1-bromopropane

Evaluation of the carcinogenicity and genotoxicity

To: the Minister of Social Affairs and Employment
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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-broompropaan 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 informatie over de taken van deze vaste commissie van de Gezondheidsraad. De samenstelling van de commissie is te vinden achterin dit advies.

Over 1-broompropaan

De stof 1-broompropaan wordt gebruikt bij de productie van onder andere pesticiden, smaakstoffen en geurstoffen. Daarnaast wordt 1-broompropaan onder meer gebruikt als schoonmaakmiddel, lijmspray en als oplosmiddel voor vetten, wax en hars.

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

Er zijn geen onderzoeksgegevens beschikbaar over mutageniteit van 1-broompropaan bij mensen. Uit laboratoriumstudies en dierstudies komen onvoldoende aanwijzingen dat 1-broompropaan mutageen is. In een bacteriële mutageniteitstest vertoonde 1-broompropaan weliswaar mutageniteit in twee stammen, maar in een latere studie werd dit niet gevonden. In één test met cellen van muizen (muizenlymfoom test) vertoonde 1-broompropaan mutageniteit. In andere genotoxiciteitstests (zowel laboratorium-

studies als dierexperimenten) werden geen mutagene effecten gevonden.

Er zijn geen onderzoeksgegevens beschikbaar over gevallen van kanker bij mensen door blootstelling aan 1-broompropaan. In twee dierexperimenten veroorzaakte de stof tumor-vorming. In ratten werden goedaardige tumoren waargenomen. Er werden geen overtuigende aanwijzingen gevonden voor kwaadaardige tumorvorming in ratten. In muizen werd een toename waargenomen van zowel goedaardige als kwaadaardige longtumoren.

Advies

De commissie adviseert om 1-broompropaan:

- niet te classificeren voor mutageniteit in geslachtscellen;
- te classificeren als een stof die ervan verdacht wordt kankerverwekkend te zijn voor de mens (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-bromopropane 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-bromopropane

1-bromopropane is used as a chemical intermediate in the production of amongst others pesticides, flavours and fragrances. Moreover, 1-bromopropane is amongst others used for immersion cleaning, spray adhesive applications and solvent for fats, waxes and resins.

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

In a bacterial mutagenicity test, one study reported mutagenicity of 1-bromopropane in two strains. However, other studies did not confirm these findings. A mouse lymphoma assay also showed mutagenic effects of 1-bromopropane. No effects were found in other genotoxicity (both laboratory and animal) tests.

No data on the carcinogenicity of 1-bromopropane in humans were available. A US National Toxicology Program study showed an increase in benign tumours in rats, but did not yield sufficient evidence for malignant tumour development. However, mice exposed to 1-bromopropane demonstrated an increase in both benign and malignant lung tumours.

Recommendation

The Committee recommends:

- not to classify 1-bromopropane 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 of 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-bromopropane.

1.2 Committee and procedure

The assessment 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 (IARC). A summary of IARC's 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-bromopropane, its chemical synonyms and EEC/CAS-numbers. These terms were combined with general terms regarding genotoxicity, carcinogenicity and occupational exposure. The literature search was completed by consulting the registration dossiers on 1-bromopropane 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 March 2021.

In the case of 1-bromopropane, 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-bromopropane, 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. 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-bromopropane is outlined in the RIVM document (2020) and IARC Monograph (2016). A summary is given below.

1-bromopropane (CAS number 106-94-5; EC/EINECS number 203-445-0) is a colourless, volatile liquid at room temperature which is slightly soluble in water (2450 mg/L). Several analytical methods are available for measuring 1-bromopropane in air using gas chromatography. Internal exposure can be assessed by measuring levels of 1-bromopropane, bromide ion and 1-bromopropane metabolites in urine. 1-bromopropane is used primarily as a chemical intermediate in the production of pesticides, flavours, fragrances, quaternary ammonium compounds, and pharmaceuticals in closed processes. Moreover, 1-bromopropane is used as a solvent for fats, waxes and resins and for vapour degreasing and immersion cleaning, fabric dry cleaning, liquid and spray adhesive applications, and aerosol spray products.

The European Commission has classified 1-bromopropane as a highly flammable liquid and vapour (H225) that causes skin irritation (H315) and serious eye irritation (H319). Furthermore, it may cause respiratory irritation (H335), drowsiness or dizziness (H336) and it may damage fertility and/or the unborn child (H360Fd). Finally, it may cause damage to organs through prolonged or repeated exposure (H373). IARC classified 1-bromopropane as *possibly carcinogenic to humans* (Group 2B).

Data on the kinetics of 1-bromopropane are obtained from both human studies and animal studies. 1-bromopropane can be absorbed dermally and via inhalation; dermal absorption is fast but dependent on type and duration of exposure. No human data are available on the distribution of 1-bromopropane in the body. The major metabolite of 1-bromopropane is *N*-acetyl-*S*-propylcysteine, which was detected in urine, and increased with increasing exposure levels. 1-bromopropane can also be excreted unchanged and as bromide ion. Both are significantly correlated with exposure levels.

Studies in rats and mice show that 1-bromopropane is well absorbed after inhalation, and oral and intraperitoneal administration. In the urine of rodents, no unchanged 1-bromopropane was reported. The major metabolic pathways are conjugation with GSH and oxidation reactions. Several reactive metabolites of 1-bromopropane have been identified, that are possibly carcinogenic.[4]

03 genotoxicity

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

Data on mutagenicity are summarised in the RIVM document[1] and the IARC Monograph.[5] The Committee did not find additional or new data in the literature. Tables 1 to 5 show a summary of the findings.

3.2 Mutagenicity

An overview of mutagenicity studies is presented in Table 1. The study of Barber et al. (1981) showed a dose-related statistically significant increase in mutant frequency compared to control in Salmonella typhimurium strains TA100 and TA1535, in both the presence and absence of a metabolic activation system (S9 mix). Concentrations of 0-20.3 µmol (≈2,497 µg) 1-bromopropane/plate were used. No increased mutant frequency compared to control was observed for TA98, data on cytotoxicity were not reported.

These increases in mutant frequency were not confirmed in other studies. The US NTP performed two independent bacterial reverse mutation assays, using *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535 and *Escherichia coli* strain WP2 uvrA/pKM101, with and without metabolic activation system (S9 mix) and 0-10,000 µg 1-bromopropane/plate. In these assays, no biologically relevant increase in revertants compared to controls was observed. Cytotoxicity was observed at the highest concentration.

The REACH registration file presented 3 additional in vitro mutagenicity tests in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP2 uvrA. No biologically relevant increase in revertants compared to controls was observed in these studies in both the presence and absence of metabolic activation, but only a limited description of the design and/or outcome of the study was available. Cytotoxicity was observed in one of these studies.

It is important to consider the volatility of 1-bromopropane when performing in vitro tests. The study of Barber et al. (1981) specifically designed an Ames test for highly volatile substances. The US NTP study refers to a protocol by Zeiger et al., (1992)[6] for their assays, which mentions that volatile chemicals were incubated in capped tubes. However, no clear description is given in the US NTP document itself. The OECD guideline (used in the REACH studies) also demands using appropriate methods, such as sealed vessels, for testing volatile substances.

In the REACH registration file also a mouse lymphoma assay was observed. An increase in mutant frequency and an increase in the number of small colonies was observed at cytotoxic concentrations.

Table 1 Summary of in vitro mutagenicity tests.

Assay; microorganism or cell type	Concentration range	Results; cytotoxicity	Remarks
Ames test; *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535	0, 33, 100, 333, 1,000, 3,333, 10,000 µg/plate; +/-S9[a,b]; Positive controls: Sodium azide (TA100 and TA1535); 9-aminoacridine (TA97); 4-nitro-o-phenylenediamine (TA98) (-S9); 2-aminoanthracene (+S9)	No concentration-related response for all strains +/-S9; 10,000 µg/plate was cytotoxic in some trials	NTP, 2011 Comparable with OECD guideline, but 20 min incubation. No statistical analysis performed.
Ames test; *Salmonella typhimurium* strains TA98 and TA100	0, 500, 1,000, 1,500, 2,500, 3,500, 5,000, 7,500 (+S9), 10,000 (+S9) µg/plate; +/-S9[a]; Positive controls: Sodium azide (TA100); 4-nitro-o-phenylenediamine (TA98) (-S9); 2-aminoanthracene (+S9)	No concentration-related response for all strains +/-S9; 10,000 µg/plate was cytotoxic in some trials	NTP, 2011 Comparable with OECD guideline but 20 min incubation. No statistical analysis performed.
Ames test; *Escherichia coli* strain WP2 uvrA/pKM101	0, 500 (-S9), 1,500 (-S9), 2,500, 3,500, 5,000, 7,500 (+S9), 10,000 (+S9) µg/plate; +/-S9[a]; Positive controls: Methyl methanesulfonate (-S9); 2-aminoanthracene (+S9)	No concentration-related response for all strains +/-S9; 10,000 µg/plate was cytotoxic in some trials	NTP, 2011 Comparable with OECD guideline but 20 min incubation. No statistical analysis performed.
Ames test; *Salmonella typhimurium* strains TA98, TA100 and TA1535	0, 1.1, 2.3, 4.9, 9.0, 20.3 µmol/plate; +/-S9[a] (0, 135, 283, 603, 1,107, 2,497 µg/plate); Positive controls: Methyl-N-nitro-N'-nitrosoguianidine (TA100, TA1535), ICR-191 (TA98) (-S9); 2-aminoanthracene (+S9)	His+ revertants per plate TA100: -S9: 85 (control), 153, 174, - , 527, 1,616 +S9: 96 (control), 127, 102, 236, 486, 1,556; TA1535: -S9: 20 (control), 22, 30, 63, 364, 1,768 +S9: 19 (control), 23, 31, 104, - , 1,584; No dose-related response for TA98 +/-S9; Cytotoxicity was not reported	Barber et al. (1981) Closed system incubation for highly volatile substances. No statistical analysis performed.
Ames test; *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538	0, 100, 500, 1,000, 5,000, 10,000 µg/plate;+/-S9[a]; Positive controls: yes, but not specified; Negative control: 1% DMSO	No concentration-related response for all strains +/-S9; Cytotoxicity seen at 5,000 µg/plate (+S9) and 10,000 µg/plate (+S9)	Anonymous, 1994 REACH registration dossier. Comparable to OECD guideline, limited reporting (original study report not available). No statistical analysis performed.
Ames test; *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538	50, 250, 1,250, 2,500, 5,000 µg/plate; +/-S9[c]; Positive controls: yes, but not specified which substance; Negative control: DMSO	No concentration-related response for all strains +/-S9; No cytotoxicity	Anonymous, 1991 REACH registration dossier. Comparable to OECD guideline, limited reporting (original study report not available). No statistical analysis performed.

Assay; microorganism or cell type	Concentration range	Results; cytotoxicity	Remarks
Ames test; *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, *Escherichia coli* strain WP2 *uvrA*	0, 10, 100, 313, 500, 625, 1,000, 1,250, 2,500 and 5,000 µg/plate; +/-S9[c]; Positive controls: 2-aminoanthracene, 9-aminoacridine and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide; Negative control: yes, but not specified	No concentration-related response for all strains, +/-S9; Cytotoxicity was not determined.	Anonymous, 1998 REACH registration dossier. Comparable to OECD guideline, limited reporting (original study report not available). No statistical analysis performed.
Mouse lymphoma assay; Viability: Relative (to vehicle control) Cloning Efficiency (RCE_0), L5178Y cells	-S9: 0, 125, 250, 500, 1,000, 1,250, 1,500 µg/mL; +S9[a]: 0, 125, 250, 500, 1,000, 1,500, 2,000, 2,500 µg/mL; Positive controls: Methylmethanesulfonate (-S9); Cyclophosphamide (+S9); Negative control: DMSO	- S9: statistically significant increase in mutant frequency compared to control, increase in the number of small colonies at dose levels between 1,000 and 1,500 µg/mL; +S9: no increase in the mutant frequency in the first experiment. A statistically significant increase in the mutant frequency together with an increase in the number of small colonies at 1,500 and 2,000 µg/mL in the second experiment. Viability: 2,500 µg/mL; all cells dead; 3h; +/-S9; RCE_0: -S9: 1,500 µg/mL: 21/33%; 1,250 µg/mL: 46% +S9: 2,000 µg/mL: 59/9%; 1,500 µg/mL: 36%	Anonymous, 1996 REACH registration dossier. Comparable to OECD guideline, limited reporting (original study report not available).

[a] Metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat liver.
[b] Metabolic activation enzymes and cofactors from Aroclor 1254-induced male Syrian hamster liver.
[c] Metabolic activation: S9 mix derived from rat liver.

3.3 Clastogenic and aneugenic effects

The increase in small colonies reported for the mouse lymphoma assay may point towards a clastogenic effect of 1-bromopropane.

3.4 Conclusion on in vitro genotoxicity

1-bromopropane can induce mutagenic effects, based on an increase in revertants (compared to controls) observed in one study. This is supported by the increased mutant frequency and increase in small colonies seen in the mouse lymphoma test.

3.5 Summary of human data relevant for germ cell mutagenicity

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

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

In an in vivo transgenic rodent mutation assay, B6C3F1 Big Blue® transgenic mice were exposed via whole body inhalation to 0, 62,8, 125, 258 ppm (corresponding to 0, 316, 629, 1,298 mg/m^3) 1-bromopropane for 6h/day, 5 days per week for 4 weeks (Table 2). No biologically relevant increase in mutant frequency and no treatment-related clinical signs were observed.

Two rodent dominant lethal assays were performed, one in male ICR mice and one in male Sprague-Dawley rats (Table 2). A dominant lethal test investigates if a substance can cause embryonal death that results from chromosomal aberrations in germ cells. Male animals are exposed to the substance and mated to untreated females. The females are then sacrificed and the number of implants and live and dead embryos are determined. The dominant lethality is determined by comparing live implants per female in the treated group with the live implants per female in the control groups. Mice and rats were exposed to 1-bromopropane via oral gavage at 0, 300 and 600 mg/kg bw/day for 10 consecutive days (mice) or 0 and 400 mg/kg bw/day for 5 consecutive days (rats). No effects on dominant lethality were observed in either species.

The NTP performed a standard in vivo micronucleus test on male and female B6C3F1/N mice to study the induction of chromosomal aberrations by 1-bromopropane (Table 3). The animals were exposed via whole body inhalation at concentrations up to 497 ppm (2,515 mg/m^3) for 6h/day, 5 days per week for 3 months. A micronucleus test was performed on isolated normochromatic erythrocytes (NCEs). No biologically relevant increase in the frequency of micronucelated NCEs was observed in peripheral blood of male and female mice. In addition, 1-bromopropane did not affect the percentage of polychromatic erythrocytes (PCE) of male and female mice. However, according to the Committee, the applied

concentrations were probably not high enough, as no treatment-related bone marrow toxicity was observed upon exposure.

The REACH registration file reported an in vivo micronucleus test on male and female Sprague-Dawley rats. The animals were exposed via whole body inhalation at concentrations up to 1,800 ppm (9,054 mg/m^3) 1-bromopropane. No biologically relevant increase in the frequency of micronucleated PCEs was observed. It is not clear to the Committee if the applied concentrations were high enough, as data on bone marrow toxicity was not reported.

The REACH registration file also reported an in vivo mouse micronucleus test. Male and female Swiss mice were exposed intraperitoneally to 1-bromopropane at concentrations up to 800 mg/kg bw/day for 2 consecutive days. The authors report a lower PCE/NCE ratio in vehicle controls than typically observed. Five out of eight male mice died at 800 mg/kg bw in one experiment, six out of eight male mice died at the same concentration in another experiment. No biologically relevant increase in the frequency of micronucleated PCE and no change in PCE/NCE ratio was observed.

Table 2 Summary of in vivo *animal* mutagenicity tests.

Experimental period and design; species	Concentration/Dose and route	Observations and results	Remarks
In vivo transgenic rodent mutation assay; Mouse, B6C3F1, Big Blue® transgenic; female; 7/group	0, 62.8, 125, 258 ppm (0, 316, 629, 1,298 mg/m^3); Whole body inhalation; 6h/day; 5 days/week for 4 weeks; Positive control: N-Ethyl-N-nitrosourea (ENU), 40 mg/kg bw/day for 3 consecutive days, oral gavage	No significant increase in mutant frequency; no treatment-related clinical signs.	Stelljes et al., 2019; Anonymous, 2016 REACH registration dossier. According to OECD guideline. Applied concentrations were probably nog high enough; no general toxicity was noticed.
Rodent dominant lethal assay; Mating during 6 weeks. Mouse, ICR, male; 20/group; 15/group for positive control Clinical signs, gross findings, mating index, gestation index, the numbers of corpora lutea, implantations, live fetuses, resorptions and dead fetuses, pre- and post-implantation losses, and dominant lethal mutation rate were examined.	0, 300, 600 mg/kg bw/day for 10 consecutive days (oral gavage); Positive control: cyclophosphamide (i.p. 40 mg/kg bw/day for 5 days)	No effects on dominant lethality; no treatment-related clinical signs.	Yu et al., 2008 Applied dose levels were probably not high enough; no general toxicity was noticed.
Rodent dominant lethal assay; Mating during 8 weeks. Rat, Sprague-Dawley, male; 15/exposure group Number of corpora lutea, implants, live embryos and early and late embryonic death was examined and dominant lethality rate was calculated.	0, 400 mg/kg bw/day for 5 consecutive days (oral gavage); Positive control: 1,2-Dibromo-3-chloropropane (oral gavage, 50 mg/kg bw/day)	No effects on dominant lethality; no report about treatment-related clinical signs.	Saito-Suzuki et al., 1982 Not clear whether applied dose levels were high enough; findings on general toxicity were not reported.

Table 3 Summary of in vivo *animal* cytogenetic tests.

Experimental period and design; species	Concentration/Dose and route	Observations and results	Remarks
In vivo mouse micronucleus test (peripheral blood) Mouse, B6C3F1/N; 10/sex/ group; Effect parameters: determination of frequency of micronuclei in 2,000 NCEs; determination of percentage of PCEs per 1,000 erythrocytes.	0, 62.1, 124, 247, 497 ppm (0, 314, 629, 1,258, 2,515 mg/m^3); Whole body inhalation; 6h/day, 5 days/week for 3 months; Positive control: not included	No biologically relevant increases in the frequencies of micronucleated NCEs. No biologically relevant effect on percentages of PCEs.	NTP, 2011
In vivo rat micronucleus test (bone marrow) Rat, Sprague-Dawley; 10/sex/group; Effect parameter: frequency of micronuclei in 1,000 PCEs.	0, 50, 300 and 1,800 ppm (0, 252, 1,509 and 9,054 mg/m^3); Whole body inhalation; 6h/day, 5 days/week for 8 weeks; Positive control: not included	No biologically relevant increases in the frequencies of micronucleated PCEs.	Anonymous, 1998 REACH registration dossier. Original study report not available. Not clear whether applied dose levels were high enough; findings on (bone marrow) toxicity were not reported.
In vivo mouse micronucleus test (bone marrow) Mouse, Swiss; 5-8/sex/group; Effect parameters: determination of frequency of micronuclei in 2,000 PCEs; additionally, determination of ratio of PCE and NCE by scoring of 1,000 erythrocytes.	0, 100, 400, 600, 800 mg/kg bw/day; Intraperitoneal injection at 2 consecutive days; Positive control: cyclophosphamide (oral, 50 mg/kg bw/day)	PCE/NCE ratio in vehicle controls lower than typically observed. 800 mg/kg bw: Mortality 5/8 (exp. 1), 6/8 (exp.2) males. No biologically relevant increase in frequencies of micronucleated PCE and no biologically relevant change in PCE/NCE ratio in females (males not considered). 600 mg/kg bw: No biologically relevant increase in frequencies of micronucleated PCE and no biologically relevant change in PCE/NCE ratio.	Anonymous, 1995a REACH registration dossier. According to OECD guideline.

3.7 Summary of additional data

Effects of 1-bromopropane on DNA single strand breaks and DNA repair were measured in a human hepatoma cell line (HepG2) at concentrations of 25 to 500 ppm 1-bromopropane (Table 4, Hasspieler et al. 2006). No biologically relevant increase compared to control was observed on induction of DNA strand breaks and DNA repair. Cytotoxicity was observed at 500 ppm.

An in vitro comet assay was performed by Toraason et al. in human leukocytes of a single donor. The cells were exposed to 0-1 mM 1-bromopropane up to 8h. Significant increases in tail moment were observed at 1 mM 1-bromopropane after exposure for 4h and 8h. An increase in number of apoptotic cells was observed at concentrations of 0.1 mM and 1 mM.

Nepal et al. observed in vitro and in vivo DNA-adducts and GSH-adducts after exposure to 1-bromopropane (Table 4 and 5). In vitro, N^7-propylquanine was formed after exposure to up to 1 mg 1-bromopropane/mL up to 6h, independent of liver homogenate presence. GSH depletion and S-propyl GSH were detected after exposure to 1 mg 1-bromopropane/mL for 2 hours, but only in the presence of active liver homogenate. In vivo in male Sprague-Dawley rats, N^7-propylquanine, GSH depletion and S-propyl GSH formation were detected after intraperitoneal administration of up to 1,000 mg 1-bromopropane/kg bw for 3 consecutive days. N^7-propylquanine was formed in a dose- and time-dependent manner in liver>spleen>testes>lung. GSH depletion was mainly seen in liver, kidney and testes; S-propyl GSH formation in liver>testes>spleen>kidney>lung> heart.

In an in vivo human study, 64 workers exposed to 1-bromopropane were assessed for DNA damage and DNA strand breaks using a comet assay (Toraason et al., Table 5). Exposure of workers (18 male, 46 female) to 1-bromopropane was assessed for 1-3 days up to 8h/day to calculate an 8h TWA. No significant differences in level of DNA damage between high (up to 418 mg/m^3) and low (up to 25 mg/m^3) exposure groups were encountered.

Table 4 Summary of additional data on genotoxicity (in vitro).

Method; microorganism or cell type	Concentration range	Results; cytotoxicity	Remarks
DNA single strand breaks, DNA repair; Human hepatoma cell line (HepG2)	25 to 500 ppm; Positive control: 4-nitroquinoline *N*-oxide; Negative control: solvent	No biologically relevant increase in DNA strand breaks and no biologically relevant increase in DNA repair; 500 ppm: ±75% cell survival.	Hasspieler et al., 2006
In vitro comet assay; Human leukocytes derived from one donor	0, 0.01, 0.1, 1 mM 8h incubation; 1, 2, 4, 8h incubation for 1 mM; Positive control: 0.5, 1, or 2Gy of X-rays at 1 Gy/min; Negative control: DMSO	Significant increase in tail moment at 1mM upon 4h and 8h incubation; Number of apoptotic cells increased ≥0.1 mM.	Toraason et al, 2006
In vitro DNA-adduct (N^7-propyl guanine) formation, In vitro GSH-adduct (S-propyl GSH formation; Calf thymus DNA, Free GSH	0, 0.5, 1 mg/mL (DNA); 1 mg/mL (GSH); +/- liver homogenate	Formation of N^7-propyl guanine +/- liver homogenate; GSH depletion and formation of S-propyl GSH + liver homogenate.	Nepal et al., 2019

Table 5 Summary of additional data on genotoxicity (in vivo).

Experimental period and design; Species/participants	Dose and route	Observations and results	Remarks
In vivo DNA- and GSH-adduct formation; Rat, Sprague-Dawley; male; 5/group	0, 500 or 1,000 mg/kg bw; Once or daily for 3 consecutive days; intraperitoneal	N^7-propyl guanine formed in dose- and time-dependent manner in liver>spleen>testes>lung; not in heart. GSH-depletion mainly in liver; kidney; testes S-propyl GSH formation in liver>tests>spleen>kidney>lung>heart.	Nepal et al., 2019
DNA damage; DNA strand breaks (comet assay); venous blood, peripheral leukocytes; Personal air monitoring breathing zone for exposure assessment; Biomarker: Bromide in blood and urine. 64 workers (18 males/46 females) from two facilities; sprayers and other workers; no control. Subpopulation of other study	Sprayers: up to 83±85 ppm (418±428 mg/m^3) 8h TWA; Non-sprayers: up to 5±1 ppm (25±5 mg/m^3) 8h TWA	No significant differences in levels of DNA damage between groups. Significantly increased tail moments (non-sprayers) and significantly increased tail dispersion coefficients (sprayers) at the end-of week compared to start-of-week.	Toraason et al., 2006 No non-exposed population; limited number of participants.

3.8 Evaluation on germ cell mutagenicity

Classification in category 1A for germ cell mutagens requires positive evidence from human epidemiological studies. Since no data on mutagenicity in germ cells have been presented in humans, 1-bromopropane does not meet the criteria to classify the substance in category 1A. A substance can be classified in category 1B if mutagenicity is presented in germ cells in mammals in vivo or in somatic cells in mammals in vivo combined with evidence that the substance has potential to cause mutations in germ cells. In vivo animal data show no effects of 1-bromopropane in two separate dominant lethal assays. Therefore, 1-bromopropane does not meet the criteria to classify the substance in category 1B for mutagenicity.

A substance can be classified in category 2 for mutagenicity if there is positive evidence for mutagenicity from experiments in somatic cells in mammals in vivo or other in vivo somatic cell genotoxicity tests supported by in vitro data. In vivo micronucleus tests in rats and mice show no effects, although this may be explained by insufficient exposure in most studies. In vitro evidence of mutagenicity is limited to one Ames test in two strains of bacteria, supported by a mouse lymphoma assay that shows an increase in mutant frequency and an increase in small colonies. Additional data for 1-bromopropane show in vitro and in vivo DNA-adducts, GSH depletion and GSH-adducts. The Committee concludes that 1-bromopropane does not meet the criteria to classify the substance in category 2 for mutagenicity.

3.9 Recommendation on the classification for germ cell mutagenicity

The Committee recommends not classifying 1-bromopropane 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[1] and the IARC Monograph.[5] The Committee did not find additional or new data in the literature.

4.2 Observations in humans

No data on the carcinogenicity of 1-bromopropane in humans is available.

4.3 Animal carcinogenicity studies

The US National Toxicology Program (NTP) performed a well-conducted carcinogenicity study using rats and mice. In short, male and female F344/N rats and B6C3F1/N mice (N=50/sex/group) were exposed to 1-bromopropane by whole body inhalation at concentrations up to 2,515 mg/m^3, 6h/day, 5 days per week for 2 years. The main general observations were reduced survival at 2,515 mg/m^3, and head mass (incidence 1/50 0 mg/m^3; 2/50 629 mg/m^3; 5/50 1,258 mg/m^3; 9/50 2,515 mg/m^3) and torso/ventral ulcer/abscess (incidence 2/50 0 mg/m^3; 7/50 629 mg/m^3; 6/50 1,258 mg/m^3; 20/50 2,515 mg/m^3) in male rats. No treatment-related clinical findings or effects on survival rate were reported for mice.

4.3.1 Rats

Rats were exposed to 0, 125, 250 or 500 ppm (corresponding to 0, 629, 1,258 or 2,515 mg/m^3) 1-bromopropane. As depicted in Table 6a, compared to controls, a statistically significant increase in number of male rats with keratoacanthoma (1,258 and 2,515 mg/m^3), malignant mesothelioma (2,515 mg/m^3) and pancreas adenomas (all dose groups) was observed. The incidence of combined keratoacanthoma, basal cell adenoma, basal cell carcinoma or squamous cell carcinoma was significantly increased in all dose groups, and showed a concentration-response trend. Moreover, a significant increase in combined incidence of pancreas adenoma and carcinoma was observed after exposure to 629 and 1,258 mg/m^3, although all data fall within the historical control range. In female rats, a statistically significant increase in number of rats with large intestine adenomas was reported compared to control (in the group exposed to 2,515 mg/m^3), as well as a statistically non-significant increase of the combined incidence of keratocanthoma, squamous cell papilloma, basal cell adenoma or basal cell carcinoma at the highest exposure dose (see Table 6b).

Table 6a Summary incidence of neoplastic lesions in multiple tissues of male F344/N rats after exposure to various concentrations of 1-bromopropane for 2 years (source: NTP [2011]).[7]

Type of tissue/ lesion	0 mg/m³	629 mg/m³	1,258 mg/m³	2,515 mg/m³	Historical control data
Intestines / Adenoma of colon	0/50	0/50	0/50	1/50 (2%)	
Intestines / Adenoma of rectum	0/50	0/50	2/50 (4%)	0/50	
Intestines / Combined adenoma colon/ rectum	0/50	0/50	2/50 (4%)	1/50 (2%)	0/349
Skin / Basal cell adenoma	0/50	1/50 (2%)	2/50 (4%)	1/50 (2%)	4/349 (1.2%±1.1%; range 0-2%)
Skin / Basal cell carcinoma	0/50	2/50 (4%)	1/50 (2%)	2/50 (4%)	4/349 (1.1%±2.3%; range 0-6%)
Skin / Keratoacanthoma	0/50	3/50 (6%)	6/50* (12%)	6/50** (12%)	10/349 (2.9%±3.7%; range 0-8%)
Skin / Squamous cell carcinoma	1/50 (2%)	1/50 (2%)	0/50	2/50 (4%)	1/349 (0.3%±0.8%; range 0-2%)
Skin / Combined keratoacanthoma and squamous cell carcinoma	1/50 (2%)	4/50(8%)	6/50* (12%)	8/50** (16%)	11/349 (3.2%±3.5%; range 0-8%)
Skin / Combined all skin neoplasms	1/50 (2%)	7/50* (14%)	9/50** (18%)	10/50** (20%)	19/349 (5.5%±4.5%; range 0-10%)
Multiple tissues / Malignant mesothelioma	0/50	2/50 (4%)	2/50 (4%)	4/50* (8%)	5/349 (1.4%±2.2%)
Pancreas / Adenoma	0/50	5/50* (10%)	4/50* (8%)	5/50* (10%)	20/349 (5.7%±3.9%; range 0-12%)
Pancreas I Carcinoma	3/50 (6%)	7/50 (14%)	5/50 (10%)	3/50 (6%)	17/349 (4.9%±3.3%; range 2-10%)
Pancreas / Combined adenoma/carcinoma	3/50 (6%)	10/50* (20%)	9/50* (18%)	8/50 (16%)	37/349 (10.6%±4.8%; range 6-18%)

* 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 6b Summary incidence of neoplastic lesions in multiple tissues of female F344/N rats after exposure to various concentrations of 1-bromopropane for 2 years (source: NTP [2011]).[7]

Type of tissue/ lesion	0 mg/m³	629 mg/m³	1,258 mg/m³	2,515 mg/m³	Historical control data
Intestines / Adenoma of colon	0/50	1/50 (2%)	1/50 (2%)	1/50 (2%)	
Intestines / Adenoma of rectum	0/50	0/50	1/50 (2%)	4/50 (8%)	
Intestines / Combined adenoma colon/ rectum	0/50	1/50 (2%)	2/50 (4%)	5/50* (10%)	0/350
Skin / Keratoacanthoma	1/50 (2%)	0/50	1/50 (2%)	1/50 (2%)	
Skin / Squamous cell papilloma	0/50	0/50	0/50	1/50 (2%)	
Skin / Basal cell adenoma	0/50	1/50 (2%)	0/50	1/50 (2%)	
Skin / Basal cell carcinoma	0/50	0/50	0/50	1/50 (2%)	
Skin / Combined all skin neoplasms	1/50 (2%)	1/50 (2%)	1/50 (2%)	4/50 (8%)	2/350 (0.6%±1.0%; range 0-2%)

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

Nonneoplastic lesions were observed in multiple tissues of the rats after exposure to 2,515 mg/m^3 1-bromopropane (males and females). These lesions were shown to be suppurative inflammation, many with Splendore-Hoeppli (S-H) material and were predominantly located in the nose and skin, but were also present in other tissues. A concentration-related increase in the incidence of chronic suppurative inflammation that contained S-H material was seen for both male and female rats.

4.3.2 Mice

Mice were exposed to 0, 62.5, 125 or 250 ppm (corresponding to 0, 314, 629 or 1,258 mg/m^3) 1-bromopropane. As depicted in Table 7, 1-bromopropane induced a statistically significant increase (compared to control) in the number of female mice with alveolar/bronchiolar adenomas (1,258 mg/m^3) and carcinomas (314 mg/m^3 and 629 mg/m^3) and combined adenomas and carcinomas (all dose groups). A concentration-response trend was observed for both the adenomas and the adenomas and carcinomas combined. The incidence of hepatocellular carcinomas in female mice was significantly less than in the chamber controls and lower than the historical control range after exposure to 1,258 mg/m^3. Also the incidence of skin sarcoma was significantly less than in the chamber controls for this dose group. No neoplastic lesions were encountered in male mice. Nonneoplastic lesions were mainly found in the respiratory tract of both male and female mice.

Table 7 Summary incidence of neoplastic lesions in lungs of female B6C3F1/N mice after exposure to various concentrations of 1-bromopropane for 2 years (source: NTP [2011]).[7]

Type of lesion	0 mg/m^3	314 mg/m^3	629 mg/m^3	1,258 mg/m^3	Historical control data
Alveolar/bronchiolar adenoma, multiple	0/50	0/50	0/50	2/50 (4%)	
Alveolar/bronchiolar adenoma (includes multiple)	1/50 (2%)	6/50 (12%)	4/50 (8%)	10/50** (20%)	18/350 (5.1%±3.8%; range 2-12%)
Alveolar/bronchiolar carcinoma, multiple	0/50	2/50 (4%)	1/50 (2%)	1/50 (2%)	
Alveolar/bronchiolar carcinoma (includes multiple)	0/50	7/50** (14%)	5/50* (10%)	4/50 (8%)	9/350 (2.6%±2.8%; range 0-6%)
Alveolar/bronchiolar adenoma or carcinoma combined	1/50 (2%)	9/50** (18%)	8/50* (16%)	14/50** (28%)	27/350 (7.7%±3.6%; range 2-12%)

* 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

4.4 Evaluation on the carcinogenicity

No data on the carcinogenicity of 1-bromopropane in humans is available. Therefore, category 1A (*known to be carcinogenic to humans*) is not applicable.

Classification in category 1B (*presumed to be carcinogenic to humans*) 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-bromopropane was sufficient for mice (alveolar/bronchiolar adenomas/carcinomas in females). Moreover, a significant increase in mesothelioma was found in male rats, but according to the authors of Morgan *et al*. (2011), it is unclear whether the increased incidence of malignant mesothelioma was exposure related.[8] The incidence in the group exposed to 2,515 mg/m^3 was significantly increased compared to control, but only exceeded the historical control range by one neoplasm. Moreover, mesothelioma is a relatively common neoplasm in F344/N rats. A significant increase was observed in skin adenomas (keratoacanthoma) and in pancreas adenomas in male rats and in colon/rectum adenomas in female rats, but no progression into carcinomas was observed. However, adenomas in colon/rectum are rare, the incidence was exposure-related, and (significantly) higher than in unexposed animals and historical controls.[9] Although no carcinomas were observed in the study, adenomas in the intestine have been shown to progress into carcinomas in literature.[4,10,11] Therefore, the Committee considers the adenomas in the intestine of significant concern. However, as stated before, classification in category 1B requires a causal relationship between the substance and an increased incidence of *malignant* neoplasm in two or more animals species. As no malignant neoplasms have been observed in rats, the Committee concludes that 1-bromopropane 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 humans*) on the base of limited evidence for carcinogenicity in human or animal studies together with additional considerations. 1-bromopropane induced alveolar/bronchiolar carcinomas in mice and may induce mesotheliomas in male rats. Besides, multiple adenomas were observed in male rats and an increased incidence in intestine adenomas was observed in female rats, which is a rare tumour in rat. Despite a lack of epidemiological data on occupational exposure and disease development, the Committee considers the fact that exposure to 1-bromopropane can induce tumours in mice and possibly in rats an indication of its carcinogenic potential in humans. Therefore, the Committee recommends classifying the substance as suspected carcinogen in category 2.

4.5 Recommendation on the classification for carcinogenicity

The Committee recommends classifying 1-bromopropane as *suspected to be carcinogenic to humans*, which corresponds with category 2 for carcinogenicity, and to label 1-bromopropane with H351 (suspected of causing cancer).

references

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annexes

A IARC evaluation and conclusion

Cited from IARC Monographs, Volume 115, pages 66-67 (2018)[5]

Human data

No data were available to the Working Group.

Animal data

1-bromopropane was tested for carcinogenicity by inhalation in one US National Toxicology Program study in rats and mice.

In mice, inhalation of 1-bromopropane was associated with a significantly increased incidence of alveolar/bronchiolar adenoma, carcinoma and adenoma/carcinoma combined of the lung in females, but no significant increase in tumour incidence in males. In male rats, inhalation of 1-bromopropane caused a significantly increased incidence of tumours of the skin, malignant mesothelioma of the epididymis, pancreatic islet cell adenoma and pancreatic islet cell adenoma or carcinoma (combined), and was associated with a non-significant increase in the incidence of adenoma of the large intestine (colon or rectum), a tumour never observed in historical controls for inhalation studies. In female rats, inhalation of 1-bromopropane caused a significantly increased incidence of adenoma of the large intestine (colon or rectum) and was associated with a non-significant positive trend in the incidence of tumours of the skin.

Evaluation

There is *inadequate evidenc*e in humans for the carcinogenicity of 1-bromopropane.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1-bromopropane.

Overall evaluation

1-bromopropane is possibly carcinogenic to humans (Group 2B).

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).

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-bromopropane

- 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. 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).
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