Social Europe

Recommendation from the Scientific Committee on Occupational Exposure Limits for 1,4-dioxane

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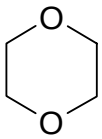
Recommendation from the Scientific Committee on Occupational Exposure Limits for 1,4-Dioxane

8 hour TWA	: 20ppm (*73 mg/m³*)
STEL (15 min)	: -
Notation	: -

Substance information

1,4-Dioxane



Synonyms : 1,4-dioxacyclohexane, diethylene dioxide, diethylene ether, diethylene-1,4-dioxide, dioxane, dioxyethylene ether, glycolethylene ether, NE 220, p-dioxane, tetrahydro-1,4-dioxane, tetrahydro-p-dioxane

EINECS No. : 204-661-8

EEC No. : 603-024-00-5

Classification : F, R11-19, Carc. Cat. 3; R36/37-40-66; S2-9-16-36/37-46

CAS No. : 123-91-1

MWt : 88 g/mol

Conversion factor (20 °C): 1 ppm = 3,6 mg/m³; 1 mg/m³ = 0,28 ppm

This document is based on the EU-RAR 2002 and Morita et al., 1998, (not cited in the EU-RAR 2002) and the references cited therein.

Physico-chemical properties

1,4-dioxane is a highly flammable liquid with a melting point of 12 °C and a boiling point of 101 °C. Its odour is etheric. The substance is miscible in water and in the most organic solvents. The flash point of 1,4-dioxane is 11 °C and the vapour pressure is 40 hPa at 20 °C.

1. Occurrence/Use

In Europe, 1,4-dioxane is at present only produced at one production site. The production volume in 1997 was estimated to be 2000-2500 tonnes with an export outside the European Community of 575 tonnes (Industry, 1998). There is no information about import volumes of 1,4-dioxane into the EU.

In 1995 the production capacity of known producers and the world wide production volume is estimated at 8,000 t/a and 10,000 t/a, respectively (BASF information). In general the world-wide production of 1,4-dioxane is decreasing because of changing use patterns. 1,4-dioxane is typically manufactured by acid-catalysed conversion of diethylene glycol by ring closure in a closed system (Weber, 1975, Dittus, 1966, BASF information).
1,4-Dioxane is used as a solvent in the production of lacquers, varnishes, cleaning and detergent preparations, adhesives, cosmetics, deodorant fumigants, emulsions and polishing compositions, pulping of wood, extraction medium for animal and vegetable oils, laboratory chemical (eluent in chromatography), cassettes, plastic and rubber, and insecticides and herbicides (BASF information; HSDB, 1996; Grant Chemicals, 1977). Further it is used as a stabiliser in 1,1,1-trichloroethane; this use is diminished considerably as a result of the restriction of the use of substances depleting the ozone layer (Grant Chemicals, 1977).
1,4-dioxane is a part of catalysts, for example in vinyl chloride polymerisation of polyvinyl chloride. It is not completely clear to what extent 1,4-dioxane is presently used as a solvent in end products.

2. Health Significance

2.1. Toxicokinetics

Absorption
Inhalation and oral
Four healthy volunteers were exposed to 50 ppm (180 mg/m^3), equivalent to 5,4 mg/kg/bw, 1,4-dioxane for 6 hours and the blood and the urine was examined (Young et al., 1977). 1,4-Dioxane was rapidly and for at least 50 % absorbed. Radiolabeled 1,4-dioxane was rapidly and almost completely absorbed after oral and inhalation exposure by rats.

Dermal
Dermal absorption occurs, but it is low, probably due to evaporation of the material. In experiments with Rhesus monkeys, 2.3 and 3.4 % of the dioxane applied non occlusively as a methanol solution or as lotion was excreted in the urine (Marzulli et al., 1981). In-vitro studies show that 3.2% of an applied dose passes through excised skin under occlusion and only 0.3% when not occluded (ECETOC, 1983).

Metabolism
The primary rote of metabolism of 1,4-dioxane, at least at relatively low doses, is via cytochrome P450-catalysed hydrolysis and then oxidation, to produce 2-hydroxyethoxyacetic acid (HEAA). There can also be oxidation of the unbroken ring to produce 1,4-dioxane-2-one, which is in equilibrium with HEAA (Woo 1977 a,b). . It
In rats it is clear that there is limited capacity to metabolise 1,4-dioxane to HEAA. A single oral dose of 10 mg/kg bw to rats was rapidly metabolised and excreted (as HEAA) via the urine, while a single oral dose of 1000 mg or repeated administration of high doses

saturated the metabolism, resulting in a decreased proportion of urinary excretion as HEAA and increased 1,4-dioxane in the expired air (Dietz et al., 1982, Reitz et al., 1990, Young et al., 1978). Furthermore, at such dose levels an alternative metabolic pathway becomes significant, that of hydroxylation, followed by oxidation, to produce the reactive metabolite
2-hydroxyethoxyacetaldehyde (HEA). In toxicity studies (see below), morphological and biochemical changes were seen at exposure concentrations which lead to this saturation of metabolism. HEA is believed to be the reactive metabolite responsible for some of the principal expressions of toxicity seen with 1,4-dioxane, including carcinogenicity in experimental animals (see below). Repeated higher exposures to 1,4 – dioxane induce cytochrome P450-oxidation in rat liver (Young et al., 1978).

Excretion
In humans exposed for 6 hours to 180 mg/m^3 1,4-dioxane in a chamber under dynamic airflow conditions 99,3% of the administered dioxane was eliminated via the urine as ß-hydroxyethoxyacetic acid (HEAA). The plasma 1,4-dioxane concentration decreased linearly, the elimination was not saturated at 50 ppm. The plasma elimination $t_{1/2}$ was 59 minutes (Young et al., 1977). A physiologically based pharmacokinetic (PB-PK) modelling study indicates that dioxane may also be excreted into human milk (Fisher et al., 1997).
1,4 -Dioxane was rapidly excreted in rats via the urine, the major metabolites were 2-hydroxyethoxyacetic acid (HEAA) and 1,4-dioxane-2-one (Woo et al., 1977a, 1977b). These two metabolites are in chemical equilibrium, dependant on the pH. At low pH the equilibrium is more shifted to 1,4-dioxan-2-one.

2.2. Acute toxicity

2.2.1. Animal data

The oral LD_{50} value of 1,4-dioxane for the rat was between 5170 and 7339 mg/kg bw. Signs of toxicity after oral administration to rats, mice and guinea-pigs included narcotic effects, coma, irritation of the gastro-intestinal mucous membranes and damage in liver and kidneys (Laug et al., 1939; Nelson, 1951; Smyth et al., 1941). In rabbits dose related narcotic effects were seen (Nelson, 1951).
The dermal LD_{50} was reported to be 7855 mg/kg b.w. for the rabbit. No toxic effects were mentioned.
With respect to inhalation the LC_{50} was 46000-52000 mg/m^3 for rats and 36700 mg/m^3 for mice. Rats showed dyspnoea, apathy, narcosis, irritation of mucous membranes (eyes, respiratory tract), eyelid-reflex loss, unkempt coat, staggering and heart dilatation and after necropsy haemorrhagic erosion of the mucous membranes of the stomach and bloody contents in stomach and intestines (BASF AG, 1980).
Acute neurotropic effects of 1,4-dioxane were investigated. Depression of tonic extension after electroshock in rats was seen at concentrations ≥ 6800 mg/m^3 and an oral administration of 1050 mg/kg b.w. caused a decrease in dopamine and serotonin levels in the hypothalamus and a decrease in serotonin in the medulla oblongata (Frantik et al., 1994).

2.3. Irritation

2.3.1. Human data

At concentrations ≥ 1000 mg/m^3 irritation of eyes, nose and throat was reported, (EU RAR, 2002). Young et al. (1977) reported in 4 healthy volunteers during an inhalation study (exposure over 6 hours) irritation of the eyes at 50 ppm (73 mg/m^3); eye irritation was a frequent complaint throughout the exposure. Perception of the odour of dioxane diminished with time. Two of the subjects could not perceive the odour after 4 and 5 hr in

the chamber, whereas the other two subjects could still detect the odour at the end of the exposure period. The subject who first lost the ability to perceive the odour of dioxane also had the highest blood plasma concentration of dioxane. No other symptoms or complaints were recorded in this study. .

2.3.2. Animal data

Skin
When applied undiluted under occlusive conditions for 1-15 minutes to rabbit skin 1,4-dioxane led to slight erythema and scale formation which was not completely reversible within 8 days (BASF, 1973; Zeller and Kühlem, 1998a). In rats and mice the lowest irritating concentration was 80% (no further information available, Sekizawa et al., 1994).
Eyes
Eye irritation (corneal opacity and conjunctival redness and slight to severe chemosis) was found after instillation of 0.05 ml into rabbit eyes, which was not completely reversible within 8 days (BASF, 1973; Zeller and Kühlem, 1998b).

Respiratory tract
Irritating effects were noted in the respiratory tract of rats, mice and guinea pigs in studies with insufficient documentation at concentrations presumably higher than 1000 ppm (EU RAR, 2002).
Conclusion: 1, 4 - dioxane is irritating to the eyes and the respiratory tract, but only probably to skin.

2.4. Sensitisation

A negative result was obtained in a guinea pig maximisation test according OECD guideline. However one positive human patch-test is reported in a man who developed dermatitis after daily dipping in a 1,4 -dioxane containing solvent (Fregert, 1974).

2.5. Repeated dose toxicity

2.5.1. Human data

Inhalation
In one case report a 21-year old worker had been exposed to 1,4-dioxane for one week at concentrations ranging from 720 mg/m^3 to 2340 mg/m^3. Moreover, he had repeatedly dipped his hands into a tub containing liquid 1,4-dioxane. The man had been an alcoholic. The signs experienced included pain in the upper abdomen, hypertonia and neurological symptoms. After one week of hospitalization the man died of kidney failure. Necropsy included renal cortex necrosis with severe interstitial haemorrhages. Severe centrilobular necrosis was found in the liver. The brain showed signs of demyelination and partial loss of nerve fibre tissue (Johnstone, 1959). Similar symptoms were observed in five patients who died after 1,4-dioxane exposure (Barber, 1934).

Dermal
Inflammatory skin changes, showing symptoms of eczema, in the upper extremities and the face were seen after dermal exposure to 1,4-dioxane for several weeks in a 47-year-old female laboratory technician (Sonneck, 1964).

2.5.2. Animal data

Inhalation
Wistar rats were exposed to 400 mg (111 ppm) 1,4-dioxane vapour/m^3 for 7 hours/day, five days a week for a total of 2 years (corresponding to 108 mg/kg bw/d). No significant treatment-related effects were seen on clinical signs, haematology or organ weights

(Kociba et al., 1974; Torkelson et al., 1974). No organ toxicity and no tumour formation were observed. Further details of this study are described under Carcinogenicity.

Oral

The studies with application of 1,4-dioxane via drinking water are summarized in Table 2. It includes also long term studies on carcinogenicity. In one long term study with rats dose related but not statistical significant spongiosis of the liver was found at the lowest dose tested of 0.02 % (Yamazaki et al., 1994), which fits to the result of the other long term study with rats, where more severe liver effects i.e. liver necrosis has been found at about 0.1 %, and no effects at lower doses (Japan Bioassay Research Center, 1998, Kociba et al., 1974). Other targets were the kidney (tubular degeneration and kidney weights) and the nose (malignant neoplasms, adenocarcinoma). The overall NOAEL, based on liver damage, can be considered to be 0.01% (equivalent to 10 mg/kg bw/d).

Special investigations

Male Sprague Dawley rats received 10 or 1000 mg 1,4-dioxane/kg bw/d via drinking water for 11 weeks (Stott et al., 1981). 7 Days prior to termination, the rats received [6-^{3}H] thymidine. 1,4-Dioxane was cytotoxic to hepatic tissue at 1000 mg/kg bw, as evidenced by an increase in liver to body weight ratio and a significant rise in hepatic DNA synthesis as measured by [6-^{3}H]-thymidine incorporation, accompanied by a minimal degree of hepatocellular swelling. The NOAEL for liver cytotoxicity is 10 mg/kg bw.

Table 1: Studies with application of 1,4-dioxane via drinking water

Species	Duration	Dose	Effects	NOAEL	Reference
Rat F344/DuCrj 10m, 10f	13 w	0, 0.064, 0.16, 0.4, 1, 2.5 % (m:60, 150, 330, 760, 1900 mg/kg bw f: 100, 200, 430, 870, 2010 mg/kg bw	≥ 0.16%: kidney f: weight ↑ nasal cavity, trachea, liver, kidney, brain m,f: non-neoplastic lesions	0.064 %	Japan Bioassay Research Center, 1998
Rat Sherman 60m, 60f	716 days	0, 0.01, 0.1, 1 % m: 0, 9.6, 94, 1015 mg/kg bw f: 0, 19, 148, 1599 mg/kg bw	≥0.1 % kidney: degeneration + necrosis of tubular epithelium, liver: degeneration necrosis, regeneration ≥ 1 % m,f: bw ↓, survival ↓, liver: weight ↑, carcinomas (10/66), cholangiomas (2/66), nose: squamous cell carcinomas (2/66)	0.01 %	Kociba et al., 1974
Rat F344/DuCrj 50 m 50f	104 w	0, 0.02, 0.1, 0.5% m: 0, 16, 81, 398 mg/kg bw f: 0, 21, 103, 514 mg/kg bw	≥0.02 % liver m: spongiosis (dose-related, not statistical significant at 0.02%) ≥0.1 % liver: m: weight ↑, m,f: hyperplasia, and spongiosis ≥0.5 %: liver m,f: adenoma and carcinoma nose m,f: malignant neoplasms skin m: mesothelioma, fibroma mammary gland m,f: fibroadenoma, adenoma	< 0.02 %	Yamazaki et al., 1994
Rat Osborne-Mendel 35 m 35f	110 w	0, 0.5, 1% m: 0, 240, 530 mg/kg bw f: 0, 350, 640 mg/kg bw	≥0.5 % nose m,f: squamous cell carcinomas (m: 0/33, 12/33, 16/34, f: 0/34, 10/35, 8/35) liver f: hepatocellular adenomas (0/31, 10/33, 11/32), m,f: cytomegaly kidney m,f: tubular degeneration stomach m,f: ulceration	< 0.5%	NCI, 1978

Species	Duration	Dose	Effects	NOAEL	Reference
Mouse Crj:BDF1 10 m, 10 f	13 w	0.064, 0.16, 0.4, 1, 2.5 % (m: 100, 260, 580, 920, 1830 f: 170, 410, 920, 1710, 2700 mg/kg bw)	≥ 0.16%: nasal cavity, trachea, lung, liver f: non-neoplastic lesion ≥ 0.4%: nasal cavity, trachea, lung, liver m: non-neoplastic lesion ≥ 1%: kidney, lung f: weight ↑ 2.5%: lung m: weight ↑		
Mouse B6C3F1 50 m 50f	90 w	0, 0.5, 1% m: 0, 720, 830 mg/kg bw f: 0, 380, 860 mg/kg bw	≥0.5 %: liver m,f: hepatic cytomegaly carcinoma respiratory tract m,f: pneumonia, rhinitis nose f: adenocarcinoma (1/50) 1 %: nose m: adenocarcinoma (1/50)	< 0.5%	NCI, 1978
Mouse Crf:BDF$_1$ 50 m 50f	104 w	0, 0.05, 0.2, 0.8% m: 0, 66, 250, 770 mg/kg bw f: 0.77, 320, 1070 mg/kg bw	≥0.05 %: liver f: adenoma and carcinoma ≥0.2 %: nose m,f: lesions in nasal cavity lung f: weight ↑ testis m: decreased minerilisation 0.8 %: nose m: esthesioneuroepithelioma (1/50) nose f: adenocarcinoma (1/50)	< 0.05%	Yamazaki et al., 1994

2.6. Genotoxicity

2.6.1. Mutagenicity in vitro

Bacterial and yeast tests
Bacterial tests with *Salmonella typhimurium* in different tester strains (among them TA 98, 100, 1535, 1537) were negative with and without metabolic activation (Haworth et al., 1983, EU-RAR, 2002). One aneuploidy test with *Saccharomyces cerevisiae* was negative (Zimmermann et al., 1985) and a DNA repair test in *E.coli* was also negative.

Mammalian tests
1,4-Dioxane did not induce gene mutation (HPGRT locus) or chromosome aberration in CHO-cells (BASF, 1991, Galloway et al., 1987). Indicator tests like unscheduled DNA synthesis, alkaline elution assay performed in rat hepatocytes revealed negative results (Goldsworthy et al., 1991, Sina et al., 1983). A sister chromatid exchange test in CHO cells was positive without and negative with metabolic activation (Galloway et al., 1987). An alkaline elution test for DNA single strand breads was positive in rat hepatocytes at cytotoxic concentrations only (Sina et al., 1983).
A cell transformation assay with Balb/3T3 cells tested without metabolic activation was positive (Sheu et al., 1988), while another test (both with and without metabolic activation) showed negative results (Microbiological Associates, 1980a/b).

2.6.2. Mutagenicity in vivo

Several micronucleus tests were performed. The majority of the MNT assays showed negative results. Mirkova, 1994 found reproducible positive results in the bone marrow assay with C57BL mice, but not with BALB/c mice. This suggests a strain-specific activity for 1,4-dioxane, although a further assay performed with C57BL mice showed negative results (Tinwell and Ashby, 1994). Quite high doses of 1,4-dioxane (more than 2 g/kg) were required to produce detectable genotoxic activity in the liver (Morita et al., 1998); the biological significance of this positive result is questionable.

A dominant lethal assay in male mouse was negative after a single i.p. injection. The rate of conception, mean number of implantations, percentage of living foetuses and mutagenicity index were unchanged (BASF, 1977). At high dosages positive results were obtained in a sex-linked recessive lethal test in *Drosophila melanogaster* (Yoon *et al.*, 1985).

Neither a single application of 1000 mg/kg bw, nor treatment with 1% 1,4-dioxane in drinking water for 2 weeks or with 2% 1,4-dioxane for 1 week did induce unscheduled DNA synthesis in primary rat hepatocytes. Negative results for unscheduled DNA synthesis were also found in rat nasal respiratory epithelial cells (from the nasoturbinate or the maxilloturbinate) after treatment of rats with 1% 1,4-dioxane in drinking water for 8 days, or after treatment with 1% in the drinking water for 8 days with an additional single gavage dose of up to 1000 mg/kg bw 1,4-dioxane (Goldsworthy et al., 1991). In an alkaline elution tests 1,4-dioxane induced DNA ss breaks in liver cells especially at dose levels higher than 2500 mg/kg (Kitchin and Brown, 1990). No DNA alkylation or increase in hepatic DNA repair in rats was observed after repeated dose administration of rather toxic doses of 1,4-dioxane (see above; Stott et al., 1981). Table 3 shows a summary of the in vivo genotoxicity tests.

Table 2: *In vivo* genotoxicity of 1,4-dioxane

Test system	Endpoint	Dose (route)*	Result	Reference
Mouse	Dominant lethal assay	2500 ml/kg bw	-	BASF, (1977)
Drosophila	Sex-linked recessive lethal	50 mg/ml (injection)	- (+ high dosages)	Yoon et al., (1985)
SD rat liver	DNA damage	1000 mg/kg (oral)	-	Stott et al., (1981)
SD rat liver	DNA damage	2550 mg/kg (oral)	+	Kitchin and Brown, (1990)
SD rat liver	DNA repair	1000 mg/kg bw (oral)	-	Stott et al., (1981)
F344 rat liver	DNA repair	1000 mg/kg (oral)	-	Goldsworthy et al., (1991)
F344 rat nasal cavity	DNA repair	1000 mg/kg bw (oral)	-	Goldsworthy et al., (1991)
B6C3F1 mouse bone marrow	Micronuclei	4000 mg/kg bw (i.p.)	-	McFee et al., (1994)
C57BL6 mouse bone marrow	Micronuclei	900 mg/kg bw (oral)	+	Mirkova (1994)
BALB/c mouse bone marrow	Micronuclei	5000 mg/kg bw (oral)	-	Mirkova (1994)
C57BL6	Micronuclei	3600 mg/kg bw (oral)	-	Tinwell et al., (1994)
CBA mouse bone marrow	Micronuclei	1800 mg/kg bw (oral)	-	Tinwell et al., (1994)
CD-1 mouse peripheral blood	Micronuclei	3200 mg/kg bw (i.p.)	-	Morita, and Hayashi (1998)
CD-1 mouse peripheral blood	Micronuclei	3000 mg/kg bw (oral	-	Morita und Hayashi (1998)
CD-1 mouse liver	Micronuclei	2000 mg/kg (oral)	+	Morita and Hayashi (1998)

*: The highest dose used in cases of negative results and the lowest effective dose in cases of positive results

2.6.3. Summary of mutagenicity

In vitro genotoxicity tests of 1,4 -dioxane were negative, with the exception of one positive sister chromatid exchange assay. The majority of *in vivo* assays were negative too. Positive results were obtained mostly at high concentrations. As micronuclei in mouse bone marrow cells may also be induced by non-genotoxic mechanisms (faulty genetic repair, faulty enucleation or differentiation of erythrocytes following enhancement of erythropoiesis, disturbance of the mitotic apparatus) 1,4 -dioxane is considered a non or either very weak genotoxic compound based on the total weight of evidence. This is further supported by the absence of DNA-adducts at hepatotoxic doses.

2.7. Carcinogenicity

2.7.1. Human data

A few epidemiological investigations are available with limited power due to relatively small numbers of study members. In a cross sectional study 74 workers (between 32 and 62

years old) employed in the 1,4-dioxane production were exposed to 1,4-dioxane concentrations of up to around 54 mg/m^3 for between 3 and 41 years. The group showed no evidence of liver or kidney damage, nor did they have a higher incidence of cancer deaths than the population. In 6 workers no increased rate of chromosome aberrations in lymphocytes compared to controls was noted (Thiess et al., 1976).

A mortality study conducted on 165 employees engaged for one month to ten years or more in 1,4-dioxane production and exposed (not continuously) to 1,4-dioxane concentrations below 90 mg/m^3 showed no significant difference in observed deaths from overall cancer compared to the expected numbers (Buffler et al., 1978).

Investigations on 80 men with potential exposure of 0.18 to 184 mg/m^3 of 1,4-dioxane showed no signs of 1,4-dioxane related health effects (NIOSH, 1977).

Animal studies
Inhalation

Wistar rats were exposed to 400 mg (111 ppm) 1,4-dioxane vapor/m^3 for 7 hours/day, five days a week for a total of 2 years (corresponding to 105mg/kg bw/d) (Torkelson et al., 1974). As stated by the author no 1,4-dioxane characteristic nasal and liver tumours, as observed after oral administration, were seen and the incidence of all observed tumours appeared to be unrelated to exposure. This study did not provide a NOAEL.

Oral - rats

The carcinogenicity studies are described in detail in table 2. The tumour incidences after oral 1,4-dioxane administration are shown in table 4.

Table 3 : Neoplastic lesions after oral 1,4-dioxane administration

Study	**Tumour incidences**	**Reference**
Sherman rats 60 m, 60 f 0, 0.01, 0.1, 1 % 716 d	Liver (0, 0.01, 0.1, 1 %) carcinoma: 0, 0, 0, 12/66 cholangioma: 0, 0, 0, 2/66 Nose (0, 0.01, 0.1, 1 %) squamous cell carcinoma: 0, 0, 0, 3/66	Kociba et al., (1974)
F344/DuCrj rats 50 m, 50 f 0, 0.02, 0.1, 0.5 % 104 w	Nose (0, 0.02, 0.1, 0.5 %) squamous cell carcinoma: m: 0/50, 0/50, 0/50, 3/50 f: 0/50, 0/50, 0/50, 7/50 other tumours: m: 0/50, 0/50, 0/50, 4/50 f: 0/50, 0/50, 0/50, 1/50 Liver (0, 0.02, 0.1, 0.5 %) adenoma: m: 0/50, 2/50, 4/49, 24/50 f: 1/50, 0/50, 5/50, 38/50 carcinoma: m: 0/50, 0/50, 0/50, 14/50 f: 0/50, 0/50, 0/50, 10/50	Yamazaki et al., (1994), Japan Bioassay research Center, 1998c
Osborne-Mendel rats 35 m, 35 f 0, 0.5, 1% 110 w	Nose (0, 0.5, 1%) squamous cell carcinoma: m: 0/33, 12/33, 16/34 f: 0/34, 10/35, 8/35 Liver (0, 0.5, 1%) adenoma: f: 0/31, 10/33, 11/32	NCI, (1978)

B6C3F1 mice 50 m, 50 f 0, 0.5, 1% 110 w	Liver (0, 0.5, 1%) adenoma and carcinoma: m: 8/49, 19/50, 28/47 f: 0/50, 21/48, 35/37 Nose (0, 0,5, 1%) adenocarcinoma: m: 0, 0, 1, f: 0, 1, 0	NCI, (1978)
Crj:BDF1 mice 50 m, 50 f 0.05, 0.2, 0.8% 104 w	Liver (0, 0.05, 0.2, 0.8%) carcinoma: m: 15/50, 20/50, 23/50, 36/50 f: 0/50, 6/50, 30/50, 45/50 Nose (0, 0.05, 0.2, 0.8%) esthesioneuroepithelioma: 0, 0, 0, 2 (1m, 1f)	Yamazaki et al., (1994), Japan Bioassay research Center, (1998c)

The non-neoplastic lesions seen after repeated dose administration of 1,4-dioxane in low dosages progressed to hepatocellular adenoma and carcinoma in rats and mice and to nasal squamous cell carcinoma in rats at higher dosages.
Liver tumours were observed at higher incidences in rats and mice at approximately ≥ 0.5 %. Neoplastic nose lesions were observed in rats at ≥ 0.5 %.

Special investigations
In a liver foci assay 1,4-dioxane showed a clear positive result (Lundberg et al., 1987), while a mouse skin papilloma test with a single dosage of 1,4-dioxane was negative (Bull et al., 1986). 1,4 - dioxane has tumour promoter, but not initiator properties.
No peroxisomal proliferation activity was observed after dosing with 1,4 dioxane (1% and 2 % in drinking water for 5 days in two studies)(Goldsworthy et al., 1991, TSCAT, 1989). The authors conclude that repair-inducing DNA adduct formation and peroxisomal proliferation in the liver do not appear to be involved in tumour formation by 1,4 – dioxane of liver tumours, the quantitative relationships between induced cell proliferation and tumourigenic potential being still established.

Conclusion on Carcinogenicity
Liver adenoma and carcinoma are seen in rats and mice after oral administration of 1,4-dioxane. In rats nasal adenomas and carcinomas were also seen. The available data indicate a non-genotoxic mechanism. It has been suggested that the nasal tumours observed in oral dosing studies studies were due to inspiration of water into the nasal cavity during exposure from sipper bottles (high doses applied directly to nasal tissue) and subsequent cytotoxicity. Dioxane was shown to act as a tumour promoter in rat liver and mouse skin carcinogenicity assays
The current classification as category 3 carcinogen (R40) is agreed by the EU-RAR. For both liver and nasal tumours, cytotoxic effects and organ damage are considered to be involved, which are subject to nonlinear kinetics, implicating a threshold. IARC (1999) concluded that there is inadequate evidence for the carcinogenicity of 1,4-dioxane in humans and that there is sufficient evidence for the carcinogenicity of 1,4-dioxane in experimental animals, and classified 1,4-dioxane as a Group 2B carcinogen (possibly carcinogenic to humans).

2.8. Reproductive toxicity

Fertility

Decreased mineralisation in the testis of Crj:BDF1 mice was observed in a carcinogenicity study at a dose of 250 mg/kg bw/d (Yamazaki et al., 1994; Japan Bioassay Research Center, 1998). In further oral 13-week studies and in the oral and inhalatory chronic toxicity/carcinogenicity studies no histopathological effects were observed in the reproductive organs of mice and rats.

Developmental toxicity

Groups of 17-20 pregnant Sprague-Dawley rats received by gavage 0, 0.25, 0.5 and 1.0 ml 1,4-dioxane/kg bw in water during days 6-15 of gestation. The animals were killed on day 21 of pregnancy (Giavini *et al.,* 1985). The females treated with 1 ml/kg bw showed a slightly smaller weight gain during treatment, which continued into the second stage of gestation. This could be due to reduced consumption of food, which especially evident in the first 2 days of treatment. However, a toxic effect of the solvent could not be excluded. Number of implantations and live fetuses did not differ compared to controls. The frequency of major malformation remained within normal limits for all groups, and no deviations were found regarding minor anomalies and variants when compared within the control group. At highest dose level a significant retardation was found in the area of the sternum. There was no indication for teratogenicity. The author stated, that the fetal retardation could be ascribed to maternal toxicity. The NOAEL in this study for maternal and embryotoxicity can be established at 0.5 ml/kg bw, equivalent to 517 mg/kg bw.

Recommendations

Torkelson et al.(1974) found in a 2 yr inhalation study in rats at an exposure level of 400 mg/m³ (111 ppm) no evidence of toxicity, including carcinogenicity. 1,4 – dioxane has been shown to be carcinogenic in several drinking water studies in rats, mice and guinea pigs. The target organs were mainly the liver and nasal cavities. The mechanism appears to be non-genotoxic, involving the saturation of one metabolic pathway and the increasing prominence of an alternative one which produces the reactive, cytotoxic metabolite
2-hydroxyethoxyacetaldehyde. Studies in human volunteers exposed to 50 ppm (180 mg/m3) 1,4-dioxane indicated almost total excretion of the inhaled dose as HEAA, with no indication of saturation of metabolism (Young et al, 1977) Human epidemiological studies did not show evidence of liver or kidney damage, nor clinical effects related to exposure of 1,4- dioxane, although the number of investigated people and the exposure was low (Thiess et al., 1976; Buffler et al. 1978). The overall death rate and the cancer death rate were not significantly increased compared to controls. The average exposures to 1,4 – dioxane were 54 mg/m³ and 90 mg/m³ respectively.

Irritation of the eye in volunteers was seen at concentration of 180 mg/m³ (50 ppm) in experimental settings (Young et al., 1977)..

On the basis of the Torkelson et al (1974) study reporting no effects in rats with lifetime exposure to 400 mg/m3 (111 ppm) and the need to avoid eye irritation (seen in human volunteers at 50 ppm; 180 mg/m3) a TWA of 20 ppm (73 mg/m³) is proposed. There is no evidence for a proposal of a STEL.

At the recommended TWA of 1,4-dioxane difficulties of air measurement are not to be foreseen.

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