p-Chloronitrobenzene

Evaluation of the carcinogenicity and genotoxicity

Dutch Expert Committee on Occupational Standards,

a committee of the Health Council of the Netherlands

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies over p-chloornitrobenzeen
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-451/JR/tvdk/459-N36
Bijlagen : 1
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Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr DGV/MBO/U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In dat kader bied ik u hierbij een advies aan over de kankerverwekkende eigenschappen van p-chloornitrobenzeen. Het is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volksgezondheid, Welzijn en Sport, de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer en de Minister van Sociale Zaken en Werkgelegenheid.

Hoogachtend,

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Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

to

the Minister and State Secretary of Social Affairs and Employment

Nr 2002/03OSH, The Hague, 16 April 2002

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..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

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

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. In het voorliggende rapport neemt de Commissie WGD van de Raad, die deze beoordelingen verricht, p-chloornitrobenzeen onder de loep. De commissie heeft haar oordeel gegoten in door de Europese Unie aangegeven termen.

De commissie concludeert dat p-chloornitrobenzeen onvoldoende is onderzocht. De commissie adviseert daarom p-chloornitrobenzeen niet te classificeren.

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the carcinogenic properties of substances at the workplace and proposes a classification with reference to the EU-directive. This evaluation is performed by the Dutch Expert Committee on Occupational Standards. The present report contains an evaluation by the committee on the carcinogenicity of p-chloronitrobenzene.

The committee concludes that p-chloronitrobenzene has been insufficiently investigated. Therefore the committee is of the opinion that p-chloronitrobenzene cannot be classified.

Chapter **1**

Scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. The Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to study the carcinogenic properties of substances and to propose a classification with reference to an EU-directive (annex F). This task is carried out by the Council's Dutch Expert Committee on Occupational Standards, hereafter called the committee.

The evaluation of the carcinogenicity of a substance is, if possible, based on IARC* evaluations. The original publications are not reviewed and evaluated in the text of the report, but the overall conclusion of the IARC on the carcinogenic properties is included (annex D).

In addition to classifying substances with respect to their possible carcinogenicity according to the EU Guidelines, the committee also assesses the genotoxic properties of the substances in question. The committee expresses its conclusions in the form of standard sentences (annex E).

* International Agency for Research on Cancer

1.2 Committee and procedures

The present report contains evaluations by the committee of the carcinogenicity of p-chloronitrobenzene. The members of the committee are listed in annex B. The first draft of this report was prepared by MI Willems, from the TNO Nutrition and Food Research in Zeist, by contract with the Ministry of Social Affairs and Employment.

In 2000 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation of the carcinogenicity of p-chloronitrobenzene has been based on an IARC evaluation (IARC96). Where relevant, the original publications were reviewed and evaluated as shown in the text.

In addition, literature has been retrieved from the online data bases Cancerlit, CA Search, Toxline, and Medline, covering the period 1994 to May 2001.

Chapter 2

p-Chloronitrobenzene

2.1 Introduction*

Name	:	p-chloronitrobenzene (p-CNB)
CAS no	:	100-00-5
CAS name	:	1-chloro-4-nitrobenzene
IUPAC name	:	1-chloro-4-nitrobenzene
EINECS no	:	202-809-6
EEC no	:	610-005-00-5
Description	:	yellowgreen crystals or powder with a sweet odour
Occurrence	:	not known to occur naturally
Use	:	as an intermediate in the manufacture of dyes, rubber, and agricultural chemicals
Chem formula	:	$C_6H_4ClNO_2$
Chem structure	:	

* Data from IARC96, Stu96

Molecular weight	:	157.6
Boiling point	:	242 °C
Melting point	:	83.6 °C
Relative density	:	1.520
Vapour pressure (30 °C)	:	0.02 kPa
Relative vapour pressure (air=1)	:	5.44
Relative density of saturated vapour/air mixture (air=1; 20 °C)	:	1.03
Solubility in water (20 °C)	:	slightly soluble (249 mg/L)
in organic solvents	:	soluble in acetone, boiling ethanol, diethyl ether, carbon disulphide; sparingly soluble in cold ethanol
Partition coefficient Log P_{ow}	:	2.4
Conversion factors (20 °C, 101.3 kPa)	:	1 ppm = 6.57 mg/m^3 1 mg/m^3 = 0.15 ppm
EC classification	:	T: toxic R23/24/25: toxic by inhalation, in contact with skin, and if swallowed R33: danger of cumulative effects

2.2 IARC conclusion

In 1996, IARC concluded that there was inadequate evidence for the carcinogenicity of p-chloronitrobenzene in humans as well as in experimental animals. p-Chloronitrobenzene was not classifiable as to its carcinogenicity to humans (Group 3).

2.3 Human data

2.3.1 IARC data

No human data were presented by IARC (IARC96).

2.3.2 Additional data

No additional data were found.

2.4 Animal data

2.4.1 IARC data

No increased tumour incidences were reported in male rats fed diets containing 0, 2 or 4 g/kg feed of p-chloronitrobenzene (p-CNB) for three months, followed by diets containing 0, 0.25 or 0.50 g/kg for two months and 0, 0.5 or 1.0 g/kg for the last thirteen months. The animals were kept on a control diet for another six months prior to sacrifice. Information on survival or body weight gain was not reported. The IARC Working Group pointed to the small number of animals (25/group), the short duration of dosing, and the limited histopathological evaluation and reporting, and considered the study to be inadequate for evaluation (IARC96).

In mice, fed diets containing 0, 3 or 6 g/kg feed of p-CNB for eighteen months, there was an increased incidence of hepatocellular carcinomas in low-dose male animals (controls: 1/14; pooled controls: 7/99; low dose: 4/14, $p<0.025$ *versus* pooled control incidence; high dose: 0/14) and an increased incidence in vascular tumours in high dose male (controls: 0/14; pooled controls: 5/99; low dose: 2/14; high dose: 4/14, $p<0.025$ *versus* pooled controls) and female mice (controls: 0/15; pooled controls: 9/102; low dose: 2/20; high dose: 7/18, $p<0.025$ *versus* concurrent and pooled controls). Information on survival or body weight gain was not reported. As for the rat study, the IARC Working Group pointed to the small number of groups (25/sex/group) and the limited histopathological evaluation and reporting, and considered the study to be inadequate for evaluation (IARC96).

2.4.2 Additional data

No additional data were found.

2.5 Mutagenicity and genotoxicity

2.5.1 IARC data

According to the data presented by IARC, p-CNB induced reverse mutations in *Salmonella typhimurium* when tested with and without metabolic activation, but no DNA damage in *Escherichia coli*. It was negative in sex-linked recessive lethal mutations assays in *Drosophila melanogaster* when fed or injected to adults or fed to larvae. p-CNB caused chromosome aberrations, at (severely) cytotoxic doses, and sister chromatid exchanges in Chinese hamster ovary cells. In non-proliferating cultured

rat hepatocytes, it induced DNA single strand breaks which were almost completely repaired within 24 hours at a concentration of 5 mM, but for only about 50% within 48 hours at a concentration of 50 mM (with most repair during the second 24 hours).

In vivo, intraperitoneal injection resulted in DNA single-strand breaks in the liver, the kidneys, and the brain of mice.

2.5.2 Additional information

p-CNB did not induce unscheduled DNA synthesis (UDS) in cultured primary rat hepatocytes (Nai84, Ste85).

p-CNB showed positive responses in the L5178Y $TK^{+/-}$ mouse lymphoma assay both with and without adding induced rat liver homogenates (concentrations tested: + S9: 42-350 µg/mL; - S9: 25-600 µg/mL) (Mit83).

In two separate experiments, p-CNB did not induce mutations in the HGPRT assay in Chinese hamster ovary cells tested with and without induced rat liver S9 at concentrations of 100-400 µg/mL (+ S9) and 100-900 µg/mL (-S9) and of 1.59 to 2.38 mM (≈250-375 µg/mL), respectively (God83, Smi80).

No increase was found in the percentage of aberrant cells, that is the number of aberrant cells/number of metaphase cells scored (gaps were not regarded as being aberrant) when tested at doses of 0.05-1.0 mmol/L (≈8-158 µg/mL) in cultured human peripheral lymphocytes (Hua95). (Note: no data on cytotoxicity were presented; the concentrations applied may have been too small to evoke a positive response.)

In vivo, no increase in the frequency of chromosomal breaks or aberrations were found in bone marrow cells of male and female rats given a single oral dose by gavage of 30, 100, or 300 mg/kg bw (harvest times: 6, 12, and 24 h). There was no effect on the mitotic index of the treated animals compared to control values.

Signs of toxicity (cyanosis, epistaxis) were seen in the animals of the mid and high dose groups, sacrificed after twelve and 24 hours (Lov85).

2.6 Evaluation

p-CNB has been tested for its carcinogenicity by dietary administration to mice and rats. The committee considers both studies inadequate for carcinogenic evaluation, because of the limited study designs.

In vitro mutagenicity and genotoxicity tests showed contradictory results. p-CNB induced mutations, but no DNA damage in bacteria. In rat hepatocytes, no induction of UDS occurred, but DNA single strand breaks were seen. It caused mutations in mouse lymphoma cells, but not in Chinese hamster ovary cells. It did not induce chromosomal aberrations in human peripheral lymphocytes, when tested at relatively low

concentrations, but in Chinese hamster ovary cells, it induced chromosomal aberrations at cytotoxic concentrations, and sister chromatid exchanges.

In vivo, it was negative in a bone marrow cytogenetics test following oral administration to rats. Intraperitoneal injection resulted in DNA single strand breaks in the liver, kidneys and brain of mice.

2.7 Recommendation for classification

p-Chloronitrobenzene is insufficiently investigated. Therefore the committee recommends that the compound should not be classified.

References

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Nai84 Naismith RW (study director). Rat hepatocyte primary culture/DNA repair test. Waverly PA, USA: Pharmacon Res Intl, Inc, 1984 (report submitted by Monsanto Co, St Louis MO, USA to USEPA; report available from NTIS, Springfield VA, USA; order no OTS0557077).

Smi80 Smith C (rapporteur). Mutagenic activity in the Chinese hamster ovary assay of 1-chloro-4-nitrobenzene with cover letter outlining current study of tetrahydrofuran toxicity dated 05/10/94 (sanitized). Newark DE, USA: EI du Pont de Nemours & Co: Haskell Lab for Toxicol and Ind Med, 1980 (submitted to USEPA; report available from NTIS, Springfield VA, USA; order no OTS0557127).

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Stu96 Studiegroep Chemiekaarten, eds. p-Chloornitrobenzeen. In: Chemiekaarten: gegevens voor het veilig werken met chemicaliën. 11th ed. Alphen a/d Rijn, The Netherlands: Samson HD Tjeenk Willink bv, 1996: 248.

A Request for advice

B The committee

C Comments on the public review draft

D IARC Monograph

E Classification of substances with respect to carcinogenicity

F Guideline 93/21/EEG of the European Union

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the

case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committee

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- P Boogaard
  toxicologist; Shell International Petroleum Company, The Hague
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  epidemiologist; Utrecht University, Utrecht
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- TM Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam
- IM Rietjens
  professor of toxicology; Wageningen University, Wageningen
- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague

- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- RA Woutersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The first draft of the present advisory report was prepared by MI Willems, from the Department of Occupational Toxicology of the TNO Nutritionand Food Research, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance was provided by mrs A van der Klugt.
Lay-out: J van Kan.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2000 for public review. The following organisations and persons have commented on the draft document:

- Dr. U Reuter, Deutsche Forschungsgemeinschaft, Germany

Annex **D**

IARC Monograph

See next pages.

Annex **E**

Classification of substances with respect to carcinogenicity

See next page.

The committee expresses its conclusions in the form of standard phrases:

Judgement of the committee	Comparable with EU class
This compound is known to be carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	1
This compound should be regarded as carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	2
This compound is a suspected human carcinogen.	3
This compound has been extensively investigated. Although there is insufficient evidence of a carcinogenic effect to warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is cause for concern.	(A)
This compound has been insufficiently investigated. While the available data do not warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is a cause for concern.	(B)
This compound cannot be classified	not classifiable

Annex **F**

Guideline 93/21/EEG of the European Union

4.2 Criteria for classification, indication of danger, choice of risk phrases

4.2.1 Carcinogenic substances

For the purpose of classification and labelling, and having regard to the current state of knowledge, such substances are divided into three categories:

Category 1:

Substances known to be carcinogenic to man.

There is sufficient evidence to establish a causal association between human exposure to a substance and the development of cancer.

Category 2:

Substances which should be regarded as if they are carcinogenic to man.

There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer, generally on the basis of:

- appropriate long-term animal studies
- other relevant information.

Category 3:

Substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

4.2.1.1 The following symbols and specific risk phrases apply:

Category 1 and 2:

T; R45 May cause cancer

However for substances and preparations which present a carcinogenic risk only when inhaled, for example, as dust, vapour or fumes, (other routes of exposure e.g. by swallowing or in contact with skin do not present any carcinogenic risk), the following symbol and specific risk phrase should be used:

T; R49 May cause cancer by inhalation

Category 3:

Xn; R40 Limited evidence of a carcinogenic effect

4.2.1.2 Comments regarding the categorisation of carcinogenic substances

The placing of a substance into Category 1 is done on the basis of epidemiological data; placing into Categories 2 and 3 is based primarily on animal experiments.

For classification as a Category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species; together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Category 3 actually comprises 2 sub-categories:

a substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.

b substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high levels exceeding the 'maximal tolerated dose'. The maximal tolerated dose is characterized by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain;
- appearance of tumours, especially at high dose levels, only in particular organs of certain species is known to be susceptible to a high spontaneous tumour formation;
- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds); if the particular target is not relevant to man;
- lack of genotoxicity in short-term tests *in vivo* and *in vitro*;
- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation;
- existence of a species - specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.

For a distinction between Category 3 and no classification arguments are relevant which exclude a concern for man:

- a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man;
- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories;
- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.