1-tert-butoxypropan-2-ol

Evaluation of the carcinogenicity and genotoxicity

To: the Minister of Social Affairs en Employment
No. 2021/45, The Hague, December 6, 2021

Health Council of the Netherlands

contents

samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid heeft de Gezondheidsraad beoordeeld of beroepsmatige blootstelling aan 1-tert-butoxypropan-2-ol een genotoxisch effect heeft en tot kanker kan leiden en op basis daarvan een classificatievoorstel opgesteld.

Het advies is tot stand gekomen in de Subcommissie Classificatie kankerverwekkende stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS). Op www.gezondheidsraad.nl staat meer informatie over de taken van deze vaste commissie van de Gezondheidsraad.
De samenstelling van de commissie is te vinden achterin dit advies.

Over 1-tert-butoxypropan-2-ol
De stof 1-tert-butoxypropan-2-ol wordt gebruikt als oplosmiddel in onder meer allesreinigers, deklagen, inkt, lijm en in lak- en latexverf.

Blootstelling op de werkvloer kan plaatsvinden op plekken waar 1-tert-butoxypropan-2-ol wordt geproduceerd of gebruikt.

Beoordeling kankerverwekkende en mutagene eigenschappen
De commissie beoordeelt aan de hand van de beschikbare wetenschappelijk literatuur of er aanwijzingen zijn dat een stof genotoxisch en kankerverwekkend is en hoe groot de bewijskracht daarvoor is. Genotoxische stoffen met mutagene eigenschappen kunnen het erfelijk materiaal in de cel blijvend veranderen (mutatie of genafwijking). Hierdoor kunnen zij kankerverwekkend zijn. Aan de hand van de bewijskracht doet de commissie vervolgens voorstellen om de stof te classificeren in gevarencategorieën: één die aangeeft hoe groot de bewijskracht is dat de stof mutageen is in geslachtscellen, en één die aangeeft hoe groot de bewijskracht is dat de stof tot kanker kan leiden. De categorieën zijn gebaseerd op de criteria die ook gebruikt worden in EU-verordening (EG) 1272/2008 over de classificatie van stoffen. Op basis van de voorstellen van de commissie kan de minister besluiten om de stof al dan niet als mutageen in geslachtscellen en/of als kankerverwekkend aan te merken.

Beschikbaar onderzoek
In laboratoriumstudies vertoonde 1-tert-butoxypropan-2-ol mutageniteit. Er zijn geen experimenten met geslachtscellen gedaan.

Er zijn geen onderzoeksgegevens beschikbaar over gevallen van kanker in mensen door blootstelling aan 1-tert-butoxypropan-2-ol. Er zijn wel gegevens uit dierexperimenten. In die studies werden geen overtuigende aanwijzingen gevonden voor kwaadaardige tumorvorming in

ratten. In muizen werd een toename van levertumoren waargenomen.

Advies

De commissie adviseert om 1-tert-butoxypropan-2-ol:

- niet te classificeren voor mutageniteit in geslachtscellen;
- te classificeren als een stof die ervan verdacht wordt kankerverwekkend voor de mens te zijn (overeenkomend met een classificatie in categorie 2) en te kenmerken met H351 (verdacht van het veroorzaken van kanker).

executive summary

The Health Council of the Netherlands assessed whether occupational exposure to 1-tert-butoxypropan-2-ol may induce genotoxic effects and may cause cancer. The assessment is performed by the Subcommittee on Classifying carcinogenic substances of the Dutch Expert Committee on Occupational Safety of the Health Council. On the website www.gezondheidsraad.nl, more information can be found on the tasks of this Committee. The composition of the Committee can be found on the last page of this assessment.

About 1-tert-butoxypropan-2-ol

1-tert-butoxypropan-2-ol is used as a solvent in all-purpose cleaning products, coatings, inks, adhesives, lacquers and latex paints. There is a potential for occupational exposure in places where 1-tert-butoxypropan-2-ol is produced or used.

Assessment of genotoxicity and carcinogenicity

Based on the available scientific literature, the Committee assesses the potential genotoxic and carcinogenic properties of the substance in question. If there are indications for such properties, it recommends classifying the substance in two hazard categories, which represent the weight of evidence that the substance is mutagenic in germ cells (a measure for genotoxicity), and that the substance is carcinogenic. The categories are based on the globally harmonized system criteria for assessing hazard categories, which are also used by the European Commission (EU-guideline (EG) 1272/2008). The recommendation can be used by the Minister of Social Affairs en Employment to decide whether the substance should be listed as mutagenic in germ cells and/or carcinogenic.

Evaluation of the data

No data on the carcinogenicity in humans are available. Mutagenicity was observed in vitro. No experiments in germ cells have been conducted. An NTP study did not yield sufficient evidence for carcinogenicity in rats. Mice exposed to 1-tert-butoxypropan-2-ol demonstrated an increase in liver malignancies.

Recommendation

The Committee recommends:

- not to classify 1-tert-butoxypropan-2-ol as a germ cell mutagen;
- to classify the substance as *suspected to be carcinogenic in humans*, which corresponds with a classification in category 2 and label with H351 (suspected of causing cancer).

01 scope

1.1 Background

In the Netherlands, a special policy is in force with respect to occupational use and exposure to carcinogenic substances. In light of 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. In addition to this classification, the Health Council also assesses the genotoxic properties of the substance in question, and proposes a classification on germ cell mutagenicity. A letter of the request can be found on the website of the Health Council.

This report contains the evaluation of the genotoxicity and carcinogenicity of 1-tert-butoxypropan-2-ol.

1.2 Committee and procedure

The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances – hereafter called the Committee – of the Dutch Expert Committee on Occupational Safety of the Health Council. The members of the Committee, including the consulted experts, are listed on the last page of this report.

In May 2021, the President of the Health Council released a draft of the report for public review. The Committee has taken these comments into account in preparing the final version of the report. The comments, and the replies by the committee, can be found on the website of the Health Council.

1.3 Data

The evaluation and recommendation of the Committee are based on scientific data that are publicly available.

A literature summary published by the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM), which was prepared at request of the Health Council, is used as a starting point for the evaluation.[1] The report can be found as a background document on the website of the Health Council. Another important source of information is the evaluation by the International Agency for Research on Cancer. A summary of IARCs' conclusion can be found in Annex A.

Additionally, data published after the publication of the RIVM-document and the IARC Monograph were retrieved from the online databases PubMed (NIH), Web of Science and Embase, using the key words 1-tert-butyoxypropan-2-ol, its chemical synonyms and EEC/CAS-numbers. These terms were combined with general terms in the field genotoxicity, carcinogenicity and occupational exposure. The literature search was

completed by consulting the registration dossiers on 1-tert-butoxypropan-2-ol in the database of the European Chemicals Agency (ECHA), and by consulting websites of various scientific bodies that are known to evaluate the toxicity of chemical substances (*e.g.*, ATSDR, NIOSH, Anses, DFG, AGS, NEG). The last search was performed in February 2021.

In the case of 1-tert-butoxypropan-2-ol, the committee did not find additional data, other than already summarised in the RIVM document and IARC Monograph.

1.4 Quality assessment

For the assessment of the genotoxic and carcinogenic properties of 1-tert-butoxypropan-2-ol, the Committee retrieved the individual studies summarised in the RIVM document and the IARC Monograph. Subsequently, the Committee evaluated the selected studies on their quality. Study quality may vary and therefore, the Committee judges the quality of the study on reliability (quality of methodology and reporting), on the relevance for the purpose of the assessment, and on adequacy (usefulness), according to the current views in the scientific community. The quality evaluation is performed to assess the weight of evidence for an association between substance exposure and genotoxicity and/or risk of cancer development.

1.5 Criteria for classification

The classification systems on mutagenicity and carcinogenicity are based on a weight of evidence assessment, in which more weight is given to evidence obtained from human data than to evidence obtained from animal studies or laboratory data. Furthermore, the weight of evidence depends on the number of reliable studies that show clear associations between exposure and the occurrence of genotoxicity or carcinogenicity. This implies that studies with significant shortcomings contribute to a lesser extent to the overall weight of evidence.

Mutagenic substances. In the European Union, the classification as a mutagenic substance is based on the evidence of mutagenicity in germ cells. For recommending such a classification, the Committee uses the criteria described in Section 3.5 of Annex I of the European regulation No. 1272/2008. Although the criteria mentioned in the regulation are set for substances that are evaluated according to the Classification, Labelling and Packaging (CLP)-regulation, the Committee considers them useful in recommending a mutagenicity classification in germ cells for substances, mixtures and emissions, for which the CLP regulation does not apply. The criteria are based on the Globally Harmonized System and can be universally applied.

Carcinogenic substances. In 2010, the Health Council published a Guideline to the classification of carcinogenic compounds. This is a guide

for classifying substances in terms of their carcinogenic properties, and for assessing the genotoxic mode of action.[2] The criteria and the classification are based on the Globally Harmonized System, which is also used by the European Union for the classification, labelling and packaging of substances and mixtures.[3]

Annexes B and C summarise the classification system for mutagenic substances and carcinogenic substances, respectively, as used by the Committee.

The recommendations for classification are expressed in standard sentences, combined with a category number.

02 general information

Information on the identification, physicochemical properties, monitoring, manufacturing and use, international classifications, and (toxico)kinetics of 1-tert-butoxypropan-2-ol is outlined in the RIVM document (2020) and IARC Monograph (2019).[1,4] A summary is given below.

1-tert-butoxypropan-2-ol (CAS number 57018-52-7; EC/EINECS number 406-180-0; IUPAC name 1-(tert-butoxy)propan-2-ol) is a colourless liquid at room temperature, which readily mixes with water. No internationally validated methods exist to measure exposure levels of the substance in the air or in biological samples. The substance is manufactured to be used as a solvent in, for instance, all-purpose cleaning products, coatings, inks, adhesives, nail polish, lacquers and latex paints.

The European Commission has classified 1-tert-butoxypropan-2-ol as a flammable liquid (H266) that causes serious eye damage upon exposure (H318). In the European Union, no classification or labelling as a mutagen or carcinogen is set. IARC classified 1-tert-butoxypropan as *possibly carcinogenic to humans* (Group 2B).

Data on the kinetics of 1-tert-butoxypropan-2-ol is obtained from animal data only. In rats, which were given radiolabelled 1-tert-butoxypropan-2-ol orally by gavage (concentrations up to 377 mg/kg bw), the substance distributed throughout the whole body. Within 3 days after administration most of the substance was excreted from the body via the kidneys (as glucuronide and to a lesser extent sulphate conjugates), and exhalation. 1-tert-butoxypropan-2-ol is also taken up into the body via inhalation.

03 genotoxicity

3.1 Summary and relevance of the provided information on (germ cell) mutagenicity

Data on genotoxicity are summarised in the RIVM document and the IARC Monograph.[1,4] The original data were published by the NTP.[5]

The Committee did not find additional or new data in the literature.

Table 1 shows a brief summary of the findings.

3.2 Mutagenicity

The NTP performed a bacterial reverse mutation assay, using *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537, and 1-tert-butoxypropan-2-ol at 0 to 10,000 µg 1-tert-butoxypropan-2-ol/plate. Increased mutant frequency was observed only in strain TA97 in the absence of a metabolic activation system. The effect increased with increasing concentration. No cytotoxicity was observed at any of the concentrations tested.

3.3 Clastogenic and aneugenic effects

A chromosomal aberration test in Chinese hamster ovary cells did not show effects. A micronucleus test in mice showed an increased number of cells with micronuclei in females but not in males. The increase is small, but statistically significant. However, it is unknown how the data compare with historical data.

3.4 Sister chromatid exchanges

A sister chromatid exchange test in Chinese hamster ovary cells did not show an increase in sister chromatid exchanges.

3.5 Conclusion on in vitro genotoxicity

Per the Ames test, 1-tert-butoxypropan-2-ol induced mutations in a dose-dependent manner in one of six strains of Salmonella typhimurium.

Table 1 Summary of genotoxicity tests (data obtained from NTP 2004 and Doi et al. 2004).[5]

Assay; microorganism or cell type	Concentration range	Results; Cytotoxicity	Remarks
Ames test; *Salmonella typhimurium* strains: TA97, TA98, TA100, TA1535, TA1537	0, 100, 333, 1,000, 3,333, 10,000 µg/plate; +/-S9; Positive controls: sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537) and 4-nitro-o-phenylenediamine (TA98) (-S9); 2-aminoanthracene (+S9)	Mutations induced in TA97 (-S9): Trial 1:135±3.8 (control), 123±3.8, 131±8.3, 150±5.5, 248±18.3, 325±18.9; no cytotoxicity; Trial 2: 149±0.3 (control), 146±8.4, 163±5.0, 227±22.3, 247±10.7, 318±26.6; no cytotoxicity; No effect in TA97 (+S9), TA98 (+/-S9), TA100 (+/-S9), TA1535 (+/-S9), TA1537 (-S9); No cytotoxicity observed; sufficient exposure confirmed by test result in TA97	Comparable with OECD guideline but 20 min incubation. No statistical analysis performed.
Chromosomal aberration test, Chinese hamster ovary cells; Effect parameter: percentage cells with aberrations	0, 1,081, 2,325, 5,000 µg/ml;+/-S9; Positive control: mitomycin-C (-S9), cyclophosphamide (+S9)	No concentration-related response. Cytotoxicity was not reported.	Statistical analysis conducted on the slopes of the dose-response curve.
Sister chromatid exchange test, Chinese hamster ovary cells; Effect parameter: frequency of SCEs per cell	0, 167, 500, 1,667, 5,000 µg/ml; +/-S9; Positive control: mitomycin-C (-S9), cyclophosphamide (+S9)	No concentration-related response. Cytotoxicity was not reported.	Statistical analysis conducted on the slopes of the dose-response curve and individual dose points.
In vivo mouse micronucleus test (peripheral blood), Mouse, B6C3F1, 10/sex/group; Effect parameters: determination of frequency of micronuclei in 2,000 NCEs; determination of percentage of PCEs	0, 406, 811, 1,622, 3,244, 6,488 mg/m^3; Whole body inhalation; 6h/day, 5 days/week for 14 weeks; Positive control not included	Increase in the frequency of micronucleated NCEs in peripheral blood of females (trend: p=0.021); 0.70±0.15 (control), 0.95±0.20, 0.75±0.20, 0.60±0.18, 1.00±0.15, 1.25±0.17. No effect in males; no effect on percentage of PCEs in males and females. Pathology observed after exposure in high doses.	Statistical analysis using on trend and pairwise comparison between each exposed group and the control group.

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3.6 Summary of human data relevant for germ cell mutagenicity

A review of the literature did not reveal any human data.

3.7 Summary of genotoxicity tests in mammalian somatic or germ cells in vivo

The NTP performed a standard in vivo micronucleus test on male and female mice to study the induction of chromosomal aberrations by 1-tert-butoxypropan-2-ol. The animals inhaled the substance at concentrations of up to 6,488 mg/m^3 (1,200 ppm) for 6 hours per day, 5 days per week for 14 weeks. A micronucleus test was performed on isolated normochromatic erythrocytes (NCEs). In NCEs obtained from exposed female mice, a statistically significant exposure-related trend ($p=0.021$) in the frequency of micronucleated NCEs was observed; the higher the exposure, the higher the frequency. In male mice no effect was found. In addition, 1-tert-butoxypropan-2-ol did not induce an increase in frequency of micronuclei in isolated polychromatic erythrocytes (PCEs) in any of the female or male animals. The lack of effect could indicate that the exposure levels were too low to induce an effect. However, the Committee noted that the authors reported on pathological changes in mice exposed to the highest concentrations. This suggests that the exposure levels were sufficiently high.

3.8 Evaluation on germ cell mutagenicity

Evidence of mutagenicity is limited to one Ames test in one strain of bacteria. 1-tert-butoxypropan-2-ol did not induce chromosomal aberrations in in vitro assays. In vivo, an increase in the frequency of micronucleated NCEs was observed in females. Lack of effects in in vitro test systems may be explained by insufficient exposure. No experiments have been conducted on germ cells.

3.9 Recommendation on the classification for germ cell mutagenicity

The Committee recommends not classifying 1-tert-butoxypropan-2-ol as a germ cell mutagen, due to insufficient evidence.

04 carcinogenicity

4.1 Summary and relevance of the provided information on carcinogenicity

Data on carcinogenicity are summarised in the RIVM document and the IARC Monograph.[1,4] The original data were published by the NTP. The committee did not find additional or new data in the literature.

4.2 Observations in humans

No data on the carcinogenicity of 1-tert-butoxypropan-2-ol in humans are available.

4.3 Animal carcinogenicity studies

The US National Toxicology Programme (NTP) performed a well-conducted carcinogenicity study using rats and mice.[5] Details are summarised in the RIVM document and IARC Monograph.[1] The study was performed according to the OECD guidelines. In short, male and female F344/N rats and B6C3F1 mice (N=50/sex/group) were exposed to 1-tert-butoxypropan-2-ol by whole-body inhalation at concentrations of 0, 406, 1,622 or 6,488 mg/m^3, 6h/day, 5 days per week for 2 years.
The main general observations were: significant reduced survival in male rats at 1,622 mg/m^3; significant reduced body weight in male and female rats, and in female mice, at 6,488 mg/m^3.

4.3.1 Rats

1-tert-butoxypropan-2-ol did not induce a statistically significant increase in carcinomas or other malignant neoplasia in any exposure group (see Table 2). The authors observed a non-significant increase in number of male rats with renal tubule adenomas (in groups exposed to 1,622 or 6,488 mg/m^3), and hepatocellular adenomas (in the group exposed to 1,622 mg/m^3), compared to the chamber and historical controls.

Histopathologic analyses revealed statistically increased number of male rats with hyaline droplet accumulation in the proximal renal tubules, and renal tubule hyperplasia (in groups exposed to 1,622 or 6,488 mg/m^3). The Committee observed that in male rats, the incidence of chronic nephropathy was very high for all exposure groups, including the non-exposed chamber control group. Also, almost all female rats, whether or not exposed, showed chronic nephropathy.

Table 2a Summary incidence of neoplastic lesions in the kidneys and the liver of male F344/N rats after exposure to various concentrations of 1-tert-butoxypropan-2-ol for 2 years.[5]

Type of tissue/lesion	0 mg/m³	406 mg/m³	1,622 mg/m³	6,488 mg/m³	Historical control data
Kidneys / Renal tubule adenoma	1/50 (2%)	2/50 (4%)	5/49 (10%)	4/50 (8%)	3/299 (1±1%; range 0-2%)
Kidneys / Combined renal tubule adenoma and carcinoma	1/50 (2%)	2/50 (4%)	5/49 (10%)	5/50 (10%)	4/299 (1.3±1.0%; range 0-2%)
Liver / Hepatocellular adenoma	3/50 (6%)	0/50	2/49 (4%)	6/50 (12%)	4/299 (1.3±2.4%; range 0-6%)
Liver / Cholangiosarcoma	0/50	0/50	0/49	1/50 (2%)	not reported

Table 2b Summary incidence of neoplastic lesions in the kidneys and the liver of female F344/N rats after exposure to various concentrations of 1-tert-butoxypropan-2-ol for 2 years.[5]

Type of tissue/lesion	0 mg/m³	406 mg/m³	1,622 mg/m³	6,488 mg/m³	Historical control data
Renal tubule adenoma	0/49	0/50	0/50	1/50 (2%)	not reported
Hepatocellular adenoma	1/49 (2%)	0/50	0/50	2/50 (4%)	not reported

The observed hyaline droplets accumulation in the proximal renal tubule epithelium of male rats might indicate that 1-tert-butoxypropan-2-ol induces renal tubule nephropathy and neoplasia by a α2u-globulin mechanism, a mechanism only seen in male rats and not in female rats, other rodents or humans. This would indicate that the findings on kidney effects in male rats is not relevant for humans. Earlier, the NTP performed a 2-week (F344/N and NBR rats), and a 3-month (F344/N rats) study, using male and female animals, to evaluate the possible involvement of a α2u-globulin mechanism as a key event.[5] These studies showed histopathological end-points associated with α2u-globulin nephropathy in F344/N male rats only. Earlier, IARC (1999) established seven criteria that should be met to conclude whether a substance causes kidney tumours in male rats by a α2u-globulin mechanism.[6] Based on the data presented by the NTP, IARC concluded that for 1-tert-butoxypropan three criteria were met (i.e., induction of histopathological changes associated with α2u-globulin accumulation; identification of the accumulating protein as α2u-globulin; and, induction of sustained increases in cell proliferation in the renal cortex). However, the other criteria were not met, in that there were signs of genotoxic activity; nephropathy was not specific for male rats; lack of data on the binding of the substance to α2u-globulin; and, no similar dose-response relationships when tumour development was compared with histopathological end-points associated with α2u-globulin nephropathy. Overall, the Committee is of the opinion that no definite conclusion can be made on a α2u-globulin mechanism as a key event in tumour development in male rats.

The Committee noted that in the highest exposed group, one male rat developed cholangiosarcoma, a malignant tumour of the connective tissue of the bile ducts. From the data it is not extractable whether this type of tumour was caused by exposure to 1-tert-butoxypropan-2-ol, or by

coincidence. No other types of malignant neoplasia in the liver were observed.

Overall, the committee concludes that there is insufficient evidence for carcinogenicity in rats.

4.3.2 Mice

In male and female mice, hepatocellular adenomas and carcinomas were observed in all exposure groups, including in the (non-exposed) chamber control group and historical controls. Only for the adenomas in the groups exposed to the highest concentration, the incidence was statistically significantly increased and showing a concentration-response trend (see Table 3).

In male mice, exposure to 1-tert-butoxypropan-2-ol at a concentration of 6,488 mg/m^3 (the highest concentration tested) resulted in a statistically significant increase in incidence of hepatoblastomas compared to controls, a rare type of malignant tumour. According to the authors, this effect showed a concentration-response trend. This type of tumour was also observed in male mice in the second highest exposure group, and in female mice in the highest exposure group. However, the incidence in these two groups did not reach statistical significance.

Table 3a Summary incidence of hepatic neoplastic lesions in male B6C3F1 mice after exposure to 1-tert-butoxypropan-2-ol for two-years.[5]

Type of tissue / lesion	0 mg/m^3	406 mg/m^3	1,622 mg/m^3	6,488 mg/m^3	Historical control data
Liver / Hepatocellular adenomas	18/50 (36%)	23/49 (47%)	26/50 (52%)	36/50 (72%)**	95/250 (38±6.8%; range 30-46%)
Liver / Hepatocellular carcinomas	9/50 (18%)	8/49 (16%)	13/50 (26%)	11/50 (22%)	60/250 (24±5.8%; range 18-32%)
Liver / Combined hepatocellular adenoma and carcinoma	25/50 (50%)	26/49 (53%)	33/50 (66%)	41/50 (82%)**	139/250 (55.6±7.3%; range 50-68%)
Liver / Hepatoblastoma	0/50	0/49	1/50 (2%)	5/50 (10%)*	0/250

* Significantly different ($p \leq 0.05$) from the chamber control group by the Poly-3 test
** Significantly different ($p \leq 0.01$) from the chamber control group by the Poly-3 test

Table 3b Summary incidence of hepatic neoplastic lesions in female B6C3F1 mice after exposure to 1-tert-butoxypropan-2-ol for two-years.[5]

Type of tissue / lesion	0 mg/m^3	406 mg/m^3	1,622 mg/m^3	6,488 mg/m^3	Historical control data
Liver / Hepatocellular adenomas	14/49 (28%)	8/50 (16%)	10/50 (20%)	37/49 (74%)**	48/248 (19.4±6.9%; range 12-29%)
Liver / Hepatocellular carcinomas	4/49 (8%)	8/50 (16%)	7/50 (14%)	10/49 (20%)	26/248 (10.5±2.1%; range 8-12%)
Liver / Combined hepatocellular adenoma and carcinoma	18/49 (37%)	14/50 (28%)	16/50 (32%)	41/49 (84%)**	72/248 (29±6.8%; range 22-37%)
Liver / Hepatoblastoma	0/49	0/50	0/50	2/49 (4%)	0/248

** Significantly different ($p \leq 0.01$) from the chamber control group by the Poly-3 test

4.4 Evaluation on the carcinogenicity

No data on the carcinogenicity of 1-tert-butoxypropan-2-ol in humans is available. Therefore, category 1A (*known to be carcinogenic In man*) is not applicable.

Classification in category 1B (*presumed to be carcinogenic in man*) requires a causal relationship between the substance and an increased incidence of malignant neoplasm in two or more animal species. In a well performed study by the NTP, evidence for carcinogenicity of 1-tert-butoxypropan-2-ol in rats was insufficient, whereas in mice sufficient evidence for carcinogenicity (hepatoblastomas) was found. Therefore, 1-tert-butoxypropan-2-ol does not meet the criteria to classify the substance in carcinogenic category 1B.

A substance can be classified in category 2 (*suspected to be carcinogenic to man*) on the base of limited evidence of carcinogenicity in human or animal studies together with additional considerations. 1-tert-butoxypropan-2-ol clearly induced exposure-related hepatoblastomas in male mice. In humans, hepatoblastoma is a rare tumour type normally found in children only. Despite a lack of epidemiological data on occupational exposure and disease development, the committee considers the fact that exposure to 1-tert-butoxypropan-2-ol can induce an otherwise rare tumour type in mice an indication of its carcinogenic potential in humans. Therefore, the committee finds it justified to classify the substance as suspected in category 2.

4.5 Recommendation on the classification for carcinogenicity

The committee recommends classifying 1-tert-butoxypropan-2-ol as *suspected to be carcinogenic to man*, which corresponds with category 2 for carcinogenicity, and to label 1-tert-butoxypropan-2-il with H351 (suspected of causing cancer).

references

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3 Official Journal of the European Union. *Regulation (EC) No 1272/2008 - classification, labelling and packaging of substances and mixtures (CLP) on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.* 2008. p. 1-1355.

4 Iarc Working Group on the Evaluation of Carcinogenic Risks to Humans. *Some chemicals that cause tumours of the urinary tract in rodents*. International Agency for Research on Cancer © International Agency for Research on Cancer 2019; 119.

5 Doi AM, Roycroft JH, Herbert RA, Haseman JK, Hailey JR, Chou BJ, et al. *Inhalation toxicology and carcinogenesis studies of propylene glycol mono-t-butyl ether in rats and mice.* Toxicology 2004; 199(1): 1-22.

6 IARC Scientific Publications. *Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis.* 1999; 147: 1-111.

annex A

IARC evaluation and conclusion

Cited from IARC Monographs, Volume 119, pages 49-50 (2019).

Human data

No data were available to the Working Group.

Animal data

In one well-conducted study that complied with good laboratory practice (GLP) in male and female mice treated by whole-body inhalation, 1-tert-butoxypropan-2-ol significantly increased the incidence (with a significant positive trend) of hepatocellular adenoma, of hepatocellular adenoma or carcinoma (combined), and of hepatoblastoma in males; and significantly increased the incidence (with a significant positive trend) of hepatocellular adenoma, and of hepatocellular adenoma or carcinoma (combined) in females.

In a second well-conducted GLP study in male and female rats treated by whole-body inhalation, 1-tert-butoxypropan-2-ol caused a significant positive trend in the incidence of hepatocellular adenoma, and the occurrence of rare neoplasms of the renal tubules in males.

Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 1-tert-butoxypropan-2-ol.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1-tert-butoxypropan-2-ol.

Overall evaluation

1-tert-butoxypropan-2-ol is possibly carcinogenic to humans (Group 2B).

annex B
classification on germ cell mutagenicity

Source: Section 3.5 (Germ cell mutagenicity) of Regulation No. 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances and mixtures (Version November 14, 2020).[3]

3.5.1. Definitions and general considerations

3.5.1.1. A mutation means a permanent change in the amount or structure of the genetic material in a cell. The term ‘mutation’ applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term ‘mutagenic’ and ‘mutagen’ will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

3.5.1.2. The more general terms ‘genotoxic’ and ‘genotoxicity’ apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxicity test results are usually taken as indicators for mutagenic effects.

3.5.2. Classification criteria for substances

3.5.2.1. This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class.

3.5.2.2. For the purpose of classification for germ cell mutagenicity, substances are allocated to one of two categories as shown in Table 3.5.1.

Table 3.5.1 Hazard categories for germ cell mutagens.

Categories	Criteria
CATEGORY 1:	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.
Category 1A:	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
Category 1B:	The classification in Category 1B is based on: • positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or • positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/ genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or • positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
CATEGORY 2:	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans The classification in Category 2 is based on: positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: • somatic cell mutagenicity tests in vivo, in mammals; or • other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays. *Note*: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

3.5.2.3 Specific considerations for classification of substances as germ cell mutagens

3.5.2.3.1. To arrive at a classification, test results are considered from experiments determining mutagenic and/or genotoxic effects in germ and/ or somatic cells of exposed animals. Mutagenic and/or genotoxic effects determined in in vitro tests shall also be considered.

3.5.2.3.2. The system is hazard based, classifying substances on the basis of their intrinsic ability to induce mutations in germ cells. The scheme is, therefore, not meant for the (quantitative) risk assessment of substances.

3.5.2.3.3. Classification for heritable effects in human germ cells is made on the basis of well conducted, sufficiently validated tests, preferably as described in Regulation (EC) No 440/2008 adopted in accordance with Article 13(3) of Regulation (EC) No 1907/2006 (‘Test Method Regulation’) such as those listed in the following paragraphs. Evaluation of the test results shall be done using expert judgement and all the available evidence shall be weighed in arriving at a classification.

3.5.2.3.4. In vivo heritable germ cell mutagenicity tests, such as:

- rodent dominant lethal mutation test;
- mouse heritable translocation assay.

3.5.2.3.5. In vivo somatic cell mutagenicity tests, such as:

- mammalian bone marrow chromosome aberration test;
- mouse spot test;
- mammalian erythrocyte micronucleus test.

3.5.2.3.6. Mutagenicity/genotoxicity tests in germ cells, such as:

a. mutagenicity tests:

- mammalian spermatogonial chromosome aberration test;
- spermatid micronucleus assay;

b. genotoxicity tests:

- sister chromatid exchange analysis in spermatogonia;
- unscheduled DNA synthesis test (UDS) in testicular cells.

3.5.2.3.7. Genotoxicity tests in somatic cells such as:

- liver Unscheduled synthesis test (UDS) in vivo;
- mammalian bone marrow Sister Chromatid Exchanges (SCE).

3.5.2.3.8. In vitro mutagenicity tests such as:

- in vitro mammalian chromosome aberration test;
- in vitro mammalian cell gene mutation test;
- bacterial reverse mutation tests.

3.5.2.3.9. The classification of individual substances shall be based on the total weight of evidence available, using expert judgement (See 1.1.1). In those instances where a single well-conducted test is used for classification, it shall provide clear and unambiguously positive results. If new, well validated, tests arise these may also be used in the total weight of evidence to be considered. The relevance of the route of exposure used in the study of the substance compared to the route of human exposure shall also be taken into account.

3.5.3 Classification criteria for mixtures

3.5.3.1. Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.5.3.1.1. The mixture shall be classified as a mutagen when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 mutagen and is present at or above the appropriate generic concentration limit as shown in Table 3.5.2 for Category 1A, Category 1B and Category 2 respectively.

Table 3.5.2 Generic concentration limits of ingredients of a mixture classified as germ cell mutagens that trigger classification of the mixture.

Ingredient classified as:	Concentration limits triggering classification of a mixture as:		
	Category 1A mutagen	Category 1B mutagen	Category 2 mutagen
Category 1A mutagen	≥0,1 %	-	-
Category 1B mutagen	-	≥0,1 %	-
Category 2 mutagen	-	-	≥1,0 %

Note. The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).

3.5.3.2. Classification of mixtures when data are available for the complete mixture

3.5.3.2.1. Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients classified as germ cell mutagens. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of germ cell mutagenicity test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.5.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles

3.5.3.3.1. Where the mixture itself has not been tested to determine its germ cell mutagenicity hazard, but there are sufficient data on the individual ingredients and similar tested mixtures (subject to paragraph

3.5.3.2.1), to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.5.4. Hazard communication

3.5.4.1. Label elements shall be used in accordance with Table 3.5.3, for substances or mixtures meeting the criteria for classification in this hazard class.

Table 3.5.3 Label elements of germ cell mutagenicity.

Classification	Category 1A or Category 1B	Category 2
GHS Pictograms		
Signal word	Danger	Warning
Hazard Statement	H340: May cause genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H341: Suspected of causing genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
Precautionary Statement Prevention	P201, P202, P281	P201, P202, P281
Precautionary Statement Response	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405
Precautionary Statement Disposal	P501	P501

3.5.5. Additional classification considerations

It is increasingly accepted that the process of chemical-induced tumorigenesis in humans and animals involves genetic changes for example in proto-oncogenes and/or tumour suppresser genes of somatic cells. Therefore, the demonstration of mutagenic properties of substances

in somatic and/or germ cells of mammals in vivo may have implications for the potential classification of these substances as carcinogens (see also Carcinogenicity, section 3.6, paragraph 3.6.2.2.6).

annex C

classification on carcinogenicity

In 2010, the Committee published a guideline for classifying substances in terms of their carcinogenic properties, and for assessing their genotoxicity.[2] The classification on carcinogenic properties is based on the Globally Harmonized System, which is also used by the European Union for the classification, labelling and packaging of substances and mixtures (Regulation EC 1272/2008, Section 3.6 Carcinogenicity).

The Committee expresses its conclusions in standard phrases:

Category	Judgement by the Committee	Comparable with EU Category
1A	*The compound is known to be carcinogenic to humans.* • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1A
1B	*The compound is presumed to be carcinogenic to humans.* • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1B
2	*The compound is suspected to be carcinogenic to man.*	2
(3)	*The available data are insufficient to evaluate the carcinogenic properties of the compound.*	not applicable
(4)	*The compound is probably not carcinogenic to man.*	not applicable

Committee and consulted expert

The membership of the Subcommittee on Classifying Carcinogenic Substances for the evaluation of the genotoxicity and carcinogenicity of 1-tert-butoxypropan-2-ol

- Prof. Dr. H.P.J. te Riele, Professor of molecular biology, VU University Amsterdam, and Netherlands Cancer Institute, Amsterdam, *chairman*
- Dr. R.W.L. Godschalk, genetic toxicologist and molecular epidemiologist, Maastricht University
- Dr. E. de Rijk, Toxicologic pathologist, Charles River Laboratories, ‘s Hertogenbosch
- Dr. J.J. Vlaanderen, Epidemiologist, Institute for Risk Assessment Sciences, Utrecht
- Dr. J. van Benthem, Genetic toxicologist, RIVM, Bilthoven, *structurally consulted expert*

Observer

- M. Woutersen, Bureau REACH, RIVM, Bilthoven

Scientific secretaries

- Dr. D. Dezentje, The Health Council of the Netherlands, The Hague (until 1 September 2021)
- Dr. E.E.J. Kasteel, The Health Council of the Netherlands, The Hague
- Dr. J.M. Rijnkels, The Health Council of the Netherlands, The Hague

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).
The Health Council receives most requests for advice from the Ministers of Health, Welfare and Sport, Infrastructure and Water Management, Social Affairs and Employment, and Agriculture, Nature and Food Quality. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.
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The reports are available to the public.

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