The MAK-Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

1,4-Dioxane

MAK Value Documentation, supplement – Translation of the German version from 2019 – Erratum

A. Hartwig[1,*] MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *E-Mail: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords

mechanism of action, toxicokinetics, metabolism, irritation, reproductive toxicity, developmental toxicity, peak limitation, germ cell mutagenicity, sensitization, skin absorption, genotoxicity, carcinogenicity, maximum workplace concentration, MAK value, toxicity, hazardous substance

Citation Note:
Hartwig A, MAK Commission. 1,4-Dioxane. MAK Value Documentation, supplement – Translation of the German version from 2019 – Erratum. MAK Collect Occup Health Saf. 2020 May;5(1):Doc023. DOI: 10.34865/mb12391e5_1err1

Manuscript completed:
21 Mar 2018

Publication date:
11 May 2020

Published with erratum:
04 Jun 2020

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated 1,4-dioxane [123-91-1] considering all toxicological endpoints. Available publications and unpublished study reports are described in detail.

The critical effect is nasal toxicity and irritation as well as carcinogenic effects in the nose, liver, and kidneys. New carcinogenicity studies with 1,4-dioxane in drinking water confirm the previous tumour findings in rats and mice. Squamous cell carcinomas in the rat nose, also occurring in a long-term rat inhalation study at 1250 ml/m³, are a result of direct tissue contact with 1,4-dioxane in the drinking water. At 50 ml/m³ (LOAEC, lowest observed adverse effect level), no increase in tumour incidences, but nuclear enlargement, atrophy, and respiratory metaplasia in the nasal cavity were noted. The mechanisms involved in the tumour development in the nose are most likely cytotoxicity, inflammation, regenerative cell proliferation and hyperplasia. As the primary mode of action is non-genotoxic and genotoxic effects play no or at most a minor part at cytotoxic doses, 1,4-dioxane remains in Carcinogen Category 4.

A NAEC of 16.67 ml/m³ (LOAEC / 3) for effects in the nose was calculated from the long-term rat inhalation study, which is in the same range as the NOAEC of 20 ml/m³ from studies with 2- to 8-hour inhalation exposure of volunteers. To provide additional protection from tumour induction in the nose, the MAK value is lowered to 10 ml/m³.

As the critical effect of 1,4-dioxane is local and no irritation was observed in the study with 2-hour exposure of volunteers to 20 ml/m³, Peak Limitation Category I and the excursion factor of 2 are retained.

There is an adequate margin between the NOAEC for developmental toxicity and the MAK value. Therefore, damage to the embryo or foetus is unlikely when the MAK value is not exceeded and 1,4-dioxane remains assigned to Pregnancy Risk Group C.

In the original version of the article (DOI 10.34865/mb12391e5_1) the given authors are incorrect. In this erratum the authors are named correctly.

Because skin contact is expected to contribute significantly to systemic toxicity, the substance remains designated with “H”.

Limited data do not show a sensitizing potential.

MAK value (2018)	**10 ml/m³ (ppm) ≙ 37 mg/m³**
Peak limitation (2018)	**Category I, excursion factor 2**
Absorption through the skin (1966)	**H**
Sensitization	**–**
Carcinogenicity (1998)	**Category 4**
Prenatal toxicity (2006)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value (2012)	**400 mg 2-hydroxyethoxy acetic acid/g creatinine**
Solubility	miscible with water (ECHA 2018)
log K_{OW}	−0.42 (ECHA 2018)
1 ml/m³ (ppm) ≙ 3.656 mg/m³	**1 mg/m³ ≙ 0.273 ml/m³ (ppm)**

Documentation was published in 2003 (Greim 2003), followed by a supplement for prenatal toxicity in 2007 (Greim 2007, available in German only). A re-evaluation has become necessary as a result of new studies that investigated inhalation exposure, the carcinogenic potential of the substance and its metabolism and mechanism of action.

1,4-Dioxane is used mainly as a solvent because it is readily miscible with other solvents and is relatively inert.

The blood:air partition coefficient is 1666 (Sweeney et al. 2008). Therefore, the increased respiratory volume at the workplace has to be taken into account for the derivation of limit values that are based on systemic effects.

Toxic Effects and Mode of Action

The earlier findings have been confirmed by long-term drinking water studies that were published after the 2003 documentation (Greim 2003). 1,4-Dioxane induced squamous cell carcinomas in the nose and hepatocellular adenomas and carcinomas in F344 rats. Hepatocellular adenomas and carcinomas were found in mice. Nuclear enlargement was observed in the nasal epithelium. In drinking water studies, the nasal tissue comes into direct contact with 1,4-dioxane. Therefore, the development of nasal tumours in the drinking water studies may have been caused by local exposure in addition to possible systemic exposure. In a new carcinogenicity study with inhalation exposure of rats, 1,4-dioxane likewise induced squamous cell carcinomas in the nose at the high concentration of 1250 ml/m³. Tumours were not found at the concentration of 50 ml/m³; however, an increased incidence of nuclear enlargement in the respiratory and olfactory epithelium and atrophy and respiratory metaplasia of the olfactory epithelium were observed in the nose, the target organ.

Genotoxicity studies showed that 1,4-dioxane induced DNA strand breaks and micronuclei in vivo only at cytotoxic concentrations, in most cases close to or above the recommended limit dose of 2000 mg/kg body weight and day.

New clinical findings have not been obtained in humans for the sensitizing effects of 1,4-dioxane on the skin. A maximization test in guinea pigs yielded negative results. There are no data available for the sensitizing effects of 1,4-dioxane on the respiratory tract.

Mechanism of Action

A large number of mechanisms of action were investigated and reviewed in the 2003 documentation (Greim 2003). The critical carcinogenic metabolite has to date not been elucidated. The metabolism of 1,4-dioxane is still inadequately understood. 2-Hydroxyethoxyacetic acid is regarded as a detoxification product and the main urinary metabolite, but it is in equilibrium with the lactone dioxan-2-one. A conceivable activation pathway would be the directly inducible alpha-oxidation of 1,4-dioxane to dioxan-2-ol, which is a hemiacetal that is in equilibrium with a hydroxyaldehyde. This could be clarified by determining the dose-dependence of the formation of a critical metabolite or its reaction products (Greim 2003).

Only the new studies that investigated the mechanism of action and were published since the 2003 documentation (Greim 2003) are described below.

Nasal tumours

A specific study was carried out to investigate the open question of whether the nasal epithelium has contact with drinking water if animals are exposed to 1,4-dioxane via the drinking water. Groups of 5 rats were given drinking water without any additions (control group), drinking water containing a fluorescent dye (group 1) or drinking water containing fluorescent dye and 0.5% 1,4-dioxane (group 2). The animals were exposed overnight. Another animal was given 2 ml drinking water containing fluorescent dye by gavage twice at an interval of 30 minutes. The amount of drinking water containing fluorescent dye that was consumed by group 1 corresponded to that taken up by the control group, whereas the presence of 1,4-dioxane in the drinking water (group 2) reduced the amount consumed to on average 62% of that of the controls. In the drinking water groups, the fluorescent dye was detected in the oral cavity and nasal airways of the animals. The presence of 1,4-dioxane had no effect on the amount of dye detected. The fluorescent dye was found in numerous areas of the anterior third of the nose; these were the same areas in which nasal irritation and tumours were identified in long-term studies (see *Section "Subacute, subchronic and chronic toxicity"* and *"Carcinogenicity"*). After gavage administration, fluorescent dye was not detected in the nose. The results show that exposure of the nasal tissue in drinking water studies occurs through direct contact in addition to a possible systemic exposure (Sweeney et al. 2008).

Irritation of the nasal epithelium resulting in cytotoxicity, inflammation, regenerative cell proliferation and hyperplasia is assumed to be the mechanism by which nasal tumours develop after inhalation.

Liver tumours

The metabolite 1,4-dioxan-2-one did not induce pre-neoplastic foci in the liver after 12-week oral exposure of F344 rats. The rats were given doses of 0.02 g/rat (15% of the LD_{50}) on 3 days in week 1 (treatment every second day) and 0.04 g/rat in the subsequent 11 weeks according to the same pattern. As expected, the positive control aflatoxin induced pre-neoplastic foci (Koissi et al. 2012).

A study that investigated the mechanism of action underlying the development of liver tumours supported an earlier hypothesis that attributed the hepatocellular adenomas and carcinomas induced by 1,4-dioxane to cytotoxicity and subsequent regenerative hyperplasia of the liver. The authors of this study evaluated the available subchronic and chronic studies in rats and mice as regards this mechanism and concluded that metabolic saturation of 1,4-

dioxane initiates the cytotoxic effects on the liver as evidenced by increased AST (aspartate aminotransferase) and ALT (alanine aminotransferase) levels in the blood and corresponding dose and time-dependent histopathological findings. The hepatotoxicity was first observed at 9.6 to 42 mg/kg body weight and day in rats and at 57 to 66 mg/kg body weight and day in mice. In rats, enlargement of hepatocytes, hypertrophy of the liver and an increase in organ weights were observed at 42 to 55 mg/kg body weight and day; this induced necrosis at doses of 94 to 219 mg/kg body weight and day, hyperplasia and the development of foci at 55 to 389 mg/kg body weight and day, and subsequently the development of adenomas and carcinomas at 274 to 1015 mg/kg body weight and day. It is not possible to establish such a dose-dependent listing for mice because hepatocellular adenomas and carcinomas were observed even at the lowest dose tested of 66 mg/kg body weight and day. The metabolic saturation that underlies the cytotoxicity of 1,4-dioxane has been investigated in detail in animal studies and humans. At low dose levels, the 1,4-dioxane metabolism follows non-saturated, first-order kinetics leading to the main metabolite 2-hydroxyethoxyacetic acid with a pH-dependent reversal to 1,4-dioxan-2-one. Saturation starts at higher dose levels, followed by non-linear kinetics. It is expected to occur at about 30 to 100 mg/kg body weight in rats and at 200 mg/kg body weight and day and above in mice. In rodent studies, 1,4-dioxan-2-one did not induce pre-neoplastic foci; therefore, the authors concluded that 1,4-dioxane itself is the toxic agent that accumulates after metabolic saturation (Dourson et al. 2017).

However, the theory that 1,4-dioxane itself is the hepatotoxic agent requires critical review. Firstly, the molecule has no reactive functional group and secondly, metabolic saturation is found only after a single, but not after repeated administration because 1,4-dioxane induces its own metabolism in the latter case (Dietz et al. 1982). Thus, after exposure of rats to 1000 mg/kg body weight for 1 day, the body burden is 900 times higher than after exposure to 10 mg/kg body weight; however, after exposure for 17 days, the ratio is reduced to 100, the factor calculated from the doses (1000 mg/kg body weight/10 mg/kg body weight). In addition, the course of the plasma levels of 1,4-dioxane in the 13-week inhalation study in rats was completely linear at 400 ml/m^3 and above, whereas the concentration–effect relationship for the liver effects (necrosis) was sublinear (Kasai et al. 2008).

Renal tumours

Increased exposure to oxygen radicals resulting from the induction of CYP2E1 after long-term exposure to 1,4-dioxane is regarded as a possible mechanism of the carcinogenicity in the kidneys (see *Section "Enzyme induction"*; Nannelli et al. 2005).

Fibroadenomas in the rat mammary gland

As in humans, fibroadenomas of the rat mammary gland are benign tumours composed of well-differentiated epithelial and fibrous connective tissue. The spontaneous incidence of fibroadenomas in female rats is between 20% and 40% for most rat strains. The period with the highest risk is between 31 and 36 months of age, and the incidence decreases again in older rats. Chemically induced mammary gland tumours in rats are generally hormone-dependent adenocarcinomas. Fibroadenomas are not regarded as a premalignant lesion in rats or humans (Russo 2015).

Fibromas of the subcutis

Carcinogenicity studies in rats (Kano et al. 2009; Kasai et al. 2009) were carried out with the F344/DuCrj strain; the strain has a relatively high spontaneous incidence of subcutaneous fibromas of 6% and higher (no other details; Takanobu et al. 2015; English summary of the Japanese study). A higher sensitivity to chemicals is known also for other tumour localizations with a high spontaneous incidence, one example being the high spontaneous tumour incidence and high sensitivity for the induction of liver adenomas and carcinomas in B6C3F1 mice (Haseman et al. 1998; Maronpot 2009). The late occurrence of only benign tumours at sites with a high spontaneous incidence was probably caused by growth-promoting effects (US EPA 2005) and, after carefully reviewing each case, is therefore regarded as not relevant for humans.

Enzyme induction

In male rats, cytochrome P450 (CYP) was induced after oral exposure to a 1,4-dioxane dose of 2000 mg/kg body weight and day on 2 days or exposure to 1.5% in the drinking water for 10 days (about 1800 mg/kg body weight and day; conversion factor: 0.12 (subacute) according to EFSA (2012)). Treatment led to the induction of CYP2B1/2 and CYP2C11 activities (pentoxyresorufin-*O*-dependentylase and 2-alpha-testosterone hydroxylase) in the liver and CYP2E1 activities (*p*-nitrophenol hydroxylase) in the liver, kidneys and nose. The induction of CYP enzymes in the lungs was not observed. 1,4-Dioxane did not change the plasma alanine aminotransferase activity (ALT) or the glutathione level (GSH) in the liver of rats that had increased CYP2B1/2 and CYP2E1 activities after pre-treatment with phenobarbital or after fasting. According to the authors, this suggests that reactive or toxic intermediates did not form during the metabolism of 1,4-dioxane. Likewise, exposure to 1,4-dioxane for 10 days did not induce palmitoyl CoA oxidase, the marker enzyme of peroxisome proliferation. In the liver, the induction of CYP2E1 was most relevant after prolonged treatment, whereas the induction of CYP2B1 and CYP3A1/2 was most relevant after acute treatment. The induction of CYP2B1/2, CYP2E1 or CYP1A is often accompanied by a decrease in CYP2C11, which is under hormonal control. However, CYP2C11 was induced in this case; this may indicate a change in growth hormone levels in the plasma. According to the authors, increased exposure to oxygen radicals resulting from the induction of CYP2E1 after long-term exposure to 1,4-dioxane is a possible mechanism of the cell proliferation in the liver and the carcinogenicity in the liver, kidneys and nose (Nannelli et al. 2005). The induction of CYP2B1/2 suggests the involvement of the CAR receptor (constitutive androstane receptor); its activation likewise causes increased cell proliferation in the liver of rats (Elcombe et al. 2014).

In the liver microsomes of rats that were given intraperitoneal injections of 1,4-dioxane at 500 mg/kg body weight and day on 3 days, the activities of CYP2E and CYP2B were significantly increased and those of CYP2C were significantly reduced (Takano et al. 2010).

Replicative DNA synthesis

In an in vivo/in vitro study in isolated rat hepatocytes, 1,4-dioxane induced cytotoxicity at doses of 1000 or 2000 mg/kg body weight and increased replicative DNA synthesis at the dose level of 2000 mg/kg body weight. However, the results were regarded as questionable (Uno et al. 1994).

Inhibition of RNA polymerases A and B

Male Sprague Dawley rats were given intravenous injections of 1,4-dioxane in doses of 0 and about 50 or 500 mg/kg body weight (10 or 100 mg/rat) and the hepatocytes from 6 animals per dose were isolated at various times. At 50 mg/kg body weight, RNA polymerase A and B activities in the isolated hepatocytes after 10 minutes were about 75% and 50%, respectively, of the control values; after 1 hour, the inhibition of polymerase B was reversible, whereas the polymerase A activity was up to 40% higher than the control value. Both polymerases were inhibited once again to about 65% of the control value after 2 and 4 hours. The polymerase B activity recovered almost completely within 24 hours after administration of the substance, whereas the polymerase A activity was 35% higher than the control value after 24 hours. A similar course was observed after exposure to 500 mg/kg body weight, but the inhibition was more pronounced and, unlike the polymerase A activity, the polymerase B activity did not return to the control values (Kurl et al. 1981).

Toxicokinetics and Metabolism

Absorption, distribution, elimination

1,4-Dioxane is absorbed completely by rats after oral exposure. In addition, there is evidence of absorption by inhalation in rats and humans (Greim 2003).

A study investigated the toxicokinetics of 1,4-dioxane in humans exposed by inhalation at rest and during physical stress. The cohort of 18 volunteers (8 men and 10 women) was divided into 3 groups that were exposed to 20 ml/m³ (73 mg/m³) for 8 hours. Group 1 was exposed at rest, whereas the volunteers in groups 2 and 3 exercised on an ergometer for 10 minutes every hour, corresponding to work-loads of 50 W (experiment 2) and 75 W (experiment 3). Blood samples were taken after 4 and 8 hours, and urine samples were collected over 24 hours and analysed for 1,4-dioxane and the 2-hydroxyethoxyacetic acid metabolite. At about 1 mg/l, the 1,4-dioxane concentration in the blood and urine was only slightly above the limit of detection. About 53% ± 15% of the theoretically inhaled 1,4-dioxane dose was eliminated as 2-hydroxyethoxyacetic acid within 24 hours. As almost all of the amount of the metabolite was excreted with the urine, this approximately corresponds to the pulmonary retention. Pulmonary retention of 60.5% can be assumed from the empirically derived relationship between pulmonary absorption and the blood:air partition coefficient for water-soluble substances. The maximum amount of the 2-hydroxyethoxyacetic acid metabolite in the urine was reached 9.8 ± 1.9 hours after the beginning of exposure. Depending on the work-load, the maximum elimination rate increased significantly from 23.2 ± 7.7 in group 1 to 30.4 ± 7.2 and 41.8 ± 23.8 mg/hour in groups 2 and 3, respectively. Likewise, the cumulative excretion of 2-hydroxyethoxyacetic acid with the urine was increased by exercise. The average maximum 2-hydroxyethoxyacetic acid concentration was between 378 and 451 mg/g creatinine and increased with work-load. The half-life of 2-hydroxyethoxyacetic acid was 3.4 ± 0.5 hours. As a 2-hydroxyethoxyacetic acid concentration of 31 to 51 mg/g creatinine was detected 24 hours after the beginning of exposure, the authors assumed only low accumulation during a working week (Göen et al. 2016).

There are new studies available for dermal absorption of the substance. In an in vitro study, a 1,4-dioxane concentration of 200 µl/cm² was applied occlusively to freshly excised human skin in static Franz cells. The receptor medium consisted of physiological saline. A flux of 1.4 mg/cm² and hour was calculated from the amounts of 1,4-dioxane found in the receptor phase after 1 hour and after 2, 3 and 4 hours (Dennerlein et al. 2013).

A similar study with non-occlusive application of 100 µl 1,4-dioxane to 0.64 cm² human skin for 1 hour reported a cumulative amount of 309 µg in the receptor medium and of 6 µg in the epidermis and dermis after 8 hours. Absorption was almost complete after 8 hours. Only 63% of the amount applied was recovered; this was attributed to the evaporation of the substance (Dennerlein et al. 2015). About 984 mg of 1,4-dioxane would be absorbed after the exposure of a 2000 cm² surface area of skin for 1 hour.

Metabolic saturation was demonstrated in rats (Greim 2003). A recent study revealed that this is relevant also in the case of B6C3F1 mice. Groups of 27 animals were given a single oral dose of 20, 200 or 2000 mg/kg body weight and day. After 0, 30 and 60 minutes and 2, 3, 6, 9, 12 and 24 hours, the blood was analysed for 1,4-dioxane and its main metabolite 2-hydroxyethoxyacetic acid. At the middle and high doses, the maximum blood concentration of 1,4-dioxane was reached after 1 hour, whereas the concentration was close to the detection limit in the low dose group at all sampling times. In all 3 groups, the metabolite reached its maximum concentration between 30 minutes and 2 hours. The highest percentage of the metabolite was determined at 20 mg/kg body weight. The metabolite was still detected after 12 and 24 hours only in the high dose group. A comparison of the AUCs (blood concentration–time curves) of 1,4-dioxane and 2-hydroxyethoxyacetic acid suggested non-linear metabolism, as demonstrated by a disproportional increase in 1,4-dioxane compared with the given dose and a concurrent decrease in the ratio of the metabolite to the parent substance in the blood. In addition to the evidence of metabolic saturation, the study in mice revealed very rapid metabolism after a single oral dose of 20 mg/kg body weight; only trace amounts of 1,4-dioxane could be detected in the blood. Metabolic saturation was reached at about 200 mg/kg body weight and above (Sweeney et al. 2008).

As there was some uncertainty with regard to the predictions of the first physiologically based pharmacokinetic (PBPK) models of 1,4-dioxane and its main metabolite 2-hydroxyethoxyacetic acid from the 1990s, the model was improved for the evaluation of the carcinogenic risk according to today's standards. Studies were carried out to fill data gaps and reduce uncertainties as regards the pharmacokinetics of 1,4-dioxane and 2-hydroxyethoxyacetic acid, such as partition coefficients and the time course in the blood of mice, and in vitro studies were performed in rat, mouse and human hepatocytes; updated PBPK models were developed based on these new data. In mice, the

optimized rate of metabolism was significantly higher than the value previously estimated, whereas in rats, it was similar to that in the models from the 1990s. Model predictions in humans were consistent with the findings of the study in workers, but not with those of the study in volunteers. The data showed that after external exposure to 50 ml/m³, the blood levels of 1,4-dioxane in humans (10 to 20 mg/l) were twice as high as those in rats (about 7 mg/l) (Sweeney et al. 2008).

Metabolism

At low concentrations (50 ml/m³) or doses (10 mg/kg body weight), more than 90% of 1,4-dioxane is metabolized by humans and rats to 2-hydroxyethoxyacetic acid and excreted with the urine. In rats, dioxan-2-one was reported as the main metabolite when a different method of isolation was used; this substance exists in pH-dependent equilibrium with 2-hydroxyethoxyacetic acid (Greim 2003).

Three metabolic pathways were postulated for the formation of the main metabolite 2-hydroxyethoxyacetic acid (Figure 1):

a) Oxidation by CYP; further hydroxylation leads to the formation of 2-hydroxyethoxyacetic acid from the cyclic ketone. Dioxan-2-one was detected by a method of acidic isolation and at low concentrations of about 50 ml/m³.

b) Oxidation by CYP, ring opening to yield diethylene glycol and oxidation to form 2-hydroxyethoxyacetic acid (this step is known from diethylene glycol metabolism, but has not been demonstrated for 1,4-dioxane).

c) Possible metabolism at saturation of a): α-hydroxylation to form dioxan-2-ol, ring opening to yield 2-hydroxyethoxy acetaldehyde and oxidation to form 2-hydroxyethoxyacetic acid (here, too, there is no experimental evidence for the aldehyde intermediate). Dioxan-2-ol is in equilibrium with the aldehyde (von Helden 2013).

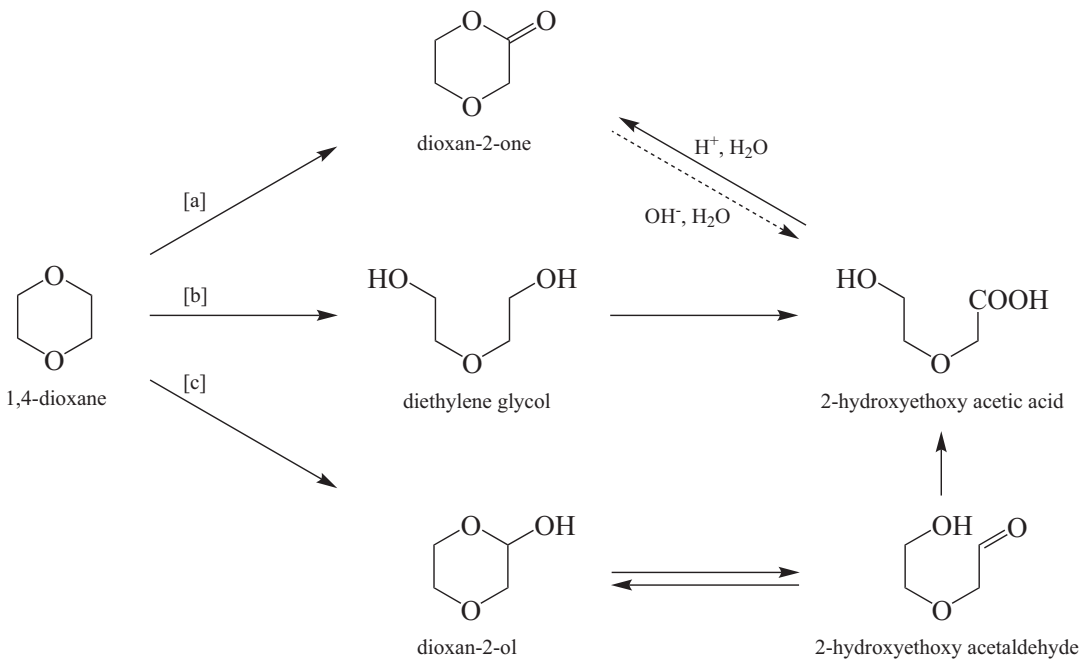

Fig. 1 Possible pathways for the metabolism of 1,4-dioxane to 2-hydroxyethoxyacetic acid (von Helden 2013)

However, it has been demonstrated that dioxan-2-one is not stable at physiological pH and hydrolyses to 2-hydroxyethoxyacetic acid at pH 7 with a half-life of 2 hours. Likewise, the equilibrium is far to the side of 2-hydroxyethoxyacetic acid in the acidic medium (Koissi et al. 2012). Thus, dioxan-2-one is rapidly metabolized to 2-hydroxyethoxyacetic acid if it is formed as a metabolite of 1,4-dioxane. It is possibly only an artefact of the method of acidic isolation used.

CO_2 was identified as another metabolite (Greim 2003).

The metabolism of 1,4-dioxane can be induced by phenobarbital or by 1,4-dioxane itself (Greim 2003).

Effects in Humans

There are no new data available for allergenic effects or reproductive toxicity.

Single exposures

In a volunteer study, 6 men and 6 women were exposed to stepwise increasing concentrations of 1,4-dioxane for 10 minutes each of 1, 2, 5, 10 and 20 ml/m³ (3.6, 7.2, 18, 36 and 72 mg/m³). Irritant and general symptoms were rated in a questionnaire with 10 questions: 1) discomfort in the eyes: burning, irritated or watery eyes, 2) discomfort in the nose: burning, irritated or runny nose, 3) discomfort in the throat or airways, 4) breathing difficulties, 5) solvent smell, 6) headache, 7) fatigue, 8) nausea, 9) dizziness, and 10) feeling of intoxication. The standardized rating of the severity of the symptoms was based on a visual analogue scale (VAS) from 0 to 100 mm with 7 grades of assessment (not at all, hardly at all, somewhat, rather, quite, very, almost unbearable). The evaluation of the results of these tests showed that the level of 1,4-dioxane exposure had no significant effects on any parameter (Ernstgård et al. 2006).

On the basis of the reported results, a 1,4-dioxane concentration of 20 ml/m³ was chosen for the main study, and 6 male and 6 female volunteers were exposed to 1,4-dioxane at 0 or 20 ml/m³ for 2 hours. In addition to the analysis with the above-mentioned questionnaire, the following examinations were carried out: the pulmonary function was determined by measuring vital capacity (VC), forced VC (FVC), forced expiratory volume (FEV), forced expiratory volume in 1 second (FEV1) and peak expiratory flow (PEF). Possible nasal swelling was assessed by acoustic rhinometry during or after exposure. The blinking frequency was monitored throughout the 2-hour exposure by electromyography. C-Reactive protein and interleukin-6 were determined as inflammatory markers in the blood of the volunteers. The evaluation of the results of these tests showed that the level of 1,4-dioxane exposure had no significant effects on any symptom listed in the questionnaire except for “solvent smell”. The ratings for solvent smell were significantly increased after 3, 60 and 118 minutes. There were no significant changes in the parameters of pulmonary function, nasal swelling, blinking frequency or C-reactive protein. Compared with the values before exposure, the interleukin-6 concentrations in the blood of the volunteers were reduced by 33% in the control group and by 37% after exposure to 20 ml/m³. These changes were significant over time, but not with regard to the exposure. Therefore, the changes were caused by the stay in the exposure chamber, but not by 1,4-dioxane. In summary, there was no significant change in any parameter at 20 ml/m³. Therefore, this value corresponds to the NOAEC (no observed adverse effect concentration) for 2-hour exposure to 1,4-dioxane (Ernstgård et al. 2006).

A toxicokinetics study with exposure of volunteers to 20 ml/m³ at rest or during physical exercise for 8 hours did not report adverse effects on the respiratory tract or the eyes (Göen et al. 2016), unlike in the toxicokinetics study with exposure to 50 ml/m³ for 6 hours, in which eye irritation was a frequent complaint (Greim 2003).

Genotoxicity

In lymphocytes obtained from 6 workers employed in 1,4-dioxane production who were exposed to unspecified concentrations of the substance in the air for 6 to 15 years, no increase in the incidence of chromosomal aberrations was found compared with that in the controls (Greim 2003).

Carcinogenicity

In a small prospective mortality study of 165 workers who had been exposed to low concentrations of 1,4-dioxane since 1954, 7 deaths had occurred in the manufacturing department by 1975; 2 of them were from cancer. The expected numbers, based on the mortalities in Texas, were 4.9 and 0.9, respectively. In the processing department, 5 deaths were observed compared with the statistically expected 4.9; 1 of them was from cancer (0.8 statistically expected) (Greim 2003). As the exposure concentration was not reported and exposure to a mixture of substances is assumed, the study has not been included in the evaluation of carcinogenicity.

Animal Experiments and in vitro Studies

Subacute, subchronic and chronic toxicity

Inhalation

In the 2003 documentation (Greim 2003), a NOAEC of 111 ml/m^3 (7 hours a day on 5 days a week) was derived from a long-term inhalation study in Wistar rats (Torkelson et al. 1974). Neither organ toxicity nor tumour formation was observed. The nose was not investigated in this study.

However, the 2-year inhalation study in F344 rats published in 2009 yielded a LOAEC (lowest observed adverse effect concentration) of 50 ml/m^3 (180 mg/m^3), which was the lowest concentration tested. At this concentration and above, an increased incidence of nuclear enlargement in the respiratory and olfactory epithelium as well as atrophy and respiratory metaplasia of the olfactory epithelium were observed in the nose as the target organ (see Table 1; Kasai et al. 2009; see also *Section "Carcinogenicity"*). The data are not suitable for benchmark modelling because the end point "nuclear enlargement" was already observed in all animals of the low concentration group, and because the incidence for the end point "olfactory atrophy" was 80% in the low concentration group and thus far higher than the usual benchmark response of 5%; this means that the BMD is dependent only on the model. In view of these uncertainties, the US EPA (2013) did not use benchmark modelling to derive the RfC (0.0322 mg/m^3) for 1,4-dioxane.

The most sensitive systemic effect observed at 250 ml/m^3 was nuclear enlargement in the kidneys. Nuclear enlargement was not regarded as adverse by the US EPA (2013). However, at this concentration, increased incidences of hydropic changes in the kidneys and centrilobular necrosis in the liver were observed; these increases were not significant, but they were dose-dependent. Therefore, initial effects were observed at 250 ml/m^3.

The concentration of 16.67 ml/m^3 (LOAEC/3) is assumed to be the NAEC (no adverse effect concentration) of the chronic rat study.

Tab. 1 Effects on the nose in the 2-year study with inhalation exposure of rats to 1,4-dioxane (Kasai et al. 2009)

	Exposure concentration (ml/m³)			
only ♂ animals examined	**0**	**50**	**250**	**1250**
nose:				
respiratory epithelium				
nuclear enlargement	0/50 (0%)	50/50 (100%)**	48/50 (96%)**	38/50 (76%)**
olfactory epithelium				
nuclear enlargement	0/50 (0%)	48/50 (96%)**	48/50 (96%)**	45/50 (90%)**
atrophy	0/50 (0%)	40/50 (80%)**	47/50 (94%)**	48/50 (96%)**
respiratory metaplasia	11/50 (22%)	34/50 (68%)**	49/50 (98%)**	48/50 (96%)**
inflammation	0/50 (0%)	2/50 (4%)	32/50 (64%)**	34/50 (68%)**
nasal cavity				
hydropic changes of the lamina propria	0/50 (0%)	2/50 (4%)	36/50 (72%)**	49/50 (98%)**

*$p \leq 0.05$; **$p \leq 0.01$

In preparation for the long-term study, the same research group carried out a 13-week range-finding study in F344/DuCrj rats. Groups of 10 male and 10 female animals were exposed whole-body to 1,4-dioxane concentrations of 0, 100, 200, 400, 800, 1600, 3200 or 6400 ml/m³ for 6 hours a day on 5 days a week. All animals exposed to the high concentration died by the end of the first week. Renal failure and congestion in the lungs were the main findings. The body weights determined at the end of the study were significantly reduced in the males of the groups exposed to 200 and 3200 ml/m³ and in the females exposed to 200 ml/m³ and at 800 ml/m³ and above. Significant increases in the relative liver weights were observed in both sexes at 800 ml/m³ and above, and the relative kidney weights were significantly increased in the males exposed to 3200 ml/m³ and in the females exposed to 800 ml/m³ and above. The relative lung weights were significantly increased in the males exposed to 200 ml/m³ and at 1600 ml/m³ and above and in the females at 200 ml/m³ and above. AST levels were slightly increased in females at 200 ml/m³ and 3200 ml/m³, and ALT levels were increased in both sexes at 3200 ml/m³. Triglycerides, blood glucose and urinary protein were reduced only in the males of the group exposed to 3200 ml/m³. Nuclear enlargement in the nasal respiratory epithelium was observed in the males and females at the lowest concentration tested of 100 ml/m³ and above, followed by enlarged nuclei in the olfactory epithelium at 200 ml/m³ and above. The increased incidences of degenerative changes in the olfactory epithelium were statistically significant in the males at 400 ml/m³ and above and in the females at 800 ml/m³ and above, and in the bronchial epithelium at 1600 ml/m³ and above. 1,4-Dioxane induced effects on the liver only at higher concentrations of 1600 ml/m³ and above. The lesions were characterized by single cell necrosis and centrilobular swelling of hepatocytes in males and females. GST-P-positive foci (placental glutathione *S*-transferase; pre-neoplastic marker) in the liver were observed in the females at 1600 ml/m³ and above and in the males at 3200 ml/m³. The plasma concentration of 1,4-dioxane increased linearly with the exposure concentration at 400 ml/m³ and above. This means that metabolic saturation did not occur in this study, presumably as a result of the induction of metabolizing enzymes. The LOAEC in this study was 100 ml/m³ because of the nuclear enlargement in the nasal respiratory epithelium. It was not possible to derive a NOAEC (Kasai et al. 2008).

The studies with inhalation exposure are summarized in Table 2.

Tab. 2 Studies with inhalation exposure of rats to 1,4-dioxane

Species, strain, number of animals/group	Duration, concentration	Findings	References
rat, F344/DuCrj, 10 ♂, 10 ♀	**13 weeks** 0, 100, 200, 400, 800, 1600, 3200 or 6400 ml/m³ 5 days/week, 6 hours/day whole-body exposure purity: > 99%	**100 ml/m³: LOAEC** **100 ml/m³ and above:** ♂, ♀: nose: incidence of nuclear enlargement in the respiratory epithelium ↑; **200 ml/m³:** ♂, ♀: body weights ↓, ♂: relative lung weights ↑, AST ↑; **200 ml/m³ and above:** ♂, ♀: nose: incidence of nuclear enlargement in the olfactory epithelium ↑, ♀: relative lung weights ↑; **400 ml/m³ and above:** ♂: nose: degenerative changes in the olfactory epithelium; **800 ml/m³ and above:** ♂, ♀: relative liver weights ↑, ♀: body weights ↓, relative liver weights ↑, nose: degenerative changes in the olfactory epithelium; **1600 ml/m³ and above:** ♂, ♀: lungs: degenerative changes in the bronchial epithelium, ♂: relative lung weights ↑, ♀: liver: GST-P-positive foci; **3200 ml/m³:** ♂, ♀: liver: single cell necrosis and centrilobular swelling of hepatocytes, ALT ↑, ♂: body weights ↓, relative liver weights ↑, liver: GST-P-positive foci, triglycerides and blood glucose ↓, urinary protein ↓, ♀: AST ↑; **6400 ml/m³:** ♂, ♀: mortality 10/10 by the end of week 1 (renal failure and congestion in the lungs)	Kasai et al. 2008
rat, Wistar, 288 ♂, 288 ♀ control animals: 192 ♂, 192 ♀	**2 years** 0, 111 ml/m³ 5 days/week, 7 hours/day whole-body exposure	**111 ml/m³: NOAEC** no unusual findings: growth, mortality, haematological and clinico-chemical parameters, gross-pathological and histopathological examinations nose not examined	Greim 2003; Torkelson et al. 1974
rat, F344/DuCrj, 50 ♂, 50 ♀	**2 years** 0, 180, 900, 1800 mg/m³ (0, 50, 250, 1250 ml/m³) 5 days/week, 6 hours/day whole-body exposure purity: > 99%	**50 ml/m³: LOAEC** **50 ml/m³ and above:** nose: incidence of nuclear enlargement and inflammation ↑, atrophy and respiratory metaplasia of the olfactory epithelium, hydropic changes and sclerosis of the lamina propria, proliferation of the glands; **250 ml/m³ and above:** mortality ↑, nose: squamous cell metaplasia, kidneys: incidence of nuclear enlargement ↑; **1250 ml/m³:** body weights ↓, nose: squamous cell hyperplasia, kidneys: hydropic changes of the renal proximal tubules, liver: relative weights ↑, incidence of nuclear enlargement ↑, necrosis of hepatocytes, changes in cell foci, plasma: ALT, AST and γ-GTP activities ↑	Kasai et al. 2009

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GST-P: placental glutathione *S*-transferase (pre-neoplastic marker); GTP: glutamyl transpeptidase

Oral administration

In the 2003 documentation (Greim 2003), a NOAEL (no observed adverse effect level) of 0.01% 1,4-dioxane in the drinking water was derived from the long-term studies in rats (daily dose of about 9.6 and 19 mg/kg body weight in males and females, respectively). 1,4-Dioxane induced hepatotoxicity and nephrotoxicity and a slight increase in hepatocellular tumours at a dose level of about 100 mg/kg body weight and day. Inflammation of the nasal cavities with squamous cell carcinomas were observed at about 500 mg/kg body weight and day (Greim 2003).

The effects in mice were almost identical with those described in rats; therefore, the target organs were the liver, kidneys and nasal cavities. The incidence of hepatocellular adenomas and carcinomas was already markedly increased at the lowest concentration tested of about 50 mg/kg body weight and day (Greim 2003). For this reason, it was not possible to derive a NOAEL for mice.

The new long-term studies have confirmed the previous data. In rats, 1,4-dioxane induced nuclear enlargement in the olfactory epithelium in the females at 83 mg/kg body weight and day and above. The NOAEL was 11 and 18 mg/kg body weight and day for males and females, respectively. The systemic NOAEL was 83 mg/kg body weight and day (Kano et al. 2009; see also *Section "Carcinogenicity"*).

When mice were given 1,4-dioxane with the drinking water, the increased incidences of hepatocellular adenomas and carcinomas were statistically significant in both sexes, beginning in females at the lowest dose of 49 mg/kg body weight and day and above (Kano et al. 2009; see also *Section "Carcinogenicity"*). For this reason, it is not possible to derive a NOAEL for mice.

The same research group carried out 13-week range-finding studies in male and female F344 rats and BDF1 mice in preparation of the respective long-term studies. The animals were given 1,4-dioxane in the drinking water at concentrations of 0%, 0.064%, 0.16%, 0.4%, 1% or 2.5%. The NOAEL was 0.064% for rats and mice on the basis of the nuclear enlargement in the nasal respiratory epithelium in both species, the centrilobular enlargement of hepatocytes in rats and the nuclear enlargement in the bronchial epithelium of mice at 0.16%. The NOAEL is equivalent to about 52 and 83 mg/kg body weight and day in male and female rats, respectively, and 170 and 231 mg/kg body weight and day in female and male mice, respectively (Kano et al. 2008; for the findings see Table 3).

Tab. 3 13-week studies in rats and mice with 1,4-dioxane in the drinking water (Kano et al. 2008)

Species, strain, number of animals/dose	Duration, dose	Findings
rat, F344/DuCrj, 10 ♂, 10 ♀	**13 weeks** 0%, 0.064%, 0.16%, 0.4%, 1% or 2.5% in the drinking water (0, 52, 126, 274, 657, 1554 mg/kg body weight and day in males and 0, 83, 185, 427, 756, 1614 mg/kg body weight in females) purity: 99%	**52/83 mg/kg body weight: NOAEL** ♂, ♀; **126/185 mg/kg body weight and above:** ♂, ♀: nose: nuclear enlargement in the respiratory epithelium ↑, ♂: liver: centrilobular enlargement of hepatocytes ↑, ♀: relative liver weights ↑, relative kidney weights ↑; **274/427 mg/kg body weight and above:** ♂, ♀: water consumption ↓, nose: nuclear enlargement in the olfactory epithelium ↑, trachea: nuclear enlargement in the epithelium ↑, ♂: relative kidney weights ↑, urinary pH ↓, liver: single-cell necrosis ↑, ♀: body weights ↓; liver: single cell necrosis ↑; **657/756 mg/kg body weight and above:** ♂, ♀: kidneys: nuclear enlargement in the proximal tubular epithelial cells, ♂: body weights ↓, relative liver weights ↑, liver: centrilobular vacuoles ↑, ♀: feed consumption ↓, relative lung weights ↑, urinary pH ↓, liver: centrilobular enlargement of hepatocytes ↑; **1554/1614 mg/kg body weight:** ♂, ♀: ruffled and discoloured fur, blood glucose levels ↓, kidneys: hydropic changes in the proximal tubular epithelial cells, brain: vacuolar changes in the cerebrum, ♂: feed consumption ↓, relative lung weights ↑, erythrocytes, haemoglobin and haematocrit ↑, plasma AST and ALT ↑, ♀: lethal for 1 of 10 (renal failure), plasma AST ↑, liver: single cell necrosis and centrilobular vacuoles ↑, lungs: nuclear enlargement of the bronchial epithelium ↑
mouse, Crj:BDF$_1$, 10 ♂, 10 ♀	**13 weeks** 0%, 0.064%, 0.16%, 0.4%, 1% or 2.5% in the drinking water (0, 86, 231, 585, 882, 1570 mg/kg body weight and day in males and 0, 170, 387, 898, 1620, 2669 mg/kg body weight in females) purity: 99%	**170 mg/kg body weight: NOAEL** ♀; **231 mg/kg body weight: NOAEL** ♂; **387 mg/kg body weight and above:** ♀: lungs: nuclear enlargement in the bronchial epithelium ↑; **585/898 mg/kg body weight and above:** ♂, ♀: nose: nuclear enlargement in the olfactory epithelium ↑, trachea: nuclear enlargement in the epithelium ↑, liver: single cell necrosis and enlargement of the centrilobular hepatocytes ↑, ♂: lungs: nuclear enlargement in the bronchial epithelium ↑; **882/1620 mg/kg body weight and above:** ♂, ♀: water consumption ↓, urinary pH ↓, ♀: relative lung weights ↑, ALT ↑, glucose ↓, lungs: degeneration in the bronchial epithelium ↑; **1570/2669 mg/kg body weight:** ♂, ♀: nose: nuclear enlargement in the respiratory epithelium ↑, vacuolar changes in the olfactory epithelium ↑, relative kidney weights ↑, plasma AST and ALT ↑, ♂: lethal for 1 of 10, ruffled fur, body weights ↓, feed consumption ↓, relative lung weights ↑, erythrocytes, haemoglobin and haematocrit ↑, glucose ↓, lungs: degeneration in the bronchial epithelium ↑

* $p < 0.05$
ALT: alanine aminotransferase; AST: aspartate aminotransferase

Allergenic effects

In a maximization test with female Dunkin Hartley guinea pigs, a 5% aqueous formulation of 1,4-dioxane was applied for intradermal induction and undiluted 1,4-dioxane for topical induction and challenge. None of the 10 animals reacted to the challenge treatment after 24 hours; later readings were not carried out (ECHA 2018).

Reproductive and developmental toxicity

Fertility

Fertility or generation studies were not carried out with 1,4-dioxane.

The inhalation studies with 13-week or 2-year exposure of F344/DuCrj rats carried out according to OECD Test Guideline 413 or 453 did not reveal effects on the reproductive organs up to the highest concentration tested of 6400 ml/m³ (13 weeks) or 1250 ml/m³ (2 years) (Kasai et al. 2008, 2009).

Likewise, in the 13-week and 2-year drinking water studies carried out according to OECD Test Guideline 408 and 451 in male and female F344/DuCrj rats and Crj:BDF1 mice, respectively, no effects on the reproductive organs were observed up to the highest doses tested of about 1550 to 2700 mg/kg body weight and day (13 weeks) and about 270 to 960 mg/kg body weight and day (2 years) (Kano et al. 2008, 2009).

In a 2-generation study in ICR Swiss mice in which 1,4-dioxane was used as a stabilizer for 1,1,1-trichloroethane, no toxic effects on reproduction were found up to the highest 1,4-dioxane dose tested of 30 mg/kg body weight and day (Greim 2003; Greim 2007, available in German only; Lane et al. 1982).

Developmental toxicity

In a prenatal developmental toxicity study similar to OECD Test Guideline 414, groups of 17 to 20 Sprague Dawley rats were given gavage doses of 1,4-dioxane (purity: 99%) of 0, 0.25, 0.5 or 1.0 ml/kg body weight and day, corresponding to 0, 260, 520 or 1035 mg/kg body weight and day, from days 6 to 15 of gestation. The body weight gains of the dams and the body weights of the foetuses were reduced at 1035 mg/kg body weight and day. Teratogenic effects were not observed (Giavini et al. 1985; Greim 2003; Greim 2007, available in German only). The NOAEL for developmental and maternal toxicity was 520 mg/kg body weight and day.

Developmental toxicity studies also used 1,4-dioxane as a stabilizer for 1,1,1-trichloroethane. These studies did not report toxic effects on development after the exposure of rats or mice by inhalation up to the highest 1,4-dioxane concentration tested of 32 ml/m³ (Schwetz et al. 1975). Likewise, no developmental toxicity was observed in Sprague Dawley rats in a drinking water study with 3% 1,4-dioxane in the drinking water. The high dose corresponded to 3.5 mg 1,1,1-trichloroethane/kg body weight and day or 0.1 mg 1,4-dioxane/kg body weight and day (George et al. 1989).

Genotoxicity

The findings of genotoxicity studies reviewed in the 2003 documentation (Greim 2003) led to the conclusion that cytotoxic concentrations of 1,4-dioxane caused DNA strand breaks in rat liver both in vitro and in vivo.

In a number of studies in vivo, 1,4-dioxane caused micronuclei, but did not induce any mutagenic effects (Greim 2003).

In vitro

No new studies of genotoxicity in vitro have become available that are relevant for the evaluation.

In vivo

In the 3 micronucleus tests in mouse bone marrow that were described in the 2003 documentation (Greim 2003), a positive result was obtained after oral treatment with 900 to 3600 mg/kg body weight, but not after the exposure of male C57BL6 mice to 450 mg/kg body weight. Positive results were obtained after exposure of female C57BL6 mice at 5000 mg/kg body weight. A range-finding study yielded an LD_{50} between 4000 and 5000 mg/kg body weight and day (0/6 animals at 3000, 2/6 animals at 4000 and 4/6 animals at 5000 mg/kg body weight) (Mirkova 1994). Cytotoxicity was not determined in this study.

In another micronucleus test in partially hepatectomized CD1 mice that was described in the 2003 documentation (Greim 2003), micronuclei were observed in the liver after oral exposure to 2000 mg/kg body weight, but not in the peripheral blood at 3000 mg/kg body weight. There was no change in the percentage of reticulocytes in the

peripheral blood. Partial hepatectomy induces cell proliferation in the liver and possibly also in the bone marrow (Morita and Hayashi 1998).

The authors cited another study, which is available only as an abstract in Japanese; according to this study, micronuclei were induced in rat liver when F344 rats were given a 1,4-dioxane dose of 1000 mg/kg body weight by intraperitoneal injection (Morita and Hayashi 1998). The authors assumed a non-genotoxic mechanism for 1,4-dioxane resulting from errors in DNA repair followed by the induction of cell proliferation (Morita and Hayashi 1998).

The in vivo studies that have been published since the 2003 documentation (Greim 2003) are shown in Table 4.

Tab. 4 Genotoxicity of 1,4-dioxane in vivo (studies published since the 2003 documentation)

Test		Dose	Result	References
MN, bone marrow follow-up study of Morita and Hayashi (1998)	CD-1 mouse male 5/group	0, 1500, 2500, 3500 mg/kg body weight 5 × oral (5 days, gavage)	+ at cytotoxic doses; cytotoxicity statistically significant at 1500 mg/kg body weight and above	Roy et al. 2005
MN, liver follow-up study of Morita and Hayashi (1998)	CD-1 mouse male 5/group	0, 1500, 2500, 3500 mg/kg body weight 5 × oral (5 days, gavage)	+ cytotoxicity at 1500 mg/kg body weight and above, but statistically significant only at 3500 mg/kg body weight	Roy et al. 2005
gene mutation in transgenic rats liver	gpt delta transgenic rats male 30 animals in total (number per group not reported)	0%, 0.02%, 0.1%, 0.5% in the drinking water for 16 weeks	– (0.02%–0.1%) + (0.5%) cells additionally GST-P-positive (pre-neoplastic marker) and PCNA-positive (marker for cell proliferation)	Fukushima et al. 2009
meiotic non-disjunction	Drosophila melanogaster	0%, 1%, 1.5%, 2%, 3%, 3.5% in the diet, oocytes examined 24 and 48 hours after mating	+ (2% and above), no dose dependence; at toxic doses (mortality 0%, 0%, 2.4%, 8.1%, 51.7%, 82.8%)	Muñoz and Mazar 2002

A recent study for meiotic non-disjunction in Drosophila melanogaster yielded positive results that were not dose-dependent and were observed at toxic doses. According to the authors, these results may have been caused by cytotoxicity induced by non-specific disturbances in cell division (Muñoz and Mazar 2002).

In a follow-up study of the micronucleus test of Morita and Hayashi (1998) (see Greim 2003) with oral exposure of mice, the number of cells with micronuclei was significantly increased at 1500 mg/kg body weight and above and the concurrent 16% decrease in the PCE/NCE ratio at 1500 and 2500 mg/kg body weight and day or 37% at 3500 mg/kg body weight and day was statistically significant. This is evidence of the cytotoxicity and bioavailability of the substance in the bone marrow. The test yielded positive results in proliferating hepatocytes and negative results in non-proliferating hepatocytes at the same doses. Reduced cell division, which was demonstrated by BrdU staining, was observed here, too, but it was significant only at 3500 mg/kg body weight. The micronuclei resulted from chromosome breaks as no centromers were detected in 80% to 90% of the micronuclei by means of CREST or FISH staining (Roy et al. 2005).

The positive results obtained in micronucleus tests of Mirkova (1994), Morita and Hayashi (1998) and Roy et al. (2005) provided evidence of the induction of micronuclei close to or at cytotoxic concentrations together with cell proliferation in the liver and in the bone marrow. However, in micronucleus tests with negative results, also the bioavailability of the substance in the bone marrow was demonstrated in the form of reduced PCE/NCE ratios (McFee et al. 1994; Mirkova 1994; Tinwell and Ashby 1994; see Greim 2003).

A test for mutations in transgenic rats yielded negative results in the liver up to 0.1% in the drinking water. At 0.5%, an increased incidence of mutations of the *gpt* transgene was detected, and the hepatocytes were GST-P-positive (marker for pre-neoplastic changes) and PCNA-positive (marker for cell proliferation). The study is available only as an abstract (Fukushima et al. 2009).

Summary As reported in the 2003 documentation (Greim 2003), most in vivo studies showed that cytotoxic concentrations of 1,4-dioxane induced DNA strand breaks and micronuclei, in most cases close to or above the recommended limit dose of 2000 mg/kg body weight and day. Cell proliferation was induced concurrently. Cytotoxicity with subsequent cell proliferation was detected as a possible mechanism in the liver and in the bone marrow. Genotoxic effects were not induced below or at 1000 mg/kg body weight and day.

A dominant lethal test in mice yielded negative results after a single intraperitoneal injection of 2500 µl/kg body weight (about 2580 mg/kg body weight) (see Greim 2003).

Carcinogenicity

Short-term studies

Cell transformation tests

Cell transformation tests in BALB/3T3 cells yielded negative results for 1,4-dioxane in one test without cytotoxicity, and cell transformation with concurrent cytotoxicity in another. The metabolite 1,4-dioxan-2-one induced cell transformations in BALB/3T3 cells even without cytotoxicity (Greim 2003).

Inhibition of metabolic co-operation

1,4-Dioxane inhibited metabolic co-operation in V79 cells (thioguanine nucleotide transfer) (Greim 2003).

Initiation and promotion studies

1,4-Dioxane did not function as an initiator on the skin of mice that were subsequently treated with tetradecanoylphorbol acetate (TPA); however, tumour-promoting activity (γ-GT foci) was observed in the liver of male Sprague Dawley rats (Greim 2003).

Short-term studies

In short-term studies in mice, intraperitoneal injections of 1,4-dioxane led to a significant increase in the incidence of lung tumours in one study, whereas a questionable result was obtained in another study because of a lack of dose-dependence (Greim 2003).

Long-term studies

Inhalation

An inhalation study in rats from the 1970s that was described in the 2003 documentation (Greim 2003) did not report any carcinogenic effects at the only concentration tested of 111 ml/m^3 (400 mg/m^3; doses of about 100 mg/kg body weight and day). In this study, 288 male and 288 female Wistar rats were exposed in inhalation chambers for 7 hours a day, on 5 days a week, for 2 years. Growth, mortality, haematological and clinico-chemical parameters, and the gross-pathological and histopathological findings did not differ from those of the 192 males and 192 females of the control group. Tumours did not develop, nor were liver or kidney lesions observed. In this study, the nose was not examined and the MTD was not reached (Torkelson et al. 1974).

In a valid new study, groups of 50 male F344/DuCrj rats were exposed whole-body to 1,4-dioxane concentrations of 0, 50, 250 or 1250 ml/m^3 (0, 180, 900 or 1800 mg/m^3) for 6 hours a day, on 5 days a week, for 104 weeks. Only male animals were used because mesotheliomas had not been observed in the female animals after oral administration in

the 2-year study of Kano et al. (2009) (see following *Section "Oral administration"*). The increases in the incidences of hepatocellular adenomas and nasal squamous cell carcinomas in the high concentration group and peritoneal mesotheliomas in the middle and high concentration groups were statistically significant. Subcutaneous fibromas were detected only in the middle concentration group. The peritoneal mesotheliomas originated from the scrotal sac (Kasai et al. 2009). They are not relevant for human risk evaluation because they develop only in male F344 rats as a consequence of a sex, strain and species-specific susceptibility (Maronpot et al. 2015). An increased incidence of fibroadenomas in the mammary gland was observed, but this finding is not regarded as a pre-malignant lesion and the increase was not statistically significant (Russo 2015; see also *Section "Mechanism of Action"*). Renal cell carcinomas and adenomas in the Zymbal gland were found in the highest concentration group, but they were not statistically significant; however, the kidneys are one of the target organs of toxicity (Kasai et al. 2009). Tumours in organs that occur only in animals, but not in humans, are generally not relevant for humans if they are observed in isolated cases; however, they may be evidence of a carcinogenic potential of a substance. The Zymbal gland is one such example (ECHA 2015). Subcutaneous fibromas were not dose-dependent, but they were found also in the oral carcinogenicity study. A relatively high spontaneous incidence of these tumours was observed in studies of the JBRC (Japan Bioassay Research Center) in the F344/DuCrj rat strain (Takanobu et al. 2015; see also *Section "Mechanism of Action"*); therefore, they are of questionable human relevance.

Details of the study and the tumour incidences are shown in Table 5.

Tab. 5 Carcinogenicity study with inhalation exposure of rats to 1,4-dioxane (Kasai et al. 2009)

Author:	Kasai et al. 2009
Substance:	1,4-dioxane (purity: > 99%)
Species:	**rat**, F344/DuCrj, 50 ♂
Administration route:	inhalation
Concentration:	0, 50, 250, 1250 ml/m^3 (0, 180, 900, 1800 mg/m^3)
Duration:	2 years, 5 days/week, 6 hours/day
Toxicity:	50 ml/m^3 and above: nose: incidence of nuclear enlargement and inflammation ↑, atrophy and respiratory metaplasia of the olfactory epithelium, hydropic changes and sclerosis of the lamina propria, proliferation of the glands 250 ml/m^3 and above: mortality ↑, nose: squamous cell metaplasia, kidneys: incidence of nuclear enlargement ↑ 1250 ml/m^3: body weights ↓, nose: squamous cell hyperplasia, kidneys: hydropic changes of the renal proximal tubules, liver: relative weights ↑, incidence of nuclear enlargement ↑, necrosis of hepatocytes, changes in cell foci, plasma: ALT, AST and γ-GTP activities ↑

	exposure concentration (ml/m^3)			
	0	50	250	1250
surviving animals	37/50 (74%)	37/50 (74%)	29/50 (58%)	25/50 (50%)
tumours and pre-neoplastic changes				
nose:				
respiratory epithelium				
nuclear enlargement	0/50 (0%)	50/50 (100%)**	48/50 (96%)**	38/50 (76%)**
inflammation	13/50 (26%)	9/50 (18%)	7/50 (14%)	39/50 (78%)**
squamous cell metaplasia	0/50 (0%)	0/50 (0%)	7/50 (14%)*	44/50 (88%)**
squamous cell hyperplasia	0/50 (0%)	0/50 (0%)	1/50 (2%)	10/50 (20%)**

Tab. 5 *(continued)*

olfactory epithelium				
nuclear enlargement	0/50 (0%)	48/50 (96%)**	48/50 (96%)**	45/50 (90%)**
atrophy	0/50 (0%)	40/50 (80%)**	47/50 (94%)**	48/50 (96%)**
respiratory metaplasia	11/50 (22%)	34/50 (68%)**	49/50 (98%)**	48/50 (96%)**
inflammation	0/50 (0%)	2/50 (4%)	32/50 (64%)**	34/50 (68%)**
nasal cavity				
hydropic changes of the lamina propria	0/50 (0%)	2/50 (4%)	36/50 (72%)**	49/50 (98%)**
sclerosis of the lamina propria	0/50 (0%)	0/50 (0%)	22/50 (44%)**	40/50 (80%)**
proliferation of the glands	0/50 (0%)	1/50 (2%)	0/50 (0%)	6/50 (12%)*
squamous cell carcinomas	0/50 (0%)	0/50 (0%)	1/50 (2%)	6/50 (12%)*
liver:				
nuclear enlargement, centrilobular	0/50 (0%)	0/50 (0%)	1/50 (2%)	30/50 (60%)**
acidophilic cell foci	5/50 (10%)	10/50 (20%)	12/50 (24%)	25/50 (50%)**
basophilic cell foci	17/50 (34%)	20/50 (40%)	15/50 (30%)	44/50 (88%)**
clear cell foci	15/50 (30%)	17/50 (34%)	20/50 (40%)	23/50 (46%)
mixed cell foci	5/50 (10%)	3/50 (6%)	4/50 (8%)	14/50 (28%)
spongiosis hepatis	7/50 (14%)	6/50 (12%)	13/50 (26%)	19/50 (38%)**
necrosis, centrilobular	1/50 (2%)	3/50 (6%)	6/50 (12%)	12/50 (24%)**
hepatocellular adenomas	1/50 (2%)	2/50 (4%)	3/50 (6%)	21/50 (42%)**
hepatocellular carcinomas	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
kidneys:				
nuclear enlargement, proximal tubule	0/50 (0%)	1/50 (2%)	20/50 (40%)**	47/50 (94%)**
hydropic changes, proximal tubule	0/50 (0%)	0/50 (0%)	5/50 (10%)	6/50 (12%)*
renal cell carcinomas	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
peritoneum:				
mesotheliomas	2/50 (4%)	4/50 (8%)	14/50 (28%)**	41/50 (82%)**
mammary gland:				
fibroadenomas	1/50 (2%)	2/50 (4%)	3/50 (6%)	5/50 (10%)
adenomas	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Zymbal gland:				
adenomas	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
subcutis:				
fibromas	1/50 (2%)	4/50 (8%)	9/50 (18%)**	5/50 (10%)

*$p \leq 0.05$; **$p \leq 0.01$

Oral administration

Two new carcinogenicity studies have been published since the last documentation (Greim 2003) with exposure of rats or mice to 1,4-dioxane with the drinking water for 2 years (Kano et al. 2009).

In rats of the high dose group, the increase in squamous cell carcinomas in the nose of females, adenomas and carcinomas in the liver of both sexes, peritoneal mesotheliomas in males, and adenomas of the mammary gland in females observed after exposure to 1,4-dioxane was statistically significant, while the increase in subcutaneous fibromas was not significant (Kano et al. 2009). The peritoneal mesotheliomas are not relevant for human risk evaluation because they develop only in male F344 rats as a consequence of a sex, strain and species-specific susceptibility (Maronpot et al. 2015). Fibroadenomas of the mammary gland are not regarded as a pre-malignant lesion in rats and humans (see above and *Section "Mechanism of Action"*). Subcutaneous fibromas were not significantly increased in male rats and were not dose-dependent in female rats; however, they were found also in the chronic inhalation study with rats and are (see above and *Section "Mechanism of Action"*) of questionable human relevance.

In mice, increases in tumour incidences with statistical significance were observed only in the liver; these were hepatocellular adenomas and carcinomas in the females at the low dose and above, hepatocellular carcinomas in the males at the middle dose and above and hepatocellular carcinomas in the high dose group. In addition, 2 nasal tumours were found in the high dose group: an adenocarcinoma in one female and an aesthesioneuro-epithelioma in one male animal. According to the authors, both tumours are very rare; thus, these tumours did not occur in any of the 1846 males or 1847 females of the historical control animals of the Japan Bioassay Research Center (JBRC). The tumours were demonstrated in section level 3 (Kano et al. 2009).

The studies are shown in Table 6.

Tab. 6 Drinking water studies that investigated the carcinogenicity of 1,4-dioxane in rats and mice (Kano et al. 2009)

Author:		Kano et al. 2009			
Substance:		1,4-dioxane (purity: 99%)			
Species:		**rat**, F344/DuCrj, 50 ♂, 50 ♀			
Administration route:		drinking water			
Concentration:		0, 200, 1000, 5000 mg/l (corresponds to 0, 11, 55, 274 and 0, 18, 83, 429 mg/kg body weight and day in males and females, respectively)			
Duration:		2 years, continuous			
Toxicity:		274/429 mg/kg body weight: mortality ↑, body weights and body weight gains ↓, relative liver weights ↑ (♀)			
		exposure dose (mg/kg body weight and day) ♂/♀			
		0	11/18	55/83	274/429
surviving animals	♂	40/50 (80%)	45/50 (90%)	35/50 (70%)	22/50 (44%)
	♀	38/50 (76%)	37/50 (74%)	38/50 (76%)	24/50 (48%)
tumours and pre-neoplastic changes					
nose:					
squamous cell carcinomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	7/50 (14%)**
aesthesioneuro-epitheliomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
rhabdomyosarcomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
undifferentiated sarcomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)

Tab. 6 *(continued)*

respiratory epithelium					
nuclear enlargement	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	26/50 (52%)**
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	13/50 (26%)**
squamous cell metaplasia	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	31/50 (62%)**
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	35/50 (70%)**
squamous cell hyperplasia	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	5/50 (10%)
olfactory epithelium					
nuclear enlargement	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	38/50 (76%)**
	♀	0/50 (0%)	0/50 (0%)	28/50 (56%)**	39/50 (78%)**
liver:					
acidophilic cell foci	♂	12/50 (24%)	8/50 (16%)	7/50 (14%)	5/50 (10%)
	♀	1/50 (2%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
basophilic cell foci	♂	7/50 (14%)	11/50 (22%)	8/50 (16%)	16/50 (32%)
	♀	23/50 (46%)	27/50 (54%)	31/50 (62%)	8/50 (16%)
clear cell foci	♂	3/50 (6%)	3/50 (6%)	9/50 (18%)	8/50 (16%)
	♀	1/50 (2%)	1/50 (2%)	5/50 (10%)	4/50 (8%)
mixed cell foci	♂	2/50 (4%)	8/50 (16%)	14/50 (28%)	13/50 (26%)
	♀	1/50 (2%)	1/50 (2%)	3/50 (6%)	11/50 (22%)
hepatocellular adenomas	♂	3/50 (6%)	4/50 (8%)	7/50 (14%)	32/50 (64%)**
	♀	3/50 (6%)	1/50 (2%)	6/50 (12%)	48/50 (98%)**
hepatocellular carcinomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	14/50 (28%)**
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	10/50 (20%)**
hepatocellular adenomas and carcinomas	♂	3/50 (6%)	4/50 (8%)	7/50 (14%)	39/50 (78%)**
	♀	3/50 (6%)	1/50 (2%)	6/50 (12%)	48/50 (98%)**
peritoneum:					
mesotheliomas	♂	2/50 (4%)	2/50 (4%)	5/50 (10%)	28/50 (54%)**
	♀	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
mammary gland:					
fibroadenomas	♂	1/50 (2%)	1/50 (2%)	0/50 (0%)	4/50 (8%)
	♀	3/50 (6%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
adenomas	♂	0/50 (0%)	1/50 (2%)	2/50 (4%)	2/50 (4%)
	♀	6/50 (12%)	7/50 (14%)	10/50 (20%)	16/50 (32%)*
adenomas and fibroadenomas	♂	1/50 (2%)	2/50 (4%)	2/50 (4%)	6/50 (12%)
	♀	8/50 (16%)	8/50 (16%)	11/50 (22%)	18/50 (34%)*
subcutis:					
fibromas	♂	5/50 (10%)	3/50 (6%)	5/50 (10%)	12/50 (24%)
	♀	0/50 (0%)	2/50 (4%)	1/50 (2%)	0/50 (0%)

* $p \leq 0.05$; ** $p \leq 0.01$ Fisher's exact test (DECOS 2015)

Tab. 6 *(continued)*

Author:		Kano et al. 2009			
Substance:		1,4-dioxane (purity: 99%)			
Species:		**mouse**, Crj:BDF$_1$, 50 ♂, 50 ♀			
Administration route:		drinking water			
Concentration:		0%, 0.5%, 0.2%, 0.8% (corresponds to 0, 49, 191, 677 and 0, 66, 278, 964 mg/kg body weight and day in males and females, respectively)			
Duration:		2 years, continuous			
Toxicity:		191/278 mg/kg body weight and above: mortality ↑ (only ♀), body weights and body weight gains ↓, relative liver weights ↑ (only ♂) 677/964 mg/kg body weight: feed and water consumption ↓, relative liver weights ↑ (♀)			
		exposure dose (mg/kg body weight and day) ♂/♀			
		0	49/66	191/278	677/964
surviving animals	♂	31/50 (62%)	33/50 (66%)	25/50 (50%)	26/50 (52%)
	♀	29/50 (58%)	29/50 (58%)	17/50 (34%)	5/50 (10%)
tumours and pre-neoplastic changes					
nose:					
adenocarcinomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
aesthesioneuro-epitheliomas	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
respiratory epithelium					
nuclear enlargement	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	31/50 (62%)**
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	41/50 (82%)**
olfactory epithelium					
nuclear enlargement	♂	0/50 (0%)	0/50 (0%)	9/50 (0%)	49/50 (98%)**
	♀	0/50 (0%)	0/50 (0%)	41/50 (82%)**	33/50 (66%)**
liver:					
hepatocellular adenomas	♂	9/50 (18%)	17/50 (34%)	23/50 (46%)**	11/50 (22%)
	♀	5/50 (10%)	31/50 (62%)**	20/50 (40%)**	3/50 (6%)
hepatocellular carcinomas	♂	15/50 (30%)	20/50 (40%)	23/50 (46%)	36/50 (72%)**
	♀	0/50 (0%)	6/50 (12%)*	30/50 (60%)**	45/50 (90%)**
hepatocellular adenomas and carcinomas	♂	23/50 (46%)	31/50 (62%)	37/50 (74%)**	40/50 (80%)**
	♀	5/50 (10%)	35/50 (70%)**	41/50 (82%)**	46/50 (92%)**

* $p \leq 0.05$; ** $p \leq 0.01$ Fisher's exact test (DECOS 2015)

Summary: tumours relevant for humans and the evaluation

Inhalation, rat: Exposure to 1,4-dioxane by inhalation induced squamous cell carcinomas in the nose, hepatocellular adenomas and a slight increase in the incidence of renal cell carcinomas without statistical significance at the highest concentration tested of 1250 ml/m^3; subcutaneous fibromas were observed only at the middle concentration. Although the findings lacked statistical significance, renal cell carcinomas are regarded as relevant for the evaluation because the kidneys are a target organ of toxicity. Initial findings were observed in the nose as the target organ (increased incidences of nuclear enlargement in the respiratory and olfactory epithelium, atrophy and respiratory metaplasia of the olfactory epithelium) even at the lowest concentration tested of 50 ml/m^3 (180 mg/m^3) and above (Kasai et al. 2009).

Oral uptake, rat and mouse: In drinking water studies in rats, 1,4-dioxane induced hepatotoxicity and nephrotoxicity and a slight increase in hepatocellular adenomas or carcinomas at a dose of about 100 mg/kg body weight and day; inflammation of the nasal cavities with squamous cell carcinomas were observed at about 500 mg/kg body weight and day (Greim 2003).

In a long-term study in mice, hepatocellular adenomas and carcinomas developed at the lowest concentration tested in the drinking water, which corresponds to a 1,4-dioxane dose of about 50 mg/kg body weight and day; inflammation of the nasal cavities with squamous cell carcinomas were reported at about 500 mg/kg body weight and day (Greim 2003).

These studies demonstrated a clear dose-dependence for the carcinogenic effects. A NOAEL of 10 mg/kg body weight and day was determined for toxicity and carcinogenicity in rats (Greim 2003).

The new carcinogenicity studies of Kano et al. (2009) have confirmed the findings from earlier studies.

In female rats, the increased incidence of squamous cell carcinomas induced by 1,4-dioxane in the nose at the high dose of 429 mg/kg body weight and day was statistically significant. This finding did not occur in male rats, probably because the highest dose tested in males of 274 mg/kg body weight and day was not high enough to cause this effect. Nuclear enlargement in the olfactory epithelium was observed in the females at 83 mg/kg body weight and above and in the males at 274 mg/kg body weight and above. Hepatocellular adenomas and carcinomas were found in males and females at 274 and 429 mg/kg body weight and day, respectively. The tumour incidences were not increased at 55 or 83 mg/kg body weight and day; this dose is also the systemic NOAEL (Kano et al. 2009).

When mice were given 1,4-dioxane with the drinking water, only the increased liver tumour incidences were statistically significant. They included hepatocellular adenomas and carcinomas in both sexes, beginning in females at the lowest dose tested of 66 mg/kg body weight and day and above. In addition, a rare tumour was reported in the nose in 1 male and 1 female at 677 and 964 mg/kg body weight and day, respectively. Nuclear enlargement in the nasal epithelium was observed at the middle dose of 278 mg/kg body weight and day and above (Kano et al. 2009). Mice are considerably more sensitive than rats; this may be due to the higher spontaneous incidence of liver tumours in mice.

As the nasal tissue comes into direct contact with 1,4-dioxane in drinking water studies (Sweeney et al. 2008), the development of nasal tumours in these studies may have been caused by local exposure in addition to possible systemic exposure.

Manifesto (MAK value/classification)

The critical effects are nasal toxicity, irritation and the carcinogenic effects on the nose, liver and kidneys as the target organs.

Carcinogenicity. As reported in the 2003 documentation (Greim 2003), most in vivo studies of genotoxicity found that cytotoxic concentrations of 1,4-dioxane induced DNA strand breaks and micronuclei, in most cases close to or above the recommended limit dose of 2000 mg/kg body weight and day. Cell proliferation was induced concurrently. Cytotoxicity with subsequent cell proliferation was detected as a possible mechanism in the liver and in the bone marrow. Genotoxic effects were not induced below or at 1000 mg/kg body weight and day.

The earlier data have been confirmed by the drinking water carcinogenicity studies that were published since the 2003 documentation. 1,4-Dioxane induced nasal squamous cell carcinomas and hepatocellular adenomas and carcinomas in F344 rats. Hepatocellular adenomas and carcinomas were found in mice (Kano et al. 2009). As the nasal tissue comes into direct contact with 1,4-dioxane in drinking water studies (Sweeney et al. 2008), the development of nasal tumours in these studies may have been caused by local exposure in addition to possible systemic exposure.

In a carcinogenicity study with inhalation exposure of male rats, 1,4-dioxane induced squamous cell carcinomas in the nose at the high concentration of 1250 ml/m³. At 50 ml/m³, no tumours were found, but initial findings were already observed in the nose as the target organ (increased incidences of nuclear enlargement, atrophy and respiratory metaplasia of the olfactory epithelium) at the lowest concentration of 50 ml/m³ (180 mg/m³) and above (Kasai et al. 2009).

As described in the 2003 documentation (Greim 2003), non-linear toxicokinetics and accumulation of the substance were demonstrated experimentally at high doses (see *Section "Mechanism of Action"*); this is explained by metabolic saturation. It is assumed that toxicity, which additionally causes carcinogenic effects in the liver and kidneys, occurs only after the saturation of metabolism. However, irritation of the nose, which is observed below saturation, likewise induces carcinomas. Irritation of the nasal epithelium resulting in cytotoxicity, inflammation, regenerative cell proliferation and hyperplasia is assumed to be the mechanism that leads to the development of nasal tumours following inhalation exposure.

As genotoxic effects play a subordinate role in carcinogenicity and are observed only at cytotoxic doses, if at all, 1,4-dioxane continues to be classified in Carcinogen Category 4.

MAK value. 1,4-Dioxane was one of the first substances for which non-linear toxicokinetics and the accumulation of the substance at high doses were demonstrated experimentally and were explained by metabolic saturation (Greim 2003).

Since then, the increased incidence of nuclear enlargement in the kidneys at 250 ml/m³ was found to be the most sensitive systemic effect in a chronic inhalation study in rats (Kasai et al. 2009). Thus, the systemic NOAEC is 50 ml/m³. A MAK value of 10 ml/m³ is calculated from this concentration taking into consideration the increased respiratory volume (1:2), the extrapolation from animal studies to humans (1:2) and the preferred value approach. On the basis of this study, irritation of the nose is the most sensitive local effect in rats because an increased incidence of nuclear enlargement in the respiratory and olfactory epithelium, and atrophy and respiratory metaplasia of the olfactory epithelium were observed as the initial effects in the nose as the target organ at the lowest concentration tested of 50 ml/m³ (180 mg/m³) (see Table 1). The concentration of 16.67 ml/m³ (LOAEC/3) is assumed to be the NAEC of the chronic rat study. It is thus in the same range as the NOAEC of 20 ml/m³ determined for sensory irritation in volunteers after 2 to 8 hours at a LOAEC of 50 ml/m³ after 6 hours. As 1,4-dioxane induced genotoxic effects only at very high doses, irritation of the nasal epithelium resulting in cytotoxicity, inflammation, regenerative cell proliferation and hyperplasia is probably the mechanism that leads to the development of nasal tumours after inhalation exposure.

It is assumed that avoiding sensory irritation also provides protection against histopathologically detected irritation and cytotoxicity in the nasal epithelium (Brüning et al. 2014). As 1,4-dioxane caused carcinogenic effects in the nose, a higher level of protection against irritation is necessary than would be the case for a non-carcinogenic substance; therefore, the MAK value has been lowered to 10 ml/m³. As described above, systemic effects are not expected to occur at 10 ml/m³.

Peak limitation. 1,4-Dioxane continues to be classified in Peak Limitation Category I because local irritation is its critical effect. An excursion factor of 2 has been established because irritation was not observed in the study with 2-hour exposure of volunteers to 20 ml/m³.

Prenatal toxicity. A prenatal developmental toxicity study with 1,4-dioxane in Sprague Dawley rats reported decreased foetal body weights with low maternal toxicity (reduced body weight gains) at 1035 mg/kg body weight and day. The NOAEL for developmental toxicity is 520 mg/kg body weight and day.

The following toxicokinetic data are used to extrapolate the NOAEL for developmental toxicity to a concentration in workplace air: the species-specific correction value of 1:4 for the rat determined on the basis of the toxicokinetic data, the oral absorption of 100% demonstrated in rats, and the body weight of 70 kg, the respiratory volume of 10 m³ during 8 working hours and the 60% absorption by inhalation of the person. This results in a 1,4-dioxane

concentration in air of 1520 mg/m³, which is 41 times as high as the MAK value of 37 mg/m³. As this margin is sufficient and teratogenic effects were not observed, classification in Pregnancy Risk Group C has been confirmed.

Germ cell mutagenicity. As reported in the 2003 documentation (Greim 2003), most in vivo studies demonstrated that cytotoxic concentrations of 1,4-dioxane induced DNA strand breaks and micronuclei, in most cases close to or above the recommended limit dose of 2000 mg/kg body weight and day. Cell proliferation was induced concurrently. Cytotoxicity with subsequent cell proliferation was detected as a possible mechanism in the liver and in the bone marrow. Genotoxic effects were not induced at doses of up to 1000 mg/kg body weight and day. A dominant lethal test in mice yielded negative results after intraperitoneal injection of 2580 mg/kg body weight. Therefore, no data are available that would justify classification in one of the categories for germ cell mutagens.

Absorption through the skin. In an in vitro study, the maximum amount dermally absorbed was estimated to be 984 mg for humans after exposure to undiluted 1,4-dioxane under standard conditions (2000 cm² surface area of skin and 1-hour exposure).

The systemic NOAEC for long-term exposure by inhalation is 50 ml/m³ (180 mg/m³) in rats. The following toxicokinetic data are used to extrapolate this concentration to humans: the respiratory volume during 8 hours (10 m³), the extrapolation from animal studies to humans (1:2) and the increased respiratory volume at the workplace (1:2). This was used to calculate a systemically tolerable amount of 450 mg.

The amount absorbed through the skin (984 mg) is thus more than 25% of the systemically tolerable amount, and 1,4-dioxane continues to be designated with an “H” (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Only a few earlier clinical findings of questionable reliability are available in humans and no positive findings have been reported from animal studies for the sensitizing effects of 1,4-dioxane on the skin. Likewise, there are no findings available for sensitizing effects on the respiratory tract. 1,4-Dioxane is therefore not designated with either “Sh” or “Sa” (for substances which cause sensitization of the skin or airways).

References

Brüning T, Bartsch R, Bolt HM, Desel H, Drexler H, Gundert-Remy U, Hartwig A, Jäckh R, Leibold E, Pallapies D, Rettenmeier AW, Schlüter G, Stropp G, Sucker K, Triebig G, Westphal G, van Thriel C (2014) Sensory irritation as a basis for setting occupational exposure limits. Arch Toxicol 88: 1855–1879. DOI: 10.1007/s00204-014-1346-z

DECOS (Dutch Expert Committee on Occupational Standards) (2015) 1,4-Dioxane. Re-evaluation of the carcinogenicity and genotoxicity, publication no 2015/26. Health Council of the Netherlands, The Hague. https://www.healthcouncil.nl/documents/advisory-reports/2015/11/13/14-dioxane-re-evaluation-of-the-carcinogenicity-and-genotoxicity, accessed 30 Oct 2017

Dennerlein K, Schneider D, Göen T, Schaller KH, Drexler H, Korinth G (2013) Studies on percutaneous penetration of chemicals – Impact of storage conditions for excised human skin. Toxicol In Vitro 27: 708–713. DOI: 10.1016/j.tiv.2012.11.016

Dennerlein K, Jäger T, Göen T, Kilo S, Schaller KH, Drexler H, Korinth G (2015) Evaluation of the effect of skin cleaning procedures on the dermal absorption of chemicals. Toxicol In Vitro 29: 828–833. DOI: 10.1016/j.tiv.2015.03.001

Dietz FK, Stott WT, Ramsey JC (1982) Nonlinear pharmacokinetics and their impact on toxicology: illustrated with dioxane. Drug Metab Rev 13: 963–981. DOI: 10.3109/03602538208991372

Dourson ML, Higginbotham J, Crum J, Burleigh-Flayer H, Nance P, Forsberg ND, Lafranconi M, Reichard J (2017) Update: Mode of action (MOA) for liver tumors induced by oral exposure to 1,4-dioxane. Regul Toxicol Pharmacol 88: 45–55. DOI: 10.1016/j.yrtph.2017.02.025

ECHA (European Chemicals Agency) (2015) Guidance on the application of the CLP criteria – Guidance to regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 4.1, June 2015. https://echa.europa.eu, accessed 06 Mar 2017

ECHA (European Chemicals Agency) (2018) Information on registered substances. Dataset on 1,4-dioxane (CAS Number 123-91-1), joint submission, first publication 02 Mar 2011, last modification 02 Oct 2017. http://echa.europa.eu/web/guest/information-on-chemicals, accessed 24 Jan 2018

EFSA (European Food Safety Authority) (2012) Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA J 10: 2579. DOI: 10.2903/j.efsa.2012.2579

Elcombe CR, Peffer RC, Wolf DC, Bailey J, Bars R, Bell D, Cattley RC, Ferguson SS, Geter D, Goetz A, Goodman JI, Hester A, Jacobs A, Omiecinski CJ, Schoeny R, Xie W, Lake BG (2014) Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit Rev Toxicol 44: 64–82. DOI: 10.3109/10408444.2013.835786

Ernstgård L, Iregren A, Sjögren B, Johanson G (2006) Acute effects of exposure to vapours of dioxane in humans. Hum Exp Toxicol 25: 723–729. DOI: 10.1177/0960327106073805

Fukushima S, Wei M, Omori M, Morimura K, Kinoshita A, Musumura K (2009) Carcinogenicity and in vivo mutagenicity of 1,4-dioxane in gpt delta rats. Exp Toxicol Pathol 61: 282. DOI: 10.1016/j.etp.2009.02.039

Giavini E, Vismara C, Broccia ML (1985) Teratogenesis study of dioxane in rats. Toxicol Lett 26: 85–88. DOI: 10.1016/0378-4274(85)90189-4

George JD, Price CJ, Marr MC, Sadler BM, Schwetz BA, Birnbaum LS, Morrissey RE (1989) Developmental toxicity of 1,1,1-trichloroethane in CD rats. Fundam Appl Toxicol 13: 641–651. DOI: 10.1016/0272-0590(89)90322-9

Göen T, von Helden F, Eckert E, Knecht U, Drexler H, Walter D (2016) Metabolism and toxicokinetics of 1,4-dioxane in humans after inhalational exposure at rest and under physical stress. Arch Toxicol 90: 1315–1324. DOI: 10.1007/s00204-015-1567-9

Greim H (ed) (2003) 1,4-Dioxane. MAK Value Documentation, 2003. In: Occupational Toxicants, vol 20. Wiley-VCH, Weinheim, 105–133. Also available from DOI: 10.1002/3527600418.mb12391e0020

Greim H (ed) (2007) 1,4-Dioxan. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 43. Lieferung. Wiley-VCH, Weinheim. Also available from DOI: 10.1002/3527600418.mb12391d0043

Haseman JK, Hailey JR, Morris RW (1998) Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. Toxicol Pathol 26: 428–441. DOI: 10.1177/019262339802600318

von Helden F (2013) Standardisierte Expositionen gegenüber 1,4-Dioxan zur Etablierung eines Biomonitorings. Dissertation, Justus-Liebig-Universität, Gießen. http://geb.uni-giessen.de/geb/volltexte/2014/11161/pdf/HeldenFranziska_2014_10_23.pdf, accessed 06 Feb 2018

Kano H, Umeda Y, Saito M, Senoh H, Ohbayashi H, Aiso S, Yamazaki K, Nagano K, Fukushima S (2008) Thirteen-week oral toxicity of 1,4-dioxane in rats and mice. J Toxicol Sci 33: 141–153. DOI: 10.2131/jts.33.141

Kano H, Umeda Y, Kasai T, Sasaki T, Matsumoto M, Yamazaki K, Nagano K, Arito H, Fukushima S (2009) Carcinogenicity studies of 1,4-dioxane administered in drinking-water to rats and mice for 2 years. Food Chem Toxicol 47: 2776–2784. DOI: 10.1016/j.fct.2009.08.012

Kasai T, Saito M, Senoh H, Umeda Y, Aiso S, Ohbayashi H, Nishizawa T, Nagano K, Fukushima S (2008) Thirteen-week inhalation toxicity of 1,4-dioxane in rats. Inhal Toxicol 20: 961–971. DOI: 10.1080/08958370802105397

Kasai T, Kano H, Umeda Y, Sasaki T, Ikawa N, Nishizawa T, Nagano K, Arito H, Nagashima H, Fukushima S (2009) Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. Inhal Toxicol 21: 889–897. DOI: 10.1080/08958370802629610

Koissi N, Shah NH, Ginevan B, Eck WS, Roebuck BD, Fishbein JC (2012) Lactone metabolite common to the carcinogens dioxane, diethylene glycol, and N-nitrosomorpholine: aqueous chemistry and failure to mediate liver carcinogenesis in the F344 rat. Chem Res Toxicol 25: 1022–1028. DOI: 10.1021/tx3000076

Kurl RN, Poellinger L, Lund J, Gustafsson JA (1981) Effects of dioxane on RNA synthesis in the rat liver. Arch Toxicol 49: 29–33. DOI: 10.1007/BF00352068

Lane RW, Riddle BL, Borzelleca JF (1982) Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice. Toxicol Appl Pharmacol 63: 409–421. DOI: 10.1016/0041-008X(82)90270-8

Maronpot RR (2009) Biological basis of differential susceptibility to hepatocarcinogenesis among mouse strains. J Toxicol Pathol 22: 11–33. DOI: 10.1293/tox.22.11

Maronpot RR, Thoolen RJMM, Hansen B (2015) Two-year carcinogenicity study of acrylamide in Wistar Han rats with in utero exposure. Exp Toxicol Pathol 67: 189–195. DOI: 10.1016/j.etp.2014.11.009

McFee AF, Abbott MG, Gulati DK, Shelby MD (1994) Results of mouse bone marrow micronucleus studies on 1,4-dioxane. Mutat Res 322: 145–148. DOI: 10.1016/0165-1218(94)90096-5

Mirkova ET (1994) Activity of the rodent carcinogen 1,4-dioxane in the mouse bone marrow micronucleus assay. Mutat Res 322: 142–144. DOI: 10.1016/0165-1218(94)90095-7

Morita T, Hayashi M (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. Environ Mol Mutagen 32: 269–280. DOI: https://doi.org/10.1002/(SICI)1098-2280(1998)32:3%3C269::AID-EM10%3E3.0.CO;2-8

Muñoz E, Mazar B (2002) The rodent carcinogens 1,4-dioxane and thiourea induce meiotic non-disjunction in Drosophila melanogaster females. Mutat Res 517: 231–238. DOI: 10.1016/S1383-5718(02)00083-9

Nannelli A, De Rubertis A, Longo V, Gervasi PG (2005) Effects of dioxane on cytochrome P450 enzymes in liver, kidney, lung and nasal mucosa of rat. Arch Toxicol 79: 74–82. DOI: 10.1007/s00204-004-0590-z

Roy S, Thilagar A, Eastmond D (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. Mutat Res 586: 28–37. DOI: 10.1016/j.mrgentox.2005.05.007

Russo J (2015) Significance of rat mammary tumors for human risk assessment. Toxicol Pathol 43: 145–170. DOI: 10.1177/0192623314532036

Schwetz BA, Leong KJ, Gehring PJ (1975) The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats. Toxicol Appl Pharmacol 32: 84–96. DOI: 10.1016/0041-008X(75)90197-0

Sweeney LM, Thrall KD, Poet TS, Corley RA, Weber TJ, Locey BJ, Clarkson J, Sager S, Gargas ML (2008) Physiologically based pharmacokinetic modeling of 1,4-dioxane in rats, mice, and humans. Toxicol Sci 101: 32–50. DOI: 10.1093/toxsci/kfm251

Takano R, Murayama N, Horiuchi K, Kitajima M, Shono F, Yamazaki H (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. J Health Sci 56: 557–565. DOI: 10.1248/jhs.56.557

Takanobu K, Aiso S, Umeda Y, Senoh H, Saito M, Katagiri T, Ikawa N, Ishikawa H, Mine T, Take M, Haresaku M, Matsumoto M, Fukushima S (2015) [Background data of spontaneous tumors in F344/DuCrlCrlj rats]. Sangyo Eiseigaku Zasshi 57: 85–96. DOI: 10.1539/sangyoeisei.E14006

Tinwell H, Ashby J (1994) Activity of 1,4-dioxane in mouse bone marrow micronucleus assay. Mutat Res 322: 148–150. DOI: 10.1016/0165-1218(94)90097-3

Torkelson TR, Leong BKJ, Kociba RJ, Richter WA, Gehring PJ (1974) 1,4-Dioxane. II. Results of a 2-year inhalation study in rats. Toxicol Appl Pharmacol 30: 287–298. DOI: 10.1016/0041-008X(74)90100-8

Uno Y, Takasawa H, Miyagawa M, Inoue Y, Yoshikawa K (1994) An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens. Mutat Res 320: 189–205. DOI: 10.1016/0165-1218(94)90046-9

US EPA (US Environmental Protection Agency) (2005) Guidelines for carcinogen risk assessment, March 2005. US EPA, Washington, DC. https://www3.epa.gov/airtoxics/cancer_guidelines_final_3-25-05.pdf, accessed 06 Feb 2018

US EPA (US Environmental Protection Agency) (2013) Toxicological review of 1,4-dioxane (with inhalation update) (CAS No. 123-91-1). US EPA, Washington, DC. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0326tr.pdf, accessed 29 Jan 2018