Health Council of the Netherlands

1,4-Dioxane

Health-based recommended occupational exposure limit

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *1,4-Dioxane*
Uw kenmerk : DGV/MBO/U-932342
Ons kenmerk : U 6562/AvdB/fs/459-G65
Bijlagen : 1
Datum : 24 juni 2011

Geachte staatssecretaris,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan 1,4-Dioxaan.

Dit advies maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige advieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Het genoemde advies is opgesteld door de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. L.J. Gunning-Schepers,
voorzitter

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1,4-Dioxane

Health-based recommended occupational exposure limit

Dutch Expert Committee on Occupational Safety
A Committee of the Health Council of the Netherlands

to:

the State Secretary of Social Affairs and Employment

No. 2011/09, The Hague, June 24, 2011

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research...” (Section 22, Health Act).

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Preferred citation:
Health Council of the Netherlands. 1,4-Dioxane. Health-based recommended occupational exposure limit. The Hague: Health Council of the Netherlands, 2011; publication no. 2011/09.

ISBN: 978-90-5549-842-0

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Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en Beroepsmatige Blootstelling aan stoffen (GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in lucht waaraan mensen beroepsmatig blootgesteld kunnen worden. Deze aanbevelingen vormen de basis voor wettelijke grenswaarden, vast te stellen door de minister, waarmee de gezondheid van werknemers beschermd kan worden.

In het voorliggende advies bespreekt de commissie de gevolgen van blootstelling aan 1,4-dioxaan en beveelt zij een gezondheidskundige advieswaarde voor die stof aan. Haar conclusies zijn gebaseerd op wetenschappelijke publicaties die vóór maart 2011 zijn verschenen.

Fysische en chemische eigenschappen

1,4-dioxaan is een snel ontvlambare stof met een smeltpunt van 12°C en een kookpunt van 101°C. De stof is mengbaar met water en de meeste organische oplosmiddelen.

Monitoring

Voor 1,4-dioxane ontbreken actuele gegevens over het monitoren van de blootstelling.

Huidige grenswaarden

In 1987 heeft de voormalige Werkgroep van Deskundigen (WGD) een gezondheidkundige advieswaarde voorgesteld van 40 mg/m^3 (11 ppm) voor 8 uur blootstelling. De Europese SCOEL (Scientific Committee on Occupational Exposure Limits) heeft in 2004 een 8-uur grenswaarde geadviseerd van 73 mg/m^3 (20 ppm).

Kinetiek

Bij werknemers is duidelijk aangetoond dat 1,4-dioxaan kan worden opgenomen in het lichaam na inhalatoire blootstelling. Dit is bevestigd in een experimentele studie waarin vrijwilligers zijn blootgesteld aan dioxaan. 1,4-Dioxaan wordt opgenomen en gemetaboliseerd zowel in mensen als in ratten. De meest voorkomende metaboliet in mensen is β-hydroxyethoxy acetic acid (HEAA). Deze metaboliet wordt voornamelijk via de urine weer uitgescheiden. Ratten zetten 1,4-dioxaan slechts beperkt om tot HEAA. Voornamelijk blootstelling aan lage concentraties dioxaan resulteert in de vorming van HEAA. Bij hogere blootstelling speelt een andere metabole route een belangrijkere rol. Dit resulteert in de vorming van hydroxyethoxyazijnzuur (HEA).

Effecten

Epidemiologische studies geven geen aanwijzingen voor lever of nierschade als gevolg van blootstelling aan dioxaan. Echter de zeggingskracht van deze epidemiologische studies is beperkt omdat het aantal onderzochte mensen klein is. Daarnaast was de blootstelling in deze studies laag. In een experimentele studie waar vrijwilligers werden blootgesteld aan 180 mg/m^3 dioxaan, werd irritatie van de ogen gerapporteerd.

In dierexperimentele onderzoeken is beperkte acute toxiciteit na orale, dermale en inhalatoire blootstelling waargenomen. De stof irriteert de ogen en de adem-

halingsorganen en is slechts matig irriterend voor de huid. Er zijn geen aanwijzingen gevonden voor sensibiliserende eigenschappen van 1,4-dioxaan.

Er zijn twee carcinogeniteitsstudies beschikbaar die de gevolgen van inhalatoire blootstelling aan dioxaan in kaart brengen. In de eerste studie zijn ratten chronisch blootgesteld aan 400 mg/m^3 (111 ppm). In deze studie zijn geen aanwijzigen voor systemische toxiciteit of kankerverwekkende effecten gevonden. In een recentere studie zijn mannelijke ratten gedurende twee jaar blootgesteld aan verschillende concentraties dioxaan (180, 900 and 4500 mg/m^3 (50, 250 and 1250 ppm)). In deze studie is een verhoging van het aantal tumoren in de neus '*cell carcinomen*' en in de lever (adenomen) waargenomen na blootstelling aan 4500 mg/m^3. Bij een blootstelling aan 180 mg/m^3 zijn niet-neoplastische effecten in de neus gevonden: 'nuclear enlargement' van het respiratoir en olfactorisch epitheel, atrofie and respiratoire metaplasie van het olfactorisch epitheel.

Daarnaast veroorzaakt dioxaan ook kanker in de neus en de lever van ratten na blootstelling aan dioxaan via het drinkwater.

Dioxaan is overwegend negatief in genotoxiciteitstesten. In enkele studies zijn positieve resultaten waargenomen echter alleen bij niet relevante, hoge, blootstellingsniveaus.

De Subcommissie Classificatie Kankerverwekkende stoffen heeft geconcludeerd dat dioxaan beschouwd moet worden als kankerverwekkend voor de mens, en geadviseerd dioxaan te classificeren in categorie 1B. Bovendien heeft de subcommissie geoordeeld dat de kankerverwekkende effecten ontstaan via een niet genotoxisch werkingsmechanisme.

De commissie beschouwt de niet neoplastische effecten in de neus als gevolg van levenslange inhalatoire blootstelling aan 180 mg/m^3 als het kritische effect. De commissie is van mening dat dit het laagst waargenomen nadelig effect niveau (LOAEL) is.

Bij het vaststellen van een gezondheidskundige advieswaarde houdt de commissie rekening met verschillende onzekerheden. In het algemeen hanteert de commissie een factor van 3 om te extrapoleren van een LOAEL naar een 'geen effect niveau' (NAEL). Omdat er sprake is van een lokaal effect in de neus, meent de commissie dat het niet nodig is te compenseren voor de verschillen tussen proefdier en mens (zogenaamde interspecies variatie). Tot slot hanteert de commissie een onzekerheidsfactor van 3 om te compenseren voor interindividuele verschillen (tussen mensen).

Uitgaande van een LOAEL van 180 mg/m^3 en gebruikmakend van een onzekerheidsfactor van 9 (3*3), om te compenseren voor de extrapolatie van LOAEL naar NAEL en voor de compensatie van inter-individuele verschillen, beveelt de commissie een gezondheidskundige advieswaarde aan van 20 mg/m^3 (6 ppm) gedurende 8 uur per dag. Met een advieswaarde van 20 mg/m^3 is de commissie van mening dat de effecten van blootstelling aan dioxaan op de werkplek voorkomen kunnen worden.

Gezondheidskundige advieswaarde

De commissie beveelt een gezondheidskundige advieswaarde voor beroepsmatige blootstelling voor dioxaan aan van 20 mg/m^3 (6 ppm), gemiddeld over een acht urige werkdag (t.g.g. 8 uur).

Summary

Scope

At request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, recommends health based occupational exposure limits for airborne substances to which people are exposed in the air at the workplace. These recommendations serve as a basis in setting legally binding limit values by the Minister.

In the present report, the Committee discusses the effects of exposure to 1,4-dioxaan and recommends an health based occupational exposure limit for this substance. The Committee's conclusions are based on scientific publications from prior to March 2011.

Physical and 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 with water and in the most organic solvents.

Monitoring

Recent environmental monitoring data for 1,4-dioxane are lacking.

Current limit values

In 1987, the former DECOS has recommended an health based occupational exposure limit of 40 mg/m^3 (11 ppm) for 8 hours exposure. SCOEL recommended an 8-hour exposure limit value of 73 mg/m^3 (20 ppm) in 2004.

Kinetics

Absorption of 1,4-dioxane following inhalation exposure has been qualitatively demonstrated in workers and human volunteers. 1,4-Dioxane is extensively absorbed and metabolized in both humans and rats. The metabolite most often measured and reported in man is β-hydroxyethoxy acetic acid (HEAA), which is predominantly excreted in the urine. The capacity of rats to metabolize 1,4-dioxane to HEAA is rather limited and predominantly occurring at low exposure levels. At higher exposure levels, an alternative metabolic pathway becomes more significant in rats, resulting in the production of hydroxyethoxyacetic acid (HEA).

Effects

Human epidemiological studies did not show evidence of liver or kidney damage, nor clinical effects related to exposure of 1,4-dioxane. However, the number of investigated people and the exposure was low. Irritation of the eye in volunteers was seen after exposure to a concentration of 180 mg/m^3 (50 ppm) in experimental settings.

In studies with experimental animals, 1,4-dioxane was not very toxic by the oral, dermal or inhalation route. The substance was irritating to the eyes and the respiratory tract, and slightly irritating to skin. In a guinea pig maximisation test, no skin sensitising properties were apparent.

Two carcinogenicity studies were available. The first study showed rats which were chronically exposed to 400 mg/m^3 (111 ppm) of 1,4-dioxane vapour by inhalation. There were no signs of systemic toxicity or carcinogenicity. In a more recent study, male rats were exposed to 1,4-dioxane concentrations of 180, 900 and 4,500 mg/m^3 (50, 250 and 1250 ppm) for 2 years, 6 h/day, 5 days/wk. In this study, an increased incidence of cell carcinoma in the nasal cavity and hepatocellular adenoma in the liver was observed after exposure to the highest exposure level of 4,500 mg/m^3. Non-neoplastic and pre-neoplastic changes in the

nasal cavity (nuclear enlargement of respiratory and olfactory epithelial, atrophy and respiratory metaplasia of the olfactory epithelium) were already observed at the lowest exposure level, 180 mg/m^3, and above. 1,4-Dioxane has been shown to be carcinogenic (liver and nasal cavities) in some drinking water studies in rats, mice and guinea pigs as well.

1,4-Dioxane showed predominantly negative results in several *in vitro* mutagenicity assays. Nevertheless, some studies demonstrated genotoxic properties for 1,4-dioxane, although only at elevated dose levels.

DECOS' Subcommittee on the classification of carcinogenic substances concluded that 1,4-dioxane is a presumed human carcinogen, and recommends classifying the compound in category 1B (*Should be regarded as carcinogenic to humans)*. In addition, the Subcommittee is of the opinion that a non-genotoxic mode of action is involved in the development of the carcinogenicity in liver and nasal epithelium.

DECOS considers the nasal lesions in rats after lifetime exposure to 180 mg/m^3 (50 ppm) 1,4-dioxane as the critical effect. The committee is of the opinion that this level is the lowest observed adverse exposure limit (LOAEL). As a standard procedure, DECOS applies an factor (3) to compensate for extrapolation of the LOAEL to a NAEL. An extrapolation factor to compensate for the differences between rats and humans is unnecessary, as the critical effect is a local (non systemic) effect. Finally, a factor of 3 is used to compensate for the inter-individual differences.

Hazard Assessment and recommended occupational exposure limit.

DECOS recommends an HBROEL TWA 8 hours for 1,4-dioxane of 20 mg/m^3 (6 ppm).

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Part I

Health based recommended occupational exposure limit of 1,4-dioxane: Evaluation and recommendation

Chapter 1

Scope

1.1 Background

At the request of the Minister of Social Affairs and Employment (Annex A), the Dutch Expert Committee on Occupational Safety (DECOS), a Committee of the Health Council of the Netherlands, performs scientific evaluations of the toxicity of substances to which man can be exposed at the workplace. The purpose of these evaluations is to recommend a health-based recommended occupational exposure limit (HBROEL) for the concentration of the substance in air, provided the database allows the derivation of such value.

In 1987, DECOS published an advice on the toxicity of 1,4-dioxane. An health based occupational exposure limit was recommended[1]. Several years later, the European Scientific Committee on Occupational Exposure Limits (SCOEL) published an evaluation on the toxicity of 1,4-dioxane as well.[2]

In the present advice, the Committee reconsiders the former Health Based Occupational Exposure Limits for 1,4-dioxane based on the previous report of the Committee, the advice of the SCOEL published in 2004 and additional published studies from 2004 till March 2011.

1.2 Committee and method of work

The present document contains the re-assessment of the toxicity of 1,4-dioxane by DECOS. The members of the DECOS are listed in Annex B.

In 2010, the DECOS released a draft version of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. DECOS has taken these comments into account in finalising its report.

1.3 Data

In part I of the present document, the Committee evaluates the toxicity of dioxane and recommends, if possible, a health based occupational exposure limit for dioxane. This evaluation is based on the data described in Part II of the present report.

Part II of the present document contains an update of a Health Council report, issued in 1987.[1] The 1987 report was, however, in the Dutch language. Therefore, the present report, is based on relevant data from the more recent SCOEL report on 1,4-dioxane[2] published in 2004. Additional data were retrieved from the literature published since January 2002 using the online databases Toxline, Medline and Chemical Abstracts (CAPlus), using “1,4-dioxane” and CAS no 123-91-1 as keywords. The last search was performed in March 2011.

Chapter 2

Hazard Assessment

This chapter contains a short summary of the relevant data on the effects of exposure to 1,4-dioxane, based on the data summarized in Part II of the present report. In addition, the Committee evaluates the toxicity of 1,4-dioxane and recommends an health based occupational exposure limit for 1,4-dioxane.

2.1 Hazard identification

2.1.1 Metabolism in man and experimental animals

A study with human volunteers exposed for 6 hours to 180 mg/m^3 (50 ppm) 1,4-dioxane, indicated an almost total excretion of the inhaled dose as β-hydroxyethoxy acetic acid (HEAA). There was no indication of saturation of the metabolism. The capacity of rats to metabolize 1,4-dioxane to HEAA is rather limited and predominantly occurring at low exposure levels. At higher exposure levels, an alternative metabolic pathway becomes more significant in rats, resulting in the production of hydroxyethoxyacetic acid (HEA).[2]

2.1.2 Observations in humans

Eye irritation was a frequent complaint throughout exposure in this study. In another study with volunteers, no irritation of eyes, nose or throat, or respiratory effects resulted from exposure to 72 mg/m^3 (20 ppm) 1,4-dioxane during 2 hours.

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.

A cross-sectional study with 1,4-dioxane production workers did not show evidence of liver or kidney damage, nor clinical effects after exposure to concentrations up to 54 mg/m^3. However, the number of investigated people and the exposure levels were low. In this study, the overall cancer mortality was not increased compared to controls. A mortality study in employees exposed to concentrations up to 90 mg/m^3 dioxane did not show an increased mortality (from overall cancer) as well.

2.1.3 *Animal data*

Two carcinogenicity studies have been conducted, in which rats were exposed by inhalation to 1,4-dioxane vapour. Torkelson *et al.*[3] exposed rats to a single dose of 400 mg/m^3 (111 ppm) for 2 years, 7 h/day, 5 days/wk. There were no signs of systemic toxicity or carcinogenicity. In a more recent study by Kasai *et al.*[4], male rats were exposed to 1,4-dioxane concentrations of 180, 900 and 4,500 mg/m^3 (50, 250 and 1,250 ppm) for 2 years, 6 h/day, 5 days/wk (see Table 1). In this study, an increased incidence of cell carcinoma in the nasal cavity and hepatocellular adenoma in the liver was observed after exposure to the highest level of 4,500 mg/m^3. In addition, an increase in the number of peritoneal mesotheliomas (from the mesothelium of the scrotal sac) was observed as well. However, the Subcommittee on classifying carcinogenic substances is of the opinion that these tumours are not relevant for the human risk assessment as it has been recognized that the peritoneal mesothelioma is a commonly observed, spontaneous neoplasm in the male F344 rats arising from the tunica vaginalis. Non-neoplastic and pre-neoplastic changes in the nasal cavity (nuclear enlargement of respiratory and olfactory epithelial, atrophy and respiratory metaplasia of the olfactory epithelium) were already observed at the lowest exposure level, 180 mg/m^3, and above.

1,4-Dioxane has been shown to be carcinogenic in several drinking water studies in rats, mice and guinea pigs[5,6] as well. The target organs were the liver and nasal cavities.

1,4-Dioxane showed to be negative in several *in vitro* mutagenicity assays. In recent genotoxicity studies[7,8], it was demonstrated that 1,4-dioxane has genotoxic properties, although only at elevated dose levels.

Table 1 Incidences of selected histopathological lesions in male F344 rats by inhalation to 1,4-dioxane.[4]

	Control	50 ppm 180 mg/m³	250 ppm 900 mg/m³	1,250 ppm 4,500 mg/m³
Number of animals examined	50	50	50	50
Neoplastic lesions				
Nasal cavity, squamous cell carcinoma	0	0	1	6[a]
Liver, hepatocellular adenoma	1	2	3	21[b]
hepatocellular carcinoma	0	0	1	2
Kidney, renal cell carcinoma	0	0	0	4
Peritoneum, mesothelioma	2	4	14[b]	41[b]
Mammary gland, fibroadenoma	1	2	3	5
adenoma	0	0	0	1
Zymbal gland, adenoma	0	0	0	4
Subcutis, fibroma	1	4	9[b]	5
Pre- and nonneoplastic lesions				
Nasal cavity: Respiratory epithelium				
Nuclear enlargement	0	50[b]	48[b]	38[b]
Squamous cell metaplasia	0	0	7[a]	44[b]
Squamous cell hyperplasia	0	0	1	10[b]
Inflammation	13	9	7	39[b]
Nasal cavity: Olfactory epithelium				
Nuclear enlargement	0	48[b]	48[b]	45[b]
Atrophy	0	40[b]	47[b]	48[b]
Respiratory metaplasia	11	34[b]	49[b]	48[b]
Inflammation	0	2	32[b]	34[b]
Proliferation of nasal gland	0	1	0	6[a]
Liver:				
Nuclear enlargement	0	0	1	30[b]
Acidophilic cell foci	5	10	12	25[b]
Basophilic cell foci	17	20	15	44[b]
Spongiosis hepatis	7	6	13	19[b]
Necrosis: centrilobular	1	3	6	12[b]
Kidney:				
Nuclear enlargement: proximal tubule	0	1	20[b]	47[b]
Hydropic change: proximal tubule	0	0	5	6[a]

[a] significantly different from control at p≤0,05.
[b] significantly different from control at p≤0,01.

From the available data on carcinogenicity, DECOS' Subcommittee on the classification of carcinogenic substances concludes that 1,4-dioxane is a presumed human carcinogen, and recommends classifying the compound in category 1B (*Should be regarded as carcinogenic to humans)*. In addition, the Subcommittee is of the opinion that a non-genotoxic mode of action is involved

in the development of the carcinogenicity in liver and nasal epithelium (see Annex D for further details on the Subcommittees opinion).

2.2 Risk assessment

DECOS considers the nasal lesions (increased incidence of nuclear enlargement in the respiratory and olfactory epithelia) found in rats after lifetime exposure to 1,4-dioxane as the critical effect. In addition, the Committee is of the opinion that these nasal lesions in the epithelium are precursor events in the development of nasal tumors. The nasal lesions are found at lower levels of exposure than the levels causing nasal tumors. Therefore, the Committee assumes that preventing increased nuclear enlargement in the respiratory and olfactory epithelia, will prevent the development of nasal tumours as well.

In deriving a health based recommended occupational exposure limit (HBROEL), the Committee preformed a bench mark dose analyses on the critical effects. However, a BMD analysis of the relevant data revealed the BMD approach not to be applicable. Therefore, the Committee takes the LOAEL of 180 mg/m^3 (50 ppm) found in the chronic inhalation study of Kasai *et al.*[4] as a starting point. For the establishment of the HBROEL, uncertainty factors are applied to compensate for the extrapolation from a LOAEL to a NAEL*, for differences between rats and humans, for the duration of exposure, and for inter-individual differences. For the extrapolation of the LOAEL to a NAEL, a factor of 3 is applied. An uncertainty factor to compensate for the differences between rats and humans is unnecessary, as the critical effect is a local (non systemic) effect and nasal flux in rats is higher than in humans leading to higher exposure of nasal respiratory and olfactory epithelia. A factor compensating for the difference in duration of exposure (6 hours per day in the Kasai *et al.* study versus an 8-hour working day), is not deemed necessary as the rat is more sensitive to the observed effects than man. Finally, a uncertainty factor of 3 is used to compensate for the inter-individual differences.

Considering all these aspects, starting from a LOAEL of 180 mg/m^3 (50 ppm) and using an extrapolation factor of 9, the Committee recommends an HBROEL TWA 8 hours for 1,4-dioxane of 20 mg/m^3 (6 ppm). The Committee is of the opinion that this exposure limit will protect against the development of liver tumours as well.

* NAEL: No Adverse Effect Level.

2.3 Skin notation

The absorption rate of 1,4-dioxane through human skin *in vitro* is approximately 0.36 µg/cm/h.[9] When both hands and underarms (surface area 2000 cm^2) are exposed during eight hours the quantity absorbed would amount to (0.36x2000x8) 6 mg. Via the inhalatory route an amount of 200 mg is absorbed during 8 h exposure (10 m^3) to the recommended exposure limit of 20 mg/m^3, assuming 100% absorption. Therefore, skin absorption of 1,4-dioxane does not add considerably to the body burden. Therefore, a skin notation is not considered necessary by the Committee.

2.4 Groups at extra risk

No groups at extra risk could be identified.

2.5 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health based occupational exposure limit of 20 mg/m^3 (6 ppm) as an 8-hour Time Weighted Average concentration.

2.6 HBROEL DECOS versus SCOEL

In 2004, the European Scientific Committee on Occupational Exposure Limits (SCOEL) recommended an occupational exposure limit of 73 mg/m^3 (20 ppm). However, the critical study in the evaluation of DECOS, ie. Kasai *et al.*[4] was not available in 2004.

In 1987, DECOS recommended an HBROEL of 40 mg/m^3 based on the study of Torkelson *et al.*[3] Again, the critical study in the present evaluation of DECOS, ie. Kasai *et al.*[4] was not available in 1987.

References

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- G.J. Mulder, *chairman*
  Emeritus Professor of Toxicology, Leiden University, Leiden
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- J.J.A.M. Brokamp, *advisor*
  Social and Economic Council, The Hague
- D.J.J. Heederik
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The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of this advisory report was released in 2011 for public review. The following organisations and persons have commented on the draft:

- National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA.

Annex **D**

Advice of the Subcommittee on Classifying Carcinogenic Substances

D.1 Scope

On request of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council, the Subcommittee on the Classification of Carcinogenic Substances evaluates in this advice the carcinogenic properties of dioxane.

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification with reference to an EU-directive (see Annex A). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question.

The members of the Subcommittee on Classifying Carcinogenic Substances are listed at the end of this Annex. This evaluation is based on the data summarized in part II of the present report of DECOS.

D.2 Carcinogenicity of dioxane

In 1999, IARC* 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. Therefore, IARC classified 1,4-dioxane as a Group 2B carcinogen (possibly carcinogenic to humans).

Only a few epidemiological studies are available concerning the carcinogenic properties of 1,4-dioxane; they show no indications for carcinogenicity. However, as these studies have limited power, the Subcommittee is of the opinion that the human data are insufficient for conclusions.

Two carcinogenicity studies have been conducted, in which rats were exposed by inhalation to 1,4-dioxane vapour. Torkelson *et al.* (1974) exposed Dow Wistar rats (male and female) to a single dose of 400 mg/m^3 (111 ppm) for 2 years, 7 h/day, 5 days/wk. There were no signs of systemic toxicity or carcinogenicity. In a more recent study (Kasai *et al*. 2009), male F344/DuCrj rats were exposed to 1,4-dioxane concentrations of 180, 900 and 4,500 mg/m^3 (50, 250 and 1,250 ppm) for 2 years, 6 h/day, 5 days/wk. In this study, an increased incidence of squamous cell carcinoma in the nasal cavity and hepatocellular adenoma in the liver was observed after exposure to 4,500 mg/m^3. Moreover, the incidence of peritoneal mesothelioma was statistically significantly increased (dose dependently) after exposure to 900 and 4,500 mg/m^3 as well. However, the prevalence of these mesotheliomas in the control animals is unusual high (2/50) for other rat species.

Non-neoplastic and pre-neoplastic changes in the nasal cavity (nuclear enlargement of the olfactory and respiratory epithelium, and atrophy and metaplasia of the olfactory epithelium) were observed at the lowest exposure level, 180 mg/m^3, and above.

1,4-Dioxane has been shown to be carcinogenic in several drinking water studies in rats, mice and guinea pigs (Kano *et al*. 2008, 2009). The target organs were the liver, and nasal cavities, while also peritoneal mesotheliomas were induced. The relevance of the effects on the nasal cavity for humans after exposure via drinking water was questioned by Stickney *et al.* (2003). Although the nasal lesions and nasal tumours were consistently seen after exposure to

* IARC: International Agency for Research on Cancer.

1,4-dioxane through the drinking water, such lesions could result from water entering the nasal cavity when the animals drink from sipper bottles (Sweeney *et al.* (2008)). However, because nasal tumours were also observed after inhalatory exposure in rats, these are considered relevant for humans by the Subcommittee.

The Subcommittee is of the opinion that the studies of Kasai *et al.* 2009 en Kano *et al*. 2008, 2009 show consistent carcinogenic effects (hepatocellular adenomas, squamous cell carcinomas in the nasal cavity and peritoneal mesotheliomas) after exposure to dioxane by inhalation and via drinking water respectively. A clarification for the negative findings in the study of Torkelson *et al.* 1974 was not found. However, the Subcommittee noticed that Torkelson *et al* did not examine the nasal cavity. Therefore, because of the sound positive studies of Kasai *et al.* 2009 and Kano *et al*. 2008, 2009, the Subcommittee recommends to classify 1,4-dioxane in category 1B (‘*the compound should be regarded as carcinogenic to humans’*).

1,4-Dioxane is negative in most *in vitro* mutagenicity assays (eg. salmonella mutagenicity test, thymidine kinase tests in mouse lymphoma cells etc) and clastogenicity tests (chromosomal aberrations, micronucleus formation etc.). In a few micronuclei assays, however, 1,4-dioxane showed a positive result in liver and bone marrow. Recently, Roy *et al.* 2005; reported a positive result on both liver and bone marrow of Young CD-1 mice using CREST staining. In addition, it was found that 1,4-dioxane elevated micronuclei originating from chromosomal breakage (Fukushima *et al.* 2009). However, these results were obtained after exposure to very high concentrations of dioxane (exceeding the maximal tolerable dose) and therefore not considered relevant by the Subcommittee.

Overall, the Subcommittee judges that 1,4-dioxane is not genotoxic. The Subcommittee is of the opinion that the nasal tumours found after exposure to 1,4-dioxane are possibly associated with a non-genotoxic mechanism of action ie. the injury of cells in the respiratory and olfactory epithelium. In addition, the Subcommittee assumes that the hepatocellular adenomas are associated with a non genotoxic mechanism as well, ie hepatocellular injury (necrosis of hepatocytes). These peritoneal mesotheliomas in F344 rats in the study of Kasai *et al* are not considered relevant for the human evaluation.

D.3 Recommendation for classification

Based on the available data, the Subcommittee recommends classifying 1,4-Dioxane in category 1B (*the compound should be regarded as carcinogenic to humans)* and considers the substances as a non genotoxic carcinogen.

D.4 References

Fukushima S, Wei M, Omori M, Morimura K, Kinoshita A, Masumura K, *et al*. Carcinogenicity and in vivo mutagenicity of 1,4-dioxane in gpt delta rats. Experimental and Toxicologic Pathology 2009; 61: 282.

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Kano H, Umeda Y, Saito M, Senoh H, Ohbayashi H, Aiso S, *et al.* Thirteen-week oral toxicity of 1,4-dioxane in rats and mice. J Toxicol Sci 2008; 33(2): 141-153.

Kasai T, Kano H, Umeda Y, Sasaki T, Ikawa N, Nishizawa T, *et al.* Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. Inhal Toxicol 2009; 21(11): 889-897.

Roy SK, Thilagar AK, Eastmond DA. 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 2005; 586(1): 28-37.

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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. Appt. Pharmacol. 30: 287-298.

D.5 The Committee (December 2010)

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Part II

Data on 1,4-dioxane

Chapter 1

Identity, properties and monitoring

If not stated otherwise, information in this chapter is retrieved from the SCOEL report on dioxane*.

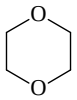
1.1 Chemical identity

Chemical name	1,4-Dioxane
CAS registry number	123-91-1
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

1,4-Dioxane is a colourless liquid with a characteristic etheric odour. Ignition of the vapour is possible at distance. The substance can form peroxides. Due to the poor electrical conductivity, electrostatic charges may be generated when flowing, upon agitation, etc. In addition, 1,4-dioxane violently reacts with strong oxidizers with risk of fire and explosion.

* For references etc. see: References part I: SCOEL SUM112/2004.

1.2 Physical and chemical properties

molecular formula	$C_4H_8O_2$
structure	
molecular weight	88.12
boiling point (100 kPa)	101.3 °C
melting point	11.8 °C
vapour pressure (20 °C/1 bar)	41.3 mbar (40 hPa)
relative density of the saturated vapour in air (air = 1)	1.08
vapour percentage in saturated air (20 °C, 1 bar)	4.1 %
density of the liquid (20 °C) (water = 4 °C)	1.0356
flash point	11 °C
solubility in water, in alcohol, in ether	miscible with water, alcohol, ether, acetone, benzene, acetic acid and other organic solvents
odour threshold	
detection	10 mg/m^3
recognition	21 mg/m^3 (NIOSH, cited in Health Council 1987)
conversion factors (25 °C, 760 mmHg)	1 ppm = 3.6 mg/m^3 air 1 mg/m^3 air = 0.278 ppm

1.3 Analytical methods

1.3.1 Environmental monitoring

See Health Council 1987.

1.3.2 Biological monitoring

See Health Council 1987.

Chapter 2

Sources of exposure

If not stated otherwise, information in this chapter is retrieved from the SCOEL report on dioxane*.

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 worldwide production volume is estimated at 8,000 t/a and 10,000 t/a, respectively (BASF information). In general, the worldwide 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;

* For references etc. see: References part I: SCOEL SUM112/2004.

HSDB 1996; Grant Chemicals 1977). Further it is used as a stabiliser for 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).

Chapter 3

Environmental levels and human exposure

No information available.

Chapter 4

Kinetics

If not stated otherwise, information in this chapter is retrieved from the SCOEL report on dioxane*.

4.1 Absorption

Inhalation and oral

Four healthy volunteers were inhalatory exposed to 50 ppm (180 mg/m^3) 1,4-dioxane for 6 hours and the blood and the urine was examined (SCOEL: 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 (SCOEL: Marzulli *et al.* 1981). In-vitro studies show that 12% of an

* For references etc. see: References part I: SCOEL SUM112/2004.

applied dose passes through excised skin under occlusion and only 0.3% when not occluded (SCOEL: ECETOC 1983).

Additional information

The skin penetration rate of 1,4-dioxane in water was 0.36 ± 0.03 µg $cm^{-2}hr^{-1}$ (ATSDR 2007).[1]

4.2 Distribution

No information available.

4.3 Biotransformation

Mixed-function oxidation enzymes, and cytochrome P-450 in particular, are critical to the metabolism of 1,4-dioxane.The primarily route of metabolism of 1,4-dioxane results in the formation of hydroxyethoxyacetic acid (HEAA). Induction of the cytochrome P-450 enzymes increases the rate HEAA formation, and inhibition decreases HEAA formation. There can also be oxidation of the unbroken ring to produce 1,4-dioxane-2-one, which is in equilibrium with HEAA (SCOEL: Woo 1977a,b).

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 (SCOEL: Dietz *et al.* 1982, SCOEL: Reitz *et al.* 1990, SCOEL: 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 β-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) (SCOEL: Young *et al.* 1978).

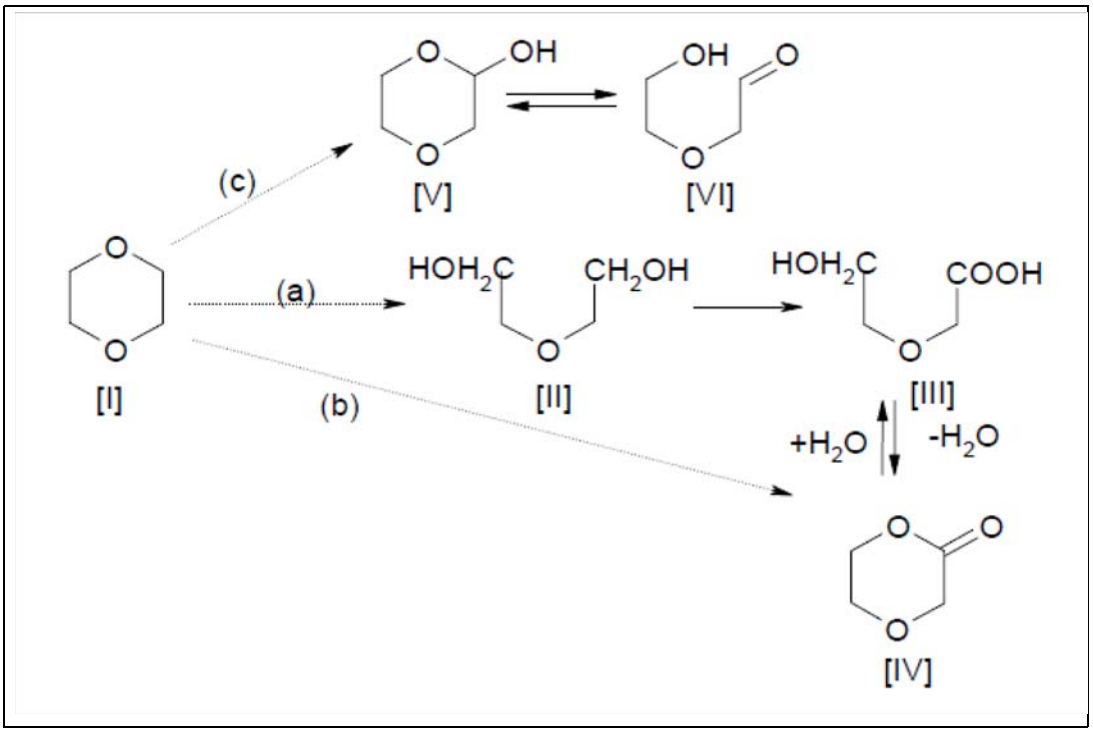


Figure 1 Suggested metabolic pathways of 1,4-dioxane in the rat (SCOEL: Woo *et al.* 1977a). (I 1,4-dioxane; II diethylene glycol; III β-hydroxyethoxy acetic acid (HEAA); IV 1,4-dioxane-2-one; V 1,4-dioxane-2-ol, VI β-hydroxyethoxy acetaldehyde) *(from European Commission 2002).*

4.4 Elimination

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 t½ was 59 minutes (SCOEL: Young *et al.* 1977). A physiologically based pharmacokinetic (PB-PK) modelling study indicates that dioxane may also be excreted into human milk (SCOEL: 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 (SCOEL: Woo *et al.* 1977a, 1977b). These two metabolites are in chemical equilibrium,

dependent on the pH. At low pH the equilibrium is more shifted to 1,4-dioxan-2-one.

4.5 Biological monitoring

See Health Council 1987. Recent environmental monitoring data for 1,4-dioxane are lacking.

Chapter 5

Effects

If not stated otherwise, information in this chapter is retrieved from the SCOEL report on dioxane*.

5.1 Observations in humans

5.1.1 Irritation and sensitisation

At concentrations 1,000 mg/m^3 irritation of eyes, nose and throat was reported (SCOEL: European Commission 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 (180 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 (SCOEL Young *et al*.).

Inflammatory skin changes, showing symptoms of eczema, in the upper extremities and the face were seen after dermal exposure to 1,4-dioxane for

* For references etc. see: References part I: SCOEL SUM112/2004.

several weeks in a 47 year-old female laboratory technician (SCOEL Sonneck 1964). A positive human patch-test is reported in a man who developed dermatitis after daily dipping in a 1,4-dioxane containing solvent (SCOEL Fregert 1974).

Additional information

Exposure of 12 volunteers (6 males and 6 females) to 72 mg/m^3 (20 ppm) 1,4-dioxane for 2 hours caused no irritation of eyes, nose or throat (burning, irritation, or running eyes or nose) or respiratory effects during exposure or up to 3 hours after exposure in a study by Ernstgård *et al.*[2] Subjective symptoms were assessed with a questionnaire, but respiratory function was also assessed by spirometry at 0 and 3 hours after exposure. Nasal swelling and markers of inflammation in blood (CRP and IL-6) were also measured.

5.1.2 Acute toxicity

No information available.

5.1.3 Short-/long term exposure

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 2,340 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 (SCOEL Johnstone 1959). Similar symptoms were observed in five patients who died after 1,4-dioxane exposure (SCOEL Barber 1934).

5.1.4 Epidemiological studies

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 (SCOEL 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 (SCOEL 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 (SCOEL: NIOSH 1977).

5.2 Animal experiments

5.2.1 Irritation and sensitisation

Irritation

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 (SCOEL BASF 1973; Zeller and Kühlern 1998a). In rats and mice the lowest irritating concentration was 80% (no further information available, SCOEL 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 (SCOEL BASF 1973; SCOEL Zeller and Kühlern 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 1,000 ppm (3,600 mg/m^3) (European Commission 2002).

Sensitisation

A negative result was obtained in a guinea pig maximisation test according OECD guideline (SCOEL Fregert 1974).

5.2.2 *Acute toxicity*

The oral LD_{50} value of 1,4-dioxane for the rat was between 5,170 and 7,339 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 (SCOEL Laug *et al.* 1939; SCOEL Nelson 1951; SCOEL Smyth *et al.* 1941). In rabbits dose related narcotic effects were seen (SCOEL Nelson 1951).

The dermal LD_{50} was reported to be 7,855 mg/kg bw for the rabbit. No toxic effects were mentioned.

With respect to inhalation the LC_{50} was 46,000-52,000 mg/m^3 for rats and 36,700 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 (SCOEL BASF AG 1980).

Acute neurotropic effects of 1,4-dioxane were investigated. Depression of tonic extension after electroshock in rats was seen at concentrations ≥ 6,800 mg/m^3 and an oral administration of 1,050 mg/kg bw caused a decrease in dopamine and serotonin levels in the hypothalamus and a decrease in serotonin in the medulla oblongata (SCOEL Frantik *et al.* 1994).

5.2.3 *Short-term toxicity*

Inhalation

Wistar rats were exposed to 400 mg 1,4-dioxane vapour/m^3 (111 ppm) 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 (SCOEL Kociba *et al.* 1974; SCOEL Torkelson *et*

al. 1974). No organ toxicity and no tumour formation was observed.
Further details of this study are described under Carcinogenicity.

Additional information

Male and female F344/DuCrj rats (10/sex/group) were whole-body exposed by inhalation to 1,4-dioxane vapour (concentrations 0, 100, 200, 400, 800, 1,600, 3,200 and 6,400 ppm [v/v]) for 6 h/day, 5 days/wk, for 13 weeks. Glutathione S-transferase placental form (GST-P) positive liver foci, a preneoplastic marker, were examined for detecting early stages of development of nasal and hepatic tumours.

All the 6,400-ppm-exposed rats of both sexes died in the first week of the study, renal failure and lung congestion being the primary causes of death. All remaining animals survived the treatment period, showing no abnormal clinical signs. Body weight was decreased in males at 200 and 3,200 ppm and in females at 200 ppm, 800 ppm and above. Relative liver weight was increased in both sexes at exposure levels ≥ 800 ppm. Relative kidney weight was higher in males at 3200 ppm and in females at 800 ppm and above. Relative lung weight was increased in males exposed to 200 ppm and 1,600 ppm and above and in females exposed to 200 ppm and above. There was a slight increase of plasma AST at 200 and 3200 ppm in females and of ALT at 3,200 ppm in both sexes. Histopathological examination revealed lesions of the upper and lower respiratory tracts and in the liver of males and females, and in the kidney of female animals.

Lesions in the respiratory tract were characterised by a dose dependent increase of enlarged nuclei in epithelial cells at all exposure levels. The incidence of degenerative changes in olfactory sensory cells, was increased at ≥ 400 ppm in males (vacuolisation) and ≥ 800 in females (vacuolisation and decreased number of cells). In males exposed to 1,600 ppm, vacuolic changes also occurred in bronchial epithelium.

Liver toxicity was characterised by increased incidences of single cell necrosis and centrilobular swelling of hepatocytes in males and females at 3,200 ppm. In addition, GST-P positive liver foci of hepatocytes were detected in 3/10 males and in 2/10 females at 3,200 ppm and in 4/10 females at 1,600 ppm.

The incidence of hydropic change in renal proximal tubules was increased in the 3,200 ppm exposed females. Kasai *et al.* concluded that the LOAEL was 100 ppm (360 mg/m^3) in this study, based on an increased incidence of nuclear enlargement in the nasal respiratory epithelium of both male and female rats at the lowest exposure level.[8]

Oral

The studies with application of 1,4-dioxane via drinking water are summarized in Table 1. 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 % (SCOEL: 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 (SCOEL: Japan Bioassay Research Center 1998; SCOEL: 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 1,000 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 1,000 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 (from SCOEL 2004; additional information in italics, taken from the published studies).

species	duration	dose	effects	NOAEL	reference
Rat F344/DuCrj 10m, 10f	13 w	0,0.064,0.16,0.4, 1, 2.5 % m: 52,126, 274, 657, 1,554 mg/kg bw f: 83, 185, 427, 756, 1,614 mg/kg bw	0.16%: liver, kidney f: weight nasal cavity, trachea, liver, kidney, brain m,f: non-neoplastic lesions	0.064%	Japan Bioassay research Center 1998; Kano *et al.* (2008)[6]
Rat Sherman 60m, 60f	716 days	0, 0.01, 0.1, 1% m: 0, 9.6, 94, 1,015 mg/kg bw f: 0, 19, 148, 1,599 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 50m, 50f	104 w	0, 0.02, 0.1, 0.5% m: 0, 11, 55, 274 mg/kg bw f: 0, 18, 83, 429 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: *nuclear enlargement of olfactory epithelium cells, squamous cell metaplasia and hyperplasia in respiratory epithelium and* malignant neoplasms skin m: mesothelioma, fibroma mammary gland m,f: fibroadenoma, adenoma	< 0.02%	Yamazaki *et al.* (1994); Kano *et al.* (2009)[5]
Rat Osborne-Mendel 35m,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
Mouse Crj:BDF1 10m, 10f	13 w	0.064, 0.16, 0.4, 1, 2.5% m: 86, 231, 585, 882, 1570 mg/kg bw f: 170, 387, 898, 1,620, 2,669 mg/kg bw	0.16%: trachea, lung f: non-neoplastic lesion 0.4%: nasal cavity, trachea, lung, liver m,f: non-neoplastic lesion 1%: lung f: weight 2.5%: lung, kidney m: weight	0.064%	Kano *et al.* (2008)[6]
Mouse B6C3F1 50m, 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 Crj:BDF1 50m, 50f	104 w	0, 0.05, 0.2, 0.8% m: 0, 49, 191, 677 mg/kg bw f: 0, 66, 278, 964 mg/kg bw	0.05%: liver f: adenoma and carcinoma 0.2%: nose m,f: lesions in nasal cavity *(nuclear enlargement)* lung f: weight testis m: decreased mineralisation 0.8%: nose m: esthesioneuroepithelioma (1/50) nose f: adenocarcinoma (1/50)	< 0.05%	Yamazaki *et al.* (1994); Kano *et al.* (2009)[5]

m = male
f = female

5.2.4 *Long-term toxicity and carcinogenicity*

Inhalation

Wistar rats were exposed to 111 ppm 1,4-dioxane vapour (400 mg/m^3) for 7 hours/day, five days a week for a total of 2 years (corresponding to 105 mg/kg bw/d) (SCOEL: 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.

Additional information

Male* F344/DuCrj rats (50/group) were whole-body exposed by inhalation to 1,4-dioxane vapour (concentrations 0, 50, 250 and 1,250 ppm [v/v]) for 6 h/day, 5 days/wk, for 104 weeks. Glutathione S-transferase placental form (GST-P) positive liver foci, a preneoplastic marker, were examined for detecting early stages of development of nasal and hepatic tumours.

Survival rate was decreased in the 250 and 1,250 ppm groups. In the high dose group, the primary cause of death were peritoneal mesotheliomas, with nasal tumours contributing to the mortality. There were no differences in food consumption. In the 1,250 ppm exposed group, terminal body weight was decreased (<10%), relative liver weight was 27% higher, and plasma ALT, AST and gamma-GT enzyme activities were increased.

Incidences of nuclear enlargement in the respiratory and olfactory epithelia were significantly increased in all 1,4-dioxane exposed groups, while significantly increased incidences of nuclear enlargement were observed in the liver of the 1,250 ppm exposed group and in the kidney of the 250 ppm and 1,250 ppm exposed groups. Inflammation in the respiratory and olfactory epithelia and atrophy in the olfactory epithelium, hydropic change and sclerosis of lamina propria, and proliferation in the nasal gland occurred at a statistically significant level in the 1,4-dioxane exposed groups with different exposure concentrations. Necrosis of hepatocytes in the centrilobular region, spongiosis hepatise in the liver, and hydropic change in the renal proximal tubule also occurred in the 1,250

* The reason for selecting male animals only was the absence of mesotheliomas in females in a previous 2-year oral study with 1,4-dioxane administration via drinking water (Kano *et al.* 2009).[7]

ppm exposed group. As preneoplastic lesions, squamous cell hyperplasia in the nasal cavity and altered cell foci in the liver were observed in the 1,250 ppm-exposed group. Squamous cell metaplasia, characterized by replacement of transitional and respiratory epithelia by squamous epithelium with or without keratinization occurred in rats exposed to 250 ppm and above.

A dose-dependent and statistically significant increase was observed in the incidences of nasal squamous cell carcinomas, hepatocellular adenomas and peritoneal mesotheliomas. Incidences of neoplastic lesions are shown in Table 2.

Table 2 Incidences of neoplastic lesions in rats (n=50/group) exposed to 1,4-dioxane vapour for 2 years (Kasai *et al.* 2009).[7]

	control	50 ppm	250 ppm	1250 ppm
Nasal cavity, squamous cell carcinoma	0	0	1	6[a]
Liver, hepatocellular adenoma	1	2	3	21[b]
hepatocellular carcinoma	0	0	1	2
Kidney, renal cell carcinoma	0	0	0	4
Peritoneum, mesothelioma	2	4	14[b]	41[b]
Mammary gland, fibroadenoma	1	2	3	5
adenoma	0	0	0	1
Zymbal gland, adenoma	0	0	0	4
Subcutis, fibroma	1	4	9[b]	5

[a] statistically significant from control at $p \leq 0.05$.
[b] statistically significant from control at $p \leq 0.01$.

The increased incidences of nuclear enlargement in the respiratory and olfactory epithelia, and atrophy and respiratory metaplasia in the olfactory epithelium, noted in the nasal cavity of male rats exposed at ≤ 50 ppm 1,4-dioxane, were not reported by Torkelson *et al.* 1974 (single dose of 111 ppm). However, the nasal cavity was not listed in the organs subjected to microscopic examination in Torkelson's study.

The authors derived a chronic LOAEL of 50 ppm (180 mg/m^3) from this study, based on an increased incidence of nuclear enlargement in the nasal respiratory epithelium at the lowest exposure level (Kasai *et al.* 2009).[7]

Oral administration - rats

Chronic toxicity and carcinogenicity studies, in which 1,4-dioxane was administered via drinking water, are listed in Table 1. Neoplastic lesions in these studies are described in detail in Table 3.

Table 3 Neoplastic lesions after oral 1,4-dioxane administration (from SCOEL 2004; additional information in italics, taken from the published studies).

species	duration	dose	effects	reference
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	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)
Rat F344/DuCrj 50m, 50f	104 w	0, 0.02, 0.1, 0.5% m: 0, 11, 55, 274 mg/kg bw f: 0, 18, 83, 429 mg/kg bw	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 Peritoneum (0, 0.02, 0.1, 0.5%) mesothelioma: m: 2/50, 2/50, 5/50, 28/50	Yamazaki *et al.* (1994), Japan Bioassay Research Center (1998c), *Kano et al. (2009)*[5]
Rat Osborne-Mendel 35m,35f	110 w	0, 0.5, 1% m: 0, 240, 530mg/kg bw f: 0, 350, 640 mg/kg bw	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
Mouse B6C3F1 50m, 50f	90 w	0, 0.5, 1% m: 0, 720, 830 mg/kg bw f: 0, 380, 860 mg/kg bw	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
Mouse Crj:BDF1 50m, 50f	104 w	0, 0.05, 0.2, 0.8% m: 0, 49, 191, 677 mg/kg bw f: 0, 66, 278, 964 mg/kg bw	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), *Kano et al. (2009)*[5]

m = male
f = female

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 %.

In a liver foci assay 1,4-dioxane showed a clear positive result (SCOEL Lundberg *et al.* 1987), while a mouse skin papilloma test with a single dosage of 1,4,-dioxane was negative (SCOEL Bull *et al.* 1986).

No peroxisomal proliferation activity was observed after dosing with 1,4 dioxane (1% and 2% in drinking water for 5 days in two studies)(SCOEL: Goldsworthy *et al.* 1991, SCOEL: TSCAT 1989).

5.2.5 *Mutagenicity*

Bacterial and yeast tests, in vitro

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

Mammalian tests, in vitro

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 (SCOEL: 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 (SCOEL: Microbiological Associates 1980a,b).

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 (SCOEL: 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 (SCOEL: Morita *et al.* 1998).

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 (SCOEL: 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 (SCOEL: 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 (SCOEL: 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; SCOEL: Stott *et al.* 1981). Table 4 shows a summary of the *in vivo* genotoxicity tests.

Additional information

Recent studies in rats and mice demonstrated that 1,4-dioxane has genotoxic properties in vivo, although at elevated dose levels only.[10,3] Roy *et al.*[10] demonstrated that the micronuclei, induced in both the bone marrow and the liver of young male CD-1 mice, resulted primarily from chromosome breakage implying a genotoxic mechanism. In a poster abstract, Fukushima *et al.*[3] reported an increased mutation frequency in liver cells of a transgenic rat at a 1,4-dioxane concentration of 5,000 ppm in drinking water, but not at 1,000 ppm and lower concentrations.

Table 4 In vivo genotoxicity of 1,4-dioxane (from SCOEL 2004; additional information in italics).

test system	endpoint	dose (route)[a]	result	reference
Mouse	Dominant lethal assay	2,500 mg/kg bw	-	BASF (1977)
Drosophila	Sex-linked recessive lethal	50 mg/ml (injection)	- (+ high dosages)	Yoon *et al.* (1985)
SD rat liver	DNA damage	1,000 mg/kg (oral)	-	Stott *et al.* (1981)
SD rat liver	DNA damage	2,550 mg/kg (oral)	+	Kitchin and Brown (1990)
SD rat liver	DNA repair	1,000 mg/kg bw (oral)	-	Stott *et al.* (1981)
F344 rat liver	DNA repair	1,000 mg/kg (oral)	-	Goldsworthy *et al.* (1991)
F344 rat nasal cavity	DNA repair	1,000 mg/kg bw (oral)	-	Goldsworthy *et al.* (1991)
B6C3F1 mouse bone marrow	Micronuclei	4,000 mg/kg bw (ip)	-	McFee *et al.* (1994)
C57BL6 mouse bone marrow	Micronuclei	900 mg/kg bw (oral)	+	Mirkova (1994)
BALB/c mouse bone marrow	Micronuclei	5,000 mg/kg bw (oral)	-	Mirkova (1994)
C57BL6	Micronuclei	3,600 mg/kg bw (oral)	-	Tinwell *et al.*(1994)
CBA mouse bone marrow	Micronuclei	1,800 mg/kg bw (oral)	-	Tinwell *et al.* (1994)
CD-1 mouse peripheral blood	Micronuclei	3,200 mg/kg bw (ip)	-	Morita and Hayashi (1998)
CD-1 mouse peripheral blood	Micronuclei	3,000 mg/kg bw (oral)	-	Morita and Hayashi (1998)
CD-1 mouse liver	Micronuclei	2,000 mg/kg (oral)	+	Morita and Hayashi (1998)
Additional information				
Drosophila	Meiotic non-disjunction	1% (food)	+	Muñoz and Barnett (2002)[9]
gpt delta transgenic rat liver	GST-placental positive foci (pre-neoplastic marker), liver cell proliferation and mutation frequency of gpt transgene gene	0, 200, 1,000 and 5,000 ppm (drinking water, 16 weeks)	- at 200 and 1,000 ppm + at 5,000 ppm	Fukushima *et al.* (2009)[3]
CD-1 mouse bone marrow	Micronuclei	1,500 mg/kg bw (oral, 5 days)	+	Roy *et al.* (2005)[10]
CD-1 mouse liver	Micronuclei	2,500 mg/kg bw (oral, 5 days)	+	Roy *et al.* (2005)[10]

[a] The highest dose used in cases of negative results and the lowest effective dose in cases of positive results.

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.

5.2.6 Reproduction 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 (SCOEL: Yamazaki et al 1994; SCOEL: 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 (SCOEL: 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 was 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 malformations remained within normal limits for all groups, and no deviations were found regarding minor anomalies and variants when compared with the control group. At the highest dose level a significant retardation was found in the area of the sternum. There was no indication of teratogenicity. The authors stated that the fetal retardation could be ascribed to maternal toxicity. The NOAEL for maternal and embryotoxicity in this study was 0.5 ml/kg bw, equivalent to 517 mg/kg bw.

5.3 Summary

Studies in human volunteers exposed to 180 mg/m^3 (50 ppm) 1,4-dioxane indicated almost total excretion of the inhaled dose as HEAA, with no indication

of saturation of metabolism (SCOEL: 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 (SCOEL: Thiess *et al.* 1976; SCOEL: Baffler *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/m^3 and 90 mg/m^3 respectively.

Irritation of the eye in volunteers was seen at concentration of 180 mg/m^3 (50 ppm) in experimental settings (Young *et al.* 1977). Skin dermatitis was reported for men after repeated skin contact with 1,4-dioxane (SCOEL: Sonneck 1964; SCOEL: Fregert 1974).

In studies with experimental animals, 1,4-dioxane was not very toxic by the oral, dermal or inhalation route. The substance was irritating to the eyes and the respiratory tract, and slightly irritating to skin. In a guinea pig maximisation test, no skin sensitising properties were apparent.

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. In a 2-yr inhalation study in rats, Torkelson *et al.* (1974) found no evidence of toxicity, including carcinogenicity at an exposure level of 400 mg/m^3 (111 ppm).

Additional information

In a recent 2-yr inhalation study in male rats, nasal squamous cell carcinomas and pre-neoplastic lesions such as atrophy, metaplasia, and nuclear enlargement were observed (Kasai *et al.* 2009).[7] In this study, an increased incidence of neoplastic lesions was noted at 250 and 1,250 ppm, whereas non-neoplastic and pre-neoplastic changes were observed at the lowest exposure level, 50 ppm, and above.

The mechanism for carcinogenicity appears to be primarily 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 (SCOEL 2004). 1,4-Dioxane-induced nasal tumours are considered to result primarily from the injury of cells in the respiratory and olfactory epithelia and subsequent regenerative cell proliferation,

as evidenced by squamous cell metaplasia and hyperplasia which can be regarded as a pre-neoplastic lesion (Kasai *et al.* 2009).[7] An increased incidence of hepatocellular tumours was only seen at high exposure levels and together with hepatocellular injury, evidenced by the necrosis of hepatocytes and enhanced cytolytic release of liver enzymes such as AST, ALT, ALP, and gamma-GT into plasma. The European Commission (2002) and SCOEL (2004) concluded that 1,4-dioxane-induced carcinogenesis is primarily driven by a cytotoxic/proliferative, non-genotoxic mode of action. However, at elevated dose levels genotoxic activity is also apparent, based upon repeated positive findings in micronucleus assays in liver and bone marrow of several strains of mice (SCOEL: Mirkova 1994; SCOEL: Tinwell *et al.* 1994; SCOEL: Morita and Hayashi 1998; Roy *et al.* 2005[10]). Roy *et al.* (2005)[10] demonstrated that the increased frequency of micronuclei is primarily due to chromosome breakage.

Chapter 6

Existing guidelines, standards and evaluations

6.1 General population

No information available.

6.2 Working populations

In the following Table occupational exposure limits from several countries are presented.

Table 5 Occupational exposure limits.

country	year	TLV	source
Germany	2009	73 mg/m^3 (20 ppm) classified in group B (carcinogens)	TRGS 900
UK	2007	91 mg/m^3 (25 ppm) STEL = 366 mg/m^3 (100 ppm)	EH40/2005
USA (ACGIH)	2003	70 mg/m^3 (20 ppm) [skin notation] Group A3 carcinogenicity classified	ACGIH
USA (OSHA)	2004	360 mg/m^3 (100 ppm) [skin notation]	29 CFR 1910.1000
USA (NIOSH)	2004	3.6 mg/m^3 (1 ppm - 30 m) [skin notation]	NIOSH

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).

The current classification as category 3 carcinogen (R40) is agreed by the European Commission (2002). For both liver and nasal tumours, cytotoxic effects and organ damage are considered to be involved, implicating a threshold.

In the Netherlands, a TLV of 40 mg/m^3 (8-h TWA) and a STEL of 80 mg/m^3 (15-min TWA) were established in 1992. The basis for these limit values was a report by the Health Council (1987), recommending a TLV of 40 mg/m^3 (11 ppm), based on the study by Torkelson *et al.* (1974) showing no effects in rats with lifetime exposure to 400 mg/m^3 (111 ppm), applying an interspecies factor of 10 for extrapolation of animals to humans. An additional factor for interindividual differences in sensitivity was not considered necessary. The official limit values in the Netherlands ceased to exist on 1 January 2007.

SCOEL (2004) recommended a TLV of 73 mg/m^3 (20 ppm) on the basis of the same chronic toxicity study in rats and on the need to avoid eye irritation (seen in human volunteers at 180 mg/m^3; 50 ppm, Young *et al.* 1977). SCOEL concluded that a STEL proposal would not be needed.

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