# Methyl isobutyl ketone

(4-Methylpentan-2-one)
Evaluation of the carcinogenicity and genotoxicity

To: the State Secretary of Social Affairs and Employment
No. 2020/26, The Hague, December 8, 2020

Health Council of the Netherlands

# contents

# samenvatting

De Gezondheidsraad heeft beoordeeld of beroepsmatige blootstelling aan methylisobutylketon (MIBK) een genotoxisch effect heeft en tot kanker kan leiden en op basis daarvan een classificatievoorstel opgesteld. Het advies is opgesteld door de Subcommissie Classificatie kankerverwekkende stoffen – hierna aangeduid als de commissie – een subcommissie van de vaste 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.

## Toepassingen MIBK

MIBK wordt voor verschillende doeleinden gebruikt bij het vervaardigen van producten. In uiteenlopende industrieën kunnen mensen tijdens hun werk met de stof in aanraking komen. Het wordt bijvoorbeeld gebruikt als oplosmiddel in cosmetische producten, verven en lakken. Ook wordt het gebruikt bij de bereiding van sommige geneesmiddelen. MIBK kent ook een toepassing als synthetische geurstof en het wordt gebruikt als component in voedselverpakkingsmaterialen en als component in rubberen (auto)banden.

## 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 voor mensen 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 gebruikt worden in EU-verordening (EG) 1272/2008 over de classificatie van stoffen. Op basis van de voorstellen van de commissie kan de staatssecretaris 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 MIBK bij mensen.
Uit dierstudies en laboratoriumstudies komen onvoldoende aanwijzingen dat MIBK mutageen is in geslachtscellen.

Er zijn geen gegevens uit onderzoeken bij mensen beschikbaar over het optreden van kanker door blootstelling aan MIBK. In twee

experimenten bij dieren veroorzaakte de stof tumoren. In mannelijke ratten zijn niertumoren gevonden, die zijn veroorzaakt door een mechanisme dat niet relevant is voor de mens. In muizen zijn levertumoren gevonden, waarvan een in de literatuur voorgestelde carcinogene werkingsmechanisme onvoldoende is onderzocht om te kunnen beoordelen in hoeverre de bevindingen in muizen relevant zijn voor de mens. Over het geheel genomen concludeert de commissie dat er beperkt bewijs is voor carcinogeniteit in dierexperimenten.

**Advies**

De commissie adviseert de stof MIBK

- niet te classificeren voor mutageniteit;
- te classificeren als kankerverwekkend in gevarencategorie 2*: stoffen die ervan verdacht worden kankerverwekkend te zijn voor de mens.*

# executive summary

The Health Council of the Netherlands assessed whether occupational exposure to methyl isobutyl ketone (MIBK) may induce genotoxic effects and may cause cancer. 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 Health Council has a permanent task in the protection of employees to harmful health effects of substances to which they may be exposed during work. More information on this task can be found on the website www.gezondheidsraad.nl.

**Methyl isobutyl ketone**

MIBK is used for various purposes in manufacturing products. Examples are: as solvent in cosmetic products, paints and lacquers; in the manufacturing of certain medicines; as synthetic flavouring; as component in food contact materials; and, as component in rubber tyres.

**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 grade 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 criteria for assessing hazard categories, as set by the European Commission (EU-guideline (EG) 1272/2008). The recommendation can be used by the State Secretary to decide whether the substance should be listed as mutagenic in germ cells and/or carcinogenic.

**Recommendation**

There are insufficient indications that MIBK is a mutagen. Therefore, the Committee recommends not classifying MIBK as a germ cell mutagen.

No data were available on the carcinogenicity of MIBK in humans. The substance induced tumours in two animal experiments, one experiment in rats, the other in mice. In male rats, kidney tumours were found, but the carcinogenic mechanism through which they are induced is not relevant for humans. In mice, MIBK induced liver tumours, of which a proposed carcinogenic mode of action is insufficiently investigated to conclude whether or not the findings in mice are relevant to humans. Overall, the committee concludes that there is limited evidence for carcinogenicity of MIBK in animals. It, therefore, recommends classifying the substance as *suspected to be carcinogenic in man*, which corresponds with carcinogenic category 2.

# 01 scope

## 1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification. In addition to classifying substances as carcinogenic, 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 mutagenicity and carcinogenicity of methyl isobutyl ketone (MIBK).

## 1.2 Committee and procedure

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

In December 2019, the President of the Health Council released a draft of the report for public review. The committee has taken these comments into account in deciding on 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 is standardly based on scientific data, which are publicly available. The starting points of the committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are re-reviewed only by the committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of MIBK, such an IARC-monograph is available, of which the summary and conclusion is inserted in Annex A.

Data published after the last IARC evaluation were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in November 2020. The literature search was based on the following key words: 4-methylpentan-2-one, methyl isobutyl ketone, CAS number, occupational exposure, cancer, carcinog*, mutag*, genotox*. All genotoxicity and carcinogenicity data retrieved (i.e., data from the IARC Monograph and new data) were summarized in tables in the annexes of the present advisory report. Other data (i.e., data on physico-chemical properties, monitoring, use, kinetics) are retrieved from secondary sources, such as evaluations by other scientific bodies.

## 1.4 Quality assessment

The Committee evaluates the data retrieved on reliability and quality, by using criteria set by others, and by expert judgment. For animal experiments and in vitro assays, the criteria set by Klimisch et al. (1997) are used.[1] For epidemiological studies, the reliability criteria set by Money et al. (2013) are used.[2] A summary of the reliability criteria is given in Annex B and C, respectively.

In the Chapters 7 and 8, studies with sufficient reliability (with or without restrictions) are described, and taken into account for the hazard assessment. Studies with lower quality are incorporated in the summary tables in the annexes, but not considered for the hazard assessment.

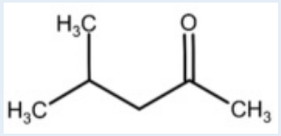
## 1.5 Criteria for classification

For recommending a classification on mutagenicity in germ cells, the Committee uses the criteria described in Section 3.5 of Annex I of the European regulation No. 1272/2008 (see annex D), in combination with expert judgement.[3] Although the criteria mentioned in the regulation are set for substances that are evaluated according to the CLP-regulation, the Committee considers them useful in recommending a classification as mutagenic in germ cells for substances, mixtures and emissions, for which the regulation does not apply. The criteria are based on the Globally Harmonized System, and can be universally applied.

In 2010, the Health Council published a *Guideline to the classification of carcinogenic compounds*, for classifying substances in terms of their carcinogenic properties, and for assessing the genotoxic mode of action.[4] The criteria and 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).[3] Annex E summaries the classification system for carcinogenic substances, as used by the Committee. For the assessment of the carcinogenicity, the Committee used four categories of evidence. These categories are described in detail in the *Guideline to the classification of carcinogenic compounds* (Health Council, 2010). The proposal for a classification is expressed in standard sentences, combined with a category number.

# 02 identity of the substance

## 2.1 Name and other identifiers

| IUPAC name | 4-methylpentan-2-one |
|---|---|
| CAS number | 108-10-1 |
| EC name | 4-methylpentan-2-one; methyl isobutyl ketone |
| EC number | 203-550-1 |
| Synonyms | methyl isobutyl ketone (MIBK); isobutyl methyl ketone; 2-pentanone,-4-methyl; isopropyl acetone; methyl isobutyl acetone; isohexanone; hexone |
| CLP Annex VI Index number | 606-004-00-4 |
| Molecular formula | $C_6H_{12}O$ |
| Molecular weight | 100.16 |
| Structural formula |  |
| Conversion ppm = mg/m$^3$ | 1 ppm = 4.15 mg/m$^3$<br>0.24 ppm = 1 mg/m$^3$ |

## 2.2 Composition of the substance

Not applicable.

## 2.3 Physico-chemical properties

| Properties | Value | Ref. | Comment |
|---|---|---|---|
| State of the substance | liquid | 5, 6 | Colourless liquid with sweet odour |
| Melting/freezing point | -84 °C, -80.26 °C | 5, 6 | |
| Boiling point | 117 to 118 °C | 5, 6 | |
| Relative density | 0.80 g/cm$^3$ at 20 °C | | |
| Vapour pressure | 19.9 mm Hg at 25 °C | 5, 6 | |
| Surface tension | 23.6-24 dynes/cm (20 °C) | 5, 6 | |
| Water solubility | 19.1 g/L in water at 20 °C and miscible with most organic solvents, soluble in chloroform | 5, 6 | |
| Partition coefficient n-octanol/water | 1.31 | 5, 6 | |
| Flash point | 14°C | 5, 6 | |
| Flammability | Lower explosive limit: 1%<br>Upper explosive limit: 8% | 7, 8 | Highly flammable liquid and vapour (H225) |
| Explosive properties | Sensitive to air (can form explosive peroxides), reacts violently with strong reducing agents and strong acids. | 9 | |
| Self-ignition temperature | 448 to 460 °C | 8 | |
| Oxidising properties | None | | Based on chemical structure |
| Granulometry | Not applicable | | 4-methylpentan-2-one is a liquid at room temperature |
| Dissociation constant (pKa) | No dissociation expected | | Based on chemical structure |
| Viscosity | 0.585 mPa.s at 20 °C | 8 | |

# 03 international classification

## 3.1 European Commission

MIBK is not classified by the European Commission for mutagenicity in germ cells, or for carcinogenicity.[3] In 2019, the Committee for Risk Assessment (RAC) of the European Chemicals Agency, adopted an opinion on the proposal for a harmonized classification and labelling of MIBK at EU level.[10] The RAC concluded that no classification as mutagenic in germ cells for 4-methylpentan-2-one is warranted, as negative results were mostly observed, and noted that the overall database on genotoxicity is limited and particularly on direct gene mutagenicity. In addition, it proposes to classify MIBK as carcinogenic in category 2.

## 3.2 IARC

In 2013, the IARC concluded that, although no data regarding carcinogenicity following exposure of humans to MIBK was available, there was sufficient evidence from *in vivo* studies for classification. It concluded that MIBK was carcinogenic in experimental animals. Therefore, the IARC classified MIBK as possibly carcinogenic to humans (Group 2B; see Annex A).[5]

## 3.3 The Health Council of the Netherlands

Not evaluated.

# 04 monitoring

## 4.1 Environmental exposure monitoring

In his evaluation on the toxicity of MIBK, Johnson (2004) reported on various analytical methods to measure the substance in the air.[6] These include: gas chromatography with or without flame ionization detection or mass spectrometry; high-resolution capillary gas chromatography, and: infrared spectroscopy. The US National Institute for Occupational Safety and Health (NIOSH) refers to NIOSH methods 1300, 2555, and 2027. These methods are based on gas chromatography, and differ in sorbent for collection of air samples.[11]

## 4.2 Biological exposure monitoring

Kawai et al. (2003), Gobba et al. (1997), and Ogata et al. (1990, 1995) measured unmodified MIBK in the urine of exposed workers by using gas chromatographic analyses, with or without using mass selective detectors. They used this method as a biological marker of occupational exposure to low concentrations of MIBK.[12-15]

# 05 manufacture and uses

## 5.1 Manufacture

MIBK is usually produced from acetone by a three-step process. First, acetone is condensed using a solid alkaline catalyst to yield diacetone alcohol. Next, diacetone alcohol is dehydrated in the presence of an acid catalyst, thereby producing mysityl oxide which in turn is hydrogenated to prepare 4-methylpentan-2-one. Alternatively, MIBK can be produced in a mixed ketones process from isopropanol, with acetone and diisobutyl ketone as co-products.[5, 6, 16]

In 2002, MIBK was produced by 9 companies in Europe.[5] Furthermore, MIBK is manufactured and/or imported in Europe at 10,000 to 100,000 tonnes per year.[7]

## 5.2 Identified uses

The major uses of MIBK are as denaturant and solvent in cosmetic products, as denaturant in denatured alcohol, and as an excipient in drugs. Additionally, it is used as a component of synthetic flavouring substances and adjuvants, and as a component of adhesives which are included in food contact materials. Further uses of MIBK include its application as a solvent for resin-based and cellulose-based coatings, paint and lacquers and its inclusion in rubber chemicals for the production of tyres.[5, 6]

# 06 summary of kinetics

The data presented below is a summary from evaluations and reviews by IARC (2013), EPA (2003), NTP (2007) and Johnson (2004).[5, 6, 17, 18]

## 6.1 Absorption, distribution and elimination

Following human exposure by inhalation, the relative uptake of MIBK via the respiratory tract is around 60%, independent of exposure levels (up to 200 mg/m³). A linear exposure level-dependency was observed in the total respiratory uptake. In rats, MIBK absorption following exposure by inhalation is rapid and exposure level-related. Similar results were found following oral administration in rats, and additionally uptake via the dermal route was demonstrated in a guinea pig model.

The blood/air partition coefficient of MIBK in humans is 70 to 90. MIBK is highly protein bound and is presumably rapidly distributed in the body. As a result of its high lipid solubility, the tissue distribution also includes lipid-rich tissues. Distribution of MIBK was determined in two workers, exposed via inhalation during spray painting, who died from a fall and cerebral oedema respectively. MIBK was detected in the brain, lung, liver, kidney, blood and vitreous fluid. Another study demonstrated the presence of the substance in human maternal blood, collected immediately after delivery. Distribution studies in rats also indicated rapid distribution, as MIBK and its metabolites were detected in the lung, liver and plasma following inhalation or within 1 hour following oral administration. Intraperitoneal administration of mice showed that MIBK is rapidly detectable in the brain but was completely eliminated from the brain 90 minutes post exposure.

Elimination of MIBK after human exposure by inhalation mainly occurs via exhalation. In the study by Kawai et al. (2003), air sampling and collected urine samples from 27 furniture-making workers, and 11 non-exposed controls, showed that approximately 0.12% of the inhaled MIBK was excreted into the urine.[13] Elimination occurs in a biphasic manner, i.e. a rapid phase (0 to 30 minutes post exposure) followed by a slow phase (60 to 70 minutes post exposure). Furthermore, a study with 98 male and female volunteers indicated that most of the absorbed compound was eliminated from the body 90 minutes after inhalation. Similar results were found in mice that were administered with an intraperitoneal injection. In guinea pigs, exposed to a single intraperitoneal dose of MIBK, a half-life in serum of 66 minutes was reported. The metabolite of MIBK, methyl isobutyl carbinol, was cleared from the blood within 16 hours post exposure.

## 6.2 Metabolism

MIBK can be reduced to a secondary alcohol, methyl isobutyl carbinol (synonym 4-methyl-2-pentanol), or oxidized to a hydroxylated ketone, 4-hydroxy-4-methyl-2-pentanone (HMP). These metabolites have been identified in tissues of rats and the blood of guinea pigs, but were found to be below the detection limit in human urine. No data on quantification of

metabolite levels in human blood are available. Animal studies further showed induction of cytochrome P450 expression (in rat and rabbit) and glutathione S-transferase activity (in rat) after inhalation of MIBK. It is suggested that in humans, MIBK can be further metabolized by conjugation reactions such as sulphation or glucuronidation or may enter intermediary metabolism resulting in elimination as $CO_2$. Alternatively, the metabolite methyl isobutyl carbinol may be incorporated in tissues, a process which might indicate accumulative potential.

# 07 germ cell mutagenicity

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

### 7.1.1 Summary of genotoxicity tests in vitro

Detailed information on the individual studies is given in Annex F.

*Mutagenicity*

A number of bacterial reverse mutation assays (Ames tests) have been performed to investigate the mutagenic properties of MIBK. The tests had negative outcomes, indicating that the substance is not mutagenic when using the standard *S. typhimurium* and *E. coli* strains.

The mutagenic activity of MIBK was also tested in mammalian cell gene mutation assays, using L5178Y $TK^{+/-}$ mouse lymphoma cells.[19, 20] Without a metabolic activation system, variable outcomes on mutant frequency were reported: the mutant frequency was more than doubled compared to solvent control at 1.8 and 3.2 µg MIBK/mL, but not at 2.4 µg MIBK/mL (survival at these concentrations was higher than 10%). Repeating the test at comparable concentrations also resulted in variable outcomes on mutant frequency: it was more than doubled compared to solvent control at 2.2 µg MIBK/mL, but not at 2.9 µg MIBK/mL (survival at these concentrations was higher than 10%). The mean mutant colonies per plate did not change at any concentration in the main or repeated study. With a metabolic activation system, no MIBK-related effects on mutant frequency and the number of mean mutant colonies per plate were observed. Overall, the committee considers the results of these studies equivocal.

*Clastogenic and aneugenic effects*

MIBK did not induce a biologically relevant increase of rat liver cells with chromosome aberrations.[21, 22] No other data are available on the potential clastogenic or aneugenic effects of the substance.

*Miscellaneous*

As shown in Annex F, MIBK did not induce unscheduled DNA-synthesis in vitro.[19, 20] In general, the committee considers unscheduled DNA-synthesis of less relevance, because this test gives no proof of a genotoxic potential. Rather, it is marker of exposure at molecular level.

*Conclusion on in vitro genotoxicity*

In general, MIBK did not show genotoxic activity leading to mutations in in vitro test systems with bacterial or mammalian cells.

### 7.1.2 Summary of human data relevant for germ cell mutagenicity

A review of the literature did not reveal any human data relevant for germ cell mutagenicity or other human data relevant for other genotoxic endpoints.

### 7.1.3 Summary of genotoxicity tests in mammalian somatic or germ cells in vivo

Overall, data on genotoxic effects in vivo is limited. In Annex G, details are given of one micronucleus bone marrow assay. The study concerns a single administration to mice by intraperitoneal injection of MIBK at a dose of 0.73 mL/kg bw/day.[19, 20] In the study, no exposure-related increase in micronucleated erythrocytes were observed.

No data are available on in vivo heritable germ cell genotoxicity.

## 7.2 Evaluation on germ cell mutagenicity

For MIBK, no data have been found on germ cell mutagenicity in human or animal studies. One in vivo somatic cell mutagenicity test in mice has been performed, but no MIBK-related mutagenic activity was observed. Since there is a lack of in vitro and in vivo genotoxic data on germ cells, and no indications for genotoxic activity have been observed, a classification in category 1 or 2 does not apply for the substance.

## 7.3 Recommendation on the classification for germ cell mutagenicity

The Committee recommends not classifying MIBK as a germ cell mutagen.

# 08 carcinogenicity

## 8.1 Summary and relevance of the provided information on carcinogenicity

### 8.1.1 Observations in humans

A review of the literature did not reveal any case reports or epidemiological studies concerning the association between exposure to MIBK and cancer risk in humans.

### 8.1.2 Animal carcinogenicity studies

The US National Toxicology Programme (NTP) performed a two-year animal carcinogenicity inhalation study on mice and rats.[18, 23] Details of the study are given in Annex H.

*Mice*. In summary, in male and female B6C3F$_1$ mice, a statistically significant increase of the incidence of hepatic adenomas and carcinomas (combined) was observed at the highest exposure level (1,800 ppm, comparable with 7,447 mg MIBK/m$^3$), as were hepatocellular and multiple adenomas in the liver. No exposure-related differences in clinical findings and body weight were observed among the negative control and exposure groups. In addition, no exposure-related tumours were observed at other sites of the body.

*Rats*. Survival in the highest exposed male group was lower than in the control group, and in the highest exposed female group. In male F344/N rats, statistically significantly increased incidences of renal tubule adenomas, and combined adenomas and carcinomas, were observed in the highest exposed group (1,800 ppm, comparable with 7,447 mg MIBK/m$^3$). This was accompanied with a slight increase in nephropathy (Chronic Progressive Nephropathy (CPN)), papilla mineralization, and renal tubule hyperplasia. Also, in male rats a non-significant trend of leukaemia development was observed. The committee noted the already high incidence of leukaemia in the negative control group (25 of the 50 control animals), as such that no conclusion can be made on this type of cancer. In male rats, no exposure-related tumours were observed at the other sites of the body. Concerning female rats, two of the fifty females in the highest exposure group, developed malignant mesenchymal kidney tumours. No such tumours were observed in the other exposure groups. Since this type of non-epithelial kidney tumour is reported to occur spontaneously in several strains of rats, and the type of tumour is particularly observed in rats, the committee considers this finding of low relevance.[24] Furthermore, in the female groups, no exposure-related tumours were found at the other sites of the bodies.

Overall, the committee is of the opinion that there is clear evidence that chronic inhalation of MIBK induces liver cancer in mice, and kidney cancer in male rats.

### 8.1.3 Other data relevant for the assessment of carcinogenicity

**Liver tumours**

Hughes et al. (2016) suggested that the liver tumours, which were observed in mice in the NTP study (see details in the previous section), could be induced by a so-called CAR/PXR nuclear receptor activation, a non-genotoxic mode of action.[25] The xenobiotic CAR (Constitutive Androstane Receptor) and PXR (Pregnane X Receptor) receptors function as sensors of toxic by-products of exogenous chemicals to enhance their elimination. To study this hypothesis, Hughes et al. performed a single-dosed animal experiment, in which wild type B6C3F$_1$ mice (N=8/sex/group), wild type C57BL/6 mice (N=8/sex/group), and C57BL/6 CAR/PXR knock out mice (N=5-8/sex/group), inhaled (whole body) MIBK at concentrations of 0 or 1,800 ppm for 6 hours a day, 5 days a week for 2 weeks. No positive control compound was used. During exposure the animals received 5-bromo-2-deoxyuridine via a pump system, which was subcutaneously implanted. After two weeks, the animals were sacrificed, and blood and liver samples were taken to test for hepatocyte proliferation, changes in clinical chemistry parameters, and gene expression responses of hepatic metabolic enzymes. The CAR/PXR knock-out mice showed a lack of response on liver hypertrophy, hepatocyte proliferation, and induction of the cyp2b10 metabolic enzyme in the liver, compared to the two wild type mouse strains. According to the authors this would suggest a similar response to MIBK as to other known CAR activators, such as phenobarbital (see text box below), which is consistent with a CAR-mediated hepatocarcinogenic mode of action.

**CAR/PXR mechanism in cancer development: phenobarbital and chlordane**

Data on the CAR/PXR mechanism in cancer development is mainly available for phenobarbital and chlordane (Elcombe et al. 2014; Andersen et al. 2014; Felter et al. 2018).[26-28] To summarize from these reviews, in the literature it is considered likely that phenobarbital activates CAR/PXR nuclear receptors, which leads to liver tumours in mice and rats. This is supported by data on CAR knockout mice, which did not develop tumours after phenobarbital exposure, whereas wild type mice did. Other genotoxic data support the suggestion that phenobarbital acts as a non-genotoxic carcinogen and a tumour promoter. Subsequently it is under discussion to what extent this non-genotoxic mode of action is relevant for humans, because available epidemiological studies do not show any association between phenobarbital treatment and increased cancer risk. Humans also possess CAR/PXR nuclear receptors. In the case of phenobarbital, when cultured human hepatocytes were exposed to the substance, no mitogenic, anti-apoptotic activities, and inhibition of gap junctional intercellular communication have been observed, whereas in wild type mouse and rat liver cells such effects were observed. This would indicate that in humans the CAR/PXR mediated nuclear receptor activation does not induce or stimulate tumour development (at least for phenobarbital). However, in three mechanistic studies in which mouse models expressing human CAR and/or PXR genes were used, no clear indications for this suggestion were found. In the first model expressing human CAR/PXR genes, phenobarbital or chlordane induced hepatocellular hypertrophy, but no increased replicative DNA synthesis or cell proliferation (Ross et al. 2010).[29] In a second model expressing human CAR genes only, phenobarbital induced cell proliferation in the liver, and

suppressed UV-induced apoptosis (Huang et al. 2005).[30] It is unclear why in the model by Ross et al. cell proliferation was not observed, whereas in the model by Huang et al. it was. Possible explanations are differences in treatment and study design. In a third model, Haines et al. (2018) studied the hepatic effects of sodium phenobarbital in male C57BL/6J wild type mice and in humanized mice (hCAR/hPXR mice).[31] They also performed experiments in cultured male C57BL/6J and CD-1 mouse, male Sprague-Dawley rat, and male and female human hepatocytes. The treatment of wild type and hCAR/hPXR mice with 186-984 ppm sodium phenobarbital in the diet for 7 days resulted in increased relative liver weight, hypertrophy and induction of cytochrome P450 (CYP) enzyme activities. The treatment also produced dose-dependent increases in hepatocyte replicative DNA synthesis, with the effect being more marked in wild type than in hCAR/hPXR mice. While the treatment of cultured C57BL/6J and CD-1 mouse, Sprague-Dawley rat and human hepatocytes with 100 and/or 1,000 µM sodium phenobarbital for 4 days induced CYP enzyme activities, increased replicative DNA synthesis was only observed in mouse and rat hepatocytes. However, as a positive control, epidermal growth factor increased replicative DNA synthesis in hepatocytes from all three species. In summary, although human hepatocytes are refractory to the mitogenic effects of sodium phenobarbital, treatment with sodium phenobarbital induced replicative DNA synthesis in vivo in hCAR/hPXR mice, which is presumably due to the human CAR and PXR receptors operating in a mouse hepatocyte regulatory environment. As the response of the hCAR/hPXR mouse to the CAR activator sodium phenobarbital differs markedly from that of human hepatocytes, Haines et al. conclude that the hCAR/hPXR mouse is thus not a suitable animal model for studies on the hepatic effects of nongenotoxic rodent CAR activators. In none of the three mouse models, data were obtained on cancer development, since they were not used to study possible long-term effects after chronic exposure.

The committee notes that Hughes et al. (2016) is the only study suggesting that the CAR/PXR nuclear receptor activation could explain MIBK-induce liver tumour development in mice. According to Peffer et al. (2018), a CAR-mediated mode of action for rodent liver tumors could be demonstrated in a short-term dosing study (e.g., 1–28 days) that shows a robust dose concordance between the dose levels that produce each of the early key events (in the short-term study), and those that produce the eventual adverse outcome (in the carcinogenicity study).[32] In the Hughes-study no carcinogenicity data were available. Furthermore, presuming that MIBK induced liver tumours in mice by a CAR/PXR mechanism, it is not clear to the committee whether the CAR/PXR nuclear receptors react differently between mice and humans, because mechanistic human data are not available for MIBK. In addition, no epidemiological studies have been performed on MIBK and cancer risk.

In conclusion, there is insufficient data to unambiguously assign the CAR/PXR nuclear receptor activation as the sole cause of MIBK induced liver tumours in mice. In addition, the relevance of this mode of action for humans has not been investigated. Until more data are available, the committee leaves open the possibility that other mechanisms may have played a role.

**Kidney tumours**

In male rats of the NTP-study, treatment-related increases in incidence of kidney tumours were observed, but not in female rats or in mice.

This could indicate that the renal tubule tumours in male rats are induced by an α2μ-globulin nephropathy mode of action, a carcinogenic mechanism known to occur in male rats only. Such a mode of action was suggested by the NTP (Stout et al. 2008; Doi et al. 2007), but the study was not designed to give a decisive answer on this possibility.[23, 33]
Scientific criteria are set by Swenberg and Lehman-McKeeman (1999) to assess the plausibility that this mechanism may be responsible for the kidney tumours after exposure of a number of chemicals.[34] Using these criteria, IARC (2013) concluded that "while α2μ-globulin nephropathy may contribute to the renal tumour response, the critical component(s) of the nephropathy most closely associated with the development of tumours has not been identified. Thus, the strength of the evidence that male rat kidney tumours arose through a α2μ-globulin nephropathy mechanism is weak".[5] Further evidence for the induction of α2μ-globulin nephropathy by MIBK in male rats is given by Borghoff et al. (2015).[35] Male and female F344 rats (N = 9 - 10 animals/group/sex) inhaled MIBK at concentrations of 0, 450, 900 and 1,800 ppm for 6 hours a day, for 4 days or 4 weeks (week 1 through 3, 5 days/week; week 4, 4 days). The study included a positive control (D-limonene, orally administered, males only). At the highest exposure level, the terminal kidney weights in male and female rats were statistically significantly increased compared to the non-exposed animals, whereas absolute body weight did not differ. Slight signs of chronic progressive nephropathy was observed in male rats only after 4 weeks of exposure to 900 and 1,800 ppm. In addition, in male rats only, a significant exposure-related increase in hyaline droplet accumulation was observed after 4 days and 4 weeks exposure. In male kidney tissues, accumulation of protein droplets positive α2μ-globulin was observed in exposed males, but not in female rats. In kidney homogenates, no changes in total protein were observed in any of the animals, whereas the α2μ-globulin concentrations were statistically significantly increased in males rats only at all exposure levels compared to non-exposed animals, in a dose-related manner. The MIBK induced renal effects in males rats were accompanied by renal cell proliferation (BrdU labelling index, mitotic index), which was not observed in female rats.
In a separate *in vitro* test, the binding capacity of MIBK to α2μ-globulin was tested by using kidney tissue and D-limonene oxide, a metabolite of D-limonene with high affinity to bind to α2μ-globulin. When D-limonene oxide was added, less MIBK was bound to α2μ-globulin. This was observed only in male kidney tissues and not in female kidney tissues. According to the Committee, this study gives further evidence for a α2μ-globulin nephropathy mode of mechanism in males rats, suggesting a mode of action which is not relevant for humans.
In the literature, suggestions are made that chronic progressive nephropathy, which is observed in rats in the carcinogenicity study, might be a secondary carcinogenic mechanism in MIBK-induced kidney tumours.[10, 36-38] However, the Committee considers this unlikely, since the exposure-response relationship did not parallel the exposure-response relationship of the kidney tumours, and CPN was also observed in the

non-exposed groups and in female rats. CPN is known as a spontaneous renal disease of rats, and, the pathological characteristics of CPN do not resemble the pathology normally seen in human nephrotoxicity.[24, 39] Considering these arguments, the committee considers CPN not relevant for humans.

## 8.2 Evaluation on the carcinogenicity

No data on the carcinogenicity of MIBK in humans is available. Therefore, category 1A is not applicable.

Classification in category 1B requires a causal relationship between the substance and an increased incidence of malignant neoplasm in two or more animal species. For MIBK, a well-performed carcinogenicity inhalation study in rats and mice is available. Male and female mice developed liver tumours due to exposure to MIBK. In male rats, exposure led to an increased incidence of kidney tumours. In female rats no such type of tumours were observed.

The Committee considered the suggestion that the liver tumours in mice could have been induced by CAR/PXR nuclear receptor activation, a non-genotoxic mode of action. However, whether this mode of action has indeed played a role in MIBK induced liver tumours in mice, has insufficiently been investigated. In addition, the relevance for humans has not been investigated. Until more data are available, the committee considers it possible that other mechanisms may have played a role, and thus that the findings in mice could be relevant to humans.

In addition, the relevance of MIBK-induced kidney tumours in male rats for humans is questioned, because most likely these type of tumours were induced by an α2μ-globulin nephropathy mode of action. This non-genotoxic mechanism is known to occur in male rats only. Furthermore, the committee does not consider it likely that chronic progressive nephropathy, another proposed mode of action for carcinogenicity, played a role, since the aetiology is unclear and this type of nephropathy is frequently observed in non-exposed rats. Overall, the Committee considers the kidney tumours found in male rats not relevant for humans.

To conclude, no data are available on cancer in humans. There is limited evidence for carcinogenicity in animal experiments. According to the criteria, MIBK should therefore be classified as "*suspected to be carcinogenic to man*", which corresponds to classification in carcinogenicity category 2.

## 8.3 Recommendation on the classification for carcinogenicity

Based on the limited evidence for carcinogenicity, the committee recommends classifying MIBK as *suspected to be carcinogenic to man*, which corresponds with carcinogenic category 2.

# 9

# references

[1] Klimisch HJ, Andreae M and Tillmann U. *A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data*. Regulatory toxicology and pharmacology 1997; 25(1): 1-5.

[2] Money CD, Tomenson JA, Penman MG, Boogaard PJ and Jeffrey Lewis R. *A systematic approach for evaluating and scoring human data*. Regulatory toxicology and pharmacology 2013; 66(2): 241-7.

[3] European Commission. *Regulation (EC) No 1272/2008 of the European Parliamant and of the Council on classification, labelling and packaging of substances and mixtures*. 2009.

[4] The Health Council of the Netherlands. *Guideline to the classification of carcinogenic compounds. Guide for classifying compounds in terms of their carcinogenic properties and for assesing their genotoxicity*. The Hague, 2010; A10/07E.

[5] International Agency for Cancer Research. *Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-Water*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Volume 101, pages 305-23. Lyon, France: 2013.

[6] Johnson W, Jr. *Safety assessment of MIBK (methyl isobutyl ketone)*. Int J Toxicol 2004; 23 Suppl 1: 29-57.

[7] European Chemicals Agency. *Registered Substances. Database on information on registered substances under REACH by search on CAS No.* 2017. http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances]

[8] European Chemicals Agency. *C & L Inventory Database. Information on ECHA's website by search on CAS No.* . 2017. http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances]

[9] ChemicalBook. *Chemical Search Engine by search on CAS No.* 2017. http://www.chemicalbook.com]

[10] Committee for Risk Assessment. *Opinion proposing harmonised classification and labelling at EU level of 4-methylpentan-2-one; isobutyl methyl ketone, EC number: 203-550-1, CAS number 108-10-1*. European Chemicals Agency, Helsinki, Finland. website: echa.europe.eu. CLH-O-0000001412-86-295/F, Adopted 20 September, 2019.

[11] National Institute for Occupational Health and Safety. *Pocket guide hexone*. NIOSH Pocket Guide to Chemical Hazards, Centers for Disease Control and Prevention, Washington D.C. USA. https://www.cdc.gov/niosh/npg/npgd0326.html]. Consulted: March 6, 2019

[12] Gobba F, Ghittori S, Imbriani M, Maestri L, Capodaglio E and Cavalleri A. *The urinary excretion of solvents and gases for the biological monitoring of occupational exposure: a review*. Sci Total Environ 1997; 199(1-2): 3-12.

[13] Kawai T, Zhang ZW, Takeuchi A, Miyama Y, Sakamoto K, Higashikawa K, et al. *Methyl isobutyl ketone and methyl ethyl ketone in urine as biological markers of occupational exposure to these solvents at low levels*. Int Arch Occup Environ Health 2003; 76(1): 17-23.

[14] Ogata MT, Taguchi T and Horike T. *Evaluation of exposure to solvents from their urinary excretions in workers coexposed to toluene, xylene, and methyl isobutyl ketone*. Applied Occupational and Environmental Hygiene 1995; 10(11): 913-20.

[15] Ogata MT, Taguchi T and Hirota N. *Database for biological monitoring of armotatic solvents*. Biological monitoring of exposure to industrial chemicals: 119-26. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; 1990.

[16] Lei Z, Li J, Li C and Chen B. *Improvement of separation process of synthesizing MIBK by the isopropanol one-step method*. Korean Journal of Chemical Engineering 2006; 23(2): 264-70.

[17] Environmental Protection Agency (EPA). *Toxicological Review of Methyl Isobutyl Ketone (CAS No. 108-10-1): In Support of Summary Information on the Integrated Risk Information System (IRIS)*. Washington DC: U.S. Environmental Protection Agency; 2003.

[18] National Toxicology Program. *Toxicology and carcinogenesis studies of methyl isobutyl ketone (CAS No. 108-10-1) in F344/N rats and B6C3F1 mice (inhalation studies)*. Research Triangle Park, NC.: U.S. Department of Health and Human Services (NTP Technical Report Series No. 538. NIH Publication No. 07-4476), 2007.

[19] Chemical Manufacturers Association. *Lists of final reports submitted summaries of MEK and MIBK testing programs, with cover letter to EPA*. Washington, DC.: Washington, DC, US Environmental Protection Agency, Office of Toxic Substances (NTIS Report No. OTS0507470), 1984.

[20] O'Donoghue JL, Haworth SR, Curren RD, Kirby PE, Lawlor T, Moran EJ, et al. *Mutagenicity studies on ketone solvents: methyl ethyl ketone, methyl isobutyl ketone, and isophorone*. Mutat Res 1988; 206(2): 149-61.

[21] Brooks TM, Meyer AL and Hutson DH. *The genetic toxicology of some hydrocarbon and oxygenated solvents*. Mutagenesis 1988; 3(3): 227-32.

[22] Shell Oil Company. *Mitigation of chemical spills with cover letter. Toxicity studies with chemical solvents: short-term in vitro tests for genotoxic activity with methyl isobutyl ketone*. Washington, DC.: Washington, DC, US Environmental Protection Agency, Office of Toxic Substances (NTIS Report No. OTS0206218), 1980.

[23] Stout MD, Herbert RA, Kissling GE, Suarez F, Roycroft JH, Chhabra RS, et al. *Toxicity and carcinogenicity of methyl isobutyl ketone in F344N rats and B6C3F1 mice following 2-year inhalation exposure*. Toxicology 2008; 244(2-3): 209-19.

[24] Hard GC, Banton MI, Bretzlaff RS, Dekant W, Fowles JR, Mallett AK, et al. *Consideration of rat chronic progressive nephropathy in regulatory evaluations for carcinogenicity*. Toxicol Sci 2013; 132(2): 268-75.

[25] Hughes BJ, Thomas J, Lynch AM, Borghoff SJ, Green S, Mensing T, et al. *Methyl isobutyl ketone-induced hepatocellular carcinogenesis in B6C3F1 mice: A constitutive androstane receptor (CAR)-mediated mode of action*. Regulatory toxicology and pharmacology 2016; 81: 421-9.

[26] Andersen ME, Preston RJ, Maier A, Willis AM and Patterson J. *Dose-response approaches for nuclear receptor-mediated modes of action for liver carcinogenicity: Results of a workshop*. Crit Rev Toxicol 2014; 44(1): 50-63.

[27] Elcombe CR, Peffer RC, Wolf DC, Bailey J, Bars R, Bell D, et al. *Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator*. Crit Rev Toxicol 2014; 44(1): 64-82.

[28] Felter SP, Foreman JE, Boobis A, Corton JC, Doi AM, Flowers L, et al. *Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action*. Regular Toxicology and Pharmacology 2018; 92: 1-7.

[29] Ross J, Plummer SM, Rode A, Scheer N, Bower CC, Vogel O, et al. *Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo*. Toxicol Sci 2010; 116(2): 452-66.

[30] Huang W, Zhang J, Washington M, Liu J, Parant JM, Lozano G, et al. *Xenobiotic stress induces hepatomegaly and liver tumors via the nuclear receptor constitutive androstane receptor*. Mol Endocrinol 2005; 19(6): 1646-53.

[31] Haines C, Elcombe BM, Chatham LR, Vardy A, Higgins LG, Elcombe CR, et al. *Comparison of the effects of sodium phenobarbital in wild type and humanized constitutive androstane receptor (CAR)/pregnane X receptor (PXR) mice and in cultured mouse, rat and human hepatocytes*. Toxicology 2018; 396-397: 23-32.

[32] Peffer RC, LeBaron MJ, Battalora M, Bomann WH, Werner C, Aggarwal M, et al. *Minimum datasets to establish a CAR-mediated mode of action for rodent liver tumors*. Regular Toxicology and Pharmacology 2018; 96: 106-20.

[33] Doi AM, Hill G, Seely J, Hailey JR, Kissling G and Bucher JR. *alpha 2u-globulin nephropathy and renal tumors in national toxicology program studies*. Toxicol Pathol 2007; 35(4): 533-40.

[34] Swenberg JA and Lehman-McKeeman LD. *alpha 2-Urinary globulin-associated nephropathy as a mechanism of renal tubule cell carcinogenesis in male rats*. IARC Sci Publ 1999; 147: 95-118.

[35] Borghoff SJ, Poet TS, Green S, Davis J, Hughes B, Mensing T, et al. *Methyl isobutyl ketone exposure-related increases in specific measures of alpha2u-globulin (alpha2u) nephropathy in male rats along with in vitro evidence of reversible protein binding*. Toxicology 2015; 333: 1-13.

[36] Hard GC, Betz LJ and Seely JC. *Association of advanced chronic progressive nephropathy (CPN) with renal tubule tumors and precursor hyperplasia in control F344 rats from two-year carcinogenicity studies*. Toxicol Pathol 2012; 40(3): 473-81.

[37] Melnick RL, Burns KM, Ward JM and Huff J. *Chemically exacerbated chronic progressive nephropathy not associated with renal tubular tumor induction in rats: an evaluation based on 60 carcinogenicity studies by the national toxicology program*. Toxicol Sci 2012; 128(2): 346-56.

[38] Hard GC, Cohen SM, Ma J, Yu F, Arnold LL and Banton MI. *Histopathology re-examination of the NTP toxicity/carcinogenicity studies of tert-butyl alcohol to identify renal tumor and toxicity modes of action*. Regul Toxicol Pharmacol 2019; 102: 65-73.

[39] Hard GC, Johnson KJ and Cohen SM. *A comparison of rat chronic progressive nephropathy with human renal disease-implications for human risk assessment*. Crit Rev Toxicol 2009; 39(4): 332-46.

[40] Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K. *Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals*. Environ Mol Mutagen 1992; 19 Suppl 21: 2-141.

[41] Goodyear Tire & Rubber Co. *Mutagenicity Evaluation of Methyl Isobutyl Ketone (MIBK) with cover letter*. Akron, OH.: Washington, DC, US Environmental Protection Agency, Office of Toxic Substances (NTIS report No. OTS0205942), 1982.

[42] Litton Bionetics Inc. *Letter concerning the enclosed Ames salmonella/microsome plate tests on methyl ethyl ketone and methyl isobutyl ketone with attachments*. Washington, DC.: Washington, DC, US Environmental Protection Agency, Office of Toxic Substances (NTIS Report No. OTS0534320), 1991.

# annexes

# A IARC evaluation and conclusion

*Source:* IARC. Some Chemicals present in industrial and consumer, food and drinking-water. Monograph on the evaluation of carcinogenic risks in humans, Volume 101, pp 305-324, 2013.[5]

## 6 Evaluation

### 6.1 Cancer in humans

No data were available to the Working Group

### 6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of methyl isobutyl ketone.

### 6.3 Overall evaluation

Methyl isobutyl ketone is possibly carcinogenic to humans (Group 2B).

# B reliability testing of animal and in vitro studies

To assess the reliability of animal and in vitro studies, the Committee uses the criteria set by Klimisch et al. 1997.[1] A summary of the criteria of the reliability scores is given below. Only studies with a reliability score of 1 or 2 are considered in assessing genotoxicity and carcinogenicity.

*Reliability 1 (reliable without restriction)*
For example, guideline study (OECD, etc.); comparable to guideline study; test procedure according to national standards (DIN, etc.).

*Reliability 2 (reliable with restrictions)*
For example, acceptable, well-documented publication/study report which meets basic scientific principles; basic data given: comparable to guidelines/standards; comparable to guideline study with acceptable restrictions.

*Reliability 3 (not reliable)*
For example, method not validated; documentation insufficient for assessment; does not meet important criteria of today standard methods; relevant methodological deficiencies; unsuitable test system.

*Reliability 4 (not assignable)*
For example, only short abstract available; only secondary literature (review, tables, books, etc.).

# C reliability testing of epidemiological studies

To assess the reliability of epidemiological studies, the Committee uses the criteria set by Money et al.(2013).[2] A summary of the reliability categories set by Money et al. is given below. Only studies with a reliability score of 1 or 2 are considered in assessing genotoxicity and carcinogenicity.

**Reliability 1 (reliable without restriction)**

*Chronic, non-specific outcomes*

Appropriate study design to research question.

(1) Selected subjects or persons at risk represent appropriate exposure distributions. Adequate procedures of follow-up and reduction of loss to follow up were performed.

(2) Exposure assessment was made independent of outcome with validated methods, preferentially with individual exposure data.

(3) Effect data were collected independently from exposure status, using standardized data collection procedures/registries.

(4) The possibility of serious bias has been reduced by design, controlled through statistical adjustment, and/or quantified through sensitivity analyses.

(5) The sample/exposure range was sufficient to study the question under investigation, so that effects estimates are not constrained by high imprecision.

(6) The data were analysed using appropriate statistical techniques to address the research questions and model assumptions.

(7) The methodology and results were comprehensively and transparently reported according to relevant guidelines (*e.g.*, the STROBE guidelines for observational data, Von Elm et al. 2007).

*Acute or specific outcomes*

The same principles should be applied as for chronic, non-specific outcomes. The focus lies more with how well exposure has been characterised, and the disease outcome is defined.

**Reliability 2 (reliable with restrictions)**

*Chronic, non-specific outcomes*

Applies to studies which possess most of the qualities of studies with reliability 1. The overall quality is comprised due to minor, but obvious, methodological limitations. Examples include well-designed and conducted studies, but with limited measurement data, possibility of some residual confounding, some imprecision due to small sample size or low exposure range.

*Acute or specific outcomes*

The same principles should be applied as for chronic, non-specific outcomes. Examples of shortcomings may include a lack of individual exposure data, and effects derived from self-reported outcomes.

Note: some studies with serious methodological limitations may provide reliable information for an acute or specific outcome.

**Reliability 3 (not reliable)**

The studies fail to meet one or more of the most basic standards necessary to interpret epidemiologic research, such as appropriate study design to the research question. Shortcomings may include using census job titles as a surrogate for exposure.

**Reliability 4 (not assignable)**

This includes studies or data which do not give sufficient details about methodology used, or which are short listed in abstracts or secondary literature.

# D 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 10 August 2009 on classification, labelling and packaging of substances.[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.

3.5.2.3 Specific *consid*erations 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.

**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:<br>- positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or<br>- 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<br>- 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:<br>- positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:<br>- somatic cell mutagenicity tests in vivo, in mammals; or<br>- other in vivo somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.<br>*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. |

3.5.2.3.3. 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).

# E classification on carcinogenicity

In 2010, the Committee published a guideline for classifying substances in terms of their carcinogenic properties, and for assessing their genotoxicity.[4] 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).[3]

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.*<br>It acts by a stochastic genotoxic mechanism.<br>It acts by a non-stochastic genotoxic mechanism.<br>It acts by a non-genotoxic mechanism.<br>Its potential genotoxicity has been insufficiently investigated.<br>Therefore, it is unclear whether the compound is genotoxic. | 1A |
| 1B | *The compound is presumed to be as carcinogenic to humans.*<br>It acts by a stochastic genotoxic mechanism.<br>It acts by a non-stochastic genotoxic mechanism.<br>It acts by a non-genotoxic mechanism.<br>Its potential genotoxicity has been insufficiently investigated.<br>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 |

# F genotoxicity in vitro

## Mutagenicity assays

Note: all test were performed without and with a metabolic activation system (-/+ S9).

Bacterial reverse mutation assay

| Microorganism, reference | Concentration range of MIBK | Results | Remarks and quality |
|---|---|---|---|
| *S. typhimurium:*<br>TA1535, TA1537, TA1538, TA98, TA100; preincubation method<br><br>O'Donoghue et al. (1988)[20] | *Concentrations*: 0.04, 0.1, 1.0 and 4 µl/plate, -/+S9<br><br>Test included negative and positive controls | *Outcome*: negative for all strains and tested concentrations<br><br>No data on cytotoxicity | Comparable to OECD 471 guideline study<br><br>Klimisch score 1 |
| *S. typhimurium:*<br>TA1535, TA1537, TA1538, TA98, TA100; *E. coli*: WP2, WP2 uvrA, WP2 uvrA pKM101; *S. cerevisiae*: JD1; preincubation method<br><br>Brooks et al. (1988)[21] | Concentrations selected at 2-fold intervals up to 8,000 µg/plate, -/+ S9<br><br>Test includes negative and positive controls, all tests performed in triplicate<br><br>Preliminary cytotoxicity test performed | *Outcome*: negative for all strains and tested concentrations | Compliant to OECD 471 guideline study<br><br>No positive control for most strains<br><br>Klimisch score 2 |
| *S. typhimurium*: TA98, TA100, TA1535, TA1537, TA1538; *S. cerevisiae* JDI; preincubation method;<br><br>Shell Oil Company 1980[22] | *S. typhimurium*:<br>31.25 to 4,000 µg/plate (8 concentrations)<br><br>*S. cerevisiae*:<br>0.2 to 50 mg/mL<br>(5 concentrations) | *Outcome*: negative for all tested strains (-/+ S9)<br><br>Cytotoxicity not specified. | Non-GLP compliant comparable to OECD 471 guideline<br><br>Klimisch score 2 |
| *S. typhimurium* strains: TA97, TA98, TA100, TA1535; preincubation method<br><br>NTP (2007),[18] Zeiger et al. 1992[40] | 100 to 6,667 µg/plate<br>(5 concentrations) | *Outcome*: negative for all tested strains (-/+ S9)<br><br>Slight cytotoxicity observed at the top dose | Comparable to OECD 471 guideline study<br><br>Klimisch score 2 |

| Microorganism, reference | Concentration range of MIBK | Results | Remarks and quality |
|---|---|---|---|
| *S. typhimurium* strains: TA98, TA100, TA1535, TA1537, TA1538; preincubation method<br><br>Chem. Man. Ass. 1984,[19] | 0.04 to 4 µL/plate<br>(5 concentrations) | *Outcome*: negative for all tested strains (-/+ S9)<br><br>Cytotoxicity not specified for main test; pre-test demonstrated a normal background bacterial lawn at 1.7 µL/plate, a slightly reduced background lawn at 5.2 µL/plate, and an absent background lawn at and higher than 17 µL/plate | Comparable to OECD 471 guideline; longer incubation time compared to guideline<br><br>Klimisch score 2 |
| *S. typhimurium:*<br>TA1535, TA1537, TA1538, TA98, TA100; *E. coli*: WP2, WP2 uvrA, WP2 uvrA pKM101; *S. cerevisiae*: JD1;<br>Plate incorporation method<br><br>Brooks et al. (1988)[21] | Concentrations selected at 2-fold intervals up to 8,000 µg/plate, -/+ S9<br><br>Test included negative and positive control, all tests performed in triplicate<br><br>Preliminary cytotoxicity test performed | *Outcome*: negative for all strains and tested concentrations | Compliant to OECD 471 guideline study<br><br>Klimisch score 1 |
| *S. typhimurium* strains: TA98, TA100, TA1535, TA1537, TA1538; Plate incorporation method<br><br>Goodyear Tire & Rubber Company 1982[41] | 0.1 to 2,000 µg/plate<br>(4 concentrations) | Outcome: negative for all tested strains (-/+ S9)<br><br>Cytotoxicity not specified | Comparable to OECD 471 guideline study; insufficient number of test concentrations<br><br>Klimisch score 2 |
| *S. typhimurium* strains: TA98, TA100, TA1535, TA1537, TA1538; *S. cerevisiae* D4; Plate incorporation method<br><br>Litton Bionetics 1991[42] | 0.01 to 10 µL/plate<br>(5 concentrations) | *Outcome*: negative for all tested strains (-/+ S9)<br><br>Cytotoxicity not specified | Comparable to OECD 471 guideline study<br><br>Klimisch score 2 |

*Mammalian cell gene mutation assays*

| Cell type, reference | Concentration range of MIBK | Results | Remarks and quality |
|---|---|---|---|
| L5178Y TK$^{+/-}$ mouse lymphoma cells<br><br>O'Donoghue et al. (1988)[20] | *Concentrations*:<br>- Main study: 0.32 up to 4.2 µL/mL (10 concentrations), -/+S9<br>- Repeat study: 0.6 up to 3.7 µL/mL (5 concentrations, -S9)<br>- Repeat study: 1.4 up to 3.4 µL/mL (5 concentrations, +S9)<br><br>Test included negative (solvent, DMSO) and positive (ethyl methane-sulfonate) controls<br><br>*Endpoints*: mean mutant colonies/plate, mutant frequency, survival rate; 2-fold increase compared to control is considered significant. | *Outcome main study*:<br>-S9: increased mutant frequency (frequency doubled compared to solvent control) at 1.8 and 3.2 µL/mL (survival 58 and 31%, respectively), but not at 2.4 µL/mL (survival 52%); the results at 4.2 µL/mL are not relevant, because the survival was too low (3%); no significant change in mean mutant colonies per plate compared to solvent control<br>+S9: no change in mutant frequency and mean mutant colonies per plate compared to solvent control<br><br>*Outcome repeat studies*:<br>-S9: increased mutant frequency (frequency doubled compared to solvent control) at 2.1 µL/mL (survival 31%); equivocal results between duplicates at 2.9 µL/mL (one increased, one not significantly increased; survival 32% and 42%, respectively); results at 3.7 µL/mL are not relevant, because the survival was too low (4%); no significant change in mean mutant colonies per plate at any dose applied compared to solvent control<br>+S9: no change in mutant frequency and mean mutant colonies per plate compared to solvent control | GLP compliant OECD 490 study<br><br>Note: according to the renewed guideline, the number of colonies is relevant in assessing mutagenic activity, and no longer the mutant frequency; relative survival or relative total growth should not be less than 10%.<br><br>Klimisch score: 1 |
| L5178Y TK$^{+/-}$ mouse lymphoma cells<br><br>Chem. Man. Ass. (1984)[19]<br><br>Note: most likely data are also presented in O'Donoghue et al. (1988)[20] | *Main test*:<br>-/+ S9: 0.13 to 10 µL/mL<br>*Cloned cultures*:<br>-/+ S9: 0.13 to 4.2 µL/mL<br>(10 concentrations)<br><br>*Repeat test*:<br>-S9: 0.6 to 6 µL/mL<br>+S9: 0.4 to 4 µL/mL<br>*Cloned cultures*:<br>-S9: 0.6 to 3.7 µL/mL<br>+S9: 1.4 to 3.4 µL/mL<br>(5 concentrations)<br><br>Negative control: DMSO (solvent)<br><br>*Endpoints*: mutant frequency, relative cell growth, cell death; 2-fold increase compared to control is considered significant. | *Outcome main test*:<br>-S9: Equivocal, near significant increase in mutant frequency at 1.8 and 3.2 µL/mL); results at 4.2 µL/mL are not relevant, because the survival was too low (3%); no clear dose-response effect observed; 3 to 175% total relative growth compared to solvent control<br>+S9: No significant increase in mutant frequency observed compared to control; 23 to 95% total relative growth compared to solvent control<br><br>*Outcome repeat test*:<br>-S9: Equivocal, near significant increases in mutant frequency at 2.1 and 2.9 µL/mL, which are not consistent within duplicates; results at 3.7 µL/mL are not relevant, because of 96 to 99% cell death; 1 to 80% total relative growth compared to solvent control<br>+S9: No significant increase in mutant frequency observed compared to solvent control; 28 to 63% total relative growth compared to solvent control | GLP compliant OECD 490 study<br><br>Note: relative survival or relative total growth should not be less than 10%.<br><br>Klimisch score: 1 |

## Other genotoxicity assays

### *Chromosome aberration test*

| Cell type, reference | Concentration range of MIBK | Results | Remarks and quality |
|---|---|---|---|
| Rat liver (RL4) cell line, and Chinese Hamster Ovary (CHO) cells<br><br>Brooks et al. (1988)[21] | *Concentrations selected*:<br>- Rat liver cells: 0.5, 0.25 and 0.125 of the $GI_{50}$ (50% growth inhibition) (up to 8,000 µl/ml) (-S9)<br>- CHO cells: 1, 0.5 and 0,25 of the $GI_{50}$ (+S9)<br><br>Tests included negative and positive controls | *Outcome*: no biologically relevant increase in cells with chromosomal aberrations observed at any dose applied for both cell types, compared to negative control | Compliant to OECD 473 guideline study<br><br>Klimisch score 1 |
| Rat liver (RL4) cell line<br><br>Shell Oil Company 1980[22] | *Concentrations applied*: 250, 500 and 1,000 µg/mL | *Outcome*: no biologically relevant increase in cells with chromosomal aberrations observed at any dose applied, compared to negative control<br><br>No cytotoxicity observed. | Comparable to OECD 473 guideline; longer incubation time compared to guideline<br><br>Klimisch score: 2 |

## Miscellaneous

### *Unscheduled DNA synthesis*

| Cell type, reference | Concentration range of MIBK | Results | Remarks and quality |
|---|---|---|---|
| Primary hepatocytes derived from Sprague-Dawley rats<br><br>O'Donoghue et al. (1988)[20] | Concentrations applied: 0.010 to 100 µL/mL (5 concentrations)<br><br>Negative and positive control included | *Outcome*: no significant effects on relative survival, and average net grains/nucleus; concentrations of 10 and 100 µL/mL were too toxic to count. | Compliant to OECD 482 guideline study<br><br>Klimisch score 1 |
| Primary hepatocytes (from male Sprague-Dawley rats)<br><br>Chem. Man. Ass. 1984[19] | Concentrations applied: 0.010 to 100 µL/mL (5 concentrations) | *Outcome*: no significant induction of unscheduled DNA synthesis observed_<br><br>*Cytotoxicity*: 98 to 100% at the two highest doses | GLP compliant OECD 482 study<br><br>Klimisch score 1 |

# G genotoxicity in vivo

| Method (reference) | Animal | Exposure conditions | Results | Remarks and quality |
|---|---|---|---|---|
| Micronucleus assay (bone marrow)<br><br>Chem. Man. Ass. 1984[19], TSCA 1984, O'Donoghue et al. (1988)[20] | Male and female Swiss CD-1 mice (N=5/sex/dose/ time point); one additional animal/time point as replacement animal | Single intraperitoneal injection; 0.73 mL/kg bw/day ($LD_{20}$; purity: 99.56%); dose is based on toxicity in range-finding study (0.2 to 1 mL/kg bw/day, 8 dose levels, single application)<br><br>Negative control: vehicle (corn oil); positive control: triethyl-enemelamine<br><br>Sacrifice at 12, 24 or 48 hours after exposure for treated and negative control animals; positive control animals sacrificed 24 hours after exposure<br><br>Statistical analyses: One-way ANOVA, Duncan's multiple range test | *Outcome*: no significant changes in number of micronucleated polychromatic erythrocytes, or in the ratio of polychro-matic to monochromatic erythrocytes<br><br>*General toxicity*: 2/18 males and 4/18 females died; treated animals appeared heavily sedated following administration<br><br>*General toxicity in range-finding study*: no mortality up to 0.65 mL/kg; 1/10, 2/10, 10/10 and 10/10 animals died at 0.70, 0.75, 0.80 and 1.00 mL/kg, respectively | GLP compliant study comparable to OECD 474 guideline; single dose only<br><br>Studies are reliable with restriction<br><br>Klimisch score 2 |

# H animal carcinogenicity studies

| Species (references) | Design | Exposure | Observations | Remarks and Quality |
|---|---|---|---|---|
| Male and female B6C3F1 mice (N=50/sex/group;<br>5-6 weeks old at study initiation<br><br>NTP (2007), Stout et al. (2008)[18, 23] | Two-year carcinogenicity inhalation study<br><br>Purity: > 99% (GC-MS) | Whole body inhalation<br><br>*Concentration levels*:<br>0, 450, 900 and 1,800 ppm (0, 1,861, 3,724 and 7,447 mg/m$^3$; concentrations used are based on chronic toxicity studies in literature)<br><br>*Duration of exposure*:<br>6 hours plus 12 min (T90; time needed to achieve 90% of target concentration after beginning of vapour generation in chamber) per day, 5 days a week for 105 weeks<br><br>*Effect endpoint(s)*:<br>Following death, complete necropsies and microscopic examinations were performed on all animals.<br><br>*Statistical analysis*: survival analysis; product-limit procedure of Kaplan and Meier, Cox's method and Tarone's life table test; analysis of neoplasm and nonneoplastic lesion incidences: (continuity-corrected) Poly-k test; analysis of continuous variables: parametric multiple comparison procedures of Dunnett and Williams, Mann-Whitney U test. | *Outcome*: Exposure induced a statistically significant increase in incidence of liver cancer<br><br>*General toxicity*: No treatment-related clinical findings observed; 1,800 ppm females had lower mean body weights after week 17; survival males: 40/50, 42/50, 35/50, 37/50; survival females: 35/50, 37/50, 39/50, 38/50<br><br>*Incidence of neoplasms*:<br>*Liver, males*<br>- hepatocellular adenoma or carcinoma: 27/50, 34/50, 28/50, 37/50 (P=0.019), trend observed (P=0.028)<br>- hepatocellular adenoma:<br>17/50, 25/50, 23/50, 34/50 (P<0.001), trend observed (P<0.001)<br>- hepatocellular carcinoma: 12/50, 12/50, 10/50, 9/50<br>*Liver, females*<br>- hepatocellular adenoma or carcinoma: 17/50, 17/50, 22/50, 27/50 (P=0.035), trend observed (P=0.013)<br>- hepatocellular adenoma: 13/50, 15/50, 20/50, 23/50 (P=0.033), trend observed (P=0.016)<br>- hepatocellular carcinoma: 6/50, 5/50, 6/50, 11/50<br>No other significant exposure-related neoplasia observed<br><br>*Nonneoplastic lesions*:<br>Liver (eosinophilic foci) in<br>- males: 3/50, 4/50, 5/50, 8/50<br>- females: 4/50, 11/50 (P≤0.01), 10/50, 14/50 (P≤0.05) | Study is well performed (OECD 451 guideline; GLP compliant study) and reliable without restrictions<br><br>Quality score 1 |

| Species (references) | Design | Exposure | Observations | Remarks and Quality |
|---|---|---|---|---|
| Male and female F344/N rats (N=50/sex/group); approximately 6 weeks old at study initiation<br><br>NTP (2007), Stout et al. (2008)[18, 23] | See above | See above | *Outcome*: Exposure induced a statistically significant increase in incidence of kidney cancer<br><br>*General toxicity*: 900 and 1,800 ppm males had lower mean body weights after respectively week 97 and 89, and appeared thin and lethargic<br>Survival males: 32/50, 28/50, 25/50, 19/50 (P=0.015); Survival females: 35/50, 34/50, 26/50, 32/50<br><br>*Incidence of neoplasms*:<br>*The kidneys, males*<br>- renal tubule adenoma[a]: 2/50, 3/50, 3/50, 10/50 (P=0.009), trend observed (P=0.002)<br>- renal tubule carcinoma[a]: 0/50, 1/50, 0/50, 2/50<br>- renal tubule adenoma or carcinoma[a]: 2/50, 4/50, 3/50, 11/50 (P=0.004), trend observed (P<0.001)<br>*The kidneys, females*<br>- malignant mesenchymal tumour: 0/50, 0/50, 0/50, 2/50, trend observed (P=0.043)<br>*Leukaemia, males* ((mononuclear cell): 25/50, 26/50, 32/50, 35/50 (P=0.027), trend observed (P=0.016) (note: findings uncertain because of the strength of the response)<br>No other significant exposure-related neoplasia observed<br><br>*Nonneoplastic lesions*:<br>*The kidneys, males*<br>- renal tubule hyperplasia)[a]: 1/50, 14/50 (P≤0.05), 7/50 (P≤0.05), 21/50 (P≤0.01)<br>- nephropathy Chronic Progressive Nephropathy (CPN): 42/50, 45/50, 47/50, 50/50 (P≤0.05)<br>- papilla mineralization: 1/50, 6/50 (P≤0.05), 22/50 (P≤0.01), 29/50 (P≤0.01)<br>- transitional epithelium hyperplasia: 1/50, 5/50, 6/50 (P≤0.05), 19/50 (P≤0.01)<br>- adrenal medulla hyperplasia: 13/50, 18/48, 18/50, 24/50 (P≤0.05)<br>*The kidneys, females*<br>- nephropathy (CPN): 19/50, 35/50, 38/50, 44/50 (P≤0.01, all concentrations)<br><br>[a] Combined results from single sections and step sections (extended evaluation) | |

## The Committee and consulted experts

**The membership of the Subcommittee on Classifying Carcinogenic Substances for the evaluation of the genotoxicity and carcinogenicity of MIBK**

- H.P.J. te Riele, Professor of molecular biology, VU University Amsterdam, and Netherlands Cancer Institute, Amsterdam, *chairman*
- R.W.L. Godschalk, Genetic toxicologist and molecular epidemiologist, Maastricht University (member since January 1, 2020)
- M.J.M. Nivard, Molecular biologist and genetic toxicologist, Leiden University Medical Center, Leiden (member up to December 31, 2019)
- E. de Rijk, Toxicologic pathologist, Charles River Laboratories, ‘s Hertogenbosch
- J.J. Vlaanderen, Epidemiologist, Institute for Risk Assessment Sciences, Utrecht

**Consulted experts**

- J. van Benthem, Genetic toxicologist, RIVM, Bilthoven, *structurally consulted expert*
- P.J. Boogaard, Professor of environmental health and human biomonitoring, Wageningen University and Research Centre, and toxicologist, SHELL International BV, The Hague, *incidental consulted expert*

**Observer**

- M. Woutersen, Bureau REACH, RIVM, Bilthoven

**Scientific secretary**

- 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.
Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity.
The reports are available to the public.

This publication can be downloaded from www.healthcouncil.nl.

Preferred citation:
Health Council of the Netherlands. Methyl isobutyl ketone (4-Methylpentan-2-one).
The Hague: Health Council of the Netherlands, 2020; publication no. 2020/26.