Committee for Risk Assessment

RAC

Opinion on scientific evaluation of occupational exposure limits for

1,4-dioxane

ECHA/RAC/OEL-O-0000007101-89-01/F

18/03/2022

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON THE EVALUATION OF THE OCCUPATIONAL EXPOSURE LIMITS (OELs) FOR 1,4-DIOXANE

Commission request

The Commission, in view of the preparation of the proposals for amendment of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens mutagens or reprotoxic substacnes at work (CMRD), and in line with the 2017 Commission Communication '*Safer and Healthier Work for All' - Modernisation of the EU Occupational Safety and Health Legislation and Policy*[1], asked the advice of RAC to assess the scientific relevance of occupational exposure limits for some carcinogenic chemical substances.

Therefore, the Commission made a request on 11 December 2020 to ECHA in accordance with the Service Level Agreement (SLA) (Ares(2019)18725), to evaluate, in accordance with the Directive 2004/37/EC, 1,4-dioxane.

1,4-dioxane was previously classified as a category 2 carcinogen in CLP legislation, but has a new classification as a category 1B carcinogen bringing it into the scope of the CMD. 1,4-dioxane already has an IOELV under CAD and as a result of its reclassification it is necessary to review the current IOELV and to replace it with an OEL under CMD.

I PROCESS FOR ADOPTION OF THE OPINION

Following the above request from the European Commission, RAC was requested to draw up an opinion on the evaluation of the scientific relevance of occupational exposure limits (OELs) for 1,4-dioxane with a deadline of 30 June 2022.

Chemical name(s): 1,4-dioxane

In support of the Commission's request, ECHA prepared a scientific report concerning occupational limit values for 1,4-dioxane at the workplace. In the preparatory phase of making this report, a call for evidence was started on 23 March 2021 to invite interested parties to submit comments and evidence on the subject by 22 June 2021. The scientific report was made publicly available[2] on 27 September 2021 and interested parties were invited to submit comments by 26 November 2021.

RAC developed its opinion on the basis of the scientific report submitted by ECHA. During the preparation of the opinion, the scientific report was further developed as an Annex to the RAC opinion.

The RAC opinion includes a recommendation to the Advisory Committee on Safety and Health at Work (ACSH) in line with the relevant Occupational Safety and Health legislative procedures.

[1] http://ec.europa.eu/social/main.jsp?langId=en&catId=148&newsId=2709&furtherNews=yes

[2] https://echa.europa.eu/documents/10162/ffebd37d-e38c-0b15-7376-229481dd9619

II ADOPTION OF THE OPINION OF THE RAC

Rapporteurs, appointed by RAC: **Gerlienke Schuur** and **Andrea Hartwig**.

The opinion of RAC was adopted by **consensus** on **18 March 2022.**

RAC Opinion of the assessment of the scientific relevance of OELs for 1,4-dioxane

RECOMMENDATION

The opinion of RAC on the assessment of the scientific relevance of Occupational Exposure Limits (OELs) for 1,4-dioxane is set out in the table below and in the following summary of the evaluation.

SUMMARY TABLE

The table presents the outcome of the RAC evaluation to derive limit values for 1,4-dioxane.

Derived Limit Values

OEL as 8-hour TWA:	7.3 mg/m^3 (2 ppm)
STEL:	73 mg/m^3 (20 ppm)
BLV:	45 mg 2-hydroxyethoxyacetic acid/g creatinine
BGV:	-

Notations

Notations:	skin

RAC OPINION

Background

This opinion concerns 1,4-dioxane (See section 1 of Annex 1).

1,4-dioxane already has an Indicative Occupational Exposure Limit Value (IOELV) of 73 mg/m^3 (20 ppm) under the Chemical Agents Directive. Because 1,4-dioxane has recently been reclassified under the Classification Labelling and Packaging Regulation (EC) 1272/2008 from a category 2 carcinogen to a category 1B carcinogen, the current IOELV is being reviewed for replacement with a binding OEL under CMD.

This evaluation takes previous reviews into account, in particular:

- DECOS, (2011 and 2015)
- ATSDR (Wilbur et al., 2012)
- CLH dossier and RAC CLH opinion on 1,4-dioxane (ECHA, 2019)
- DFG (Eckert et al., 2020; Hartwig, 2020)

Key conclusions of the evaluation

- 1,4-dioxane is used for the most part as a solvent in industrial settings. Other uses are as a solvent in laboratories and in a polymerisation process in an industrial setting. Occupational exposure is expected to occur during production, processing and use, via inhalation (ranging from estimated concentrations of 0.03 mg/m^3 up to around 26 mg/m^3 as reported in the REACH registration dossier) and the dermal route.
- 1,4-dioxane has a harmonised classification as Carcinogen 1B under CLP, based on animal studies in which 1,4-dioxane was shown to be a multiple-site carcinogen.
- Mutagenicity assays were all negative *in vitro.* Some results *in vivo* were positive, mostly demonstrated at high doses and also showing cytotoxicity. Recent studies point at clastogenic effects, potentially caused by cytotoxicity and oxidative stress.
- Although 1,4-dioxane may have genotoxic potential, and therefore could be considered a genotoxic carcinogen, there is evidence for indirect DNA damage (from oxidative stress) as the main mechanism in tumour formation. Also, cytotoxicity, irritation and inflammation appear to be associated with tumor formation, e.g. in the nasal epithelium and liver. These thresholded mechanisms support a non-linear dose-response relationship.
- 1,4-dioxane is rapidly and almost completely absorbed after inhalation and oral exposure. At relatively low doses, the primary route of metabolism of 1,4-dioxane is via cytochrome P450-catalysed hydrolysis and then oxidation, to produce 2-hydroxyethoxyacetic acid (HEAA), considered to be a detoxification product. There can also be oxidation of the unbroken ring to produce 1,4-dioxane-2-one, which is in equilibrium with HEAA. Excretion is primarily in the urine as HEAA. At higher doses, saturation of metabolism has been observed in rodents, and human data suggest saturation could also be plausible in humans.
- Acute toxicity of 1,4-dioxane is low. 1,4-dioxane is an eye and respiratory tract irritant and may cause skin dryness or cracking by removing the skin fat protective layer. After repeated dose exposure, the main target organs are the respiratory tract (a.o. nasal cavity), liver and kidney.
- No reproductive effects were observed in rodent studies.
- *In vitro* studies provide a range of dermal absorption rates. Taking into account the most recent ones, a skin notation is proposed.

Carcinogenicity and mode of action (see sections 7.7 and 8.1 of Annex 1 for full discussion)

1,4-dioxane is a multiple tissue site carcinogen in rats following exposure through inhalation and drinking water. The most consistent findings following both routes of administration were nasal cavity and liver tumours, accompanied by pre-neoplastic lesions. Tumours were also observed in the peritoneum, mammary gland and subcutis (both routes) and in the kidney and Zymbal's gland (inhalation only). In mice, mainly liver tumours were identified following drinking water exposure.

No indications of carcinogenicity were observed in the few epidemiological studies available. However, the limited quality of the studies (due to limited information on exposure levels and potential confounding factors) does not allow a conclusion on the carcinogenicity potential of 1,4-dioxane in humans.

The mode of action (MoA) leading to tumour formation is not fully resolved. There are potentially a variety of ways in which 1,4-dioxane could induce cancer, given the various tissue sites where it was experimentally seen to have induced tumours in animals.

When looking at its genotoxicity profile (see section 7.6 of Annex 1 for full details), *in vitro* tests in bacteria and mammalian cells are negative. 1,4-dioxane was tested in six reverse mutation assays in bacterial cells, in three gene mutation assays, one micronucleus assay and two chromosome aberrations tests in mammalian cells. Also negative results were reported in two unscheduled DNA synthesis assays, two sister chromatid exchange assays (one positive without information on cytoxicity), a DNA damage assay and an aneuploidy assay. A Comet assay with rat primary hepatocytes was positive, however at cytotoxic concentrations. Induction of micronuclei was increased in some *in vivo* studies, however mostly at dose levels above the limit dose of 2000 mg/kg bw. One Comet assay showed a dose-related increase in DNA single-strand breaks at high doses in rats. No induction of DNA alkylation was shown in another rat study. *In vivo* studies on unscheduled DNA synthesis in rat liver and nasal epithelials cells were negative. Further, a study on the measurement of DNA alkylation in liver cells, and the measurement of cell proliferation by the replicative DNA synthesis assay in two studies were negative.

Until now, it was concluded that 1,4-dioxane was not genotoxic (SCOEL, 2001; DECOS, 2011, 2015; DFG (Hartwig) 2020). In the CLH opinion (2019) on 1,4-dioxane, RAC concluded based on these data to not classify for mutagenicity.

Since that time, five new studies on genotoxicity have become available (see section 7.6 of Annex 1 for full details on these oral studies). The study by Gi et al. (2018) demonstrated increased mutation frequency in a transgenic (gpt delta) rat model after 16 weeks of exposure. Dose-dependent mutagenic potency was demonstrated in the liver without cytotoxicity. In wild-type F344 rats, significantly increased numbers of GST-P positive foci and increased cell proliferation were observed at ≥222 and 560 mg/kg bw/day, respectively. In a follow-up study (Totsuka et al., 2020) DNA adducts were increased, especially 8-oxo-dG, and shown to be formed after induction of oxidative stress. Further, Itoh & Hattori (2019) performed liver and bone marrrow micronucleus tests showing induction of genotoxicity in the liver at ≥2000 mg/kg bw, and not in the bone marrow. They also performed a Pig-a gene mutation assay in rat peripheral blood, which was negative. Furihata et al. (2018) performed a RNA sequencing on 11 marker genes in liver cells for comparison of the effects of 1,4-dioxane with the profile of known genotoxic and non-genotoxic hepatocarcinogens. The authors concluded that 1,4-dioxane has an intermediate profile of gene expression between a genotoxic and a non-genotoxic substance. Chen et al. (2022) looked at redox changes linked to cytotoxicity and genotoxicity in mice, concluding that oxidative stress (by redox dysregulation) could be a candidate mechanism of 1,4-dioxane liver carcinogenicity.

These recent studies confirm the possibility that 1,4-dioxane might have some genotoxic potential, involving DNA damage, cytotoxicity and oxidative stress. However, this is reported at doses higher than tumours are reported.

In general, substances that cause tumours at multiple tissue sites most commonly have a DNA-reactive MoA. The question in the case of 1,4-dioxane would be whether DNA adduct formation is a consequence of oxidative stress or occurs via direct DNA binding. Overall, there might be more clues to indirect genotoxicity via cytotoxicity and oxidative stress.

Other, non-genotoxic mechanisms MoAs to be noted:

The non-genotoxic mechanism of action with regard to the nasal cavity tumours found in animals is assumed to involve irritation of the nasal epithelium, resulting in cytotoxicity, inflammation, regenerative cell proliferation and hyperplasia.

Systemic toxicity (liver tumours) is considered to occur only after saturation of metabolism, which is shown in some animal studies (Young et al., 1978a/b; Sweeney et al., 2008; Dietz et al., 1982). For example, Sweeney et al. (2008) observed saturation above 200 mg/kg bw. Dourson et al. (2014; 2017) proposed a regenerative hypoplasia mode of action model with four steps as follows:

1. metabolic saturation and consequently accumulation of 1,4-dioxane.
2. Liver hypertrophy
3. Hepatocellular cytotoxicity
4. Regenerative cell proliferation leading to liver tumour formation

Two recent articles further explored and supported this MoA. Lafranconi et al. (2021) looked at earlier time course in the events, and the results supported that a mitogenic response precedes the development of cytotoxicity and regenerative hyperplasia. Chappel et al. (2021) conducted a transcriptomics analysis on liver tissue, supportive of a non-mutagenic, threshold-based, mitogenic MoA for 1,4-dioxane-induced liver tumors.

Cancer Risk Assessment (see sections 8.1, 9.1 and 9.2 of Annex 1 for full discussion)

The available human epidemiological studies are descriptive occupational studies, mainly from the 1970s, with no dose-response risk estimates. Although some uncertainty on the mode of action remains, the carcinogenicity of 1,4-dioxane is considered to be related to a non-genotoxic mechanism, involving saturation of metabolic capacity, irritation at high exposure levels and formation of liver tumours by regenerative proliferation. Even though a mode of action-based threshold is assumed for the carcinogenic effects of 1,4-dioxane, some uncertainties with regard to residual cancer risk remain. However, the level of uncertainty is considered to be low, in view of the evidence that only above saturation levels of metabolism (which in humans is above 180 mg/m^3; EU, 2002) are tumours formed. Therefore, in this case, no additional dose-response for carcinogenicity (i.e.cancer risk estimates) is provided for the purpose of this report.

Derived Limit Values (see section 9.2 of Annex 1 for full discussion)

Evaluations to date (SCOEL, 2004; DECOS, 2011; DFG, 2020) have assumed the primary MoA to be non-genotoxic. DECOS and DFG have taken the nasal pre-neoplastic lesions as a critical effect, and thus the LOAEC of 180 mg/m^3 (50 ppm) in a 2-year rat study by Kasai et al. (2009) as the point of departure. In its derivation of a drinking water guideline, Health Canada (2021) concluded that the pattern is inconsistent with an MoA where genotoxicity is an early, direct and influential key event in the carcinogenic MoA.

In weighing all the current evidence, RAC is of the opinion that at low doses, 1,4-dioxane is not mutagenic. Liver and nasal cavity tumours are reported following saturation of 1,4-dioxane metabolism or elimination. Several studies support a non-genotoxic MoA involving cytotoxicity (oxidative stress) followed by regenerative hyperplasia and

stimulation of endogenously formed mutations. A non-linear (threshold) risk assessment approach is considered appropriate.

OEL - 8h-TWA

OEL derivation

OELs are calculated for local effects as well as systemic effects, after which the lowest OEL is selected.

Local effects

The key study is the carcinogenicity study in rats exposed to 1,4-dioxane for 2 years (5 days/week, 6 hr/day) at doses of 0, 180, 900, and 4500 mg/m^3 (0, 50, 250, 1250 ppm) by Kasai et al. (2009). The initial nasal effects are observed at all dose levels, thus resulting in a LOAEC of 180 mg/m^3 (50 ppm). The peritoneal mesothelioma was reported from 900 mg/m^3 (250 ppm) and higher. Liver tumours and pre-neoplastic lesions in liver were reported at 4500 mg/mg^3 (1250 ppm). The effect in kidneys was nuclear enlargement starting from 250 ppm, and renal cell carcinomas were found in the highest dose group.

- Adjusting the LOAEC for nasal effects with respect to differences in human and experimental exposure conditions is deemed not necessary, as the toxic effects (local irritation) is driven by the concentration.
- To extrapolate from the LOAEC to the NAEC, a default assessment factor of 3 is taken. Although almost all animals showed local irritation in the nose at 183 mg/m^3 (50 ppm), in human volunteer studies, no irritation was seen at 73 mg/m^3 (20 ppm), while it was reported in another study at 183 mg/m^3 (50 ppm). Therefore, taking into account animal and human data, RAC considers a factor 3 is sufficient.
- For interspecies extrapolation, allometric scaling is not applied as the effects are considered local effects.
- The default assessment factor for remaining uncertainties with regard to dynamic differences is 2.5.
- In the review of Brüning et al. (2014) the authors conclude on an iEF (interspecies extrapolation factor) of 3 for extrapolating animal data to humans concerning local sensory irritating effects. Results from short-term studies with human volunteers showed no sensory irritation effects upon exposure at 20 ppm for 6 hr (Ernstgård et al., 2006) and only eye irritation at 50 ppm for 6 hr (Young et al. 1977). As these human studies are short-term, and the local irritation effects in the nose were found in a 2-year study, RAC considers a factor of 2.5 for interspecies necessary.
- For intraspecies differences, an assessment factor of 3 is chosen.

The total assessment factor would thus be 22.5 (3x1x3x2.5). This results in an OEL (8 hr TWA) of 8.1 mg/m^3 ppm (2.2 ppm).

Systemic effects

With regard to the systemic effects, in repeated dose toxicity studies after inhalation, the target organs were liver, kidney, and nose. When looking at the systemic effects, a NOAEC of 180 mg/m^3 (50 ppm) can be derived for kidney effects (nuclear enlargement of the proximal tubule in 20 of 50 animals) from the same inhalation carcinogenicity study with rats (Kasai et al. 2009). The dose-related effects in liver (centrilobular necrosis) already started at the lowest dose concentration, but were not statistically significant untill the highest dose. Altogether, 50 ppm was identified as NOAEC for all endpoints with respect to systemic effects.

From human data, there is only some evidence from old case studies (Barber, 1934; Johnstone, 1959) after exposure to high concentrations of 1,4 dioxane in the air for 1 or 2 weeks, resulting in death. Post-mortem findings showed extensive lesions in kidneys (hemorrhagic necrosis of the kidney cortex), next to hepatic necrosis and perivascular widening in the brain. No information in humans is available after chronic exposure.

For the derivation of an OEL, the NOAEC from the rat study should be converted with regard to the exposure conditions. The NOAEC of 50 ppm is converted from rat to human, taking into account differences in breathing volume (x 6.7 m^3/10 m^3), to 33.5 ppm, and adjusted for 6 hours exposure (5 days/week, 2 years) to 8 hours, resulting in a converted NOAEC of 25.1 ppm (92 mg/m^3).

Using default assessment factors, a total assessment factor of 12.5 is applied (2.5 for interspecies differences, 5 for intraspecies differences, none for exposure duration), resulting in an OEL of 7.3 mg/m^3 (2 ppm).

Therefore, RAC proposes an OEL of 7.3 mg/m^3 (2 ppm) based on the systemic effects in kidney, which is also protective of the nasal irritation effects leading to carcinogenicity and the effects found in liver.

Even though the proposed limit value assumes a mode of action-based threshold for the carcinogenic effects of 1,4-dioxane, some uncertainties with regard to residual cancer risk remain. However, provided that the proposed OEL is complied with, the level of uncertainty is considered to be low, in view of the evidence that only above saturation levels of metabolism (which is in humans at least above 180 mg/m^3; EU, 2002) tumours are formed. Therefore, in this case, no additional dose-response for carcinogenicity (i.e. cancer risk estimates) is provided for the purpose of this report.

No analytical difficulties are foreseen as 1,4-dioxane can be measured in air in low concentrations (LOQ 0.047 mg/m^3) using a sorbent tube and GC analysis after extraction by desorption on carbon disulfide.

Short term limit value (STEL) (see section 7.4.1, and 9.2.3 of Annex 1 for full discussion)

Several studies with human exposure (to different but high levels of 1,4-dioxane and for different short durations) report on irritation of the nose, throat and eyes. In a study with human volunteers (Ernstgård et al., 2006), exposure to 0 or 73 mg/m^3 (0 or 20 ppm) of 1,4-dioxane vapour, no effects were observed after exposure to 73 mg/m^3 (20 ppm) for 2 hrs. In a further study with four male volunteers exposed to 180 mg/m^3 (50 ppm) for 6 hrs, only eye irritation was reported (Young et al., 1977).

RAC recommends a STEL (15 minutes) of 73 mg/m^3 (20 ppm).

Biological guidance and limit values

Biological limit value (see section 6.2.2, 7.1.2 and 9.2.4 of Annex 1 for full discussion)

For the metabolism of 1,4-dioxane, three metabolic pathways are hypothesized, leading to the main metabolite: 2-hydroxyethoxyactic acid (HEAA) (see Figure 1 in Annex 1). Monitoring information is available from three human volunteer studies with inhalation exposure to 6 mg/m^3 (1.6 ppm), 183 mg/m^3 (50 ppm) and 73 mg/m^3 (20 ppm) respectively. Based on these studies, the DFG (Eckert et al. 2020) established a relationship (three data points) between the mean urinary HEAA levels after the end of exposure (extrapolated to 8 hrs) and the air concentration of 1,4-dioxane. This function is:

> urinary HEAA level (in mg/g creatinine) at end of exposure = 17.82 x (air concentration of 1,4-dioxane in ppm) + 9.58.

This correlation is proposed to be used in setting a BLV. With an OEL of 7.3 mg/m^3 (2 ppm), this leads to the recommendation by RAC for **a BLV of 45 mg HEAA in urine/g creatinine** (rounded-off). Sampling needs to take place at the end of exposure or end of shift.

Biological Monitoring (see section 6.2.1 and 9.2.5 of Annex 1 for full discussion)

There are no data on 1,4-dioxane or its metabolite levels in the general population available, thus, no BGV is proposed. It is expected that the BLV will be well above the levels in the general population.

Notations

Information available for dermal absorption is hampered by the quick evaporation of 1,4-dioxane, but it is available.

Reviews (ATSDR, 2012; DECOS, 2011; SCCS, 2015 and older) before 2015 used the Bronaugh and Marzulli et al. studies, and concluded on limited dermal absorption and a not relevant contribution to the total exposure by the dermal route. The recent *in vitro* human skin studies by Dennerlein et al. show about a 1000-fold higher *in vitro* dermal penetration rate compared to those in the Bronaugh *in vitro* study, also performed in human skin, and no obvious deviations between the studies (although Bronaugh misses some details in the description). There is also a discrepancy with the *in vivo* Marzulli et al. (1981) study, which showed a low absorption percentage <4%.

The guidance document R8-17 (2019) notes "*Usually, a skin notation is applied where it can be assumed that dermal exposure may contribute to about 10% or more of the body burden by inhalation exposure at the OEL*".

In this case, the data are equivocal with regard to the amount of dermal absorption. Putting some more weight on the recent studies, a penetration rate of 984 mg/2000 cm^2/h has been calculated. It can be estimated that about 7800 mg 1,4-dioxane could be dermally absorbed (two hands 2000 cm^2 x 984 mg x 8 hr) upon exposure for 8 hours, in comparison with an amount of 73 mg (OEL of 7.3 mg/m^3 x 10 m^3 in 8 hr and 100% absorption) absorbed via inhalation during a work-day at an air concentration of 7.3 mg/m^3. Therefore, dermal exposure is considered relevant and a skin notation is proposed.

ATTACHMENTS:

The Annex (Annex 1) gives the detailed scientific grounds for the opinion.

RCOM (Annex 2): Comments received on the ECHA scientific report, and responses provided by ECHA and RAC (excluding confidential information).