National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Pyridine: an overview of available data on mutagenicity and carcinogenicity

RIVM letter report 2021-0191
W. Chen | D. Zijtveld

National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Pyridine: an overview of available data on mutagenicity and carcinogenicity

RIVM letter report 2021-0191
W. Chen | D. Zijtveld

Colophon

RIVM attaches a great deal of importance to the accessibility of its products. However, it is at present not yet possible to provide this document in a completely accessible form. If a part is not accessible, it is mentioned as such. Also see www.rivm.nl/accessibility.

DOI 10.21945/RIVM-2021-0191

W. Chen (author), RIVM
D. Zijtveld (author), RIVM

Contact:
Andre Muller
Centrum voor Veiligheid van Stoffen en Producten
andre.muller@rivm.nl

This investigation was performed by order, and for the account, of the Health Council of the Netherlands, within the framework of the project 'Literature reviews for use in advisory reports on substances'

Published by:
National Institute for Public Health and the Environment, RIVM
P.O. Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

Synopsis

Pyridine: an overview of available data on mutagenicity and carcinogenicity.

RIVM performed a literature search with a focus on the mutagenic and carcinogenic properties of pyridine which is used as a solvent for paint, rubber, polycarbonate resins and textile water repellents and for the production of substituted pyridines, piperidine, agrochemicals, pharmaceuticals and other products.

The data found was summarised. At the request of the Dutch Minister of Social Affairs and Employment, the Health Council of the Netherlands will use the summaries to assess the mutagenic and carcinogenic properties and to provide a recommendation for its classification.

The assessment will be performed by the Health Council's Subcommittee on Classifying Carcinogenic Substances. This subcommittee falls under the Dutch Expert Committee on Occupational Safety, which focuses on health risks associated with occupational exposure of workers to chemicals.

Keywords: pyridine, mutagenicity, carcinogenicity

Publiekssamenvatting

Pyridine: een overzicht van de beschikbare data over mutageniteit en carcinogeniteit

De stof pyridine wordt gebruikt als oplosmiddel voor een breed scala aan producten. Het zit bijvoorbeeld in verf, rubber, waterafstotende textielstoffen, geneesmiddelen en vitaminen en smaakstoffen voor levensmiddelen. Het RIVM heeft in de wetenschappelijke literatuur onderzocht wat er bekend is over twee mogelijke schadelijke eigenschappen van deze stof. De vraag is of pyridine kankerverwekkend is en erfelijke veranderingen kan veroorzaken door schade aan het DNA (mutageen).

De gevonden informatie is samengevat. De Gezondheidsraad gebruikt de samenvattingen om de mutagene en kankerverwekkende eigenschappen te beoordelen. De Gezondheidsraad gebruikt ze ook om een advies op te stellen voor classificatie van de stof. Dit gebeurt op verzoek van de minister van Sociale Zaken en Werkgelegenheid (SZW).

De uiteindelijke beoordeling wordt uitgevoerd door de Subcommissie Classificatie van carcinogene stoffen van de Gezondheidsraad. Deze subcommissie valt onder de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS). De GBBS richt zich op gezondheidsrisico’s door blootstelling aan chemische stoffen op de werkplek.

Kernwoorden: pyridine, mutageniteit, carcinogeniteit

Contents

Summary

RIVM performed a literature search with a focus on the mutagenic and carcinogenic properties of pyridine and summarized the relevant studies. Pyridine is used as a solvent and in paint, rubber, polycarbonate resins, textile water repellents, substituted pyridines, piperidine, agrochemicals, pharmaceuticals and other products.

Available data on *in vitro* mutagenicity testing of pyridine included a bacterial mutagenicity test with Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 and L5178Y mouse lymphoma cells, *in vitro* cytogenetic testing of pyridine with a sister chromatid exchange test in and two chromosome aberration tests in Chinese hamster ovary cells and human peripheral blood lymphocytes. *In vivo* mutagenetic tests of pyridine included chromosomal aberrations and formation of micronucleate erythrocytes in bone marrow of mice. No human mutagenicity data are available on pyridine. No data on the carcinogenicity of pyridine in humans were found. In vivo carcinogenicity studies with pyridine administered via drinking water were available in F334/N rats, Wistar rats and B6C3F1 mice.
All studies were considered of sufficient quality.

The data found was summarised. At the request of the Dutch Minister of Social Affairs and Employment, the Health Council of the Netherlands will use the summaries to assess the mutagenic and carcinogenic properties and to provide a recommendation for its classification.

The assessment will be performed by the Health Council's Subcommittee on Classifying Carcinogenic Substances. This subcommittee falls under the Dutch Expert Committee on Occupational Safety, which focuses on health risks associated with occupational exposure of workers to chemicals.

1 Introduction

The aim of current research is to identify and summarize the available data from studies with laboratory models, test animals and humans on the substance pyridine. The focus of current literature review will be on the mutagenic and carcinogenic properties of this substance.
At the request of the Dutch Minister of Social Affairs and Employment, the Health Council of the Netherlands will use the summaries to assess the mutagenic and carcinogenic properties and to provide a recommendation for its classification. The assessment will be performed by the Health Council's Subcommittee on Classifying Carcinogenic Substances. This subcommittee falls under the Dutch Expert Committee on Occupational Safety, which focuses on health risks associated with occupational exposure of workers to chemicals.

The current RIVM-report does not include an assessment of the reported mutagenic and carcinogenic effects of pyridine, nor does it include a conclusion regarding classification of the substance based on the CLP-criteria.

The literature search strategy which forms the basis of current literature overview is presented in chapter 2. In chapter 3 the substance identity of pyridine is provided. Chapter 4 presents information on international classifications of pyridine. Available information on monitoring (*i.e.* environmental and biological exposure monitoring) and manufacture and use is presented in chapters 5 and 6, respectively. A summary of the (toxico)kinetics of pyridine is described in chapter 7. Chapter 8 describes an overview of the data on mutagenicity. Finally, the data on carcinogenicity are presented in chapter 9.

2 Literature search strategy

A literature search for publications on mutagenicity and carcinogenicity of pyridine has been performed using various databases up to August 2021. Below the literature search strategy and its results is presented. Given the low number of records, the searches for pyridine focused primarily on synonyms and its CAS-number. For that reason also no specific search terms for environmental and biological exposure monitoring and (toxico)kinetics were included in the search strategy.

2.1 Embase

Table 1 presents the search terms and the results for the database Embase.

Table 1 Search strategy and result for Embase.

Query	Search terms	Number of records
#1	'110-86-1':rn	6,830
#2	'pyridine'/exp/mj	1,775
#3	'toxicity'/mj OR 'genotoxicity'/exp OR 'genotoxicity assay'/exp OR 'mutagenicity'/exp OR 'mutagen testing'/exp	94,787
#4	'carcinogenecity' OR 'carcinogen testing'/exp OR 'carcinogenesis'/exp	263,612
#5	'toxic*':ti OR 'carcinogen*':ti OR 'mutagan*':ti OR 'mutat*':ti OR 'genotox*':ti OR 'epigen*':ti OR 'genetic*':ti	848,661
#6	'micronucl*':ti,ab OR 'transgen*':ti,ab	232,220
#7	#3 OR #4 OR #5 OR #6	1,320,408
#8	#2 AND #7	54
#9	'toxicokinetics'/exp OR 'toxicokinet*':ti,ab	14,150
#10	'bioaccessibility'/exp OR 'bioaccessibility':ti OR 'bioelut*':ti,ab	1,702
#11	(('environment*' OR 'human*' OR 'biologic*') NEAR/3 'exposure monitor*'):ti,ab	125
#12	#2 AND (#9 OR #10 OR #11)	6
#13	'xenobiotic metabolism'/exp OR 'metal metabolism'/mj OR 'metabolism'/mj	235,131
#14	'metabolism':ti OR 'adme':ti,ab OR 'absorption distribution metabolism excretion':ti,ab	240,360
#15	#13 OR #14	447,016
#16	#2 AND #15	46
#17	#8 OR #12 OR #16	94

2.2 PubMed

Table 2 presents the search terms and the results for the database Pubmed.

Table 2 Search strategy and result for Pubmed.

Query	Search terms	Number of records
#1	Search "pyridine"[Supplementary Concept] OR "Pyridines/toxicity"[MAJR:NoExp] AND "pyridin*"[tw]	3,583
#2	Search "Toxicity Tests"[Mesh] OR "Toxicology"[Mesh:NoExp] OR "Toxicokinetics"[Mesh]	142,140
#3	Search "Carcinogenesis"[Mesh] OR "Mutagenesis"[Mesh]	341,846
#4	Search "toxic*"[ti] OR "carcinogen*"[ti] OR "mutagen*"[ti] OR "mutat*"[ti] OR "genotox*"[ti] OR "epigen*"[tw] OR "genetic*"[ti] OR "micronucle*"[tw] OR "transgen*"[tw]	993,852
#5	Search #2 OR #3 OR #4	1,366,858
#6	Search #1 AND #5	474
#7	Search "Toxicokinetics"[Mesh] OR "Toxicological Phenomena"[Mesh] OR "toxicokinetic*"[tw] OR "bioaccessib*"[tw] OR "bioelut*"[tw]	464,001
#8	Search "exposure monitor*"[tw] AND ("environment*"[tw] OR "human"[tw] OR "biologic*"[tw])	532
#9	Search "Metabolism"[Majr:NoExp] OR "metabolism"[ti] OR "adme"[tw] OR "absorption-distribution-metabolism-excretion"[tw]	222,216
#10	Search #7 or #8 or #9	681,892
#11	Search #1 and #10	394
#12	Search #6 or #11	685

2.3 Scopus

The following search terms were used for the database Scopus:
(CASREGNUMBER (110-86-1) OR TITLE ({pyridine})) AND (TITLE-ABS-KEY (toxic* OR carcinogen* OR mutagen* OR mutat* OR genotox* OR epigen* OR genetic* OR micronucle* OR transgen* AND toxicokinetic* OR bioaccessib* OR bioelut* OR ((environment* OR human OR biologic*) W/3 exposure-monitor*) OR adme OR absorption-distribution-metabolism-excretion) OR TITLE (metabolism))

This resulted in 289 records.

2.4 Toxcenter

A search was performed in Toxcenter based on its CAS number 110-86-1. This resulted in 494 records. Table 3 presents the search terms and the results for the database Toxcenter.

Table 3 Search strategy and result for Toxcenter.

L1	10875 SEA 110-86-1
L2	5344557 SEA TOXIC? OR CARCINOGEN? OR MUTAGEN? OR MUTAT? OR GENOTOX? OR EPIGEN? OR GENETIC? OR MICRONUCLE? OR TRANSGEN?
L3	2800 SEA L1 AND L2
L4	162 SEA L3/HUM,ANI
L5	2638 SEA L3 NOT L4
L6	527 SEA L5 AND PYRIDINE/TI
L7	137007 SEA ADME OR ABSORPTION DISTRIBUTION METABOLISM EXCRETION OR METABOLISM/TI
L8	5272 SEA BIOACCESSIB? OR BIOELUT? OR (ENVIRONMENT? OR HUMAN OR BIOLOGIC?)(3W)EXPOSURE MONITOR?
L9	139 SEA L1 AND (L7 OR L8)
L10	790 SEA L4 OR L6 OR L9 D L4 TI NOH 1-162 D L9 TI NOH 1-139
L11	494 SEA L6 NOT (L4 OR L9) D L11 TI NOH 1-494

2.5 ECHA database

The REACH registration dossier of pyridine (publicly available on ECHA website) was consulted[1].

2.6 Secondary sources

Secondary sources were consulted. These included e.g. IARC, SCOEL, WHO, IPCS, ATSDR, DFG; primarily consulted via echemportal[2]. Also RIVM-reports and evaluations and the RIVM-website 'Risico's van stoffen'[3] were consulted as well.

2.7 Overall evaluation of results literature search

The obtained records were evaluated, duplicates were removed, and records were included if considered relevant based on title and abstract. Additionally, publications cited in the selected publications, but not selected during the primary search, were added if considered appropriate.
With respect to human health endpoints evaluated in current report (i.e. mutagenicity and carcinogenicity), this resulted in twelve studies for mutagenicity and four studies for carcinogenicity.

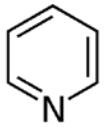
[1] https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/9228
[2] https://www.echemportal.org
[3] https://rvs.rivm.nl/

3 Substance identification

3.1 Name and other identifiers of the substance

The identity of pyridine is presented in Table 4 below.

Table 4 Substance identity and information related to molecular and structural formula of pyridine.

Name(s) in the IUPAC nomenclature or other international chemical name(s)	**Pyridine**
Other names (usual name, trade name, abbreviation)	Azabenzene, Azine, Azinine 1-Azacyclohexa-1,3,5-diene
ISO common name (if available and appropriate)	N/A
EC/EINECS number (if available and appropriate)	203-809-9
EC name (if available and appropriate)	Pyridine
CAS number	110-86-1
Other identity code (if available)	N/A
Molecular formula	C_5H_5N
Structural formula	
SMILES notation (if available)	C1CCNCC1
Molecular weight or molecular weight range	79.1
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	N/A
Description of the manufacturing process and identity of the source (for UVBC substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	N/A

N/A: Not applicable

3.2 Physico-chemical properties

The physical-chemical properties of pyridine are presented in Table 5 below.

Table 5 Summary of physicochemical properties

Properties	Value	Reference
State of the substance at normal temperature and pressure	Colorless liquid with disagreeable odor at 20C and 101.2 kPa	(2)
Melting/freezing point	-41.6 °C (at 101.3 kPa)	(2)
Boiling point	115.2 °C (at 101.3 kPa)	(2)
Relative density	0.982 (at 20°C)	(2)
Vapour pressure	26.7 hPa (at 20 °C)	(2)
Surface tension	36.56 nM/m (at 25°C)	(2)
Water solubility	1,000 g/L (at 20 °C)	(2)
Partition coefficient n-octanol/water	0.64 (at 20 °C)	(2)
Flash point	20 °C (at 101.3 kPa)	(2)
Flammability	Flammable Lower flammability limit: 1.8%, upper limit: 12.4%	
Explosive properties	Non-explosive	(2)
Self-ignition temperature	900 °C (at 101.3 kPa)	(2)
Oxidising properties	No	(2)
Granulometry	-	
Stability in organic solvents and identity of relevant degradation products	-	
Dissociation constant (pKa)	5.2 (at 20 °C)	(2)
Viscosity	0.879 mPa·s dynamic (at 20 °C)	(2)

4 International classifications

4.1 European Commission

Pyridine has currently a harmonized classification in Annex VI of the CLP-Regulation (EC) 1272/2008 (entry number 613-002-00-7) as:

- Flam. Liq. 2 (H225: Highly flammable liquid and vapour)
- Acute Tox 4* (H332: Harmful if inhaled)
- Acute Tox. 4* (H312: Harmful in contact with skin)
- Acute Tox. 4* (H302: Harmful if swallowed)

4.2 The Health Council

Pyridine has not previously been evaluated by the Health Council of the Netherlands.

4.3 IARC

IARC has evaluated pyridine in 2019 (3). IARC considered that there is inadequate evidence in humans for the carcinogenicity of pyridine, and that there is sufficient evidence in experimental animals for the carcinogenicity of pyridine. Overall, IARC concluded in 2019 that pyridine is possibly carcinogenic to humans (Group 2B).

4.4 Other countries

Pyridine has the following classification in Japan[4]:

- Flam. Liq. Cat. 2 (H225: Highly flammable liquid and vapour)
- Skin corr./irrit. Cat. 1 (H314: Causes severe skin burns and eye damage)
- Serious eye damage/Eye Irrit. Cat. 1 (H318: Causes serious eye damage)
- Acute tox. (oral) Cat. 4 (H302: Harmful if swallowed)
- Acute tox. (dermal) Cat. 4 (H312: Harmful in contact with skin)
- Acute tox. (inhalation: vapour) Cat. 4 (H332: Harmful if inhaled)
- Carcinogenic Cat. 2 (H351: Suspected of causing cancer)
- Specific target organ tox. (single exposure) Cat. 1 (central nervous system), Cat. 3 (respiratory tract irritation, anesthetic action): (H370: Causes damage to central nervous system, H335: May cause respiratory irritation, H336: May cause drowsiness or dizziness)
- Specific target organ tox. (repeated exposure) Cat. 1 (nervous system, liver, kidney, blood system) (H372: Causes damage to nervous system, liver, kidney, blood system)
- Aspiration hazard 1 (H304: May be fatal if swallowed and enters airways)

Pyridine has the following classification in Australia[5]:

- Flam. Liq. 2 (H225: Highly flammable liquid and vapour)
- Acute tox. 4 (H302: Harmful if swallowed)
- Acute tox. 4 (H312: Harmful in contact with skin)
- Acute tox. 4 (H332: Harmful if inhaled)

[4] https://www.nite.go.jp/chem/ghs/17-mhlw-2105.html
[5] http://hcis.safeworkaustralia.gov.au/HazardousChemical/Details?chemicalID=3731

- Skin corr. 1C (H314: Causes severe skin burns and eye damage)
- Specific target organ tox. (repeated exposure) 2 (H373: May cause damage to organs through prolonged or repeated exposure)

In Germany, pyridine is not included in the list of additional CMR substances in the context of worker protection.[6]

In the state of California, pyridine is considered a substance to cause cancer.[7]

The substance pyridine is not included in the Report on Carcinogens (14th edition).[8]

[6] https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/TRGS-905.pdf?__blob=publicationFile
[7] https://oehha.ca.gov/media/downloads/proposition-65//p65list091319.pdf
[8] https://ntp.niehs.nih.gov/whatwestudy/assessments/cancer/roc/index.html#toc1

5 Monitoring

5.1 Environmental exposure monitoring

Selected methods for the analysis of pyridine in various matrices are given in Table 6. (4)

Table 6 Selected methods for the analysis of pyridine

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb (charcoal); desorb (dichloromethane)	GC/FID	0.02 mg/sample	(5)
Water, soil, municipal waste	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	5 µg/L	(6)
Solid waste matrices[b]	Solvent extraction or direct injection (with azeotropic distillation) into capillary GC column	GC/FID	9–21 µg/L (aqueous matrices); 0.08–0.20 mg/kg (solid matrices)	(7)
	Direct injection (with azeotropic distillation) into capillary GC column	GC/MS	4 µg/L	(8)

[a] Abbreviations: GC, gas chromatography; FID, flame ionization detection; MS, mass spectrometry

[b] Includes: groundwater, sludges, caustic and acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils and sediments

5.2 Biological exposure monitoring

No methods were found for biological monitoring of exposure to pyridine.

6 Manufacture and uses

There are few selective commercial processes for preparing pyridine and its derivatives. Almost all manufacturing processes produce pyridine along with a series of alkylated pyridines in a mixture. The reaction of aldehydes or ketones with ammonia is the most general synthetic reaction for the manufacture of pyridine bases, and allows the preparation of various pyridine derivatives. Reaction of acetaldehyde and formaldehyde with ammonia is the most widely used method for pyridine production (9).
Pyridine can also be prepared from cyclopentadiene by ammoxidation, or from 2-pentenenitrile by cyclization and dehydrogenation. Furfuryl alcohol or furfural reacts with ammonia in the gas phase to give pyridine (9).

Pyridine is widely used as a solvent in organic chemistry and in industrial practice. Pyridine is an effective, basic solvent that is relatively unreactive, which makes it a good acid scavenger. As a solvent, it is amongst others used for acylation and dehydrochlorination reactions, for paint, rubber, pharmaceuticals, polycarbonate resins and textile water repellents.
Large amounts of pyridine are used as an intermediate in the manufacture of substituted pyridines, piperidine, agrochemicals (herbicides: diquat, paraquat; insecticide: chlorpyrifos; fungicide: pyrithione), pharmaceuticals and other products (9, 10-12).

7 (Toxico)kinetics

7.1 Human data

The fate of pyridine was examined in two healthy male subjects. They received an oral dose of 3.4 mg [^{14}C]pyridine [approx. 0.01 mg/kg bw] in orange juice. In the 0–24-h urine, a total of 65 and 68% of the ^{14}C-dose was recovered, respectively. In addition, two metabolites were identified: pyridine N-oxide, which accounted for 32% of the dose, and N-methylpyridinium ion, accounting for 6 and 12% of the dose, respectively. Approximately 25% of the dose was not characterized (13-14). This is the only data found on humans.

7.2 Animal data

In animals, pyridine is metabolized by oxidation at the nitrogen atom, giving pyridine N-oxide, and at all carbon atoms of the ring, giving 2- and 4-pyridone and 3-hydroxypyridine. In addition, it undergoes N-methylation, yielding the quaternary ammonium ion N-ethylpyridinium. The relative contributions of these pathways to the overall fate of pyridine were examined by Damani et al. (1982), who administered [^{14}C]pyridine intraperitoneally to rats, mice, guinea-pigs, hamsters, gerbils, rabbits and cats, at a dose of 7 mg/kg bw (14). At least 50% of the administered ^{14}C was recovered in the urine of the animals. The amounts of the various specific metabolites differed markedly between the species, as shown in Table 7. Only small amounts (0.4–5% of dose) of unchanged pyridine were found in most species, but cats and rabbits excreted 14% and 25% of the dose in the unchanged form. The extent of N-oxidation varied widely between species, from 0.3% of the dose applied in rats to 39% in hamsters. Pyridine N-oxide was not detected in rabbit urine. The excretion of N-methylpyridinium also varied between species, being lowest in gerbils (~2% of dose) and highest in cats (51% of dose). The major oxidation product was 4-pyridone (from 4% of the dose applied in hamsters to 19% in rabbits). 2-Pyridone and 3-hydroxypyridine were minor metabolites in all species, the former being absent from the metabolic profile in rabbits. Mice did not oxidize pyridine. The authors assumed that the occurrence of additional metabolic pathways is suggested by the excretion of unidentified products, accounting for up to
37% of the dose, in all species except guinea-pigs and cats (14). These pathways include glucuronidation of 3-hydroxypyridine, previously observed in rabbits (15).

There is a dose-dependence in the metabolism of pyridine. At low doses of pyridine, N-methylation may be the preferred route of biotransformation. At higher doses, such as 40 mg/kg bw, the extent of N-oxidation varied from some 10% in rats to 20–40% in mice, hamsters, guinea-pigs, rabbits and ferrets (14). In rats, the formation of N-methylpyridinium ion fell from 10 to 0.8% (as a percentage of the administered dose) with increasing dose over the range 1–500 mg/kg bw (13). The occurrence of N-methylation was similar whether pyridine was given orally or by intraperitoneal injection.

In contrast, guinea-pigs excreted 31% of a dose as N-methylpyridinium independently of dose (either 1 or 7 mg/kg bw, as for rats): this was unaffected by the route of administration but the excretion decreased to 2% when the intraperitoneal dose was 500 mg/kg bw. The low N-methylation capacity of the rat was not enhanced by pre-treatment and dietary supplementation with DL-methionine, the precursor of the methyl donor S-adenosylmethionine.
D'Souza et al. (1980) examined the further metabolism of N-methylpyridinium. Rats and guinea-pigs given N-methyl[^{14}C]pyridinium by intraperitoneal injection excreted 53% and 85% respectively of the dose in the 0–24-h urine. In both species, > 95% of urinary ^{14}C was present as unchanged N-methylpyridinium (13).

Table 7 Species variations in the metabolic C- and N-oxidation and N-methylation of [^{14}C]pyridine in various laboratory animals in vivo

	Total ^{14}C recovery (%)	**% of dose in 0–24-h urine**						
		Pyridine	**N-Methyl pyridinium[a]**	**2-Pyridone**	**3-Hydroxypyridine**	**4-Pyridone**	**Pyridine N-oxide[b]**	**Unknown(s)**
Rat	48	2	4(5)	1	2	10	0.5(0.3)	28
Mouse	66	2	21(12)	ND	ND	ND	5(6)	37
Guinea-pig	66	5	31(30)	2	2	18	9(8)	0
Hamster	67	0	17(26)	1	0.3	4	39(37)	6
Gerbil	52	0.4	1(2and3)	1	1	7	8(10)	34
Rabbit[c]	77	25	13(15)	0	4	19	0	17
Cat[d]	75	14	51(40)	2	1	10	3	0
Human	66	ND	(6 and 12)	ND	ND	ND	(32)	-25

From (13). Values obtained by high-performance liquid chromatography
ND, not determined
[a] Values in parentheses obtained by reverse isotope dilution
[b] Values in parentheses obtained by gas chromatography
[c] 0–72-h urine
[d] 0–48-h urine

8 Germ cell mutagenicity

8.1 Summary of in vitro mutagenicity tests

Data on *in vitro* mutagenicity testing of pyridine are presented in Table 8.

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 (16). Further, no significant increase in mutant frequencies was observed in L5178Y mouse lymphoma cells, tested with and without S9 metabolic activation (17).

8.2 Summary of in vitro cytogenetic tests

Data on *in vitro* cytogenetic testing of Pyridine are presented in Table 9.

In cytogenetic tests with cultured Chinese hamster ovary cells, pyridine did not induce an increase of cells with sister chromatid exchange (SCEs) or chromosome aberration (Abs), with or without S9. At the highest viable concentration (1,673 µg/mL) tested for SCE induction in the absence of S9, pyridine induced marked cell cycle delay. In this test, an extended culture time (31 hours) was used to allow sufficient cells to accumulate for analysis (18-20).

8.3 Summary of human data on mutagenicity

No human mutagenicity data are available on Pyridine.

8.4 Summary of in vivo mutagenicity tests

Data on *in vivo* animal mutagenicity testing of pyridine are presented in Table 10.

In Drosophila melanogaster, the results were positive for induction of sex-linked recessive lethal mutations following injection of pyridine. However, in the same test, the substance did not induce reciprocal translocations in germ cells (18, 21-24)

8.5 Summary of in vivo cytogenetic tests

Data on *in vivo* animal cytogenetic testing of pyridine are presented in Table 11.

In vivo assays for chromosomal effects were conducted with male mice. No increase in bone marrow cells with chromosomal aberrations was noted at either of two sampling times (400-600 mg/kg pyridine; single injection), and no increase in the frequency of number of micronucleated PCEs was noted in bone marrow after intraperitoneal injection of pyridine (up to 500 mg/kg administered three times at 24-hour intervals).
An unscheduled DNA synthesis (UDS) assay in hepatocytes from male mice administered the chemical once by oral gavage at 175, 350 or 700 mg/kg bw and the animals were euthanised two or 16 hours after the last exposure. No significantly increase of the UDS response was observed in hepatocytes isolated from the treated animals, as measured by the incorporation of [^{3}H]thymidine.

Table 8 Summary table of in vitro mutagenicity tests with pyridine

Reference	Method	Microorganism or cell type	Concentration range	Results	Remark
Micro-organisms					
(16, 18)	*Salmonella Typhimurium* mutagenicity test Effect parameter: number of histidine-independent (revertant) colonies Statistical analysis: not used	*Salmonella typhimurium* strains: TA98, TA100, TA1535, and TA1537	0, 100, 333.3, 1,000 3,333.3, 10,000 µg pyridine/plate; Purity: ≥99%; 20 min incubation; +/-S9[a,b]; 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)	Well-performed study; GLP; non-guideline; appropriate results were obtained with negative (solvent) and positive controls.
Mammalian cells					
(17-18)	Mouse Lymphoma mutagenicity test Effect parameter: mutant frequencies Statistical analysis: All data were evaluated statistically for trend and peak responses.	L5178Y mouse lymphoma cells	(-)S9[c] Trial 1: 0, 625, 1,250, 2,500, 5,000 pyridine µg/mL Trial 2: 0, 1,000, 2,000, 3,000, 4,000 5,000 pyridine µg/ mL Trial 3: 0, 2,000, 3,000, 4,000, 5,000 pyridine µg/ mL (+)S9 Trial 1: 0, 1,000, 2,000, 3,000, 4,000	No significant increase in mutant frequencies was observed in L5178Y mouse lymphoma cells (+/-S9) The high dose of pyridine of 5,000 µg/mL did not induce cytotoxicity.	Well-performed study; GLP; non-guideline; appropriate results were obtained with negative (solvent) and positive controls.

Reference	Method	Microorganism or cell type	Concentration range	Results	Remark
			5,000 pyridine µg/ mL Trial 2: 0, 2,000, 3,000, 4,000, 5,000 pyridine µg/ mL, incubated with Pyridine for 4 h. Positive control: Methyl methanesulfonate		

[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 enzymes and cofactors from Aroclor 1254-induced male 344 rats liver

Table 9 Summary table of in vitro cytogenetic tests with pyridine

Reference	Method	Microorganism or cell type	Concentration range	Results	Remark
Mammalian cells					
(18-19)	Sister chromatid exchange test Effect parameter: frequency of SCEs per cell Statistical analysis conducted on the slopes of the dose-response curve and individual dose points	Chinese hamster ovary cells	(-S9 [a]) 0, 167, 502, 1,673, 5,020 µg pyridine/mL; (+S9); 0, 167, 502, 1,673, 5,020 µg pyridine/mL; Purity: ≥99%; 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.	No effect on frequency of SCEs per cell observed	Well-performed study; GLP; non-guideline; appropriate results were obtained with negative (solvent) and positive controls.

Reference	Method	Microorganism or cell type	Concentration range	Results	Remark
			+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 +S9: cyclophosphamide		
(18-19)	Chromosomal aberration test Effect parameter: percentage cells with aberrations Statistical analysis conducted on the slopes of the dose-response curve	Chinese hamster ovary cells	0, 1,081, 2,325, 5,000 µg pyridine/ml; +/-S9[a]; Purity: ≥99%; 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 effect on percentage cells with aberrations	Well-performed study; GLP; non-guideline; appropriate results were obtained with negative (solvent) and positive controls.

Reference	Method	Microorganism or cell type	Concentration range	Results	Remark
(20)	Chromosomal aberrations test Effect parameter: percentage of cells with breaks and pulverization Statistical analysis: Not used	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 concentration of pyridine showed an increase of cells with breaks and pulverization of chromosomes in concentration dependent manner.	Well-performed study; non-GLP; non-guideline; appropriate results were obtained with negative (solvent) and positive controls.

[a] metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat liver

Table 10 Summary table of in vivo animal mutagenicity tests with pyridine

Reference	Species	Experimental period and design	Dose and route	Observations and results	Remark
(18, 21)	Adult male, wild-type Canton-S flies (D. melanogaster)	Sex-Linked Recessive Lethal Mutation Test Effect parameters: Frequency of SLRL mutations Statistical analysis: Binomial test with normal approximation	Feed: 0, 600, 700 ppm pyridine; Injection: 0, 7,000 ppm pyridine; Positive controls: N-nitrosodimethylamine (DMN) and β-propiolactone (22)	Administration of pyridine by injection (7,000 ppm in aqueous 0.7% saline solution) gave no effects (P=0.225). Feeding (600 and 700 ppm pyridine in aqueous 5% sucrose) produced a non-significant increase in (cells with) recessive lethal mutations (P=0.043). No treatment-related clinical signs.	GLP; non-guideline, appropriate results were obtained with positive controls. Applied dose levels were probably not high enough, given that no general toxicity was noticed.

Reference	Species	Experimental period and design	Dose and route	Observations and results	Remark
(18, 23)	Adult male, wild-type Canton-S flies (D. melanogaster)	Sex-Linked Recessive Lethal Mutation Test Effect parameters: Frequency of SLRL mutations Statistical analysis: Binomial test with normal approximation	Feed: 0, 729 ppm pyridine Injection: 0, 500 ppm pyridine Positive controls: N-nitrosodimethylamine (DMN) and β-propiolactone (22)	Both injection (500 ppm) and feeding (729 ppm) yielded no effects. No treatment-related clinical signs.	GLP; non-guideline, appropriate results were obtained with positive controls. Applied dose levels were probably not high enough, given that no general toxicity was noticed.
(18, 24)	Adult male, wild-type Canton-S flies (D. melanogaster)	Sex-Linked Recessive Lethal Mutation Test Effect parameters: Frequency of SLRL mutations Statistical analysis: Binomial test with normal approximation	Feed: 0, 500 ppm pyridine Injection: 0, 4,300 ppm pyridine Positive controls: N-nitrosodimethylamine (DMN) and β-propiolactone (Woodruff, 1984)	Feeding (500 ppm) experiment did not induce increase in the frequency of number of cells with SLRL mutations, (P=0.998); injection (4,300 ppm) induced a significant increase in the frequency of number of cells with SLRL mutations (P=0.008). No treatment-related clinical signs.	GLP; non-guideline, appropriate results were obtained with positive controls. Applied dose levels were probably not high enough, given that no general toxicity was noticed.

Reference	Species	Experimental period and design	Dose and route	Observations and results	Remark
	Adult male, wild-type Canton-S flies (D. melanogaster)	Reciprocal Translocation Test Effect parameter: Statistical analysis: Conditional binomial response test	Injection:4,300 ppm pyridine; purity ≥99%;	No effect No treatment-related clinical signs.	GLP; non-guideline. Applied dose levels were probably not high enough, given that no general toxicity was noticed.
(25)	Adult female, wild-type Canton-S flies (D. melanogaster)	Chromosomal nondisjunction test Effect parameter: X chromosome nondisjunction Statistical analysis: Binomial test with normal approximation	0.05, 0.1, 0.2, 0.3 or 0.4% pyridine	Pyridine induced significant increase in disjunction broods arising from nearly mature oocytes, but not early-stage or mature oocytes. There was no dose-response relationship.	

Table 11 Summary table of in vivo animal cytogenetic tests with pyridine

Reference	Species	Experimental period and design	Dose and route	Observations and results	Remark
(18, 26)	Male B6C3F1 mice 10/dose group	*In vivo* mouse bone marrow Chromosomal Aberrations Test; Effect parameters: Fifty first-division metaphase cells; Statistical analysis: Trend test.	0, 400, 500, 600 mg/kg pyridine purity ≥99%; intraperitoneal injection, single, volume: 0.4 mL; Positive control: Mitomycin-C	No induction of aberrations was noted in bone marrow cells at either of two sampling times (17 and 36 hours) No treatment-related clinical signs.	GLP; non-guideline. Applied dose levels were probably not high enough, given that no general toxicity was noticed.

Reference	Species	Experimental period and design	Dose and route	Observations and results	Remark
(18, 27)	Male B6C3F1 mice 10/exposure concentration (chamber control or exposed);	In vivo mouse bone marrow Micronucleus Test; Effect parameters: determination of frequency of micronuclei in 2,000 PCEs; determination of percentage of PCEs. Statistical analysis using a one-tailed Cochran-Armitage trend test, followed by pairwise comparison between each exposed group and the control group.	0, 31.25, 62.5, 125, 250, 500 mg/kg pyridine; purity ≥99%; intraperitoneal injection, three times at 24-hour intervals; total dosing volume: 0.4 mL Positive control: cyclophosphamide	No increase in the frequency of 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.	GLP; non-guideline. Applied dose levels were probably not high enough, given that no general toxicity was noticed.
(28)	B6C3F1 Mice 4/dose group Only the first three successful perfusions in each dose group were analyzed for UDS.	*In vivo* DNA repair assay Effect parameter: Unscheduled DNA synthesis(UDS) Statistical analysis: Not used	0, 175, 350 and 700 mg/kg Pyridine, Purity: ≥99%; Administered in water by gavage, 2 or 16 h prior to the scheduled sacrifice. Positive control: Dimethylnitrosamine	No evidence of an increase in UDS inB6C3F1 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)of pyridine.	Well-performed study; GLP; Guideline for Testing of Chemicals, No. 486

PCE: polychromatic erythrocytes

9 Carcinogenicity

9.1 Observations in humans

No data on the carcinogenicity of pyridine in humans were found.

9.2 Animal experiments

The carcinogenicity studies of pyridine in experimental animals are summarized in Table 12. In these studies, animals were exposed to the substance via drinking water. No dermal or inhalation carcinogenicity studies were available.

Table 12 Summary of animal carcinogenicity studies on Pyridine.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
(17)	Rat, F344/N, male and female 50/sex/ exposure concentration (chamber control or exposed)	Carcinogenicity study Statistical analysis tumour incidences: the Poly-κ test (with κ =3) was used to assess neoplasm and nonneoplastic lesion prevalence	Concentration in drinking water: 0, 100, 200, 400 ppm pyridine (result in an average daily dose of 0, 7, 14, 33 mg/kg) purity ≥99%; Drinking water were 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,	Well-performed study; GLP; non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				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) *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 Leukemia (200 ppm, females, p=0.043; 400 ppm, females, p=0.020)	
(17)	Rat, Wistar, male 50/ exposure concentration (chamber control or exposed)	Carcinogenicity study Statistical analysis tumour incidences: the Poly-κ test	Concentration in drinking water: 0, 100, 200, 400 ppm Pyridine (result in an average daily dose of 0, 8, 17, 36 mg/kg)	<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;	Well-performed study; GLP; non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
		(with κ =3) was used to assess neoplasm and nonneoplastic lesion prevalence	purity ≥99%; Drinking water were given for 104 weeks.	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).	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				Bone: Fibrous osteodystrophy (100 ppm, P≤0.05) 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.	
(17)	Mouse, $B6C3F_1$, male and female 50/sex/ exposure concentration (chamber control or exposed)	Carcinogenicity study Statistical analysis tumour incidences: the Poly-κ test (with κ =3) was used to assess neoplasm and nonneoplastic lesion prevalence	Concentration in drinking water: (male) 0, 250, 500, or 1,000 ppm pyridine resulted in average daily doses of 0, 35, 65, or 110 mg/kg (female) 0, 125, 250, or 500 ppm pyridine resulted in average daily doses of 0, 15, 35, or 70 mg/kg purity ≥99%; Drinking water were 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.	Well-performed study; GLP; non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				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)	
(29)	Rat, F344/N, male and female 40, 30, 20 and 10/sex/ exposure concentration (high to low)	Carcinogenicity study Statistical analysis: not used	0, 3, 10, 30 and 100 mg/kg pyridine Administered subcutaneously twice a week for 52 weeks	<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 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.	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				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)	

In a 2-year GLP study, male and female F344/N rats (50/sex/dose) were exposed to Pyridine (purity≥99%) via drinking water at concentrations of 0, 100, 200, 400 ppm Pyridine (result in an average daily dose of 0, 7, 14, 33 mg/kg) for 104 (males) and 105 weeks (females), respectively.
Mean body weights of 400 ppm males and females were generally less than those of controls throughout the study, and those of 200 ppm males and females were generally less during the second year of the study. There were no treatment-related clinical findings (17).

Tables 13 and 14 present a summary of the nonneoplastic and neoplastic lesions, respectively. In females only liver effects were observed, including a decrease in basophilic foci, and increases in clear cell focus, centrilobular cytomegaly, vacuolization cytoplasmic, centrilobular degeneration, bile duct hyperplasia, and pigmentation.
In to dose males there was an increase in renal tubule hyperplasia, and glandular mineralization in the stomach. Liver effects included a decrease in basophilic foci, centrilobular cytomegaly, cytoplasmic vacuolization, periportal fibrosis, fibrosis, centrilobular degeneration, centrilobular necrosis, pigmentation.
Some nonneoplastic incidences were already significantly increased at lower dose groups: clear cell focus, eosinophilic focus, and pigmentation were observed in male rats at 100 ppm, it is worth emphasizing that the first two incidences only occur at 100 ppm; basophilic foci, centrilobular cytomegaly, vacuolization cytoplasmic and pigmentation were observed at 200 ppm in male rats. For female rats, a decrease in basophilic foci and an increase in bile duct hyperplasia were observed from 100 ppm and 200 ppm onward, clear cell focus were observed from 200 ppm.

There were no statistically significant increases in the incidences of hepatocellular neoplasms in exposed F344/N rats. However, a significant dose-dependent increase of kidney renal tubule adenoma (or carcinoma) in male, and mononuclear cell leukemia in female F334/N rats were observed.

Table 13 Nonneoplastic lesions in male and female F344/N rats exposed Pyridine exposure via drinking water for 2 years[a] (17).
A: Males

	Exposure concentration			
ppm:	**0**	**100**	**200**	**400**
mg/kg bw/d:	**0**	**7**	**14**	**33**
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* (1.7)	17/50** (2.4)
Periportal fibrosis	0	0	2/50 (2.5)	29/50** (1.8)

	Exposure concentration			
ppm:	**0**	**100**	**200**	**400**
mg/kg bw/d:	**0**	**7**	**14**	**33**
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)

B: Females

	Exposure concentration			
ppm:	**0**	**100**	**200**	**400**
mg/kg bw/d:	**0**	**7**	**14**	**33**
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 14 Neoplastic lesions in male and female F344/N rats exposed to Pyridine exposure via drinking water for 2 years (17).
A. Males

	Exposure concentration				**Historical control data[a]**
ppm:	**0**	**100**	**200**	**400**	
mg/kg bw/d	**0**	**7**	**14**	**33**	
Kidney					
Renal tubule adenoma[b]					1/327; 0.3% ± 0.8% (range 0%-2%)
- Single sections	1/50	0/48	2/50	6/49*	
- Single and step sections	2/50	3/48	6/50	10/49**	
Renal tubule adenoma or carcinoma[c]					1/327; 0.3% ± 0.8% (range 0%-2%)
- Single sections	1/50	1/48	2/50	6/49*	
- Single and step sections	2/50	4/48	6/50	10/49**	

B. Females

	Exposure concentration				**Historical control data[d]**
ppm:	**0**	**100**	**200**	**400**	
mg/kg bw/d	**0**	**7**	**14**	**33**	
All organs:					
Mononuclear Cell Leukemia	12/50	16/50	22/50*	23/50**	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 leukemias

In a 2-year GLP study, male Wistar rats (50/dose) were exposed to Pyridine (purity≥99%) via drinking water at concentrations of 0, 100, 200, 400 ppm Pyridine for 104 weeks.
Survival of rats exposed to 200 or 400 ppm was significantly less than that of the controls. Mean body weights of rats exposed to 100, 200, or 400 ppm were significantly less than controls, the mean body weights were 91%, 83% and 84%, respectively of the control group at week 103. Water consumption by exposed rats was similar to the controls (17).

Tables 15 and 16 present a summary of the nonneoplastic and neoplastic lesions, respectively. Incidences of centrilobular degeneration and pigmentation of the liver were significantly increased in all the exposed groups. Incidences of eosinophilic focus, fibrosis and periportal fibrosis in the liver were significantly increased in the 200 and 400 ppm groups. The incidences of stomach glandular mineralization and parathyroid gland hyperplasia were significantly increased in the 100

and 200 ppm groups. The incidences of single sections renal tubule hyperplasia and bone fibrous osteodystrophy were significantly increased in the 100 ppm group. The incidence of testes adenoma was significantly increased only in the 400 ppm group.

Table 15 Nonneoplastic lesions in male Wistar rats exposed Pyridine exposure via drinking water for 2 years (17).

	Exposure concentration			
ppm:	**0**	**100**	**200**	**400**
mg/kg bw/d:	**0**	**8**	**17**	**36**
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* (2.5)	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 (1.5)	15/50* (1.3)	34/50** (1.8)	42/50** (1.8)

* 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 16 Neoplastic lesion in male Wistar rats exposed to Pyridine exposure via drinking water for 2 years (17).

	Exposure concentration			
ppm:	**0**	**100**	**200**	**400**
mg/kg bw/d	**0**	**8**	**17**	**36**
Testes				
Adenoma	5/50	6/49	4/49	12/50*

* Significantly different (P≤0.05) from the control group by the Poly-3 test

In a 2-year GLP study, male B6C3F$_1$ mice (50/dose) were exposed to Pyridine (purity≥99%) via drinking water at concentrations of 0, 250, 500, or 1,000 ppm for 104 weeks, and female B6C3F$_1$ mice (50/dose) were exposed to Pyridine (purity≥99%) via drinking water at concentrations of 0, 100, 200, or 500 ppm for 105 weeks (17).

Reduced body weight were observed in 250 and 500 ppm females. Water consumption was increased in low and mid dose males in the 2nd year of the study, and decreased in high dose males. Water consumption by females, was lower in the 1st year and higher in the 2nd year than in the controls.

No significant treatment-related nonneoplastic lesions were observed. Tables 17 present a summary of the neoplastic lesions. The incidences of hepatocellular adenoma (multiple) and hepatocellular carcinoma (multiple) were significantly increased in all the exposed groups, except the high dose female group.

Table 17 Neoplastic lesions in (A) male and (B) female B6C3F$_1$ mice exposed to Pyridine exposure via drinking water for 2 years (17).
A. Males

	Exposure concentration				**Historical control data[a]**
ppm:	**0**	**250**	**500**	**1,000**	
mg/kg bw/d:	**0**	**35**	**65**	**110**	
Liver:					
Hepatocellular adenoma, multiple	16/50	29/50*	29/49*	28/50*	179/289; 61.9% ± 9.1%, (range: 47%-70%)
Hepatocellular carcinoma, multiple	3/50	19/50**	26/49**	18/50**	80/289; 27.7% ± 11.7%, (range: 10%-42%)
Hepatoblastoma, multiple	1/50	4/50	6/49*	2/50	9/289; 3.1% ± 5.0%, (range: 0%-12%)
Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma	38/50	47/50*	46/49*	47/50*	212/289; 73.4% ± 11.7%, (range: 53%-81%)

B. Females

	Exposure concentration				**Historical control data[a]**
ppm:	**0**	**125**	**250**	**500**	
mg/kg bw/d:	**0**	**15**	**35**	**70**	
Liver:					
Hepatocellular adenoma, multiple	24/49 (49%)	34/50* (68%)	37/50** (74%)	30/50 (69%)	150/289; 51.9% ± 20.8%, (range: 26%-80%)
Hepatocellular carcinoma, multiple	3/49 (6%)	11/50* (22%)	14/50** (28%)	30/50** (30%)	55/289; 19.0% ± 13.7%, (range: 8%-42%)
Hyperblastoma	1/49 (2%)	2/50 (4%)	9/50* (18%)	16/50* (32%)	
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] Historical Data as of 1 August 1997

In a 1-year chronic study, male and female F344/N rats (40, 30, 20 and 10/sex/dose (high to low)) were exposed to pyridine via subcutaneous administration at concentrations of 0, 3, 10, 30, 100 mg/kg for 52 weeks (29). At the highest dose, an average retardation of weight gain of 11% (5-16%) was observed. At lower doses the retardation of weight gains were less significant. Survival of the rats was not affected.

No significant increase in treatment-related tumour incidence was observed. Table 18 presents a summary of the identified tumours.

Table 18 Tumour incidence in male and female F344/N rats upon subcutaneous exposure to pyridine for 1 year (29).

		Tumour bearing rats			Tumour types		
Dose (mg/kg)	**Group size (male/ female)**	**Male**	**Female**	**Both**	**Fibroma or sarcoma (%)**	**Mammary (%)**	**Others (%)**
100.0	40/40	2 (5%)	2 (5%)	4 (5%)	2 (3%)	0 (0%)	2 (3%)
30.0	30/30	1 (3%)	7 (23%)	8 (13%)	0 (0%)	2 (3%)	6 (10%)
10.0	20/20	0 (0%)	2 (10%)	2 (5%)	0 (0%)	0 (0%)	2 (5%)
3.0	10/10	0 (0%)	1 (10%)	1 (5%)	0 (0%)	1 (5%)	0 (0%)
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%)

9.3 Additional information

The liver and kidney have previously been reported as target organs in rats administered pyridine in feed at 0.34% to 1.0% for up to 4 months (30). Liver toxicity was observed in Sprague-Dawley rats administered 50 mg/kg bw/d pyridine by oral gavage for 13 weeks (31).

In male F344/N rats from the 13-week study of pyridine, kidney changes consistent with α2u-globulin inducers were observed in the 1,000 ppm group and to a lesser extent in the 500 ppm group. These changes included a very subtle increase in the amount of hyaline droplets which appeared positive for α2u-globulin by immunohistochemistry and one to three small granular casts in 1,000 and 500 ppm males; at the next lowest exposure concentration (250 ppm) no changes were observed consistent with hyaline droplet nephropathy.

In the 2-year studies there was a marginal increase in the incidence of renal tubule adenomas in the 400 ppm male F344/N rats. An extended evaluation of the entire kidney by step sectioning confirmed a significant exposure-related increase in the incidences of renal tubule adenomas in this group. Slight increases in the incidences of renal tubule hyperplasia were also observed for 400 ppm male F344/N rats and 100 ppm Wistar rats.

Establishing causation between neoplastic outcome and the α2u-globulin response in male rats requires demonstration of similar exposure-

response relationships between renal tubule neoplasm incidence and α2u-globulin accumulation (as determined by histopathology and immunohistochemistry), reversible binding of the chemical or its metabolite to α2u-globulin, and sustained cell proliferation in the renal cortex. In studies in which the association between hyaline droplet nephropathy and neoplasm development was clearly demonstrated, the severities of hyaline droplets and granular casts exceeded those observed in the present study. Moreover, the rat renal tubule neoplastic response occurred mainly at an exposure concentration (400 ppm) lower than the concentration at which only subtle lesions characteristic of α2u-globulin inducers were observed (500 ppm). Additionally, six renal tubule neoplasms occurred in the 200 ppm group compared with two in the control group. No evidence of α2u-globulin nephropathy was observed at 250 ppm or below in the 13-week studies. In the F344/N rats in this study of pyridine, there was no significant exacerbation of nephropathy after 2 years, nor were there any significant increases in the incidences of parathyroid gland hyperplasia or fibrous osteodystrophy, two common changes in NTP studies with chemical-exacerbated chronic progressive nephropathy. There were also no liner foci of mineralization within the renal medulla in this study. By contrast to the findings in the F344/N rat, there was evidence (parathyroid gland hyperplasia, fibrous osteodystrophy, and glandular stomach mineralization) that chronic progressive nephropathy was more severe after 2 years in Wistar rats receiving 100 and 200 ppm, although there was no evidence of hyaline droplet nephropathy in male Wistar rats in the 13-week study. All of these considerations combined suggest that the neoplastic response to pyridine in the male F344/N rat kidney was not attributable to α2u-globulin.

Liver lesions in F344/N rats were characterized by centrilobular cytomegaly, degeneration, and necrosis; cytoplasmic vacuolization; foci of cellular alteration; fibrosis; and pigmentation in Kupffer's cells and macrophages. Bile duct hyperplasia was observed in all exposed groups of males and females and the incidences were significantly increased in exposed females compared to controls. Periportal fibrosis was a prominent lesion in 400 ppm males. There were no statistically significant increases in the incidences of hepatocellular neoplasms in exposed F344/N or Wistar rats.
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 zetaglobinv-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. 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 theTg·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 (32).

10 References

1 REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

2 European Chemicals Agency (ECHA). Registration dossier of Pyridine https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/13681. Consulted: May 27th, 2020.

3 International Agency for Research on Cancer. (2019). Some Chemicals That Cause Tumours Of The Urinary Tract In Rodents. IARC monographs on the evaluation of carcinogenic risks to humans ; volume 119

4 International Agency for Research on Cancer. (2000). Pyridine summary & evaluation. IARC IPCS Inchem, 77, 503.

5 Eller, P.M., ed. (1994) NIOSH Manual of Analytical Methods (DHHS (NIOSH) Publ. No. 94-113), 4th Ed., Cincinnati, OH, United States National Institute for Occupational Safety and Health [Method 1613]

6 Environmental Protection Agency (1995) Method 1665. Semi-volatile Organic Compounds Specific to the Pharmaceutical Manufacturing Industry by Isotope Dilution GC/MS, Washington DC.

7 Environmental Protection Agency (1996a) Method 8015B – Nonhalogenated Organics Using GC/FID, Washington DC.

8 Environmental Protection Agency (1996b) Method 8260B. Volatile organic compounds by gas chromatography/mass spectrometry (GC/MS) [Rev. 2]. In: Test Methods for Evaluating Solid Waste– Physical/Chemical Method 9US EPA No. SW 846), Washington DC, Office of Solid Waste.

9 Scriven, E. F., & Murugan, R. (2000). Pyridine and pyridine derivatives. Kirk - Othmer Encyclopedia of Chemical Technology.

10 Santodonato, J., Bosch, S., Meylan, W., Becker, J. & Neal, M. (1985) Monograph on Human Exposure to Chemicals in the Workplace: Pyridine (Report No. SRC-TR-84-1119), Syracuse, New York, Syracuse Research Corporation, Center for Chemical Hazard Assessment.

11 Agency for Toxic Substances and Disease Registry (1992) Toxicological Profile for Pyridine (TP-91/24), Atlanta, GA, Department of Health and Human Services, Public Health Service.

12 Shimizu, S., Watanabe, N., Kataoka, T., Shoji, T., Abe, N., Morishita, S. & Ichimura, H. (1993) Pyridine and pyridine derivatives. In: Elvers, B., Hawkins, S., Russey, W. & Schulz, G., eds, Ullmann's Encyclopedia of Chemical Technology, 5th rev. Ed., Vol. A22, New York, VCH Publishers, pp. 399–430.

13 D'Souza, J., Caldwell, J. & Smith, R.L. (1980) Species differences in the N-methylation and quaternization of [14^C]pyridine. Xenobiotica, 10, 151–157.

14 Damani, L.A., Crooks, P.A., Shaker, M.S., Caldwell, J., D'Souza, J. & Smith, R.L. (1982) Species differences in the metabolic C- and N-oxidation, and N-methylation of [14^C]pyridine *in vivo*. Xenobiotica, 12, 527–534.

15 Smith, J.N. (1953) Studies in detoxication. 53. The glucuronic acid conjugation of hydroxyquinolines and hydroxypyridines in the rabbit. Biochem. J., 55, 156–160.
16 Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. 5 (Suppl. 1), 3-142.
17 McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C., and Caspary, W.J. (1988). Responses of the L5178Y tk+/tk mouse lymphoma cell forward mutation assay: III. 72 codedchemicals. Environ. Mol. Mutagen. 12, 85-154.
18 National Toxicology Program. (2000). NTP Toxicology and Carcinogenesis Studies of Pyridine (CAS No. 110-86-1) in F344/N Rats, Wistar Rats, and B6C3F1 Mice (Drinking Water Studies). National Toxicology Program technical report series, 470, 1.
19 Galloway, S.M., Armstrong, M.J., Reuben, C., et al., (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ. Mol. Mutagen. 10 (Suppl. 10), 1-175.
20 Emelnsia, A. D., & Rather, I. A. (2016). In vitro genotoxicity of pyridine in human lymphocytes. Pakistan journal of pharmaceutical sciences, 29.
21 Valencia, R., Mason, J. M., Woodruff, R. C., & Zimmering, S. (1985). Chemical mutagenesis testing in Drosophila. III. Results of 48 coded compounds tested for the National Toxicology Program. Environmental mutagenesis, 7(3), 325-348.
22 Woodruff, R. C., Mason, J. M., Valencia, R., & Zimmering, S. (1984). Chemical mutagenesis testing in Drosophila: I. Comparison of positive and negative control data for sex - linked recessive lethal mutations and reciprocal translocations in three laboratories. Environmental mutagenesis, 6(2), 189-202.
23 Foureman, P., Mason, J. M., Valencia, R., & Zimmering, S. (1994). Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the national toxicology - program. Environmental and molecular mutagenesis, 23(3), 208-227.
24 Mason, J. M., Valencia, R., & Zimmering, S. (1992). Chemical mutagenesis testing in Drosophila: VIII. Reexamination of equivocai results. Environmental and molecular mutagenesis, 19(3), 227-234.
25 Muñoz, E.R. Barnett, B.M. (2003). Chromosome malsegregation induced by the rodent carcinogens acetamide, pyridine and diethanolamine in Drosophila melanogaster females. Mutat Res. 539 (1-2). 137-44.
26 Margolin, B.H., Resnick, M.A., Rimpo, J.Y., Archer, P., Galloway, et al., (1986). Statistical analyses for *in vitro* cytogenetic assays using Chinese hamster ovary cells. Environ. Mutagen. 8, 183-204.
27 Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. Environ. Mol. Mutagen. 21, 160-179.
28 Macgregor, J. A., Hamilton, C. M., Kubicek, J. E., & Mirsalis, J. C. (2000). Pyridine does not induce unscheduled DNA synthesis (UDS) in hepatocytes of male B6C3F1 mice treated in vivo. Journal of Applied Toxicology: An International Journal, 20(5), 389-393.

29 Mason, J.M., Cate, C.C., Baker, J. (1971). Toxicology and carcinogenesis of various chemicals used in the preparation of vaccines. Clin Toxicol. 4(2), 185-204.
30 Baxter, J.H. (1948). Hepatic and renal injury with calcium deposits and cirrhosis produced in rats by pyridine. Am. J. Pathol. 24, 503-525.
31 Anderson, R.C. (1987). 90-Day Subchronic Oral Toxicity in Rats. Test Material: Pyridine. Vol. I. Report to Dynamac Corporation, Rockville, MD, by Arthur D. Little, Inc., Cambridge, MA.
32 Spalding, J.W., French, J.E., Stasiewicz, S., Furedi-Machacek, M., Conner, F., Tice, R.R., et al., (2000) Responses of transgenic mouse lines p53(+/-) and Tg.AC to agents tested in conventional carcinogenicity bioassays. Toxicol Sci. 53(2). 213-23.

RIVM
Committed to *health and sustainability*