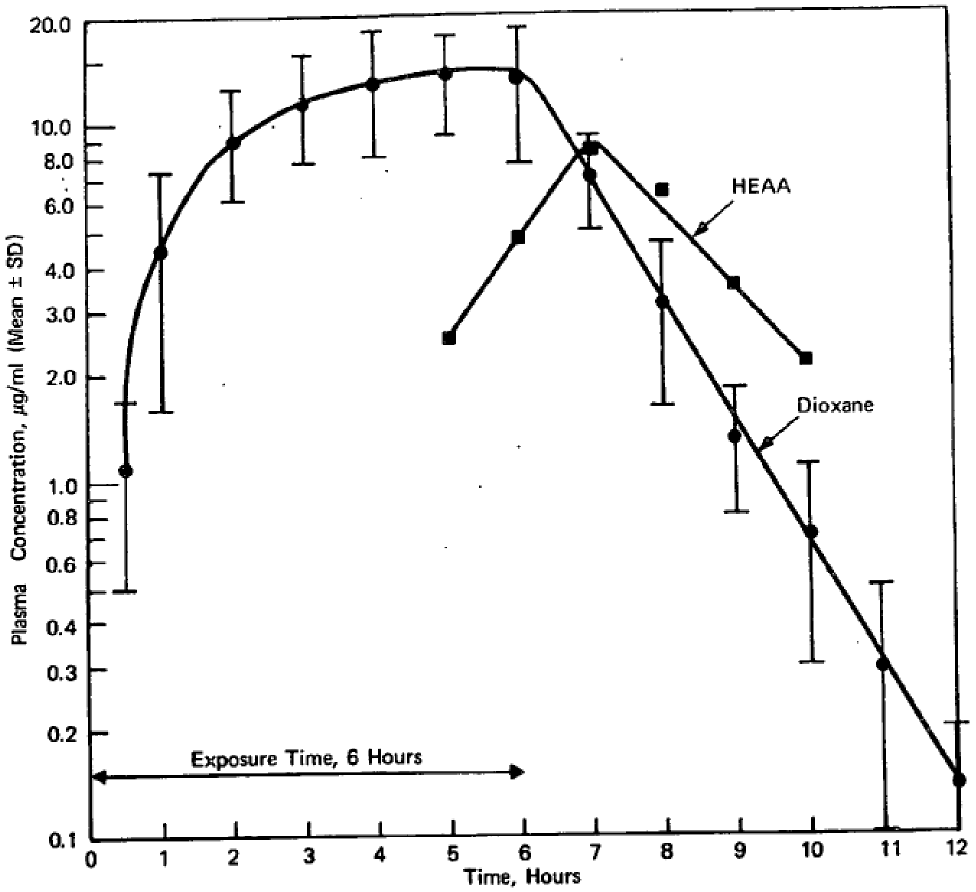


FIGURE 1. Plasma concentration-time curves for dioxane and HEAA for humans exposed to 50 ppm dioxane vapor for 6 hr. Dioxane concentrations are mean ± SD, $n = 4$. HEAA concentrations are averages for two to three individuals.

Physiologically-based pharmacokinetic (PB-PK) models were developed by Reitz *et al.* and Leung and Paustenbach (Reitz *et al*. 1990; Leung and Paustenbach 1990), which were further improved by Sweeney *et al*. (Sweeney *et al*. 2008). The plasma concentrations as well as HEAA urinary excretion after exposure to 1,4-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 . Only the urinary excretion data of Young *et al*. were well predicted by the model (Young, Braun, and Gehring 1978). A physiologically based pharmacokinetic modelling study indicates that 1,4-dioxane may also be excreted into human milk (Fisher *et al*. 1997).

1,4-Dioxane is rapidly excreted in rats via the urine. The major metabolite is HEAA (Woo, Arcos, and Argus 1977; Woo, Argus, and Arcos 1978). At low pH, HEAA is rearranged (reversibly) to 1,4-dioxan-2-one.

Metabolism

1,4-Dioxane is metabolized by cytochrome P-450’s, possibly of the 2A and 2D family (Sweeney *et al*. 2008). Induction of the cytochrome P-450 enzymes increases the rate of HEAA formation, whereas inhibition decreases HEAA formation (Woo, Arcos, and Argus 1977; Woo, Argus, and Arcos 1978). Repeated oral administration of 1,000 mg/kg of 1,4-dioxane induced 1,4-dioxane metabolism in rats, but at doses of 10 mg/kg no such effect was observed (Young, Braun, and Gehring 1978).

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 (i.e., nonlinear metabolism/pharmacokinetics) seemed to occur above 200 mg/kg (Sweeney *et al*. 2008).

In rats, the plasma concentration of 1,4 dioxane and the main metabolite HEAA were measured every 8h. 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 or 1000 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, Stott, and Ramsey 1982; Reitz *et al*. 1990; Young, Braun, and Gehring 1978).

Young, Braun, and Gehring (1978). observed a statistically significant increase of $^{14}CO_2$ excretion at multiple oral doses of ^{14}C-labelled 1,4-dioxane compared to the control; it is unclear as yet how this mechanistically reflects metabolism of 1,4-dioxane . It has been suggested by the Scientific Committee on Occupational Exposure Limits (SCOEL) that at high dose another, presumably reactive metabolite of 1,4-dioxane, β-hydroxyethoxyacetaldehyde (HEA) might be responsible for (cyto)toxicity: in the toxicity studies, morphological and biochemical changes were observed at exposure concentrations which lead to saturation of the metabolism (SCOEL 2004). 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 (SCOEL 2004).

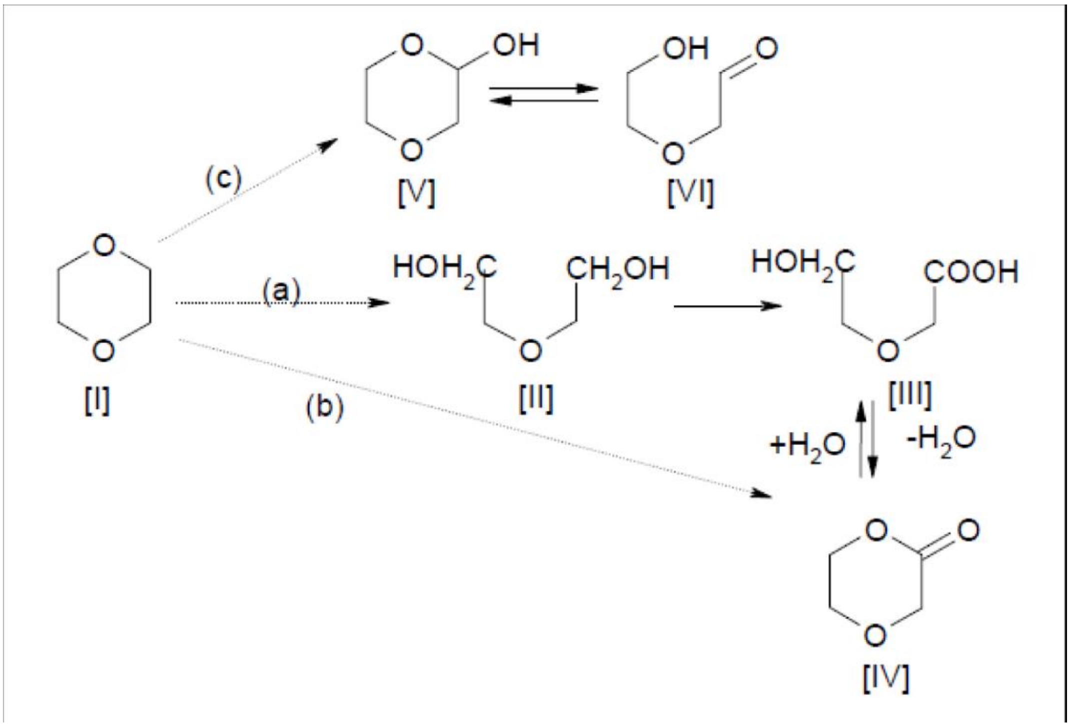


Figure 2 Suggested metabolic pathways of 1,4-dioxane in the rat (Woo et al. 1977). [I], 1,4-dioxane; [II], diethylene glycol; [III], -hydroxyethoxy acetic acid (HEAA); [IV], 1,4-dioxane-2-one; [V], 1,4-dioxane-2-ol; [VI] -hydroxyethoxy acetaldehyde (HEA). Note: Metabolite [V] is a likely intermediate 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.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated in this dossier.

10.2 Acute toxicity - dermal route

Not evaluated in this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

10.4 Skin corrosion/irritation

Not evaluated in this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

Not evaluated in this dossier.

10.8 Germ cell mutagenicity

10.8.1 Non-human information

In vitro data

The data on *in vitro* mutagenicity testing as summarized in Table 9 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.

Table 9: Summary of *in vitro* mutagenicity studies

Method	**Cell type**	**Concentration range***	**Results** **- negative** **+ positive**	**Klimisch Score****	**References**
Micro-organisms					
Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537 E. coli WP2uvrA and WP2	0, 156, 313, 625, 1,250, 2,500, and 5,000 µg/plate +/- preincubation	-	2	(Morita and Hayashi 1998)
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, Quast, and Watanabe 1981)
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)
Reverse mutation	S. typhimurium TA100, TA1535	0, 10, 31, 103 mg/plate preincubation	-	3 (only two strains; methodological deficiencies)	(Nestmann et al. 1984)
Reverse mutation	S. typhimurium TA98, TA100, TA1530, TA1535, TA1537	Dose levels not provided	-	3 (dose levels not provided)	(Khudolei, Mizgirev, and Pliss 1986)
Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	4, 20, 100, 500, 2,500 µg/plate	-	2	(ECHA 2015)
Mammalian cells					
Gene mutation	Mouse lymphoma L5178Y cells, tk locus	0, 1,250, 2,500 and 5,000 µg/ml: 3 and 24 hr exposure	- (slight decrease in relative survival at 5,000 µg/ml +S9)	2	(Morita and Hayashi 1998)
Gene mutation	Mouse lymphoma L5178Y cells, tk locus	0, 312.5, 625, 1,250, 2,500, 5,000 µg/ml (-S9) 0, 1,000, 2,000, 3,000, 4,000, 5,000 µg/ml (+S9)	-	2	(McGregor et al. 1991)
Gene mutation	Chinese hamster ovary, K1 cells	0.05, 0.1, 0.5, 1.0, 5.0, 10.0 mg/ml	-	2	(ECHA 2015)

Method	**Cell type**	**Concentration range***	**Results - negative + positive**	**Klimisch Score****	**References**
Micronucleus	Chinese hamster ovary, K1 cells	0, 1,250, 2,500 and 5,000 µg/ml: 5 and 44 hr exposure (+/-S9)	-	2	(Morita and Hayashi 1998)
Chromosome aberration	Chinese hamster ovary, K1 cells	0, 1,250, 2,500 and 5,000 µg/ml (+/-S9)	-	2	(Morita and Hayashi 1998)
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 cytotoxicit y)	(Galloway et al. 1987)
Other supporting studies					
Sister chromatid exchange	CHO-K1 cells	0, 1250, 2,500 and 5,000 µg/ml (+/- S9) 3 and 26 hr exposure	- (dose-related cytotoxicity observed)	2	(Morita and Hayashi 1998)
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); - (+S9)	3 (no data on purity, negative control or cytotoxicit y)	(Galloway et al. 1987)
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)
UDS	Rat primary hepatocytes	10-8 to 1 M	-	3 (methodolo gical deficiencie s)	(Stott, Quast, and Watanabe 1981)
'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 (methodolo gical deficiencie s)	(Sina et al. 1983)
DNA damage (Mutatox assay)	Photobacterium phosphoreum M169 (strain sensitive to DNA damaging agents, DNAintercalating agents, DNA-synthesis inhibitors, and direct mutagens).	Not specified; -S9 only	-	4 (no standard test, relevance unknown; concentrati ons not specified)	(ATSDR 2012)
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)	(Zimmermann et al. 1985)

* + or - S9, with or without metabolic activation system. ** (Klimisch, Andreae, and Tillmann 1997) *** Comet assay and alkaline elution assay: DNA single and double strand breaks, DNA cross-links.

In vivo data

Data on the *in vivo* mutagenicity testing are presented in Table 10.

Table 10: Summary of *in vivo* mutagenicity studies (animal studies)

Method	**Animal**	**Exposure conditions**	**Results**	**Klimisch Score***	**References**
Somatic cell mutagenicity					

Method	Animal	Exposure conditions	Results	Klimisch Score*	References
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)
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)
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)
	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, Thilagar, and Eastmond 2005)
	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)
	CBA mice, male bone marrow; 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 hepatectomy 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	3 (method not validated: partial hepatectomy to stimulate mitosis)	(Morita and Hayashi 1998)

Method	Animal	Exposure conditions	Results	Klimisch Score*	References
			positive and negative controls		
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)
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)
Meiotic nondisjunction	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)	(Muñoz and Mazar Barnett 2002)
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)	(ECHA 2015)
Other supporting studies					
UDS	Male rat liver F344 and primary hepatocytes	1% (1,500 mg/kg bw/day) in drinking water for 1 week (pre-treatment 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)
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	
UDS	SD rat liver; 4 rats/group	1,000 mg/kg bw (14C oral gavage)	-	3 (no positive control; (methodological deficiencies)	(Stott, Quast, and Watanabe 1981)
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) 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)
Replicative DNA synthesis (marker	Male F344 rats; 4/group; hepatocytes isolated after	Gavage; 1,000, 1,500, 2,000 and 4,000 mg/kg bw; 24 hr and 48 hr	+ (24 hr-response time: dose-related increase	2	(Miyagawa et al. 1999)

Method	Animal	Exposure conditions	Results	Klimisch Score*	References
for cell proliferation)	exposure for testing	response time; thymidine and BrdU incorporation	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) (48 hr-response time; no hepatocytotoxicity)		
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)
DNA alkylation	SD rat liver; 4-6 males/group	1,000 mg/kg bw 14C (gavage); DNA isolation from hepatocytes and HPLC analysis	-	3 (positive control missing; (methodological deficiencies; limited study)	(Stott, Quast, and Watanabe 1981)
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.)
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)	(Hellmér and Bolcsfoldi 1992)

* (Klimisch, Andreae, and Tillmann 1997) ** 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.

Germ cells

No acceptable 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).

Somatic cells

As summarized in Table 10, 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.

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). 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 1994) reported a statistically significant dose-related increase in bone marrow cells with micronuclei from 900 mg/kg bw/day and (Roy, Thilagar, and Eastmond 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. Decreases in bone marrow cell proliferation were also observed. (Roy, Thilagar, and Eastmond 2005) also observed that the induced micronuclei are formed primarily from chromosomal breakage.

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).

The majority of the animal studies reported no data on cytotoxicity, 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. Nevertheless, statistically significant dose-related positive findings were observed in micronuclei in bone marrow at doses below the limit dose of 2,000 mg/kg bw (Mirkova 1994; Roy, Thilagar, and Eastmond 2005), indicating that 1,4-dioxane may have genotoxic potential.

Other *in vivo* studies have also been summarized in Table 10. (Kitchin 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. 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, Quast, and Watanabe 1981). 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. 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.

10.8.2 Human information

In Table 11 data are shown on 1,4-dioxane exposure in humans.

Table 11: Summary of human studies

Methods	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, Tress, and Fleig 1976)

* (Klimisch, Andreae, and Tillmann 1997)

10.8.3 Summary and discussion of mutagenicity

Below, only data are summarized of reliable experimental design according to the Klimisch criteria 1 and 2 (Klimisch, Andreae, and Tillmann 1997). Notably, no studies have been found investigating the mutagenic potential of the (proposed) metabolites of 1,4 dioxane.

Germ cell genotoxicity
As no genotoxicity studies of 1,4-dioxane in germ cells were found, it is not possible 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. 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 9) 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. As these positive findings cannot be ignored, 1,4-dioxane may also have a genotoxic potential. Aneuploidy was not observed. The majority of the supportive *in vivo* genotoxicity tests (Table 10) 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 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 genotoxic mechanism as secondary mode of action cannot be excluded. In conclusion, 1,4-dioxane is mutagenic *in vivo* in mammalian cells.

10.8.4 Comparison with the CLP criteria

According to the criteria in Annex VI of the European regulation No. 1272/2008, 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 acceptable data have been presented on human or animal germ cell mutagenicity. 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”. 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, it is recommended to classify 1,4-dioxane in category 2.

10.8.5 Conclusions on classification and labelling for germ cell mutagenicity

Based on the available data, it is recommended to classify 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).

10.9 Carcinogenicity

Data on animal carcinogenicity studies are summarized in Table 12.

Table 12: Summary table of animal studies on carcinogenicity

Species	Design	Exposure levels	Observations and remarks (Klimisch score)*	Reference
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 According to OECD TG 453 *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)	0, 50, 250, 1,250 ppm (v/v) (calculated as 0, 180, 900 and 4,500 mg/ m3) by inhalation (whole body vaporization technique); Actual exposure levels were: 50.2 + 1.4 250.9 + 3.2 1,247.5 + 18.6 ppm	Klimisch-score: 1 *Neoplastic lesions:* + Significant induction of nasal squamous cell carcinomas, hepatocellular adenomas, peritoneal mesotheliomas and subcutis fibroma (see Table 13). *General:* The terminal survival rates of the control, 50, 250, and 1,250 ppm-exposed groups were 37/50, 37/50, 29/50, and 25/50, respectively. At 1,250 ppm terminal body weights decreased, relative liver weight increased and plasma ALT, AST and gamma-GTP enzyme activities increased. *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 propria and proliferation nasal gland within exposed groups. At 1,250 ppm necrosis of hepatocytes and	(Kasai *et al.* 2009)

Species	Design	Exposure levels	Observations and remarks (Klimisch score)*	Reference
			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 *Neoplastic lesions*: - No substance-related tumours found. *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)
Oral administration				
Rat F344/DuCrj	50 animals/sex/group; study duration 104 weeks; haematology, clinical biochemistry, gross necropsy and histopathological examination According to OECD TG 451	0, 0.02, 0.1, 0.5% (w/w) in drinking water (*ad libitum*) Actual dose levels: m: 0, 11, 55, 274 mg/kg bw/day; f: 0, 18, 83, 429 mg/kg bw/day	Klimisch-score: 2 *Neoplastic lesions:* + 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 14). *General:* Significantly decreased survival rates at 0.5% (m: 22/50; f: 24/50), 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	(Kano *et al*. 2009)
Mouse Crj:BDF1	50 animals/sex/group; study duration 104 weeks; haematology, clinical biochemistry, gross necropsy and histopathological examination According to OECD TG 451	0, 0.05, 0.2, 0.8% w/w) in drinking water (*ad libitum*). Actual dose levels: m: 0, 49, 191, 677 mg/kg bw/day; f: 0, 66, 278, 964 mg/kg bw/day	Klimisch-score: 2 *Neoplastic lesions:* + Significant induction of hepatocellular tumours in both sexes. Two nasal tumours in the highest dose groups for tumour incidences (see Table 15). *General:* Significantly decreased survival rates at 0.2 and 0.8% (29/50). 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	(Kano *et al*. 2009)
Rat Sherman	60 animals/sex/ group; study duration 716 days; haematology, gross necropsy and histopathological examination	0, 0.01, 0.1, 1% in drinking water (*ad libitum*) Actual dose levels m: 0, 9.6, 94, 1,015 mg/kg bw/day f: 0, 19, 148, 1,599 mg/kg bw/day	Klimisch-score: 2 *Neoplastic lesions:* + Treatment related hepatocellular carcinomas and nasal squamous cell carcinomas (see Table 16). *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 (66/120, 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	(Kociba, McCollister, and Park 1974)

Species	Design	Exposure levels	Observations and remarks (Klimisch score)*	Reference
			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%. No difference between control and exposed animals on haematology	
Rat 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*). Actual dose levels m: 0, 240, 530 mg/kg bw f: 0, 350, 640 mg/kg bw	Klimisch-score: 2 *Neoplastic lesions:* + Significant induction of nasal squamous cell carcinomas in males and females and hepatocellular adenomas in females (see Table 17). *General:* survival rate males: 33/35 high dose group, 26/35 low dose group, females: 29/35 high dose group, 30/35 low dose group; no clinical signs other than fluctuations in mean body weights of males probably due to mortality. *Histopathology*: Tubular degeneration in kidney Liver cytomegaly Gastric ulceration of stomach: - m: 0/33, 5/28, 5/30 Pneumonia: - m: 8/30, 15/31, 14/33 - f: 6/30, 5/34, 25/32	(NCI 1978)
Mouse 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*). Actual dose levels m: 0, 720, 830 mg/kg bw/day f: 0, 380, 860 mg/kg bw/day	Klimisch-score: 2 *Neoplastic lesions:* + Significant induction of hepatocellular adenomas or carcinomas in females and males (see Table 18). *General:* survival rates males: 45/50 high dose group, 46/50 low dose group, females: 28/50 high dose group, 39/50 low dose group. Pneumonia: - m: 1/49, 9/50, 17/47 - f: 2/50, 33/47, 32/36 Rhinitis: - m: 0/49, 1/50, 1/49 - f: 0/50, 7/48, 8/39 No clinical signs other than altered body weights	(NCI 1978)
Rat 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,	Klimisch-score: 3 *Neoplastic lesions:* - *Non-neoplastic lesions:* Nasal cavity, squamous cell carcinomas (0, 0.75, 1.0, 1.4, 1.8%): 0/30, 1/30,1/30, 2/30, 2/30	(Hoch-Ligeti, Argus, and Arcos 1970)

Species	Design	Exposure levels	Observations and remarks (Klimisch score)*	Reference
		803, 1,032 mg/kg bw/day)		
Rat 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 *Neoplastic lesions*: *(0 and 1%, respectively)*: - Lymphosarcoma: 1/9, 0/26 - Liver tumours: 0/9, 6/26 - Kidney cell carcinoma: 0/9, 1/26 Histological changes in liver	(Argus, Arcos, and Hoch-Ligeti 1965)
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 *Neoplastic lesions*: - *General*: Survival rate male rats high dose: 24/35, low dose: 26/35, female rats high dose: 20/35, low dose: 32/35; Survival rate male mice high dose: 50/50, low dose: 49/50, female mice high dose: 49/50, low dose: 49/50; increased weight gain in male rat and mice; histopathological lesions of lung and liver in rats only	(King, Shefner, and Bates 1973)
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 Oral: 0 and 24,000 mg/kg bw 3 applications/wk for 8 weeks, followed by 16 wks observation	Klimisch-score: 3 *Neoplastic lesions:* Intraperitoneal, lung tumours (0, 4,800, 12,000, 24,000, respectively): - m: 1/14, 1/16, 6/16, 2/11 - f: 7/15, 3/16, 5/16, 3/13 Oral, lung tumours (0 and 24,000, respectively): - m: 51/135 and 4/15 - f: 32/131 and 5/14 General: survival rate males ip: 4,800 mg/kg bw: 16/16, 12,000 mg/kg bw: 16/16, 24,000 mg/kg bw: 11/16, females ip: 4,800 mg/kg bw:16/16, 12,000 mg/kg bw: 16/16, 24,000 mg/kg bw: 13/16; survival rate males oral: 15/16, females: 14/16	(Stoner *et al*. 1986)
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 *Neoplastic lesions:* Lung tumours in %(0, 400, 1,000, and 2,000 respectively): 33, 17, 48, and 62	(Maronpot *et al*. 1986)
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 *Neoplastic lesions*: no papilloma, one malignant lymphoma. One suspected carcinoma (f) and one subcutaneous tumour (m) *General*: increase in male body weight	(King, Shefner, and Bates 1973)
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 *General*: Mortality only in rats; increased weight gain in male rat and mice. Histopathologic lesions in the lung and liver in rats only.	(King, Shefner, and Bates 1973)

*(Klimisch, Andreae, and Tillmann 1997)

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 (0, 50, 250, and 1250 ppm (v/v), respectively), for 6 hours a day, 5 days/week for 104 weeks (Kasai *et al*. 2009). Details on tumour incidences are shown in Table 13, a comprehensive summary is presented in Annex I. Shortly, 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. In the study by Torkelson *et al*. Wister 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). The substance did not induce neoplastic lesions.

Table 13: Tumour incidences in male rats exposed to 1,4-dioxane for 2 years (Kasai *et al*. 2009)

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: * $p \leq 0.05$, ** $p \leq 0.01$

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 12). 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 14 to 18. In addition, 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. A comprehensive summary of the study by (Kasai et al. 2009) is shown in Annex I.

Table 14: Tumour incidences in rats exposed to 1,4-dioxane for 2 years (Kano *et al*. 2009)

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 15: Tumour incidences in mice exposed to 1,4-dioxane for 2 years (Kano *et al*. 2009)

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 16: Tumour incidences in male and female rats (combined) exposed to 1,4-dioxane for 2 years (Kociba, McCollister, and Park 1974)

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 17: Tumour incidences in rats exposed to 1,4-dioxane for 2 years (NCI 1978)

Exposure level (%, w/w, 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 18: Tumour incidences in mice exposed to 1,4-dioxane for 2 years (NCI 1978)

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

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

Carcinogenicity: dermal exposure and other routes of exposure

Considering the low quality of the animal carcinogenicity studies on dermal exposure and intraperitoneal injection, these studies are not included in assessing the carcinogenic properties of 1,4-dioxane.

Human carcinogenicity

Data on human carcinogenicity are shown in Table 19. It should be noted that the quality of these studies are low, data is frequently obtained from secondary sources, and study details are missing. In addition, the size of the cohorts, and thus the power of the studies, is low. In none of the studies evidence for carcinogenicity due to occupational exposure to 1,4-dioxane could be assessed.

Table 19: Summary table of human data on carcinogenicity

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 or 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, Tress, and Fleig 1976)
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)
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)	(NIOSH 1977)

Other relevant information

As summarized in Table 20, 1,4-dioxane is 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 is negative (Bull, Robinson, and Lauri 1986). No peroxisomal proliferation activity was observed after oral dosing with 1% and 2% 1,4-dioxane in drinking water for 5 days followed by hepatocyte incubation (Goldsworthy *et al*. 1991).

Table 20: Summary table of other studies relevant for carcinogenicity

Method	**Cell type**	**Concentration**	**Results and remarks**	**Klimisch score***	**Reference**
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, Robinson, and Lauri 1986)
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 rat 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)

Method	Cell type	Concentration	Results and remarks	Klimisch score*	Reference
Mice, Swiss-Webster	30/sex/group; study duration 78 weeks. Gross necropsy and histopathology	50 µg DMBA (dimethylbenzanthracene) 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. Skin tumours increased sharply after 10 weeks. No skin tumours observed after dermal application in absence of DMBA initiation (Table 11). *General*: mortality up to 25/36 after 60 weeks	3 (limited test design no haematology clinical biochemistry; minimal reported; purity not specified)	(King, Shefner, and Bates 1973)
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)
	Balb/3T3 cells	+ and -S9	- (with and without S9)	4	(EU 2002)
Liver preneoplastic marker (glutathione Stransferase, placental form); cell proliferation (PCNA-positive index).	*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) + (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)
Other supporting studies					
Unscheduled DNA synthesis test (UDS)	Male rat liver F344 and primary oocytes	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)

*(Klimisch, Andreae, and Tillmann 1997)

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

A few human 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 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 the study by Kasai et al. (2009), male F344/DuCrj rats were exposed to 1,4-dioxane concentrations of 180, 900 and 4,500 mg/m^3 (which equals 50, 250 and 1,250 ppm, respectively) for 2 years, 6 h/day, 5 days/wk. 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. Nonneoplastic 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 *et al*., 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). The substance did not induce neoplastic lesions, probably because the exposure level was too low. Moreover, the nasal cavity was not examined. Therefore, 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*. 2009; Kano *et al*. 2008). Although the target organs were liver and nasal cavities, peritoneal mesothelioma were also induced. The relevance of the effects on the nasal cavity for humans after exposure via drinking water was questioned by Stickney *et al*. (Stickney *et al*. 2003). 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). However, as nasal tumours were also observed after inhalatory exposure in rats, these are considered relevant for humans.

In summary, three tumour types have been observed in the literature: peritoneal mesothelioma, hepatocellular adenoma/carcinoma and squamous cell carcinoma. Significant effects have been observed for all three endpoints in the highest dose groups. Peritoneal mesothelioma’s are already observed to a significant extent in the mid-dose group of rats (exposure by inhalation, 250ppm). When exposed to 1,4 dioxane via the drinking water, the incidence of hepatocellular adenoma’s and carcinoma’s is higher compared to peritoneal mesothelioma’s in contrast to exposure via inhalation. The incidence of squamous cell carcinoma’s in the nasal cavity seems similar for both exposure routes. Additionally, the incidence of liver tumours is statistically increased in the mid-dose group of the study with mice (orally exposed) by Kano *et al*. 2009. However no peritoneal mesothelioma’s were reported.

To help determine the carcinogenic potency of 1,4 dioxane and set substance specific exposure limits, T25 values according to EC (1999) were determined. The most sensitive endpoint in the studies by Kano et al. (2009) and Kasai et al. (2009) were used as these are the key studies available.

Kasai et al (2009).
Species and exposure route: Rats, inhalation.
Endpoint: Peritoneal mesothelioma in 14/50 (28%) rats at 250 ppm by inhalation (second highest dose is closer to T25 than the incidence in the high dose group), and 2/50 (4%) at 0 mg/kg bw/day
Net incidence: 14x((100/50)-2x(100/50))/(100-2x(100/50)) = 25%
Daily dose: 250.9 ppm, at a rate of 6 l/h 6h/day with a weight of 500g (males only). The air concentration in mg/m^3 is 250.9 ppm x 0.0409 x 88.12 g/mol = 904.27 mg/m^3. Therefore the exposure is 904.27/1000 x 6l/h x 6h/day = 32.6 mg/day equal to 65.2 mg/kg bw/day in the male rats assuming 100% uptake.
Exposure frequency: 5/7 days per weeks
Exposure duration: 104 weeks is considered the general life span of the rats, therefore no correction is necessary.
T25: 65.2 mg/kg bw/day x 25/25x 5/7 x 104/104 = 46.6 mg/kg bw/day

Kano et al (2009)
Species and exposure route: Rats, oral administration.
Endpoint: Hepatocellular adenoma and carcinoma, 48/50 (96%) (f) at 429 mg/kg bw/day and 39/50 (78%)(m) at 274 mg/kg bw/day and 3/50 at 0 mg/kg bw/day (m/f)
Net incidence females: 48x((100/50)-3x(100/50))/(100-3x(100/50)) = 93.8%
Net incidence males: 39x((100/50)-3x(100/50))/(100-3x(100/50)) = 76.6%
Daily dose females: 429 mg/kg bw/day, Daily dose males: 274 mg/kg bw/day
Exposure frequency: 7 days/week
Exposure duration: 104 weeks
T25 females: 429 mg/kg bw/day x 25/93.8 x 7/7 x 104/104 = 114.3 mg/kg bw/day
T25 males: 274 mg/kg bw/day x 25/76.6 x 7/7 x 104/104 = 89.4 mg/kg bw/day

Kano et al (2009)
Species and exposure route: Mice, oral administration.
Endpoint: Hepatocellular adenoma and carcinoma, 35/50 (70%) (f) at 49mg/kg bw/day and 37/50 (74%)(m) at 191 mg/kg bw/day. Control incidence was 5/50, 10% (f) or 23/50, 46% (m)
Net incidence females: 35x((100/50)-5x(100/50))/(100-5x(100/50)) = 66.7%
Net incidence males: 37x((100/50)-23x(100/50))/(100-23x(100/50)) = 51.8%
Daily dose females: 49 mg/kg bw/day, Daily dose males: 191 mg/kg bw/day
Exposure frequency: 7 days/week

Exposure duration: 104 weeks
T25 females: 49 mg/kg bw/day x 25/66.7 x 7/7 x 104/104 = 18.4 mg/kg bw/day
T25 males: 191 mg/kg bw/day x 25/51.8 x 7/7 x 104/104 = 92.2 mg/kg bw/day

Note that for the calculations of T25 it was assumed the potency is linearly related to the exposure which may not necessarily be true. However, the T25 calculated from the inhalation study are in the same potency range as listed by the EC (1999). Therefore no influence of a possible non-linear relationship between dose and effect is expected. Significant tumor incidence was only observed in the highest dose group of the orally dosed mice (Kano et al. 2009). Therefore the calculated T25 may be less accurate since the tumor incidence was larger than 25% (51-96% depending on species and gender).
According to the EC (1999) and the criteria of the previous Directive 67/548/EEC, the carcinogenic potency of 1,4 dioxane should be medium (between 1-100 mg/kg bw/day) and since the substance induces several tumor types across genders via multiple exposure routes it can be considered of medium potency. Therefore, no SCL is required.

10.9.2 Comparison with the CLP criteria

According to the criteria in Annex I of the European regulation No. 1272/2008 substance classification as a known human carcinogen (Category 1A) is warranted if there are human studies that establish a causal relationship between human exposure to a substance and the development of cancer. As no evidence of carcinogenicity was observed in humans, classification as Cat. 1A is not warranted.
Classification as Cat. 1B carcinogen is usually based on animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). The studies by Kasai et al. (2009) and Kano et al. (2009)show consistent carcinogenic effects (hepatocellular adenoma, squamous cell carcinoma in the nasal cavity and peritoneal mesothelioma) in rodents after exposure to 1,4-dioxane by inhalation and via drinking water, respectively . Histopathological effects were observed in the liver and the nasal epithelium in the repeated dose toxicity tests (see section 10.12) and in the chronic tests. No non-tumor histopathological effects were reported for the rat peritoneum. Notably, this tissue is not normally assessed in toxicological studies. The available data suggest a contribution of cell proliferation which may be secondary to local necrosis. However, the available data is limited especially for organs other than liver (EPA, 2013). Therefore, it has to be assumed that the observed increase in tumors is relevant for humans. Because of these sound positive studies in several species in multiple organs it is recommended to classify 1,4-dioxane as a substance that is “presumed to have carcinogenic potential for humans”, which corresponds to classification in category 1B.
Carcinogen cat. 2 can be proposed if the evidence available is indicative only and not very clear. The new data presented here is clear evidence of carcinogenic effects in multiple species in multiple organs, therefore 1,4-dioxane should be categorized as Carc. 1B, rather than Carc. 2.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the available data, it is concluded that 1,4-dioxane is presumed to be carcinogenic to man, and recommended to classify the substance for carcinogenicity in category 1B. An SCL is not required.

10.10 Reproductive toxicity

Not evaluated in this dossier.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Note that the studies summarized here are supportive for the carcinogenicity studies and assessment while classification for STOT RE is not assessed in this dossier.

Table 21 Summary table of animal data on STOT RE.

Species	Design	Exposure levels	Observations and remarks (Klimisch score)*	Reference
Inhalation				
Rat F344/Du Crj	10 rats/sex/ dose group; study duration: 6 h/day, 5 days/wk for 13 weeks; hematology, clinical biochemistry, gross necropsy and histopathological examination Substance: 1,4 dioxane, purity >99% Study performed according to OECD TG 413 (1981)	0, 100, 200, 400, 800, 1600, 3200 or 6400 ppm (v/v) (calculated as 0, 360, 72, 1440, 2880, 5760, 11420 or 230400 mg/ m3) by inhalation (vapour)	Klimisch-score: 2 *General:* All 6400 ppm exposed males died in the first week, primarily caused by renal failure. All other dose groups survived until week 13 without abnormal clinical signs. Terminal body weights significantly decreased in 200 & 3200 ppm (m) and 200 and ≥800 ppm (f). Relative liver weight increased at ≥800 ppm (m/f), relative kidney weight was increased at ≥800 ppm (f) and at ≥3200 ppm (m). Relative lung weights were increased in 200 and ≥1600 ppm (m) and ≥200 ppm (f). ALT was increased in 3200 ppm (m/f) and AST was increased in 200 and 3200 ppm (f). Glucose and trygliceride levels were decreased in 3200 ppm males. *Histopathology:* Increased incidences of nuclear enlargement in respiratory (at >100 ppm) , olfactory (at >200 ppm) and Trachea epithelia (at ≥1600 ppm) (m/f). Nuclear enlargement also at ≥ 1600 ppm (m) or ≥3200 ppm (f) in Bronchial epithelium. Single cell necrosis of hepatocytes was found in males at 3200 ppm. Centrilobular swelling of hepatocytes was found (m/f) at 3200 ppm. GST-P positive liver foci were observed in 3/10 males and 2/10 females at 3200 ppm and in 4/10 females at 1600 ppm. Hydropic changes in renal proximal tubule were observed at 3200 ppm (f)	(Kasai et al. 2008)
Rat F344/Du Crj	10 animals/sex/group; study duration 13 weeks; haematology, clinical biochemistry, gross necropsy and histopathological examination. Substance: 1,4 dioxane, purity >99% Study performed according to OECD TG 408 (1981)	0, 640, 1600, 4000, 10000 and 25000 ppm (w/w) in drinking water Actual dose levels: m: 0, 52, 126, 274, 657 and 1554 mg/kg bw/day; f: 0, 83, 185, 427, 756 and 1614 mg/kg bw/da.y	Klimisch-score: 2 *General:* one female died in the highest dose group during the second week due to renal failure. Food consumption decreased at 25000 ppm (m) and ≥10000 ppm (f). Water consumption was decreased at ≥4000 ppm (m/f). Terminal body weights reduced in ≥10000 ppm (m) and ≥4000 ppm (f). Relative kidney weight increased in ≥4000 ppm (m) and ≥1600 (f). Absolute kidney weight only increased at the highest dose (m/f). Relative lung weight increased at 25000 ppm (m) and at ≥10000 ppm in females. *Hematology*: RBC, haemoglobin, HTC, AST and ALT increased in the highest dose group (m). Decrease in blood glucose was seen in ≥10000 ppm (m+f). AST increased in females at 25000 ppm. Urinary pH decreased in ≥4000 ppm (m) and ≥10000 ppm (f). *Histopathology;* nuclear enlargement in nasal respiratory epithelium at ≥1600 ppm (m/f) followed by enlarged nuclei of epithelial cells in olfactory epithelium and tracheal and bronchial epithelium. Centrilobular swelling occurred at ≥1600 ppm (m) and single cell necrosis and inflammatory cell infiltration increased in 4000 and 25000 ppm (m) and 25000 ppm (f). GST-P positive foci were found in all animals (m/f) of	(Kano et al. 2008)

Species	Design	Exposure levels	Observations and remarks (Klimisch score)*	Reference
			the highest dose group. Nuclear enlargement of renal proximal tubule epithelial cells at ≥10000 ppm (m/f) and hydropic change in the proximal tubules was seen at 25000 ppm (m/f). Vacuolic changes in the cerebrum were noted at 25000 ppm (m/f) in the corpus callosum, hippocampus and dentate gyrus.	
Mouse Crj:BDF 1	10 animals/sex/group; study duration 13 weeks; haematology, clinical biochemistry, gross necropsy and histopathological examination. Substance: 1,4 dioxane, purity >99% Study performed according to OECD TG 408 (1981)	0, 640, 1600, 4000, 10000 and 25000 ppm (w/w) in drinking water Actual dose levels: m; 0, 86, 231, 585, 882, 1570 mg.kg bw/day. f; 0, 170, 387, 898, 1620, 2669 mg/kg bw/day	Klimisch-score: 2 *General:* One male mouse died at 25000 ppm during the second week (unknown cause). Food consumption decreased at 25000 ppm (m) and water consumption decreased ≥10000 ppm (m/f). Terminal body weights only reduced at 25000 ppm (m). Relative kidney and lung weight was increased in 25000 ppm (m/f). *Hematology: RBC, Hb and HTC increased in high dose males.* AST was increased at 25000 ppm (m/f) and ALT was increased at ≥10000 ppm (f). decreases in glucose levels were observed at 10000 ppm (f) and 25000 ppm (m/f). Urinary pH was decreased at ≥10000 ppm (m/f. *Histopathology:* nuclear enlargement in bronchial epithelium at ≥1600 ppm (f) and at ≥4000 ppm (m). Nuclear enlargement in olfactory epithelium at ≥4000 ppm (m/f), and respiratory epithelium at 25000 ppm (f). single cell necrosis was increased at ≥4000 ppm (m/f). Centrilobular swelling occurred at ≥4000 ppm (m/f) and vacuolic change in the olfactory nerve (lamina propria), was observed at 25000 ppm (m/f)	(Kano et al. 2008)

*(Klimisch, Andreae, and Tillmann 1997)

10.12.1 Short Summary and overall relevance of the provided information on Repeated dose toxicity and carcinogenicity

Two subchronic repeated dose studies are available from Kasai et al. (2008) (inhalation route in F344 rats, OECD TG 413) and Kano et al. (2008) (oral administration in BDF 1 mice and F344 rats, OECD TG 408), that served as dose range finder studies for their long term carcinogenicity studies. Both studies found nuclear enlargement in several epithelial tissues along the respiratory tract (olfactory, respiratory, tracheal and bronchial). However, there is a clear difference in the location of the 1,4-dioxane induced enlarged nuclei of the nasal epithelial cells between the exposure routes (inhalation or oral administration) (Kasai et al. 2008). The respiratory epithelial area having the enlarged nuclei was expanded from the anterior portion to the entire region with inhaled 1,4 dioxane. On the other hand, the oral administration of 1,4-dioxane-formulated drinking water uniformly produced the nuclear enlargement over the entire region of the respiratory epithelium without any anterior–posterior gradient along the nasal passage. This difference in the route of exposure can be accounted for in terms of a first-pass effect such that the inhaled 1,4-dioxane comes into first contact with the anterior portion of the respiratory epithelium, while the orally administered 1,4-dioxane is conveyed to the respiratory epithelial cells through the nasal blood flow after its first entrance in the gastrointestinal system including the liver (Kasai et al. 2008). In line with this, centrilobular swelling of hepatocytes was observed at lower estimated body doses to 1,4 dioxane via the oral route (≥126 mg/kg bw/day) in comparison to exposure via inhalation (3200 ppm corresponding to an estimated 2336 mg/kg bw/day).
Other histopathological findings in the liver include single cell necrosis and GST-P positive liver foci at predominantly high doses. The summarized histopathological findings in the liver and nasal cavity, are in line with the observed carcinomas found in the longer term (2-year) studies reported by Kano et al. (2009) and Kasai et al. (2009).

10.12.2 Comparison with CLP criteria

Not evaluated in this dossier.

10.13 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Not evaluated in this dossier.

11.2 Environmental transformation of metals or inorganic metals compounds

Not evaluated in this dossier.

11.3 Environmental fate and other relevant information

Not evaluated in this dossier.

11.4 Bioaccumulation

Not evaluated in this dossier.

11.5 Acute aquatic hazard

Not evaluated in this dossier.

11.6 Long-term aquatic hazard

Not evaluated in this dossier.

11.7 Comparison with the CLP criteria

Not evaluated in this dossier.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Not evaluated in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 ADDITIONAL LABELLING

14 REFERENCES

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15 ANNEXES

15.1 Annex I

15.1.1 Summary of the study by (Kasai et al. 2009), adapted from (EPA 2013)

Groups of male 6-week-old F344/DuCrj rats (50/group) weighing 120 ± 5g (mean ± SD) at the beginning of the study were exposed via inhalation to nominal concentrations of 0 (clean air), 50, 250, and 1,250 ppm (0, 180, 900, and 4,500 mg/m3, respectively) of vaporized 1,4-dioxane (>99% pure) for 6 hours/day, 5 days/week, for 104 weeks (2 years) in whole body inhalation chambers (Kasai et al. 2009). Each inhalation chamber housed male rats individually in stainless-steel wire hanging cages. The authors stated female counterparts were not exposed given data illustrating the absence of induced mesotheliomas following exposure to 1,4-dioxane in drinking water (Yamazaki et al. 1994). During exposure, the concentration of 1,4-dioxane vapour was determined every 15 minutes by gas chromatography and animals received food and water ad libitum. In addition, during the 2-year exposure period, clinical signs and mortality were recorded daily. BW and food intake were measured once weekly for the first 14 weeks of exposure, and thereafter, every 4 weeks. At the end of the 2-year exposure period or at the time of an animal's death during exposure, all organs were collected, weighed, and evaluated for macroscopic lesions. Additional examinations were completed on rats sacrificed at the end of the 2-year exposure period. Endpoints examined included: 1) measurement of hematological and clinical chemistry parameters using blood collected from the abdominal aorta of rats following an overnight fasting at the end of the 2-year exposure period; 2) measurement of urinary parameters using Ames reagent strips during the last week of the exposure period; and 3) histopathological evaluations of organs and tissues outlined in the OECD test guideline which included all tissues of the respiratory tract. For measured hematological and clinical chemistry parameters, analyses included: red blood cell count, hemoglobin, hematocrit, MCV, mean corpuscular hemoglobin (MCH), AST, ALT, ALP, and γ-GTP. Organs and tissues collected for histopathological examination were fixed in 10% neutral buffered formalin with the exception of nasal cavity samples. Nasal tissue was trimmed transversely at three levels after decalcification and fixation in a formic acid-formalin solution. The levels were demarcated at the following points: at the posterior edge of the upper incisor teeth (level 1), at the incisive papilla (level 2), and at the anterior edge of the upper molar teeth (level 3). All tissue samples were embedded in paraffin, and then sectioned (at 5 μm thickness) and stained with hematoxylin and eosin (H&E). Dunnett's test, χ^2 test, and Fisher's exact test were used by study authors to determine statistical differences (*p*-value of 0.05) between 1,4-dioxane exposed and clean air exposed group data.

Deformity in the nose was the only clinical sign reported in this study. This deformity was seen at exposure weeks 74 and 79 in one rat each, exposed to 250 ppm and 1,250 ppm of 1,4-dioxane, respectively. Both of these rats did not survive the 2-year exposure with deaths caused by malignant nasal tumors.

Growth rates and survival rates were analyzed. Growth rates were not significantly affected by 1,4-dioxane exposures, but a decreasing trend in growth was observed during the latter half of the 2-year exposure period for all exposure doses (i.e., 50, 250, and 1,250 ppm). Survival rates were significantly decreased following 91 weeks of exposure to 1,250 ppm of 1,4-dioxane. The authors attributed these deaths to increased incidences of peritoneal mesotheliomas, but also noted that nasal tumors could have been a contributing factor. Terminal survival rates were 37/50, 37/50, 29/50, and 25/50 for 0, 50, 250, and 1,250 ppm exposed groups, respectively.

Exposure-related effects on final BWs, organ weights, and hematological and clinical chemistry parameters were reported. Changes in these effects, as compared to control are outlined in Table 21 and Table 22. Briefly, at 1,250 ppm terminal BWs were significantly decreased and relative liver and lung weights were significantly increased. It is of note that the observed change in terminal body weight was not an effect of food consumption, which was determined by the study authors to be unaltered. Altered hematological and clinical chemistry parameters were also observed with significant changes at 1,250 ppm. Altered endpoints included decreased hemoglobin, MCV, and MCH, and increased AST, ALT, ALP, and γ-GTP ($p \leq 0.01$) levels. In addition, urine pH was significantly decreased in 1,250 ppm exposed rats.

Histopathology findings of pre- and nonneoplastic lesions associated with 1,4-dioxane treatment were seen in the nasal cavity, liver, and kidneys (Table 23). At the highest concentration of 1,250 ppm, all pre- and nonneoplastic lesions were significantly increased, as compared to controls, with the exception of clear and mixed cell foci in the liver. At the lowest concentration of 50 ppm, nuclear enlargement of the respiratory epithelium was the most sensitive lesion observed in the nasal cavity. Based on this finding, the study authors identified a LOAEL of 50 ppm in male rats.

Tumor development was observed in the nasal cavity (squamous cell carcinoma), liver (hepatocellular adenoma and carcinoma), peritoneum (peritoneal mesothelioma), kidney (renal cell carcinoma), mammary gland (fibroadenoma and adenoma), Zymbal gland (adenoma), and subcutaneous tissue (subcutis fibroma). Tumor incidences with a dose-dependent, statistically significant positive trend (Peto's test) included nasal squamous cell carcinoma, hepatocellular adenoma, peritoneal mesothelioma, mammary gland fibroadenoma, and Zymbal gland adenoma. Renal cell carcinoma was also identified as statistically significant with a positive dose-dependent trend; however, no tumor incidences were reported at 50 and 250 ppm. At 1,250 ppm, significant increases in nasal squamous cell carcinoma, hepatocellular adenoma, and peritoneal mesothelioma were observed. At 250 ppm, significant increases in peritoneum mesothelioma and subcutis fibroma were observed. Table 24 presents a summary of tumor incidences found in this study. Further characterizations of neoplasms revealed nasal squamous cell carcinoma occurred at the dorsal area of the nose (levels 1-3) marked by keratinization and the progression of growth into surrounding tissue. Peritoneal mesotheliomas were characterized by complex branching structures originating from the mesothelium of the scrotal sac. Invasive growth into surrounding tissues was occasionally observed for peritoneal mesotheliomas.

Table 21: Terminal body and relative organ weights of F344/DuCrj male rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

	Males			
	1,4-dioxane vapor concentration (ppm)			
	0 (clean air)	50	250	1,250
Number of animals examined	**37**	**37**	**29**	**25**
Body weight (g)	383 ± 50	383 ± 53	376 ± 38	359 ± 29[b]
Lung (%)	0.45 ± 0.25	0.49 ± 0.27	0.45 ± 0.18	0.46 ± 0.07[a]
Liver (%)	3.57 ± 0.66	3.86 ± 1.05	3.58 ± 0.52	4.53 ± 0.71[b]
Kidneys (%)	0.87 ± 0.21	0.93 ± 0.32	0.81 ± 0.13	0.86 ± 0.12

[a]$p \leq 0.01$ by Dunnett's test.
[b]$p \leq 0.05$ by Dunnett's test.

Table 22: Hematology and clinical chemistry of F344/DuCrj male rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

	Males			
	1,4-dioxane vapor concentration (ppm)			
	0 (clean air)	50	250	1,250
Number of animals examined	**35**	**35**	**28**	**25**
Red blood cell (106/μL)	7.4 ± 1.8	6.8 ± 1.8	7.9 ± 1.0	7.0 ± 1.8
Hemoglobin (g/dL)	12.5 ± 3.5	12.0 ± 3.1	13.4 ± 1.9	10.9 ± 2.8[b]
Hematocrit (%)	38.6 ± 8.7	36.9 ± 7.9	40.7 ± 5.1	34.3 ± 7.6
MCV (fL)	52.4 ± 5.7	55.6 ± 8.7	51.8 ± 2.3	49.4 ± 4.0[b]
MCH (pg)	16.9 ± 2.2	17.8 ± 2.4	17.1 ± 1.2	15.5 ± 1.3[a]
AST (IU/L)	67 ± 31	95 ± 99	95 ± 116	98 ± 52[a]
ALT (IU/L)	37 ± 12	42 ± 21	49 ± 30	72 ± 36[a]
ALP (IU/L)	185 ± 288	166 ± 85	145 ± 71	212 ± 109[a]
γ-GTP (IU/L)	6 ± 3	8 ± 5	10 ± 8	40 ± 26[a]
Urinary pH	7.1 ± 0.6	7.1 ± 0.6	7.1 ± 0.6	6.6 ± 0.4[b]

[a]$p \leq 0.01$ by Dunnett's test.
[b]$p \leq 0.05$ by Dunnett's test.

Table 23: Incidence of pre-and nonneoplastic lesions in male F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

	1,4-dioxane vapor concentration (ppm)			
Effect	**0 (clean air)**	**50**	**250**	**1,250**
Nuclear enlargement; nasal respiratory epithelium	0/50	50/50[a]	48/50[a]	38/50[a]
Squamous cell metaplasia; nasal respiratory epithelium	0/50	0/50	7/50[b]	44/50[a]
Squamous cell hyperplasia; nasal respiratory epithelium	0/50	0/50	1/50	10/50[a]
Inflammation; nasal respiratory epithelium	13/50	9/50	7/50	39/50[a]
Nuclear enlargement; nasal olfactory epithelium	0/50	48/50[a]	48/50[a]	45/50[a]
Respiratory metaplasia; nasal olfactory	11/50	34/50[a]	49/50[a]	48/50[a]

Effect	1,4-dioxane vapor concentration (ppm)			
	0 (clean air)	50	250	1,250
epithelium				
Atrophy; nasal olfactory epithelium	0/50	40/50[a]	47/50[a]	48/50[a]
Inflammation; nasal olfactory epithelium	0/50	2/50	32/50[a]	34/50[a]
Hydropic change; lamina propria	0/50	2/50	36/50[a]	49/50[a]
Sclerosis; lamina propria	0/50	0/50	22/50[a]	40/50[a]
Proliferation; nasal gland	0/50	1/50	0/50	6/50[b]
Nuclear enlargement; liver centrilobular	0/50	0/50	1/50	30/50[a]
Necrosis; liver centrilobular	1/50	3/50	6/50	12/50[a]
Spongiosis hepatis; liver	7/50	6/50	13/50	19/50[a]
Clear cell foci; liver	15/50	17/50	20/50	23/50
Basophilic cell foci; liver	17/50	20/50	15/50	44/50[a]
Acidophilic cell foci; liver	5/50	10/50	12/50	25/50[a]
Mixed-cell foci; liver	5/50	3/50	4/50	14/50
Nuclear enlargement; kidney proximal tubule	0/50	1/50	20/50[a]	47/50[a]
Hydropic change; kidney proximal tubule	0/50	0/50	5/50	6/50[a]

[a]$p \leq 0.01$ by $\chi 2$ test.
[b]$p \leq 0.05$ by $\chi 2$ test.

Table 24: Incidence of tumors in male F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

Effect	1,4-dioxane vapor concentration (ppm)			
	0 (clean air)	50	250	1,250
Nasal squamous cell carcinoma	0/50	0/50	1/50	6/50[b,c]
Hepatocellular adenoma	1/50	2/50	3/50	21/50[a,c]
Hepatocellular carcinoma	0/50	0/50	1/50	2/50
Renal cell carcinoma	0/50	0/50	0/50	4/50[c]
Peritoneal mesothelioma	2/50	4/50	14/50[a]	41/50[a,c]
Mammary gland fibroadenoma	1/50	2/50	3/50	5/50[d]
Mammary gland adenoma	0/50	0/50	0/50	1/50
Zymbal gland adenoma	0/50	0/50	0/50	4/50[c]
Subcutis fibroma	1/50	4/50	9/50[a]	5/50

[a]$p \leq 0.01$ by Fisher's exact test.
[b]$p \leq 0.05$ by Fisher's exact test.
[c]$p \leq 0.01$ by Peto's test for dose-related trend.
[d]$p \leq 0.05$ by Peto's test for dose-related trend.

15.1.2 Summary of the study by (Kano et al. 2009), adapted from (EPA 2013)

Groups of F344/DuCrj rats (50/sex/dose level) were exposed to 1,4-dioxane (>99% pure) in the drinking water at levels of 0, 200, 1,000, or 5,000 ppm for 2 years. Groups of Crj:BDF1 mice (50/sex/dose level) were similarly exposed in the drinking water to 0, 500, 2,000, or 8,000 ppm of 1,4-dioxane. The high doses were selected based on results from the (Kano et al. 2008) 13-week drinking water study so as not to exceed the maximum tolerated dose (MTD) in that study. Both rats and mice were 6 weeks old at the beginning of the study. Food and water were available ad libitum. The animals were observed daily for clinical signs of toxicity; and BWs were measured once per week for 14 weeks and once every 2 weeks until the end of the study. Food consumption was measured once a week for 14 weeks and once every 4 weeks for the remainder of the study. The investigators used data from water consumption and BW to calculate an estimate of the daily intake of 1,4-dioxane (mg/kg-day) by male and female rats and mice. (Kano et al. 2009) reported a calculated mean ± standard deviation for the daily doses of 1,4-dioxane for the duration of the study. Male rats received doses of approximately 0, 11 ± 1, 55 ± 3, or 274 ± 18 mg/kg-day and female rats received 0, 18 ± 3, 83 ± 14, or 429 ± 69 mg/kg-day. Male mice received doses of 0, 49 ± 5, 191 ± 21, or 677 ± 74 mg/kg-day and female mice received 0, 66 ± 10, 278 ± 40, or 964 ± 88 mg/kg-day. For the remainder of this document, including the dose-response analysis, the mean calculated intake values are used to identify dose groups. The study was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) Principles for Good Laboratory Practice (GLP).

For rats, growth and mortality rates were reported for the duration of the study. Both male and female rats in the high dose groups (274 and 429 mg/kg-day, respectively) exhibited slower growth rates and terminal body weights that were significantly different ($p < 0.05$) compared to controls. A statistically significant reduction in terminal BWs was observed in high-dose male rats (5%, $p < 0.01$) and in high-dose female rats (18%, $p < 0.01$). Food consumption was not significantly affected by treatment in male or female rats; however, water consumption in female rats administered 18 mg/kg-day was significantly greater ($p < 0.05$). In female rats, relative liver weight was increased at 429 mg/kg-day. Significantly increased incidences of liver tumors (adenomas and carcinomas) and tumors of the nasal cavity occurred in high-dose male and female rats (Table 25 and Table 26) treated with 1,4-dioxane for 2.

For mice, growth and mortality rates were reported for the duration of the study. Similar to rats, the growth rates of male and female mice were slower than controls and terminal body weights were lower for the mid (p < 0.01 for males administered 191 mg/kg-day and p < 0.05 for females administered 278 mg/kg-day) and high doses (p < 0.05 for males and females administered 677 and 964 mg/kg-day, respectively). (Kano et al. 2009) reported a 10% incidence rate for hepatocellular adenomas and a 0% incidence rate for hepatocellular carcinomas in control female BDF1.

Table 25: Incidence of nasal cavity, peritoneum, and mammary gland tumors in F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 2 years

Effect	Males				Females			
Dose (mg/kg-day)	**0**	**11**	**55**	**274**	**0**	**18**	**83**	**429**
Nasal cavity								
Squamous cell carcinoma	0/50	0/50	0/50	3/50[a]	0/50	0/50	0/50	7/50[a,b]
Sarcoma	0/50	0/50	0/50	2/50	0/50	0/50	0/50	0/50
Rhabdomyosarcoma	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50
Esthesioneuroepithelioma	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
Peritoneum								
Mesothelioma	2/50	2/50	5/50	28/50[a,b]	1/50	0/50	0/50	0/50
Mammary gland								
Fibroadenoma	1/50	1/50	0/50	4/50[a]	3/50	2/50	1/50	3/50
Adenoma	0/50	1/50	2/50	2/50	6/50	7/50	10/50	16/50[a,c]
Either adenoma or fibroadenoma	1/50	2/50	2/50	6/50[a]	8/50	8/50	11/50	18/50[a,c]

[a]Statistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).
[b]Significantly different from control by Fisher's exact test ($p < 0.01$).
[c]Significantly different from control by Fisher's exact test ($p < 0.05$).

Table 26: Incidence of liver tumors in F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 2 years

Effect	Males				Females			
Dose (mg/kg-day)	**0**	**11**	**55**	**274**	**0**	**18**	**83**	**429**
Hepatocellular adenoma	3/50	4/50	7/50	32/50[a,b]	3/50	1/50	6/50	48/50[a,b]
Hepatocellular carcinoma	0/50	0/50	0/50	14/50[a,b]	0/50	0/50	0/50	10/50[a,b]
Either adenoma or carcinoma	3/50	4/50	7/50	39/50[a,b]	3/50	1/50	6/50	48/50[a,b]

[a]Significantly different from control by Fisher's exact test ($p < 0.01$).
[b]Statistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).

15.1.3 Summary of the study by (NCI 1978), adapted from (EPA 2013)

Groups of Osborne-Mendel rats (35/sex/dose) and B6C3F1 mice (50/sex/dose) were administered 1,4-dioxane (≥ 99.95% pure) in the drinking water for 110 or 90 weeks, respectively, at levels of 0 (matched controls), 0.5, or 1%.

Solutions of 1,4-dioxane were prepared with tap water. The report indicated that at 105 weeks from the earliest starting date, a new necropsy protocol was instituted. This affected the male controls and high-dose rats, which were started a year later than the original groups of rats and mice. Food and water were available ad libitum. Endpoints monitored in this bioassay included clinical signs (twice daily), BWs (once every 2 weeks for the first 12 weeks and every month during the rest of the study), food and water consumption (once per month in 20% of the animals in each group during the second year of the study), and gross and microscopic appearance of all major organs and tissues (mammary gland, trachea, lungs and bronchi, heart, bone marrow, liver, bile duct, spleen, thymus, lymph nodes, salivary gland, pancreas, kidney, esophagus, thyroid, parathyroid, adrenal, gonads, brain, spinal cord, sciatic nerve, skeletal muscle, stomach, duodenum, colon, urinary bladder, nasal septum, and skin). Based on the measurements of water consumption and BWs, the investigators calculated average daily intakes of 1,4-dioxane of 0, 240, and 530 mg/kg-day in male rats, 0, 350, and 640 mg/kg-day in female rats, 0, 720, and 830 mg/kg-day in male mice, and 0, 380, and 860 mg/kg-day in female mice. According to the report, the doses of 1,4-dioxane in high-dose male mice were only slightly higher than those of the low-dose group due to decreased fluid consumption in high-dose male mice.
During the second year of the study, the BWs of high-dose rats were lower than controls, those of low-dose males were higher than controls, and those of low-dose females were comparable to controls. The fluctuations in the growth curves were attributed to mortality by the investigators; quantitative analysis of BW changes was not done. Mortality was significantly increased in treated rats, beginning at approximately 1 year of study. Analysis of Kaplan-Meier curves (plots of the statistical estimates of the survival probability function) revealed significant positive dose-related trends (p < 0.001, Tarone test). In male rats, 33/35 (94%) in the control group, 26/35 (74%) in the mid-dose group, and 33/35 (94%) in the high-dose group were alive on week 52 of the study. The corresponding numbers for females were 35/35 (100%), 30/35 (86%), and 29/35 (83%). Nonneoplastic lesions associated with treatment with 1,4-dioxane were seen in the kidneys (males and females), liver (females only), and stomach (males only). Kidney lesions consisted of vacuolar degeneration and/or focal tubular epithelial regeneration in the proximal cortical tubules and occasional hyaline casts. Elevated incidence of hepatocytomegaly also occurred in treated female rats. Gastric ulcers occurred in treated males, but none were seen in controls. The incidence of pneumonia was increased above controls in high-dose female rats. The incidence of nonneoplastic lesions in rats following drinking water exposure to 1,4-dioxane is presented in Table 27. EPA identified the LOAEL in rats from this study as 240 mg/kg-day for increased incidence of gastric ulcer and cortical tubular degeneration in the kidney in males; a NOAEL was not established.

Table 27: Incidence of nonneoplastic lesions in Osborne-Mendel rats exposed to 1,4-dioxane in drinking water

	Males (mg/kg-day)			Females (mg/kg-day)		
	0	**240**	**530**	**0**	**350**	**640**
Cortical tubule degeneration	0/31[a]	20/31[b] (65%)	27/33[b] (82%)	0/31[a]	0/34	10/32[b] (31%)
Hepatocytomegaly	5/31 (16%)	3/32 (9%)	11/33 (33%)	7/31[a] (23%)	11/33 (33%)	17/32[b] (53%)
Gastric ulcer	0/30[a]	5/28[b] (18%)	5/30[b] (17%)	0/31	1/33 (3%)	1/30 (3%)
Pneumonia	8/30 (27%)	15/31 (48%)	14/33 (42%)	6/30[a] (20%)	5/34 (15%)	25/32[b] (78%)

[a]Statistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.05$) performed for this review.
[b]Incidence significantly elevated compared to control by Fisher's Exact test ($p < 0.05$) performed for this review.

Neoplasms associated with 1,4-dioxane treatment were limited to the nasal cavity (squamous cell carcinomas, adenocarcinomas, and one rhabdomyoma) in both sexes, liver (hepatocellular adenomas) in females, and testis/epididymis (mesotheliomas) in males. The first tumors were seen at week 52 in males and week 66 in females. The incidence of squamous cell carcinomas in the nasal turbinates in male and female rats is presented in Table 28. Squamous cell carcinomas were first seen on week 66 of the study. Morphologically, these tumors varied from minimal foci of locally invasive squamous cell proliferation to advanced growths consisting of extensive columns of epithelial cells projecting either into free spaces of the nasal cavity and/or infiltrating into the submucosa. Adenocarcinomas of the nasal cavity were observed in 3 of 34 high-dose male rats, 1 of 35 low-dose female rats, and 1 of 35 high-dose female rats. The single rhabdomyoma (benign skeletal muscle tumor) was observed in the nasal cavity of a male rat from the low-dose group. A subsequent re-examination of the nasal tissue sections by Goldsworthy et al. (Goldsworthy et al. 1991) concluded that the location of the tumors in the nasal apparatus was consistent with the possibility that the nasal tumors resulted from inhalation of water droplets by the rats.

Table 28: Incidence of nasal cavity squamous cell carcinoma and liver hepatocellular adenoma in Osborne-Mendel rats exposed to 1,4-dioxane in drinking water

	Males (mg/kg-day)[a]		
	0	240[b]	530
Nasal cavity squamous cell carcinoma	0/33 (0%)	12/33 (36%)	16/34 (47%)[e]
Hepatocellular adenoma	2/31 (6%)	2/32 (6%)	1/33 (3%)
	Females (mg/kg-day)[a]		
	0	**350**	**640**
Nasal cavity squamous cell carcinoma	0/34 (0%)[d]	10/35 (29%)[c]	8/35 (23%)[c]
Hepatocellular adenoma	0/31 (0%)[f]	10/33 (30%)[e]	11/32 (34%)[e]

[a]Tumor incidence values were not adjusted for mortality.
[b]Group not included in statistical analysis by NCI because the dose group was started a year earlier without appropriate controls.
[c]$p \leq 0.003$ by Fisher's Exact test pair-wise comparison with controls.
[d]$p = 0.008$ by Cochran-Armitage test.
[e]$p \leq 0.001$ by Fisher's Exact test pair-wise comparison with controls.
[f]$p = 0.001$ by Cochran-Armitage test.

The incidence of hepatocellular adenomas in male and female rats is presented in Table 28. Hepatocellular adenomas were first observed in high-dose females in week 70 of the study. These tumors consisted of proliferating hepatic cells oriented as concentric cords. Hepatic cell size was variable; mitoses and necrosis were rare. Mesothelioma of the vaginal tunics of the testis/epididymis was seen in male rats (2/33, 4/33, and 5/34 in controls, low-, and high-dose animals, respectively). The difference between the treated groups and controls was not statistically significant. These tumors were characterized as rounded and papillary projections of mesothelial cells, each supported by a core of fibrous tissue. Other reported neoplasms were considered spontaneous lesions not related to treatment with 1,4-dioxane.
In mice, mean BWs of high-dose female mice were lower than controls during the second year of the study, while those of low-dose females were higher than controls. In males, mean BWs of high-dose animals were higher than controls during the second year of the study. According to the investigators, these fluctuations could have been due to mortality; no quantitative analysis of BWs was done. No other clinical signs were reported. Mortality was significantly increased in female mice ($p < 0.001$, Tarone test), beginning at approximately 80 weeks on study. The numbers of female mice that survived to 91 weeks were 45/50 (90%) in the control group, 39/50 (78%) in the low-dose group, and 28/50 (56%) in the high-dose group. In males, at least 90% of the mice in each group were still alive at week 91. Nonneoplastic lesions that increased significantly due to treatment with 1,4-dioxane were pneumonia in males and females and rhinitis in females. The incidences of pneumonia were 1/49 (2%), 9/50 (18%), and 17/47 (36%) in control, low-dose, and high-dose males, respectively; the corresponding incidences in females were 2/50 (4%), 33/47 (70%), and 32/36 (89%). The incidences of rhinitis in female mice were 0/50, 7/48 (14%), and 8/39 (21%) in control, low-dose, and high-dose groups, respectively. Pair-wise comparisons of low-dose and high-dose incidences with controls for incidences of pneumonia and rhinitis in females using Fisher's Exact test (done for this review) yielded p-values < 0.001 in all cases. Incidences of other lesions were considered to be similar to those seen in aging mice. The authors stated that hepatocytomegaly was observed in dosed and control mice but did not comment on the significance of the effect. EPA concluded the LOAEL for 1,4-dioxane in mice was 380 mg/kg-day based on the increased incidence of pneumonia and rhinitis in female mice; a NOAEL was not established in this study.
As shown in Table 29, treatment with 1,4-dioxane significantly increased the incidence of hepatocellular carcinomas or adenomas in male and female mice in a dose-related manner. Tumors were first observed on week 81 in high-dose females and in week 58 in high-dose males. Tumors were characterized by parenchymal cells of irregular size and arrangement, and were often hypertrophic with hyperchromatic nuclei. Mitoses were seldom seen. Neoplasms were locally invasive within the liver, but metastasis to the lungs was rarely observed.

Table 29: Incidence of hepatocellular adenoma or carcinoma in B6C3F1 mice exposed to 1,4-dioxane in drinking water

	Males (mg/kg-day)[a]		
	0	720	830
Hepatocellular carcinoma	2/49 (4%)[b]	18/50 (36%)[c]	24/47 (51%)[c]
Hepatocellular adenoma or carcinoma	8/49 (16%)[b]	19/50 (38%)[d]	28/47 (60%)[c]
	Females (mg/kg-day)[a]		
	0	**380**	**860**
Hepatocellular carcinoma	0/50 (0%)[b]	12/48 (25%)[c]	29/37 (78%)[c]
Hepatocellular adenoma or carcinoma	0/50 (0%)[b]	21/48 (44%)[c]	35/37 (95%)[c]

[a]Tumor incidence values were not adjusted for mortality.
[b]$p < 0.001$, positive dose-related trend (Cochran-Armitage test).
[c]$p \leq 0.001$ by Fisher's Exact test pair-wise comparison with controls.

[d] $p = 0.014$.

In addition to liver tumors, a variety of other benign and malignant neoplasms occurred. However, the report (NCI 1978) indicated that each type had been encountered previously as a spontaneous lesion in the B6C3F1 mouse. The report further stated that the incidences of these neoplasms were unrelated by type, site, group, or sex of the animal, and hence, not attributable to exposure to 1,4-dioxane. There were a few nasal adenocarcinomas (1/48 in low-dose females and 1/49 in high-dose males) that arose from proliferating respiratory epithelium lining of the nasal turbinates. These growths extended into the nasal cavity, but there was minimal local tissue infiltration. Nasal mucosal polyps were rarely observed. The polyps were derived from mucus-secreting epithelium and were otherwise unremarkable. There was a significant negative trend for alveolar/bronchiolar adenomas or carcinomas of the lung in male mice, such that the incidence in the matched controls was higher than in the dosed groups. The report (NCI 1978) indicated that the probable reason for this occurrence was that the dosed animals did not live as long as the controls, thus diminishing the possibility of the development of tumors in the dosed groups.

15.1.4 Summary of several *in vivo* micronuclei tests

Roy et al. (Roy, Thilagar, and Eastmond 2005) examined micronucleus formation in male CD1 mice exposed to 1,4-dioxane to confirm the mixed findings from earlier mouse micronucleus studies and to identify the origin of the induced micronuclei. Mice were administered 1,4-dioxane by gavage at doses of 0, 1,500, 2,500, and 3,500 mg/kg-day for 5 days. The mice were also implanted with 5-bromo-2-deoxyuridine BrdU-releasing osmotic pumps to measure cell proliferation in the liver and to increase the sensitivity of the hepatocyte assay. The frequency of micronuclei in the bone marrow erythrocytes and in the proliferating BrdU-labeled hepatocytes was determined 24 hours after the final dose. Significant dose-related increases in micronuclei were seen in the bone-marrow at all the tested doses (≥ 1,500 mg/kg-day). In the high-dose (3,500-mg/kg) mice, the frequency of bone marrow erythrocyte micronuclei was about 10-fold greater than the control frequency. Significant dose-related increases in micronuclei were also observed at the two highest doses (≥ 2,500 mg/kg-day) in the liver. Antikinetochore (CREST) staining or pancentromeric fluorescence in situ hybridization (FISH) was used to determine the origin of the induced micronuclei. The investigators determined that 80–90% of the micronuclei in both tissues originated from chromosomal breakage; small increase in micronuclei originating from chromosome loss was seen in hepatocytes. Dose-related statistically significant decreases in the ratio of bone marrow polychromatic erythrocytes (PCE):normochromatic erythrocytes NCE), an indirect measure of bone marrow toxicity, were observed. Decreases in hepatocyte proliferation were also observed. Based on these results, the authors concluded that at high doses 1,4-dioxane exerts genotoxic effects in both the mouse bone marrow and liver; the induced micronuclei are formed primarily from chromosomal breakage; and 1,4-dioxane can interfere with cell proliferation in both the liver and bone marrow. The authors noted that reasons for the discrepant micronucleus assay results among various investigators was unclear, but could be related to the inherent variability present when detecting moderate to weak responses using small numbers of animals, as well as differences in strain, dosing regimen, or scoring criteria.

Table 30: The peripheral reticulocyte micronucleus test in CD-1 male after double *i.p.* dosing with 1,4-dioxane, results (Table 1) from (Morita 1994)

Dose (mg/kg)	Sampling time (h)[a]	MNRETs / 1000 RETs assessed per animal: Individual animal data[b]	Group mean ± SD (%)
500 x 2	0	0, 1, 3, 3, 0	0.14 ± 0.14
	24	1, 1, 1, 1, 2	0.10 ± 0.07
	48	0, 2, 0, 1, 2	0.10 ± 0.10
	72	0, 1, 2, 3, 1	0.14 ± 0.11
1000 x 2	0	3, 1, 1, 0, 3	0.16 ± 0.13
	24	3, 1, 1, 3, 0	0.16 ± 0.13
	48	1, 1, 2, 0, 1	0.10 ± 0.07
	72	1, 2, 2, 0, 2	0.14 ± 0.09
2000 x 2	0	0, 2, 1, 0, 2	0.10 ± 0.10
	24	2, 1, 5, 3, 3	0.28 ± 0.15
	48	3, 1, 2, 3, 0	0.18 ± 0.13
	72	1, 1, 0, 3, 0	0.10 ± 0.12
3200 x 2	0	1, 2, 1, 1, 1	0.12 ± 0.04
	24	3, 1, 1, 2, 3	0.20 ± 0.10
	48	2, 2, 1, 1, 1	0.14 ± 0.05
	72	-[c], 1, 1, 0, 2	0.10 ± 0.08
0.5 x 2[d]	0	3, 1, 0, 4, 0	0.16 ± 0.18
	24	33, 17, 10, 14, 13	1.74 ± 0.91**
	48	16, 11, 12, 3, 5	0.94 ± 0.53**
	72	6, 2, 3, 3, 4	0.36 ± 0.15

a, 0 h: just before dosing (negative control, 0.14 ± 0.12 %, n=25), 24, 48, and 72 h: after the second dosing. b, Each column per treatment group corresponds to one mouse. c, Mouse died. d, Positive control, Mitomycin C. ** p<0.01 (Kastenbaum and Bowman).

MNRETs: micronucleas reticulocytes

Table 31: Micronucleated PCEs and proportion of PCEs in marrow of mice given 3 daily injections of 1,4-dioxane (Oak Ridge), results (Table 1) from (McFee et al. 1994)

Daily dose (mg/kg)	*n*	PCE scored for MN	MN-PCE/ 1000 PCE	Pairwise significance	% PCE Among erythrocytes	Pairwise significance
Trial I						
0	5	10,000	3.9 ± 0.6 [a]	–	38.9 ± 6.3 [a]	–
500	5	10,000	4.5 ± 0.8	0.256	44.9 ± 4.3	0.775
1,000	5	10,000	2.9 ± 0.4	0.888	49.8 ± 5.6	0.914
2,000	5	10,000	2.0 ± 0.6	0.993	34.4 ± 4.7	0.281
	Trend test	*p* value	0.998			
MMC, 0.2	5	10,000	6.7 ± 1.2	0.003 *	16.8 ± 1.72	< 0.001 *
Trial II						
0	5	10,000	3.1 ± 0.7	–	32.9 ± 2.9	–
500	5	10,000	2.9 ± 0.3	0.602	40.0 ± 3.8	0.891
1000	5	10,000	2.7 ± 0.5	0.701	37.1 ± 4.0	0.771
2000	5	10,000	4.4 ± 0.7	0.066	18.3 ± 3.8	0.002 *
	Trend test	*p* value	0.039			
MMC, 0.2	5	10,000	8.3 ± 1.8	< 0.001 *	34.6 ± 5.7	0.764

[a] Means ± SE based on animals.
* Significantly different from control value.

PCEs: polychromatic erythrocytes (reticulocytes; immature erythrocytes)

Table 32: Micronucleated PCEs and proportion of PCEs in marrow of mice sampled 24 or 48 h after single injections of 1,4-dioxane, results (Table 2) from (McFee et al. 1994)

Sample time	Dose (mg/kg)	n	PCE Scored for MN	MN-PCE/ 1000 PCE	Pairwise significance	% PCE Among erythrocytes	Pairwise Significance
24 h	0	6	12,000	2.4 ± 0.3 [a]	–	30.8 ± 3.9	–
	2,000	6	12,000	4.2 ± 0.8	0.009 *	25.3 ± 3.6	0.108
	3,000	6	12,000	1.5 ± 0.3	0.946	21.0 ± 1.8	0.013 *
	4,000	6	12,000	1.7 ± 0.3	0.901	17.0 ± 2.1	0.001 *
		Trend test	p value	0.966			
	MMC, 0.5	6	12,000	17.2 ± 1.6	< 0.001 *	38.6 ± 2.78	0.994
48 h	0	6	12,000	2.7 ± 0.4	–	37.7 ± 3.3	–
	2,000	6	12,000	2.8 ± 0.5	0.424	25.2 ± 2.4	0.001 *
	3,000	6	12,000	3.3 ± 0.8	0.260	30.7 ± 3.6	0.050
	4,000	6	12,000	2.8 ± 0.8	< 0.462	18.0 ± 1.4	< 0.001 *
		Trend test	p value	0.386			
	MMC, 0.5	6	12,000	5.5 ± 0.9	< 0.001 *	19.3 ± 3.4	< 0.001 *

[a] Means ± SE based on animals.
* Significantly different from control values.

Table 33: Micronucleated PCEs and proportion of PCEs in marrow of mice given 3 daily injections of 1,4-dioxane (Lexington), results (Table 3) from (McFee et al. 1994)

Daily dose (mg/kg)	n	PCE scored for MN	MN-PCE/ 1000 PCE	Pairwise significance	% PCE Among erythrocytes
Trial I					
0	5	10,000	1.4 ± 0.3 [a]	–	37.1 ± 5.0
500	5	10,000	2.0 ± 0.4	0.222	37.8 ± 2.1
1000	5	10,000	2.1 ± 1.0	0.189	41.3 ± 2.4
2000	5	10,000	2.8 ± 0.4	0.054	35.1 ± 3.2
	Trend test	p value	0.056		
MMC, 0.2	5	10,000	10.9 ± 1.9	< 0.001 *	35.9 ± 2.1
Trial II					
0	5	10,000	2.0 ± 0.2	–	28.5 ± 1.7
500	5	10,000	3.9 ± 0.9	0.007 *	33.0 ± 1.9
1000	5	10,000	3.9 ± 0.5	0.007 *	32.2 ± 2.0
2000	5	10,000	3.4 ± 1.0	0.028	28.2 ± 2.7
	Trend test	p value	0.097		
MMC, 0.2	5	10,000	11.4 ± 2.8	< 0.001 *	29.6 ± 2.6

[a] Means ± SE based on animals.
* Significantly different from control value.

Table 34: Results of mouse bone marrow micronucleus assays of 1,4-dioxane, results (Table 1) from (Mirkova 1994)

Exp	Strain and sex of mice	Dose Levels (mg/kg)	Sampling Time (h)	No. of Animals/ Group	MPE/1000PE Based on 2000 PE assessed per slide/animal: Individual Animal Values	Mean ± SD
1	Control C57BL6 Males	10 ml/kg	24	4	4, 4, 3, 3	3.5 ± 0.6
		DX				
		3600	24	4	12, 10, 8.5, 11	10.4 ± 1.5 **
		1800	24	4	6.5, 8.5, 7, 12	9.4 ± 2.1 **
		900	24	4	8, 6.5, 5.5, 6.5	6.6 ± 1.03 **
		CP				
		62.5	24	3	16, 13.5, 14	14.5 ± 1.3 **
		Control	48	4	2.5, 1, 4, 2.5	2.5 ± 1.2
		DX				
		3600	48	4	6.5, 7.5, 9, 8	7.7 ± 1 **
2	C57BL6 Males	Control 10 ml/kg	24	10	1.5, 3, 2, 3.5, 3.5, 3.5, 1.5, 2.5, 3, 2.5	2.6 ± 0.8
		DX				
		3600	24	10	12.5, 11, 8, 11, 10.5, 12, 11.5, 11, 14, 14.5	11.6 ± 1.8 **
		1800	24	10	8, 9, 7.5, 9, 9, 10, 5, 8, 7.5, 6, 9	8.3 ± 1.2 **
		900	24	10	5.5, 8, 8, 7.5, 4, 7, 9, 6, 4, 7.5	6.6 ± 1.7 **
		450	24	10	4.5, 3, 2.5, 2, 5.5, 4, 3, 3.5, 2.5, 1.5	3.2 ± 1.2
		Control	48	5	3.5, 1.5, 3.5, 4, 2	2.9 ± 1.1
		DX				
		3600	48	5	7.5, 7.5, 6.5, 8.5, 6	7.2 ± 1 **
3	C57BL6 Females	Control 10 ml/kg	24	5	3.5, 2, 3, 2, 0.5	2.2 ± 1.1
		DX				
		5000	24	5	11.5, 10, 7, 6, 5.5	8 ± 2.6 **
		Control 10 ml/kg	48	5	0.5, 1, 1.5, 0, 1.5	0.9 ± 0.6
		DX				
		5000	48	5	5, 9.5, 5, 8.5, 7	7 ± 2 **
4	C57BL6 Males	Control 10 ml/kg	24	10	3.5, 4.5, 2.5, 4, 4.5, 2, 1, 5, 2, 5, 3, 3,	3.1 ± 1
		DX				
		3600	24	10	8, 5, 5, 5, 5, 6.5, 5, 9, 7.5, 5	6.1 ± 1.5 **
		CP				
		62.5	24	3	14, 19, 22	18.3 ± 4 **
5	BALB/c Males	Control 10 ml/kg	24	6	0.5, 2, 2.5, 1, 3.5, 1	1.7 ± 1.1
		DX				
		5000	24	5 [a]	4.5, 4, 4, 1, 1.5	3 ± 1.6

** $p < 0.01$ (one-sided Student's *t*-test).
[a] 1/6 animals in the test group found dead at 24 h.

MPE: micronucleated polychromatic erythrocytes; PE: polychromatic erythrocytes

Table 35: Results from 3 independent mouse bone marrow micronucleus assays testing 1,4-dioxane, results (Table 1) from (Tinwell and Ashby 1994)

Results from 3 independent mouse bone marrow micronucleus assays testing 1,4-dioxane (DIOX)

Expt. No. (mouse strain/stain)	Compound	Dose (mg/kg)	No. of animals	MPE/100PE based on 2000PE assessed per animal		PE/NE ± SD
				Individual Animal Data	Group Mean ± SD	
1 (CBA Giemsa)	Dist. water	10 ml	4	1, 1, 2, 3	1.75 ± 1.0	0.9 ± 0.2
	CP	65	3	4.5, 8, 9	5.8 ± 4.6	0.4 ± 0.2 **
	DX	1800	4	0.5, 2.5, 3, 4	2.5 ± 1.5	0.6 ± 0.05 **
2 (CBA AO)	Dist. Water	10 ml	5	3.5, 3.5, 2.5, 5, 0.5	3 ± 1.7	1.0 ± 0.1
	CP	65	2	20.5, 20	20.25 **	0.5 **
	DX	1800	8	1.5, 2, 0.5, 1.5 1, 2.5, 2, 5.5	2.1 ± 1.5	0.9 ± 0.1
3 (C57Bl6 AO)	Dist. water	10 ml	4	1, 6.5, 3, 4.5	3.8 ± 2.3	0.8 ± 0.2
	CP	65	2	24, 30.5	27.27 **	0.6 **
	DX	3600	4	7, 8.5, 3, 7	6.4 ± 2.4	1.0 ± 0.3

Expts. 1 and 2 employed male CBA mice whereas Expt. 3 involved male C57Bl6 mice. Data were assessed for statistical significance using a one-sided Students *t*-test; ** $p < 0.01$

CP: cyclophosphamide (used as positive control); DX: 1,4-dioxane; PE/NE: ratio of PEs to normocytes