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Pyridine

Evaluation of the carcinogenicity and genotoxicity

Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety, a Committee of the Health Council of the Netherlands

The Health Council would like to give you the opportunity to comment on the draft advisory report. The draft has been presented to the Working Conditions Committee of the Social Economic Council of the Netherlands, and to experts of employer's organizations and trade unions. Other interested parties or persons are also invited to comment. The comments will be taken into account when drafting the final version of the advisory report.

Please follow the instructions for review, see www.healthcouncil.nl

Comments may be submitted until **June 1, 2023**
By e-mail: draftOSH@gr.nl

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Subcommittee on the Classification of Carcinogenic Substances
The Health Council of The Netherlands

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid heeft de Gezondheidsraad beoordeeld of blootstelling aan pyridine 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 pyridine

De stof pyridine wordt gebruikt bij de productie van onder andere pesticiden, geneesmiddelen, waterafstotende middelen voor textiel en geurstoffen. Daarnaast wordt pyridine onder meer gebruikt als oplosmiddel voor verf, rubber 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.

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Beschikbaar onderzoek

De meeste *in vitro* mutageniteitstesten waren negatief. Alleen één *in vitro* chromosoom aberratie test in humane lymfocyten was positief; een tweede chromosoom aberratie test met CHO cellen was negatief. In de *in vivo* studies werd geen mutageniteit waargenomen. Er zijn geen mutageniteitstesten in geslachtscellen uitgevoerd. Aangezien de resultaten van *in vivo* genotoxiciteitstesten zwaarder wegen dan de resultaten van *in vitro* testen, kan pyridine als een niet-genotoxische verbinding beschouwd worden.

Er zijn geen betrouwbare onderzoeksgegevens beschikbaar over gevallen van kanker in mensen door blootstelling aan pyridine.

Er zijn gegevens beschikbaar over mogelijke kankerverwekkende eigenschappen van pyridine in ratten en muizen. In muizen is een dosis-afhankelijke verhoging van het aantal kwaadaardige levertumoren beschreven. Een US National Toxicology Program (NTP) studie in mannelijke F344 ratten rapporteert een dosis-afhankelijke toename van niertumoren, waaronder een verhoging van slechts één kwaadaardige tumor in de lage doseringsgroep. In deze studie worden ook een verhoging van het aantal gevallen van leukemie in vrouwelijke F344 ratten beschreven, waarvan onduidelijk is of deze veroorzaakt zijn door blootstelling aan pyridine. De commissie oordeelt dat er geen overtuigend verband is aangetoond tussen de blootstelling aan pyridine en een toename van kwaadaardige tumoren in een tweede diersoort, en adviseert derhalve om pyridine te classificeren als een verdacht kankerverwekkende stof voor de mens.

Advies

De commissie adviseert om pyridine:

- 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).

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Executive summary

The Health Council of the Netherlands assessed whether exposure to pyridine 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 pyridine

Pyridine is used as a chemical intermediate in the production of amongst others pesticides, pharmaceuticals, textile water repellents, and flavours. Moreover, pyridine is amongst others used as a solvent for the production of paint, rubber, 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 to decide whether the substance should be listed as mutagenic in germ cells and/or carcinogenic.

Evaluation of the data

Most in *vitro* genotoxicity tests were negative. Exclusively, one *in vitro* chromosome aberration tests in human lymphocytes was positive; a second chromosome aberration test in CHO cells was negative. No mutagenicity was observed in *in vivo* studies. No experiments in common germ cell mutagenicity tests have been conducted. Since results of *in vivo* genotoxicity tests overrule the results of *in vitro* tests, pyridine can be considered a non-genotoxic compound based on both *in vitro* and *in vivo* genotoxicity tests.

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There is no reliable data on the carcinogenicity of pyridine in humans available.

There is data available on increased malignant tumour development in both rats and mice. A dose-dependent increase in the number of malignant liver tumours has been described in mice. A US National Toxicology Program (NTP) study in male F344 rats reports a dose-dependent increase in single kidney tumours, among which only a single malignant tumor in the low-dose group. This study also describes cases of leukemia in female F344 rats, of which it is unclear whether they were caused by pyridine exposure. The committee considers these observations in rats as insufficient proof for an increased incidence of carcinomas in a second animal species, and therefore proposes to classify pyridine as a substance suspected to be carcinogenic to humans.

Recommendation

The Committee recommends

- not to classify pyridine as a germ cell mutagen;
- to classify pyridine as *suspected to be carcinogenic to humans*, which corresponds with category 2 for carcinogenicity, and to label pyridine with H351 (suspected of causing cancer).

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1 Scope

1.1 Background

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

This report contains the evaluation of the genotoxicity and carcinogenicity of pyridine.

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 November 2022, the President of the Health Council released a draft of the report for public review.

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] Another important source of information is the evaluation by the International Agency for Research on Cancer (IARC).[2] A summary of IARC's conclusion can be found in Annex B.

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,

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and Embase, using the key words pyridine, 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 pyridine 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 September 2022. In the case of pyridine, 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 pyridine, the Committee retrieved the individual studies summarised in the RIVM document and the IARC Monograph. Subsequently, the Committee evaluated the selected studies on their quality. Study quality may vary and therefore, the Committee judges the quality of the study on reliability (quality of methodology and reporting), on the relevance for the purpose of the assessment, and on adequacy (usefulness), according to the current views in the scientific community. The quality evaluation is performed to assess the weight of evidence for an association between substance exposure and genotoxicity and/or risk of cancer development.

1.5 Criteria for classification

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

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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. 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]. This guideline is currently under revision.

Annexes B and C summarize 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.

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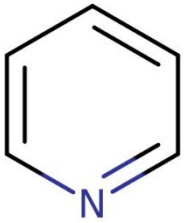

2 General information

Information on the identification, physicochemical properties, monitoring, manufacturing and use, international classifications, and (toxico)kinetics of pyridine is outlined in the RIVM document (2021) and IARC Monograph (2019). A summary is given below.

Pyridine (C_5H_5N; CAS number 110-86-1; EC/EINECS number 203-809-9) is a colourless liquid at room temperature which is highly soluble in water (1000 g/L). Several analytical methods are available for measuring pyridine in air using gas chromatography. Measuring levels of pyridine metabolites in urine might be used to assess internal exposure, but no validated methods are available. Pyridine is used as a solvent for the production of paint, rubber, pharmaceuticals and polycarbonate resins, and as a denaturant in alcohol and antifreeze mixtures. Moreover, pyridine is used as an intermediate in the manufacture of pesticides and pharmaceuticals, dyes, textile water repellents and flavours.

The European commission has classified pyridine as a highly flammable liquid and vapour (H225) that is harmful if swallowed (H302), harmful in contact with skin (H312) and harmful if inhaled (H332). IARC classified pyridine as *possibly carcinogenic to humans* (Group 2B).

Data on kinetics of pyridine are obtained from both human and animal studies. Pyridine can be absorbed after oral, dermal and inhalation exposure. Pyridine is metabolised to mainly pyridine N-oxide, 2-pyridone, 4-pyridone, 3-hydroxypyridine and N-metylpyridinium ions. The proportion of the dose excreted as each of these metabolites is species-dependent.[4] Two healthy male volunteers received an oral dose of 3.4 mg [14C]pyridine (~0.04 mg/kg bw) in orange juice.[4,5] Of the total dose, 32% was recovered as pyridine N-oxide and 5.5% and 12% as N-methylpyridinium ion in the collected urine after 24 hours). The major metabolic pathways are initiated by CYP2E1 enzymes.

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3 Genotoxicity

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

Data on mutagenicity are summarized in the RIVM document (2021) and IARC monograph (2019). The Committee did not find additional or new data in the literature. Tables 1 (*in vitro* mutagenicity tests) and 2 (*in vivo* mutagenicity tests) show summaries of the findings.

3.2 *In vitro* genotoxicity

An overview of mutagenicity studies is presented in Table 1. The study of Haworth et al. (1983) showed that pyridine (100-10,000 µg/plate) was not mutagenic in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without S9 metabolic activation enzymes. No significant increase in mutant frequencies was observed in L5178Y mouse lymphoma cells using concentrations up to 5000 µg/mL, tested with and without S9 metabolic activation.[6]

Table 1. Summary of *in vitro* mutagenicity tests

Assay; microorganism or cell type	**Concentration range**	**Results; cytotoxicity**	**References/remarks**
In vitro mutagenicity tests - micro-organisms			
Ames test; *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537.	(+/-S9[a,b]): 0, 100, 333.3, 1,000, 3,333.3, 10,000 µg/plate; 20 min incubation Positive controls: -S9: sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-o-phenylenediamine (TA98) +S9: 2-aminoanthracene (all strains)	No increase in histidine-independent (revertant) colonies for TA98 (+/-S9), TA100 (+/-S9), TA1535 (+/-S9), TA1537 (+/-S9)	[7], [8] Well-performed study according to GLP; not according to OECD TG 471. Appropriate results were obtained with negative (solvent) and positive controls No statistical analysis performed
Ames test; *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537.	(+/-S9[a,b]): 0, 237 µg/plate	No increase in histidine-independent (revertant) colonies for TA98 (+/-S9), TA100 (+/-S9), TA1535 (+/-S9), TA1537 (+/-S9)	[9] Not according to OECD TG 471. Appropriate results were obtained with negative (solvent) and positive controls

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			No statistical analysis performed
In vitro mutagenicity tests - Mammalian cells			
Mouse Lymphoma assay; L5178Y cells	(-)S9[c] Trial 1: 0, 625, 1,250, 2,500, 5,000 µg/mL Trial 2: 0, 1,000, 2,000, 3,000, 4,000, 5,000 µg/ mL Trial 3: 0, 2,000, 3,000, 4,000, 5,000 µg/ mL (+)S9 Trial 1: 0, 1,000, 2,000, 3,000, 4,000, 5,000 µg/ mL Trial 2: 0, 2,000, 3,000, 4,000, 5,000 µg/ mL, incubated with Pyridine for 4h Positive control: methyl methanesulfonate	No significant increase in mutant colonies was observed in L5178Y mouse lymphoma cells (+/-S9) compared to untreated control cells The high dose of pyridine of 5,000 µg/mL did not induce cytotoxicity.	[6], [8] Well-performed study according to GLP; not according to OECD TG 490. Appropriate results were obtained with negative (solvent) and positive controls Statistical analysis: All data were evaluated statistically for trend and peak responses.
In vitro cytogenicity tests – mammalian cells			
Sister chromatid exchange test; Chinese hamster ovary cells	(-S9[a]): 0, 167, 502, 1,673, 5,020 µg/mL (+S9): 0, 167, 502, 1,673, 5,020 µg/mL Incubation: -S9: 26 hour incubation with the test chemical; BrdU was added 2 hours after culture initiation. After 26 hours, medium was removed and fresh BrdU and Colcemid was added for 2 hours +S9: 2 hour incubation with test chemical. After removal of test chemical, BrdU was added for an additional 26 hour incubation; Colcemid was added during the final 2 hours Positive control: -S9: mitomycin-C	No effect on frequency of SCEs per cell observed	[10], [8] Well-performed study according to GLP; appropriate results were obtained with negative (solvent) and positive controls Statistical analysis conducted on the slopes of the dose-response curve and individual dose points

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	+S9: cyclophosphamide		
Chromosomal aberration test; Chinese hamster ovary cells	(+/-S9[a]): 0, 1,081, 2,325, 5,000 µg pyridine/ml Incubation: -S9: 11.5 hours incubation with test chemical; Colcemid was added and incubation continued for 2 hours +S9: 2 hours incubation with test chemical; after removal of test chemical, fresh medium was added for 11.5 hours with Colcemid present for the final 2 hours Positive control: -S9: mitomycin-C +S9: cyclophosphamide	No increase in number of cells with chromosomal aberrations	[10], [8] Well-performed study according to GLP; Not according to OECD TG 473 ; appropriate results were obtained with negative (solvent) and positive controls Statistical analysis: conducted on the slopes of the dose-response curve
Chromosomal aberrations test; Human peripheral blood lymphocytes	0.002, 0.02, 0.2, 3.25 µg pyridine/mL The lymphocyte cultures were incubated at 37°C for 72 hours. Test chemicals were added 48 hours after initiating the culture Positive control: Cyclophosphamide	Positive The four different concentrations of pyridine showed an increase in the number of cells with chromosomal aberrations compared to untreated cells in a concentration dependent manner.	[11] Well-performed study according to GLP; not according to OECD TG 473. Appropriate results were obtained with negative (solvent) and positive controls No statistical analysis performed

[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.
PCE: polychromatic erythrocytes

In a chromosomal aberration test using human peripheral blood lymphocytes with concentrations up to 3.25 µg pyridine/mL a concentration dependent increase in cells with chromosomal aberrations was found at all concentrations tested. However, statistical analysis was not performed. In a second chromosomal aberration test with Chinese hamster ovary (CHO) cells with higher concentrations up to 5000 µg pyridine/mL but with shorter incubation times, no statistically or biologically relevant increases in cells with chromosomal aberrations were found.

A sister chromatid exchange test in Chinese hamster ovary cells did not show an increase in sister chromatid exchanges (SCEs), with or without S9-mix.[8,10,11]

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Pyridine induced marked cell cycle delay after exposure to the highest viable concentration (1,673 µg/mL), in the absence of S9-mix. In this test, an extended culture time (31 hours) was used to allow sufficient cells to accumulate for analysis.

3.3 Conclusion on *in vitro* genotoxicity

Pyridine was not mutagenic in a gene mutation test with bacteria (Ames test) nor in a gene mutation test with L5178Y mouse lymphoma cells. Pyridine did induce an increase in chromosomal aberrations in a chromosomal aberration test with human lymphocytes. A chromosomal aberration test with CHO cells was negative. Pyridine did not induce SCEs in cultured CHO cells.

3.4 Summary of human data relevant for germ cell mutagenicity

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

3.5 Summary of genotoxicity tests in mammalian somatic or germ cells *in vivo*

In a *Drosophila melanogaster* sex-linked recessive lethal mutation test following feeding of pyridine a non-significant increase in cells with recessive lethal mutations were found whereas after injection no increase was observed.[8,12] In a second test with *Drosophila melanogaster* after injection with a lower dose, a significant increase in cells with recessive lethal mutations were observed whereas after feeding with a somewhat lower dose, no increase was observed. [8,13] In a third *Drosophila melanogaster* sex-linked recessive lethal mutation test, the substance did not induce reciprocal translocations both after injection and feeding.[8,14]

In a reciprocal translocation test in *Drosophila melanogaster* no increase in translocation nor in clinical signs were observed after injection with pyridine.[13]

In *in vivo* mouse bone marrow tests in B6C3F1 mice, intraperitoneally injected pyridine did not induce an increase in bone marrow cells with chromosomal aberrations after 17 and 36 hours (400-600 mg/kg pyridine; single injection) exposure (Table 2).[8,15] An increase in the number of micronucleated polychromatic erythrocytes (PCEs) was also not observed in a micronucleus test after intraperitoneal injection of pyridine in B6C3F1 mice (up to 500 mg/kg administered three times at 24-hour intervals).[16]

In an *in vivo* unscheduled DNA synthesis (UDS) assay in male B6C3F1 mice that were orally exposed to 175, 350 or 700 mg/kg bw by gavage, no significant increase of UDS

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response was observed in hepatocytes harvested 2 and 16 hours after dosing, as measured by the incorporation of [3H]thymidine.[8,17]

The Committee notes that with the exemption of the OECD-guideline UDS test, the applied doses in the in vivo genotoxicity studies were too low as no general toxicity has been 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 mutagenicity tests			
Sex-Linked Recessive Lethal Mutation Test; adult male, wild-type Canton-S flies (*D. melanogaster*)	Feed: 0, 600, 700 ppm Injection: 0, 7,000 ppm Positive controls: N-nitrosodimethylamine (DMN) and β-propiolactone[18]	Administration by injection (7,000 ppm in aqueous 0.7% saline solution) caused no effects (P=0.225). Feeding (600 and 700 ppm in aqueous 5% sucrose) produced a significant increase in cells with recessive lethal mutations (P=0.043). No treatment-related clinical signs.	[12], [8] Well-performed study according to GLP; appropriate results were obtained with positive controls Applied dose levels were probably not high enough, given that no general toxicity was noticed. Statistical analysis: binomial test with normal approximation
Sex-Linked Recessive Lethal Mutation Test; adult male, wild-type Canton-S flies (D. melanogaster)	Feed: 0, 729 ppm Injection: 0, 500 ppm Positive controls: N-nitrosodimethylamine (DMN) and β-propiolactone[18]	Both injection (500 ppm) and feeding (729 ppm) yielded no effects. No treatment-related clinical signs.	[14], [8] Well-performed study according to GLP; appropriate results were obtained with positive controls Applied dose levels were probably not high enough, given that no general toxicity was noticed. Statistical analysis: binomial test with normal approximation
Sex-Linked Recessive Lethal Mutation Test; adult male, wild-type Canton-S flies (D. melanogaster)	Feed: 0, 500 ppm Injection: 0, 4,300 ppm Positive controls: N-nitrosodimethylamine	Feeding (500 ppm) experiment did not induce an increase in the frequency of number of cells with SLRL mutations, (P=0.998) Injection (4,300 ppm) induced a significant increase in the	[13], [8] Well-performed study according to GLP; appropriate results were obtained with positive controls

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	(DMN) and β-propiolactone (Woodruff, 1984)	frequency of number of cells with SLRL mutations (P=0.008). No treatment-related clinical signs.	Applied dose levels were probably not high enough, given that no general toxicity was noticed. Statistical analysis: Binomial test with normal approximation
Reciprocal Translocation Test; adult male, wild-type Canton-S flies (*D. melanogaster*)	Injection:4,300 ppm	No treatment-related increase in translocation was found after injection with pyridine. No treatment-related clinical signs.	[13] Well-performed study according to GLP; non-guideline Applied dose levels were probably not high enough, given that no general toxicity was noticed. Statistical analysis: conditional binomial response test
Chromosomal nondisjunction test; adult female, wild-type Canton-S flies (*D. melanogaster*)	0.05, 0.1, 0.2, 0.3 or 0.4%	Pyridine induced significant increase in disjunction broods arising from nearly mature oocytes, but not early-stage or mature oocytes. No dose-response relationship.	[19] Guideline unknown Statistical analysis: binomial test with normal approximation
In vivo animal cytogenicity tests			
In vivo mouse bone marrow Chromosomal Aberrations Test; Male B6C3F1 mice 10/dose group	0, 400, 500, 600 mg/kg Intraperitoneal injection, single, volume: 0.4 mL Positive control: Mitomycin-C	No induction of chromosomal aberrations was noted in bone marrow cells at either of two sampling times (17 and 36 hours). No treatment-related clinical signs.	[15], [8] Well-performed study according to GLP; non-guideline Applied dose levels were probably not high enough, given that no general toxicity was noticed. Statistical analysis: trend test
In vivo mouse bone marrow Micronucleus Test; male B6C3F1 mice 10/exposure concentration (chamber control or exposed)	0, 31.25, 62.5, 125, 250, 500 mg/kg Intraperitoneal injection, three times at 24-hour intervals; total dosing volume: 0.4 mL Positive control: cyclophosphamide	No increase in the number of cells with micronucleated PCEs was noted in bone marrow after intraperitoneal injection of pyridine (up to 500 mg/kg administered three times at 24-hour intervals). No treatment-related clinical signs.	[16], [8] Well-performed study according to GLP; non-guideline Applied dose levels were probably not high enough, given that no general toxicity was noticed.

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			Statistical analysis: a one-tailed Cochran-Armitage trend test, followed by pairwise comparison between each exposed group and the control group.
Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo; B6C3F1 Mice 4/dose group Only the first three successful perfusions in each dose group were analyzed for UDS.	0, 175, 350 and 700 mg/kg Administered in water by gavage, 2 or 16 h prior to the scheduled sacrifice. Positive control: Dimethylnitrosamine	No evidence of an increase in UDS in B6C3F1 hepatocytes following *in vivo* exposures up to the maximum tolerated dose of pyridine. In the UDS assay, some mildly adverse, reversible clinical signs were seen in mice given the high dose (700 mg/kg).	[17] Well-performed study according to GLP; guideline for Testing of Chemicals, No. 486 No statistical analysis performed

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3.6 Conclusion on *in vivo* genotoxicity

Pyridine exposure resulted in mixed results in 3 different sex-linked recessive lethal mutation tests in *Drosophila melanogaster.* Also, a reciprocal translocation test in adult male, wild-type Canton-S flies (D. melanogaster) was negative. The positive results found in an *in vitro* chromosomal aberration test were not confirmed under *in vivo* conditions. In the *in vivo* chromosomal aberration test in male B6C3F1 mice, a biologically relevant increase in cells with aberrations was not observed. Finally, an unscheduled DNA synthesis test in B6C3F1 mice was also negative.
The *in vivo* genotoxicity test results are insufficient to draw conclusions on the cell mutagenic properties of pyridine.

3.7 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, pyridine 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 did not show reliable positive effects of pyridine in three sex-linked recessive lethal mutation tests in Drosophila melanogaster. Therefore, pyridine 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* tests for both induction of chromosomal aberrations or micronuclei in male mice did not show positive effects, although this may be explained by insufficient exposure in most studies. Therefore, the Committee concludes that pyridine does not meet the criteria to classify the substance in category 2 for mutagenicity.

3.8 Recommendation on the classification for germ cell mutagenicity

The Committee recommends not classifying pyridine as a germ cell mutagen due to lack of sufficient data.

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4 Carcinogenicity

4.1 Summary and relevance of the provided information on carcinogenicity

Data on carcinogenicity are summarised in the RIVM document (2021) and the IARC Monograph (2019).

4.2 Observations in humans

A cohort study was performed in England in 729 men manufacturing 4,4′-bipyridyl [4,4′-bipyridine] (Paddle et al., 1991; as cited from IARC, 2019). For all cancers combined, the standardized mortality ratio (SMR) was 1.1 (95% CI, 0.7-1.5; 29 deaths) and for cancer of the lung it was 1.2 (95% CI, 0.7–2.1; 13 deaths). In an earlier case-series study of skin lesions in the same plant a total of 6 cases of Bowen's disease and 6 cases of squamous cell carcinoma were observed (Bowra et al., 1982; as cited from IARC, 2019). No cancer risk data were reported and no quantitative exposure data were available to study the associations between cancer risk and exposure to pyridine.

4.3 Animal carcinogenicity studies

No carcinogenicity studies in which animals were exposed via inhalation or the skin are available. The carcinogenicity studies in which animals were exposed to pyridine via the oral or subcutaneous route are summarized in Table A1 in appendix A.

4.3.1 Rats

Male and female F344/N rats (N=50/sex/dose) were exposed to pyridine ad libitum via drinking water at concentrations of 0, 100, 200 or 400 ppm (corresponding to 0, 7, 14 and 33 mg/kg bw/d) for 104 (males) and 105 (females) weeks (according to GLP).[8] Nonneoplastic lesions in male and female F344/N rats are briefly summarized in appendix A, Table A2.1 and A2.2 respectively. Mean body weights of rats exposed to 400 ppm were generally lower than those of the control group throughout the study. Body weights of rats exposed to 200 ppm were generally lower during the second year of the study. As depicted in Table 3a, compared to the controls, there was a statistically significant increase in number of male rats with renal tubule adenomas and adenomas and carcinomas combined at the highest dose tested (33 mg/kg bw/d). The increased kidney tumours were reflected in a dose-dependent increased incidence in renal tubular hyperplasia (14 and 33 mg/kg bw/d) (see appendix A, Table A2).

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Tumour incidence was observed even in the absence of alpha 2u-globulin, hence eliminating its possible role in the reported nephropathy. In female rats, a statistically significant increase in number of rats with mononuclear cell leukaemia was reported compared to control (in the groups exposed to 14 and 33 mg/kg bw/d) (Table 3b). Although the increase was statistically significant, the absolute increment in incidence was small compared to the wide range observed in the historical control data set.

Table 3a. *Number of male F344/N rats* with *neoplastic lesions after exposure to pyridine via drinking water for 2 years.*[8]

Type of tissue/lesion	**0 ppm (0 mg/kg bw/d)**	**100 ppm (7 mg/kg bw/d)**	**200 ppm (14 mg/kg bw/d)**	**400 ppm (33 mg/kg bw/d)**	**Historical control data[a]**
Number of animals					
Kidney					
Renal tubule adenoma[b] - Single sections - single and step sections	 1/50 (2%) 2/50 (4%)	 0/48 (0%) 3/48 (6%)	 2/50 (4%) 6/50 (12%)	 6/49* (12%) 10/49* (20%)	 1/327; 0.3% ± 0.8% (range 0-2%)
Renal tubule adenoma or carcinoma[c] - Single sections - single and step sections	 1/50 (2%) 2/50 (4%)	 1/48 (2%) 4/48 (8%)	 2/50 (4%) 6/50 (12%)	 6/49* (12%) 10/49** (20%)	 1/327; 0.3% ± 0.8% (range 0-2%)

Table 3b. *Number of female F344/N rats with neoplastic lesions after exposure to pyridine via drinking water for 2 years.*[8]

Type of tissue/lesion	**0 ppm (0 mg/kg bw/d)**	**100 ppm (7 mg/kg bw/d)**	**200 ppm (14 mg/kg bw/d)**	**400 ppm (33 mg/kg bw/d)**	**Historical control data[d]**
All organs					
Mononuclear cell leukaemia	12/50 (24%)	16/50 (32%)	22/50* (44%)	23/50* (46%)	102/330; 30.9% ± 10.0% (range: 16-44%)

* Significantly different (P≤0.05) from the control group by the Poly-3 test
** Significantly different (P≤0.01) from the control group by the Poly-3 test
[a] Historical Data as of 1 August 1997.
[b] For extended evaluation of renal proliferative lesions in male rats, kidneys were step sectioned at 1-mm intervals, and four additional sections were obtained from each kidney.
[c] combined incidence of renal tubule adenoma or carcinoma
[d] Data as of 1 August 1997; includes data for lymphocytic, monocytic, mononuclear cell, and undifferentiated leukaemias

Male Wistar rats (N=50/sex/dose) were exposed to pyridine ad libitum via drinking water at concentrations of 0, 100, 200 or 400 ppm (corresponding to 0, 8, 17 and 36 mg/kg bw/d) for 104 weeks (according to GLP).[8] Nonneoplastic lesions in male Wistar

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rats are briefly summarized in appendix A, Table A3. Compared to the controls mean body weights were significantly lower in all dosed groups (91%, 83% and 84%, respectively). A statistically significant increase in interstitial cell adenomas in the testes was observed in the high dose group (Table 4). No historical background data were available. However, it is described that in Wistar rats the background incidences of interstitial cell adenomas are highly dependent on the breeder and mean incidences can vary from 2.8% to 39.9%.[20]

Table 4. *Number of Wistar rats with neoplastic lesion after exposure to pyridine via drinking water for 2 years.*[8]

Type of tissue/lesion	0 ppm (0 mg/kg bw/d)	100 ppm (8 mg/kg bw/d)	200 ppm (17 mg/kg bw/d)	400 ppm (36 mg/kg bw/d)	Historical control data[a]
Testes					
Interstitial cell adenoma	5/50 (10%)	6/49 (12%)	4/49 (8%)	12/50* (24%)	N.A.

a Historical Data as of 1 August 1997
N.A. not available

4.3.2 Supplemental chronic study in rats

Male and female F344 rats were exposed to 0, 3, 10, 30 or 100 mg/k bw/day pyridine by subcutaneous administration for 52 weeks.[21] An average reduction in weight gain of 11% (5-16%) was observed at the highest dose. No significant increase in treatment-related tumour incidence was observed (Table 5).

Table 5. *Tumour incidence in male and female F344/N rats upon subcutaneous exposure to pyridine for 1 year.*[21]

		Tumour bearing rats			**Tumour types**		
Dose (mg/kg bw/day)	Group size (male/female)	Male	Female	Both	Fibroma or sarcoma (%)	Mammary (%)	Others (%)
3	10/10	0 (0%)	1 (10%)	1 (5%)	2 (3%)	1 (5%)	0 (0%)
10	20/20	0 (0%)	2 (10%)	2 (5%)	0 (0%)	0 (0%)	2 (5%)
30	30/30	1 (3%)	7 (23%)	8 (13%)	0 (0%)	2 (3%)	6 (10%)
100	40/40	2 (5%)	2 (5%)	4 (5%)	0 (0%)	0 (0%)	2 (3%)
Negative control	50/50	5 (10%)	9 (18%)	14 (14%)	1 (1%)	1 (1%)	12 (12%)
Vehicle control (saline)	50/50	3 (6%)	9 (18%)	12 (12%)	0 (0%)	3 (3%)	9 (9%)

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4.3.3 Mice

Male B6C3F1 mice (50/dose) were exposed for 104 weeks to 0, 250, 500 or 1000 ppm (corresponding to 0, 35, 65 or 110 mg/kg bw/d) pyridine via drinking water.[8] As depicted in Table 6a, pyridine induced a statistically significant increase (compared to control) in the number of male mice with hepatocellular carcinomas and hepatoblastomas. Combinations of hepatocellular adenomas, hepatocellular carcinomas and hepatoblastomas were significantly increased in males in all dose groups. Female B6C3F1 mice (50/dose) were exposed to 0, 125, 250 or 500 ppm pyridine (corresponding to 0, 15, 35 or 70 mg/kg bw/d) via drinking water. Reduced body weights were observed in females after exposure to 35 or 70 mg/kg bw/day. In females, the incidence of hepatocellular carcinomas, hepatoblastomas, or a combination of these tumours was dose-dependently increased in all dose groups (Table 6b).

Table 6a. *Number of male B6C3F1 mice with neoplastic lesions after exposure to pyridine via drinking water for 2 years.*[8]

Type of tissue/lesion	0 ppm (0 mg/kg bw/d)[a]	250 ppm (35 mg/kg bw/d)	500 ppm (65 mg/kg bw/d)	1000 ppm (110 mg/kg bw/d)	Historical control data[b]
Liver					
Hepatocellular adenoma: - Single - Multiple - Total	 13/50 (26%) 16/50 (32%) 29/50 (58%)*	 11/50 (22%) 29/50 (58%) 40/50 (80%)**	 5/49 (10%) 29/49 (59%) 34/49 (69%)	 11/50 (22%) 28/50 (56%) 39/50 (78%)*	179/289; 61.9% ± 9.1% (range: 47-70%)
Hepatocellular carcinoma: - Single - Multiple - Total	 12/50 (24%) 3/50 (6%) 15/50 (30%)**	 16 (32%) 19/50 (38%) 35/50 (70%)**	 15 (31%) 26/49 (53%) 41/49 (84%)**	 22 (44%) 18/50 (36%) 40/50 (80%)**	80/289; 27.7% ± 11.7% (range: 10-42%
Hepatoblastoma: - Single - Multiple - Total	 1/50 (2%) 1/50 (2%) 2/50 (4%)**	 14/50 (28%) 4/50 (8%) 18/50 (36%)**	 16/49 (33%) 6/49* (12%) 22/49 (45%)**	 13/50 (26) 2/50 (4%) 15/50 (30%)**	9/289; 3.1% ± 5.0% (range: 0-12%)
Hepatocellular adenoma or carcinoma	37/50 (74%)**	45/50 (90%)**	45/49 (92%)**	47/50 (94%)**	N.A.
Hepatocellular carcinoma or hepatoblastoma	17/50 (34%)**	42/50 (84%)**	45/49 (92%)**	42/50 (84%)**	N.A.

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Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma	38/50 (76%)**	47/50 (94%)**	46/49 (94%)**	47/50 (94%)**	212/289; 73.4% ± 11.7% (range: 53-81%)

Table 6b. *Number of female B6C3F1 mice with neoplastic lesions after exposure to pyridine via drinking water for 2 years.*[8]

Type of tissue/lesion	0 ppm (0 mg/kg bw/d)[a]	125 ppm (15 mg/kg bw/d)	250 ppm (35 mg/kg bw/d)	500 ppm (70 mg/kg bw/d)	Historical control data[b]
Liver					
Hepatocellular adenoma: - Single - Multiple - Total	 13/49 (27%) 24/49 (49%) 37/49 (76%)	 5/50 (10%) 34/50 (68%) 39/50 (78%)	 6/50 (12%) 37/50 (74%) 43/50 (86%)*	 4/50 (8%) 30/50 (69%) 34/50 (68%)	 150/289; 51.9% ± 20.8% (range: 26-80%)
Hepatocellular carcinoma: - Single - Multiple - Total	 10/49 (20%) 3/49 (6%) 13/49 (27%)**	 12/50 (24%) 11/50 (22%) 23/50 (46%)*	 19/50 (38%) 14/50 (28%) 33/50 (66%)**	 11/50 (22%) 30/50 (30%) 41/50 (82%)**	 55/289; 19.0% ± 13.7% (range: 8-42%)
Hepatoblastoma: - Single - Multiple - Total	 1/49 (2%) - 1/49 (2%)**	 2/50 (4%) - 2/50 (4%)	 6/50 (12%) 3/50 (6%) 9/50 (18%)**	 12/50 (24%) 11/50 (22%) 16/50 (32%)**	 0/289
Hepatocellular adenoma or carcinoma	41/49 (84%)*	42/50 (84%)	44/50 (88%)	44/50 (88%)*	N.A.
Hepatocellular carcinoma or hepatoblastoma	13/49 (27%)**	23/50 (46%)*	36/50 (72%)**	43/50 (86%)**	N.A.
Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma	41/49 (84%)**	42/50 (84%)	45/50 (90%)*	44/50 (88%)*	173/289; 59.9% ± 21.3% (range: 32-82%)

* Significantly different (P≤0.05) from the control group by the Poly-3 test.
** Significantly different (P≤0.01) from the control group by the Poly-3 test.
[a] Beneath the control incidence are the P values associated with the trend test
[b] Historical Data as of 1 August 1997
N.A. Not available

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4.4 Summary of additional data

Pyridine was tested in two transgenic mouse models for evidence of treatment-related lesions. Assessment of the p53+/− mouse model, that responds to genotoxic chemicals, and the zeta globin-Ha-ras (Tg·Ac) model, that was reported to respond to genotoxic and non-genotoxic carcinogens, was based on gross necropsy on all animals at 26 weeks.[22] Pyridine was delivered in water ad libitum 7 days/week to p53+/−mice for 26 weeks at doses of 0, 250, 500 and 1000 ppm for males and doses of 0, 125, 250 and 599 ppm for females. Doses of 0, 1.5, 3.0 and 6.0 mg pyridine were administered topically to Tg·Ac mice for 20 weeks. Dose groups in the Tg·Ac studies comprised 15–20 female mice. Dose groups in the p53+/− studies comprised 10 female and 10 male mice. Tissues from multiple organs of control mice and mice given the highest dose were examined microscopically. In addition, in the Tg.Ac model, a section of the skin at the site of application was examined microscopically. No significant increase in the incidence of neoplasms was observed in either of the transgenic mouse models exposed to pyridine.

4.5 Evaluation on the carcinogenicity

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

In a well performed study by the National Toxicology Program (NTP), renal tubule adenomas and carcinomas, mononuclear cell leukaemia, and testicular adenomas were found in rats. The Committee notes that for renal tubule adenomas, and adenomas and carcinomas combined, a statistically significant dose-dependent increase was observed in male rats. It should be noted that in the whole study only one renal carcinoma was observed at the lowest dose. A modestly dose-dependent increased incidence was observed for mononuclear cell leukaemia in F344 female rats. However, the Committee considers the findings in the F344 rat carcinogenicity study of limited value given their high spontaneous background incidence and species-specific biology, resulting in a lack of relevance in predicting human carcinogenicity as described by Maronpot et al. 2016.[23]
A statistically significant increase in interstitial cell adenomas in the testes was observed in the high dose group in male rats, but no progression to malignancy was apparent.

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In male mice, pyridine significantly increased the number of hepatocellular carcinomas and hepatoblastomas compared to the control mice. The combination of hepatocellular adenomas, hepatocellular carcinomas, and hepatoblastomas was significantly increased in male mice. In female mice, the incidence of hepatocellular carcinomas, hepatoblastomas, or a combination of these tumours was increased in all dose groups. Although these increased incidences of the combination of liver tumours showed variation and the control incidence was high (albeit within historical control values), the increase in malignant liver tumour incidences showed a convincing dose-relationship.

Overall, the Committee concludes that there is insufficient evidence for carcinogenicity in two or more animal species, which is needed for classification in category 1B. Based on the dose-dependent increase in hepatocellular carcinomas in mice, the Committee recommends classifying pyridine as a suspected carcinogen.

4.6 Recommendation on the classification for carcinogenicity

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

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Annexes

A Summary Tables for carcinogenicity
B IARC evaluation and conclusion
C Classification criteria on germ cell mutagenicity
D Classification system on carcinogenicity

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A Summary Tables for carcinogenicity

Table A1. *Summary of carcinogenicity tests.*

Experimental design	Concentration and route	Results	Remarks
Carcinogenicity Study Rat, F344/N male and female 50/sex/exposure concentration (chamber control or exposed)	Concentration in drinking water: 0, 100, 200, 400 ppm (results in an average daily dose of 0, 7, 14, 33 mg/kg) Drinking water was given for 104 (males) or 105 (females) weeks.	<u>Observations</u> Twice daily observation; Clinical findings were recorded at 4-week intervals, and body weights were recorded at the start of the study, weekly for the first 13 weeks, every 4 weeks until week 92, and then once every 2 weeks until study termination; Complete necropsies and histopathologic examinations were performed on all core study rats. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were processed and stained with H&E for microscopic examination. In an extended evaluation of the kidneys for renal proliferative lesions, the residual wet kidney tissue of male rats was step sectioned at 1 mm intervals to obtain three to four additional sections from each kidney with a maximum of eight additional sections per animal. <u>Results</u> Survival: not significantly different from controls. Clinical findings: no treatment-related clinical findings Nonneoplastic lesions: *Kidney*: Single Sections renal tubule hyperplasia (400 ppm males; P≤0.05) *Liver*: basophilic foci (200 and 400 ppm males and females, P≤0.01; 100 ppm females, p≤0.05), clear foci (400 ppm females, P≤0.01; 100 ppm males and 200 ppm females, P≤0.05), Eosinophilic Focus (100 ppm, males, P≤0.05), Centrilobular Cytomegaly (200 ppm males and 400 ppm females, P≤0.01; 400 ppm males, P≤0.05), Vacuolization Cytoplasmic (400 ppm males, P≤0.01; 200 ppm males and 400 ppm females, P≤0.05); Periportal Fibrosis (400 ppm males, P≤0.01), Fibrosis (400 ppm males, P≤0.01), Centrilobular Degeneration (400 ppm males and females, P≤0.05), Centrilobular Necrosis (400 ppm males, P≤0.05), Bile Duct Hyperplasia (200 ppm females, P≤0.01, 100 and 300 ppm females, p≤0.05), Pigmentation (200 ppm males, 400 ppm males and females P≤0.01; 100 ppm males, P≤0.05)	[8] Well-performed study according to GLP; non-guideline Statistical analysis tumour incidences: the Poly-κ test (with κ =3) was used to assess neoplasm and nonneoplastic lesion prevalence

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		Stomach: glandular mineralization 400 ppm males; P≤0.01) Neoplastic lesions: *Kidney*: Single sections, renal tubule adenoma and combined adenoma/carcinoma (400 ppm males, p=0.042); Single sections and Step sections (Combined), renal tubule adenoma and combined adenoma/carcinoma (400 ppm males, p=0.008) *Liver*: hepatocellular neoplasms were not significantly increased in exposed rats compared to controls. All organs: Mononuclear Cell Leukaemia (200 ppm, females, p=0.043; 400 ppm, females, p=0.020)	
Rat, Wistar males 50/exposure concentration (chamber control or exposed)	Carcinogenicity Study Concentration in drinking water: 0, 100, 200, 400 ppm (results in an average daily dose of 0, 8, 17, 36 mg/kg) Drinking water was given for 104 weeks	<u>Observations</u> Twice daily observation; Clinical findings were recorded at 4-week intervals, and body weights were recorded at the start of the study, weekly for the first 13 weeks, every 4 weeks until week 88 and then once every 2 weeks until study termination; Complete necropsies and histopathologic examinations were performed on all core study rats. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were processed and stained with H&E for microscopic examination. In an extended evaluation of the kidneys for renal proliferative lesions, the residual wet kidney tissue of male rats was step sectioned at 1 mm intervals to obtain three to four additional sections from each kidney with a maximum of eight additional sections per animal. <u>Results</u> Survival: 200 or 400 ppm, significantly less than that of the controls. Clinical findings: no treatment-related clinical findings Nonneoplastic lesions: *Kidney*: Single sections renal tubule hyperplasia (100 ppm males; P≤0.01) *Liver*: Eosinophilic focus (400 ppm, P≤0.01; 200 ppm, P≤0.05), Centrilobular degeneration (100, 200 and 400 ppm, P≤0.01), Centrilobular necrosis (400 ppm, P≤0.01), Fibrosis (200 and 400 ppm, P≤0.01), Periportal Fibrosis (400 ppm, P≤0.01; 200 ppm, P≤0.05), Pigmentation (200 and 400 ppm, P≤0.01; 100 ppm, P≤0.05) Stomach: glandular mineralization (100 ppm P≤0.01; 200 ppm, P≤0.05). *Parathyroid gland*: Hyperplasia (100 and 200 ppm, p≤0.01). Bone: Fibrous osteodystrophy (100 ppm, P≤0.05)	[8] Well-performed study according to GLP; non-guideline Statistical analysis tumour incidences: the Poly-κ test (with κ =3) was used to assess neoplasm and nonneoplastic lesion prevalence

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		Neoplastic lesions: *Testes*: Adenoma (400 ppm, males, p=0.012) *Kidney*: no significant treatment-related increase in incidences of renal tubule hyperplasia, adenoma, or carcinoma. *Liver*: hepatocellular neoplasms were not significantly increased in exposed rats compared to controls	
Mouse, B6C3F1 male and female 50/sex/exposure concentration (chamber control or exposed)	Carcinogenicity Study Concentration in drinking water: *males*: 0, 250, 500, or 1,000 ppm (results in an average daily doses of 0, 35, 65, or 110 mg/kg) *females*: 0, 125, 250, or 500 ppm results in an average daily doses of 0, 15, 35, or 70 mg/kg Drinking water was given for 104 (males) or 105 (females) weeks.	<u>Observations</u> Twice daily observation; Clinical findings were recorded at 4-week intervals, and body weights were recorded at the start of the study, weekly for the first 13 weeks, every 4 weeks until week 96, and then once every 2 weeks until study termination; Complete necropsies and histopathologic examinations were performed on all core study mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were processed and stained with H&E for microscopic examination. <u>Results</u> Survival: not affected Clinical findings: reduced body weight in females (250 and 500 ppm), increased water consumption in males in the 2nd year of the study (250 and 500 ppm), decreased water consumption in males (1, 000 ppm), water consumption by females, lower (the 1st year) and higher (2nd year) than the controls. Nonneoplastic lesions: No significant treatment-related lesions Neoplastic lesions: *Liver*: hepatocellular adenoma (include multiple) (250 ppm females, P≤0.01; 250, 500 and 1,000 ppm males, 125 ppm females P≤0.05;), hepatocellular carcinoma (include multiple) (250, 500 and 1,000 ppm males, 250 and 500 ppm, females, P≤0.01; 125 ppm females, P≤0.05), hepatoblastoma (include multiple) (250, 500 and 1,000 ppm, males and 500 ppm females, P<0.001; 250 ppm, females, p=0.007), hepatocellular adenoma, hepatocellular carcinoma, or Hepatoblastoma (1,000 pm males, p<0.001; 250 ppm, males, p=0.002; 500 ppm, males, p=0.003; 250 ppm, females, p=0.042; 500 ppm, females, p=0.045)	[8] Well-performed study according to GLP; non-guideline Statistical analysis tumour incidences: the Poly-κ test (with κ =3) was used to assess neoplasm and nonneoplastic lesion prevalence
Rat, F344/N, male and female 40, 30, 20 and 10/sex/exposure	Carcinogenicity Study	<u>Observations</u> Animals were examined daily and all abnormalities were reported immediately. A weekly record was kept of animal weights, injection volumes, and gross observations. All	[21] Non-guideline study

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concentration (high to low)	0, 3, 10, 30 and 100 mg/kg Administered subcutaneously twice a week for 52 weeks	experimental animals were necropsied after they died or were sacrificed. Organ weights were obtained and selected tissues preserved for histopathologic study. <u>Results</u> Survival: not affected Weight gain: at highest dose a reduced gain in body weight of 5-16% was observed. At lower doses the retardation of weight gain was less significant Tumour incidence (% of group): 0 mg/kg: male 5/50 (10), female 9/50 (18) 3 mg/kg: male 0/10 (0), female 1/10 (10) 10 mg/kg: male 0/20 (0), female 2/20 (10) 30 mg/kg: male 1/30 (3), female 7/30 (23) 100 mg/kg: male 2/40 (5), female 2/40 (5)	No statistical analysis performed

Table A2.1. *Number of male F344/N rats with nonneoplastic lesions after exposure to pyridine via drinking water for 2 years.*[8]

Type of tissue/lesion	**0 ppm (0 mg/kg bw/d)**	**100 ppm (7 mg/kg bw/d)**	**200 ppm (14 mg/kg bw/d)**	**400 ppm (33 mg/kg bw/d)**
Kidney				
Single sections renal tubule, hyperplasia	1/50 (1.0)[a]	0	4/50 (3.0)	7/49* (1.7)
Liver				
Basophilic focus	12/50	5/49	0**	1/50**
Clear cell focus	7/50	1/49*	7/50	4/50
Eosinophilic focus	14/50	23/49*	23/50	13/50
Centrilobular cytomegaly	0	4/49 (1.3)	8/50** (1.3)	6/50* (2.0)
Vacuolization cytoplasmic	4/50 (1.5)	6/49 (1.8)	13/50*	17/50** (2.4)
Periportal fibrosis	0	0	2/50 (2.5)	29/50** (1.8)
Fibrosis	1/50 (2.0)	1/49 (2.0)	1/50 (1.0)	10/50** (1.6)
Centrilobular degeneration	1/50 (2.0)	3/49 (2.3)	2/50 (2.0)	8/50* (2.1)
Centrilobular necrosis	0	3/49 (1.7)	0	5/50* (2.2)
Pigmentation	4/50 (1.0)	11/49* (1.3)	20/50** (1.3)	25/50** (2.0)
Stomach				
Glandular mineralization	0	2/49 (2.0)	2/50 (1.5)	8/50** (2.0)

Table A2.2. *Number of female F344/N rats with nonneoplastic lesions in female F344/N rats after exposure to pyridine via drinking water for 2 years.*[8]

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Type of tissue/lesion	0 ppm (0 mg/kg bw/d)	100 ppm (7 mg/kg bw/d)	200 ppm (14 mg/kg bw/d)	400 ppm (33 mg/kg bw/d)
Liver				
Basophilic focus	38/50	28/50*	11/50**	0**
Clear cell focus	4/50	9/50	11/50*	16/50**
Centrilobular cytomegaly	0	1/50 (1.0)	4/50 (1.0)	20/50** (1.4)
Vacuolization cytoplasmic	10/50 (1.8)	7/50 (1.0)	9/50 (1.8)	18/50* (1.6)
Centrilobular degeneration	1/50 (2.0)	2/50 (2.5)	2/50 (1.5)	7/50* (1.1)
Bile duct hyperplasia	20/50 (1.0)	29/50* (1.1)	34/50** (1.0)	29/50* (1.0)
Pigmentation	6/50 (1.5)	2/50 (1.5)	6/50 (2.3)	17/50** (1.6)

* Significantly different (P≤0.05) from the control group by the Poly-3 test
** Significantly different (P≤0.01) from the control group by the Poly-3 test
a Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Table A3. *Number of male Wistar rats with nonneoplastic lesions in after exposure to pyridine via drinking water for 2 years.*[8]

Type of tissue/lesion	0 ppm (0 mg/kg bw/d)	100 ppm (8 mg/kg bw/d)	200 ppm (17 mg/kg bw/d)	400 ppm (36 mg/kg bw/d)
Kidney				
Single sections renal tubule, hyperplasia	6/50 (1.7)[a]	17/50** (2.1)	8/50 (2.48)	5/50 (2.6)
Stomach				
Glandular mineralization	8/49 (2.8)	25/50** (2.8)	16/48* (3.0)	6/48 (2.7)
Parathyroid gland				
Hyperplasia	16/48 (3.3)	32/47** (3.2)	29/48** (3.0)	12/47 (2.5)
Bone				
Fibrous Osteodystrophy	10/50 (2.8)	21/50* (2.8)	16/50 (2.9)	6/50 (1.7)
Liver				
Eosinophilic Focus	14/50	12/50	4/50*	2/50**
Centrilobular Degeneration	1/50 (1.0)	15/50** (1.8)	25/50** (2.1)	33/50** (2.4)
Centrilobular Necrosis	5/50 (2.8)	6/50 (2.0)	4/50 (2.8)	23/50** (2.5)
Fibrosis	1/50 (2.0)	5/50 (1.4)	26/50** (1.6)	31/50** (1.8)
Periportal Fibrosis	0	0	5/50* (2.0)	7/50** (2.4)
Pigmentation	6/50	15/50*	34/50**	42/50**

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	(1.5)	(1.3)	(1.8)	(1.8)

1 * Significantly different (P≤0.05) from the control group by the Poly-3 test
2 ** Significantly different (P≤0.01) from the control group by the Poly-3 test
3 [a] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
4

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B IARC evaluation and conclusion

Cited from IARC Monographs, Volume 119, pages 173-203 (2019).

Human data

One small cohort study of mortality in workers exposed to pyridine and numerous other chemicals did not show any excess of mortality from cancer of the lung or all cancers combined. Six cases of squamous cell carcinoma of the skin were observed in the study population, but no risk data were reported.

Animal data

In one well-conducted good laboratory practice (GLP) study in male and female mice given drinking-water containing pyridine, there was a significant increase, with a significant positive trend, in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and the combination of these tumours in males and females.

In another well-conducted GLP drinking-water study in male and female F344/N rats, pyridine significantly increased the incidence of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in males, and of mononuclear cell leukaemia in females, with a significant positive trend. In a third well-conducted GLP drinking-water study in male Wistar rats, pyridine significantly increased the incidence of testicular cell adenoma with a significant positive trend.

One study in male and female rats given pyridine by subcutaneous injection gave negative results. One feeding study and one skin-application study in transgenic mice gave negative results.

Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of pyridine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of pyridine

Overall evaluation

Pyridine is *possibly carcinogenic to humans (Group 2B)*.

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C 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.

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.

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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.

3.5.2.3.3. 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.

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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.

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

	Concentration limits triggering classification of a mixture as:		
Ingredient classified 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.

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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).

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D Classification on carcinogenicity

In 2010, the Committee published a guideline for classifying substances in terms of their carcinogenic properties, and for assessing their genotoxicity[24]. 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

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The Committee

The members of the Subcommittee on the Classification of Carcinogenic Substances

- H.P.J. te Riele, Professor of molecular biology, VU University Amsterdam, and Netherlands Cancer Institute, Amsterdam, *chairman*
- Prof. dr. M.L. de Bruin, Professor Drug Regulatory Science, Utrecht University, Department Farmaceutical Sciences
- Dr. R.W.L. Godschalk, Genetic Toxicology and Molecular Epidemiology, Maastricht University
- E. de Rijk, Toxicologic pathologist, Charles River Laboratories, 's Hertogenbosch
- dr. P.T.J. Scheepers, Associate Professor Molecular Epidemiology and Risk Assessment, Radboud University
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