2,4-Dichloro-1-nitrobenzene
1,4-dichloro-2-nitrobenzene

Evaluation of the carcinogenicity and genotoxicity

To: the State Secretary of Social Affairs en Employment
No. 2018/24, The Hague, December 11, 2018

Health Council of the Netherlands

contents

samenvatting

Op verzoek van de Minister van Sociale zaken en Werkgelegenheid (SZW) heeft de Gezondheidsraad de genotoxische en kankerverwekkende eigenschappen beoordeeld van 2,4-dichloor-1-nitrobenzeen en 1,4-dichloor-2-nitrobenzeen (in dit advies kortweg aangeduid als dichloornitrobenzenen). Dit advies is tot stand gekomen in de Subcommissie Classificatie van carcinogene stoffen, een subcommissie van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS).
Op www.gezondheidsraad.nl staat informatie over de taken van deze vaste commissie van de Gezondheidsraad. De samenstelling van de commissie is te vinden achterin dit advies.

Gebruik van dichloornitrobenzenen

2,4-Dichloor-1-nitrobenzeen wordt onder meer gebruikt als intermediair in de synthese van pigmenten, pesticiden en medicinale drugs. 1,4-Dichloor-2-nitrobenzeen wordt gebruikt als intermediair in de synthese van pigmenten, pesticiden en UV absorbers.

Classificeren op basis van bewijskracht voor schadelijk effect

De commissie beoordeelt of er aanwijzingen zijn dat de stof genotoxisch en kankerverwekkend is voor mensen. Als dat zo is, stelt de commissie voor om de stof in te delen in gevarencategorieën, één die aangeeft hoe groot de bewijskracht is dat de stof mutageen is in de geslachtscellen (dat wil zeggen: erfelijk overdraagbare mutaties kan veroorzaken) en één die aangeeft hoe groot de bewijskracht is dat de stof tot kanker kan leiden. De categorieën zijn afgeleid van EU-verordening (EG) 1272/2008.

Advies aan de staatssecretaris

Op grond van de beschikbare wetenschappelijke gegevens concludeert de commissie dat 2,4-dichloor-1-nitrobenzeen en 1,4-dichloor-2-nitrobenzeen beschouwd moeten worden als kankerverwekkend voor de mens. Ze adviseert de stof in te delen in gevarencategorie 1B[a]. De twee stoffen kunnen kanker veroorzaken via een stochaststisch genotoxisch werkingsmechanisme.

Voor mutageniteit adviseert de commissie om de stoffen niet in te delen in een gevarencategorie. Er zijn onvoldoende gegevens beschikbaar over de mutagene effecten van de stoffen in geslachtscellen.

[a] Zie Annex A (carcinogeniteit) en B (mutageniteit) voor classificatiesysteem.

executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the genotoxic and carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the SubCommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee. In this report, the Committee evaluated 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene.

Identified uses

2,4-Dichloro-1-nitrobenzene is used as an intermediate for the synthesis of pigments, pesticides and medicinal drugs. 1,4-Dichloro-2-nitrobenzene is used as an intermediate for the synthesis of pigments, pesticides and UV absorbents.

Classification according to strength of evidence for toxic effect

For the judgement on the carcinogenic properties of a substance, the Committee uses a classification system, which is largely based on EU-Directive 1272/2008. In addition to classifying substances on carcinogenicity, the Committee also assesses the genotoxic properties.

Recommendations to the State Secretary

Based on the available scientific data, the Committee concludes that 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene are presumed to be carcinogenic to man, and recommends classifying both compounds in category 1B[a] (the substances are presumed to be carcinogenic to humans). The subsances may act by a stochastich genotoxic mechanism of action.

Based on insufficient data, the Committee does not recommend a classification as germ cell mutagens.

[a] See Annex A (carcinogenicity) and B (mutagenicity) for the classification system.

01 scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification. In addition to classifying substances as carcinogenic, the Health Council also assesses the genotoxic properties of the substance in question, and proposes a classification on germ cell mutagenicity. Both classifications are based on the criteria set by the European Parliament (EU Regulation No. 1272/2008), and expressed in the form of standard sentences (see Annex A and B for carcinogenicity and mutagenicity, respectively).

This report contains the evaluation of the carcinogenicity and germ cell mutagenicity of 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene.

1.2 Committee and procedure

The evaluation is performed by the Subcommittee on Classifying carcinogenic substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee. The members of the Committee are listed on the last page of this advisory report .

In 2018, the President of the Health Council released a draft of the report for public review. The Committee has taken the comments received into account in deciding on the final version of the report. These comments, and the replies by the Committee, can be found on the website of the Health Council.

1.3 Data

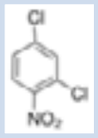
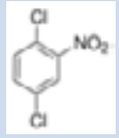
The evaluation and recommendation of the Committee is standardly based on scientific data, which are publicly available. The starting point of the Committees' reports is, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the Committee when these are considered relevant in assessing the carcinogenicity and genotoxicity of the substance in question. However, in the case of 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene, such an IARC-monograph is not available. In addition, published data were retrieved from the online databases Medline, Toxline, and Chemical Abstracts covering the period to June 2018 using 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene as keywords, in combination with keywords representative for carcinogenesis and mutagenesis.

02 identity of the substances

2.1 Name and other identifiers of the substances

The identity and some physicochemical properties of 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene are given in Tables 1 and 2 below.[1-3]

Table 1. Substance identity and information related to molecular and structural formula of the substances

	2,4-dichloro-1-nitrobenzene	1,4-dichloro-2-nitrobenzene
Chemical name	2,4-dichloro-1-nitrobenzene	1,4-dichloro-2-nitrobenzene
IUPAC name	2,4-dichloro-1-nitrobenzene	1,4-dichloro-2-nitrobenzene
EC number	210-248-3	201-923-3
RTECS number	CZ5420000 [RTECS]	CZ5260000 [RTECS]
CAS registry number:	611-06-3	89-61-2
Synonyms	2,4-dichloronitrobenzene, 1,3-dichloro-4-nitrobenzene asym.-Nitro-*m*-dichlorobenzene	2,5-dichloronitrobenzene Nitro-*p*-dichlorobenzene
Molecular formula	$C_6H_3Cl_2NO_2$	$C_6H_3Cl_2NO_2$
Structural formula		

2.2 Physico-chemical properties

Table 2. Summary of physicochemical properties

Property	2,4-dichloro-1-nitrobenzene Value	1,4-dichloro-2-nitrobenzene Value	Comment	Ref.
Physical description and colour	Pale yellow needles	Plates or prisms		1-3
Molar mass	192.0 g/mol	192.0 g/mol		1-3
Melting point	34 °C	53-56 °C		1-3
Boiling point	258 °C at1.013 hPa	261-267 °C at 1.013 hPa		1-3
Relative density (air = 1)	1.4790 at 80 °C; 1.54 at 15 °C	1.669 at 22 °C		1-3
Solubility in water	68.9 mg/L at 25 °C; 200 mg/L at 25 °C (meas.)	95 mg/L at 25 °C (meas.)		1-3
Solubility in organic solvents	Soluble in ethanol and ether; slightly soluble in chloroform	Soluble in ethanol, ether, benzene, carbon disulfide; slightly soluble in carbon tetrachloride		1-3
Log P (n-octanol/ water)	3.07 (not further specified); 2.90 at 25 °C (meas.)	2.93 at 25 °C (meas.)		1-3
Vapour pressure	1.0 Pa at 25 °C (meas.); 1.91 Pa at 25 °C (calc)	0.51 Pa at 25 °C (meas.)		1-3
Conversion factor (20 °C, 101.3 kPa)	1 ppm = 7.85 mg/m³ air at 20 °C; 1 mg/m³ = 0.127 ppm	1 ppm = 7.85 mg/m³ air at 20 °C; 1 mg/m³ = 0.127 ppm		1-3

2.3 International classifications

European Commission

No harmonized classification is available for 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene.

IARC

IARC has not evaluated 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene. In the report of the Advisory Group to Recommend Priorities for IARC Monographs during 2015-2019, it is recommended to evaluate 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene with high priority.[4]

03
manufacture and uses

3.1 Manufacture

Not relevant for classification.

3.2 Identified uses

2,4-Dichloro-1-nitrobenzene is used as an intermediate for the synthesis of pigments, pesticides and medicinal drugs.[3] 1,4-Dichloro-2-nitrobenzene is used as an intermediate for the synthesis of pigments, pesticides and UV absorbents.[2]

04 summary of toxicokinetics

No human and animal data were found on the absorption, distribution and excretion of 2,4-dichloro-1-nitrobenzene and 1,4-dichloro-2-nitrobenzene.

Ohnishi et al. postulated a metabolism for tumour formation for both substances based on cysteine metabolites found in the urine of male rats[5]. They suggested that both 1,4-dichloro-2-nitrobenzene and 2,4-dichloro-1-nitrobenzene are conjugated by glutathione, degraded to the cysteine equivalent by gamma-glutamyltransferase, and subsequently cleaved by renal β-lyase to produce a toxic thiol which caused localized kidney damage.

05
2,4-dichloro-1-nitrobenzene

5.1 Genotoxicity

5.1.1 Non-human information

In vitro data

Data on in vitro genotoxicity testing of 2,4-dichloro-1-nitrobenzene are presented in Table 3.

Table 3. Summary table of genotoxicity tests in vitro with 2,4-dichloro-1-nitrobenzene (CAS 611-06-3)

Method	Micro-organism or cell type	Concentration range	Results	Klimisch score	References
Micro-organisms					
reverse mutation	*Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, TA1538	0, 51, 102, 205, 410, 819, 1,638, 3,277 and 6,554 µg/plate; - S9; pre-incubation Purity: 99%	positive for TA100:181±23 (control), 195±18, 226±25, 231±32, 277±48, 303±58, 424±88, 468±109, His^+ revertants/plate; cytotoxic at 6,554 µg/plate negative for TA98, TA1535, TA1537 and TA1538	Klimisch 2	Shimizu et al.1983[6]
	Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537	0, 3.3, 10, 33, 100 and 215 µg/plate; +/-S9[a]; pre-incubation Purity 99%	positive for TA100 (+S9): rat liver: 114±3.5 (control), 123±1.9, 135±2.3, 162±4.9, 254±32.1, 369±20.9, no cytotoxicity. hamster liver: 123±9.5 (control), 371±22.4, 472±55.0, 765±48.9, 1,027±13, 1,111±29.7 no cytotoxicity negative for TA98, TA1535 and TA1537	Klimisch 2	Haworth et al. 1983; NTP[7,8]
	Salmonella. typhimurium strains: TA100	1 µg/plate;-S9; pre-incubation	negative	Klimisch 3 (limited information on design en results: only one strain used; not tested +S9, no data on cytotoxicity)	Black et al. 1985[9]
Mammalian cells					
chromo-somal aberration	Chinese hamster lung cells	0, 0.04, 0.07 and 0.14 mg/mL; +/-S9 Purity 99.6%	positive: 4.0% cells with aberrations at 0.14 mg/ml[b] (-S9 with 24 h exposure); no polyploidy	Klimisch 1	Study report MLHW 1996 (Japanese);[10] Morita et al. 2012[11]
chromo-somal aberration	Chinese hamster lung cells	0, 0.04, 0.07 and 0.14 mg/mL; +/-S9 Purity: >93%	negative; no polyploidy	Kimisch 2	Study report MLHW 1996 (Original data, Japanese);[10] Kusakabe et al. 2002;[12] OECD 1996[2]

chromo-somal aberration	Chinese hamster ovarian cells	0, 50, 100, 250 and 500 µg/ml without S9; 0, 1,000, 1,500, 2,100 and 3,000 µg/ml with S9	negative	Klimisch 2	NTP[8]
sister chromatid exchange	Chinese hamster ovarian cells	0, 5, 16.7, 50, 167 and 500 µg/ml; +/- S9	negative	Klimisch 2	NTP[8]

[a] rat and hamster S9 was used

[b] significantly different from solvent control at p<0.05

2,4-Dichloro-1-nitrobenzene was found to be positive with *Salmonella typhimurium* TA100, once in the presence of metabolic activation and once without.[6-8] The results showed a dose related response. Mutagenicity was not observed in strains TA98, 1535, 1537 and 1538. Furthermore, 2,4-dichloro-1-nitrobenzene did not induce chromosomal aberrations in Chinese hamster lung cells in vitro according to Kusakabe et al., NTP and the OECD SIDS. In contrast, the original Japanese report (tables in English) shows a statistically significant increase in chromosomal aberrations at the highest dose, in the absence of metabolic activation (24 h-exposure; no data on cytotoxicity for 24h-exposure).[8,10,12] A sister chromatid exchange test was also negative with and without metabolic activation.[8]

In vivo data

Tabel 4. Summary table of mutagenecity tests in mammalian somatic of germ cells in vivo with 2,4-dichloro-1-nitrobenzene (CAS 611-06-3)

Method	Organism or cell type	Concentration range	Results	Klimisch score	References
Sex-linked recessive lethal mutations	*Drosophila melanogaster*	Male: 0, 20, 30 ppm feeding; 0, 30 ppm injection	negative	Klimisch 3 (classification is based on studies in mammalians; no OECD guideline anymore);	Yoon et al. 1985;[13] NTP[8] .

In Table 4 in vivo mutagenicity studies with 2,4-dichloro-1-nitrobenzene identified in the literature have been summarized. 2,4-dichloro-1-nitrobenzene was negative in the sex-linked recessive lethal mutation test in *Drosophila melanogaster*.[8,13] However, the Committee considers this test species not relevant for humans.

5.1.2 Human information

No studies on humans were retrieved.

5.1.3 Summary and discussion on mutagenicity

Below, only data are summarized of a reliable experimental design according to the Klimisch criteria 1 and 2.[14]

Germ cell genotoxicity

As no relevant genotoxicity studies of 2,4-dichloro-1-nitrobenzene in germ cells were found, the Committee is not able to make a conclusion whether the substance is mutagenic in germ cells.

Somatic cell genotoxicity

2,4-Dichloro-1-nitrobenzene was investigated only in in vitro genotoxicity tests for reverse mutations and chromosomal aberrations. The substance was shown to be mutagenic in vitro in bacteria both in the absence and the presence of metabolic activation. In two in vitro tests, 2,4-dichloro-1-nitrobenzene did not increase chromosomal aberrations, in a third test only at a cytotoxic concentration. Hence, 2,4-dichloro-1-nitrobenzene might be considered to be potentially genotoxic based on in vitro reverse mutation tests. No reliable in vivo studies were available to confirm the positive findings in in vitro tests (Table 4).

Overall the Committee concludes that 2,4-dichloro-1-nitrobenzene is mutagenic in vitro, and may act by a stochastic genotoxic mechanism of action.

5.1.4 Comparison with criteria

According to the criteria in Annex VI of the European regulation No. 1272/2008 (see Annex B), classification as *a* mutagen in category 1 is warranted when positive evidence for in vivo heritable germ cell mutagenicity in humans (1A) or mammals (1B) has been reported. No data have been presented on human germ cell mutagenicity. Overall, due to a lack of data the Committee concludes that there is no evidence for in vivo heritable germ cell mutagenicity of 2,4-dichloro-1-nitrobenzene.

In addition, substances may be categorized in 1B if there are *"positive results 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."* The latter may be based on a) "*supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo*", or b) "*by demonstrating the ability of the substance or its metabolites to interact with the genetic material of germ cells*" (see Annex B). No evidence has been found for in vivo mutagenicity testing in mammals. Regarding the second part of the criterion, there is no evidence that 2,4-dichloro-1-nitrobenzene is genotoxic in germ cells.

If substances do not meet the criteria for classification in category 1, they may be classified in category 2 if there is "*positive evidence 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*". (see Annex B). The Committee concludes that the positive results of the reverse mutation tests may indicate a dose-response, but this finding is too limited to classify 2,4-dichloro-1-nitrobenzene in category 2.

Overall, the Committee concludes that there is insufficient data to recommend a classification as a germ cell mutagen.

5.1.5 Conclusions on classification

Due to a lack of data, the Committee does not recommend to classify 2,4-dichloro-1-nitrobenzene as a germ cell mutagen.

5.2 Carcinogenicity

5.2.1 Non-human information

The carcinogenicity studies of 2,4-dichloro-1-nitrobenzene in experimental animals are summarized in Table 5. In these studies, the substance was administrated by diet. No inhalation carcinogenicity studies were available.

In a chronic toxicity and carcinogenicity study using rats, 2,4-dichloro-1-nitrobenzene was administered at 0, 36, 75 and 154 mg/kg bw/day for males, and 0, 43, 91 and 183 mg/kg bw/day for females, in the diet for 2 years.[15] This study was conducted with reference to the OECD Guideline for Testing of Chemicals 451 “Carcinogenicity Studies” (OECD, 1981), and was carried out in conformity with the OECD Principle of Good Laboratory Practice (OECD, 1998). A dose-related decreased terminal body weight was seen at the mid- and high doses in both sexes. No effect on survival was noted. Relative liver and kidney weights were increased in all males and in females at 91 and 183 mg/kg bw/day. Plasma levels of total cholesterol and phospholipids were increased in all groups in both sexes. Blood urea nitrogen was increased in all males and in females at 91 and 183 mg/kg bw/d. As shown in Table 5, increased pre-neoplastic lesions in the form of atypical tubular hyperplasia of the proximal tubules were seen in the kidneys of both sexes at all dose levels; in males no dose-response relationship was seen in incidence of atypical tubular hyperplasia, but the severity of the hyperplasia increased with dose, while in females both incidence and severity were related to the dose. The incidence of eosinophilic droplets in the proximal tubule was increased in all treated groups. Chronic progressive nephropathy was seen in all groups, including the controls, and the incidence of marked and severe grades of chronic progressive nephropathy was increased in all males and in females at 43 and 91 mg/kg bw/day. The incidence of urothelial hyperplasia of the pelvis and mineralization in the papilla was increased in all males. The substance induced renal cell adenomas and carcinomas at the mid- and high dose in both sexes in a dose-related manner; statistically significant in males at both doses and in females at the highest dose. The incidence of preputial gland adenomas was increased at 154 mg/kg bw/day in males only. No metastasis was observed.

In a chronic toxicity and carcinogenicity study using rats, 2,4-dichloro-1-nitrobenzene was administered at 0, 36, 75 and 154 mg/kg bw/day for males, and 0, 43, 91 and 183 mg/kg bw/day for females, in the diet for 2 years.[15] This study was conducted with reference to the OECD Guideline

Table 5. Summary of animal carcinogenicitystudies on 2,4-dichloro-1-nitrobenzene (CAS 611-06-3) exposure

Species	Design	Exposure levels	Observations and remarks (Klimisch score)	References
F344/ DuCrj rat	Chronic toxicity/ carcinogenicity study; 50/sex;	Diet; 0, 750, 1,500 and 3,000 ppm (=0, 36, 75 and 154 mg/kg bw/d for males and 0, 43, 91 and 183 mg/kg bw/d for females) Xpo: 104 weeks Xpe: 104 weeks Purity: 99.4% Gross necropsy and histopathological examination	Klimisch 2 *General*: no difference in survival; decreased body weight, increased liver and kidney weight; increased incidence of chronic progressive nephropathy; M: urothelial hyperplasia in the pelvis and mineralization in the papilla; *Atypical tubular hyperplasia incidence*[a] in control, 750, 1,500 and 3,000 ppm: Males: 0/50, 46/50[##], 46/50[##], 48/50[##] (n.a.) Females: 0/50, 28/50[##], 40/50[##], 50/50[##](n.a.) *Tumour incidence* in control, 750, 1,500 and 3,000 ppm: *Males:* Renal cell adenoma: 0/50, 0/50, 3/50, 26/50** (↑↑) Renal cell carcinoma: 0/50, 0/50, 2/50, 23/50** (↑↑) Combined incidence[b]: 0/50, 0/50, 5/50*, 38/50** (↑↑) Preputial gland adenoma: 1/50, 4/50, 2/50, 7/50* (↑) No metastasis observed. *Females:* Renal cell adenoma: 0/50, 0/50, 3/50, 26/50**(↑↑) Renal cell carcinoma: 0/50, 0/50, 0/50, 12/50**(↑↑) Combined incidence[b]:0/50, 0/50, 3/50, 32/50** (↑↑)	Kano et al. 2012[15]
Crj:BDF$_1$ mouse	Chronic toxicity/ carcinogenicity study; 50/sex;	Diet; 0, 750, 1,500 and 3,000 ppm (=0, 82, 172 and 355 mg/kg bw/d) for males and 0, 1,500, 3,000 and 6,000 ppm (= 203, 416 and 942 mg/kg bw/d) for females Xpe: 104 weeks Xpo: 104 weeks Gross necropsy and histopathological examination	Klimisch score: 2 *General:* decreased survival rate in males at 3,000 ppm and females at 3,000 and 6,000 ppm; decreased body weight at ≥1,500 ppm and food consumption (high dose); increased liver weight; increased centrilobular hepatocellular hypertrophy, increased acidophilic cell foci in liver, deposition of brown pigment in nasal cavity, respiratory metaplasia in olfactory epithelium and submucosal gland, F: increased eosinophillic globules in olfactory and respiratory epithelia, increased eosinophilic globules in nasopharynx. *Tumour incidence in control, 750, 1,500 and 3,000 ppm in males resp.:* Hepatocellullar adenoma: 18/50, 34/50**, 30/50*, 43/50** (↑↑) Hepatocellular carcinoma: 7/50, 7/50, 11/50, 15/50* (↑↑) Hepatoblastoma: 1/50, 5/50, 16/50**, 27/50** (↑↑) Combined incidence[c]: 19/50, 39/50**, 41/50**, 45/50** (↑↑) Peritoneum, haemangiosarcoma: 1/50, 0/50, 2/50, 5/50 (↑↑) *Tumour incidence in control, 1,500, 3,000 and 6,000 ppm in females resp.*: Hepatocellullar adenoma: 8/49, 25/50**, 42/50**, 45/50** (↑↑) Hepatocellular carcinoma: 1/49, 2/50, 11/50**, 21/50** (↑↑) Hepatoblastoma: 0/49, 2/50, 7/50**, 7/50** (↑↑) Peritoneum, haemangiosarcoma: 0/49, 3/50, 7/50**, 17/50** (↑↑) Hepatocellular carcinomas and hepatoblastomas metastasized to the lung, peritoneum, lymph node, stomach, ovary and pancreas.	Kano et al. 2012[15]

*, ** $p < 0.05, 0.01$ with Fisher exact test, respectively

#, ## $p < 0.05, 0.01$ with Chi-square test, respectively

↑, ↑↑ $p < 0.05, 0.01$ with Peto's test (dose-response relationship), respectively; n.a. = not analyzed

[a] Average severity grade index of the lesion: 1.2, 1.6 and 1.9 for treated males and 1.1, 1.2 and 1.8 for treated females.

[b] combined incidence of renal cell adenoma and/or carcinoma

[c] combined incidence of hepatocellular adenoma, carcinoma and/or hepatoblastoma

for Testing of Chemicals 451 “Carcinogenicity Studies” (OECD, 1981), and was carried out in conformity with the OECD Principle of Good Laboratory Practice (OECD, 1998). A dose-related decreased terminal body weight was seen at the mid- and high doses in both sexes. No effect on survival was noted. Relative liver and kidney weights were increased in all males and in females at 91 and 183 mg/kg bw/day. Plasma levels of total cholesterol and phospholipids were increased in all groups in both sexes. Blood urea nitrogen was increased in all males and in females at 91 and 183 mg/kg bw/d. As shown in Table 5, increased pre-neoplastic lesions in the form of atypical tubular hyperplasia of the proximal tubules were seen in the kidneys of both sexes at all dose levels; in males no dose-response relationship was seen in incidence of atypical tubular hyperplasia, but the severity of the hyperplasia increased with dose, while in females both incidence and severity were related to the dose. The incidence of eosinophilic droplets in the proximal tubule was increased in all treated groups. Chronic progressive nephropathy was seen in all groups, including the controls, and the incidence of marked and severe grades of chronic progressive nephropathy was increased in all males and in females at 43 and 91 mg/kg bw/day. The incidence of urothelial hyperplasia of the pelvis and mineralization in the papilla was increased in all males. The substance induced renal cell adenomas and carcinomas at the mid- and high dose in both sexes in a dose-related manner; statistically significant in males at both doses and in females at the highest dose. The incidence of preputial gland adenomas was increased at 154 mg/kg bw/day in males only. No metastasis was observed.

In a chronic toxicity and carcinogenicity study in mice, 2,4-dichloro-1-nitrobenzene was administered via the diet at 0, 82, 172 and 355 mg/kg bw/day to males and at 0, 203, 416 and 942 mg/kgbw/day to females in the diet for 2 years.[15] A decreased survival rate was noted at and above 355 mg/kg bw/day in both sexes. This was related to death due to tumours. The terminal body weight was decreased in males at 172 and 355 mg/kg bw/day, and in all females. Food consumption was decreased in the high dose males and females. Relative liver weight was increased dose-dependently in males at 172 and 355 mg/kg bw/day and in all females. The incidence of hepatocellular adenoma as well as the size and the incidence of metastasis were increased dose-dependently in all groups in both sexes. Decreased red blood cell count and hemoglobin, and increased mean cell volume were noted in males at 416 mg/kg bw/day. Total cholesterol levels were increased in all treated groups of both sexes. Phospholipid was increased in all groups, except the males at 82 mg/kg bw/day. Alkaline phosphatase was increased in all groups of both sexes, and alanine transaminase and aspartate transaminase were increased in all groups of both sexes, except in females given 203 mg/kg bw/day. Gamma-GTP was increased in males at 355 mg/kg bw/day and females at 416 and 942 mg/kg bw/day. LDH and creatine kinase were increased in all groups of both sexes, except 82 mg/kg bw/day males.

Blood urea nitrogen was increased in females at 416 and 942 mg/kg bw/day. Total bilirubin was increased in males at 172 and 355 mg/kg bw/day and in females at 942 mg/kg bw/day. Neoplastic lesions are described in detail in Table 5. An increased incidence of hepatocellular adenomas was seen in the liver of both sexes at all dose levels. The substance induced hepatocellular carcinomas at 355 mg/kg bw/day in males, and 416 and 942 mg/kg bw/day in females. Hepatoblastomas were considered test substance related at all dose levels in both sexes (clear dose response for males only). An increased incidence of centrilobular hepatocellular hypertrophy was noted in the liver of males at all dose levels, and in females at 942 mg/kg bw/day only. An increased incidence of acidophilic cell foci in the liver with dose-related response was seen in females at 416 and 942 mg/kg bw/day. The hepatocellular carcinomas and hepatoblastomas metastasized predominantly to the lung, followed by the peritoneum, lymph node, stomach, ovary and pancreas (not further specified). An increased incidence of hemangiosarcoma was seen in high dose males and in females at all dose levels (dose-related, statistically significant at and above 355 mg/kg bw/day).

In the nasal cavity, brown pigment was deposited in all groups. Respiratory metaplasia in the olfactory epithelium and submucosal gland were increased in both sexes. In addition, the incidence of eosinophilic globules in the olfactory and respiratory epithelia was increased in females. An increased incidence of eosinophilic globules in the nasopharynx occurred in males at 355 mg/kg bw/day and all females.

5.2.2 Observations in humans

No data on carcinogenicity in humans were found.

5.2.3 Other relevant information

No relevant data were found.

5.2.4 Summary and discussion on carcinogenicity

The renal tumours observed in F344 rats exposed to 2,4-dichloro-1-nitrobenzene were observed at 75 and 154 mg/kg bw/day in males and 91 and 183 mg/kg bw/day in females.[15] As stated in Chapter 4, for both dichloronitrobenzenes a cysteine conjugate was detected in the urine of treated male rats. These cysteine conjugates were postulated to be responsible for the increased incidence in renal tumours induced in rats by 2-year dietary administration of 2,4-dichloro-1-nitrobenzene.[16] The β-lyase activity responsible for cleaving the cysteine off to form a reactive thiol is present in humans as well, albeit the activity in the rat is 8 to 30 times higher than the activity in humans.[5] The metabolites formed from these substance may play a role in formation of renal tumours, but have not been related to the other tumours observed.

In mice, the incidence in hepatocellular adenomas was statistically significantly increased at all dose levels in both sexes. In addition, the incidence in hepatocellular carcinomas was statistically significantly increased at 355 mg/kg bw/day in males and at 416 and 942 mg/kg bw/day in females. The incidence of hepatoblastoma was

statistically significantly increased at 172 and 355 mg/kg bw/day in males and 426 and 942 mg/kg bw/day in females. Furthermore, an increased incidence of hemangiosarcomas in the peritoneum was seen in males at 355 mg/kg bw/day and females at 416 and 942 mg/kg bw/day.

Conclusion

2,4-Dichloro-1-nitrobenzene was shown to be carcinogenic in rats and mice. Increased incidences in renal adenomas and carcinomas were seen in male and female rats, and adenomas in the preputial gland in male rats. In mice, an increased incidence in hepatocellular adenomas and carcinomas, and in hepatoblastomas were observed. Hepatic tumours metastasized to other organs in both sexes. Also an increased incidence in haemangiosarcomas were observed in the peritoneum of mice.

As both renal and liver tumours do occur in humans and are not species-specific, the Committee considered these tumours observed in experimental animals as relevant for humans.

Based on these findings, the Committee concludes that there is sufficient evidence for carcinogenicity of 2,4-dichloro-1-nitrobenzene in animals.

5.2.5 Comparison with criteria

No data on the carcinogenicity of 2,4-dichloro-1-nitrobenzene in humans was available. Therefore category 1A is not applicable.

Classification in category 1B requires a causal relationship between the substance and an increased incidence of malignant neoplasm in two or more species. Adequate animal data were available for the oral route of exposure. In these studies 2,4-dichloro-1-nitrobenzene induced tumours in the liver and the peritoneum of mice, and the kidneys of rats. The Committee considers the kidney and liver tumours relevant for humans. According to the CLP criteria, 2,4-dichloro-1-nitrobenzene should therefore be classified as “presumed to be as carcinogenic to humans”, which corresponds to classification in category 1B.

5.2.6 Conclusions on classification

The Committee concludes that 2,4-dichloro-1-nitrobenzene is “presumed to be carcinogenic to man”, and recommends classifying the substance in category 1B. The substance may cause cancer by a stochastic genotoxic mechanism of action.

06
1,4-dichloro-2-nitrobenzene

6.1 Genotoxicity

6.1.1 Non-human information

In vitro data

Data on in vitro genotoxicity testing of 1,4-dichloro-2-nitrobenzene is presented in Table 6 .

Table 6. Summary table of genotoxicity tests in vitro with 1,4-dichloro-2-nitrobenzene (CAS 89-61-2)

Method	Cell type	Concentration range	Results	Klimisch score	References
Micro organisms					
severse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, E.coli WP2 uvrA	0,78, 156, 312, 625, 1,250, 2,500, 5,000 µg/plate; -S9; pre-incubation Purity>99.5%	positive for TA98 (-S9): 18±1.2 (control), not done (78µg/plate), 27±5.6, 45±13.6, 43±7.8, 40±3.8, 42±6.5 His^+ revertants plate, cytotoxicity at 5,000 µg/plate positive for TA100 (-S9); 100±8.0 (control), 226±25.3, 335±11.0, 567±58.7, 730±27.6, 779±51.4 revertants/ plate, cytotoxicity at 2,500 µg/plate TA100 (+S9): 121±16.9 (control), 215±13.2, 279±18.8, 384±37.1, 545±9.0, 665±46.8 revertants/plate, cytotoxicity at 2,500 µg/plate negative for TA1535, TA1537 and E.coli WP2 uvrA	Kimisch 1	Study report MLHW (Japanese, Tables in English);[17]
Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	0, 51, 102, 205, 410, 819, 1,638, 3,277 and 6,554 µg/plate; -S9; pre-incubation Purity: 99.6%	positive for TA98: 28±6 (control), 39±5, 56±9, 71±11, 105±32, 130±38, 124±45, 101±39 His^+ revertants/ plate, cytotoxicity at 6,544 µg/plate positive for TA100; 181±23 (control), 170±16, 355±23, 499±68, 742±83, 852±87, 938±119, 1,252±236 His^+ revertants/plate, cytotoxicity at 6,544 µg/plate negative for TA1535, TA1537 and TA1538	Klimisch 1	Shimizu et al. 1983[6]

Reverse mutation	*Salmonella typhimurium* strains TA100	1 µg/plate; -S9; pre-incubation 3-150 µg/plate: -S9 preincubation Purity: > 98%	negative positive: frequency of revertants 3 to 13-fold of the negative control, plateau at 75 ug/plate	Klimisch 3 (limited information on design en results: only one strain used; not tested +S9, no data on cytotoxicity)	Black et al. 1985[9]
Mammalian cells					
Chromo-somal aberra-tion	Chinese hamster lung cells	0, 0.024-0.15 mg/mL; +/S9 (continuous 24 and 48h) Purity: 99.5%	negative; only positive at cytotoxic level (0.15 mg/ml), no polyploidy	Klimisch 2	Study report MLHW (original data, Japanese, tables in English);[18] Kusakabe et al. 2002;[12] Morita et al. 2012;[11] OECD 1996[3]
Other studies					
SOS res-ponse	*Salmonella typhimurium* strains TA1535/ pSK1002 (*umuC'-'lacZ*)	0, 50, 500, 1,250, 2,500 and 12,250 µg/mL; metabolic activation not indicated	Positive at 1,000 µg/mL and higher[b], toxic at 12,250 µg/mL, increased *umu* expression	Klimisch 4 (limited data)	Jin and Qian 1991[19]

[a] LEC = lowest effective concentration

[b] A 2-fold increase in colonies per plate and β-galactosidase activity above the control levels was defined as positive.

In Table 6 in vitro mutagenicity studies with 1,4-dichloro-2-nitrobenzene, which were identified in the literature, have been summarized. 1,4-Dichloro-2-nitrobenzene was found to be mutagenic in the *Salmonella typhimurium* strains TA98 and TA100, in the absence of metabolic activation only.[6,17] Furthermore, 1,4-dichloro-2-nitrobenzene induced chromosomal aberrations in Chinese hamster lung cells in the absence of metabolic activation (48 hours exposure only), but this was observed at a concentration with more than 50% cytotoxicity and only 104 cells could be analysed at this concentration.[3,11,12,18]

In vivo data

No in vivo data on 1,4-dichloro-2-nitrobenzene were retrieved.

6.1.2 Human information

No studies on humans were retrieved

6.1.3 Summary and discussion on mutagenicity

Below, only data are summarized of a reliable experimental design according to the Klimisch criteria 1 and 2 (See Annex C).[14]

Germ cell genotoxicity

As no relevant genotoxicity studies of 1,4-dichloro-2-nitrobenzene in germ cells were found, the Committee is not able to draw a conclusion on whether the substance is mutagenic in germ cells.

Somatic cell genotoxicity

1,4-Dichloro-2-nitrobenzene was investigated only in in vitro genotoxicity tests for reverse mutations and chromosomal aberrations. 1,4-Dichloro-2-nitrobenzene was shown to be mutagenic in vitro in bacteria in the absence, but not in the presence, of metabolic activation. Exposure to 1,4-dichloro-2-nitrobenzene did result in an increase in cells with chromosomal aberrations, but only at cytotoxic levels. Hence, 1,4-dichloro-2-nitrobenzene might be considered to be genotoxic based on in vitro reverse mutation. No in vivo studies were available to confirm the positive findings in in vitro tests.

Overall the Committee concludes that 1,4-dichloro-2-nitrobenzene is mutagenic in vitro.

6.1.4 Comparison with criteria

According to the criteria in Annex VI of the European regulation No. 1272/2008 (see Annex B), classification as a mutagen in category 1 is warranted when positive evidence for in vivo heritable germ cell mutagenicity in humans (1A) or mammals (1B) has been reported. No data have been presented on human germ cell mutagenicity.

In addition, substances may be categorized in 1B if *there are positive results 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*. The latter may be based on

a) "*supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo*", or

b) "*by demonstrating the ability of the substance or its metabolites to interact with the genetic material of germ cells*" (see Annex B). Overall, due to lack of data on germ cell mutagenicity, the Committee is of the opinion that it is not possible to recommend a classification as a germ cell mutagen.

If substances do not meet the criteria for classification in category 1, they may be classified in category 2 if *there is positive evidence from experiments in mammals and/or in some cases from in vitro experiments obtained from*

a) somatic cell mutagenicity tests in vivo, in mammals or

b) other in vivo somatic cell genotoxicity tests, which are supported by positive results from in vitro mutagenicity assays. (see Annex B). The Committee concludes that the positive results of the reverse mutation tests indicate a dose-response. Due of a lack of data, the Committee does not recommend a classification as a germ cell mutagen.

Tabel 7. Summary of animal carcinogenicity studies on 1,4-dichloro-2-nitrobenzene (CAS 89-61-2) exposure

Species	Design	Exposure levels	Observations and remarks (Klimisch score)	References
F344/DuCrj; rat	Chronic toxicity/ carcinogenicity study 50/sex	Diet; 0, 320, 800 and 2,000 ppm (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) Xpe: 104 weeks Xpo: 104 weeks Gross necropsy and histopathological examination	Klimisch 2 *General*: no difference in survival; decreased body weight; increased liver and kidney weight; severity of chronic progressive nephropathy increased dose-related (M), urothelial hyperplasia in the pelvis and mineralization in the papilla (M); increased haematopoiesis of bone marrow (F). Incidence of atypical tubular cell hyperplasia in control, *320, 800 and 2,000 ppm resp: 0/50, 46/50, 46/50, 48/50* *Tumour incidence and pre-neoplastic lesions: in control, 320, 800 and 2,000 ppm resp.* Males: Hepatocellular adenoma: 0/50, 1/50, 0/50, 6/50* (↑↑) Hepatocellular carcinoma: 0/50, 0/50, 1/50, 2/50 Combined incidence[a]: 0/50, 1/50, 1/50, 8/50* (↑↑) Basophilic hepatocellular foci: 21/50, 22/50, 32/50##, 40/50## (n.a.) Renal cell adenoma: 0/50, 0/50, 0/50, 2/50 Renal cell carcinoma: 0/50, 1/50, 0/50, 1/50 Combined incidence[b]: 0/50, 1/50, 0/50, 3/50 (↑) Zymbal gland: 0/50, 0/50, 0/50, 4/50 (↑↑) *Tumour incidence and pre-neoplastic lesions: in control, 17, 44 and 109 ppm resp:* Females: No tumours observed Basophilic hepatocellular foci: 26/50, 20/50, 18/50, 25/50 (n.a.) No renal cell carcinoma metastasized to the lung.	Yamazaki et al. 2006[16]
Crj:BDF$_1$; mouse	Chronic toxicity/ carcinogenicity study; 50/sex;	Diet; 0, 320, 800 and 2,000 ppm (equivalent to 32, 80 and 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) Xpe: 104 weeks Xpo: 104 weeks Gross necropsy and histopathological examination	Klimisch 2 *General:* decreased survival rate in males and females at 2,000 ppm; decreased body weight; increased liver and kidney weight; increased incidence liver nodules; increased centrilobular hypertrophy of hepatocytes, increased hemosiderin deposit in kidney(M), increased erythropoiesis of bone marrow (M). *Tumour incidence at: control, 320, 800 and 2,000 ppm resp.* Males: Hepatocellullar adenoma: 17/49, 21/50, 20/50, 16/50 Hepatocellular carcinoma: 15/49, 15/50, 23/50, 31/50** (↑↑) Hepatoblastoma: 1/49, 10/50**, 12/50**, 25/50** (↑↑) Combined incidence[c]: 26/49, 34/50, 41/50**, 45/50** (↑↑) Acidophilic hepatocellular foci: 0/49, 2/50, 7/50#, 11/50## Hepatocellular carcinomas and hepatoblastomas metastasized to lungs. *Tumour incidence at: control, 41, 80 and 200 ppm resp.* Females: Hepatocellullar adenoma: 5/50, 5/50, 17/50**, 16/50** (↑↑) Hepatocellular carcinoma: 1/50, 3/50, 15/50**, 31/50** (↑↑) Hepatoblastoma: 0/50, 0/50, 0/50, 2/50 Combined incidence[c]:6/50, 8/50, 29/50**, 39/50** (↑↑) Acidophilic hepatocellular foci: 1/50, 7/50, 3/50, 3/50 (n.a.)	Yamazaki et al. 2006[16]

*,** p < 0.05, 0.01 with Fisher exact test respectively; #, ## p < 0.05, 0.01 with Chi-square test, respectively; ↑, ↑↑ p < 0.05, 0.01 with Peto's test (dose-response relationship), respectively; n.a. = not analyzed

[a] combined incidence of hepatocellular adenoma and/or carcinoma

[b] combined incidence of renal cell adenoma and/or carcinoma

[c] combined incidence of hepatocellular adenoma, carcinoma and/or hepatoblastoma.

6.1.5 Conclusions on classification

Due to a lack of data, the Committee does not recommend to classify 1,4-dichloro-2-nitrobenzene as a germ cell mutagen.

6.2 Carcinogenicity

6.2.1 Non-human information

Data on carcinogenicity of 1,4-dichloro-2-nitrobenzene are summarized in Table 7. In these studies 1,4-Dichloro-2-nitrobenzene was administred by diet. No inhalation carcinogenicity studies were available.

In a chronic toxicity and carcinogenicity study in rats 1,4-dichloro-2-nitrobenzene was administered at 0, 10, 25 and 63 mg/kg bw/day for males, and 0, 17, 44 and 109 mg/kg bw/day for females in the diet for 2 years.[16] No difference in survival was noted. Terminal body weight was decreased in all males, and in females at 109 mg/kg bw/day. In females, the haematocrit was decreased at 109 mg/kg bw/day, and haemoglobin was decreased at 44 and 109 mg/kg bw/day. Gamma-GTP was increased in all groups of both sexes. Total cholesterol, phospholipids and blood urea nitrogen were increased in males at 25 and 63 mg/kg bw/day, and in all females. Triglyceride was increased in males at 25 and 63 mg/kg bw/day. Total protein and albumin were increased in females at 44 and 109 mg/kg bw/d, and glucose was increased in females at 109 mg/kg bw/day. Urine analysis showed in males that protein was present at 63 mg/kg bw/day, and that the urinary pH was lowered at 25 and 63 mg/kg bw/day. Relative liver weight was increased in all animals. Relative kidney weight was increased in all males, and in females at 44 and 109 mg/kg bw/day.

The incidence of hepatocellular adenomas was increased in males at 63 mg/kg bw/day. Chronic progressive nephropathy was seen in all groups including the controls, but marked and severe chronic nephropathy was increased compared to the control in all male groups in a dose-related manner. Mineralization in the papilla was increased in males at 25 and 63 mg/kg bw/day. Urothelial hyperplasia in the pelvis was increased in all males. An increased haematopoiesis of the bone marrow was observed in females at 109 mg/kg bw/day.

As shown in Table 7, only in males increased tumour incidences were found. An increased incidence of pre-neoplastic lesions in the form of basophilic cell foci was seen in the liver of males at 25 and 63 mg/kg bw/day with a dose-related response. This finding correlated with hepatocellular adenoma at 63 mg/kg bw/day, one hepatocellular carcinoma observed at 25 mg/kg bw/day, and two hepatocellular carcinomas at 63 mg/kg bw/day. The two renal cell adenomas and carcinomas at 63 mg/kg bw/day in males were not statistically significant and not considered related to treatment. Moreover, the incidence of renal cell carcinoma was within the historical control range. The incidence of Zymbal gland adenomas was non-statistically significantly increased at 63 mg/kg bw/day in males (outside historical control). No metastasis was observed.

In a chronic toxic and carcinogenicity study in mice, 1,4-dichloro-2-nitrobenzene was administered at 0, 32, 80 and 200 mg/kg bw/day for males, and 0, 41, 103 and 257 mg/kg bw/day for females in the diet for 2 years.[16] A decrease in survival rate at 200 mg/kg bw/day in males, and to a less extent in females was noted. Terminal body weight was decreased at 80 and 200 mg/kg bw/day in males, and 257 mg/kg bw/day in females. Relative liver weight was increased at the mid and high dose groups in both sexes. Relative kidney weight was increased at 80 and 200 mg/kg bw/day in males, and 257 mg/kg bw/d in females. Red blood cell count, haemoglobin and haematocrit were increased in females at 257 mg/kg bw/day. Alanine transaminase, aspartate transaminase, LDH and alkaline phosphatase were increased in males at 80 and 200 mg/kg bw/day, and alkaline phosphatase was increased in females at 103 and 257 mg/kg bw/day. Gamma-GTP was increased at the high dose in both sexes. Total cholesterol was increased in all males and in females at 103 and 257 mg/kg bw/day. Phospholipid was increased in both sexes at the mid and high dose. Blood urea nitrogen was increased in females at 257 mg/kg bw/day. Glucose was decreased in males at 200 mg/kg bw/day and females at 257 mg/kg bw/day. No consistent, dose-related change in urinary parameters was observed. An increased erythropoiesis of the bone marrow was seen in males at 200 mg/kg bw/day. An increased incidence of centrilobular hypertrophy of hepatocytes was noted in all groups of both sexes. An increased incidence of haemosiderin deposit in the kidney was observed in males at 200 mg/kg bw/day. The incidence of acidophilic cell foci in the liver, not considered pre-neoplastic, was increased dose-dependently in males at 80 and 200 mg/kg bw/day. Neoplastic lesions and relevant putative pre-neoplastic lesions are described in detail in Table 7. An increased incidence of hepatocellular adenomas was seen in females at 103 and 257 mg/kg bw/day, without a clear dose-response relationship. An increased incidence of hepatocellular carcinomas was noted in males at 200 mg/kg bw/day and females at 103 and 257 mg/kg bw/day (dose-related). The incidence of hepatoblastoma was increased at all dose levels in males, in a dose related trend. In females. the incidence of hepatoblastoma was increased at 257 mg/kg bw/day in females (outside historical control; not statistically significant). The hepatocellular carcinomas and hepatoblastomas metastasized to the lung (not further specified).

6.2.2 Human information

No data on carcinogenicity in humans were found.

6.2.3 Other relevant information

No relevant data were found.

6.2.4 Summary and discussion on carcinogenicity

In rats an increased incidence of hepatocellular adenomas and carcinomas was seen only in males at 63 mg/kg bw/day.

The slightly increased incidence of renal cell adenoma at 63 mg/kg bw/day is restricted to male rats. Alpha-2mu-globulin was shown to be accumulated in the tubular epithelial cells in the 13-week study with this substance and is associated with development of renal tumours after long-term exposure of F344 rats. Therefore, the increased incidence of renal adenomas in male rats is considered not relevant for humans.
In mice the incidence of hepatocellular adenoma and carcinoma was increased in females at 103 and 257 mg/kg bw/day, and the incidence of hepatocellular carcinoma was increased at 200 mg/kg bw/day in males. An increased incidence of hepatoblastoma was found in males at all dose levels and females at 257 mg/kg bw/day.

Conclusion
In rats of both sexes, 1,4-dichloro-2-nitrobenzene statistically significantly induced an increased incidence in hepatocellular adenomas and carcinomas. In male rats, also increased incidences in adenomas were induced in the liver, kidneys and Zymbal glands. In mice, the incidence of hepatocellular adenomas was increased in females, but not in males. Increased incidences in hepatocellular carcinomas and hepatoblastomas were induced in both sexes with metastasis to the lungs.
As both renal and liver tumours do occur in humans and are not species-specific, the Committee considered the observed tumours in experimental animals as relevant for humans. Based on these findings, the Committee concludes that there is sufficient evidence for carcinogenicity of 1,4-dichloro-2-nitrobenzene in animals.

6.2.5 Comparison with criteria

No data on the genotoxicity and carcinogenicity of 1,4-dichloro-2-nitrobenzene in humans were available. Therefore category 1A is not applicable.
Classification in category 1B requires a causal relationship between the substance and an increased incidence of malignant neoplasm in two or more species. Adequate animal data were available for the oral route. In these studies 1,4-dichloro-2-nitrobenzene induced tumours in the liver of mice and rats. The substance also induced tumours in the kidneys of male rats. The Committee considered the liver and kidney tumours relevant for humans.
According to the CLP criteria, 1,4-dichloro-2-nitrobenzene should therefore be classified as “presumed to be as carcinogenic to humans”, which corresponds to classification in category 1B.

6.2.6 Conclusions on classification

The Committee concludes that 1,4-dichoro-2-nitrobenzene is “presumed to be carcinogenic to man”, and recommends classifying the substance in category 1B. The substance may cause cancer by a stochastic genotoxic mechanism of action.

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annexes

A classification on carcinogenicity

The Committee expresses its conclusions in the form of standard phrases[20]:

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category1	
		(before 16 December 2008)	(as from 16 December 2008)
1A	*The compound is known to be carcinogenic to humans.* • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	*The compound is presumed to be as carcinogenic to humans.* • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	*The compound is suspected to be carcinogenic to man.*	3	2
(3)	*The available data are insufficient to evaluate the carcinogenic properties of the compound.*	not applicable	not applicable
(4)	*The compound is probably not carcinogenic to man.*	not applicable	not applicable

[a] See Section 3.6 (Carcinogenicity) of Regulation No. 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances.

B classification on mutagenicity

Source: Section 3.5 (Germ cell mutagenicity) of Regulation No. 1272/2008 of the European Parliament and of the council of 16 December 2008 on classification, labelling and packaging of substances.

3.5.1. Definitions and general considerations

3.5.1.1. A mutation means a permanent change in the amount or structure of the genetic material in a cell. The term 'mutation' applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term 'mutagenic' and 'mutagen' will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

3.5.1.2. The more general terms 'genotoxic' and 'genotoxicity' apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxicity test results are usually taken as indicators for mutagenic effects.

3.5.2. Classification criteria for substances

3.5.2.1. This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class.

3.5.2.2. For the purpose of classification for germ cell mutagenicity, substances are allocated to one of two categories as shown in Table 3.5.1.

3.5.2.3 Specific considerations for classification of substances as germ cell mutagens

3.5.2.3.1. To arrive at a classification, test results are considered from experiments determining mutagenic and/or genotoxic effects in germ and/or somatic cells of exposed animals. Mutagenic and/or genotoxic effects determined in in vitro tests shall also be considered.

3.5.2.3.2. The system is hazard based, classifying substances on the basis of their intrinsic ability to induce mutations in germ cells. The scheme is, therefore, not meant for the (quantitative) risk assessment of substances.

Table 3.5.1 Hazard categories for germ cell mutagens

Categories	Criteria
CATEGORY 1:	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Substances known to induce heritable mutations in the germ cells of humans.
Category 1A:	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
Category 1B:	The classification in Category 1B is based on: — positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or — positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/ genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
CATEGORY 2:	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: - positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: - somatic cell mutagenicity tests in vivo, in mammals; or - other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays. *Note*: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

3.5.2.3.3. Classification for heritable effects in human germ cells is made on the basis of well conducted, sufficiently validated tests, preferably as described in Regulation (EC) No 440/2008 adopted in accordance with Article 13(3) of Regulation (EC) No 1907/2006 ('Test Method Regulation') such as those listed in the following paragraphs. Evaluation of the test results shall be done using expert judgement and all the available evidence shall be weighed in arriving at a classification.

3.5.2.3.4. In vivo heritable germ cell mutagenicity tests, such as:
— rodent dominant lethal mutation test;
— mouse heritable translocation assay.

3.5.2.3.5. In vivo somatic cell mutagenicity tests, such as:
— mammalian bone marrow chromosome aberration test;
— mouse spot test;
— mammalian erythrocyte micronucleus test.

3.5.2.3.6. Mutagenicity/genotoxicity tests in germ cells, such as:
(a) mutagenicity tests:
- *mammalian spermatogonial chromosome aberration test;*
- *spermatid micronucleus assay;*

(b) Genotoxicity tests:

- *sister chromatid exchange analysis in spermatogonia;*
- *unscheduled DNA synthesis test (UDS) in testicular cells.*

3.5.2.3.7. Genotoxicity tests in somatic cells such as:
— liver Unscheduled synthesis test (UDS) in vivo;
— mammalian bone marrow Sister Chromatid Exchanges (SCE);

3.5.2.3.8. In vitro mutagenicity tests such as:
— in vitro mammalian chromosome aberration test;
— in vitro mammalian cell gene mutation test;
— bacterial reverse mutation tests.

3.5.2.3.9. The classification of individual substances shall be based on the total weight of evidence available, using expert judgement (See 1.1.1). In those instances where a single well-conducted test is used for classification, it shall provide clear and unambiguously positive results. If new, well validated, tests arise these may also be used in the total weight of evidence to be considered. The relevance of the route of exposure used in the study of the substance compared to the route of human exposure shall also be taken into account.

3.5.3 Classification criteria for mixtures

3.5.3.1. Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.5.3.1.1. The mixture shall be classified as a mutagen when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 mutagen and is present at or above the appropriate generic concentration limit as shown in Table 3.5.2 for Category 1A, Category 1B and Category 2 respectively.

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

	Concentration limits triggering classification of a mixture as:		
Ingredient classified as:	Category 1A mutagen	Category 1B mutagen	Category 2 mutagen
Category 1A mutagen	≥ 0,1 %	-	-
Category 1B mutagen	-	≥ 0,1 %	-
Category 2 mutagen	-	-	≥ 1,0 %

Note. The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).

3.5.3.2. Classification of mixtures when data are available for the complete mixture

3.5.3.2.1. Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients classified as germ cell mutagens. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual ingredients. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of germ cell mutagenicity test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.5.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles

3.5.3.3.1. Where the mixture itself has not been tested to determine its germ cell mutagenicity hazard, but there are sufficient data on the individual ingredients and similar tested mixtures (subject to paragraph 3.5.3.2.1), to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.5.4. Hazard communication

3.5.4.1. Label elements shall be used in accordance with Table 3.5.3, for substances or mixtures meeting the criteria for classification in this hazard class.

Table 3.5.3 Label elements of germ cell mutagenicity

Classification	Category 1A or Category 1B	Category 2
GHS Pictograms		
Signal word	Danger	Warning
Hazard Statement	H340: May cause genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H341: Suspected of causing genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)
Precautionary Statement Prevention	P201, P202, P281	P201, P202, P281
Precautionary Statement Response	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405
Precautionary Statement Disposal	P501	P501

3.5.5. Additional classification considerations

It is increasingly accepted that the process of chemical-induced tumorigenesis in humans and animals involves genetic changes for example in proto-oncogenes and/or tumour suppresser genes of somatic cells. Therefore, the demonstration of mutagenic properties of substances in somatic and/or germ cells of mammals in vivo may have implications for the potential classification of these substances as carcinogens (see also Carcinogenicity, section 3.6, paragraph 3.6.2.2.6).

C criteria for testing reliability of animal and in vitro studies

To assess the reliability of animal and in vitro studies, the Committee uses the criteria set by Klimisch et al. 1997.[14] A summary of the criteria of the reliability scores is given below. Only studies with a reliability score of 1 or 2 are considered in assessing genotoxicity and carcinogenicity.

Reliability 1 (reliable without restriction)

For example, guideline study (OECD, etc.); comparable to guideline study; test procedure according to national standards (DIN, etc.).

Reliability 2 (reliable with restrictions)

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

Reliability 3 (not reliable)

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

Reliability 4 (not assignable)

For example, only short abstract available; only secondary literature (review, tables, books, etc.).

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The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research...” (Section 22, Health Act).
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