CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Chemical name:

1,4-Dichloro-2-nitrobenzene

EC Number: **201-923-3**

CAS Number: **89-61-2**

Index Number: **N/A**

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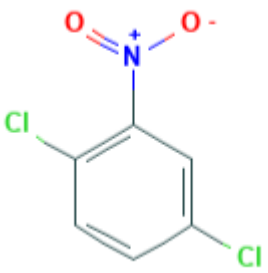
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1,4-dichloro-2-nitrobenzene
Other names (usual name, trade name, abbreviation)	Benzene, 1,4-dichloro-2-nitro- 2,5-Dichloronitrobenzene 2,5-Dichloro-1-nitrobenzene Benzene, 2,5-dichloronitro- Nitro-p-dichlorobenzene
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	201-923-3
EC name (if available and appropriate)	1,4-dichloro-2-nitrobenzene
CAS number (if available)	89-61-2
Other identity code (if available)	ICSC Number: 1618 RTECS Number: CZ5260000 UN number: 1578 PubChemCID: 6977
Molecular formula	$C_6H_3Cl_2NO_2$
Structural formula	
SMILES notation (if available)	C1=CC(=C(C=C1Cl)[N+](=O)[O-])Cl
Molecular weight or molecular weight range	192 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 80 wt %

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP) by registrants*
1,4-dichloro-2-nitrobenzene, CAS number: 89-61-2 EC number: 201-923-3	Confidential information, see confidential annex	No harmonised classification available	Acute Tox. 4 (H302) Skin Irrit. 2 (H315) Skin Sens. 1 (H317) Eye Irrit. 2 (H319) Muta. 2 (H341) Aquatic Chronic 3 (H412)

* ECHA C&L Inventory, 2021, Information on Chemicals - Classification & Labelling Inventory, European Chemicals Agency. Online: http://echa.europa.eu/information-on-chemicals/cl-inventory

1,4-Dichloro-2-nitrobenzene is a mono-constituent substance (CAS number: 89-61-2). The current self-classification by the registrants is given in Table 2. The frequency of hazard classifications among all notifications was retrieved from PubChem on 02/02/2021 and is given below. In total, 89 companies provided notifications with hazard classifications (10 aggregated notifications).

One company reported 1,4-dichloro-2-nitrobenzene as not meeting GHS hazard criteria.

Hazard classifications occurring in at least 10% of notifications:

Hazard code	Hazard statement	% of notifications
H302	Harmful if swallowed	62.5
H319	Causes serious eye irritation	57.95
H411	Toxic to aquatic life with long lasting effects	93.18

Except for the self-classification by the registrant, no CMR properties were notified.

The test substance is 1,4-dichloro-2-nitrobenzene in all studies where the test substance was explicitly stated. The purity is given in the study records below if available.

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No information on impurities available	Confidential information			

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No information on additives available					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	TBD	1,4-dichloro-2-nitrobenzene	201-923-3	89-61-2	Carc. 1B	H350	GHS08 Dgr	H350		-	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	data inconclusive	Yes
Carcinogenicity	harmonised classification proposed: Carc 1B H350	Yes
Reproductive toxicity	hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification and labelling available for 1,4-dichloro-2-nitrobenzene. The substance has not been included in former activities on harmonised classification.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at community level.

The substance has CMR properties (germ cell mutagenicity and carcinogenicity). Harmonised classification and labelling for CMR is a community-wide action under article 36 of the CLP regulation.

5 IDENTIFIED USES

1,4-Dichloro-2-nitrobenzene is used as an intermediate in the production of fine chemicals, pharmaceuticals, pigments, pesticides, and ultraviolet absorbers and as a laboratory agent (ECHA Dissemination, 2021; HCN, 2018; IARC, 2020; OECD, 1996). In the European Union, it is registered for use at industrial sites including the production of pulp, paper, and paper products and textiles, leather, and fur as well as the inclusion into/onto articles like fabrics, textiles, and apparel (ECHA Dissemination, 2021). According to ECHA disseminated database, the source of exposure of professional workers and consumers to 1,4-dichloro-2-nitrobenzene are cleaning and maintenance products (e.g., washing and cleaning products) (ECHA Dissemination, 2021).

6 DATA SOURCES

Systematic searches for publications and other relevant data were performed based on the following databases:

- U.S. National Library of Medicine, Pubmed.gov
- TOXNET, ChemIDplus, IPCS, eChemPortal
- Medline, SciSearch, Biosis, PQscitech, Chemical Abstracts (HCA), Embase (at host STN International)

The REACH registration dossier for 1,4-dichloro-2-nitrobenzene (last modified: 1 September 2020) publicly available from ECHA's disseminated database (ECHA Dissemination, 2021) has been analysed for study references, which then have been considered as data sources for this CLH report.

Relevant reviews and monographs with toxicological risk assessments on 1,4-dichloro-2-nitrobenzene were analysed for study references. Used reviews are from the International Agency for Research on Cancer (IARC) (2020), Health Council of the Netherlands (HCN) (2018), Organisation for Economic Co-operation and Development Screening Information Data Sets (OECD SIDS) (1996), and GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA report BUA (1991). The respective primary sources within the reviews were retrieved when possible.

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7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid, crystalline	ECHA Dissemination (2021)	visual observation
Melting/freezing point	53.1 °C	ECHA Dissemination	measured, at 97.21 kPa

Property	Value	Reference	Comment (e.g. measured or estimated)
		(2021)	
Boiling point	241.3 °C	ECHA Dissemination (2021)	measured, at 97.21 kPa
Relative density	1.262 g/cm³	ECHA Dissemination (2021)	density measured, at 20 °C
Vapour pressure	0.673 Pa	ECHA Dissemination (2021)	reported from handbook, measured
Surface tension		ECHA Dissemination (2021)	waived
Water solubility	84.095 mg/l	ECHA Dissemination (2021)	measured, at 25 °C
Partition coefficient n-octanol/water	2.87	ECHA Dissemination (2021)	measured, at 25 °C and pH 6.5
Flash point	114 °C	ECHA Dissemination (2021)	measured, at 97.13 kPa
Flammability	No data	ECHA Dissemination (2021)	Information requirement waived
Explosive properties	No data	ECHA Dissemination (2021)	Information requirement waived
Self-ignition temperature	465 °C	ECHA Dissemination (2021)	reported from secondary source (peer-reviewed data base), measured
Oxidising properties	No data	ECHA Dissemination (2021)	Information requirement waived
Granulometry	D50: > 53 ≤ 150 µm	ECHA Dissemination (2021)	measured
Stability in organic solvents and identity of relevant degradation products	No data	ECHA Dissemination (2021)	
Dissociation constant	No data	ECHA Dissemination (2021)	
Viscosity	No data	ECHA Dissemination (2021)	

8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Repeated dermal toxicity study/absorption; Rabbits were dermally exposed to	1,4-dichloro-2-nitrobenzene is absorbed after repeated dermal exposure; signs of systemic toxicity were present (reduction	Original study report not available, only secondary source	(BUA, 1991)

Method	Results	Remarks	Reference
100, 200, 400 mg /kg bw 1,4-dichloro-2-nitrobenzene (purity unknown) once daily for up to 15 days. Non guideline, non-GLP.	of erythrocytes and haemoglobin level, hyperaemia, erythropoiesis, and iron pigmentation in spleen) and mortalities occurred.		
Metabolism study; Groups of female rabbits (6-10 animals per group) were given 0.4 g/kg bw 1,4-dichloro-2-nitrobenzene (purity unknown) once orally via gavage administration as aqueous suspension. Urine samples were collected for 72 hours and analysed by paper chromatography and absorption spectra. Non guideline, non-GLP.	Main urinary metabolites were determined to be excreted as mercapturic acid (9-33%), glucuronide (8-56%) and sulphate (3-21%). Dichloroaniline was excreted to a lesser degree (free: 10-19%, combined: 1%). Of the mercapturic acid metabolites, the isolated metabolites could be assigned to 2,5-dichloroaniline (13%), N-acetyl-S-(4-chloro-2-nitrophenyl)-l-cysteine (2%), and 4-amino-2,5-dichlorophenol (1%). Recovery in urine: 92% of administered dose		Bray et al. (1957) BUA (1991)
Metabolism study; A group of 3 male F344/DuCrj rats was exposed for 2 days to 1,4-dichloro-2-nitrobenzene (purity unknown) orally via diet containing 1% 1,4-dichloro-2-nitrobenzene. Urine samples of all three animals were collected for 24 hours, pooled as one sample, and analysed by UV, nuclear overhauser effect (NOE) method of ^{1}H-NMR, and liquid chromatography (LC)-MS/MS. Non guideline, non-GLP.	Clear yellow coloured urine observed in treated rats versus clear colourless urine in controls Main urinary metabolite was determined to be an N-acetylcysteine conjugate, namely N-acetyl-S-(4-chloro-3-nitrophenyl)-l-cysteine		Ohnishi et al. (2004) ECHA Dissemination (2021)
In vitro studies investigated the conjugation of glutathione to 1,4-dichloro-2-nitrobenzene by microsomal glutathione S-transferase purified from rat and human liver. Morgenstern et al. (1988) reported that 1,4-dichloro-2-nitrobenzene with a purity of 99% was used. Keen et al. (1976) performed a purification of 1,4-dichloro-2-nitrobenzene by distillation under pressure, however the purity is not provided. Activities were determined in an enzyme assay in potassium phosphate at pH 6.5-7.0.	1,4-Dichloro-2-nitrobenzene is a substrate of glutathione S-transferase but it can also react to thioether in the absence of the enzyme.		BUA (1991) Morgenstern et al. (1988) Keen et al. (1976)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

There is no information for 1,4-dichloro-2-nitrobenzene from humans or guideline toxicokinetic studies available. Only limited data on absorption, metabolism, and excretion was identified from animal studies conducted in rabbits and rats.

Absorption of 1,4-dichloro-2-nitrobenzene after single oral or repeated dermal exposure was observed in rabbits (Bray et al., 1957; BUA, 1991; Ohnishi et al., 2004). After oral administration of 0.4 g 1,4-dichloro-2-nitrobenzene/kg body weight (bw) by gavage to female rabbits 92% of the administered dose was recovered as metabolites in the urine (Bray et al., 1957; BUA, 1991). The main urinary metabolites detected were: mercapturic acid (9-33% of applied dose), glucuronide (8-56% of applied dose) and sulphate (3-21% of applied dose). The isolated metabolites of mercapturic acid metabolites were investigated further and determined to be 2,5-dichloroaniline (13% of applied dose), *N*-acetyl-*S*-(4-chloro-2-nitrophenyl)-*L*-cysteine (2% of applied dose), and 4-amino-2,5-dichlorophenol (1% of applied dose) (Bray et al., 1957).

Ohnishi et al. (2004) orally exposed three male rats to 1% 1,4-dichloro-2-nitrobenzene in diet for 2 days and collected urine samples. A clear yellow coloured urine of treated rats was observed and an *N*-acetylcysteine conjugate, namely *N*-acetyl-*S*-(4-chloro-3-nitrophenyl)-*L*-cysteine was identified as the main urinary metabolite (ECHA Dissemination, 2021; Ohnishi et al., 2004).

In vitro studies by Keen et al. (1976) and Morgenstern et al. (1988) demonstrated that 1,4-dichloro-2-nitrobenzene can react with glutathione to thioether either in the presence and absence of glutathione S-transferase (BUA, 1991).

Based on the findings of Bray et al. (1957), IARC reported a scheme as shown in Figure 1 demonstrating the metabolism of absorbed 1,4-dichloro-2-nitrobenzene to predominantly an aniline metabolite and to a phenol metabolite. Afterwards secondary conjugation of both metabolites by mercapturic acid, glucuronide, sulphate or *N*-acetylcysteine will take place (IARC, 2020).

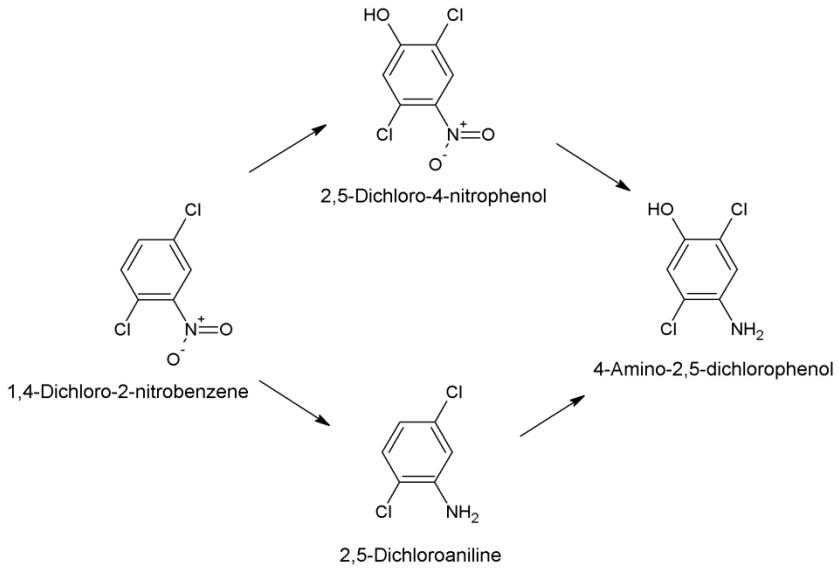

Figure 1: Identified urinary metabolites of 1,4-dichloro-2-nitrobenzene after oral exposure to rabbits, according to IARC (2020); original data by Bray et al. (1957).

To conclude, 1,4-dichloro-2-nitrobenzene is highly absorbed after oral as well as dermal exposure as indicated by the high fraction recovered from the urine. Absorbed 1,4-dichloro-2-nitrobenzene is intensively metabolised and mainly excreted via urine.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Evaluation not performed for this substance.

10.2 Acute toxicity - dermal route

Evaluation not performed for this substance.

10.3 Acute toxicity - inhalation route

Evaluation not performed for this substance.

10.4 Skin corrosion/irritation

Evaluation not performed for this substance.

10.5 Serious eye damage/eye irritation

Evaluation not performed for this substance.

10.6 Respiratory sensitisation

Evaluation not performed for this substance.

10.7 Skin sensitisation

Evaluation not performed for this substance.

10.8 Germ cell mutagenicity

Table 9: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Non-mammalian experimental systems				
Bacterial Reverse Mutation Assay According to Japanese Guideline for Screening Mutagenicity testing of chemicals; similar	1,4-dichloro-2-nitrobenzene Purity: >99.5% Impurities: <0.5% isomer of dichloronitrobenzene (no further information provided)	*Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2uvrA Plate incorporation method - preliminary cytotoxicity test (all	In the first test a precipitate of the test substance was observed on the surface of the agar plates at 1250, 2500, and 5000 µg/plate. <u>TA100:</u> positive (+/-S9); cytotoxic at	Ministry of Health and Welfare Japan (1994a), (Japanese, Tables in English)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
to OECD TG 471 Deviations: selection of positive controls, applied evaluation criteria (biological relevance poorly considered) GLP: yes Reliability: 2 (study report only available in Japanese with Tables in English; selection of positive control)	Solvent: DMSO	strains) 0, 50, 150, 500, 1500, and 5000 µg/plate with or without S9-mix Plate incorporation method - first test: TA100: 0, 78.13, 156.3, 312.5, 625, 1250, and 2500 µg/plate with or without S9-mix TA1535 and TA1537: 0, 39.06, 78.13, 156.3, 312.5, 625, and 1250 µg/plate without S9-mix; 0, 78.13, 156.3, 312.5, 625, 1250, and 2500 µg/plate with S9-mix WP2uvrA: 0, 156.3, 312.5, 625, 1250, 2500, and 5000 µg/plate with or without S9-mix TA98: 0, 156.3, 312.5, 625, 1250, 2500, and 5000 µg/plate without S9-mix; 0, 78.13, 156.3, 312.5, 625, 1250, and 2500 µg/plate with S9-mix Plate incorporation method - second test TA100, TA1535, and TA1537: 0, 78.13, 156.3, 312.5, 625, 1250, and 2500 µg/plate with or without S9-mix WP2uvrA: 0, 156.3, 312.5, 625, 1250, 2500, and 5000 µg/plate with or without S9-mix TA98: 0, 156.3,	≥1250 µg/plate in first test and at 2500 µg/plate in second test TA98: inconclusive first test negative (+/-S9); cytotoxic at ≥2500 µg/plate (-S9) and ≥1250 µg/plate (+S9); second test positive (-S9); negative (+S9); cytotoxic at 5000 µg/plate (-S9) and at ≥1250 µg/plate (+S9) TA1535: negative (+/-S9); cytotoxic at ≥625 µg/plate in first test and ≥1250 µg/plate in second test TA1537: negative (+/-S9); cytotoxic at ≥625 µg/plate (-S9) and ≥1250 µg/plate (+S9) in first test and at ≥1250 µg/plate (+/-S9) in second test WP2uvrA: negative (+/-S9); cytotoxic at 5000 µg/plate (-S9) and ≥2500 µg/plate (+S9) in first test and at ≥2500 µg/plate (+S9) in second test	HCN (2018) IARC (2020) OECD (1996)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		312.5, 625, 1250, 2500, and 5000 µg/plate without S9-mix; 0, 78.13, 156.3, 312.5, 625, 1250, and 2500 µg/plate with S9-mix +/- liver S9-mix from phenobarbital and 5,6-benzoflavone pre-treated male SD rats Media: histidine selective Plates: 3 No. replicates: 2 Positive controls: yes		
Bacterial Reverse Mutation Assay Ames test No explicit mentioning of OECD TG. Deviations: yes from OECD TG 471, not all required strains were tested, no confirmatory test performed, not tested with S9-mix, selection of positive control GLP: no Reliability: 3	1,4-dichloro-2-nitrobenzene Purity: >99.6% Impurities: not provided Solvent: DMSO	*Salmonella typhimurium* TA1535, TA1537, TA98, TA100 Pre-incubation method Test concentrations: 0, 51.2, 102.4, 204.8, 409.6, 819.2, 1638.4, 3276.8, and 6553.6 µg/plate without S9-mix No confirmatory test performed. Tests with S9-mix were only carried out if test without S9-mix were negative. Evaluation criteria: Positive, if number of revertant colonies is more than twice than the colonies on the control plate. Media: histidine	positive for TA98 and TA100 (-S9); cytotoxic at 6553.6 µg/plate positive for TA1538 (-S9); cytotoxic at ≥3276.8 µg/plate negative for TA1535 and TA1537 (-S9); no clear dose-response observed; cytotoxic at 6553.6 µg/plate	Shimizu et al. (1983) ECHA Dissemination (2021) HCN (2018) BUA (1991)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		selective Plates: 3 No. replicates: 2 Positive controls: yes		
Bacterial Reverse Mutation Assay Ames test No explicit mentioning of OECD TG or GLP. Deviations: yes from OECD TG 471, only one strain used, not tested with S9-mix, no data on cytotoxicity, only one low concentration tested, limited information on study design and results Reliability: 3	1,4-dichloro-2-nitrobenzene (named 2,5-dichloronitrobenzene) purity: commercially available, no further information (purity given in the disseminated database is for dinitrochlorobenzene) Impurities: not provided Solvent: DMSO	*Salmonella typhimurium* TA100 Plate incorporation method Test 1: test concentration of 1 µg/plate without S9-mix Media: histidine selective Plates: 4 No. replicates: 1 [Test 2: test substance named as dinitrochlorobenzene, thus the test was not considered in this evaluation] Positive control: yes	Test 1: negative TA100 (-S9); Number of revertants per plate (Mean ± SEM) were 231 ± 8 for 1,4-dichloro-2-nitrobenzene and 242 ± 10 for control Only one low concentration tested; therefore, no conclusion can be drawn	Black et al. (1985) ECHA Dissemination (2021) HCN (2018)
Bacterial Reverse Mutation Assay Ames test No explicit mentioning of OECD TG or GLP. Deviations: yes from OECD TG 471, only TA98 and TA100 strains tested, no data on cytotoxicity, limited information on study design and results as study is	1,4-dichloro-2-nitrobenzene (named 2,5-dichloronitrobenzene) purity: not provided, used purest available commercial product Impurities: not provided Solvent: DMSO	*Salmonella typhimurium* TA98 and TA100 Pre-incubation method Test concentrations: 0, 250, and 500 µg/plate with and without S9-mix Replicates: 2 Positive controls: yes Evaluation criteria: Positive, if the increase in number of	negative TA98 (+/- S9); no increase in number of revertants observed positive TA100 (+/- S9); number of revertants increased twice that of control	Kawai et al. (1987), (Japanese, Tables in English) BUA (1991) ECHA Dissemination (2021)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
in Japanese Reliability: 3		revertant colonies is more than twice than the control.		
Bacterial Reverse Mutation Assay No explicit mentioning of OECD TG or GLP. Deviations: yes from OECD TG 471, only test strain TA tested, not tested with S9-mix, very limited documentation Reliability: 3	1,4-dichloro-2-nitrobenzene (named 2,5-dichloronitrobenzol) Purity: not provided Impurities: not provided Solvent: DMSO	*Salmonella typhimurium* strain TA1535/pSK1002 Test concentrations: 0, 50, 500, 1000, 1250, 2500, 5000, and 12250 µg/mL without S9-mix Tested until limit concentration. No confirmatory test performed. Media: histidine selective Plates: 3 No. replicates: 2 Positive control: yes	positive (-S9)	Jin and Qian (1991) IARC (2020)
SOS response assay No explicit mentioning of OECD TG or GLP. Deviations: yes, limited data Reliability: 3	1,4-dichloro-2-nitrobenzene (named 2,5-dichloronitrobenzol) Purity: not provided Impurities: not provided Solvent: DMSO	*Salmonella typhimurium* strain TA1535/pSK1002 (*umu*C'-'*lac*Z) Test concentrations: 0, 10, 100, 500, and 1000 µg/mL without S9-mix No. replicates: 2 2-fold increase in colonies per plate and β-galactosidase activity above the control levels was defined as positive	positive (-S9)	Jin and Qian (1991) HCN (2018) IARC (2020)
Mammalian Cells				

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Chromosomal aberration According to Japanese Guideline for Screening Mutagenicity testing of chemicals; similar to OECD TG 473 Deviations: cytotoxicity not determined for test concentrations in main test GLP: yes Reliability: 2	1,4-dichloro-2-nitrobenzene Purity: 99.5% Impurities: <0.5% isomer of dichloronitrobenzene (no further information provided) Solvent: DMSO	Chinese hamster lung cells (CHL) Test 1 without S9-mix continuous treatment for 24 or 48 hours, test concentrations: 0, 0.04, 0.08, and 0.15 mg/mL Test 2 without S9-mix treatment for 6 h, test concentrations: 0, 0.024, 0.047, and 0.094 mg/mL Test 3 with S9-mix treatment for 6 h, test concentrations: 0, 0.024, 0.047, and 0.094 mg/mL S-9 fraction from the liver of Phenobarbital and 5,6-Benzoflavone induced male SD derived rats with NADPH-generating system Media: RPMI 1640 medium plus 10% foetal calf serum plus phytohaemagglutinin Plates/test: 2 No. replicates: 1 Positive control: yes	CLH report submitter: Test 1: No statistically significant increase in structural aberrations or number of polyploid cells observed after 24 h treatment without S9-mix. Cytotoxic at the highest test concentration (0.15 mg/mL) A statistically significant increase in structural aberrations and number of polyploid cells was observed after 48 h treatment without S9-mix in the highest concentration (0.15 mg/mL), which was cytotoxic (only 104 cells analysed instead of 200 cells). → equivocal Test 2 and 3: negative and number of polyploid cells not affected	Ministry of Health and Welfare Japan (1994b), (Japanese, Tables in English) mentioned in Kusakabe et al. (2002); Morita et al. (2012) IARC (2020) OECD (1996) HCN (2018)
Chromosome aberration study in mammalian cells No explicit mentioning of	1,4-dichloro-2-nitrobenzene Purity: not provided Impurities: not provided	Chinese hamster V79-cells Test 1 without S9-mix treatment for 4 h, test concentrations: 0, 10,	Original study not available to the dossier submitter BUA: 18 h after 4 h treatment: Test 1 and Test 2 were negative	BUA (1991)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OECD TG or GLP. Deviations: no information Reliability: 4 (secondary source)	Solvent: not provided	50, and 100 µg/mL Test 2 with S9-mix treatment for 6, test concentrations: 0, 20, 100, and 200 µg/mL	28 h after 4 h treatment: Test 1 showed a tendency to an increase in chromosome aberrations at 100 µg/mL (-S9); Test 2 a statistically significantly increase in chromosome aberrations was observed at cytotoxic concentrations without a clear concentration-response →negative without S9-mix →inconclusive with S9-mix	
HPRT assay No explicit mentioning of OECD TG or GLP. Deviations: yes, limited data Reliability: 4 (secondary source)	1,4-dichloro-2-nitrobenzene Purity: not provided Impurities: not provided Solvent: not provided	Chinese hamster V79-cells Test concentrations: 25 - 250 µg/mL with or without S9-mix Test 2 with S9-mix treatment for 6, test concentrations: 0, 20, 100, and 200 µg/mL	CLH report submitter: Original study not available thus an own assessment could not be performed BUA: negative (+/-S9)	BUA (1991)

Neither mutagenicity/genotoxicity tests in mammalian cells *in vivo* nor human data are available.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

There are only *in vitro* data available for the assessment of germ cell mutagenicity of 1,4-dichloro-2-nitrobenze. No *in vivo* data and no human data were identified.

In total 1,4-dichloro-2-nitrobenzene has been investigated in nine *in vitro* assays, which are summarised in Table 9. *In vitro* data are available from five Bacterial Reverse Mutation Assays (one Ames Test similar to OECD TG 471), a SOS response assay, two Mammalian Chromosome Aberration Assays in Chinese Hamster Lung cells (one similar to OECD TG 473) and a Mammalian Cell Forward Gene Mutation (CHL V-79/HPRT) Assay.

Due to methodological deficiencies of four Bacterial Reverse Mutation Assays reported in Table 9 only the assay reported by Ministry of Health and Welfare Japan (1994a) is regarded as reliable. In this study, conducted similar to OECD 471 and according to GLP, 1,4-dichloro-2-nitrobenzene was tested at five concentrations ranging from 39.06 to 5000 µg/plate in the presence and absence of metabolic activation. Various expert panels have differentially assessed the mutagenicity of 1,4-dichloro-2-nitrobenzene in the respective *Salmonella typh.* strains. It has to be mentioned that in its evaluation from 2020, IARC cited the results from OECD SIDS as the original documents were not accessible (IARC, 2020). Unfortunately, the applied evaluation criteria from Ministry of Health and Welfare Japan (1994a) are not clear as the study is only available in Japanese. Thus, for evaluating the results of *Salmonella typh.* strains, the dossier submitter followed the procedure established by Zeiger et al (1992) and Kier et al. (1986) and summarised in

Mortelmans and Zeiger (2000), which are in accordance with the OECD TG 471. A positive result is given if *"a reproducible, dose-related increase in the number of revertant colonies in one or more strains. […]"* are observed (Mortelmans and Zeiger, 2000). *"[…] f no dose-related increase in the number of revertant colonies is observed in at least two independent experiments […]"* then the substance is assessed as non-mutagenic (Mortelmans and Zeiger, 2000). If neither a clear positive nor negative result can be given, it is inconclusive (Mortelmans and Zeiger, 2000). Biological relevance is decisive and thus a two-fold increase in the number of revertants compared to controls is regarded as relevant in tester strains TA98, TA100, and *E. coli* WP2 uvrA. Due to a higher reversion frequency the two-fold value is too insensitive for tester strains TA1535 and TA1537 for which a three-fold rule is applied (Kier et al., 1986).

A clearly positive result, which was dose-dependent, was observed in the presence and absence of the metabolic activating rat liver S9-mix in the *Salmonella typh.* strain TA100. For the *Salmonella typh.* strain TA98 an inconclusive result was obtained without metabolic activation, as only a marginal positive result was observed in one test but not confirmed in the second one. In the presence of metabolic activation *Salmonella typh.* strain TA98 was negative. The other tested strains namely *Salmonella typh.* strain TA1535, TA1537, and *E. coli* WP2uvrA were negative with and without metabolic activation. Details on concentration-response relationship and strain-specific responses are documented in the Annex 1.

Other tests conducted with *Salmonella typh.* that are considered less reliable (reduced number of strains tested, documentation deficiencies) provide suggestive evidence as they show positive responses in some of the tested strains. However, both positive and negative results were observed for the same strain in different studies underlining that the results of these studies are ambiguous and not suitable to derive a conclusion.

The SOS response test is a bacterial genotoxicity assay approved for water and wastewater examination, which identifies primary DNA-damage caused by genotoxic substances (Oda, 2016). The induced DNA-damage cannot be repaired and leads to the expression of an enzyme which activity is determined colorimetrically. In the SOS response test conducted in *Salmonella typh.* strain 1535/pSK1002 1,4-dichloro-2-nitrobenzene is considered to be an SOS mutagen in the absence of a metabolic activating system. Due to poor data reporting and methodological deficiencies, the study was regarded to have limited reliability by the dossier submitter.

Of the three *in vitro* genotoxicity assays conducted in mammalian cells, 1,4-dichloro-2-nitrobenzene was reported to be negative in a HPRT test and not a clear clastogene in a chromosome aberration assay conducted in Chinese hamster V79-cells. Both assays are only available from a secondary source and the reliability was therefore not assignable (Reliability 4).

In a second chromosome aberration assay similar to OECD TG 473 and according to GLP (reliability 2, although some inconsistencies exist in the report), Chinese hamster lung cells were exposed to 1,4-dichloro-2-nitrobenzene either continuously for 24 or 48 hours without metabolic activation or for 6 hours with or without metabolic activation. After 6-hour treatment with or without metabolic activation 1,4-dichloro-2-nitrobenzene did not induce structural chromosome aberrations at any of the tested concentrations. After 48 hours in the continuous treatment without metabolic activation, a statistically significant increased incidence of structural aberrations was observed at the highest tested concentration. Additionally, the number of polyploid cells was statistically significantly increased. Both effects were not observed until after the 24 h-treatment at the same concentration level, which was evaluated by the authors of the study to be a cytotoxic concentration (Ministry of Health and Welfare Japan, 1994b). No data on the cytotoxicity of this test series (respective concentrations and exposure durations) was provided. Cytotoxicity was only investigated in a preliminary test, in which a cell growth of 55% was observed at 0.12 mg/mL and 0% at 0.24 mg/mL without any further concentrations tested in between (growth inhibition was less at even higher concentrations, 0.95 and 1.9 mg/mL, with growth rates between 9 and 23%, which raises doubt regarding consistency of the reported effects). Therefore, it is assumed that a treatment for 48 hours with a concentration of 0.15 mg/mL resulted in cytotoxicity that is in the borderline range at which a reliable evaluation can no longer be done, since a distinction between true genotoxicity or a genotoxic effect due to cytotoxicity is no longer possible. It also has to be noted that only 104 cells instead of planned 200 cells could be analysed at 0.15 mg/mL. Thus, the observed increased incidence of structural chromosome aberrations at the highest tested concentration after 48 hour-treatment is considered to be equivocal. Details on the performed test method and numerical values are documented in the Annex 1.

In conclusion, there are no data on germ cell mutagenicity of 1,4-dichloro-2-nitrobenzene available. For bacteria, genotoxicity assays suggest some mutagenic potential of 1,4-dichloro-2-nitrobenzene. A chromosome aberration study conducted in mammalian cells gave an equivocal result.

10.8.2 Comparison with the CLP criteria

For potential classification on germ cell mutagenicity, criteria from the CLP Regulation (EC, 2008)[1] were applied:

a) Comparison with Category 1 criteria

- *The classification in Category 1A is based on positive evidence from human epidemiological studies (EC, 2008)*

There are no epidemiological data to support classification of 1,4-dichloro-2-nitrobenzene in Category 1A.

- *The classification in Category 1B is based on positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals (EC, 2008)*
- *Classification in Category 1B can also be based on "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". (EC, 2008)*

There are neither experimental data from *in vivo* heritable germ cell mutagenicity tests in mammals nor from *in vivo* somatic cell mutagenicity tests in mammals with 1,4-dichloro-2-nitrobenzene available and thus classification in Category 1B is not supported.

b) Comparison with Category 2 criteria

- *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. (EC, 2008)

These criteria are also not met because no *in vivo* somatic cell genotoxicity tests in mammals exist for 1,4-dichloro-2-nitrobenzene.

However, in EC (2008) a note is also stated, which needs to be considered:

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. (EC, 2008)

An *in vitro* mammalian mutagenicity assay exists for 1,4-dichloro-2-nitrobenzene. As already stated in Table 9, an *in vitro* chromosome aberration study similar to OECD TG 473 conducted in CHL cells gave a negative result 24 h after continuous exposure to 1,4-dichloro-2-nitrobenzene without metabolic activation. After 48 hours without metabolic activation, an equivocal result was observed at a cytotoxic concentration. Therefore, no clear positive genotoxicity was observed in *in vitro* mammalian mutagenicity assays. The existing data from *in vitro* non-mammalian or mammalian mutagenicity assays is not regarded as sufficient to assign a classification as a category 2 mutagen.

Overall, no epidemiological data and no *in vivo* heritable germ cell / somatic cell mutagenicity tests in mammals are available. In addition, the *in vitro* bacterial assays suggest there may be some mutagenic

[1] REGULATION (EC) No 1272/2008 considering all ATPs published until January 2021

potential, but the classification criteria are not met in the absence of a clear positive response in mammalian cells.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Classification as a germ cell mutagen is not warranted because of insufficient data.

The data available also does not allow to exclude a genotoxic potential of 1,4-dichloro-2-nitrobenzene.

10.9 Carcinogenicity

Yamazaki et al. (2006) conducted chronic toxicity/carcinogenicity studies in rats and mice. The studies complied with GLP and were conducted similar to OECD TG 453, with various doses, sufficient large number of animals per treatment group, and suitable observations and examinations. Both studies were not reported in the registration dossier publicly available on the disseminated database of ECHA (ECHA Dissemination, 2021). In its evaluation from 2018, HCN assigned a reliability according to the Klimisch score of 2 (HCN, 2018). The dossier submitter agrees with this score. In the following table the results relevant for the endpoint carcinogenicity are reported. Both studies are reported in detail in Annex I.

Table 10: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Chronic toxicity and carcinogenicity study, similar to OECD TG 453 Deviations: limited reporting Reliability: 2 GLP: yes F344/DuCrj (SPF) male and female rats, 50 per sex and group	1,4-dichloro-2-nitro-benzene purity: > 98.8% 0, 320, 800 or 2000 ppm (w/w) in diet; equivalent to 10, 25 and 63 mg/kg bw/d for males and 17, 44 and 109 mg/kg bw/d for females using bw 475 g for males and 275 g for females and food intake 15 g/d cited by HCN (2018) doses were based on results from a	Incidences are always stated for doses at 0, 320, 800 and 2000 ppm (if not indicated otherwise). Statistically significant results are indicated in **bold** text/numbers as significant in trend test (**trend**) or by pairwise comparison. Survival (until termination) was 40/50, 44/50, 41/50, and 39/50 in males and 38/50, 35/50, 39/50, and 34/50 in females. No significant difference in survival rate analysis was observed between any treated groups and controls for both sexes. At 2000 ppm terminal body weights in males and females were decreased by 15% and 20% compared to their respective controls. Relative liver weight was statistically significantly (P≤0.01) increased in all treated animals of both sexes compared to controls. Relative kidney weight was statistically significantly (P≤0.01) increased in all treated males and in females at 800 and 2000 ppm compared to controls. The relative testis weight was statistically significantly (P≤0.01) increased, but the absolute testis weight was not significantly increased in males at 2000 ppm compared to controls. <u>non-neoplastic lesions:</u> Chronic progressive nephropathy (CPN, total) 46/50**, 49/50$^{\#\#}$, 50/50$^{\#\#}$, 49/50$^{\#\#}$** in males and 24/50, 23/50, 32/50, 28/50 in females Urothelial hyperplasia in pelvis 1/50, **8/50$^{\#}$, 36/59$^{\#\#}$, 39/50$^{\#\#}$** in males Mineralisation of papilla 0/50, 2/50, **47/50$^{\#\#}$, 48/50$^{\#\#}$** in males Hematopoesis in bone marrow 5/50, 9/50, 9/50, **14/50$^{\#}$** in females	Yamazaki et al. (2006) Cited also by HCN (2018) IARC (2020)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	subchronic study in rats (see Table 11) daily oral exposure via diet for 2 years (104 weeks)	No increased tumour incidences were observed in females. Males: Pre-neoplastic lesions: Basophilic hepatocellular foci: 21/50, 22/50, **32/50**$^{\#\#}$, **40/50**$^{\#\#}$ (n.a.) Neoplastic lesions: Liver: Hepatocellular adenoma: 0/50, 1/50, 0/50, **6/50*** (**trend**) Hepatocellular carcinoma: 0/50, 0/50, 1/50 (2%), 2/50 (4%) Hepatocellular adenoma and carcinoma (combined): 0/50, 1/50, 1/50, **8/50*** (**trend**) Kidney: Renal cell adenoma: 0/50, 0/50, 0/50, 2/50 (4%) Renal cell carcinoma: 0/50, 1/50 (2%), 0/50, 1/50 (2%) Renal cell adenoma and renal cell carcinoma (combined): 0/50, 1/50, 0/50, 3/50 (**trend**) Zymbal gland: Zymbal gland adenoma 0/50, 0/50, 0/50, 4/50 (8%) (**trend**) Historical control incidence in 1249 male rats (maximum incidence in any study): hepatocellular carcinoma, 0.2% (2%); renal cell adenoma, 0.16% (2%); renal cell carcinoma, 0.16% (2%); Zymbal gland adenoma, 0.2% (2%).	
Chronic toxicity and carcinogenicity study, similar to OECD TG 453 Deviations: limited reporting	1,4-dichloro-2-nitro-benzene purity: > 98.8% 0, 320, 800 or 2,000 ppm (w/w) in diet; equivalent to 32, 80 and	Incidences are always stated for doses at 0, 320, 800 and 2000 ppm (if not indicated otherwise). Statistically significant results are indicated in **bold** text/numbers as significant in trend test (**trend**) or by pairwise comparison: No significant difference in the survival rate between groups of treated mice and controls was observed in survival analysis according to Kaplan-Meier (data not shown in the publication). Survival (until termination) was 27/49, 35/50, 26/50, and 18/50 in males and 30/50, 27/50, 28/50, and 23/50 in females. After the 65th week of administration a lower survival rate was observed in mice of both sexes at 2,000 ppm. At 2000 ppm an increased number of deaths before the administration period ended was observed for mice of both sexes due to liver tumours; deaths were 7, 8,	Yamazaki et al. (2006) Cited also by HCN (2018) IARC

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Reliability: 2 GLP: yes Crj:BDF1 (SPF) male and female mice, 50 per sex and group	200 mg/kg bw/d for males and 41, 103, and 257 mg/kg bw/d for females using bw 45 g for males, 35 g for females and food intake 4.5 g/d, cited by HCN (2018) doses were based on results from a subchronic study in mice daily oral exposure via diet for 2 years (104 weeks)	11, and 23 for males and 0, 3, 4, and 6 for females. <u>Non-neoplastic lesions:</u> Centrilobular hypertrophy with nuclear atypia of hepatocytes 0/49[a], **38/50[##], 39/50[##], 40/50[##]** in males and 0/50, **15/50[##], 29/50[##], 35/50[##]** in females Hemosiderin deposition in kidney 1/49, 6/50, 6/50, **25/50[##]** in males Erythropoiesis in bone marrow 7/49, 7/50, 14/50, **23/50[##]** in males <u>Pre-neoplastic lesions:</u> Acidophilic cell foci: 0/49, 2/50, **7/50[#], 11/50[##]** in males <u>Neoplastic lesions:</u> Males: Hepatocellullar adenoma: 17/49, 21/50, 20/50, 16/50 Hepatocellular carcinoma: 15/49, 15/50, 23/50, **31/50** (trend)** Hepatoblastoma: 1/49, **10/50**, 12/50**, 25/50** (trend)**, historical control data: 5/1,047 in 21 studies Hepatocellular adenoma, hepatoblastoma and carcinoma (combined): 26/49, 34/50, **41/50**, 45/50** (trend)** Hepatocellular carcinoma and hepatoblastoma metastasized to lungs. Females: Hepatocellular adenoma: 5/50, 5/50, **17/50*, 16/50* (trend)** Hepatocellular carcinoma: 1/50, 3/50, **15/50*, 31/50* (trend)** Hepatoblastoma: 0/50, 0/50, 0/50, 2/50, historical control data: 0/1,047 in 21 studies Hepatocellular adenoma, hepatoblastoma and carcinoma (combined): 6/50, 8/50, **29/50*, 39/50* (trend)**	(2020)

[#] and [##] significantly different at P≤0.05 and P≤0.01 by Chi-square test

[*] and [**] significantly different at P≤0.05 and P≤0.01 by Fisher's exact test

Trend test performed by Peto test.

a: one male control mouse died accidentally during administration

Table 11: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Subchronic toxicity study, similar to OECD TG 408 Reliability: 2 GLP: yes F344/DuCrj (SPF) male and female rats, 10 per sex and group	1,4-dichloro-2-nitro-benzene purity: > 99.9%	0, 1481, 2222, 3333, 5000 or 7500 ppm (w/w) in diet (0, 93, 135, 207, 316 or 474 mg/kg bw/d for males and 0, 106, 162, 238, 342 or 458 mg/kg bw/d for females) doses were based on results from a subacute study in rats daily oral exposure via diet for 90 days	Incidences are always stated for doses at 0, 1481, 2222, 3333, 5000 or 7500 ppm (if not indicated otherwise). Statistically significant results are indicated in **bold**. All rats of treated and control groups survived the administration period and no significant difference in survival rate analysis was observed. Feed intake was statistically significantly lower at 2222 ppm and above in males and 3333 ppm in females. A statistically significant reduced terminal body weight in males and females was observed at 2222 ppm and above, respectively. Only doses 2222 ppm or less did not lead to a body weight decrement above 10%, except in females dosed at 2222 ppm (13%). A dose-dependent retardation of growth was seen in treated animals of both sexes, being more notable in treated males. Yellow coloured urine was observed in treated rats of both sexes. Increase in absolute and relative liver weights was seen in all treated rats of both sexes, except for absolute live weights of high-dosed male rats. In all treated rats of both sexes the relative kidney weights was significantly increased. Absolute and relative testes weights were decreased in rats at 2222 ppm and above, but ovary weight was not affected. In male rats at 2222 ppm and above and in female rats at 5000 and above relative spleen weights were significantly increased. At necropsy, three males at 5000 ppm and all males of the high dose group had accentuated lobular structures of the liver. Increased methaemoglobin levels were observed in 5000 ppm-dosed females and high-dosed males. Haematological parameters were changed at different doses in rats. In males, significant increases gamma-GTP and alanine aminotransferase levels were seen at 5000 ppm and above and at 7500 ppm, respectively. In female mice, gamma-GTP was increased at 3333 ppm and above. Total cholesterol, phospholipid, total protein, and albumin were significantly increased in all treated rats, except total protein in high dosed rats and albumin in high-dosed male rats. In male rats dosed up to 3333 ppm blood urea nitrogen was increased and in female rats at 5000 ppm and above. <u>Incidences for histopathological lesions in males:</u> Centrilobular hypertrophy hepatocytes in liver: 0/10, **6/10*, 10/10**, 10/10**, 9/10**, 8/10**** Centrilobular vacuolar changes in liver: 0/10,	Yamazaki et al. (2005)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			0/10, 0/10, **6/10*, 10/10**, 10/10**** Total hyaline droplets in kidney: 10/10 (consisting of 8 animals with moderate and 2 with marked droplets), **10/10** (all marked), 10/10** (all marked), 10/10** (all marked), 10/10** (all marked), 9/10* (5 slight, 4 moderate)** Granular casts in kidney: 0/10, **10/10**, 10/10**, 10/10**,** 0/10, 0/10 Cytoplasmic basophilia in kidney: 0/10, **10/10**, 10/10**, 10/10**, 1/10, 0/10 Eosinophilic droplet: proximal tubule in kidney: 0/10, 0/10, 0/10, 0/10, 0/10, 0/10 Deposition of hemosiderin in spleen: 0/10, 1/10, **10/10**, 10/10**, 10/10**, 10/10**** Increased extramedullary haematopoiesis in spleen: 0/10, 0/10, 1/10, 4/10, **9/10**, 10/10**** Germ cell necrosis in testis: 0/10, 0/10, **6/10*, 10/10**, 10/10**, 10/10**** Debris of spermatic elements in epididymis: 0/10, 0/10, **6/10*, 10/10**, 10/10**, 10/10**** Disappearance of sperm in epididymis: 0/10, 0/10, 0/10, **10/10**, 10/10**, 10/10**** <u>Incidences for histopathological lesions in females:</u> Centrilobular hypertrophy hepatocytes in liver: 0/10, **10/10**, 10/10**, 10/10**, 10/10**, 10/10**** Centrilobular vacuolar changes in liver: 0/10, 0/10, 0/10, 0/10, 0/10, **8/10**** Total hyaline droplets in kidney: 0/10, 0/10, 0/10, 0/10, 0/10, 0/10 Eosinophilic droplet: proximal tubule in kidney: 0/10, **8/10**, 10/10**, 10/10**, 10/10**, 4/10 Deposition of hemosiderin in spleen: 0/10, **8/10**, 10/10**, 10/10**, 10/10**, 9/10**** Increased extramedullary haematopoiesis in spleen: 0/10, 0/10, 0/10, 2/10, **8/10**, 9/10****	
Subchronic toxicity study, similar to OECD TG 408 Reliability:	1,4-dichloro-2-nitro-benzene purity: > 99.9%	0, 1481, 2222, 3333, 5000 or 7500 ppm (w/w) in diet (0, 245, 374, 530, 775 or 1647 mg/kg bw/d for males and 0, 284, 428, 613, 936 or 1601 mg/kg bw/d for females); doses were based on	Incidences are always stated for doses at 0, 1481, 2222, 3333, 5000 or 7500 ppm (if not indicated otherwise). Statistically significant results are indicated in bold. During the administration period, at 5000 ppm one male mice and at 7500 ppm 4 male and 4 female mice died (causes could not be confirmed). One accidental death of a male mice	Yamazaki et al. (2005)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
2 GLP: yes Crj:BDF1 (SPF) male and female mice, 10 per sex and group		results from a subacute study in mice daily oral exposure via diet for 90 days	at 1481 ppm occurred. Feed intake was statistically significantly lower at 7500 in mice of both sexes. A statistically significantly reduced terminal body weight in males and females was observed at 7500 ppm. Yellow coloured urine was observed in treated mice of both sexes. Increase in absolute and relative liver weights was seen in all treated mice of both sexes, except for absolute liver weights of 1481 ppm dosed female mice. In males at 3333 ppm and above and in females at 5000 ppm and above, the relative kidney weights were significantly increased. Absolute and relative testes weights were decreased in mice at 7500 ppm, but ovary weight was not affected. In mice of both sexes at 2222 ppm and above relative spleen weights were significantly increased. Animals found dead or in moribund state had thymus atrophy. Increased methaemoglobin levels were observed in 7500 ppm-dosed mice of both sexes. Haematological parameters were changed at different doses in mice. Significant increased alanine aminotransferase and aspartate aminotransferase levels were seen at 2222 ppm and above and at 3333 ppm and above in female and male mice, respectively. Total cholesterol, phospholipid, total protein, and albumin were also increased in treated mice but to a lesser extent compared to rats, except total protein in high dosed rats and albumin in high-dosed male rats. Blood urea nitrogen was increased in female mice at 5000 ppm and above. <u>Incidences for histopathological lesions in males:</u> Focal necrosis in liver: 1/10, 1/9, 2/10, 3/10, 5/10, 1/10 Single cell necrosis in liver: 1/10, 1/9, 3/10, **8/10**, 10/10**, 10/10**** Deposit of needle-like body in liver: 0/10, 0/9, **5/10*, 9/10**, 10/10**, 9/10**** Centrilobular hypertrophy hepatocytes in liver: 0/10, **9/9**, 10/10**, 9/10**, 10/10**, 10/10**** Deposition of hemosiderin in spleen: 2/10, 6/9, **10/10**, 9/10**, 10/10**,** 7/10 Increased extramedullary haematopoiesis in spleen: 0/10, 1/9, **5/10*, 9/10**, 10/10**, 6/10*** Germ cell necrosis in testis: 0/10, 0/9, 0/10, 0/10, 0/10, **10/10**** Debris of spermatic elements in epididymis: 0/10,	

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			0/9, 0/10, 0/10, 0/10, **10/10**** Disappearance of sperm in epididymis: 0/10, 0/9, 0/10, 0/10, 1/10, **6/10*** <u>Incidences for histopathological lesions in females:</u> Focal necrosis in liver: 0/10, 0/10, 0/10, 0/10, 0/10, 0/10 Single cell necrosis in liver: 0/10, 2/10, 4/10, **10/10**, 10/10**, 6/10*** Deposit of needle-like body in liver: 0/10, 0/10, **10/10**, 10/10**, 10/10**, 9/10**** Centrilobular hypertrophy hepatocytes in liver: 0/10, **10/10**, 10/10**, 10/10**, 10/10**, 10/10**** Deposition of hemosiderin in spleen: 0/10, **10/10**, 7/10**, 10/10**, 10/10**, 8/10**** Increased extramedullary haematopoiesis in spleen: 0/10, 2/10, 4/10, **10/10**, 10/10**, 5/10***	

* and ** significantly different at P≤0.05 and P≤0.01 by Chi-square test

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

There are no human data available for assessing the carcinogenic potential of 1,4-dichloro-2-nitrobenzene. Inhalation carcinogenicity studies were not identified for 1,4-dichloro-2-nitrobenzene.

Reliable oral chronic toxicity/carcinogenicity studies conducted in rats and mice, which were complying to GLP and similar to OECD TG 453 were available. Carcinogenic effects were observed in these studies (see Table 10) including:

- liver adenoma and carcinoma in male rats
- renal cell adenoma and carcinoma in male rats
- Zymbal gland adenoma in male rats
- liver adenoma in female mice as well as liver carcinoma and hepatoblastoma in male and female mice

In female rats no neoplastic lesions were observed.

Regarding classification and labelling for carcinogenicity it is necessary to assess if the observed neoplastic lesions in experimental animals are relevant for humans. This will be discussed for each observed tumour type separately, taking into account the sparsely available information on the Mode of Action (MoA).

10.9.1.1 Liver tumours in male rats and male and female mice

In a chronic toxicity/carcinogenicity study, Yamazaki et al. (2006) reported a statistically significant increase in incidences of hepatocellular adenoma as well as hepatocellular adenoma and carcinoma (combined) in male rats exposed to 1,4-dichloro-2-nitrobenzene. In the highest exposure group (2000 ppm) the incidence of hepatocellular carcinoma was higher than the upper range of the historical control data. Basophilic cell foci,

a pre-neoplastic lesion, was also statistically significantly and dose-related increased. Metastisation of liver tumours to other organs or tissues was not observed.

In this study, general toxicity after chronic exposure was characterised by decrease in terminal body weight, changes in blood, and blood biochemistry parameters as well as absolute and relative organ weights (for details see Table 10 and Annex I). Hepatotoxicity was observed as increased relative liver weights in rats of both sexes and centrilobular hypertrophy of hepatocytes in mice of both sexes. Although these effects were also noted in low dose exposure groups their occurrence and severity is limited and thus not regarded as excessive toxicity.

In mice hepatocarcinogenicity of 1,4-dichloro-2-nitrobenzene was evident as significantly increased incidences of liver adenoma in female mice, liver carcinoma in male and female mice, and liver adenoma, carcinoma, and hepatoblastomas (combined) in male and female mice were observed (Yamazaki et al., 2006). An increase in mice dying due to liver tumours before the end of the treatment period was observed (7, 8, 11, and 23 males and 0, 3, 4, and 6 females for 0, 320, 800, and 2000 ppm). Statistically significantly dose-relatedly increased incidences of acidophilic cell foci, a pre-neoplastic and proliferative lesion, was observed in male mice.

Hepatoblastoma is a tumour type with a low background rate in mice. Incidences of hepatoblastomas were statistically significantly increased in all exposed male animals and increased in female mice of the highest exposure group compared to controls (1 and 0 affected animals for male and female controls, resp.) and also compared to historical control data (5 cases out of 1047 males; no case out of 1047 females). Hepatocellular carcinoma of male and female mice as well as hepatoblastomas of male mice metastasised into the lung.

Data from subchronic oral toxicity studies with 1,4-dichloro-2-nitrobenzene conducted in rats and mice identified the liver and kidney as the target organs (for details see Table 11) (Yamazaki et al., 2005). Toxic effects observed in the liver included increased liver weights and centrilobular hypertrophy of hepatocytes in both species as well as single cell hepatic necrosis and elevated serum activity levels of liver-associated transaminases in mice. Clear histopathological changes in hepatocytes were only observed in mice with markedly enlarged cytoplasm and various sizes and shapes of nuclei.

From the literature it is known that continuous hepatocellular proliferation can be a driver for the development of substance-induced hepatocarcinogenicity. Regarding neoplastic findings in murine liver, Takahashi et al (2002) reported progressive lesion development from first appearing of vinyl carbamate-induced hepatocellular foci to adenoma and subsequently carcinoma, which can be regarded as a biological and morphological continuum.

Yamazaki et al. (2006) suggested for 1,4-dichloro-2-nitrobenzene that *"A genotoxic mode of action is thought to operate in the DCNB-induced hepatocarcinogenicity"*. As mentioned in section 10.8.1, there is evidence from an *in vitro* bacterial genotoxicity study that 1,4-dichloro-2-nitrobenzene can act as a mutagen. However, due to lack of data on *in vivo* heritable germ cell or somatic cell mutagenicity tests in mammals, there is insufficient data to support a genotoxic MoA.

In its evaluation from 2020, IARC assessed the carcinogenic potential of various nitrobenzenes by comparing their activity in standardised biochemical and cell-based assays across a few endpoints that are linked to common characteristics of carcinogens. In case of 1,4-dichloro-2-nitrobenzene, ToxCast and/or Tox21 high-throughput screening assays found to be active in 3 out of 54 assay endpoints investigated concerning *"Modulates receptor-mediated effects"*. The three induced reporter transcripts were *"human aryl hydrocarbon receptor response element (AhRE, responsive to AhR), human nuclear receptor subfamily 1, group I, member 2 response element (PXRE, responsive to NR1I2); and human peroxisome proliferator-activated receptor gamma (PPARγ) transcription factors in the HepG2 human liver cell line"*, whereas the latter was regarded as the most sensitive one (IARC, 2020). By comparing typical key characteristics of carcinogens with 1,4-dichloro-2-nitrobenzene IARC resumed that there is *"weak evidence that 1,4-dichloro-2-nitrobenzene is genotoxic"* (IARC, 2020).

Although the exact MoA is not known, data from carcinogenicity studies in rats and mice indicate clearly that exposure to 1,4-dichloro-2-nitrobenzene induced tumours in the liver. As liver tumours also occur in humans and no data exist that link 1,4-dichloro-2-nitrobenzene to a MoA which is not relevant for humans

(e.g., via PPARα), 1,4-dichloro-2-nitronbenzene's induction of liver tumours in male rats and mice of both sexes is considered as relevant for humans.

10.9.1.2 Renal tumours in male rats

After chronic exposure to 1,4-dichloro-2-nitrobenzene in rats, Yamazaki et al. (2006) reported a positive trend test for the increase in combined incidences of renal cell adenoma and carcinoma in male rats. Compared to the historical control data, a borderline increase in incidences of renal cell adenoma (4% compared to 0.16% in historical controls) in male rats of the highest exposure group was observed. In one case of a high exposed male, metastisation of the renal cell carcinoma to lung was noted. An increase in pre-neoplastic lesion was not seen in any dose group. But non-neoplastic lesions like urothelial hyperplasia in renal pelvis and renal papilla mineralisation were statistically significantly increased in a dose-dependent manner. Additionally, chronic nephropathy, which is attributed to advanced age of rats, was statistically significantly increased in all exposed dose groups.

In the subchronic toxicity study by Yamazaki et al. (2005) male rats had increased incidences of hyaline droplets and granular casts at the renal proximal tubules after subchronic oral exposure to 1,4-dichloro-2-nitrobenzene (for details see Table 11). Both observed alterations were positive for staining with α_{2u}-globulin antibody. These findings are suggesting a rat-specific α_{2u}-globulin-induced nephropathy whose MoA is characterised by α_{2u}-globulin accumulation as hyaline droplets, epithelial degeneration and necrosis, which leads to cell proliferation, chronic progressive nephropathy (often in older rats), atypical hyperplasia within the proximal tubules, and progression to renal tumours (Capen et al., 1999; Swenberg and Lehman-McKeeman, 1999). Kidney tumours in male rats associated with substances causing α_{2u}-globulin nephropathy are not considered relevant for humans (ECHA, 2017). In the literature a vast number of chemicals, e.g., hydrocarbons, 1,4-dichlorobenzene are known to cause α_{2u}-globulin-induced nephropathy. In females an increase in blood urea nitrogen level and of eosinophilic droplets in cytoplasm of proximal tubular epithelial cells, which were negative for staining with α_{2u}-globulin antibody, were observed. This indicates that the test substance causes nephrotoxicity not solely via α_{2u}-globulin pathway (Takahashi et al., 2002).

In order to identify if a substance's MoA of causing renal tumours is solely driven by α_{2u}-globulin IARC established criteria, which all must be met, and are the following:

- Lack of genotoxic activity,
- Male rat specificity for nephropathy and renal tumorigenicity,
- Indication of the characteristic sequence of histopathological changes, of which protein droplet accumulation is obligatory,
- Identification of the protein accumulating in the tubule cells as α_{2u}-globulin,
- Reversible binding of the chemical or metabolite to α_{2u}- globulin,
- Induction of sustained increased cell proliferation in the renal cortex,
- Similarities in dose-response relationship of the tumour outcome with the histopathological endpoints (protein droplets, α_{2u}-globulin accumulation, cell proliferation) (Capen et al., 1999).

IARC (2020) concluded that 1,4-dichloro-2-nitrobenzene is not fulfilling all criteria as *"criteria not met included chemical identification of the protein accumulating in tubule cells as α_{2u}-globulin, and reversible binding of 1,4-dichloro-2-nitrobenzene or a metabolite to α_{2u}-globulin"*.

Yamazaki et al. (2006) indicate that *"renal tumors in male rats that are produced by exposure to substances that produce α_{2u}-globulin nephropathy need not be considered in assessing potential neoplastic health risks to humans, when attributed exclusively to α_{2u}-globulin accumulation and non-mutagenic substances"*. As mentioned previously in section 10.8.1, 1,4-dichloro-2-nitrobenzene was mutagenic in a bacterial reverse mutation test and the result of an *in vitro* chromosome aberration assay was equivocal, thus the genotoxic MoA of 1,4-dichloro-2-nitrobenzene is unclear. However, Yamazaki et al. (2006) also refer that *"reactive metabolites of glutathione and cysteine conjugates of trichloroethylene, S-(1,2-dichlorovinyl)glutathione and*

S- (1,2-dichlorovinyl)-L-cysteine, which were biotransformed from the corresponding hepatic glutathione S-conjugate by γ-glutamyltranspeptidase and cysteine conjugate β-lyase in the kidney" and were shown to be mutagenic in an Ames test. As support Yamazaki et al (2006) made reference to a study from Elfarra et al. (1986), in which male rats were exposed to S-(1,2-dichlorovinyl)glutathione and S- (1,2-dichlorovinyl)-L-cysteine intraperitoneally, *"indicating a crucial role of the renal γ-glutamyltranspeptidase and cysteine conjugate β-lyase in the trichloroethylene-induced nephropathy"* and studies from Dekant et al. (1986) which showed that *"N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine was identified as a urinary metabolite of trichloroethylene, and that thioacylating metabolites of S-(1,2- dichlorovinyl)-L-cysteine, which were formed by renal cysteine conjugate β-lyase, might contribute to the nephrotoxic and mutagenic effects".*

1,4-Dichloro-2-nitrobenzene is excreted in urine as *N*-acetyl-S-(4-chloro-3-nitrophenyl)-*L*-cysteine (for details see section 9.1) and considering the findings of Elfarra et al. (1986) and Dekant et al. (1986) it can be assumed that it was metabolized to the glutathione conjugate in the liver, followed by reaction to *S*-(4-chloro-3-nitrophenyl)-*L*-cysteine in the kidney. Thus, Yamazaki et al. (2006) summarised *"it can be rational to infer that reactive and possibly mutagenic metabolites, which are presumably produced from S-(4-chloro-3-nitrophenyl)-L-cysteine by cysteine conjugate β-lyase in the kidneys contribute to the development of DCNB-induced nephrocarcinogenicity and chronic nephrotoxicity, although the α_{2u}-globulin-induced nephropathy can not be totally ruled out as a causative factor of nephrocarcinogenicity and nephrotoxicity".*

Taking all into account, there is evidence for 1,4-dichloro-2-nitrobenzene-induced renal tumours. However, α_{2u}-globulin-induced nephropathy might also be involved and thus the relevance of these tumours to humans is unclear.

10.9.1.3 Zymbal gland adenoma in male rats

In male rats a positive trend in increase in incidences of Zymbal gland adenoma was observed. Compared to the historical controls, the increase clearly exceeded the upper range for the high-dosed males (Yamazaki et al., 2006). Yamazaki et al. (2006) assessed the occurrence of these tumours as substance-related but only marginal due to the increase in tumour incidence.

The Guidance on the Application of the CLP criteria (ECHA, 2017) lists tumours in the Zymbal gland in context that "*some of the commonly used animal species have some tissues with no equivalent in humans"* and further describes *"Zymbal's glands are located beneath squamous epithelium at the anterior and posterior aspect of the ear canal. The external portion of the gland in rats is 3 to 5 millimetres in diameter."*. Although Zymbal glands are not occurring in humans and thus its relevance in humans seems to be low, it cannot be excluded completely. Pohl and Fouts (1983) detected cytochrome P450 activity in Zymbal glands of rats and mice, indicating that reactive metabolites of carcinogens may be formed, which can lead to tumour formation. Tumours occurring in the Zymbal gland after chronic exposure to 1,4-dichloro-2-nitrobenzene were of benign nature only. As no information on the mode of action is available for these tumours, their human relevance remains unclear.

In conclusion, the relevance of Zymbal gland tumours in male rats for the classification of 1,4-dichlor-2-nitrobenzene as carcinogenic is unclear. Overall, this evidence is therefore considered merely supportive for carcinogenicity, rather than clear evidence.

Table 12: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
F344/DuCrj (SPF) rats	**Liver** hepatocellular adenoma: Control: none (0/50) Historical control data: not reported hepatocellular carcinoma: Control: none (0/50) Historical control data: only reported for carcinoma; 3 cases (0.2%) in 1249 male rats in 25 studies with maximum incidence of 2% hepatocellular adenoma and carcinoma (combined): Control: none (0/50) Historical control data: not reported	Yes	Yes, adenoma transformed to carcinoma, see Table 10	Not applicable	Single, only observed in male rats	Not assumed to be due to excessive toxicity	Oral by diet	Relevant; discussed in detail in section 10.9.1.1
	Kidney Renal cell adenoma: Control: none (0/50) Historical control data: 2 cases (0.16%) in 1249 male rats in 25 studies with maximum incidence of 2%	Yes, one renal cell carcinoma of a high-dosed male rat metastasised to lung	Yes, adenoma transformed to carcinoma, see Table 10	Not applicable	Single, only observed in male rats	Not assumed to be due to excessive toxicity	Oral by diet	Relevance uncertain; discussed in detail in section 10.9.1.2

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	Renal cell carcinoma: Control: none (0/50) Historical control data: 2 cases (0.16%) in 1249 male rats in 25 studies with maximum incidence of 2% Renal cell adenoma and carcinoma (combined): Control: none (0/50) Historical control data: not reported							
	Zymbal gland Zymbal gland adenoma: Control: none (0/50) Historical control data: 3 cases (0.2%) in 1249 male rats in 25 studies with maximum incidence of 2%	Yes	only (non-malignant) adenoma observed	Not applicable	Single, only observed in male rats	Not assumed to be due to excessive toxicity	Oral by diet	Relevance uncertain; discussed in detail in section 10.9.1.3
Crj:BDF1 (SPF) mice	**Liver** hepatocellular adenoma Control: 17/49; 5/50 in females hepatocellular carcinoma: Control: 15/49 in males; 1/50 in females Historical control data: not reported	Yes, hepatocellular carcinoma of male and female mice as well as hepatoblastoma of male mice metastasised to lung	Yes, adenoma transformed to carcinoma, see Table 10	Not determined	Both sexes	Not assumed to be due to excessive toxicity	Oral by diet	Relevant; discussed in detail in section 10.9.1.1

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	<u>hepatoblastoma:</u> Control: 1/49 in males; 0/50 in females Historical control data: 5 cases in 1047 males and 0 cases in 1047 females <u>hepatoblastoma, adenoma, and carcinoma (combined):</u> Control: 26/49 in males; 6/50 in females Historical control data: not reported							

10.9.2 Comparison with the CLP criteria

IARC (2020) classified 1,4-dichloro-2-nitrobenzene: "*possibly* carcinogenic to humans (Group 2B)", based on *sufficient* evidence in animals and *inadequate* evidence in humans, according to the IARC criteria.

For potential classification of carcinogenicity, the criteria from the CLP Regulation (EC, 2008) supported by explanations from the Guidance on the Application of the CLP criteria (ECHA, 2017) were applied. The incidences of adenoma and carcinoma were considered. For potential classification of 1,4-dichloro-2-nitrobenzene, classification criteria were analysed accordingly.

- *The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:*

  — *human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or*

  — *animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen).*

  In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals. (EC, 2008)

The classification in Category 1A as a known human carcinogen applies for:

- *A substance may be further distinguished as Category 1A [if] classification is largely based on human evidence […].(EC, 2008)*

No epidemiological data for 1,4-dichloro-2-nitrobenzene is available, thus Category 1A is not warranted.

The classification in Category 1B as a presumed human carcinogen applies for:

- *A substance may be further distinguished […] as Category 1B [if] classification is largely based on animal evidence. (EC, 2008)*
- *Further EC regards as sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence [..](EC, 2008)*

Based on the data provided by Yamazaki et al. (2006) a causal relationship between the substance and an increased incidence of tumours in male and female mice as well as in male rats was observed. However, the criterion above is closely linked to the relevance for humans and additional considerations as discussed below.

The following additional considerations from CLP section 3.6.2.2.6 are relevant for concluding on a classification for carcinogenicity:

- (a) tumour type and background incidence;

  The tumour types observed in rodents after chronic oral exposure to 1,4-dichloro-2-nitrobenzene included hepatoadenoma and -carcinoma (male rats, and male and female mice), hepatoblastoma (male and female mice), renal cell adenoma and carcinoma (combined) (male rats), and Zymbal gland tumours (male rats). All tumour types listed above were elevated to a relevant degree compared to background incidence (or historical control data in the case of hepatoblastoma in female mice) in the individual studies.

- (b) multi-site responses;

  In rats, multi-site responses after chronic exposure to 1,4-dichloro-2-nitrobenzene were observed. Organs affected were liver, kidney, and Zymbal gland, although not all with a clear human relevance. Mice repeatedly exposed to 1,4-dichloro-2-nitrobenzene had different types of tumours in the liver.

  Metastisation of tumours to lung was observed in rodents. In mice, hepatocellular carcinoma of male and female mice as well as hepatoblastoma of male mice metastasised to lung. For renal cell carcinoma, it was observed that one case of a high-dosed male rat metastasised to lung.

- (c) progression of lesions to malignancy;

  In case of hepatocellular adenoma and renal cell adenoma the potential progress to malignancy was identified.

- (d) reduced tumour latency;

  This criterion was not addressed as the studies of Yamazaki et al. (2006) did not provide information on reduced tumour latency.

- (e) whether responses are in single or both sexes;

  Regarding the liver tumours a significant increase in hepatocellular adenoma was identified in male rats and female mice. Hepatocellular carcinoma were significantly increased in male and female mice and not statistically significantly increased in male rats. The combined incidence of hepatocellular adenoma and carcinoma was significantly increased in male rats as well as in male and female mice. Hepatoblastoma of the liver were seen in mice of both sexes. Renal cell adenoma and carcinoma (combined) as well as Zymbal gland adenoma were described in male rats only. In female rats, no tumour formation was observed.

- (f) whether responses are in a single species or several species;

  Tumour formation in the liver occurred in both rats and mice. Renal cell carcinoma and Zymbal gland adenoma were only identified in male rats.

- (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

  In its monography from 2020, IARC evaluated together with 1,4-dichloro-2-nitrobenezene the following chloronitrobenzenes: 2,4-dichloro-2-nitrobenzene (CAS No. 611-06-3), 2-chloronitrobenzene (CAS No. 88-73-3), and 4- chloronitrobenzene (CAS No.: 100-00-5) (IARC, 2020). For these chloronitrobenzenes IARC investigated their potential for carcinogenicity and concluded that they are “possibly carcinogenic to humans” (Group 2B) based on sufficient evidence in experimental animals (IARC, 2020). A harmonised classification is solely available for 4-chloronitrobenzene, which is classified for the CMR properties as a mutagen category 2 (Muta. 2) and carcinogen category 2 (Carc. 2) (ECHA C&L Inventory, 2021).

  However, the evidence for their carcinogenicity does not help to eliminate uncertainties on the mode of action and human relevance of the tumours observed in the studies conducted with 1,4-dichloro-2-nitrobenzene.

- (h) routes of exposure;

  In all available studies 1,4-dichloro-2-nitrobenzene was applied orally via diet.

- (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

  There are no indications of significant differences in the toxicokinetic data for humans and animals. However, data for humans and animals is too limited to draw a final conclusion.

- (j) the possibility of a confounding effect of excessive toxicity at test doses;

  The available critical studies gave no concern for a confounding effect by excessive toxicity at test doses. General toxicity after chronic exposure to 1,4-dichloro-2-nitrobenzene was noted by decreased terminal body weight, changes in blood and clinical biochemistry parameters, and organ weights. Adverse effects in kidney and liver were also observed in the low dose exposure group. However, these effects are not regarded as excessive toxicity caused at the tested doses.

- (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

  These aspects are discussed in detail in section 10.9.1.

Overall, there is sufficient evidence in animals for carcinogenicity with regard to tumours in the liver (multispecies and sex). A classification in Category 1B is therefore justified. The carcinogenic potential of 1,4-dichloro-2-nitrobenzene is further supported by tumour findings in other organs in the rat although their human relevance is unclear.

The classification of a substance in Category 2 as a suspected human carcinogen is based on the following, according to CLP Regulation (EC, 2008):

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (15) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

The evidence observed in animal studies as described above is not considered limited but clear evidence for carcinogenicity. The evidence is sufficiently convincing to place the substance in Category 1B. Therefore, classification in Cat 2 is not warranted.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Classification in category

Carc. 1B, 'H350: May cause cancer',

is warranted.

This conclusion is largely in accordance with the classification,

- by IARC (2020), who classify 1,4-dichloro-2-nitrobenzene as “possibly carcinogenic to humans” (Group 2B) based on sufficient evidence in experimental animals,
- by Health Council of the Netherlands (HCN, 2018), who assign the category 1B “presumed to be carcinogenic to humans” to 1,4-dichloro-2-nitrobenzene

To account for the contribution of a hazardous substance to the classification of mixtures based on the potency of the hazardous substance the concept of specific concentration limit (SCL) was established.

“Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous”. (EC, 2008)

In order to derive SCLs for carcinogens, the Guidance on the Application of the CLP criteria (ECHA, 2017) refers to the T25 concept established by Dybing et al. (1997) and a European guideline document (EC, 1999). For the calculation of T25 the endpoints with highest relevance for humans and highest incidence have to be considered. From the carcinogenicity studies performed in rodents (for details see Table 10) the statistically significantly increase in incidence of hepatoblastoma in male mice at 320 ppm (approx. 32 mg/kg bw/d) of 10/50 compared to 1/49 in the control and hepatocellular carcinoma in female mice at 800 ppm (approx. 103 mg/kg bw/d) of 15/50 compared to 1/50 in the control were used. The calculated T25 values were 44.5 mg/kg bw/d for hepatoblastoma in male mice and 92.0 mg/kg bw/d for hepatocellular carcinoma in female mice, respectively. In both cases, the T25 value is in the range of 1 mg/kg bw/d ≤ 100 mg/kg bw/d, which assigns 1,4-dichloro-2-nitrobenzene as a carcinogen of medium potency (EC, 1999). Thus, the generic concentration limit of 0.1% can be applied.

10.10 Reproductive toxicity

Evaluation not performed for this substance.

10.11 Specific target organ toxicity-single exposure

Evaluation not performed for this substance.

10.12 Specific target organ toxicity-repeated exposure

Evaluation not performed for this substance. However, evaluation of 90-day repeated dose toxicity studies in rat and mice is included in the overall assessment of the endpoint carcinogenicity in section 10.9.

10.13 Aspiration hazard

Evaluation not performed for this substance.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance.

12 EVALUATION OF ADDITIONAL HAZARDS

Evaluation not performed for this substance.

13 ADDITIONAL LABELLING

Not applicable.

14 ANNEXES

Please see separate document for Annex I and confidential Annex I.

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