

1,4-Dioxane

(CAS-NR.: 123-91-1)

Classification for Carcinogenicity:

1,4-Dioxane was shown to be carcinogenic in several drinking water studies in rats, mice and guinea pigs. The target organs were mainly liver and nasal cavities, occasionally also tumors in other tissues were recorded. The studies are comprehensively reported in widespread documents, such as the German MAK documentation (1996), the German BUA report (BUA, 1991) and the EU risk assessment report (1999). Tumor data of drinking water studies are compiled in Table 1.

The carcinogenicity of 1,4-dioxane in drinking water was recently reconfirmed in drinking water studies in rats exposed to 200; 1,000 and 5,000 ppm and in mice exposed to 500; 2,000 and 8,000 ppm (50 animals per sex and dose; Yamazaki et al., 1994). Male rats at 5,000 ppm showed nasal cavity metaplasia, proliferation and malignant tumors and increases in hepatocellular adenoma and carcinoma, peritoneal mesothelioma, fibroma of the subcutis and fibroma of the mammary glands. In female rats, at 5,000 ppm similar effects in the nasal cavities including malignant tumors were observed, furthermore increases in hepatocellular adenoma and carcinoma and adenoma of the mammary gland. At 1,000 ppm no nasal effects at all were observed in male and female rats and no hepatocellular carcinoma. Hyperplasia of liver and hepatocellular adenoma, however, were still increased in both sexes. At 200 ppm no effects were noted for the females; for males a slight increase in spongiosis hepatitis and two hepatocellular adenomas appeared to be of borderline significance in this group.

Mice responded in the top dose (8,000 ppm) with one adenocarcinoma in the nasal cavity in the female group and one esthesioneuroepithelioma in the male animals. Furthermore, a high number of hepatocellular carcinoma was obtained in both sexes, whereas the number of adenoma was in the control range. At 500 and 2,000 ppm an increase in hepatocellular adenoma and a dose-related increase of hepatocellular carcinoma was noted. Throughout all dose levels, there was an increasing trend to malignancy in liver with the dose. Thus, 500 ppm (0.05%; 66 mg/kg bw/day) were a LOAEL in this study. It should be noted, that this corresponds to a dose that was previously shown as exceeding the NOEL (10 mg/kg/day) in terms of organ toxicity, cell proliferation and metabolic saturation in rats (see below).

The overall pattern of tumorigenicity and organ toxicity is quite the same as detected in earlier drinking water studies. Toxic tissue damage and cell proliferation appear to precede the tumor formation. As was shown in an earlier report (NCI, 1978), mice appeared to be on a ppm basis somewhat more sensitive than rats. The only inhalation study that had been carried out so far employed 111 ppm to rats over two years (400 mg/m³; roughly equivalent to a dose of 72 mg/kg/day in case of a 100%

pulmonary resorption rate). An increased tumor rate was not identified in this study. (A numerical increase of reticulosos was not regarded as treatment-related by the authors; Torkelson et al., 1974). The validity of this study is somewhat limited since the MTD was not achieved and also cytotoxic effects on liver and kidney, which are a typical pattern of repeated 1,4-dioxane administration, were not observed. Furthermore, it is not certain that the authors thoroughly examined a sufficient range of sections within the nasal cavity.

Genotoxicity:

A large number of genotoxicity studies has been carried out with 1,4-dioxane (overview: MAK-Dokumentation and BUA report). According to the overall pattern the material is not genotoxic.

In a recent study a positive liver micronucleus assay was observed at 3,000 mg/kg (Moriata and Hayashi, 1998). This result appears to be compatible with earlier findings from Kitchin and Brown (1990), who have found DNA fragmentation at similar (cytotoxic) dose levels.

As a conclusion, it does not appear unlikely that under conditions of severe cytotoxicity 1,4-dioxane may exert some level of clastogenicity. However, as is shown below, these are dose levels exceeding the organisms capacity to adequately oxidize 1,4-dioxane. Under these extreme conditions 1,4-dioxane accumulates in the blood and all tissues and may produce cytotoxicity via a second metabolic pathway in those tissues with a propensity for enzyme induction.

Metabolism / Biotransformation:

Metabolism studies in rats showed that between dose levels of 10 and 100 mg/kg a saturation of the oxidative metabolism from 1,4-dioxane to β -Hydroxyethoxyacetic acid is achieved which is described by a shift from a first- to zero-order kinetics. This saturation phenomenon is equivalent to a steep and disproportional increase of the internal dose of 1,4-dioxane which is rather slowly metabolized and accumulates in the plasma (and tissues), whereas the formation and elimination of the main urinary metabolite, β -hydroxyethoxyacetic acid follows only a linear dose relation. Under these conditions a second metabolite, namely 1,4-dioxane-2-ol in equilibrium with β -hydroxyethoxyacetic aldehyde, is formed as suggested by Hecht and Young (1981); the latter being strongly protein-reactive and cytotoxic.

Time course appears to be important: Young et al. (1978a and b) demonstrated that after 17 repeated administrations of 1,000 mg/kg/day, the relative percentage of re-exhaled 1,4-dioxane decreased about 3-fold when compared to single administration, whereas the rate of exhaled CO₂ increased to the same extent. The authors therefore postulated an enzyme induction, possibly aniline hydroxylase, which may produce an additional metabolite. Enzyme induction may be conceived as a threshold-related phenomenon. A similar time course was also observed in cell proliferation studies showing a retarded onset of replicative DNA synthesis (RDS; see below).

In rodents, the nasal tissues contain significant amounts of cytochrome P450's and are therefore metabolically quite active. They may be capable of enzyme induction after repeated exposure and also prone to be target tissues for cytotoxic metabolites arising from P450-mediated oxidation. This is reflected by carcinogenic effects to nasal tissues of many chemicals after oral administration.

Cell proliferation studies:

Several studies have investigated cell proliferating effects of 1,4-dioxane in target organs. Since 1,4-dioxane (and moreover 1,4-dioxane-2-ol) has a protein-denaturing effect, one would expect cytostatic as well as proliferating effects, the latter being due to replacement of necrotic cells.

Heil and Refferscheid (1992) found inhibition of replicative DNA synthesis (RDS) in HeLa cells at 400 mM.

Miyagawa et al. (1997) used the BrdU labelling technique for the observation of replicative DNA synthesis (RDS) of 22 non-genotoxic carcinogens in rat liver. Obviously, there was only one single treatment with 2,000 mg 1,4-dioxane/kg followed by a 24 hours interval between treatment and labelling. The authors found a 3-fold increase in RDS incidence of 1,4-dioxane.

Goldsworthy et al. (1991) investigated the hepatic and nasal epithelial labelling index (LI) 24 and 48 hours following a single dose of 1,000 mg/kg or a 2-week administration via the drinking water in rats (1%; ~ 1,000 mg/kg/d). The percentage of cells in S-phase was determined by administration of ³H TdR (single injection or osmotic pump) and subsequent quantitative histoaudiography. In liver there was a 2-fold increase in LI after 2 weeks of exposure. No such effect was seen after a single administration.

Stott et al. (1981) administered 1,4-dioxane at approximately 1,000 mg/kg/day for 11 weeks in the drinking water a dose which also caused some increase in liver weights. Hepatocytes were isolated by collagenase perfusion and labeled in vitro with 6-³H-TdR. Labeling was increased at 1,000 mg/kg/day, but not at 10 mg/kg/day.

With the same in vitro labelling technique it was shown that a 1 - 3 days administration of 1,4-dioxane (2% in the drinking water) caused no increases in S-phases, whereas after 8 days and longer exposure a pronounced increase in S-phases was visible. The effect was solely dependent on the concentration of 1,4-dioxane in the drinking water (0.01 - 2%) and not on the exposure time. 0.01% (a dose clearly below metabolic saturation; Braun and Young, 1977; Young et al. 1978) were without effect; whereas 0.1% (a dose causing already a reduced clearance; Kociba et al., 1975; Young et al., loc. cit.) caused an about 3-fold increase in labeled hepatocytes (BASF, 1987).

Interspecies relations:

Inhalation studies in rats and man showed that at 50 ppm over 6 hours inhalation the metabolism still follows a kinetic of first order. Man, however, showed a 10- to 12-fold lower pulmonary resorption rate and systemic uptake than rats (Young et al., 1977).

This species difference may be due to a slower metabolic rate in humans, which in the case of 1,4-dioxane would indicate a lower sensitivity of humans in relation to rats and mice.

Conclusion:

1,4-Dioxane is considered as a typical cytotoxic, but not genotoxic carcinogen. Further-more, the cytotoxicity requires exposure levels leading to accumulation of 1,4-dioxane and enzyme induction. Without these prerequisites, no carcinogenic effects are expected.

The following criteria militate against a category 2 classification of 1,4-dioxane:

- 1,4-Dioxane is not genotoxic, unless under well-defined circumstances of excessive and cytotoxic dose levels.
- Target organ toxicity is achieved only after metabolic saturation and accumulation of the parent molecule, a phenomenon that is accessible to biomonitoring.
- Enzyme induction after metabolic overloading appears to play a role and is conceived as a threshold-related phenomenon
- Tumors arise from enforced cell proliferation rates in target organs.
- The cytotoxic mechanism has been well elucidated.

The German MAK commission has classified 1,4-dioxane as a class 4 carcinogen due to its cytotoxic mechanism (Neumann et al., 1997; 1998a and b).

1,4-Dioxane is therefore classified as a Cat. 3 carcinogen, based on the EU classification system.

An exposure standard of 20 ppm (existing German MAK value) is considered as sufficient to protect exposed persons also against irritative effects of the material.

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Table 1:**Drinking water studies with 1,4-dioxane: Tumor responses**

NCI, 1978

	Doses / concentrations		
	0%	0.5%	1%
Male mice, 90 weeks			
Hepatocellular carcinomas	2/49	18/50	24/47
Nasal adenocarcinomas	0/49	0/50	1/50
Female mice			
Hepatocellular carcinomas	0/50	12/48	29/37
Nasal adenocarcinoma	0/50	1/48	0/37

Yamazaki et al., 1994

	Doses / concentrations			
	0%	0.05%	0.2%	0.8%
Male mice, 104 weeks				
Hepatocellular carcinomas	15/50	20/50	23/50	36/50
Hepatocellular adenomas	7/50	16/50	22/50	8/50
Nasal esthesioneuroepithelioma	0/50	0/50	0/50	1/50
Female mice				
Hepatocellular carcinomas	0/50	6/50	30/50	45/50
Hepatocellular adenomas	4/50	30/50	20/50	20/50
Nasal adenocarcinoma	0/50	0/50	0/50	1/50

Hoch-Ligeti et al., 1970

	Doses / concentrations				
	0%	0.75%	1%	1.4%	1.8%
Male rats, 13 months					
Nasal cavity tumors	0/30	1/30	1/30	2/30	2/30

Kociba et al., 1974

	Doses / concentrations			
	0%	0.01%	0.1%*	1%*
60 male, 60 female rats, 102 weeks				
Liver carcinomas	1/106	0/110	1/106	10/66
Nasal carcinomas	0/106	0/110	0/106	3/66

* liver and kidney toxicity

Table 1 (Cont.):**Drinking water studies with 1,4-dioxane: Tumor responses**

NCI, 1978

	Doses / concentrations		
	0%	0.5%	1%
Male rats, 110 weeks			
Nasal squamous cell carcinomas	0/33	12/33	16/34
Female rats			
Nasal squamous cell carcinomas	0/34	10/35	8/35
Hepatocellular carcinomas	0/31	10/33	11/32

Yamazaki et al., 1994

	Doses / concentrations			
	0%	0.02%	0.1%	0.5%
Male rats				
Nasal tumors				
- squamous cell carcinoma	0/50	0/50	0/50	3/50
- sarcoma	0/50	0/50	0/50	2/50
- esthesioneuroepithelioma	0/50	0/50	0/50	1/50
- rhabdomyosarcoma	0/50	0/50	0/50	1/50
hepatocellular carcinomas	0/50	0/50	0/50	14/50
hepatocellular adenomas	0/50	2/50	4/50	24/50
peritoneal mesothelioma	2/50	2/50	5/50	28/50
Female rats				
Nasal tumors				
- squamous cell carcinoma	0/50	0/50	0/50	7/50
- esthesioneuroepithelioma	0/50	0/50	0/50	1/50
Hepatocellular carcinomas	0/50	0/50	0/50	10/50
Hepatocellular adenomas	1/50	0/50	5/50	38/50

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