ANNEX 1

in support of the Committee for Risk Assessment (RAC) for evaluation of limit values for 1,4-dioxane at the workplace

ECHA/RAC/OEL-O-0000007101-89-01/F

18 March 2022

Telakkakatu 6, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

Table of Contents

SCOPE OF THE TASK AND LITERATURE SEARCH 7

ECHA EVALUATION AND RECOMMENDATION 7

1. CHEMICAL AGENT IDENTIFICATION AND PHYSICO-CHEMICAL PROPERTIES 8

2. EU HARMONISED CLASSIFICATION AND LABELLING - CLP (EC) 1272/2008 9

3. CHEMICAL AGENT AND SCOPE OF LEGISLATION - REGULATED USES OF 1,4-DIOXANE IN THE EU 9

3.1 DIRECTIVE 98/24/EC AND DIRECTIVE 2004/37/EC 9

3.2 REACH REGISTRATIONS 9

3.3 AUTHORISED USES UNDER ANNEX XIV OF REACH 10

3.4 RESTRICTED USES UNDER ANNEX XVII OF REACH 10

3.5 PLANT PROTECTION PRODUCTS REGULATION (EC) 1107/2009 10

3.6 HUMAN AND VETERINARY MEDICINAL PRODUCTS DIRECTIVES 2001/83/EC AND 2004/28/EC RESPECTIVELY 10

3.7 BIOCIDAL PRODUCTS REGULATION (EU) 528/2012 10

3.8 OTHER LEGISLATIONS 10

4. EXISTING OCCUPATIONAL EXPOSURE LIMITS 10

5. OCCURRENCE, USE AND OCCUPATIONAL EXPOSURE 12

5.1 OCCURRENCE 12

5.2 PRODUCTION AND USE INFORMATION 12

5.3 OCCUPATIONAL EXPOSURE 14

5.4 ROUTES OF EXPOSURE AND UPTAKE 14

5.4.1 Worker exposure 14

5.4.2 General population 15

6. MONITORING EXPOSURE 15

6.1 EXTERNAL EXPOSURE 15

6.2 BIOMONITORING OF EXPOSURE (INTERNAL EXPOSURE) 16

6.2.1 Background levels 16

6.2.2 Occupational exposure 16

6.2.3 Biomonitoring analytical methods 17

7. HEALTH EFFECTS 17

7.1 TOXICOKINETICS (ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION - ADME) 17

7.1.1 Human data 18

7.1.1.1 Absorption 18
7.1.1.2 Distribution 18
7.1.1.3 Metabolism 18
7.1.1.4 Excretion 19
7.1.2 Animal data 19
7.1.2.1 Absorption 19
7.1.2.2 Distribution 20
7.1.2.3 Metabolism 21
7.1.2.4 Excretion 22
7.1.3 *In vitro* data 23
7.1.4 Toxicokinetic modelling 23
7.1.5 Summary 24
7.2 ACUTE TOXICITY 25
7.2.1 Human data 25
7.2.1.1 Acute oral toxicity 25
7.2.1.2 Acute dermal toxicity 25
7.2.1.3 Acute inhalation toxicity 25
7.2.2 Animal data 26
7.2.2.1 Acute oral toxicity 26
7.2.2.2 Acute dermal toxicity 27
7.2.2.3 Acute inhalation toxicity 27
7.2.3 *In vitro* data 27
7.2.4 Summary 27
7.3 SPECIFIC TARGET ORGAN TOXICITY/REPEATED DOSE TOXICITY 28
7.3.1 Human data 28
7.3.2 Animal data 28
7.3.2.1 Inhalation 28
7.3.2.2 Oral 30
7.3.3 *In vitro* data 31
7.3.4 Summary 31
7.4 IRRITANCY AND CORROSIVITY 31
7.4.1 Human data 31
7.4.2 Animal data 32
7.4.3 *In vitro* data 33
7.4.4 Summary 33
7.5 SENSITISATION 33
7.5.1 Human data 33
7.5.1.1 Respiratory sensitisation 33

7.5.1.2 Skin sensitisation 33
7.5.2 Animal data 33
7.5.2.1 Respiratory sensitisation 33
7.5.2.2 Skin sensitisation 34
7.5.3 *In vitro* data 34
7.5.4 Summary 34
7.6 GENOTOXICITY 34
7.6.1 Human data 34
7.6.2 Animal data (in vivo) 34
7.6.3 *In vitro* data 39
7.6.4 Summary 41
7.7 CARCINOGENICITY 41
7.7.1 Human data 41
7.7.2 Animal data 41
7.7.3 Summary 44
7.8 REPRODUCTIVE TOXICITY 44
7.8.1 Human data 44
7.8.2 Animal data 44
7.8.3 Summary 45

8. OTHER CONSIDERATIONS 45
8.1 MODE OF ACTION (MOA) CONSIDERATIONS 45
8.2 LACK OF SPECIFIC SCIENTIFIC INFORMATION 46
8.3 GROUPS AT EXTRA RISK 46

9. EVALUATION AND RECOMMENDATIONS 46
9.1 CANCER RISK ASSESSMENT 46
9.1.1 Published approaches for cancer risk assessment 46
9.1.1.1 SCOEL 46
9.1.1.2 DECOS 47
9.1.1.3 DFG 47
9.1.2 Cancer risk assessment 47
9.2 DERIVED OCCUPATIONAL EXPOSURE LIMIT (OEL) VALUES 48
9.2.1 Published approaches to establishing OELs 48
9.2.1.1 SCOEL 48
9.2.1.2 DECOS 48
9.2.1.3 DFG 48
9.2.2 Occupational Exposure Limit (OEL) - 8h TWA 48
9.2.2.1 Derivation of an OEL based on local effects 49

9.2.2.2 Derivation of an OEL based on systemic effects 49
9.2.2.3 Summary 50
9.2.3 Short Term Exposure Limit (STEL) 50
9.2.4 Biological Limit Value (BLV) 50
9.2.5 Biological Guidance Value (BGV) 50
9.3 NOTATIONS 50
REFERENCES 52

Figures

Figure 1: Metabolic pathways were postulated for the metabolization of 1,4-dioxane to its main metabolite 2 hydroxyethoxyacetic acid (HEAA) in rats (taken from Hartwig (2020)).....22

Tables

Table 1: Identity and physico-chemical properties 8

Table 2: EU classification: Summary of existing classification.....9

Table 3: REACH Registrations and tonnage 9

Table 4: Existing Occupational Exposure Limits (OELs) indicated as 8-h Time-Weighted Average (TWA) and Short-term exposure (15 min) for 1,4-dioxane 11

Table 5: Existing biological limit values 12

Table 6: Some validated methods for the measurement of 1,4-dioxane in air 15

Table 7: Human studies with external and internal exposure (1,4-dioxane/HEAA) from (Eckert, Hartwig, and Drexler 2020) 17

Table 8: Oral LD50 values 26

Table 9:Dermal LD50 values.....27

Table 10: Inhalation LC50 values 27

Table 11: Incidences of selected histopathological pre- and non-neoplastic lesions in male F344 rats exposed by inhalation to 1,4-dioxane for 2 years (Kasai et al. 2009) ..29

Table 12: Summary of *in vivo* mutagenicity studies (animal studies) 35

Table 13: *In vitro* genotoxicity studies 39

Table 14: Tumour incidences on 50 males per dose F344/DuCrj rats (Kasai et al. 2009) 42

Table 15: Tumour incidences on 50/sex/group F344/DuCrj rats (Kano et al. 2009).....42

Table 16: Tumour incidences on 50/sex/group Crj:BDF1 mice (Kano et al. 2009).....43

Table 17: Tumour incidences on 35/sex/group Osborne-Mendel rats after 110 weeks exposure (NCI 1978).....43

Table 18: Tumour incidences on 50/sex/group B6C3F1 mice after 90 weeks exposure (NCI 1978).....43

Table 19: Tumour incidences on 60/sex/group Sherman rats (Kociba et al. 1974).....43

Scope of the task and literature search

ECHA has been tasked by the European Commission to evaluate the exposure to 1,4 dioxane to assess the option of an airborne occupational exposure limit (OEL), other limit values (biological limit value (BLV)/biological guidance value (BGV)) and notations.

1,4-dioxane was previously classified as a category 2 carcinogen, but has a new classification as a category 1B carcinogen bringing it into the scope of the CMD. 1,4-dioxane already has an IOELV under CAD and as a result of its reclassification it is necessary to review the current IOELV and to replace it with an OEL under CMD.

This report is based on international assessments such as DFG (Hartwig 2020), DECOS (2011), ATSDR (Wilbur et al. 2012) and EU (BASF 1980). In addition, information was obtained from the CLH dossier on 1,4-dioxane (Committee for Risk Assessment 2019). This has been complemented by a literature search (July 2021) of published papers from the last ten years.

ECHA evaluation and recommendation

The table below presents the outcome of the scientific evaluation to derive limit values for 1,4-dioxane.

Derived Limit Values

OEL as 8-hour TWA:	7.3 mg/m^3 (2 ppm)
STEL:	73 mg/m^3 (20 ppm)
BLV:	45 mg 2-hydroxyethoxyacetic acid / g creatinine
BGV:	-

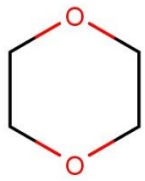
Notations

Notations:	Skin

1. Chemical Agent Identification and Physico-Chemical Properties

1,4-dioxane is a colourless liquid at ambient temperature with an ether-like odour and is soluble in water and most organic solvents.[1]

Table 1: Identity and physico-chemical properties[2]

Endpoint	Value
IUPAC Name	1,4-dioxane
Synonyms	1,4-dioxacyclohexane; diethylene ether; diethylene dioxide; [1,4]dioxane; dioxan
EC No	204-661-8
CAS No	123-91-1
Chemical structure	
Molecular formula	C4H8O2
Appearance	Liquid, colourless
Boiling point	101.2 °C (1013.25 hPa)
Density	1.0336 g/cm^3 (20 °C)
Vapour pressure	38.5 hPa (20 °C)
Partition coefficient (log Pow)	-0.42 (20 °C)
Water solubility	completely miscible at 20°C
Viscosity	1.31 mPa*s (20 °C)
Conversion factor	1 ppm = 3.66 mg/m^3 (20 °C)[3] 1 mg/m^3 = 0.273 ppm

[1] Ullmann's Encyclopaedia of industrial chemistry (2012)

[2] Physico-chemical values obtained from registration data

[3] $concentration\left[\frac{mg}{m^3}\right] = 88.1\frac{g}{mol} \cdot \frac{1.013 \cdot 10^5 Pa \cdot 1m^3}{8.314 \cdot \frac{Pa \cdot m^3}{mol \cdot K} \cdot 293.15K} \cdot 10^{-3} \cdot concentration[ppm]$

Explosion hazard

Like some other ethers, 1,4-dioxane combines with atmospheric oxygen upon prolonged exposure to air to form potentially explosive peroxides. Distillation of these mixtures is dangerous. Storage under metallic sodium could limit the risk of explosion.

2. EU Harmonised Classification and Labelling - CLP (EC) 1272/2008

Table 2: EU classification: Summary of existing classification

Index No	International chemical ID	EC No	CAS No	Annex VI of CLP hazard class and category	Hazard statement code
603-024-00-5	1,4-dioxane	204-661-8	123-91-1	Flam. Liq. 2 Carc. 1B STOT SE 3 Eye Irrit. 2	H225 H350 H335 H319

Supplementary Hazard Statements Codes: EUH019 and EUH066.
Note: D

The Commission Regulation (EU) 2021/849 (17th adaptation to technical and scientific progress) of 11 March 2021 modified the classification of 1,4-dioxane as Category 2 carcinogen to Category 1B carcinogen. The classification as Carcinogen 1B shall apply from 17 December 2022, although it can already be used for the classification and labelling.

3. Chemical Agent and Scope of Legislation - Regulated uses of 1,4-dioxane in the EU

3.1 Directive 98/24/EC and Directive 2004/37/EC

Commission Directive 2009/161/EU[4] of 17 December 2009 establishing a third list of indicative occupational exposure limit values in implementation of Council Directive 98/24/EC set an indicative OEL of 73 mg/m^3 (20 ppm) for 1,4-dioxane. At that time 1,4-dioxane had a harmonised classification as Carc. 2. As explained in section 2 the current harmonised classification is Carc. 1B thus bringing 1,4-dioxane into the scope of Directive 2004/37/EC (see preamble of this document).

3.2 REACH Registrations

Table 3: REACH Registrations and tonnage

Substance(s)		Tonnage (tonnes/annum)	
name	EC number	Full registration	intermediate use
1,4-dioxane	204-661-8	1000-10 000 (8 registrants)	-

[4] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009L0161&from=EN

3.3 Authorised uses under Annex XIV of REACH

1,4-dioxane is not currently listed in Annex XIV of REACH ("Authorisation List"). However, Germany has prepared an Annex XV dossiers for the identification of 1,4-dioxane as a Substances of Very High Concern (SVHC. This was added to the "Candidate List" on 8 July 2021 [ECHA decision on inclusion in Candidate List (europa.eu)]. The reason for inclusion is that it is carcinogenic and there is an Equivalent level of concern having probable serious effects to human health and the environment.

3.4 Restricted uses under Annex XVII of REACH

1,4-dioxane is not currently listed in Annex XVII of REACH. However, Germany has submitted an intention to submit an Annex XV restriction dossier in 2022 on 1,4-dioxane. A call for evidence was launched on the ECHA website from March to June 2021 [Previous calls for comments and evidence - ECHA (europa.eu)].

It is noted that a Risk Management Option Analysis (RMOA) was performed by the German REACH competent authority assessing the most appropriate regulatory actions under REACH and other EU legislation following the change of harmonised classification of 1,4-dioxane from Carc 2 to Carc 1B. In the conclusion document of the RMOA it was concluded that for environment and indirect exposure of the general population a restriction for specific uses would be the most appropriate regulatory option (BAuA 2020). It was further concluded that for occupational safety and health the first regulatory option to consider would be setting of a binding OEL.

3.5 Plant Protection Products Regulation (EC) 1107/2009

There are no plant protection products authorised under Regulation (EC) No 1107/2009 which are based on or include 1,4-dioxane. 1,4-dioxane is not listed as an active substance in the Annex of Commission Implementing Regulation (EU) No 540/2011.

3.6 Human and Veterinary Medicinal Products Directives 2001/83/EC and 2004/28/EC respectively

There are no authorisations for use of 1,4-dioxane in human or veterinary medicines.

3.7 Biocidal Products Regulation (EU) 528/2012

There are no biocidal products authorised under Regulation (EU) No 528/2012 which are based on or include 1,4-dioxane, nor has there been an active substance evaluation on 1,4-dioxane. 1,4-dioxane is not listed as active substance in Annex I of Regulation (EU) No 528/2012.

3.8 Other legislations

According to Annex II of the EU Regulation (EC) No 1223/2009[5] on cosmetic products, 1,4-dioxane (EC 204-661-8) is prohibited in cosmetic products.

4. Existing Occupational Exposure Limits

At EU level, there is an indicative OEL value for 1,4-dioxane of 73 mg/m^3 (20 ppm). Accordingly, EU Member States have established an OEL taking into account the EU

[5] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1223&from=EN

Value. Moreover, some Member States have established a short-term limit value (STEL) as well. Table 4 presents OEL values for several EU Members states as well as some values from outside the EU.

The list should not be considered as exhaustive.

Table 4: Existing Occupational Exposure Limits (OELs) indicated as 8-h Time-Weighted Average (TWA) and Short-term exposure (15 min) for 1,4-dioxane

Country	TWA (8 hrs)		STEL (15 min)		Remarks
	ppm	mg/m³	ppm	mg/m³	
Austria	20	73	40	146	
Belgium	20	73			Skin notation
Denmark	10	36	20	72	Skin notation
European Union	20	73			
Finland	10	36	40	150	Skin notation
France	20	73			Restrictive statutory limit values
Germany (AGS)	20	73	40	146	Skin notation
Germany (DFG)	10	37	20	73	Skin notation
Hungary		10		10	
Ireland	20	73			
Latvia	5.5	20			
Norway	5	18	10	36	Skin notation
Poland		50			
Romania	20	73			
Spain	20	73			
Sweden	10	35	25	90	
Switzerland	20	72	40	144	
USA - NIOSH			1	3.6	Ceiling limit value (30 min)
USA - OSHA	100	360			

Country	TWA (8 hrs)		STEL (15 min)		Remarks
	ppm	mg/m³	ppm	mg/m³	
United Kingdom	20	73			

Sources: Gestis database (searched June 2021): International limit values for chemical agents (Occupational exposure limits, OELs) (https://www.dguv.de/ifa/gestis/gestis-internationale-grenzwerte-fuer-chemische-substanzen-limit-values-for-chemical-agents/index-2.jsp).

MAK- und BAT-Werte-Liste 2021 https://series.publisso.de/sites/default/files/documents/series/mak/lmbv/Vol2021/Iss1/Doc001/mbwl_2021_deu.pdf

Biological limit values

Germany/DFG (Eckert, Hartwig, and Drexler 2020) has established a biological limit value using 2-hydroxyethoxy acetic acid (HEAA) in urine as a biomarker.

Table 5: Existing biological limit values

Country	BLV	Specifications	References
Germany	200 mg 2-hydroxyethoxy acetic acid/g creatinine (in urine) End of exposure or end of shift	BAT	(Eckert, Hartwig, and Drexler 2020)

BAT: biological tolerance value

5. Occurrence, Use and Occupational Exposure

5.1 Occurrence

1,4-dioxane is a manufactured chemical that does not occur naturally in the environment. It has been manufactured for several decades, and historically, around 90% of 1,4-dioxane production was used as a stabilizer in chlorinated solvents such as 1,1,1-trichloroethane (TCA) (Wilbur et al. 2012); however, the use of 1,4-dioxane has decreased since TCA was phased out by the Montreal Protocol in 1995. The occurrence of 1,4-dioxane in the environment is thought to be related to the disposal of chemical solvents containing dioxane and from disposal of 1,4-dioxane itself. Subsequent leaching of the chemicals from landfills has resulted in contamination of groundwater.

5.2 Production and Use Information

In 2021 the EU registered tonnage is approximately 3000 tonnes/year, with two thirds manufactured within one site in the EU (with a relatively small amount manufactured in a 2nd site starting in 2021), and the other third imported by 7 companies. The exposure assessments in the chemical safety reports (CSRs) have been modelled using EasyTRA 4.4.0.

There are three main types of production processes for 1,4-dioxane (EU 2002):

1. acid-catalysed conversion of diethylene glycol by ring closure in a closed system. The use of mono-, tri- and polyethylene glycol and their ethers as raw material is also reported;

2. catalysed cyclo-dimerisation of ethylene oxide on acid ion exchanger resins via oligo-ethylene sulphonates;
3. ring closure of 2-chloro-2'-hydroxyethyl ether through heating with 20% sodium hydroxide.

The second and the third processes are especially useful for the production of substituted dioxanes.

Industrially, the first production process is the most important one, and is the one used in the main production site in the EU. This production is carried out at a temperature of between 130 and 200°C and a pressure ranging from 250 to 1100 hPa. Dehydration and purification take place by distillation. For this production, sulphuric acid, phosphoric acid, p-toluenesulphonic acid and strongly acidic ion exchangers are used as catalysts. Zeolites can also be used. The continuous synthesis is carried out in a heated vessel. The raw product forms an azeotrope with water. The dioxane is separated by distillation. Water and volatile by-products are separated by extractive distillation. The main by-products are acetaldehyde and 2-methyl-1,3-dioxalane, 2-ethyl-1,3-dioxolane. At a lesser extent, glycol, crotonaldehyde and polyglycol are formed during the production. The crude 1,4-dioxane is further cleaned by heating with acids, distillation (to remove glycol and acetaldehyde), salting out with NaCl, $CaCl_2$ or NaOH and fine subsequent distillation. Manufacturing sites produce 1,4-dioxane in liquid form at concentrations greater or equal to 90%.

In the joint submission, the Lead and nearly all the Members indicate the same uses (there are no consumer uses):

- Use as solvent (use in industrial settings)
- Use in laboratories (use in industrial settings)
- Use in laboratories (use in professional settings)

Where information is available, the amount used in laboratories is minuscule compared to the industrial use as a solvent.

The technical function for all the uses (including the lab uses) is as a solvent. All these uses are described in the registrants' chemical safety reports using the Process Categories (PROCs) 1-5, 8a, 8b, 9 and 15[6]. These PROCs describe relatively controlled activities with limited exposures, with the highest exposure estimated for PROC 4 as modelled by EasyTRA 4.4.0.

One of the registrants has indicated an additional use:

- Uses at industrial sites in Polymerisation process

This use is described by PROCs 1, 2 and 3, so there is limited exposure to workers, however there is some potential for exposure due to the residual substance being present in the article (e.g. dermal exposure from shoes, estimated by the registrant using TRA Consumers 3.1). This substance can be found in products with material based

[6] Process categories (PROCs) define tasks, or process types from the occupational perspective. The PROCs are also differentiated by taking into account the exposure potential for workers during the respective tasks or process types. PROCs 1-4 describe closed or partially closed production processes, PROC 5 describes mixing, PROCs 8a, 8b and 9 describe controlled transfer activities, and PROC 15 describes small scale lab use. For more information see the ECHA Guidance Chapter R.12: Use description: https://echa.europa.eu/documents/10162/2324909/r12_guidance_draft_for_committees_201507_en.pdf

on: rubber used for articles with intense direct dermal (skin) contact during normal use (e.g. gloves, boots, clothing, rubber handles, gear lever, steering wheels).

Outside the EU, 1,4-dioxane has a wider range of applications because of its broad range of solvent properties (Wilbur et al. 2012). It has also been used as a laboratory reagent (e.g., mobile phase in chromatography); in plastic, rubber, insecticide, and herbicides; as a chemical intermediate; as part of a polymerization catalyst; and as an extraction medium of animal and vegetable oils. Other minor uses are in the manufacture of membrane filters, for measuring optical activity, and for cryoscopic determination. 1,4-dioxane has been reported to be used in the production processes of the following product categories: pharmaceuticals/pesticides, magnetic tape, and adhesives

5.3 Occupational exposure

The U.S. EPA (EPA 2020) conducted a risk evaluation for 1,4-dioxane pursuant to the Toxic Substances Control Act (TSCA), to determine whether the substance presents an unreasonable risk to health or the environment, under the conditions of use, including an unreasonable risk to a relevant potentially exposed or susceptible subpopulation. After evaluating 24 conditions of use of 1,4-dioxane, the EPA determined that 1,4-dioxane presents an unreasonable risk under 13 conditions of use. This includes an unreasonable risk to workers (those directly handling the substance) and occupational non-users (ONUs) when manufacturing or importing the chemical; processing the chemical for a variety of uses (including non-incorporative processing and use as laboratory chemicals, as per the uses in the EU); and when used in certain industrial and commercial applications.

The risk evaluation uses scientific information, technical procedures, measures, methods, protocols, methodologies and models consistent with the best available science, and the EPA has to base its decisions on the weight of the scientific evidence, including taking account of uncertainties. (EPA 2020)

Similar up-to-date studies within the EU were not found. There is an EU Risk Assessment Report (EU RAR) from 2002 (EU 2002), but this mostly describes uses that no longer occur in the EU (according to registration data), and where the uses do still occur they are most likely under conditions that are no longer applicable.

5.4 Routes of exposure and uptake

According to the ATSDR (Wilbur et al. 2012) 1,4-dioxane can be released into the air, water, and soil at places where it is produced or used mainly as a solvent.

In water, 1,4-dioxane is stable and does not break down. Compounds in the air can break down 1,4-dioxane into different compounds rapidly. In soil, 1,4-dioxane does not stick to soil particles, so it can move from soil into groundwater.

5.4.1 Worker exposure

Occupational exposure occurs during the production, processing, and use of 1,4-dioxane, via inhalation or dermal exposure.

In the REACH registration data, for all the uses where there is occupational exposure, the registrants claim that the exposure is under the IOELV of 20 ppm (73 mg/m^3). Based on Easy TRA 4.4.0 exposure estimates range from around 0.03 mg/m^3 for those activities described by PROC 1 (closed process) up to around 26 mg/m^3 for those activities described by PROC 3 (closed batch processes with occasional controlled exposure) or PROC 15 (laboratory use).

5.4.2 General population

According to the ATSDR (Wilbur et al. 2012), the general population is exposed to negligible levels of 1,4-dioxane. The primary routes of human exposure to 1,4-dioxane are:

- Inhalation of 1,4-dioxane in air,
- Oral ingestion of contaminated food (supplements, contaminated packaging etc) and drinking water containing 1,4-dioxane,
- Dermal contact with contaminated consumer products (e.g., products containing ethoxylated surfactants such as cosmetics or shampoos).

Because 1,4-dioxane may be found in tap water, human exposure to 1,4-dioxane could also occur during activities such as showering, bathing, and laundering.

In addition, as a by-product of the ethoxylation process, 1,4-dioxane can contaminate cosmetics and personal care products such as deodorants, perfumes, shampoos, toothpastes and mouthwashes. The ethoxylation process makes the cleansing agents, such as sodium laureth sulphate and ammonium laureth sulphate, less abrasive and offers enhanced foaming characteristics. The Scientific Committee on Consumer Safety (SCCS) gave an opinion in 2015 to the International Cooperation on Cosmetics Regulation (ICCR) group, that a target level of less than or equal to 10 ppm of 1,4-dioxane in finished cosmetic products should be phased in over a short transition period[7] (about 7% of 170 cosmetic and household products analysed were over this limit).

The EPA evaluated eight conditions of use of 1,4-dioxane present as a by-product in consumer products (EPA 2020). The EPA determined that these consumer uses do not present an unreasonable risk. The EPA has also evaluated exposures to the general population through surface water and determined that 1,4-dioxane does not present an unreasonable risk to the general population based on that exposure.

6. Monitoring Exposure

6.1 External exposure

There are several methods that allow the determination of 1,4-dioxane in air even in low concentrations, including concentrations below any proposed limit value. The principle of the methods is as follows: the sample is taken by passing air through a sorbent tube. The retained 1,4-dioxane is then extracted for analysis by desorption on CS_2 followed by analysis via gas chromatography with different detectors. The table below shows some of the available validated methods for measurement of 1,4-dioxane in air. The calculations of the limit of quantitation (LOQ) in air take into account the sampling times recommended in the method.

Table 6: Some validated methods for the measurement of 1,4-dioxane in air

Method	Analytical technique	LOQ and sampling volume and time
DFG (Krämer, Hebisch, and Hartwig 2016)	Gas chromatography with flame ionisation detectors (GC/FID) Desorption with CS_2	0.047 mg/m^3 (25L/ 8 hours)

[7] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_194.pdf

NIOSH 1602 (NIOSH 1994)	GC/FID Desorption with CS_2	1 mg/m^3 (10 L) (Limit of detection)

6.2 Biomonitoring of exposure (internal exposure)

The primary route of metabolism of 1,4-dioxane, at least at relatively low doses, is via cytochrome P450-catalysed hydrolysis and then oxidation, to produce 2-hydroxyethoxyacetic acid (HEAA). There can also be oxidation of the unbroken ring to produce 1,4-dioxane-2-one, which is in equilibrium with HEAA (SCOEL 2004; Woo et al. 1977; Woo, Argus, and Arcos 1977a).

1,4-dioxane and its metabolite, HEAA, were found in the urine of workers exposed to a time-weighted average air concentration of 1.6 ppm (5.9 mg/m^3) of 1,4-dioxane for 7.5 hours (Young et al. 1976). The concentration of HEAA was 414 µmol/L and that of unchanged 1,4-dioxane was only 3.5 µmol/L, suggesting rapid and extensive metabolism. 1,4-dioxane in the urine is a specific biomarker for exposure to 1,4-dioxane, but HEAA can also be produced by exposure to 1,4-dioxane-2-one and diethylene glycol. In a controlled-exposure study with volunteers exposed to 50 ppm (183 mg/m^3) 1,4-dioxane vapours for 6 hours, the half-life for elimination of 1,4-dioxane from plasma was 59 minutes (Wilbur et al. 2012; Young et al. 1977).

As a rule, only a minor quantity of the absorbed 1,4-dioxane is eliminated unchanged in the urine (less than 1%) while the main metabolite of 1,4-dioxane is HEAA (> 99%) (Kraus, Schaller, and Csanády 2007). The concentrations in blood are in the range of the analytical detection limit, thus 1,4-dioxane in blood is not suitable as indicator for biological monitoring. Moreover, the short half-life of 1,4-dioxane both in blood and in urine make the parameter unsuitable for establishing a BLV (Eckert, Hartwig, and Drexler 2020).

Besides, the half-life HEAA in urine is about 3.4 ± 0.5 h, significantly longer than that of 1,4-dioxane. It was concluded that the determination of the HEAA concentration in relation to creatinine in urine reflects very well the internal exposure to 1,4-dioxane (Eckert, Hartwig, and Drexler 2020).

6.2.1 Background levels

The primary routes of human exposure to 1,4-dioxane for the general population are inhalation of 1,4-dioxane in air, ingestion of contaminated food and drinking water containing 1,4-dioxane, and dermal contact with consumer products. Because 1,4-dioxane may be found in tap water, human exposure to 1,4-dioxane may also occur during activities such showering, bathing, and laundering (Wilbur et al. 2012).

No data on background levels of 1,4-dioxane or its metabolites in the general population have been found.

6.2.2 Occupational exposure

The evaluation carried out by DFG (Hartwig 2020) did not find any correlation between internal concentrations of 1,4-dioxane or its metabolites and health effects. No specific biomarker of effect for 1,4-dioxane was found by Wilbur et al. (2012).

However, some studies about correlations between internal and external exposure (using HEAA as biomarker) are available. The studies are summarised in the table below:

Table 7: Human studies with external and internal exposure (1,4-dioxane/HEAA) from (Eckert, Hartwig, and Drexler 2020)

	(Young et al. 1976)	(Young et al. 1977)	(Göen et al. 2016)
Number	5 workers (males)	4 volunteers (males)	18 volunteers (10 males and 8 females)
Air concentration 1,4-dioxane	1.6 ppm (1.0 to 2.0 ppm)	50 ppm	20 ppm
Exposure period	7.5 h	6 h	8 h
HEAA level at the end of exposure (mean value ± standard deviation)			
Original data from the publication	414 ± 216 µmol	118 ± 8.3 mg[a)] (after end of exposure, 6–8 h after beginning of exposure)	378 ± 115 mg/g creatinine (6 volunteers, exposure at rest)
Conversion to mg/g creatinine	35.5 ± 18.5 mg/g creatinine[b)])	674 ± 47.4 mg/g creatinine [b), c)]	378 ± 115 mg/g creatinine
Extrapolation to 8 h exposure	**37.9 ± 19.7 mg/g creatinine**	**899 ± 63.2 mg/g creatinine**	**378 ± 115 mg/g creatinine**

The DFG (Eckert, Hartwig, and Drexler 2020) used the values (corrected for 8 hours) to build a function with the relationship between the mean urinary HEAA level after the end of exposure in relation to the air concentration of 1,4-dioxane. Values of the function are the following:

$Y=17.82 \times X + 9.58$

$R^2=0.99994$

Where:

Y: urinary HEAA level after the end of exposure in mg/g creatinine

X: air concentration of 1,4-dioxane in ppm

This same correlation could be used to propose a BLV taking as a refence the OEL set. If a BLV is set the time of sampling should be at the end exposure due to the short half-life of HEAA.

Considering that there are no studies available regarding background concentration in the general population, there is no data to establish a BGV.

6.2.3 Biomonitoring analytical methods

There are analytical methods able to measure low concentration of HEAA in urine.

For instance, DFG (Leng et al. 2015) proposes a method based on gas chromatography with mass selective detection (GC–MS) that allows the determination of HEAA in urine with a detection limit 0.6 mg HEAA per litre urine.

7. Health Effects

7.1 Toxicokinetics (Absorption, distribution, metabolism and excretion - ADME)

7.1.1 Human data

7.1.1.1 Absorption

The absorption of 1,4-dioxane in human was reported in inhalation studies only (Young et al. 1976; Young et al. 1977; Göen et al. 2016). In the first study, five workers were exposed to a time-weight average of 1.6 ppm (individual averages: 1.1-2.0 ppm; 4.0-7.3 mg/m^3) for 8 hours, based on a pulmonary absorption of 100%, the absorbed dose was calculated to be 0.37 mg/kg for a 70 kg person (Young et al. 1976).

In the second study, the pharmacokinetics and metabolism of 1,4-dioxane were determined in four healthy male volunteers exposed to 50 ppm (180 mg/m^3) for 6 hours in a chamber under dynamic airflow conditions. Blood was sampled at regular intervals up to 12 hours after the start of the experiment. Urine was collected during and after exposure for a total of 48 hours. Urine and plasma were analysed for test substance and metabolites. Plasma concentrations increased rapidly within the first 2 hours after exposure, indicating an initial rapid absorption. This was followed by a gradual decrease in the rate of absorption until a plateau was reached between 3 and 6 hours, which is indicative of reaching a steady state. The plasma concentration of HEAA peaked one hour post-exposure and reached undetectable levels by 4 hours post-exposure. Based on measurements of 1,4-dioxane and HEAA in the urine, the authors calculated that the mean absorbed dose was 5.4 mg/kg bw at a mean rate of 76.1 mg/h. No saturation was identified. (Young et al. 1977).

In the most recent human study, 18 healthy volunteers (8 men and 10 women) were exposed to 20 ppm (73 mg/m^3) 1,4-dioxane for 8 hours in rest or under physical activity (10 minutes of physical activity every hour by cycling at 50 or 75 W). Blood samples were taken after 4 and 8 hours, while urine was collected after 24 hours to determine the concentration of 1,4-dioxane and its main metabolite HEAA. The spacing of the data points were not sufficient to identify any trend in 1,4-dioxane plasma uptake. The pulmonary retention was evaluated to be about 60.5% after calculation from the empirically derived relationship between pulmonary absorption and the blood:air partition coefficient for 1,4-dioxane. The authors did not compute the absorbed doses, however they found a positive correlation between workload absorption: 1.27 and 1.37 for the two increasing exercising groups with respect to the 'rest' group (Göen et al. 2016).

No human data are available on the dermal absorption of 1,4-dioxane.

7.1.1.2 Distribution

There are no data available on the distribution of 1,4-dioxane in human tissues.

7.1.1.3 Metabolism

Young et al. (1976) measured 1,4-dioxane and its metabolite HEAA in the urine of workers exposed to an average concentration of 1.6 ppm (9 mg/m^3) for 7.5 hours. The average detected concentrations were 3.5 and 414 µmol/L, for 1,4-dioxane and HEAA respectively, consequently the authors concluded that human metabolises 1,4-dioxane to the same metabolite HEAA as rats and the process is rapid at low concentrations. In addition, they speculated that low concentration of 1,4-dioxane pose a negligible hazard because, from precedent studies in rats, the toxicity of 1,4-dioxane was observed only after the metabolism to HEAA is saturated (Young et al. 1976).

In the volunteer study by Young et al. (1977), four individuals who were exposed to 50 ppm (183 mg/m^3) 1,4-dioxane for 6 hours excreted a total amount of 118 mg HEAA via urine within the first 2 h after the end of exposure. No other metabolites were mentioned. No indications of metabolic saturation were reported (Young et al. 1977).

In a recent study, blood concentrations of 1,4-dioxane were measured in 3 groups of volunteers with no or increasing physical activity. The volunteers were exposed to 20 ppm (73 mg/ m^3) for 8 hours in rest or under physical activity (10 minutes of physical activity every hour by cycling at 50 or 75 W). After 4 hours, the mean blood levels were 0.98 (± 0.10), 1.07 (± 0.15) and 1.48 (± 0.31) mg/L for the rest and increasing intensity exercises groups. After 8 hours, i.e. at the end of the exposure period, the levels were comparable or slightly higher: 1.10 (± 0.19), 1.24 (± 0.59) and 1.47 (± 0.29) mg/L for the 3 groups. Consequently, the authors conclude that a steady state was already reached after 4 hours (Göen et al. 2016).

7.1.1.4 Excretion

All the studies in human measured 1,4-dioxane and its metabolite HEAA in the urine, and in some cases in the expired air. However, no information is available on their concentration in the faeces (Young et al. 1976; Young et al. 1977; Göen et al. 2016). Despite, 1,4-dioxane was found, but not quantified, in the faeces of an ex-Soviet Union man. No other information is available, so it is not possible to estimate the exposure route (Dmitriev, Rastiannikov, and Mal'ysheva 1985).

Göen et al. (2016) tested 3 groups of volunteers exposed to 20 ppm (73 mg/m^3) 1,4-dioxane for 8 hours in rest or under increasing physical activity (cycling for 10 minutes every hour at 50 or 75 W). The percentage of 1,4-dioxane excreted unchanged in the urine was very low, between 0.2-0.3%. The maximum amount of the HEAA in the urine was reached 9.8 (± 1.9) hours after the beginning of exposure. Depending on the workload, the maximum elimination rate increased significantly from 23.2 (± 7.7) in the 'resting' group to 30.4 (± 7.2) and 41.8 (± 23.8) mg/hour in two exercising groups, respectively, which is reflective of the increased inhalation rate during physical activity. Analogously, the cumulative excretion of HEAA in the urine was increased by exercise, the average maximum level of HEAA was between 378 and 451 mg/g creatinine and increased with workload. The calculated half-life of HEAA was 3.4 (± 0.5) hours and was independent of the physical exercise levels. As low HEAA concentrations were detected 16 hours after the beginning of exposure in all 3 groups, the authors estimated that about 53% (± 15%) of the theoretically inhaled 1,4-dioxane was eliminated as HEAA within 24 hours and assumed only low accumulation during a working week. The study results revealed an increasing effect of the applied physical stress on the total eliminated amounts of HEAA as well as on the maximum HEAA levels at the end of exposure (Göen et al. 2016).

7.1.2 Animal data

7.1.2.1 Absorption

Oral administration was studied in Sprague-Dawley rats, which received by gavage doses of 10, 100, or 1000 mg/kg bw of uniformly labelled ^{14}C-1,4-dioxane as single dose or for 17 days. For all 3 doses, < 2% of the label was found in the faeces in the first 24 hours (10 mg/kg bw dose) or 72 hours (100 or 1000 mg/kg bw doses), indicating rapid and nearly-complete absorption of the compound from the gastrointestinal tract (Young, Braun, and Gehring 1978a; Young, Braun, and Gehring 1978b). Analogous results were observed after 17 days of exposure, where less than 2% of the total administered label was recovered in the faeces up to 20 days post-exposure, indicating that at least 98% absorption had occurred (Young, Braun, and Gehring 1978a; Young, Braun, and Gehring 1978b).

In the same studies, four male Sprague Dawley rats were exposed to 50 ppm (183 mg/m^3) 1,4-dioxane vapours for 6 hours (head only). The plasma 1,4-dioxane concentration peaked 6 h after the start of the exposure and decreased thereafter until it was no longer detectable 5 hours post-exposure. At the end of the exposure period, the concentration of 1,4-dioxane in the plasma was 7.3 µg/mL. Based on the measured 1,4-

dioxane and HEAA in the urine (7 µg and 21 mg, respectively), the mean absorbed dose was estimated to be 71.9 mg/kg bw (Young, Braun, and Gehring 1978a; Young, Braun, and Gehring 1978b).

In another study, male F344/DuCrj SPF rats were exposed to 250 ppm (915 mg/m^3) 1,4-dioxane vapours by inhalation in whole-body chambers for 6 hours. Blood concentration of 1,4-dioxane increased for the first 3 hours and remained constant until the end of the exposure, peaking at 22 µg/mL. Thereafter the blood concentrations declined until 1,4-dioxane was no longer detected 13 hours after the start of the experiment. The absorbed dose was not calculated (Take et al. 2012).

Dermal absorption has been studied in monkeys only. In the study, uniformly labelled ^{14}C-1,4-dioxane, dissolved in either methanol or skin lotion, was applied to the unoccluded, clipped forearm of Rhesus monkeys (4 µg/cm^2 over 3–15 cm^2) for 24 hours. Assuming a body weight of approximately 10 kg for an adult Rhesus monkey, the applied dose of 1,4-dioxane ranged from 1.2 to 4.8 mg/kg. The skin penetration of 1,4-dioxane was < 4% in all cases based on the radiotracer recovery in urine up to 5 days post-exposure. However, because the skin was unoccluded, evaporation was likely to be high and thus influenced the study results. This is supported by the fact that, between 30-50% of the absorbed dose was absorbed within the first 4 hours (Marzulli, Anjo, and Maibach 1981).

7.1.2.2 Distribution

Based on the available data in animal studies, 1,4-dioxane is expected to evenly distribute to major organs.

Take et al. (2012) reported distribution to multiple tissues (lung, liver, brain, kidney, and abdominal fat) in male F344/DuCrj SPF rats following administration via inhalation, oral, or combined inhalation and oral exposures. After a single oral gavage exposure, radiolabelled 1,4-dioxane was detected in all tested tissues (lung, liver, brain, kidney, and abdominal fat), peaked at 60 minutes to decline to non-detectable concentrations in all tissues but blood within 12 hours, in blood the concentration was non-detectable within 7 hours. Peak concentrations of radiolabelled-1,4-dioxane in the lung, liver, kidney, brain and abdominal fat were approximately 215, 185, 180, 175, and 85 µg/g tissue, respectively. The lower concentration of 1,4-dioxane in the abdominal fat implies a higher blood:abdominal fat partition coefficient than for blood versus the other tissues. After inhalation exposure of 250 ppm (915 mg/m^3), 1,4-dioxane reached steady state concentration in the tested tissues within 3 hours, its concentration remained detectable 120 minutes after exposure ended but was non-detectable after 360 minutes.

Following a single oral gavage exposure of 65 mg/kg bw deuterated 1,4-dioxane followed immediately by whole body exposure to 250 ppm (915 mg/m^3) 1,4-dioxane vapours for 360 minutes, 1,4-dioxane reached peak concentrations in all of these tissues 60 minutes after exposure and was no longer detectable in tissue 720 minutes after exposure (Take et al. 2012).

Mikheev et al. (Mikheev, Gorlinskaya Ye, and Solovyova 1990) studied the distribution of ^{14}C-1,4-dioxane in the several rats organs and tissues (blood, liver, kidney, brain, testes) for up to 6 hours after intraperitoneal (i.p.) injection of approximately one-tenth of the lethal dose, however the authors did not report the actual dose. They also did not report the actual tissue concentrations but indicated tissue:blood ratios for each tissue at six time points ranging from 5 minutes to 6 hours. The peak of radiolabel concentration was found first in the liver and kidney then in blood or the other tissues, thus the authors concluded this could be indicative of the presence of a selective membrane transport. All tissue:blood ratios were below one at all time points except in kidneys where it increased to 1 at the end of the experiment and in testes. The ratio in testes increased from 0.6 after 5 min to 1.3 at the end of the experiment. The importance of these findings is unclear, because the contribution of residual blood in the tissues was

unknown (though saline perfusion may serve to clear tissues of highly water-soluble 1,4-dioxane), no radiolabelled concentration in the tissue was given, and only a limited number of data points are available. Overall, it can be concluded that 1,4-dioxane distributes evenly among the tissues and organs studied and that accumulation does not occur.

Male Sprague Dawley rats received i.p. doses of ^{3}H-1,4-dioxane (5 mCi/kg bw) with and without an oxidase inducers pre-treatment. The main organs were collected at 1, 2, 6, and 12 hours after dosing. Blood concentrations were higher than tissue concentration at all time points, with one exception, when the kidney concentration was higher than blood 1h after dosing. The authors did not perfuse the tissues prior to analysis, thus the contribution of residual blood to radiolabel measurements is unknown, however due to 1,4-dioxane solubility in water, saline perfusion would have decreased the concentration of 1,4-dioxane from tissues. The distribution was otherwise uniform and reached peak concentration of about 20% in liver, spleen and colon, while the peak concentrations in kidneys lung and skeletal muscle about 10% were observed later after 16 hours exposure (Woo, Argus, and Arcos 1977b).

7.1.2.3 Metabolism

Young et al. (Young, Braun, and Gehring 1978a; Young, Braun, and Gehring 1978b) conducted a series of pharmacokinetic study to determine the fate of 1,4-dioxane in rats using oral, inhalation and intraperitoneal exposures. The results showed that the fate of 1,4-dioxane in Sprague Dawley rats is markedly dose-dependent due to a limited capacity to metabolization to HEAA. The pharmacokinetic data supporting these conclusions included plasma concentration-time curves for 1,4-dioxane given to rats intravenously at dose levels from 3 to 1000 mg/kg bw and an inhalation study of 50 ppm (183 mg/m^{3}) 1,4-dioxane vapours for 6 h. The plasma curves at low doses by each route were linear, with half-life values of about 1 hour for exposures between 3 to 10 mg/kg bw. As the dose was increased above 10 mg/kg bw, the plasma clearance rate decreased, the fraction of the dose excreted as HEAA decreased, and the fraction of the dose excreted as 1,4-dioxane in the urine and expired in the breath increased, the half-life was calculated at 14 hours after exposure to 1000 mg/kg bw. At saturation, the maximum velocity of the metabolism of 1,4-dioxane to HEAA was about 18 mg/kg bw/h. Multiple daily oral doses of 1000 mg/kg bw, but not 10 mg/kg bw, were excreted more rapidly than equivalent single doses, indicating that at high daily doses 1,4-dioxane induced its own metabolism. Based on these results, the authors concluded that there is an apparent threshold for the toxic effects of 1,4-dioxane which coincides with saturation of the metabolic pathway for its detoxification (Young, Braun, and Gehring 1978a; Young, Braun, and Gehring 1978b).

Sweeney et al. (2008) administered a single oral dose to of 20, 200 or 2000 mg/kg bw/day to 27 B6C3F1 mice per dose. Blood samples were collected and analysed after 0 and 30 minutes and 1, 2, 3, 6, 9, 12 and 24 hours. Blood concentrations were close to detection limits at all time points after the administration of the low dose. Instead for the mid and hight doses a peak was observed after 1 hour. In all groups, HEAA maximum concentration was measured between 30 minutes and 2 hours, and the highest rate of metabolite conversion was observed in the low dose. HEAA was still detected after 12 and 24 hours only in the high dose group. The authors proposed HEAA metabolism is non-linear based on the comparison of the AUCs (blood concentration–time curves, by the non-linear increase in 1,4-dioxane compared with the dose and the concurrent decrease in the ratio HEAA: 1,4-dioxane in blood with increasing concentration. Overall, these findings indicate metabolic saturation at high concentrations (≥ 200 mg/kg bw/day) paired with a very rapid metabolism after the admiration of the low dose (Sweeney et al. 2008).

Over the years, three metabolic pathways were postulated for the metabolization of 1,4-dioxane to its main metabolite 2-hydroxyethoxyacetic acid (HEAA) in rats (Woo, Argus, and Arcos 1977a; von Helden 2013), see figure below.

A) Oxidation by Cytochromes P450 (CYP) followed by hydroxylation to form HEAA from the cyclic ketone, dioxan-2-one. Dioxan-2-one is in a pH dependent equilibrium with HEAA and it was detected in early studies at low concentrations (~ 50 mL/m^3) when acidic isolation was used.

b) Oxidation by CYP, ring opening to form diethylene glycol, followed by oxidation to HEAA Diethylene glycol metabolization to HEAA has been observed previously, however not in the contest of 1,4-dioxane metabolism.

c) Hypothesised metabolism when pathway a) is saturated: α-hydroxylation to form dioxan-2-ol, ring opening to yield 2-hydroxy-ethoxy acetaldehyde followed by oxidation to HEAA (aldehyde intermediate has not been experimentally observed). Dioxan-2-ol is in equilibrium with the aldehyde (von Helden 2013).

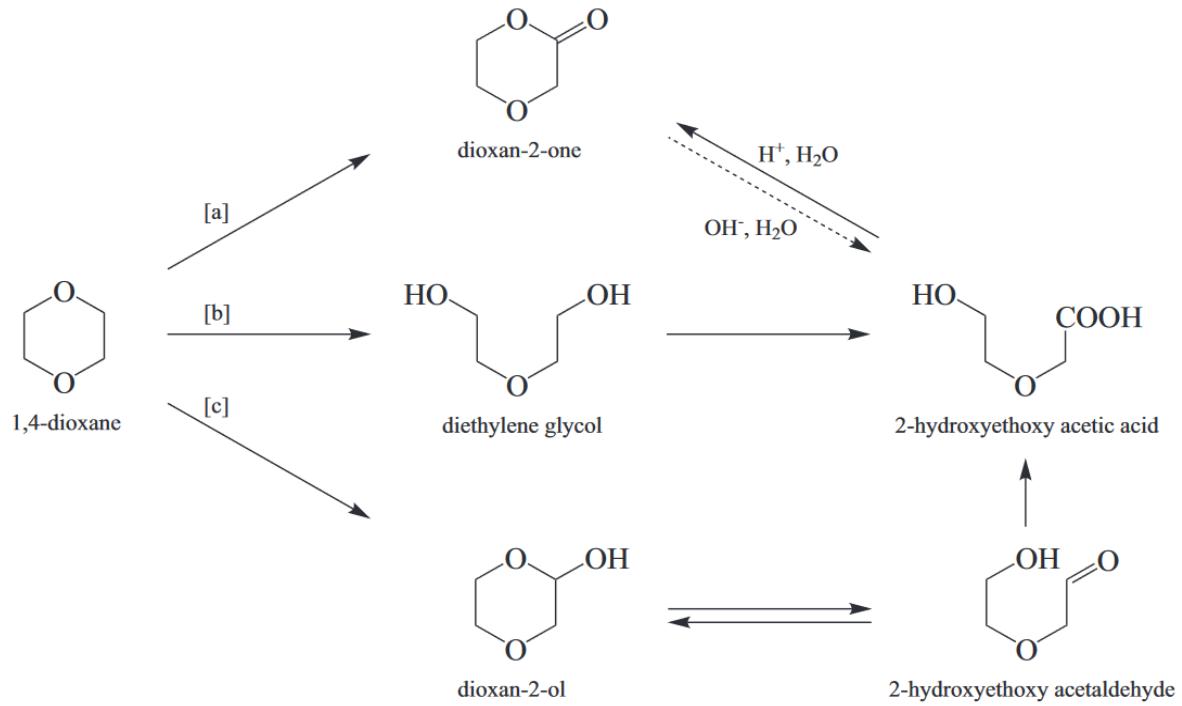


Figure 1: Metabolic pathways were postulated for the metabolization of 1,4-dioxane to its main metabolite 2 hydroxyethoxyacetic acid (HEAA) in rats (taken from Hartwig (2020)).

7.1.2.4 Excretion

Oral administration was studied in Sprague-Dawley rats, which received by gavage doses of 10, 100, or 1000 mg/kg bw of uniformly labelled ^{14}C-1,4-dioxane as single dose or for 17 days. After the single dose, the label was measured in the urine (99%, 86%, 76%), and in the expired air (< 1%, 4.7%, 25%) for the low, mid and high dose, respectively, while the percentage eliminated in the faeces or as CO_2 in in exhaled air remains low at 1 and 3% respectively (Young, Braun, and Gehring 1978a; Young, Braun, and Gehring 1978b).

In the same studies, rats were also intravenously exposed to concentration between 3 to 1000 mg/kg bw. In the expired air, the label was found as unchanged 1,4-dioxane (1.3%, 8.9%), and as CO_2 (4.1%, 7%) in animals receiving 10 and 1000 mg/kg bw/day,

respectively. Parallel to the increase elimination via exhaled air, the HEAA urinary concentration decreased from 92% to 60% of the adsorbed dose.

Elimination of 1,4-dioxane in both the expired air and in the urine appear to be first-order kinetic processes (Young, Braun, and Gehring 1978a; Young, Braun, and Gehring 1978b).

Regardless of the route of administration, the primary excretion route for 1,4-dioxane is the metabolism to HEAA and its subsequent elimination via urine. A second metabolite, cyclic lactone dioxan-2-one was found (Woo et al. 1977), however, it exists in a pH dependent equilibrium with HEAA therefore observation of cyclic lactone dioxan-2-one could have depended on experimental conditions.

7.1.3 *In vitro* data

Sweeney et al. (2008) tested 1,4-dioxane on isolated hepatocytes from Sprague–Dawley rats, B6C3F1 mice, and 3 human donors. They measured several kinetic constants, metabolic profiles and found consistency among the human donors. In addition, the human constants were similar to these measured in rats and mice hepatocytes (Sweeney et al. 2008).

Bronaugh (1982) estimated that 0.3 or 3.2% of the applied dose can be absorbed depending on the level of occlusion and noted that the percentage of absorption was low due to the rapid evaporation of 1,4-dioxane. Dermal penetration rates were estimated at 0.36, 0.23 and 0.94 µg/cm^2/h for 1,4-dioxane dissolved in water, lotion and isopropyl myristate, respectively. A permeability constant of 2.7x10^{-4} cm/h was calculated on the occluded test system. In the same study, it was estimated that about 90% of the applied dose, 1,4-dioxane in a lotion, evaporates within 15 minutes from the application, the evaporation is complete within 24 hours when a non-absorbent test material is used (Bronaugh 1982).

Based on 1,4-dioxane solubility, using the permeability coefficient according to Potts & Guy, a skin absorption rate of 0.3 mg/cm^2/h was calculated. It was noted that dermal absorption would be limited by the evaporation (NICNAS 1998).

Dennerlein (2013) described a high transdermal flux of ~1.4 mg/cm^2/h after the application of 200 µL/cm^2 (occlusive) to freshly excised human skin in static Franz cells (Dennerlein et al. 2013).

An *ex vivo* experiment was conducted with non-occlusive application of 100 µL/cm^2 1,4-dioxane to 0.64 cm^2 human skin for 1 hour using a diffusion cell technique. The study reported a cumulative amount of 309 µg in the receptor fluid, and of 6 µg in the epidermis and dermis (~2% of absorbed) after 8 hours. Absorption was almost complete after 8 hours. The cumulative recovery was low, 63%, which was attributed to the evaporation of the substance (Dennerlein et al. 2015). Based on this data, DFG (Hartwig et al. 2020) estimated that a maximum amount of 984 mg 1,4-dioxane would be absorbed after the exposure of a 2000 cm^2 surface area of skin for 1 hour (penetration rate of about 0.5 mg/cm^2/h).

7.1.4 Toxicokinetic modelling

Physiologically based pharmacokinetic (PBPK) models have been developed since the 1990s for 1,4-dioxane.

Leung and Paustenbach (1990) developed a PBPK model for 1,4-dioxane and HEAA in rats and humans based on the existing models for styrene. Their model consisted of four modelled tissue compartments and human coefficients were considered to be equal to these of rats. The metabolic constants were derived from the studies of Young et al. (Young, Braun, and Gehring 1978a; Young, Braun, and Gehring 1978b; Young et al.

1977), which were also used for the validation of the model (Leung and Paustenbach 1990).

Reitz et al. (1990) also derived their model from the existing styrene one, however used 6 compartments instead of 4 and was constructed to include oral, inhalation or intravenous exposures. The model assumed metabolization only in the liver, and human data from the Young et al. studies were again used in both the derivation of the parameters and the model validation (Reitz et al. 1990).

Fisher et al. (1997) designed a model for organic volatile compounds and claimed it could be used for 1,4-dioxane although its predictions were not tested against experimental data in rats or humans. Interesting, this model include breast milk as a compartment and predicts a significant transfer of 1,4-dioxane in milk (18%) (Fisher et al. 1997). This cannot be verified as no measurements in milk are available in rats or human.

Sweeney et al. (2008) in their study administered a single dose of 1,4-dioxane (20, 200 or 2000 mg/kg bw) by gavage to male B6C3F1 mice with the aim to collect data to update the existing pharmacokinetics models. The recalculated parameters were able to adequately predict the concentration of 1,4-dioxane in blood and exhaled air for both rats and mice for mid and high concentrations, but the prediction was poor for the low concentration. The human model predictions were in line with data from the study in workers (Young et al. 1976), but not with the blood level found in volunteers (Young et al. 1977). The authors speculated that this discrepancy could be due to a change of inhalation rate of the volunteers to the ventilation rate or 'wrong' data in the volunteer study, e.g., they speculated that to match the concentrations reported the exposure should have been 100 ppm instead of 53 ppm as reported. An explanation for this increased exposure was hypothesised to be due to the volunteers having access to food and drinks where 1,4-dioxane could have partitioned thus increasing the total dose. (Sweeney et al. 2008). . Young et al. (1977) estimated that repeated exposure at 50 ppm (8 h/day) would never result in accumulation of concentrations above those occurring after a single 8 h exposure period.

Takano et al. (2010) published a 2-compartments model, i.e. liver and a second central compartment. To develop their model, they used information from *in vivo* repeated studies in rats, *in vitro* human and rat hepatocyte and *in silico* estimation. This model predicts a slight accumulation of 1,4-dioxane in blood. However, the models can be used for oral exposure only and its validation have been limited (Takano et al. 2010).

7.1.5 Summary

1,4-dioxane is rapidly absorbed after inhalation or oral exposure. Recent data indicate significant absorption via the skin. In studies with radioactive isotopes, the substance was found to widely distribute in the body and to tend to be more concentrated in the liver and kidneys, which is compatible with the assumed liver metabolization to HEAA and subsequent elimination in the urine. The formation of the main metabolite, HEAA, is rapid and linear until saturation of the metabolic pathway occurs. Human studies have shown that urinary excretion of HEAA decreases with increased inhalation dose suggesting saturation could also be plausible in humans. No indication of saturation was reported in humans after inhalation exposure at 50 ppm (180 mg/m^3). After exposure to radioactive isotopes, radioactivity was also detected in the exhaled air to a much lower extent than detected in the urine.

Several PBPK models have been developed for 1,4-dioxane, unfortunately all have limitations in their validation or do not correctly predict the available data. Therefore, their use is limited.

7.2 Acute toxicity

7.2.1 Human data

7.2.1.1 Acute oral toxicity

No relevant data available.

7.2.1.2 Acute dermal toxicity

In a case report, Sonneck (1964) described a 47-years-old female laboratory technician working in the dioxane distillation department, who developed inflammatory skin changes in the upper arms and, to a lesser extent, in the face after several weeks of dermal exposure to 1,4-dioxane. Concentrations of 1,4-dioxane and exposure modalities were not reported. Histological examinations of the stripy skin changes showed symptoms of eczema. The involved woman had previously a burn which is a confounder in assessing the skin changes (Sonneck 1964).

A fatal case of intoxication was reported where the worker was in extensive contact with the substance dermally and orally, see 7.3.1 (Johnstone 1959).

7.2.1.3 Acute inhalation toxicity

Exposure of 12 healthy volunteers to 0 and20 ppm (73 mg/m^3) 1,4-dioxane vapour for 2 hours did not result in inflammatory changes, as measured by the levels of high sensitivity C-reactive protein and interleukin 6 in blood collected before and 3 hours after exposure (Ernstgard et al. 2006). With reference to neurological effects, self-reported ratings of headache, fatigue, nausea, and 'feeling of intoxication' during and after exposure were no different than in sham exposure.

One study reported the fatality of a worker exposed to a concrete sealant containing 1,1,1-trichloroethane (80%) and 1,4-dioxane (2.5%) (Sullivan 1994).

In one of the earliest available studies, five workers (29-38 years old) employed in an artificial silk manufacture in the UK, died within 2 weeks of exposure to high concentrations (not specified) of 1,4-dioxane vapours (Barber 1934). All deaths occurred within a two-week period after an alteration in the manufacture process, which led to an increase in potential inhalation exposure to 1,4-dioxane. However, dermal contact may have also contributed to the total body burden. No quantitative estimates of exposure levels and duration of exposure were reported. Co-exposure to other workplace processes and possibly other chemicals was mentioned, but not well described. Clinical signs of toxicity included haemorrhagic nephritis, centrilobular liver necrosis, severe epigastric pain, convulsions, and coma. Histology revealed centrilobular liver necrosis and symmetrical necrosis (outer cortex) of the kidney. Three of the subjects endured abdominal pain and vomiting before death. Autopsy revealed extensive gross and microscopic lesions to the liver and kidneys likely due to exposure to a single large dose absorbed from the stomach. Extensive lesions in the kidneys and in the liver were observed. Leukocytosis and eosinophilia were described in subjects who survived exposure to high concentrations of 1,4-dioxane. With reference to neurological effects, oedema of the brain was observed in three of the five fatal cases described. However, as suggested by NIOSH (1977), these neurological changes were likely terminal, rather than specific toxic effects of 1,4-dioxane (NIOSH 1977).

A study including four men exposed to 50 ppm (183 mg/m^3) 1,4-dioxane for 6 hours found no abnormalities in the electrocardiograms (EKG) taken 24 hours and 2 weeks after exposure compared to EKGs taken prior to the study (Young et al. 1977). The same study did not show any significant effect of exposure on haematology parameters.

Another fatality following occupational exposure to 1,4-dioxane was reported (Johnstone 1959). After 1 week of exposure to an estimated average concentration of 470 ppm

(range 208-650 ppm; 761-2380 mg/m^3) of 1,4-dioxane in air (dermal absorption was also possible), a worker using 1,4-dioxane as a solvent to remove glue, died 6 days after being admitted to hospital with severe epigastric pain. Post-mortem examination revealed hepatic (centrilobular necrosis) and renal (necrosis of cortex) lesions, and demyelination and loss of nerve fibre in the central nervous system. The author concluded that alcohol consumption may have increased the susceptibility of the worker to 1,4-dioxane intoxication but made no conclusions about the nature of the exposure (i.e., acute or cumulative) associated with the elicited effects. Co-exposure to other workplace chemicals was not assessed.

7.2.2 Animal data

7.2.2.1 Acute oral toxicity

Several acute toxicity studies have been conducted with 1,4-dioxane over the years, see table below. The lowest oral LD_{50}s are 1270, 2000, 2000, 4500 and 5170 mg/Kg bw, for guinea pig, rabbit, cat, mouse and rat respectively (BASF 1973; BUA GDCh 1994; Laug et al. 1939; Mirkova 1994; Patty et al. 1994).

Animal exposed to 1,4-dioxane orally exhibited clinical signs of central nervous system (CNS) depression such as staggered gait, narcosis, paralysis or coma, irritation of the gastrointestinal mucous membranes, hepatic and renal degeneration and necrosis (EPA 2020; Health Canada 2021; SCOEL 2004).

Table 8: Oral LD50 values

Species (strain)	Oral LD_{50} (mg/kg bw)	References
Rat (NS)	5170	(SCOEL 2004; Laug et al. 1939)
Rat (NS)	5346	(Wilbur et al. 2012; Laug et al. 1939)
Rat (Wistar)	6369 (female)	(Wilbur et al. 2012; Pozzani, Weil, and Carpenter 1959)
Rat (Wistar)	7120	(Wilbur et al. 2012; Smyth, Seaton, and Fischer 1941)
Rat (NS)	7339	(SCOEL 2004; Nelson 1951)
Mouse (NS)	4500	(Health Canada 2021; Patty et al. 1994)
Mouse (NS)	5852	(Wilbur et al. 2012; Laug et al. 1939)
Rabbit (NS)	2000	(Health Canada 2021; BUA GDCh 1994)
Cat (NS)	2000	(Health Canada 2021; Mirkova 1994)
Guinea Pig (NS)	1270	(Health Canada 2021; BASF 1973)
Guinea Pig (NS)	4033	(Wilbur et al. 2012; Laug et al. 1939)
Guinea Pig (NS)	3150	(Wilbur et al. 2012; Smyth, Seaton, and Fischer 1941)

7.2.2.2 Acute dermal toxicity

The dermal toxicity of 1,4-dioxane was tested in both rats and rabbit, the lethal dose was above 8000 mg/kg bw and 7600 mg/kg bw, respectively (Derosa et al. 1996). No effects on the rat liver were observed in this experiment.

Table 9:Dermal LD50 values

Species (strain)	Dermal LD_{50} (mg/kg bw)	References
Rat (NS)	> 8000	(NICNAS 1998; Derosa et al. 1996)
Rabbit (NS)	7600	(NICNAS 1998; Derosa et al. 1996)
Rabbit (NS)	7855	(SCOEL 2004)

7.2.2.3 Acute inhalation toxicity

Acute toxicity inhalation studies in animals conducted at relatively high concentrations of 1,4-dioxane in several species indicate that the kidneys and liver, and in some cases, the lungs, are the main targets. The LC_{50} 4 hours was calculated to be 12780 ppm (46000 mg/m^3) for rats (ECETOC 1983), 18000 (65000 mg/m^3) for mice (ECETOC 1983). However, it could be lower as 1 out of 3 mice died after 3 hours exposure to 5000 ppm (18000 mg/m^3) in a repeated dose experiment, where the animal were exposed for 1 week, 5 days/week, 3h/day to 1,4-dioxane (Fairley, Linton, and Ford-Moore 1934).

Table 10: Inhalation LC50 values

Species (strain)	Inhalation LC_{50} (ppm) [mg/L]	References
Rat (NS)	12780 [46], 2 h exposure	(NICNAS 1998; ECETOC 1983)
Rat (Wistar, F)	14250, [51] 4 h	(Wilbur et al. 2012; Pozzani, Weil, and Carpenter 1959)
Mouse (NS)	18000 [65], 2 h exposure	(NICNAS 1998; ECETOC 1983)
Guinea Pig (NS)	30000 [108] (10-540 min; death of the majority of the animals in 180 minutes)	(Wilbur et al. 2012; Yant et al. 1930)

7.2.3 *In vitro* data

No relevant data available.

7.2.4 Summary

At least three human studies, including a total of seven fatalities, reported cases following occupational inhalation exposure to 1,4-dioxane. No or limited information were available about levels and duration of exposure, and potential co-exposures to other workplace chemicals. The main reported target organ effects were liver and kidney necrosis, haemorrhagic nephritis and epigastric pain. The available information on acute dermal toxicity is limited to one case report where potential confounding factors where not addressed.

Several studies to determine the acute toxicity of 1,4-dioxane have been conducted in the past and LD_{50} have been calculated for all routes of exposures. The acute toxicity of 1,4-dioxane in animal is low (lowest LD_{50} 1270 mg/kg bw in guinea pig (BASF 1973)). When exposed orally or via inhalation, animal studies show CNS depression and effects on liver and kidneys, mainly.

7.3 Specific target organ toxicity/Repeated dose toxicity

7.3.1 Human data

An occupational mortality study included 165 Texas workers exposed for 1 month to over 20 years (mean duration of exposure less than 5 years, 43% of workers less than 2 years exposure) to 1,4-dioxane (exposure levels 0.1-17 ppm, 0.36-61 mg/m^3) (Buffler et al. 1978). Twelve deaths were identified in the cohort (6 cardiovascular, 3 malignant neoplasms, 1 non-neoplastic gastric haemorrhage, 1 chronic hepatitis and liver failure, 1 accidental). The mortality rates (all causes, age-sex-race adjusted) in both the manufacturing area (7 observed vs. 4.9 expected) and the processing area (5 observed vs. 4.9 expected) were not different from the general population ($p>0.05$). Workers were exposed to other workplace chemicals, including trichloroethylene and vinyl chloride. Smoking history was not available for all the participants. From the limited available data, duration of exposure was not significantly associated with all-cause mortality. These observations were based on a small number of deaths of employees, low levels of exposure (mainly intermittent exposure), and relatively short periods of time.

A retrospective epidemiologic study on 151 employees in a textile factory, who were exposed for 1-6 years to concentrations of up to 1,350 mg/m^3 (369 ppm) of 1,1,1-trichloroethane blended with 4% 1,4-dioxane showed no significant differences in health, particularly on ECG changes and liver damage, when compared to the control group (Kramer et al. 1978).

In a cohort study of 74 German workers (32-62 years of age) exposed to an estimated 0.02–48 mg/m^3 (0.005-13 ppm) of 1,4-dioxane for an average duration of 25 years, no clinical signs or mortality was related to the chemical exposure. High serum transaminase levels were found in 16/47 workers, but the authors concluded that these changes could have been related to habitual alcohol consumption. When the workers were compared with the general German population, no statistically significant effects were found in any studied parameter (i.e. haemoglobin concentration, erythrocyte and leukocyte counts), including age-specific mortality and cancer (Thiess, Tress, and Fleig 1976).

7.3.2 Animal data

7.3.2.1 Inhalation

Several inhalation animal studies are available. Overall, the respiratory system, kidneys and the liver are the main target organs. After exposure to concentrations from 1000 ppm of 1,4-dioxane for 1.5 h/day 5 days/week for 3–12 weeks rats, mice, guinea pigs and rabbit showed hepatocyte and renal cortex degeneration, but no lesions in the lungs (Fairley, Linton, and Ford-Moore 1934).

Female Wistar rats exhibited neurological signs, depressed avoidance response, after exposure to > 3000 ppm (11000 mg/m^3) of 1,4-dioxane for 4 h/day, 5 days/week for 2 weeks (Goldberg et al. 1964).

In a more recent study, exposure to 1,4-dioxane vapours (400 mg/m^3 (111 ppm) 7 h/day, 5 days/week for 2 years) had no significant effect on mortality or body weight gain and induced no signs of eye or nasal irritation or respiratory distress in Wistar rats (288/sex). Microscopic examination of all tissues or organs did neither reveal treatment

related effects, nor their weight was affected (Torkelson et al. 1974). The nasal cavity was not listed among the examined tissues by the authors.

F344 rats (10/sex) were exposed for 6 h/day, 5 days/week for 13 weeks to 1,4-dioxane vapours (whole body) at concentrations of 0 (control), 100, 200, 400, 800, 1600, 3200, or 6400 ppm (360, 720, 1400, 2900, 5800, 11500 or 23100 mg/m^3). All the animal exposed to the high dose died within the first week due to renal failure as in all animals marked necrosis in the renal tubules was observed. Decrease in terminal body weight, and increase in relative weights of liver, kidney, and lung were observed. AST increased in the 200 ppm and 3200 ppm exposed females, and ALT increased in the 3200 ppm exposed males and females. The repeated exposure affected the upper and lower respiratory tract and liver in both male and female, and kidneys in females. Nuclear enlargement of nasal respiratory epithelial cells occurring in the 100 ppm exposed males and females was the most sensitive, followed by the enlarged nuclei in the olfactory, tracheal, and bronchial epithelia. In particular, the incidence and severity of enlarged nuclei of epithelial cells decreased from the upper to lower respiratory tracts, thus the authors speculated that the nuclear enlargement tended to decrease with the presumably gradual decrease in the amount of 1,4-dioxane absorbed in the mucous layer of the respiratory, olfactory, tracheal, and bronchial epithelia. 1,4-dioxane induced liver lesions at 3200 ppm and were characterized by single-cell necrosis and centrilobular swelling of hepatocytes in males and females. Glutathione S-transferase placental form (GST-P) positive liver foci (a preneoplastic lesion in rat hepatocarcinogenesis) were observed in the 1600 ppm exposed females and 3200 ppm exposed males and females. Plasma levels of 1,4-dioxane increased linearly with an increase in the concentrations of exposure to 400 ppm and above and were higher in female than in male (Kasai et al. 2008).

In a carcinogenicity study, 50 male F344/DuCrj rats were exposed via inhalation to 1,4-dioxane for 6 hours for 5d/week for 2 years at concentrations of 0, 50, 250, or 1250 ppm (180, 900 or 4500 mg/m^3). Survival was statistically decrease from week 91 at the high dose and was attributed to tumours formation. In the high dose group, decrease in body weight, statistically significant increase in relative liver and lung weights were observed, as well as changes in clinical chemistry and haematology. In all treated groups, changes on the olfactory epithelium in the form of significant increase in nuclear enlargement, atrophy and respiratory metaplasia were observed. In the high dose group, significant increases of liver lesions and changes in the proximal tubule of the kidney were recorded, while significant nuclear enlargement of the proximal kidney tubule were observed in the mid and high dose groups (Kasai et al. 2009).

Table 11: Incidences of selected histopathological pre- and non-neoplastic lesions in male F344 rats exposed by inhalation to 1,4-dioxane for 2 years (Kasai et al. 2009)

	Control	50 ppm	250 ppm	1250 ppm
Nasal cavity				
Respiratory epithelium				
Nuclear enlargment	0	50**	48**	38**
Squamous cell metaplasia	0	0	7*	44**
Squamous cell hyperplasia	0	0	1	10**
inflammation	13	9	7	39**
Olfactory epithelium				
Nuclear enlargement	0	48**	48**	45**

	Control	50 ppm	250 ppm	1250 ppm
Atrophy	0	40**	47**	48**
Respiratory metaplasia	11	34**	49**	48**
Inflammation	0	2	32**	34**
Hydropic change: lamina propria	0	2	36**	49**
Sclerosis: lamina propria	0	0	22**	40**
Proliferation: nasal gland	0	1	0	6*
Liver				
Nuclear enlargement: centrilobular	0	0	1	30**
Acidophilic cell foci	5	10	12	25**
Basophilic cell foci	17	20	15	44**
Clear cell foci	15	17	20	23
Mixed cell foci	5	3	4	14
Spongiosis hepatis	7	6	13	19**
Necrosis: centrilobular	1	3	6	12**
Kidney				
Nuclear enlargement: proximal tubule	0	1	20**	47**
Hydropic change: proximal tubule	0	0	5	6*

Note: * and ** significantly different from control at $p \leq .05$ and $p \leq .01$ by χ^2 test, respectively.

7.3.2.2 Oral

Oral gavage

No relevant studies available.

Drinking water

Administration of 1,4-dioxane in drinking water resulted in degenerative changes mainly in the livers and kidneys; in several studies the respiratory tract (nose cavity, trachea or lung), the skin or the stomach were also affected. Depending on the duration of the exposure, tumours were observed in most of these organs.

1,4-dioxane was fatal rats and mice when administered in drinking water (7230 and 9812 mg/kg bw/day, respectively) for 67 days. Histological examination of surviving animals revealed severe hepatic and renal lesions (cellular degeneration, etc.) (Fairley, Linton, and Ford-Moore 1934).

In male SD rats receiving 1,4-dioxane in drinking-water at dosed of 0, 10 or 1000 mg/kg bw/day for 11 weeks increased relative liver weight and minimal degree of liver lesion at the high dose were recorded (Stott, Quast, and Watanabe 1981).

Sherman rats (60/sex/dose) were administered 100, 1000 or 10000 mg/L (9.6/19, 94/148, 1015/1599 mg/kg bw/day, males/females respectively) of 1,4-dioxane in drinking water for 2 years. After 2 months of treatment, an increase in mortality was observed on the high dose along with decreased body weight gain and water

consumption. Liver (hepatocellular degeneration and necrosis) and kidneys (tubular epithelial degeneration and necrosis) were the affected organs in the mid and high dose groups (Kociba et al. 1974).

1,4-dioxane was administered to 50 F344/DuCrj rats in drinking water for two years (11/18, 55/83 or 274/429 mg/kg bw/day), non-neoplastic findings included a slight increase in liver spongiosis hepatitis in low dosed males, hyperplasia and spongiosis from the mid dose in both sexes (Yamazaki et al. 1994).

Kano et al. (2008) administered 1,4-dioxane to both Crj:BDF1 mice and F344/DuCrj for 13 weeks at doses of 0, 640, 1600, 4000, 10000 and 25000 ppm in drinking water. Dose dependent decrease of food, water consumption and consequently of body weigh was reported in all rodents. As in the previous studies the affected organs were respiratory tract, liver and kidneys, which was established as change in relative weight (kidney and lung in rats and mice and liver in rats) and further investigated histopathologically (Kano et al. 2008).

A recent study was performed to clarify the mode of action of 1,4-dioxane in mice. A group of 10 B6D2F1/Crl female mice were administered 1,4-dioxane in water at doses of 0, 40, 200, 600, 2000 or 6000 ppm, for 7, 28 or 90 days. After taking into account body weight and water consumption, the recalculated mean effective doses were 0, 7.2, 37.3, 116, 364 or 979 mg/kg bw/day. Liver weights were increased at all time points on the highest dose group, liver to body weight was increased also at 2000 ppm after 28 or 90 days of exposure. At the high dose level (6000 ppm) after 7 days exposure, minimal to mild centrilobular hypertrophy, appearing as granular eosinophilic cytoplasm, was observed and it increased in severity with time. Analogously single cell necrosis was present after 7 days exposure with increasing in intensity to minimal or mild single cell necrosis after 90 days. 1,4-dioxane was detected in blood only at the highest dose as expected after saturation of the metabolism which is estimated to occur between 400 and 1000 mg/kg bw/day. HEAA concentrations were linear and dose-proportional, and 1,4-dioxane was detected only on the highest dose confirming the saturation of its metabolism at high doses. The authors found a correlation between the increased mitogenic response in the liver to the presence of 1,4-dioxane which was observed before the liver cytotoxicity (Lafranconi et al. 2021).

7.3.3 *In vitro* data

No data available.

7.3.4 Summary

In three occupational epidemiological studies conducted in the 1970s and assessing long-term exposure to 1,4-dioxane, no clear toxicity emerged. However, the studies are limited in size and information of exposure levels.

Hepatic effects including hepatocellular degeneration, single cell necrosis, centrilobular swelling, vacuolisations in rats and mice and some studies reported significant changes of liver enzyme activity. In the kidneys in both mice and rats the effects recorded included histopathological alterations in some experiments accompanied by increase in kidney weight, cellular swelling, vacuolar changes, nuclear enlargement of the proximal tubule and lesion to the cortex such as degeneration, necrosis haemorrhages and vascular congestions.

7.4 Irritancy and corrosivity

7.4.1 Human data

No effects were found after exposure of 12 volunteers (6 men and 6 women) to 1,4-dioxane (0,20 ppm, 0,73 mg/m^3) for 2 hours at rest. Subjective symptoms were

assessed with a questionnaire and respiratory function was assessed by spirometry. Pulmonary function and nasal swelling, as well as inflammatory markers in plasma (C-reactive protein, and interleukin-6) were measured before and at 3 hours after exposure (Ernstgard et al. 2006).

In a study of four healthy male volunteers exposed to 50 ppm (180 mg/m^3) for 6 hours, the only effect reported was eye irritation (Young et al. 1977).

A 47-year-old female laboratory technician showed inflammatory skin changes in the upper extremities and, to a lesser extent, in the face after several weeks of dermal exposure to 1,4-dioxane. Histological examinations of the stripy skin changes showed symptoms of eczema. It should be noted that the involved woman had previously a burn which is a confounder in assessing the skin changes (Sonneck, 1964).

Twelve subjects were exposed to 1,4-dioxane for 15 minutes to observe olfactory fatigue. A concentration of 20 ppm (72 mg/m^3) showed to be the highest concentration acceptable. At 300 ppm (1080 mg/m^3) irritation of eyes, nose and throat was reported, although the odour was not recognised (Silverman, Schulte, and First 1946).

Wirth and Klimmer (1936) reported a slight burning sensation on mucous membranes of the mouth following exposure to about 278 ppm for a few minutes (unspecified), in 5 subjects. Throat irritation and strong throat irritation were reported after exposure for a few minutes to 1000 or 10000 mg/m^3 (273 or 1730 ppm) respectively (Wirth and Klimmer 1936).

In a group of six individuals exposed to 2000 ppm (7320 mg/m^3) 1,4-dioxane vapours for 3 minutes in a 10 m^3 chamber, there were no complaints of nasal discomfort, but one out of four subjects exposed to 1000 ppm (3660 mg/m^3) for 5 minutes complained of constriction of the throat (Fairley, Linton, and Ford-Moore 1934). However, the exposure concentrations were not verified.

A 10 minutes exposure to 1600 ppm (5800 mg/m^3) provoked slight nose irritation and throat irritation that persisted throughout the test in a group of five individuals (Yant et al. 1930). The same five persons were exposed to 5500 ppm (~20000 mg/m^3) 1,4-dioxane for 1 minute resulted in a burning sensation to the nose and throat (Yant et al. 1930).

7.4.2 Animal data

In a study from 1973, a cotton patch was soaked with undiluted 1,4-dioxane (~0.5 ml) and then applied to the shaved back of 1 male and 1 female rabbit for 1, 5, 15 minutes or 20 hours and on the ear (20h) under occlusive condition. The skin application for 1-15 minutes led to the formation of a slight erythema after 24 hours and scale formation after 8 days. One day after the 20h exposure, slight erythema and slight oedema were noted on 1 animal and 7 days after also moderate scale formation was observed. On the ear, slight erythema was noted from 14 hours after the exposure until 8 days later (BASF 1973).

The lowest irritating concentration was determined as 80% in physiological saline when 1,4-dioxane was applied to the skin of 3 Wistar rats and 3 ddY mice per sex (Sekizawa et al. 1994).

Irritation to the eyes was tested in two White Vienna rabbits when 0.05 mL 1,4-dioxane was applied undiluted for an unreported time. A day after the exposure slight corneal opacity, conjunctival redness, slight to severe chemosis and smeary deposit were observed in both rabbits. At the end of the study on day 8, slight conjunctival redness was still present on one animal (BASF 1973).

Sprague-Dawley rats (3/sex) were exposed to 155 mg/L 1,4-dioxane for 1, 3 or 7 hours. Mortalities occurred after 3 (6 animals) and 7 hours (4 animals). Irritation of the

respiratory tract was observed, and swollen lungs recorded after histopathology (BASF 1980).

Irritation to the mucous membranes of the nose and eyes were recorded after guinea pigs were exposed to 3.6, 7.32, 10.98, 36.6 or 109.8 mg/L (980, 2000, 3000, 10000, 30000 ppm) for up to 8 hours (Yant et al. 1930). In rats, mice, guinea pigs and rabbits exposed for 8 hours to 4000 ppm (14640 mg/m^3) or 11000 ppm (40260 mg/m^3) of 1,4-dioxane marked irritation of the mucous membranes were recorded (Gingell et al. 1994).

7.4.3 *In vitro* data

Two separate isolated bovine cornea test showed irritation with changes in opacity and thickness of the cornea a concentration of 5-100% (Gautheron et al. 1992; Igarashi and Northover 1987).

7.4.4 Summary

In human studies, irritation was observed on eye, nose and throat at concentrations generally above 1000 mg/m^3. In small human volunteer studies, no irritation effects were seen after 2 or 6 hours of exposure at 20 ppm (73 mg/m^3). Young et al. (1977) reported irritation at 50 ppm (180 mg/m^3).

In old studies not conducted according to the current standards, irritation was observed on eye, respiratory tract (nose mucous membranes) and to limited extent or after repeated exposure to the skin mainly because it can cause eczema by removing the skin fat protective layer. Due to the lower concentration tested, the EU RAR consider 1,4-dioxane as eye and respiratory tract irritant as well as causing skin damage after repeated exposure (EU 2002).

7.5 Sensitisation

7.5.1 Human data

7.5.1.1 Respiratory sensitisation

No relevant data available.

7.5.1.2 Skin sensitisation

A single positive patch test response to 1,4-dioxane was reported in a worker presenting with dermatitis apparently caused by skin contact with 1,4-dioxane used as a degreasing solvent (Adams 1983).

One 52-years old man, who developed dermatitis on his left hand after daily dipping in a 1,4-dioxane containing solvent for 3 years, scored positive in a patch test (0.5% in water) (Fregert 1974).

Several weeks of dermal exposure to 1,4-dioxane resulted in inflammatory skin changes in a female laboratory technician (Sonneck 1964). That study reported that renewed exposure, some 4 weeks later, led to a relapse with clinical symptoms of eczema. However, it was concluded from negative results on 2 other volunteers that this reaction was idiosyncratic and may have been related to a previously sustained chemical burn.

7.5.2 Animal data

7.5.2.1 Respiratory sensitisation

No data available.

7.5.2.2 Skin sensitisation

In a Guinea-Pig Maximization Test performed on B6 female Pirbright White rabbits no signs of skin sensitisation were observed (BASF 1973).

7.5.3 *In vitro* data

No data available.

7.5.4 Summary

1,4-dioxane did not show sensitisation properties on a Guinea-Pig Maximization Test.

The human data are too limited to draw conclusions.

7.6 Genotoxicity

7.6.1 Human data

Chromosomal aberrations (CA) were assessed in peripheral lymphocytes in six German workers exposed to unspecified levels of 1,4-dioxane for 6-15 years. No increase in CA was reported in the workers when compared to the control group (Thiess, Tress, and Fleig 1976).

In a further study, a significant increase in mean lymphocyte chromosomal aberration frequency was found in 11 workers exposed (>20 years) to alkylene oxides (including 1,4-dioxane). Potential co-exposures to known mutagens such as ethylene oxide and propylene oxide confound any conclusions with regard to causation (Thiess et al. 1981).

7.6.2 Animal data (in vivo)

Data on the *in vivo* mutagenicity testing are presented in Table 12.

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 12, 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 at dose levels from of 2000 mg/kg bw up to 5000 mg 1,4-dioxane/kg bw (doses above the top dose recommended for genotoxicity assays by the OECD guidelines). 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 1500 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 2000 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 2000 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 12. Kitchin and Brown (1990) found a dose-related increase in DNA single-strand breaks at 2550 and 4200 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 1000 mg/kg bw (Stott, Quast, and Watanabe 1981).

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 4000 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.

Table 12: Summary of *in vivo* mutagenicity studies (animal studies)

Method	Cell type	Concentration range*	Results - negative + positive	Klimisch score**	Reference
Somatic cell mutagenicity					
Micronuclei	CD-1 mice, male peripheral blood; 5/group	0, 500, 1000, 2000 and 3200 mg/kg bw (two intraperitoneal injections, 1/day); positive and negative control	- (toxicity at 3200 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, 2000, 3000, 4000 mg/kg bw (intraperitoneal injection) 0, 500, 1000, 2000 mg/kg bw (intraperitoneal injection, 3x); two studies in two different labs	- (decreased PCE/NCE ratio) - (500 and 1000 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, 1800, 3600 mg/kg bw (oral gavage) for 24h, 3600 mg/kg bw also for 48h	+ (dose-related increase) no data on cytotoxicity	2	(Mirkova 1994)

		sampling time			
	C57BL6 mice, male bone marrow 4/group	0, 900, 1800, 3600 mg/kg bw (oral gavage) for 24h, 3600 mg/kg bw also for 48h sampling time	+ (dose-related increase) no data on cytotoxicity	2	
Micronuclei Follow-up study of Morita and Hayashi 1998	CD-1 mice, male bone marrow; 5/group	1500, 2500 and 3500 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	1500, 2500 and 3500 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 2500 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	1800 mg/kg bw (oral, gavage); Giemsa staining**	- (decreased PCE/NCE ratio)	2	(Tinwell and Ashby 1994)
Other supporting studies					
UDS	Male rat liver F344 and primary hepatocytes	1% (1500 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	1000 mg/kg bw (oral, gavage), 2 hr and 12 hr sampling time	- (cytotoxicity not observed)	2	

UDS	Male rat liver F344; 3/group	1% (1500 mg/kg bw/day) in drinking water for 2 weeks or 2% (3000 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% (1500 mg/kg bw/day) in drinking water for 8 days (pre-treatment), followed by 0, 10, 100 or 1000 mg/kg bw (single gavage dose)	- (at highest dose signs of toxicity were observed); only morphologically normal cells were scored	2	
DNA strand breaks measured by alkaline elution assay	Female SD rats, 3-5/group; histopathological examination of liver	0, 168, 840, 2550, 4200 mg/kg bw (oral gavage twice) for 21 and 4 h before sacrifice	+ (from 2550 mg/kg bw, dose-related increase; but irrelevant dose levels) Histopathology liver: 3/5 rat of 2550 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 for cell proliferation)	Male F344 rats; 4/group; hepatocytes isolated after exposure for testing	Gavage; 1000, 1500, 2000 and 4000 mg/kg bw; 24h and 48h response time; thymidine and BrdU incorporation	+ (24h-response time: dose-related increase from 1000 mg/kg bw, but no increase at 4000 mg/kg bw; relationship was bell shaped; no hepatotoxicity at any dose level) (48 hr-response time; no hepatocytotoxicity)	2	(Miyagawa et al. 1999)

* (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..

Four additional studies (described below) have been published since 2018, which concluded that the substance may be genotoxic.

A 2018 study by Gi et al. investigated the mutagenic potential on the liver of guanine phosphoribosyl transferase (*gpt*) delta transgenic F344 and wild type F344 rats(Wei et al. 2018). *Gpt* delta transgenic F344 rats received 1,4-dioxane (0, 200, 1000 or 5000 ppm; 730, 3660, 18300 mg/m^3) in drinking water for 16 weeks. The *gpt* transgene mutation frequency was increased in the 5000 (statistically different from control $p<0.05$) and 1000 ppm group. In particular, in the high dose group A:T to G:C transition and A:T to T:A transversion frequencies were significantly increased, this latter was significantly increased also in the mid dose. The number of GST-P-positive foci was

increased in the mid and high dose, reaching a statistically significant increase only on the high dose ($p<0.001$). Moreover, the relative mRNA expression of genes involved in cell proliferation [proliferating cell nuclear antigen (PCNA)], the DNA repair enzyme [O-6-methylguanine–DNA methyltransferase (MGMT)] were statistically induced in the 5000 ppm group ($p<0.05$ and $p<0.01$, respectively), while other DNA damage repair genes were induced. The wild type F344 rats received 1,4-dioxane in drinking water for 16 weeks at doses of 0, 2, 20, 200, 2000, or 5000 ppm. The number of GST-P-positive foci were statistically significantly increased at 2000 and 5000 ppm (both $p<0.001$). The authors concluded that the 5000 ppm dose exceeded the repair capacity with consequent formation of pre-neoplastic lesions, GST-P foci. The increased A:T-to-T:A transversions, observed at 1000, was attributed to the formation of adenosine adducts and was considered first consequence of excessive exposure. This because it was also observed at 5000 ppm, where A:T to G:C transitions and expression of MGMT were also increased (Gi et al. 2018).

In a follow up experiment of their 2018 study (Gi et al. 2018), Totsuka et al. (2020) analysed the DNA adducts in frozen liver samples of the F344 rats exposed to 0, 20, 200 or 5000 ppm (0, 73, 730, 18300 mg/m^3) 1,4-dioxane. A small number of DNA adducts were detected on the control and low dose group, whereas a larger number in the mid and high doses. In addition, in these two doses clusters were identified and analysed. Although precise identification of the structure of the DNA adduct was possible via LC-MS/MS spectroscopy, the most common adducts involved a thymine moiety, therefore the authors speculated that this adduct could be involved in the A:T to G:C and A:T to T:A mutations. In a second DNA adduct either cytidine or uracil were involved, and the third DNA adduct identified contained 8-oxo-dG which is produced from reactive oxygen species, thus related to oxidative stress. Based on their results, the authors speculated the mutation observed may not derive from direct DNA binding however, they could not confirm whether 1,4-dioxane binds or not directly to the DNA to form the adducts (Totsuka et al. 2020).

Furihata et al. (2018) used RNA Sequencing on 11 marker genes to compare the effects of 1,4-dioxane on liver cells with the profile of known genotoxic and non-genotoxic substances hepatocarcinogens. F344 rats received 0.5% of 1,4-dioxane in water or appropriate doses of another substance classified by IARC in group 2A, 2B or 3. The gene expression of the two genotoxic substances (groups 2A and 2B) was similar between them and distinct from that of the non-genotoxic substance (group 3), while the gene expression of 1,4-dioxane partially distinct from that of the two genotoxic molecules and appreciably distinct from the non-genotoxic one. Therefore, the authors concluded 1,4-dioxane has an intermediate profile of gene expression between a genotoxic and a non-genotoxic substance (Furihata et al. 2018).

In a 2019 study, Itoh and Hattori performed liver micronucleus, bone marrow tests and the *Pig-a* gene mutation assay using F344 male rats' peripheral blood. The liver micronucleus test was performed in three methods: one with juveniles and two with partial hepatectomy (PH), dosing before and after the PH. Groups of 4 animals were used for the micronucleus tests, five for the bone marrow or *Pig-a* assay. The animals received two oral doses in the juvenile (day 1 and 2, hepatocyte isolation on day 6), while only one dose was given to the other animals. The rats were dosed orally the day before or after the PH for the micronucleus with partial hepatectomy, the liver was removed 4 days after the partial removal, i.e. 6 or 4 days after dosing for before and after PH regimen, respectively. 1,4-dioxane was administered in 1000, 2000 or 3000 mg/kg bw, positive controls were used. In the liver micronucleus juvenile rat method, a dose dependent statistically significant increase in the incidence of micronucleated hepatocytes was observed. Treatment with 1,4-dioxane induced a dose-dependent statistically significant increase of micronucleated hepatocytes also in the liver micronucleus methods, independently of the PH performed before or after dosing. The incidence of binucleated hepatocyte in the 3000 mg/kg bw group dosing pre-PH was increased. In the bone marrow experiment, at 2000 mg/kg bw a statistically significant

increase of in the incidence of micronucleated immature erythrocytes (MNIE) was recorded one day after treatment but considered of no toxicological relevance by the authors. After 2 days treatment a statistically significant increase of in the incidence of immature erythrocytes (IE) was found in the 3000 mg/ kg bw group. No increased incidences were observed on the *Pig-a* assay. The authors speculated that 1,4-dioxane produced micronucleated hepatocytes from chromosome breakage in the liver, they considered the negative bone marrow study results supportive of the theory considering short-lived metabolite(s) or reactive oxygen species from metabolism of 1,4-dioxane important for mutagenicity. In addition, the increase in IE suggested, 1.4-dioxane (or its metabolites) reaches the bone marrow but probably not in a concentration sufficient to cause toxic effects. Overall, the authors concludes that 1,4-dioxane is clastogenic in the liver but not genotoxic in the bone marrow of rats (Itoh and Hattori 2019).

In a study from Chen et al. (Chen et al. 2022), 1,4-dioxane was administrate to wild type (WT) and *Gclm*-null (KO) mouse, 5-8 animals per group in 3 groups: (i) control, (ii) daily gavage administration of 1000 mg/kg bw for the last week at the end of the 3 months study period, and (iii) 5000 ppm in drinking water over 3 months. The overall dose was considered equivalent between the 7-day gavage and 3 months administration via drinking water. The KO mice were chosen because their natural liver glutathione (GHS) level is about 15% of that in WT mice, and consequently KO mice are more sensitive to oxidative stress leading to liver damage. The authors measured no significant differences in direct liver cytotoxicity between the WT and KO mice (mild cytotoxicity observed in both mouse types), with more significant inflammatory responses in WT compared to KO mice. The expression of several genes and proteins involved in GHS synthesis, recycling antioxidation and xenobiotic metabolism were studied. As expected, most of these were upregulated in the KO mice. The study showed that 1,4-dioxane alters the liver redox status by upregulating the synthesis of genes involved in anti-oxidative responses (e.g., persistent induction of erythroid 2-related factor 2 (NRF2), increased levels of 8-oxo-dG). Also, the CYP2E1 metabolic enzyme expression was progressively increased with time, most likely being partly responsible for the observed oxidative stress. Overall, the authors concluded that exposure to 1,4-dioxane leads to genotoxicity in the liver, in part mediated by oxidative stress, which is linked to increased levels of reactive oxygen species and is a major mutagenic mechanism leading to carcinogenicity.

7.6.3 *In vitro* data

The data on *in vitro* mutagenicity testing as summarized in Table 13 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 chromatid exchange assay.

Table 13: *In vitro* genotoxicity studies

Method	Cell type	Concentration range*	Results - negative + positive	Klimisch score**	Reference
Micro-organisms					
Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537 E. coli WP2uvrA and WP2	0, 156, 313, 625, 1,250, 2500, and 5000 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)

Method	Cell type	Concentration range*	Results - negative + positive	Klimisch score**	Reference
Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537	0, 100, 133, 1000, 1333, and 10000 µg/plate	-	2	(Haworth et al. 1983)
Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	4, 20, 100, 500, 2500 µg/plate	-	2	(ECHA 2015)
Mammalian cells					
Gene mutation	Mouse lymphoma L5178Y cells, tk locus	0, 1250, 2500 and 5000 µg/ml: 3 and 24 hr exposure	- (slight decrease in relative survival at 5000 µg/ml +S9)	2	(Morita and Hayashi 1998)
Gene mutation	Mouse lymphoma L5178Y cells, tk locus	0, 312.5, 625, 1250, 2500, 5000 µg/ml (-S9) 0, 1000, 2000, 3000, 4000, 5000 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)
Micronucleus	Chinese hamster ovary, K1 cells	0, 1250, 2500 and 5000 µg/ml: 5 and 44 hr exposure (+/-S9)	-	2	(Morita and Hayashi 1998)
Chromosome aberration	Chinese hamster ovary, K1 cells	0, 1250, 2500 and 5000 µg/ml (+/-S9)	-	2	(Morita and Hayashi 1998)
Other supporting studies					
Sister chromatid exchange	CHO-K1 cells	0, 1250, 2500 and 5000 µg/ml (+/-S9) 3 and 26 hr exposure	- (dose-related cytotoxicity observed)	2	(Morita and Hayashi 1998)
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)

* + or - S9, with or without metabolic activation system. ** (Klimisch, Andreae, and Tillmann 1997)

1,4-dioxane was studied in six reverse mutation assays in bacterial cells, in two gene mutation assays, one micronucleus assay and two chromosome aberration tests in mammalian cells. These studies showed no mutagenic activity of 1,4-dioxane. Further, negative results were also reported in the unscheduled DNA synthesis assay and the sister chromatid exchange assay. In the Comet assay and in an alkaline elution assay in rat hepatocytes 1,4-dioxane induced DNA-damage, but only at cytotoxic concentrations

(0.3 mM and higher where the following doses were tested: 0, 0.03, 0.3, 3, 10 and 30 mM).

7.6.4 Summary

The *in vitro* tests, both in bacteria and mammalian cells, are negative but some of the *in vivo* tests are positive, predominantly at doses above the limit dose of 2000 mg/kg bw. The positive results above the limit dose may be due to cytotoxicity, leading to the induction of cell proliferation. The positive results found in the tests measuring replicative DNA synthesis as a marker for cell proliferation would confirm a non-genotoxic mode of action. However, since positive results in the micronucleus tests are found at doses below the limit dose of 2000 mg/kg bw a genotoxic mechanism as a secondary mode of action cannot be excluded.

In the study by Gi et al., mutagenic effects were observed only after the DNA repair capacity was exceeded (Gi et al. 2018). However, the same group in a follow up experiment could not determine if 1,4-dioxane directly binds to the DNA or not (Totsuka et al. 2020). The mutagenic profile of 1,4-dioxane was compared to that of known mutagen and non-mutagen and showed profile of gene expression intermediate between the two (Furihata et al. 2018). A 2019 study concluded that 1,4-dioxane is clastogenic in the liver but not genotoxic in the bone marrow of rats (Itoh and Hattori 2019).

Human studies are limited due to small size, unknown exposure levels and missing information on potential exposure to other known mutagens in parallel.

7.7 Carcinogenicity

7.7.1 Human data

In a retrospective mortality study of 165 workers exposed to 1,4-dioxane during manufacture and processing, the observed cancer deaths (3) were not significantly different from the expected number (1.7) (Buffler et al. 1978). Exposure periods for tumour onset were between 1 and 4 yr. The workers concerned had apparently been exposed to less than 25 ppm (92 mg/m^3) 1,4-dioxane. Cancer deaths were reported as carcinoma of stomach, alveolar cell and mediastinal tumour. A death from chronic hepatitis/cirrhosis was also reported. Results were inconclusive according to study authors for reasons such as the small cohort size and relatively short exposure duration.

In a study from Germany, including 74 workers (age 32-62 years) exposed to 1,4-dioxane production for 5-41 years, no increased incidence in cancer was observed. The workers were exposed to 1,4-dioxane during manufacture and handling, for an average duration of 25 years, with an estimated exposure of 0.02 to 48 mg/m^3 (0.005-13 ppm). The authors concluded that increased serum transaminase levels seen in 6 of 24 workers currently exposed may have been related to alcohol consumption. Two retired workers were diagnosed with cancer (squamous epithelial carcinoma and myelofibrosis leukaemia) and died (Thiess, Tress, and Fleig 1976).

No malignancies related to exposure to 1,4-dioxane were detected in two other studies performed on workers at production plants of 1,4-dioxane or in which 1,1,1-trichloroethane was mixed with 1,4-dioxane as a stabiliser (Kramer et al. 1978; Dernhal 1976).

A retrospective study from England, including 80 factory workers potentially exposed to 0.18-184 mg/m^3 (0.05-50 ppm) of 1,4-dioxane for some years identified no exposure related health effects (Barber 1934).

7.7.2 Animal data

In a carcinogenicity study, 50 male F344/DuCrj rats were exposed via inhalation to 1,4-dioxane for 6 hours for 5d/week for 2 years at concentrations of 0, 50, 250, or 1250

ppm (180, 900 or 4580 mg/m^3). Survival was statistically decrease from week 91 at the high dose and was attributed to tumours formation. 1,4-dioxane induced a statistically significant increase in hepatocellular adenomas and nasal squamous cell carcinoma (high dose), in peritoneal mesothelioma (mid and high doses). In addition, pre-neoplastic lesions were also recorded: squamous cell metaplasia (mid ad high doses), increased incidences of nuclear enlargement in the respiratory and olfactory epithelia, atrophy and respiratory metaplasia in the olfactory epithelium in the nasal cavity of male rats (all doses) (Kasai et al. 2009). The table below shows the tumour incidences on this study.

Table 14: Tumour incidences on 50 males per dose F344/DuCrj rats (Kasai et al. 2009)

Doses (ppm)	0	50	250	1250
Nose cavity: squamous cell carcinoma	0	0	1	6*
Liver: hepatocellular 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

Fisher exact test: *p≤0.05, **p≤0.01

In an older inhalation study, Wistar rats were exposed to 400 mg/m^3 (110 ppm) for 7 hours a day, 5 d/week for 2 years. Neoplastic lesions were not observed, however the nasal cavity was not examined (Torkelson et al. 1974).

In several carcinogenicity studies, rats and mice were administered 1,4-dioxane orally in drinking water (NCI 1978; Kano et al. 2009; Kociba et al. 1974). In all studies, 1,4-dioxane induced tumours in the nasal cavity and the liver of both rats and mice. In all studies, non-neoplastic lesions progressed to hepatocellular adenoma and carcinoma and to nasal squamous carcinoma in rats but not in mice at higher dosages. Tumours in the nose were detected at higher doses (from 0.5%) and lower incidence respect to liver tumours (from 0.05%) in both rats and mice. Nasal cavity tumours were attributed to exposure while drinking (Sweeney et al. 2008). In addition, peritoneal mesotheliomas were observed (Kano et al. 2009; Kociba et al. 1974). Tumour incidences observed in these studies are summarised on the tables below.

Table 15: Tumour incidences on 50/sex/group F344/DuCrj rats (Kano et al. 2009)

Doses (mg/kg bw/day)	0	11/18	55/83	274/429
Nose cavity: squamous cell carcinoma (m/f)	0/0	0/0	0/0	3/7**
Nose cavity: esthesioneuroepithelioma (m/f)	0/0	0/0	0/0	1/1
Nose cavity: rhabdomyosarcoma (m/f)	0/0	0/0	0/0	1/0
Nose cavity: sarcoma (not otherwise specified (m/f)	0/0	0/0	0/0	2/0
Liver: hepatocellular adenoma (m/f)	3/3	4/1	7/6	32**/48**

Liver: hepatocellular carcinoma (m/f)	0/0	0/0	0/0	14**/10**
Peritoneum: mesothelioma (m/f)	2/1	2/0	5/0	6/8*
Mammary gland: fibroadenoma or adenoma (m/f)	1/8	2/8	2/11	6/18*
Subcutis: fibroma (m/f)	5/0	3/2	5/1	12/0

Fisher exact test: *p≤0.05, **p≤0.01

Table 16: Tumour incidences on 50/sex/group Crj:BDF1 mice (Kano et al. 2009)

Doses (mg/kg bw/day)	0	49/66	191/278	677/964
Nose cavity: adenocarcinoma (m/f)	0/0	0/0	0/0	0/1
Nose cavity: esthesioneuroepithelioma (m/f)	0/-	0/-	0/-	1/-
Liver: hepatocellular adenoma (m/f)	9/5	17/31**	23**/20**	11/3
Liver: hepatocellular carcinoma (m/f)	15/0	20/6*	23/30**	36**/45**

Fisher exact test: *p≤0.05, **p≤0.01

Table 17: Tumour incidences on 35/sex/group Osborne-Mendel rats after 110 weeks exposure (NCI 1978)

Doses (mg/kg bw/day)	0	240/350	530/640
Nose cavity: adenocarcinoma (m/f)	0/0	1/0	3/1
Nose cavity: squamous cell carcinoma (m/f)	0/0	12/10**	16***/8***
Nose cavity: rhabdomyosarcoma (m/f)	0/-	1/-	0/-
Liver: hepatocellular adenoma (m/f)	2/0	2/10	1/11**
Liver: hepatocellular carcinoma (m/f)	0/-	1/-	0/-
Testis/epididymis: mesothelioma (m/f)	2/-	4/-	5/-

Fisher exact test: *p≤0.05, **p≤0.01, ***p≤0,001, ****p=0.003

Table 18: Tumour incidences on 50/sex/group B6C3F1 mice after 90 weeks exposure (NCI 1978)

Doses (mg/kg bw/day)	0	720/380	830/860
Nose cavity: adenocarcinoma (m/f)	0/0	0/1	1/0
Liver: hepatocellular carcinoma (m/f)	2/0	18***/12***	24***/29***
Liver: hepatocellular adenoma or carcinoma (m/f)	8/0	19***/21***	28***/35***

Fisher exact test: *p≤0.05, **p≤0.01, ***p≤0,001, ****p=0.014

Table 19: Tumour incidences on 60/sex/group Sherman rats (Kociba et al. 1974)

Doses (mg/kg bw/day)	0	10/19	94/148	1015/1599
Nose cavity: squamous cell carcinoma	0	0	0	3***
Liver: hepatocellular carcinoma	1	0	1	10**
Liver: hepatic tumour, all types	2	0	1	12*

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

Other carcinogenicity studies were conducted in mice via intraperitoneal injection (1986) and in mice and rats via dermal exposure (1973). In these old studies tumours in the lungs and in the liver were observed, however the studies are not considered reliable.

7.7.3 Summary

A few human epidemiological studies are available concerning the carcinogenic properties of 1,4-dioxane. They show no indications of carcinogenicity. However, the quality of these studies is limited by the limited information available on potential confounding factors, and the lack of quantitative information on exposure levels, making it difficult to conclude on the carcinogenicity potential of 1,4-dioxane.

In the rodent studies, neoplastic lesions in the liver and in the nasal cavity were observed both after administration via inhalation and in drinking water studies. In addition, other types of tumours were observed in some studies, e.g., peritoneal mesothelioma and tumours in kidneys or mammary glands. Pre-neoplastic lesions were also reported on repeated dose studies in the same organs. Overall, 1,4-dioxane is considered carcinogenic to the rodents.

7.8 Reproductive toxicity

7.8.1 Human data

A Russian study, including 314 pregnant women working in the electronic industry and exposed to several chemicals (including 1,4-dioxane) reported an increased incidence of miscarriages, premature births, maternal toxicosis, foetal ossifications and decreased birth weights (Ailamazian 1990). Gonadotoxic effects, associated with 1,4-dioxane exposure, also in the electronics industry, were reported by Mikheev and Minkina (1979). However, the available data in those two studies, including the lack of exposure levels, does not allow to draw any causal relationship with respect to 1,4-dioxane exposure and the potential toxic effects observed.

7.8.2 Animal data

No generation studies have been performed with 1,4-dioxane.

In a carcinogenicity study a non-dose-dependent increased mineralisation in the testis was reported in Crj:BDF1 mice at doses ≥ 191 mg/kg bw/day, but not in F344/DuCrj rats up to the highest dose of 1025 mg/kg bw/day (Yamazaki et al. 1994).

No signs of adverse effects on the reproductive organs were observed on the 90 days or 2 years studies on either F344/DuCrj rats or Crj:BDF1 mice in drinking water (Kano et al. 2009; Kano et al. 2008), or on the 90 days or 2 years studies on F344/DuCrj rats via inhalation (Kasai et al. 2009; Kasai et al. 2008) up to the highest dosed tested.

In a prenatal developmental toxicity study similar to OECD Test Guideline 414, Sprague Dawley rats (18 to 20 per group) received 1,4-dioxane (purity: 99%) in drinking water by gavage at doses of 0, 0.25, 0.5 or 1.0 mL/kg bw/day, corresponding to 0, 257.5, 515 or 1030 mg/kg bw/day, from days 6 to 15 of gestation. The body weight gains of the dams were reduced at the highest dose. The body weights of the foetuses at day 0 and the ossification of sternebrae were significantly reduced at the highest dose. No other teratogenic effects were observed (Giavini, Vismara, and Broccia 1985).

1,4-dioxane was used as a stabiliser for 1,1,1-trichloroethane on a series of reproductive studies between 1975 and 1989. In a 2-generation study in ICR Swiss mice, no toxic effects on reproduction were found up to the highest 1,4-dioxane dose tested of 30 mg/kg bw/d (Hartwig 2020; Lane, Riddle, and Borzelleca 1982). On two developmental toxicity studies no effects were observed after the exposure of Sprague-Dawley rats and

Swiss Webster mice by inhalation up to the highest 1,4-dioxane concentration tested of 32 ppm (117 mg/m^3) for 7 hours daily between gestation days 6 to 15 (Schwetz, Leong, and Gehring 1975). No developmental toxicity or fertility effects were observed in Sprague Dawley rats dosed with 3% of 1,4-dioxane in the drinking water from 14 days pre-cohabitation, up to 13 days during the cohabitation phase, and for females up to postnatal day 21. The high dose corresponded to 3.5 mg 1,1,1-trichloroethane/kg bw/day or 0.1 mg 1,4-dioxane/kg bw/day (George et al. 1989).

7.8.3 Summary

No reproductive toxicity effects were observed in rats and mice after administration of 1,4-dioxane. However, 1,4-dioxane was studied on generation studies only as stabiliser for 1,1,1-trichloroethane.

The human studies do not allow to conclude on potential effects on reproductive toxicity.

8. Other considerations

8.1 Mode of action (MoA) considerations

1,4-dioxane is a carcinogenic substance (classified as Carc. 1B), which has been shown to cause nasal tumours in test animals as the result of direct local contact as well as systemic exposure. In addition, hepatic, renal and peritoneal tumours have been reported.

1,4-dioxane has been consistently found to be non-genotoxic (several publications and (Committee for Risk Assessment 2019)). Although recent studies provide some data on a genotoxic potential (Gi et al. 2018; Itoh and Hattori 2019; Totsuka et al. 2020), the behaviour is not fully comparable to that of a known genotoxic substance (Furihata et al. 2018), and therefore the current data is not sufficient to consider 1,4-dioxane as genotoxic. Thus, the non-genotoxic MoA, regenerative hyperplasia model, is considered as more plausible.

The assumed four events related to systemic effects (liver tumours) were summarised by Dourson et al. (Dourson et al. 2014; Dourson et al. 2017) and are explained in detail below.

1. *Metabolic saturation and consequently accumulation of 1,4-dioxane*

   The first event in the non-genotoxic MoA is the saturation of the metabolism of 1,4-dioxane to HEAA between 30 and 100 mg/kg bw/day in rats and at 200 mg/kg bw/d in mice (Dourson et al. 2017, Young, Braun, and Gehring 1978a, 1978b, Sweeney et al. 2008). The saturation level after single exposure could be lower, since it was demonstrated that 1,4-dioxane induces its own metabolism after repeated exposures (Dietz, Stott, and Ramsey 1982). Overall, the studies in animals showed that the liver toxicity is evident above the metabolic saturation, and it is thus attributed directly to 1,4-dioxane and not to a metabolite.

2. *Liver hypertrophy*

   Cellular swelling, hypertrophy and liver weight increase was observed in rats at 42 to 55 mg/kg bw/day.

3. *Hepatocellular cytotoxicity*

   Necrosis and/or inflammation in rats from 94 to 219 mg/kg bw/day.

4. *Regenerative cell proliferation leading to liver tumour formation*

   Hyperplasia and foci development in rats from 55 to 389 mg/kg bw/day, followed by adenomas and carcinomas at 274 to 1015 mg/kg bw/day.

Two 2021 articles (Lafranconi et al. 2021; Chappell, Heintz, and Haws 2021) further explored the MoA in mice. The first describes a 90-day study in mice (drinking water) and the second presents transcriptomics analyses on the livers of exposed mice. The outcome of these two studies supports the regenerative hyperplasia model MoA (Dourson et al. 2014; Dourson et al. 2017).

In their assessment of the MoA, Health Canada (2021) considered both the genotoxic and the non-genotoxic induced pathways. In their analysis of the genotoxicity MoA, there is a lack of dose concordance between the doses causing cancer or hepatic lesions and these causing micronucleus formation, ≥240, 9.6 to 94, ≥900 mg/kg bw/day respectively. Furthermore, no data are available to establish a temporal concordance. Mainly 1,4-dioxane exhibits its genotoxic effects at high doses, despite in two cases positive results were obtained in two studies at lower doses (Mirkova 1994; Suzuki et al. 1995) where cytotoxicity was not measured. Even if this genotoxic effect was not due to cytotoxicity, it could not explain the tumour formation at lower doses. From computed structure activity analysis, 1,4-dioxane could interact with DNA or protein in a non-covalent binding way. It was noted that recently, Japanese research groups (Gi et al. 2018) (Totsuka et al. 2020; Itoh and Hattori 2019), observed genotoxic properties of 1,4-dioxane and concluded that there could be a genotoxic MoA which could play a role after the DNA repair systems are saturated. Overall, Health Canada considered that it is not possible to completely exclude the contribution of a genotoxic MoA to the tumour formation, but, however, this does not appear to be the first contributing mechanism to tumour formation (Health Canada 2021).

In their documentation for OEL recommendations, DFG (Hartwig 2020) and DECOS (2011) concluded that the nasal tumours observed after exposure to 1,4-dioxane are likely to be associated with non-genotoxic mechanisms of action, involving irritation of the nasal epithelium resulting in cytotoxicity, inflammation, regenerative cell proliferation and hyperplasia. Systemic toxicity (e.g., hepatic necrosis, followed by tumour formation) was considered to occur only after the saturation of metabolism. Also, SCOEL (2004) considered that the mechanism appears to be non-genotoxic, involving the saturation of the main metabolic pathway.

Considering the available data, although there is some uncertainty on the mode of action, the carcinogenicity of 1,4-dioxane is expected to be related to non-genotoxic mechanisms, involving saturation of the metabolic capacity at high exposure levels.

8.2 Lack of specific scientific information

No specific information gaps were identified.

8.3 Groups at Extra Risk

No groups at extra risk were identified.

9. Evaluation and recommendations

9.1 Cancer risk assessment

9.1.1 Published approaches for cancer risk assessment

9.1.1.1 SCOEL

SCOEL (2004) noted that *in vitro* genotoxicity tests of 1,4-dioxane were mostly negative, and that the majority of *in vivo* assays were also negative, while the positive results were obtained mostly at high concentrations. SCOEL further considered that as micronuclei in mouse bone marrow cells may also be induced by non-genotoxic mechanisms, 1,4-dioxane is considered a non- or very weak genotoxic compound based

on the total weight of evidence. SCOEL noted that this is further supported by the absence of DNA-adducts at hepatotoxic doses. SCOEL further noted that 1,4-dioxane has been shown to be carcinogenic in several drinking water studies in rats, mice and guinea pigs and that the target organs were mainly the liver and nasal cavities. The mechanism appears to be non-genotoxic, involving the saturation of one metabolic pathway and the increasing prominence of an alternative one which produces the reactive, cytotoxic metabolite 2-hydroxyethoxyacetaldehyde. As further explained in section 9.2.1 SCOEL derived an OEL in order to avoid irritation effects.

9.1.1.2 DECOS

DECOS (2011) noted that 1,4-dioxane is negative in most *in vitro* mutagenicity assays, while a few *in vivo* micronuclei assays showed a positive result in liver and bone marrow. However, these results were obtained after exposure to very high concentrations of 1,4-dioxane (exceeding the maximal tolerable dose) and were therefore not considered relevant. Overall, DECOS concluded that 1,4-dioxane is not genotoxic and found that the nasal tumours found after exposure to 1,4-dioxane are possibly associated with a non-genotoxic mechanism of action, i.e., the injury of cells in the respiratory and olfactory epithelium. In addition, DECOS considered that the hepatocellular adenomas are associated with a non-genotoxic mechanism as well, i.e., hepatocellular injury (necrosis of hepatocytes). The LOAEL for the nasal lesions in rats after lifetime exposure to 1,4-dioxane was identified as 50 ppm (180 mg/m^3). As further explained in section 9.2.1 DECOS derived an OEL using it as a starting point.

9.1.1.3 DFG

DFG (Hartwig 2020) noted that 1,4-dioxane induced DNA strand breaks and micronuclei *in vivo* only at cytotoxic concentrations, generally above 2000 mg/kg bw/day. The primary mode of action for carcinogenesis was deemed to be non-genotoxic. Genotoxic effects were assumed to have a subordinate role in carcinogenicity and to occur only at cytotoxic doses, if at all. Non-linear toxicokinetics and the accumulation of the substance at high doses were explained by metabolic saturation. Toxicity leading to carcinogenic effects in the liver and kidneys is assumed to occur only after the saturation of 1,4-dioxane metabolism. The mechanisms involved in nasal tumour development are most likely local irritation of the nasal epithelium, followed by cytotoxicity, inflammation, regenerative cell proliferation and hyperplasia. Notably, local irritation is observed below metabolic saturation. Potential mechanisms of carcinogenesis at other cancer sites include direct liver toxicity of 1,4-dioxane induced above saturation levels and leading to enlargement of hepatocytes, hypertrophy and necrosis of the liver, as well as oxidative stress in the kidneys and in the liver, following cytochrome P450 induction. The increased incidence of nuclear enlargement in the kidneys at 250 ppm (915 mg/m^3) was found to be the most sensitive systemic effect in a chronic inhalation study in rats. As further explained in section 9.2.1, a MAK value was derived using a LOAEC of 50 ppm (180 mg/m^3) as a starting point.

9.1.2 Cancer risk assessment

The available human epidemiological studies are descriptive occupational studies, mainly from the 1970s, with no dose-response risk estimates. As discussed in section 8.1, the carcinogenicity of 1,4-dioxane is considered to be related to non-genotoxic mechanisms, involving saturation of the metabolic capacity, irritation at high exposure levels and formation of liver tumours by regenerative proliferation.

Even though a mode of action-based threshold is assumed for the carcinogenic effects of 1,4-dioxane, some uncertainties with regard to residual cancer risk remain. However, the level of uncertainty is considered to be low, in view of the evidence that only above saturation levels of metabolism (which is in humans above 180 mg/m^3; EU, 2002) tumours

are formed. Therefore, in this case, no additional dose-response for carcinogenicity (i.e. cancer risk estimates) are provided for the purpose of this report.

9.2 Derived Occupational Exposure Limit (OEL) Values

9.2.1 Published approaches to establishing OELs

9.2.1.1 SCOEL

In the SCOEL recommendation (2004) an 8h TWA of 20 ppm (73 mg/m^3) was proposed. SCOEL did not propose a STEL or any notations.

SCOEL (2004) justified the recommendation as follows: "*On the basis of the Torkelson et al (1974) study reporting no effects in rats with lifetime exposure to 400 mg/m^3 (111 ppm) and the need to avoid eye irritation (seen in human volunteers at 50 ppm; 180 mg/m^3) a TWA of 20 ppm (73 mg/m^3) is proposed*".

9.2.1.2 DECOS

DECOS (2011) recommended an 8 h TWA OEL of 6 ppm (20 mg/m^3) for 1,4-dioxane. As in the recommendation by DFG (see below) nasal lesions observed in rats after lifetime exposure to 1,4-dioxane at a concentration of 50 ppm (180 mg/m^3) (Kasai et al. 2009) were considered as the critical effect, and 50 ppm was interpreted as a LOAEL. As explained in section 9.1.1, the carcinogenicity of 1,4-dioxane was considered as being based on a non-genotoxic mode of action. The recommended OEL was obtained by applying an extrapolation factor of 3 for the conversion from LOAEL to NAEL, and a factor of 3 for interindividual differences. As the critical effect is not a systemic effect, but seen locally, there was no need to add any extrapolation factor to cover species differences. Furthermore, DECOS did not add any factor to compensate for differences in exposure duration (6 h/day in Kasai et al. (2009) and 8 h/day for occupational exposure) as the rat was considered more sensitive to nasal lesions than humans. No skin notation was proposed and no groups at extra risk were identified.

9.2.1.3 DFG

The report by DFG (Hartwig 2020) gives a MAK value (8 h TWA) of 10 ppm (37 mg/m^3). Nasal toxicity, nasal irritation and carcinogenic effects in the nose, liver and kidneys were identified as critical effects. As explained in section 9.1.1, carcinogenic effects were considered to be related to a non-genotoxic mode of action. The limit value was derived from a LOAEC of 50 ppm, identified in (Kasai et al., 2009). At this dose level, nuclear enlargement, atrophy and respiratory metaplasia in the nasal cavity were reported. No increase in tumour formation was seen. A factor of 3 was applied to convert the LOAEC to a NAEC of 16.67 ppm. Finally, with the aim to provide additional protection against tumour induction in the nose, a MAK value of 10 ppm was recommended. It was also noted that inhalation studies with human volunteers showed a NOAEC of 20 ppm for sensory irritation. In addition, DFG recommended a 15 minutes short-term value of 20 ppm (74 mg/m^3) ("Peak limitation category I and excursion factor 2").

DFG considered that "skin contact is expected to contribute significantly to systemic toxicity", and a skin notation was therefore assigned.

9.2.2 Occupational Exposure Limit (OEL) - 8h TWA

An 8 h TWA is recommended to protect workers against local and systemic effects of 1,4-dioxane. As discussed in sections 8.1 and 9.1.2, although there is some uncertainty on the mode of action, the carcinogenicity of 1,4-dioxane is considered to be related to

non-genotoxic mechanisms, involving saturation of the metabolic capacity and irritation at high exposure levels.

In addition to carcinogenicity, critical effects reported in *in vivo* studies include kidney effects, and local nasal irritation. The chronic toxicity study by Kasai et al. (2009), in which rats were exposed to 1,4-dioxane by inhalation for 2 years (5 days/week, 6 h/day) at doses of 50-1250 ppm (183-4575 mg/m^3) is identified as the key study to be used as the starting point for the derivation of an OEL.

9.2.2.1 Derivation of an OEL based on local effects

The initial nasal effects included increased incidences of nuclear enlargement of the respiratory epithelium, and nuclear enlargement, atrophy, and respiratory metaplasia of the olfactory epithelium. These effects were observed at all dose levels. Thus, the lowest dose of 50 ppm (183 mg/m^3) was identified as LOAEC for local effects.

To extrapolate from the LOAEC to a NAEC, a default assessment factor of 3 is applied. Although almost all animals showed local irritation in the nose at 183 mg/m^3 (50 ppm), in human volunteer studies, no irritation was seen at 73 mg/m^3 (20 ppm), while it was reported in another study at 183 mg/m^3 (50 ppm). Therefore, taking into account animal and human data, a factor of 3 is considered sufficient.

Adjusting the starting point (the concentration for nasal effects) with respect to differences in human and experimental exposure conditions is deemed not necessary, as the toxic effect (local irritation) is driven by the concentration.

.

For interspecies extrapolation, allometric scaling is not applied as the effects are considered local. The default assessment factor for remaining uncertainties with regard to dynamic differences is 2.5. In the review of Brüning et al. (2014) the authors concluded on an interspecies extrapolation factor of 3 for extrapolating animal data to humans concerning local sensory irritating effects. Results from short-term studies with human volunteers showed no sensory irritation effects upon exposure at 20 ppm for 6 h (Ernstgard et al., 2006) and only eye irritation at 50 ppm for 6 h (Young et al. 1977). As these human studies are short-term, and the local irritation effects in the nose of rats were found in a 2-year study, a factor of 2.5 is applied for interspecies necessary.

For intraspecies differences, an assessment factor of 3 is chosen.

The total assessment factor would thus be 22.5 (3x1x3x2.5). This results in an OEL (8 h TWA) of 8.1 mg/m^3 ppm (2.2 ppm).

9.2.2.2 Derivation of an OEL based on systemic effects

When looking at the systemic effects, a NOAEC of 50 ppm (180 mg/m^3) is identified for kidney effects (nuclear enlargement of the proximal tubule in 20 of 50 animals) from the same inhalation carcinogenicity study with rats (Kasai et al. 2009). The dose-related effects in liver (centrilobular necrosis) already started at a lower dose concentration but were not statistically significant until the highest dose. Altogether, 50 ppm was identified as NOAEC for all endpoints with respect to systemic effects.

From human data, there is only some evidence of kidney effects from old case studies (Barber, 1934; Johnstone, 1959) after exposure at high concentrations of 1,4 dioxane in the air for 1 or 2 weeks, resulting in death. Post-mortem findings showed extensive lesions in kidneys (haemorrhagic necrosis of the kidney cortex), next to hepatic necrosis and perivascular widening in the brain. No information in humans is available after chronic exposure.

For the derivation of an OEL, the NOAEC from the rat study should be converted with regard to the exposure conditions. The NOAEC of 50 ppm is converted from rat to

human, taking into account differences in breathing volume (x 6.7 m^3/10 m^3), to 33.5 ppm and adjusted from 6 hours exposure (5 days/week, 2 years) to 8 hours exposure duration, resulting in a converted NOAEC of 25.1 ppm (92 mg/m^3).

Using default assessment factors, a total assessment factor of 12.5 is applied (2.5 for interspecies differences, 5 for intraspecies differences, none for exposure duration), resulting in an OEL (8 h TWA) of 7.3 mg/m^3 (2 ppm).

9.2.2.3 Summary

An OEL of 7.3 mg/m^3 (2 ppm) is proposed based on the systemic effects in kidney, which is also protective of the nasal irritation effects leading to carcinogenicity and the effects found in liver.

Even though the proposed limit value assumes a mode of action-based threshold for the carcinogenic effects of 1,4-dioxane, some uncertainties with regard to residual cancer risk remain. However, provided that the proposed OEL is complied with, the level of uncertainty is considered to be low, in view of the evidence that only above saturation levels of metabolism (which is in humans above 180 mg/m^3; EU, 2002) tumours are formed. Therefore, in this case, no cancer risk assessments are provided.

No analytical difficulties are foreseen as 1,4-dioxane can be measured in air in low concentrations (LOQ 0.047 mg/m^3).

9.2.3 Short Term Exposure Limit (STEL)

1,4-dioxane has in humans been reported to cause irritation of the nose, eyes and throat at high concentrations. As local irritation effects of the nose may in the worst case be followed by inflammation, nasal hyperplasia and formation nasal tumours, limiting the short-term exposure is considered relevant.

In a study with human volunteers, no effects were observed upon exposure at 20 ppm for 2 hours. A STEL (15 minutes) of 73 mg/m^3 (20 ppm) is recommended.

9.2.4 Biological Limit Value (BLV)

A function showing the relationship between the mean urinary level of the metabolite 2-hydroxyethoxyacetic acid (HEAA) at the end of exposure in relation to the air concentration of 1,4-dioxane is presented in section 6.2.2. That function can be used to derive a BLV which corresponds to the OEL (8 h TWA).

If the OEL for 1,4-dioxane was 2 ppm, a calculation using the correlation explained in section 6.2.2, shows that the corresponding urinary limit value would be:

BLV=17.82 x 2 ppm+ 9.58 ≈ 45 mg HEEA / g creatinine.

Sampling should take place at the end of the exposure period or work shift.

9.2.5 Biological Guidance Value (BGV)

No data on background levels of 1,4 dioxane or its metabolites in the general population have been found and no BGV is proposed. It is expected that the BLV will be well above levels in the general population.

9.3 Notations

As presented in section 7.1, 1,4-dioxane may be absorbed via the skin in significant amounts and therefore a Skin notation is recommended.

Studying dermal absorption can be hampered by the quick evaporation of 1,4-dioxane, but data are however available.

The available data are equivocal with regard to the quantification of dermal absorption. Putting some more weight on the recent studies, a penetration rate of 984 mg/2000 cm^2/h has been calculated. It can be estimated that about 7800 mg 1,4-dioxane could be dermally absorbed (two hands 2000 cm^2; 984 mg x 8 hr) upon exposure for 8 hours, in comparison with an amount of 73 mg (OEL of 7.3 mg/m^3 x 10 m^3 in 8 hr and 100% absorption) absorbed via inhalation during a workday at an air concentration of 7.3 mg/m^3. Therefore, dermal exposure is considered relevant, and a skin notation is proposed.

REFERENCES

Adams, R. M. 1983. *Solvents and plasticizers* (New York, Grune and Stratton USA).

Ailamazian, E. K. 1990. 'Effect of ecological factors on the course of pregnancy', *Vestn Akad Med Nauk*, 7: 2.

Barber, H. 1934. 'Haemorrhagic nephritis and necrosis of the liver from dioxan poisoning', *Guy's Hospital Reports*, 84: 267-80.

BASF. 1973. *Unpublished report. Reviewed in EU RAR 2002.*

BASF. 1980. *Unpublished report. Reviewed in EU RAR 2002.*

BAuA. 2020. *Risk Management Option Analysis Conclusion Document. 1,4-dioxane.* *https://echa.europa.eu/documents/10162/010b37a1-9d0d-a69e-a703-df8626102fae* (ECHA).

Bronaugh, R. 1982. *Percutaneous absorption of cosmetic ingredients* (University of Minnesota: Minneapolis (MN)).

BUA GDCh, Advisory committee on existing chemicals of environmental relevance (BUA). 1994. "1,4-Dioxane." In, edited by Beratergremium fur Umweltrelevante Altsoffe S. Hirzel-Wissenchaftliche Verlagsgesellschaft. Stuttgart.

Buffler, P. A., S. M. Wood, L. Suarez, and D. J. Kilian. 1978. 'Mortality Follow-up of Workers Exposed to 1,4-Dioxane', *Journal of Occupational and Environmental Medicine*, 20.

Chappell, G. A., M. M. Heintz, and L. C. Haws. 2021. 'Transcriptomic analyses of livers from mice exposed to 1,4-dioxane for up to 90 days to assess potential mode(s) of action underlying liver tumor development', *Current Research in Toxicology*, 2: 30-41.

Chen, Ying, Yewei Wang, Georgia Charkoftaki, David J. Orlicky, Emily Davidson, Fengjie Wan, Gary Ginsberg, David C. Thompson, and Vasilis Vasiliou. 2022. 'Oxidative stress and genotoxicity in 1,4-dioxane liver toxicity as evidenced in a mouse model of glutathione deficiency', *Science of The Total Environment*, 806: 150703.

Committee for Risk Assessment. 2019. "Opinion proposing harmonised classification and labelling at EU level of 1,4-dioxane." In.: ECHA.

DECOS. 2011. *1,4-Dioxane. Health-based recommended occupational exposure limit. Health Council of the Netherlands.*

Dennerlein, K., T. Jäger, T. Göen, S. Kilo, K. H. Schaller, H. Drexler, and G. Korinth. 2015. 'Evaluation of the effect of skin cleaning procedures on the dermal absorption of chemicals', *Toxicology in Vitro*, 29: 828-33.

Dennerlein, K., D. Schneider, T. Göen, K. H. Schaller, H. Drexler, and G. Korinth. 2013. 'Studies on percutaneous penetration of chemicals – Impact of storage conditions for excised human skin', *Toxicology in Vitro*, 27: 708-13.

Dernhal, G.W. 1976. 'Written communication, quoted in 1,4-Dioxane', *NIOSH Criteria Document, DHeW Publication*, 77: 1.

Derosa, C. T., S. Wilbur, J. Holler, P. Richter, and Y-W. Stevens. 1996. 'Health Evaluation of 1,4-Dioxane', *Toxicology and Industrial Health*, 12: 1-43.

Dietz, F. K., W. T. Stott, and J. C. Ramsey. 1982. 'Nonlinear Pharmacokinetics and Their Impact on Toxicology: Illustrated with Dioxane', *Drug Metabolism Reviews*, 13: 963-81.

Dmitriev, MT. , EG. Rastiannikov, and AG. Mal'ysheva. 1985. 'Identification of specific micro-components of human feces', *Lab Delo*, 10: 608-14.

Dourson, M. L., J. Higginbotham, J. Crum, H. Burleigh-Flayer, P. Nance, N. D. Forsberg, M. Lafranconi, and J. Reichard. 2017. 'Update: Mode of action (MOA) for liver tumors induced by oral exposure to 1,4-dioxane', *Regulatory Toxicology and Pharmacology*, 88: 45-55.

Dourson, M., J. Reichard, P. Nance, H. Burleigh-Flayer, A. Parker, M. Vincent, and E. E. McConnell. 2014. 'Mode of action analysis for liver tumors from oral 1,4-dioxane exposures and evidence-based dose response assessment', *Regulatory Toxicology and Pharmacology*, 68: 387-401.

ECETOC. 1983. "1,4-Dioxane." In *Joint Assessment of Commodity Chemicals*. European Chemical Industry Ecology & Toxicology Centre.

ECHA. 2015. '1,4-Dioxane', ECHA data base.

Eckert, E., A. Hartwig, and H. Drexler. 2020. '1,4-Dioxane - Addendum for re-evaluation of the BAT value. Assessment Values in Biological Material. Translation of the German version from 2020.' in, *The MAK-Collection for Occupational Health and Safety*.

EPA, U.S. 2020. *Final 1,4-Dioxane Risk Evaluation.* *https://www.epa.gov/sites/default/files/2020-12/documents/1._risk_evaluation_for_14-dioxane_casrn_123-91-1.pdf*.

Ernstgard, L., A. Iregren, B. Sjogren, and G. Johanson. 2006. 'Acute effects of exposure to vapours of dioxane in humans', *Human & Experimental Toxicology*, 25: 723-29.

EU. 2002. "EU RAR. 1,4-Dioxane." In.: European Commission.

Fairley, A., E. C. Linton, and A. H. Ford-Moore. 1934. 'The Toxicity to Animals of 1:4 Dioxan', *The Journal of hygiene*, 34: 486-501.

Fisher, J., D. Mahle, L. Bankston, R. Greene, and J. Gearhart. 1997. 'Lactational transfer of volatile chemicals in breast milk', *American Industrial Hygiene Association Journal*, 58: 425-31.

Fregert. 1974. 'Allergic contact dermatitis from dioxane in a solvent for cleaning metal parts', *Contact Dermatitis Newsletter*, 15: 1.

Furihata, C., T. Toyoda, K. Ogawa, and T. Suzuki. 2018. 'Using RNA-Seq with 11 marker genes to evaluate 1,4-dioxane compared with typical genotoxic and non-genotoxic rat hepatocarcinogens', *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 834: 51-55.

Gautheron, P., M. Dukic, D. Alix, and J. F. Sina. 1992. 'Bovine corneal opacity and permeability test: An in vitro assay of ocular irritancy', *Fundamental and Applied Toxicology*, 18: 442-49.

George, J. D., C. J. Price, M. C. Marr, B. M. Sadler, B. A. Schwetz, L. S. Birnbaum, and R. E. Morrissey. 1989. 'Developmental toxicity of 1,1,1-trichloroethane in CD rats', *Fundamental and Applied Toxicology*, 13: 641-51.

Gi, M., M. Fujioka, A. Kakehashi, T. Okuno, K. Masumura, T. Nohmi, M. Matsumoto, M. Omori, H. Wanibuchi, and S. Fukushima. 2018. 'In vivo positive mutagenicity of 1,4-dioxane and quantitative analysis of its mutagenicity and carcinogenicity in rats', *Archives of Toxicology*, 92: 3207-21.

Giavini, E., C. Vismara, and M. L. Broccia. 1985. 'Teratogenesis study of dioxane in rats', *Toxicology Letters*, 26: 85-88.

Gingell, R, RJ Boatman, JS Bus, TJ Cawley, JB Knaak, WJ Krasavage, NP Skoulis, CR Stack, and TR Tyler. 1994. *Glycol ethers and other selected glycol derivatives. In: Patty's industrial hygiene and toxicology* (John Wiley & Sons).

Göen, T., F. von Helden, E. Eckert, U. Knecht, H. Drexler, and D. Walter. 2016. 'Metabolism and toxicokinetics of 1,4-dioxane in humans after inhalational exposure at rest and under physical stress', *Archives of Toxicology*, 90: 1315-24.

Goldberg, M. E., H. E. Johnson, U. C. Pozzani, and H. F. Smyth, Jr. 1964. 'Effect of Repeated Inhalation of Vapors of Industrial Solvents on Animal Behavior I. Evaluation of Nine Solvent Vapors on Pole-Climb Performance in Rats', *American Industrial Hygiene Association Journal*, 25: 369-75.

Goldsworthy, TL, TM Monticello, KT Morgan, E Bermudez, DM Wilson, R Jäckh, and BE Butterworth. 1991. 'Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes', *Arch Toxicol*, 65: 1-9.

Hartwig, A. 2020. *1,4-dioxane MAK Value Documentation, supplement – Translation of the German version from 2019 – Erratum*.

Haworth, Steve, Timothy Lawlor, Kristien Mortelmans, William Speck, and Errol Zeiger. 1983. 'Salmonella mutagenicity test results for 250 chemicals', *Environmental Mutagenesis*, 5: 3-49.

Health Canada. 2021. "Guidelines for Canadian Drinking Water Quality: Guideline Technical Document-1,4-Dioxane." In. Ottawa, ON.

Igarashi, Haruyoshl, and Ann M. Northover. 1987. 'Increases in opacity and thickness induced by surfactants and other chemicals in the bovine isolated cornea', *Toxicology Letters*, 39: 249-54.

Itoh, S., and C. Hattori. 2019. 'In vivo genotoxicity of 1,4-dioxane evaluated by liver and bone marrow micronucleus tests and Pig-a assay in rats', *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 837: 8-14.

Johnstone, R. T. 1959. 'Death due to dioxane?', *AMA Archives of Industrial Health*, 20: 445-47.

Kano, H., Y. Umeda, T. Kasai, T. Sasaki, M. Matsumoto, K. Yamazaki, K. Nagano, H. Arito, and S. Fukushima. 2009. 'Carcinogenicity studies of 1,4-dioxane administered in drinking-water to rats and mice for 2 years', *Food and Chemical Toxicology*, 47: 2776-84.

Kano, H., Y. Umeda, M. Saito, H. Senoh, H. Ohbayashi, S. Aiso, K. Yamazaki, K. Nagano, and S. Fukushima. 2008. 'Thirteen-week oral toxicity of 1,4-dioxane in rats and mice', *The Journal of Toxicological Sciences*, 33: 141-53.

Kasai, T., H. Kano, Y. Umeda, T. Sasaki, N. Ikawa, T. Nishizawa, K. Nagano, H. Arito, H. Nagashima, and S. Fukushima. 2009. 'Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats', *Inhalation Toxicology*, 21: 889-97.

Kasai, T., M. Saito, H. Senoh, Y. Umeda, S. Aiso, H. Ohbayashi, T. Nishizawa, K. Nagano, and S. Fukushima. 2008. 'Thirteen-Week Inhalation Toxicity of 1,4-Dioxane in Rats', *Inhalation Toxicology*, 20: 961-71.

Kitchin, K. T., and J. L. Brown. 1990. 'Is 1,4-dioxane a genotoxic carcinogen?', *Cancer Letters*, 53: 67-71.

Klimisch, HJ, M Andreae, and U Tillmann. 1997. 'A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data', *Regul Toxicol Pharmacol*, 25: 1-5.

Kociba, R. J., S. B. McCollister, C. Park, T. R. Torkelson, and P. J. Gehring. 1974. '1,4-Dioxane. I. Results of a 2-year ingestion study in rats', *Toxicology and Applied Pharmacology*, 30: 275-86.

Kramer, C. G., H. R. Imbus, M. G. Ott, J. E. Fulkerson, and N. Hicks. 1978. 'Health of workers exposed to 1, 1, 1,-trichloroethane: a matched-pair study', *Archives of Environmental Health*, 33: 331-42.

Krämer, W., R. Hebisch, and A. Hartwig. 2016. 'MAK Commission: Solvent mixtures [Air Monitoring Methods, 2014a]', *The MAK Collection for Occupational Health and Safety*, 1: 2127-38.

Kraus, T., K.H. Schaller, and C. Csanády. 2007. '1,4-Dioxane [BAT Value Documentation, 2007].' in, *The MAK-Collection for Occupational Health and Safety*.

Lafranconi, M., R. Budinsky, L. Corey, J. Klapacz, J. Crissman, M. LeBaron, R. Golden, and R. Pleus. 2021. 'A 90-day drinking water study in mice to characterize early events in the cancer mode of action of 1,4-dioxane', *Regulatory Toxicology and Pharmacology*, 119: 104819.

Lane, R. W., B. L. Riddle, and J. F. Borzelleca. 1982. 'Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice', *Toxicology and Applied Pharmacology*, 63: 409-21.

Laug, E. P., H. O. Calvery, H. J. Morris, and G. Woodard. 1939. 'The toxicology of some glycols and derivatives', *Journal of Industrial Hygiene and Toxicology*, 21: 173-201.

Leng, G., W. Gries, T. Göen, M. Bader, Elisabeth Eckert, and A. Hartwig. 2015. 'β-Hydroxyethoxyacetic acid (HEAA) in urine [Biomonitoring Methods, 2015].' in, *The MAK-Collection for Occupational Health and Safety*.

Leung, H. W., and D. J. Paustenbach. 1990. 'Cancer risk assessment for dioxane based upon a physiologically-based pharmacokinetic approach', *Toxicology Letters*, 51: 147-62.

Marzulli, F. N., D. M. Anjo, and H. I. Maibach. 1981. 'In vivo skin penetration studies of 2,4-toluenediamine, 2,4-diaminoanisole, 2-nitro-p-phenylenediamine, p-dioxane and N-nitrosodiethanolamine in cosmetics', *Food and Cosmetics Toxicology*, 19: 743-47.

McFee, A. F., M. G. Abbott, D. K. Gulati, and M. D. Shelby. 1994. 'Results of mouse bone marrow micronucleus studies on 1,4-dioxane', *Mutation Research/Genetic Toxicology*, 322: 145-48.

McGregor, D. B., A. G. Brown, S. Howgate, D. McBride, C. Riach, and W. J. Caspary. 1991. 'Responses of the L5178Y mouse Lymphoma cell forward mutation assay. V: 27 coded chemicals', *Environ Mol Mutagen*, 17: 196-219.

Mikheev, M. I., P. Gorlinskaya Ye, and T. V. Solovyova. 1990. 'The body distribution and biological action of xenobiotics', *Journal of Hygiene, Epidemiology, Microbiology, and Immunology*, 34: 329-36.

Mikheev, M.I. , and N.A. Minkina. 1979. 'Occupational health questions in the electronics industry', *Proceedings from the Erisman Institute of Hygiene*: 33-38.

Mirkova, E. T. 1994. 'Activity of the rodent carcinogen 1,4-dioxane in the mouse bone marrow micronucleus assay', *Mutation Research/Genetic Toxicology*, 322: 142-44.

Miyagawa, Makoto, Takashi Shirotori, Minoru Tsuchitani, and Kunie Yoshikawa. 1999. 'Repeat-assessment of 1,4-dioxane in a rat-hepatocyte replicative DNA synthesis (RDS) test: Evidence for stimulus of hepatocyte proliferation', *Experimental and Toxicologic Pathology*, 51: 555-58.

Morita, T. 1994. 'No clastogenicity of 1,4 dioxane as examined in the mouse peripheral blood micronucleus test', *Honyu Dobutsu Shiken Bunkakai Kaiho*, 2: 7-8.

Morita, T., and M. Hayashi. 1998. '1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay', *Environmental and Molecular Mutagenesis*, 32: 269-80.

NCI. 1978. 'Bioassay of 1,4-dioxane for possible carcinogenicity', *National Cancer Institute carcinogenesis technical report series*, 80: 1-123.

Nelson, N. 1951. 'Solvent toxicity with particular reference to certain octyl alcohols and dioxanes', *The Medical Bulletin*, 11: 226-38.

NICNAS, [National Industrial Chemicals Notificationand Assessment Scheme]. 1998. "1,4-Dioxane." In. Camperdown, Australia.

NIOSH. 1977. *Criteria for a recommended standard: Occupational Exposure to Dioxane* (U.S . Department of Health, Education and Welfare, Center for Disease Control, National Institute for Occupational Safety and Health.: Washington).

NIOSH. 1994. *NIOSH Manual of Analytical Methods. Method 1602* (National Institute for Occupational Safety and Health).

Patty, F. A., G. D. Clayton, F. E. Clayton, and R. E. Allan. 1994. *Patty's industrial hygiene and toxicology* (Wiley: New York [etc.]).

Pozzani, U. C., C. S. Weil, and C. P. Carpenter. 1959. 'The Toxicological Basis of Threshold Limit Values: 5. The Experimental Inhalation of Vapor Mixtures by Rats, with Notes upon the Relationship between Single Dose Inhalation and Single Dose Oral Data', *American Industrial Hygiene Association Journal*, 20: 364-69.

Reitz, R. H., P. S. McCroskey, C. N. Park, M. E. Andersen, and M. L. Gargas. 1990. 'Development of a physiologically based pharmacokinetic model for risk assessment with 1,4-dioxane', *Toxicology and Applied Pharmacology*, 105: 37-54.

Roy, S. K., A. K. Thilagar, and D. A. Eastmond. 2005. 'Chromosome breakage is primarily responsible for the micronuclei induced by 1,4-dioxane in the bone marrow and liver of young CD-1 mice', *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 586: 28-37.

Schwetz, B. A., B. K. J. Leong, and P. J. Gehring. 1975. 'The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats', *Toxicology and Applied Pharmacology*, 32: 84-96.

SCOEL. 2004. "Recommendation from Scientific Committee on Occupational Exposure Limits for 1,4-Dioxane. European Commission." In.

Sekizawa, J., K. Yasuhara, Y. Suyama, S. Yamanaka, M. Tobe, and M. Nishimura. 1994. 'A simple method for screening assessment of skin and eye irritation', *The Journal of Toxicological Sciences*, 19: 25-35.

Silverman, L., H. F. Schulte, and M. First. 1946. 'Further studies on sensory response to certain industrial solvent vapors', *The Journal of industrial hygiene and toxicology*, 28 6: 262-6.

Smyth, H.F., J. Seaton, and L. Fischer. 1941. 'The single dose toxicity of some glycols and derivatives.', *Journal of Industrial Hygiene and Toxicology*, 23: 259-68.

Sonneck, H. J. 1964. 'Kontaktekzem durch Dioxan in überwiegender lineärer Anordnung', *Dermatol. Wschr*: 24-27.

Stott, W. T., J. F. Quast, and P. G. Watanabe. 1981. 'Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat', *Toxicol Appl Pharmacol*, 60: 287-300.

Sullivan, L. J. 1994. 'Construction fatality: application of a concrete sealant.', *Applied Occupational and Environmental Hygiene*, 9: 2.

Suzuki, M., T. Noguchi, K. Noda, K. Fukuda, and T. Matsushima. 1995. "Ratliver micronucleustest with organic solvents (Japanese)." In, 151. 24th JEMS, Osaka, Japan (Abstract).

Sweeney, L. M., K. D. Thrall, T. S. Poet, R. A. Corley, T. J. Weber, B. J. Locey, J. Clarkson, S. Sager, and M. L. Gargas. 2008. 'Physiologically Based Pharmacokinetic Modeling of 1,4-Dioxane in Rats, Mice, and Humans', *Toxicological Sciences*, 101: 32-50.

Takano, R., N. Murayama, K. Horiuchi, M. Kitajima, F. Shono, and H. Yamazaki. 2010. 'Blood Concentrations of 1,4-Dioxane in Humans after Oral Administration Extrapolated from In Vivo Rat Pharmacokinetics,In Vitro Human Metabolism, and Physiologically Based Pharmacokinetic Modeling', *Journal of Health Science*, 56: 557-65.

Take, M., M. Ohnishi, S. Yamamoto, M. Matsumoto, K. Nagano, and S. Fukushima. 2012. 'Distribution of 1,4-dioxane by combined inhalation plus oral exposure routes in rats', *International Journal of Environmental Analytical Chemistry*, 92: 1715-28.

Thiess, A. M., H. Schwegler, I. Fleig, and W. G. Stocker. 1981. 'Mutagenicity study of workers exposed to alkylene oxides (ethylene oxide/propylene oxide) and derivatives', *Journal of occupational medicine. : official publication of the Industrial Medical Association*, 23: 343-7.

Thiess, A. M., E. Tress, and E. Fleig. 1976. "Examination results of persons exposed to dioxan [Arbeitsmedizinische Untersuchungsergebnisse Von Dioxan Exponier Ten Mitarbeitern]." In, edited by Sozialmedizin Arbeitsmedizin, Präventivmedizin, 36-46.

Tinwell, H., and J. Ashby. 1994. 'Activity of 1,4-dioxane in mouse bone marrow micronucleus assays', *Mutation Research/Genetic Toxicology*, 322: 148-50.

Torkelson, T. R., B. K. J. Leong, R. J. Kociba, W. A. Richter, and P. J. Gehring. 1974. '1,4-Dioxane. II. Results of a 2-year inhalation study in rats', *Toxicology and Applied Pharmacology*, 30: 287-98.

Totsuka, Y., Y. Maesako, H. Ono, M. Nagai, M. Kato, M. Gi, H. Wanibuchi, S. Fukushima, K. Shiizaki, and H. Nakagama. 2020. 'Comprehensive analysis of DNA adducts (DNA adductome analysis) in the liver of rats treated with 1,4-dioxane', *Proceedings of the Japan Academy. Series B, Physical and biological sciences*, 96: 180-87.

von Helden, F. 2013. 'Standardisierte Expositionen gegenüber 1,4-Dioxan zur Etablierung eines Biomonitorings', Justus-Liebig-Universität.

Wei, M., M. Fujioka, A. Kakehashi, T. Okuno, K. Masumura, T. Nohmi, M. Matsumoto, M. Omori, H. Wanibuchi, and S. Fukushima. 2018. 'In vivo positive mutagenicity of 1,4-

dioxane and quantitative analysis of its mutagenicity and carcinogenicity in rats', *Archives of Toxicology*, 92: 3207-21.

Wilbur, S. , D. Jones, J.F. Risher, J. Crawford, and B. Tencza. 2012. "Toxicologial profile for 1,4 dioxane." In, edited by Division of Toxicology and Environmental Medicine ATSDR, Atlanta, GA. Atlanta, Georgia.

Wirth, W., and O. Klimmer. 1936. 'On the toxicology of organic solvents. 1,4 dioxane (diethylene dioxide)', *Archiv fuer Gewerbepathologie und Gewerbehygiene*, 17: 192-206.

Woo, Y. T., J. C. Arcos, M. F. Argus, G. W. Griffin, and K. Nishiyama. 1977. 'Structural identification of p-dioxane-2-one as the major urinary metabolite of p-dioxane', *Naunyn-Schmiedeberg's Archives of Pharmacology*, 299: 283-87.

Woo, Y. T., M. F. Argus, and J. C. Arcos. 1977a. 'Metabolism in vivo of dioxane: Effect of inducers and inhibitors of hepatic mixed-function oxidases', *Biochemical Pharmacology*, 26: 1539-42.

Woo, Y.T., M.F. Argus, and J.C. Arcos. 1977b. 'Tissue and subcellular distribution of 3H-dioxane in the rat and apparent lack of microsome-catalyzed covalent binding in the target tissue', *Life Sciences*, 21: 1447-56.

Yamazaki, K., H. Ohno, M. Asakura, H. Ohbayashi, H. Fujita, M. Ohnishisi, T. Katagiri, H. Senoh, K. Yamanouchi, E. Nakayama, S. Yamamoto, T. Noguchi, K. Nagano, M. Enomoto, and H. Sakabe (ed.)^(eds.). 1994. *Two-year toxicological and carcinogenesis studies of 1,4-dioxane in F344 rats and BDF1 mice (drinking studies)* (International Center for Medical Research, Kobe University School of Medicine: Kobe, Japan).

Yant, W. P., H. H. Schrenk, C. P. Waite, and F. A. Patty. 1930. 'Acute response of guinea pigs to vapors of some new commercial organic compounds: VI. Dioxan', *Public Health Reports*, 45: 2023-32.

Yoon, J. S., J. M. Mason, R. Valencia, R. C. Woodruff, and S. Zimmering. 1985. 'Chemical mutagenesis testing in drosophila. IV. Results of 45 coded compounds tested for the national toxicology program', *Environmental Mutagenesis*, 7: 349-67.

Young, J. D., W. H. Braun, and P. J. Gehring. 1978a. 'The dose-dependent fate of 1,4-dioxane in rats', *Journal of Environmental Pathology and Toxicology*, 2: 263-82.

Young, J. D., W. H. Braun, P. J. Gehring, B. S. Horvath, and R. L. Daniel. 1976. '1,4-Dioxane and β-hydroxyethoxyacetic acid excretion in urine of humans exposed to dioxane vapors', *Toxicology and Applied Pharmacology*, 38: 643-46.

Young, J. D., W. H. Braun, L. W. Rampy, M. B. Chenoweth, and G. E. Blau. 1977. 'Pharmacokinetics of 1,4-dioxane in humans', *Journal of Toxicology and Environmental Health*, 3: 507-20.

Young, J.D., W.H. Braun, and P.J Gehring. 1978b. 'Dose-dependent fate of 1,4-dioxane in rats', *Journal of Toxicology and Environmental Health*, 4: 709-26.