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**KLK Kolb Specialties BV**

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# Derivation of the indicative Drinking Water Target Value for 1,4-dioxane

**TECHNICAL REPORT**

# Executive Summary

Wood was retained by KLK Kolb Specialties bv, hereafter Kolb, to update the substance specific drinking water quality standard for 1,4-dioxane (CAS# 123-91-1), using the formal guidance documents. Before mentioned compound is used as an industrial solvent. In the situation at Kolb, 1,4-dioxane is generated as an unwanted side product of ethoxylation of compounds like fatty acids, fatty amines and natural oils.

From an executed literature search, relevant toxicity data for 1,4-dioxane is summarized below:

- 1,4-Dioxane is not genotoxic in vitro and in vivo
- Carcinogenic effects are caused by a threshold mode of action
- 1,4-Dioxan is not developmentally toxic in rats.
- The No Observed Adverse Effect Level of 1,4-dioxane amounts 9.6 mg/kg-bw/day

The proposed drinking water target value for 1,4-dioxane is based on the TDI as established by Health Canada.

KLK Kolb Specialties bv requests the Wetenschappelijke Klankbordgroep normstelling water en lucht to evaluate and approve the proposed indicative drinking water value for 1,4-dioxane as presented in below table.

Proposed indicative drinking water target values for 1,4-dioxane are presented in below table.

| Parameter | **Proposed Drinking Water Target Value in µg/L** |
|---|---|
| 1,4-dioxane (CAS# 123-91-1) | 38 |


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# 1 Introduction

## 1.1 Preamble

KLK Kolb Specialties bv, hereafter Kolb, discharges the effluent of a wastewater treatment plant into surface water. As a result, elevated concentrations of 1,4-dioxane were observed in the Twentekanaal.
Kolb is execution an extensive program to reduce the concentration of 1,4-dioxane in the discharged effluent. As part of the overall investment to mitigate before mentioned situation, Kolb seeks confirmation of the numeric value of the drinking water target value, based on present scientific insights of the toxicity of the chemical and using the present methodology for deriving the target value.

Wood was retained by KLK Kolb Specialties bv, hereafter Kolb, to update the substance specific drinking water quality standard for 1,4-dioxane (CAS# 123-91-1), using the formal guidance documents.

## 1.2 Methodology and data mining

The Dutch National Institute for Public health and the Environment compiled a formal guidance on the derivation of substance specific drinking water parameters [RIVM, 2017] which aligns with the procedures of the European Commission. This guidance is used to derive the drinking water target value for 1,4-dioxane.

Data of existing evaluations were used as a starting point to derive the drinking water target value for 1,4-dioxane. Reportedly, Health Canada has recently derived a Tolerable Daily Intake for 1,4-dioxane. Reported TDI amounts 0.0054 mg/kg-bw/day [Health Canada, 2021].

# 2 Properties of 1,4-dioxane

Table 2.1 comprises an overview of identifiers for 1,4-dioxane.

Table 2.1 Identification of 1,4-dioxane

| Name | 1,4-dioxane |
|---|---|
| IUPAC-name | 1,4-dioxane |
| Synonyms | 1,4-diethyleene dioxide<br>Diethylene ether<br>Glycolethylene ether |
| CAS-number<br><br>EG number | 123-91-1<br><br>204-661-8 |
| Chemical group according to EPIwin | Neutral organics |
| Cramer class | III |
| Toxicity mechanism | - |
| Harmonized classification | H319 Causes serious eye irritation<br>H335 May cause respiratory irritation<br>H350 May cause cancer |

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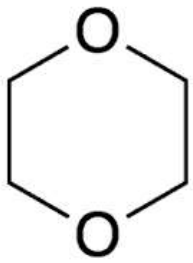
| Name | 1,4-dioxane |
|---|---|
| Molecule formula | $C_4H_8O_2$ |
| Smiles | C1COCCO1 |
| Molecule structure |  |

## 2.1 Physico-chemical properties

Table 2.2 Summary of physico-chemical properties of 1,4-dioxane

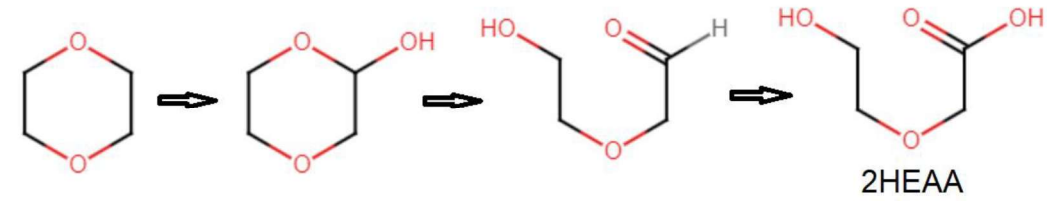
| Property | Value | Additional information | Reference |
|---|---|---|---|
| Molecular weight (g/mol) | 88.11 | | pubchem |
| Melting point (°C) | 11.75 | | ECHA, 2022 |
| Boiling point (°C) | 101.2 | | ECHA, 2022 |
| Density (kg/m$^3$) | 1.034 | | ECHA, 2022 |
| Vapor pressure (Pa) | 38500 | | ECHA, 2022 |
| Solubility in water (g/L) | 1000 | | ECHA, 2022 |
| Log P | -0.42 | | ECHA, 2022 |
| Henry-coefficient (Pa m$^3$/mol) | 0.599 | | ECHA, 2022 |
| pKa | Not applicable | | |

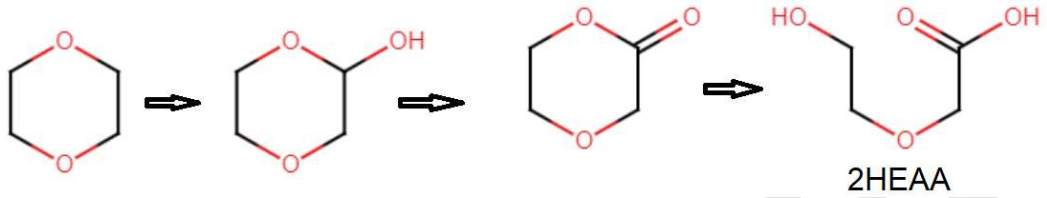
Commercial available 1,4-dioxane appears as a transparent colorless liquid.

## 2.2 Toxicokinetics

After single oral doses of $^{14}$C-1,4-dioxane in rats, 99% of the labelled compound was recovered in the urine and <1% was recovered in the expired air at 10 mg/kg; 86% of the labelled compound was recovered in the urine and 4.7% in the expired air at 100 mg/kg; and 76% of the labelled compound was found in the urine and 25% in the expired air at 1,000 mg/kg [Young et al. 1978]. Similar results were seen following 17 daily gavage doses of $^{14}$C-1,4-dioxane in rats, with 99 and 83% of the label found in the urine, 1.3 and 8.9% of the label found as expired dioxane, and 4.1 and 7% found as expired CO2 in animals receiving 10 and 1,000 mg/kg, respectively. Elimination of 1,4-dioxane in both the expired air and in the urine appear to be first-order kinetic processes [Young et al. 1978].

The oxidation by P450 enzymes starts at one of the methylene groups in 1,4-dioxane. The metabolism is presented in the figures below.




2-Hydroxyethoxyacetic acid is the final metabolite, which is excreted in the urine. The first metabolic step, oxidation of the methylene group, is saturated and follows Michaelis-Menten enzyme kinetics. This has been demonstrated by Leung et al (1990), Reitz et al [1990] and Sweeney et al [2008]. From their reports can be read, that a reasonable estimate of the Km is 32 mg/L and that the Vmax = 29.3 mg/hour at a bodyweight of 70 kg in humans. As long as the concentration of 1,4-dioxane is lower than 5 mg/L, the oxidation rate of 1,4-dioxane is 0.9 per hour and 99% of the ingested 1,4-dioxane is excreted in the urine as 2HEAA.

## 2.3 Environmental fate

According to formal OECD 301 test guidelines, 1,4-dioxane is to be considered as not readily biodegradable. Nevertheless, 1,4-dioxane is degraded in wastewater treatment plants with adapted activated sludge [Lee, 2020]. The rate of microbial degradation is limited by the oxidation of the $CH_2$-group next to the oxygen of the ring. The degradation products all are readily biodegradable [ECHA, 2022].
Wastewater with significant concentrations of 1,4-dioxane usually are (pre)treated using advanced oxidation processes. Before mentioned processes include appropriate combinations of oxidants like Ozone, Hydrogen peroxide and / or UV.

## 2.4 Use

1,4-Dioxane is used as a solvent in industrial processes as well as in laboratories. In the situation at Kolb, 1,4-dioxane is generated as an unwanted side product of ethoxylation of compounds like fatty acids, fatty amines and natural oils.

# 3 Derivation of the indicative drinking water target value

The derivation of the drinking water target value starts with the assessment of the acceptable daily intake following the technical guidance [RIVM, 2017]. Latter parameter is subsequently converted into the drinking water target value based on standard values for body weight and daily drinking water consumption.
Table 3.1 presents an overview of relevant toxicological endpoints.

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Table 3.1 Summary of toxicological endpoints for 1,4-dioxane (CAS# 123-91-1) from Appendix A

| Parameter | Test protocol | Result for 1,4-dioxane (CAS# 123-91-1) |
|---|---|---|
| LD50 oral | Mice | 4033 mg/kg-bw |
| Skin irritation | OECD404 | Not irritant under test conditions |
| Eye irritation | OECD405 | Irritating |
| Skin sensitization | EU method B.6 | Not sensitizing |
| Repeated dose toxicity oral (NOAEL) | 716-day test with rats | 9.6 mg/kg-bw/day |
| Genetic toxicity (in vitro) | | No indication |
| Genetic toxicity (in vivo) | | >1000 mg/kg-bw |
| Carcinogenicity (NOAEL) | | 11 mg/kg-bw/day |
| Developmental toxicity (NOAEL) | OECD414 | 517 mg/kg-bw/day |

Health Canada has established a Tolerable Daily Intake for 1,4-dioxane of 0,0054 mg/kg-bw/day. This value is calculated using Benchmark dose modelling (BMD) based on 95% confidence limit [Health Canada, 2021].

The indicative drinking water target value (iDTV) is calculated using equation B as referred to in section 3.7.2 of Technical Guidance 27 [EC, 2018]:

$$iDTV = 0.2 * TDI * bw / uptake_{DW}$$

with
bw = 70 kg [ECHA, 2008]
$uptake_{DW}$ = 2 liters [ECHA, 2008]

$$iDTV = 0.2 * 0.0054 * 70 / 2$$
$$= 0.0378 \text{ mg/L}$$

# 4 Discussion

The objective of this report is to derive a Drinking Water Target Value for 1,4-dioxane (CAS# 123-91-1). The derived value for this parameter amounts, rounded to two digits, 38 µg/L.
Given the properties of the concentrate, no adverse organoleptic effects on drinking water are anticipated in the case the original surface water would comprise 38 µg 1,4-dioxane/L.

The IARC classified 1,4-dioxane as "possibly carcinogenic to humans" (group 2B), like the hazard sentence H350, based on sufficient evidence in experimental animals and inadequate evidence in humans. In humans, data on 1,4-dioxane are limited to studies on the health risks associated with exposure via inhalation. The primary non-cancer health effects associated with exposure to 1,4-dioxane are reported to be on the liver and kidney; no studies have looked at the ability of 1,4-dioxane to cause cancer in humans. In animals, the

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most severe health effect associated with exposure to 1,4-dioxane is cancer. Additionally, the liver, kidney, and respiratory tract have been identified as major target organs for 1,4-dioxane toxicity.
The base of the proposed Drinking Water Target Value is the TDI based on benchmark dose modelling. The TDI is calculated using an assessment factor of 1000 to compensate for interspecies variability (AF=10), intraspecies variability (AF=10) and for database deficiencies (AF=10). The assessment factor for database deficiencies seems to be introduced because of the possible potential to promote tumors.
Thus, the proposed target value for 1,4-dioxane considers the cancer and non-cancer effects together using a threshold approach. Liver effects that are early events of cancer are the most sensitive endpoints for both cancer and non-cancer toxicity associated with oral exposure to 1,4-dioxane.

Classic derivation of the Drinking Water Target Value following the methodology described by RIVM [2015] using the lowest NOAEL as a starting point and an assessment factor of 100 to compensate for interspecies variability (AF=10) and intraspecies variability (AF=10) results in an indicative human threshold value (iHL) of 0.096 mg/kg-bw/day. The corresponding value for the Drinking Water Target Value amounts 670 µg 1,4-dioxane/L.
Based on the above, I can be concluded that the proposed Drinking Water Target Value for 1,4-dioxane of 38 µg/L, based on the preferred methodology [RIVM, 2015], offers sufficient protection to public health.

To be able to monitor the discharge into surface water, Kolb has developed a protocol for the analysis of 1,4-dioxane in the effluent of the wastewater treatment plant. Based on present experience, the detection limit for 1,4-dioxane in the effluent of the wastewater treatment plant amounts 200 µg/L. This value is higher than reported in public literature, probably due to matrix effects related to the composition of the effluent of the wastewater treatment plant. For instance, US EPA has developed an analytical method for the determination of 1,4-dioxane in drinking water based on GC/MS-SIM with a limit of detection limit tending towards 0.05 µg 1,4-dioxane/L.

# 5 References

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# Appendix A
# Toxicity data for 1,4-dioxane (CAS# 123-91-1)

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The toxicology of 1,4-dioxane (CAS# 123-91-1) has been extensively researched. In below paragraphs, the toxicological findings have been summarized.

## Acute oral toxicity

Reported single dose LD50 values in rats include 5,346 mg/kg, 5,852 mg/kg in mice and 4,033 mg/kg in guinea pigs (Laug et al. 1939).

## Skin irritation

BASF tested the skin irritation with Vienna White rabbits (1973). One ml of 1,4-dioxane was applied on a skin area of 2.5x2.5 cm. 1,4-Dioxane was removed after 1, 5 and 15 minutes and after 20 hours. The skin irritation score was recorded after 24, 48 and 72 hours and averaged. Transient desquamation was observed 6 days after application with parchment-like necrosis fallen-off. The skin irritation score was translate to the Draize score according to the OECD testing guideline 404.

According to GHS criteria 1,4-dioxane was stated not to be a skin irritant. Still, it is labelled in the EU with EUH066 "Repeated exposure may cause skin dryness or cracking". This applies to substances and mixtures which may cause concern as a result of skin dryness, flaking or cracking but which do not meet the criteria for skin irritancy according to GHS.

BASF tested the eye irritation in Vienna White rabbits (1973). An amount of 50 microliter of 1,4-dioxane was applied in the eye conjunctival sac. The eye was not washed after application. Opacity, redness and chemosis score was recorded after 1, 24, 72 and 48 hours. Mucous deposits and blood and scar were observed. The eye irritation scores were transformed to the Draize score according to the OECD testing guideline 405.
According to GHS criteria 1,4-dioxane has been classified as a category 2 eye irritant.

## Skin sensitization

The skin sensitisation potential was studied in the Guinea Pig Maximization Test according to the EU Method B.6 (Skin Sensitisation) with GLP compliance (1993). The intradermal induction was done with 5% 1,4-dioxane in water and the epidermal induction and challenge with 100% 1,4-dioxane. Upon intradermal induction well-defined signs of erythema and oedema were observed. Upon percutaneous induction incrustation, well-defined erythema and slight oedema were noted, but these were caused by the intradermal induction.
The skin reaction was read 24 hours after the challenge. Not any one of the 10 challenged guinea pigs showed a sensitisation reaction. 1,4-Dioxane is not a skin sensitiser according to GHS criteria.

## Repeated oral dose toxicity study

Subchronic oral toxicity of 1,4-dioxane was examined by administering 1,4-dioxane in drinking water at six different concentrations of 0 (control), 640, 1600, 4000, 10000 or 25000 ppm (wt/wt) to F344 rats and $BDF_1$ mice of both sexes for 13 weeks (Kano et al. 2008). Food and water consumption and terminal body weight were decreased dose-dependently in rats and mice. A dose-dependent increase in the relative weights of kidney and lung was noted in rats and mice, while the relative liver weight was increased only in rats. Increases in plasma levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and a decrease in plasma glucose were noted primarily in the rats and mice dosed 25000 ppm. Histopathological examination revealed that 1,4-dioxane affected the upper and lower respiratory tracts, liver, kidneys and brain in rats, while only the former two organs were affected in mice. Nuclear enlargement occurred in the respiratory, olfactory, tracheal and bronchial epithelia of the 1,4-dioxane-dosed rats and mice. The 1,4-dioxane-induced hepatic

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lesions were characterized by centrilobular swelling and necrosis in rats and mice and by glutathione S-transferase placental form (GST-P)-positive altered hepatocellular foci in rats, which are known as preneoplastic lesions. A no-observed-adverse-effect-level (NOAEL) was determined at 640 ppm for both rats and mice, since the nuclear enlargement in the nasal respiratory epithelium and the centrilobular swelling of hepatocytes in rats and the nuclear enlargement in the bronchial epithelium in mice were observed at 1600 ppm. The NOAEL value corresponded to the estimated 1,4-dioxane intake of 52 mg/kg/day in rats and 170 mg/kg/day in mice.

## Chronic toxicity of 1,4-dioxane by ingestion in rats

Four groups of rats, 60/sex/level, were maintained on drinking water containing 0, 1.0%, 0.1%, or 0.01% 1,4-dioxane or up to 716 days (Kociba et 1974). Male and female rats receiving 1% 1,4-dioxane (equivalent to approximately 1015 and 1599 mg/kg/day, respectively) showed decreases in body weight gains, survival rates, and water consumption. Hepatocellular and renal tubular degenerative changes, accompanied by regenerative activity, were similar to those reported in previous studies following exposure to toxic levels of 1,4-dioxane. Hepatocellular and nasal carcinomas, occurring at this dose level, were considered related to the lifetime exposure to these massive toxic dosages of 1,4-dioxane. Male and female rats receiving 0.1% 1,4-dioxane (equivalent to approximately 94 and 148 mg/kg/day, respectively) in the drinking water had variable degrees of renal and hepatic degenerative changes, but there was no indication of treatment-related tumour occurrence. Male and female rats receiving 0.01% 1,4-dioxane in the drinking water (equivalent to approximately 9.6 and 19.0 mg/kg/day, respectively) showed no evidence of tumour formation or other toxic effects considered to be related to treatment. These data indicate a very steep dose response for the toxicity of 1,4-dioxane. The NOAEL in this study was found to be 9.6 mg/kg/day in male rats and 19 mg/kg/day in female rats.

A bioassay of 1,4-dioxane for possible carcinogenicity was conducted by administering the test chemical in the drinking water to Osborne-Mendel rats and B6C3F1 mice (National Cancer Institute US, 1978). Groups of 35 rats and 50 mice of each sex were administered 1,4-dioxane at concentrations of either 0.5% or 1.0% (v/v) in the drinking water. Because of variations in intake of water, the doses of test chemical received by the high-dose groups were not precisely twice those received by the low-dose groups; in the male mice, the high dose was only slightly greater than the low dose. The rats were dosed for 110 weeks and the mice for 90 weeks. Matched controls consisted of 35 untreated rats and 50 untreated mice of each sex. All surviving rats were killed at 110-117 weeks and all surviving mice at 90-93 weeks.
The mean body weights of the rats and mice were not consistently affected by the administration of dioxane. Survival rates of the dosed groups of rats and female mice were lower than those of corresponding control groups, but sufficient numbers of animals were at risk for development of late-appearing tumors.
In male and female rats, the incidence of squamous-cell carcinomas of the nasal turbinates was statistically significant increased. In the females, but not in the males, the incidence of hepatocellular adenomas was significantly increased.
In both male and female mice, the incidence of hepatocellular carcinomas was statistically significantly increased. The incidences remained significant when hepatocellular adenomas were combined with hepatocellular carcinomas.
It was concluded that under the conditions of this bioassay, 1,4-dioxane induced hepatocellular adenomas in female Osborne- Mendel rats. 1,4-Dioxane was carcinogenic in both sexes of rats, producing squamous-cell carcinomas of the nasal turbinates, and in both sexes of B6C3F1 mice, producing hepatocellular carcinomas.

The results of these 2 drinking water studies have been summarised in Table 1 of the paper of Reitz et al (1990)

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TABLE 1

TUMOR INCIDENCES OBSERVED IN ANIMAL BIOASSAYS OF DIOXANE

| Dose | Route | Tumors | Animals | Percentage |
|---|---|---|---|---|
| Male and female rats, liver tumors | | | | |
| Controls | — | 5 | 457 | 1.1% |
| 0.01% | Water | 0 | 110 | 0.0% |
| 0.1% | Water | 1 | 106 | 0.9% |
| 0.5% | Water | 12 | 65 | 18.5% |
| 1.0% | Water | 26 | 131 | 19.8% |
| 111 ppm | Inhal | 0 | 423 | 0.0% |
| Male and female rats, nasal turbinate tumors | | | | |
| Controls | — | 0 | 462 | 0.0% |
| 0.01% | Water | 0 | 110 | 0.0% |
| 0.1% | Water | 0 | 106 | 0.0% |
| 0.5% | Water | 24 | 68 | 35.3% |
| 1.0% | Water | 31 | 135 | 23.0% |
| 111 ppm | Inhal | 0 | 423 | 0.0% |
| Male mice, liver tumors | | | | |
| Controls | — | 8 | 49 | 16.3% |
| 0.5% | Water | 19 | 50 | 38.0% |
| 1.0% | Water | 28 | 47 | 59.6% |
| Female mice, liver tumors | | | | |
| Controls | — | 0 | 50 | 0.0% |
| 0.5% | Water | 21 | 48 | 43.8% |
| 1.0% | Water | 35 | 37 | 94.6% |

*Note.* Tumor incidences (liver or nasal turbinates) in male and female rats in the studies of NCI (1978) and Kociba *et al.* (1974) are combined in this table. Liver tumor incidences for male and female mice are reported separately. No significant increases in nasal turbinate tumors were seen in either sex of mouse.

Kano et al. (2009) studied the carcinogenicity of 1,4-dioxane by giving groups of 50 F344/DuCrj rats and 50 Crj:BDF1 mice of each sex 1,4-dioxane in the drinking-water for 2 years. The concentrations of 1,4-dioxane were 0 (control), 200, 1000 and 5000 ppm (wt./wt.) for rats and 0, 500, 2000 and 8000 ppm for mice. This resulted in the actual dose levels of 0, 11, 55 and 274 mg/kg-bw/day for male rats and of 0, 18, 83 and 429 mg/kg-bw/day for female rats. In case of male mice, the actual dose levels were 0, 49, 191 and 677 mg/kg-bw/day and in case of female mice 0, 66, 278 and 964 mg/kg-bw/day. The highest dose levels did not exceed the maximum tolerated dose.

In the rat, there was significant induction of nasal squamous cell carcinomas in females and hepatocellular adenomas and carcinomas in males and females, peritoneal mesotheliomas in males, and mammary gland adenomas in females.

In the mouse, there was significant induction of hepatocellular tumors in males and females. Two nasal tumors, occurring in the 8000 ppm-dosed groups, were spontaneously rare and, thus, were attributed to 1,4-dioxane exposure. The present studies provided clear evidence of carcinogenicity in rats and mice. The NOAEL was 83 mg/kg-bw/day for nasal squamous cell carcinomas in female rats and 11 mg/kg-bw/day for hepatocellular tumors in male rats, while a LOAEL was 66 mg/kg/day for hepatocellular tumors in female mice.

## Chronic toxicity of 1,4-dioxane in rats by inhalation

Kasai et al (2009) examined the carcinogenicity and chronic toxicity of 1,4-dioxane by inhalation exposure of 50 male F344 rats to 1,4-dioxane vapor at 0 (clean air), 50, 250, or 1250 ppm (v/v) for 6 h/day, 5 days/wk, and 104 wk. Survival rates of 250 and 1250 ppm-exposed groups were decreased near the end of the 2-yr exposure period, due probably to the occurrence of malignant tumors. A statistically significant but marginal decrement of terminal body weight (<10%) was found in the 1250 ppm-exposed group, suggesting slight systemic toxicity. Significant changes in plasma levels of AST, ALT, ALP, and γ-GTP and relative weight of the liver occurred in the 1250 ppm-exposed group. Dose-dependent and statistically significant increases in incidences of nasal squamous cell carcinomas, hepatocellular adenomas, and peritoneal mesotheliomas were found primarily in the 1250 ppm-exposed group. The incidences of renal cell carcinomas, fibroadenomas in the mammary gland, and adenomas in the Zymbal gland were also increased dose-dependently. Preneoplastic lesions occurred in the nasal cavity and liver of the 1,4-dioxane-exposed groups. As nonneoplastic lesions, the significantly increased incidences of nuclear enlargement, atrophy, and respiratory metaplasia in the nasal cavity were noted at 50 ppm and above. A LOAEL (lowest observed adverse effect level) was determined at 50 ppm for the nasal endpoint of general chronic toxicity. This study provides clear evidence of carcinogenicity for 1,4-dioxane in male rats. A cytotoxic-proliferative mode of action is suggested to operate in 1,4-dioxane-induced carcinogenesis.
The inhalation dose of 50 ppm (180 mg/m3, 6 hrs/day, 5 days/week) is equivalent with a daily dose of 37 mg/kg-bw/day (0.29 m3/kg-bw 5 days per week = 0.207 m3/kg-bw for 7 days per week). It is assumed that there is 100% absorption in the respiratory tract.
Further it is worthwhile to mention, that a concentration of 180 mg/m3 in air might be in equilibrium with a water film of the mucous membrane. The aqueous equilibrium concentration in the mucous membrane, in equilibrium with an air level of 180 mg/m3 or 50 ppm on the basis of the dimensionless Henry coefficient, is estimated to be 918 mg/litre 1,4-dioxane. This is a cytotoxic level.

## Developmental toxicity study on 1,4-dioxane

Giavini et al. (1985) studied 1,4-dioxane for teratogenic potential in Sprague-Dawley rats. The compound was administered on days 6-15 of gestation by gavage (0, 0.25, 0.5 and 1.0 ml/kg/day). A slight maternal toxicity, as evidenced by reduced weight gain, was observed with 1.0 ml/kg. Animals were killed and subjected to uterine examination on day 21 of pregnancy. There were no differences between control and dioxane-treated groups in implantation numbers, live foetuses, post-implantation loss or major malformations. Embryotoxicity, manifested by reduced foetal weight, occurred only at the highest dose level.
The NOAEL for developmental effects was observed to be 0.5 ml/kg-bw/day

## Mutagenicity of 1,4-dioxane

The results of mutagenicity studies in vivo and in vitro have been summarised in the ATDSR profile of 1,4-dioxane (2012). The tables below are from this ATDSR profile (2012).

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**Table 3-4. Genotoxicity of 1,4-Dioxane *In Vitro***

| Species (test system) | End point | Results | | Reference |
|---|---|---|---|---|
| | | With activation | Without activation | |
| *Salmonella typhimurium* (TA100, TA98, TA1535, TA1537) | Gene mutation | – | – | Haworth et al. 1983 |
| *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) | Gene mutation | – | – | Stott et al. 1981 |
| *S. typhimurium* (TA100, TA1535) | Gene mutation | – | – | Nestmann et al. 1984 |
| *S. typhimurium* (TA98, TA100, TA1530, TA1535, TA1537) | Gene mutation | – | – | Khudoley et al. 1987 |
| *Photobacterium phosphoreum* | DNA damage | NT | – | Kwan et al. 1990 |
| *Escherichia coli* K-12 *uvrB/recA* | DNA damage | – | – | Hellmer and Bolcsfoldi 1992 |
| *S. typhimurium* (TA98, TA100, TA1535, TA1537) | Gene mutation | – | – | Morita and Hayashi 1998 |
| *E. coli* (WP2, WP2 *uvrA*) | Gene mutation | – | – | Morita and Hayashi 1998 |
| *Saccharomyces cerevisiae* (D61M) | Chromosomal malsegregation | NT | – | Zimmermann et al. 1985 |
| Mouse lymphoma cells | Gene mutation | – | – | Morita and Hayashi 1998 |
| CHO cells | Chromosomal aberrations | – | – | McElroy et al. 2003 |
| CHO-K1 cells | Chromosomal aberrations | – | – | Morita and Hayashi 1998 |
| CHO-K1 cells | Sister chromatid exchange | – | – | Morita and Hayashi 1998 |
| CHO-K1 cells | Micronuclei | – | – | Morita and Hayashi 1998 |
| Rat hepatocytes | DNA repair | – | – | Goldsworthy et al. 1991 |
| CHO-W-B1 cells | Chromosomal aberrations | – | – | Galloway et al. 1987 |
| CHO-W-B1 cells | Sister chromatid exchange | – | ± | Galloway et al. 1987 |
| Mouse lymphoma cells | Gene mutation | – | – | McGregor et al. 1991 |
| BALB/3T3 cells | Cell transformation | NT | + | Sheu et al. 1988 |

– = negative result; + = positive result; ± = weak positive result; CHO = Chinese hamster ovary; NT = not tested

Nearly all tests for mutagenicity of 1,4-dioxane in vitro failed to show any mutagenicity of 1,4-dioxane, except the positive cell transformation in the BALB/3T3 cells. This mutagenicity test is presently not part of the OECD testing Guidelines.

**Table 3-5. Genotoxicity of 1,4-Dioxane *In Vivo***

| Species (test system) | End point | Results | Reference |
|---|---|---|---|
| Human peripheral lymphocytes | Chromosomal aberrations | – | Thiess et al. 1976 |
| Rat hepatocytes | DNA repair | – | Goldsworthy et al. 1991 |
| Rat nasal epithelial cells | DNA repair | – | Goldsworthy et al. 1991 |
| Mouse hepatocytes | Micronuclei | + | Morita and Hayashi 1998 |
| Mouse hepatocytes | Micronuclei | + | Roy et al. 2005 |
| Mouse peripheral blood | Micronuclei | – | Morita and Hayashi 1998 |
| Rat hepatocytes | DNA alkylation or repair | – | Stott et al. 1981 |
| Rat hepatocytes | DNA damage | + | Kitchin and Brown 1990, 1994 |
| Mouse bone marrow | Micronuclei | – | Tinwell and Ashby 1994 |
| Mouse bone marrow | Micronuclei | + | Roy et al. 2005 |
| Mouse bone marrow (C57BL6) | Micronuclei | + | Mirkova 1994 |
| Mouse bone marrow (BALB/c) | Micronuclei | – | Mirkova 1994 |
| Mouse bone marrow | Micronuclei | inc | McFee et al. 1994 |
| *Drosophila* (food) | Dominant lethal | – | Yoon et al. 1985 |
| *Drosophila* (food) | Meiotic non-disjunction | + | Muñoz and Barnett 2002 |

– = negative result; + = positive result; ± = weak positive result; inc = inconclusive

The information available indicates that 1,4-dioxane is not genotoxic in in vitro tests in eukaryotic and prokaryotic cells. Tests in vivo have been mostly negative, but a few tests yielded positive results in animals treated with 1,4-dioxane in excessive high doses, many times higher than potential environmental exposures.

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