p-Nitroaniline

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *p-Nitroaniline*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-5134/JR/pg/246-G12
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Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van p-nitroaniline. Het maakt deel uit van een uitgebreide reeks waarin kankerverwekkende stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld.

Het advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. Het advies is voorgelegd aan de Commissie GBBS en vervolgens getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

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

Hoogachtend,

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p-Nitroaniline

Evaluation of the carcinogenicity and genotoxicity

Subcommittee on the classification of carcinogenic substances of the
Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2008/08OSH, The Hague, April 1, 2008

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Contents

Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens het uitoefenen van hun beroep kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie p-nitroaniline onder de loep. p-Nitroaniline wordt gebruikt als intermediair bij de productie van verschillende stoffen, zoals antioxidanten en kleurstoffen.

Op basis van de beschikbare gegevens leidt de commissie af dat p-nitroaniline onvoldoende is onderzocht. Hoewel de gegevens het niet toelaten de stof te classificeren als kankerverwekkend voor de mens of als moet beschouwd worden als kankerverwekkend voor de mens, is waakzaamheid is geboden. De commissie adviseert daarom p-nitroaniline te classificeren als *verdacht kankerverwekkend voor de mens*. Dit is vergelijkbaar met een classificatie in categorie 3 volgens de richtlijnen van de Europese Unie. Binnen deze categorie komt de situatie het meest overeen met subcategorie b.

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the 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 Standards of the Health Council, hereafter called the committee. In this report, the committee evaluated p-nitroaniline. p-Nitroaniline is used as an intermediate in the production of different substances, including antioxidants and dyes.

Based on the available information, the committee is of the opinion that p-nitroaniline has been insufficiently investigated. While the available data do not warrant a classification as carcinogenic to humans or as should be regarded as carcinogenic to humans, they indicate that there is cause for concern for man. Therefore, the committee recommends classifying p-nitroaniline as *a suspected human carcinogen*. This recommendation is comparable to the EU classification in category 3. The situation is, furthermore, comparable with subcategory b of this category.

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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. 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 (see Annex A). The assessment and the proposal for a classification are expressed in the form of standard sentences (see Annex E). The criteria used for classification are partly based on an EU-directive (see Annex F). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question.

This report contains the evaluation of the carcinogenicity of p-nitroaniline.

1.2 Committee and procedures

The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. The members of the committee are listed in Annex B. The first draft was prepared by I.A. van de Gevel and M.I. Willems, from the Department of Occupational Toxicology of the TNO Nutrition

and Food Research, by contract with the Ministry of Social Affairs and Employment.

In 2007 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 and recommendation of the committee is standardly based on scientific data, which are publicly available. The starting points of the committees' reports are, 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 most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of p-nitroaniline, such an IARC-monograph is not available.

More recently published data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in June 2007. The new relevant data were included in this report.

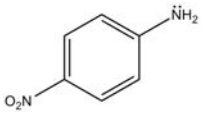
Chapter 2

General information

2.1 Identity and physico-chemical properties

p-Nitroaniline is used as an intermediate in the production of antioxidants, antiozonants, gasoline additives, and various dyes and pigments.[1,2]

Below is given the identity and some of its physical and chemical properties.[1,3,4]

Chemical name	:	1-amino-4-nitrobenzene
CAS registry no.	:	100-01-6
EINECS no.	:	202-810-1
Synonyms	:	p-nitroaniline; 4-nitroaniline; azoic diazo component 37 (C.I. 37035); developer 17; Fast Red GG Base; benzeneamine, 4-nitro
Description	:	bright yellow powder
Molecular formula	:	$C_6H_6N_2O_2$
Molecular structure	:	
Molecular weight	:	138.12
Boiling point	:	332°C
Melting point	:	148°C
Vapour pressure	:	0.00047 Pa at 25°C
Vapour density	:	4.8 (air = 1)
Solubility	:	0.8 g/L at 18.5°C (in water)

Octanol/water partition coefficient	:	2.66 (log Pow)
Stability and reactivity	:	may explode on heating. On combustion, forms toxic fumes of nitrogen oxides. The substance is a strong oxidant and reacts with combustible and reducing materials. Reacts with organic materials in presence of moisture causing fire hazard.
EU classification	:	R23/24/25: Toxic by inhalation, in contact with skin and if swallowed. R33: Danger of cumulative effects. R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. (Based on the Annex I entry 612-012-00-9, the p-nitroaniline, m-nitroaniline and o-nitroaniline)

2.2 IARC classification

IARC did not evaluate p-nitroaniline.

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

No data were available to evaluate the carcinogenicity of p-nitroaniline in humans.

3.2 Carcinogenicity studies in animals

The National Toxicology Program (NTP) published a long-term animal study on the carcinogenic effects of p-nitroaniline.[2] Groups of 70 male and 70 female $B6C3F_1$ mice were given p-nitroaniline by gavage at doses of 0, 3, 30 or 100 mg/kg bw, daily for five days a week for up to 103 weeks. In addition, ten animals per group were designated for interim evaluations at 9 and 15 months.

Survival and body weights in all dosed groups were comparable to controls. During the study, haematological effects were observed in mice given 30 or 100 mg p-nitroaniline/kg bw. These effects included: increased methaemoglobin concentration and reticulocytosis; a slight regenerative anaemia at 100 mg/kg bw only; extramedulary haematopoiesis and haemosiderin containing macrophages in the spleen; and, the presence of haemosiderin containing Kupffer cells in the liver.

Regarding tumour development, no treatment-related development of tumours was found, except for haemangiomas or haemangiosarcomas in male mice (see Table 3.1). In summary, significant increases in hepatic haemangiosar-

comas were found in male animals given 100 mg p-nitroaniline/kg bw. Furthermore, in this group, an increase in haemangiomas or haemangiosarcomas (combined) at other sites of the body were observed, with a significant positive trend; according to NTP, the incidence of these neoplasms exceeded the range for NTP historical control groups of male mice, but the incidence were not statistically significant greater than controls by pair-wise comparisons. In female mice, the incidence of haemangioma or haemangiosarcoma (combined) at all sites was slightly increased, but did not reach significance.

The investigators of the NTP study remarked that an increase in vascular tumours of the liver in male mice is seen with several other substances inducing haemolytic anaemia. Therefore, they assume that the vascular tumours observed in this study are related to p-nitroaniline exposure. Finally, it is unknown why female mice are less susceptible for p-nitroaniline exposure than male mice, but the difference might be hormone-related.[5]

Nair *et al*. (1990) studied the toxicity of p-nitroaniline in Sprague-Dawley rats.[6] Groups of 60 male and 60 female rats were daily fed (by gavage) p-nitroaniline at doses of 0, 0.25, 1.5 or 9.0 mg/kg bw, for 7 days a week for 24 months.

No clear decrease in survival was observed in any of the treated groups compared to controls. However, the average body weight was increased in the highest dosed female group, but not in other groups, nor in male groups. Regarding non-carcinogenic effects, in the high dosed groups, heamatological effects were observed, such as increased methaemoglobin levels, haemoglobin levels, red blood cell counts, and haematocrit. Furthermore, an indication of reticulocytosis was seen at the highest dose level but no increase in extramedulary haematopoiesis. In addition, dose related increases in iron pigmentation were seen in the liver sinusoidal macrophages and the reticuloendothelial cells of the spleen.

Table 3.1 p-Nitroaniline induced tumour development in B6C3F$_1$ mice.[2]

	matched controls	historic controls	exposure (mg/kg bw)		
			3	30	100
males					
hepatic haemangiosarcoma	0/50	2.1% (0-6%)	1/50	2/50	4/50 (p=0.06, Fisher)
all organ haemangioma or haemangiosarcoma	5/50	6.6% (0-12%)	3/50	4/50	10/50 (p=0.137, Fisher)
females					
all organ haemangioma or haemangiosarcoma	1/52	3.0% (0-12%)	3/50	3/51	4/51

The investigators did not find increased incidences of tumour-bearing animals, nor were there any increase in certain types of tumours observed. The committee noted that the dose applied to the animals was very low (below the MTD). This could explain the absence of vascular tumours in the liver or spleen of rats in this study. It might also explain the marginal increase in reticulocytes, and lack of increase in extramedulary haematopoiesis, compared to other methaemoglobin inducers. Therefore, the committee is of the opinion that this study is of limited value for cancer risk assessment.

Fifty male and fifty female mice were given p-nitroaniline by gavage at doses of 0, 2, 21 or 70 mg/kg bw for 2 years. Tumour incidences of treated animals did not differ from controls.[7] No further details on study design and examinations were given.

3.3 Additional information

p-Nitroaniline presents a class of single aromatic agents bearing a nitro and amino group, several of which are known carcinogens. Some of the aniline agents were, therefore, investigated on carcinogenicity as well.[2] Only *p*-chloroaniline and *o*-toluidine hydrochloride caused marginal increases in heamangiosarcomas in the liver and spleen of mice. Other type of tumours found after exposure to aniline-like agents included splenic sarcomas in rats (absent in mice).[2]

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

The outcomes of the individual *in vitro* assays are summarized in Annex D.

Overall, bacterial mutagenicity assays showed that p-nitroaniline caused frame shift mutations, in the presence and in the absence of an exogenous metabolic system. However, other mutagenicity assays were negative.

In mammalian cells and in the presence and absence of a metabolic system, p-nitroaniline also induced chromosomal aberrations, although one negative outcome was reported as well. In one assay, the agent induced sister chromatid exchanges in the presence of an activation system, but negative or equivocal outcomes were found by other investigators using the same assay.

4.2 *In vivo* assays

In the sex-linked recessive lethal mutation assay, using *Drosophila melanogaster* flies, no increased frequency of mutations in male germ cells was detected when p-nitroaniline was administered by feed (up to 5,000 ppm; larvae up to 100 ppm), or injections (up to 1,000 ppm).[2,8,9]

In addition, p-nitroaniline did not increase the frequency of unscheduled DNA synthesis in liver cells of male F344 rats.[10] The committee noted the limited reporting.

Regarding clastogenic effects, no increased frequency of micronuclei in bone marrow cells of treated CD-1 mice were found.[11] In this study, groups of five to six male and female animals received intraperitoneal injections of 0, 80, 400 or 800 mg p-nitroaniline/kg bw, two times on two consecutive days. Twenty-four hours after the last injection, and forty-eight hours for control and highest dosed animals, bone marrow cells were harvested and prepared for analysis. One of the animals of the highest dose group died during the study. Furthermore, in all treated animals clinical signs of toxicity were observed.

Topham studied sperm-head abnormalities using (CBAxBALB/c)F_1 mice, which are known to be sensitive for these kind of abnormalities.[12] Groups of five mice received intraperitoneal injections of p-nitroaniline at doses of 25 up to 500 mg/kg bw (dissolved in 0.5% Tween 80 in water), five times on five consecutive days. Five weeks after the last injection, the mice were killed and sperm was collected from the cauda epididymis.

In treated animals, exposure did not increase the incidence of sperm-head abnormities compared to control animals. The investigators also reported on lethality in the highest dose group.

4.3 Additional information

The carcinogenic mechanism through which p-nitroaniline may induce vascular tumours is not completely understood yet. Below is given the state of the art.

p-Nitroaniline is a methaemoglobin inducing agent.[1,2] Similar agents, such as aniline and substituted aniline compounds show comparable responses as those observed by p-nitroaniline. These responses are caused by reaction of the agent or its metabolites with haemoglobin, resulting in the net accumulation of methaemoglobin (an oxidized form ferrohaemoglobin).

This process may also lead to local or systemic iron overload in the body. In addition, it is hypothesized that excess of iron may promote the production of reactive oxygen species (which ultimately may lead to oxidative damage to DNA), suppression of the tumoricidal functioning of macrophages, and/or immunosuppression.[5] Related to this hypothesis are numerous reports, which suggest an association between iron overload and higher risk of cancer.[5] In case of the NTP carcinogenicity study, it is possible that there was question of iron overload, since in high-dosed male mice an increased incidence of Kupffer cell (liver macrophages) pigmentation (haemosiderosis) was observed, which is associated with iron overload.[2,5]

However, at the moment it is unclear whether there exists an association between iron overload, the presence of haemosiderosis and haemangiosarcomas, because data from other animal carcinogenicity studies indicate that certain other hemolytic agents, such as o-nitroaniline, caused increases in Kupffer cell pigmentation, but no increases in incidence of haemangiosarcomas, whereas for other agents it was the other way round.[5] No other data on the carcinogenic mechanism of p-nitroaniline is available. Therefore, it is still unclear what carcinogenic mechanism may have caused haemangiosarcomas in male mice of the NTP study.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the carcinogenicity of p-nitroaniline in humans were available.

In male mice, p-nitroaniline induced small increases in hepatic haemangiosarcomas. It furthermore increased haemangiomas and haemangiosarcomes elsewhere in the body. However, female mice showed to be less susceptible for developing p-nitroaniline-induced tumours as seen in male mice. Another carcinogenicity study on rats was of limited value for cancer risk assessment, due to low exposure levels. Overall, the available data on the carcinogenicity of p-nitroaniline is limited, but the committee is concerned about the findings in male mice.

Overall, p-nitroaniline caused mutations and clastogenic effects in some *in vitro* assays, but in other *in vitro* assays the outcomes were negative. No genotoxicity was found in *in vivo* assays. It is furthermore unclear what carcinogenic mechanism may have caused tumours as found in male mice.

5.2 Recommendation for classification

Based on the available information, the committee is of the opinion that p-nitroaniline has been insufficiently investigated. While the available data do not warrant a classification as carcinogenic to humans or as should be regarded as carcinogenic to humans, they indicate that there is cause for concern for man.

Therefore, the committee recommends classifying p-nitroaniline as *a suspected human carcinogen*. This recommendation is comparable to the EU classification in category 3. The situation is, furthermore, comparable with subcategory b of this category.

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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.

Annex **B**

The committee

- G.J. Mulder, *chairman*
  emeritus professor of toxicology, Leiden University, Leiden
- P.J. Boogaard
  toxicologist, SHELL International BV, The Hague
- Ms. M.J.M. Nivard
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  toxicologic pathologist, TNO Quality of Life, Zeist
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  professor of molecular radiation dosimetry and radiation mutagenesis, University Medical Center, Leiden
- E.J.J. van Zoelen
  professor of cell biology, Radboud University Nijmegen, Nijmegen
- J.M. Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

The committee consulted an additional expert, Prof. dr. G. Mohn, working at Department of Radiation Genetics and Chemical Mutagenesis of the University of Leiden, with respect to the genotoxic data.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2007 for public review. The following organisations and persons have commented on the draft document:

- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain;
- R.D. Zumwalde, National Institute for Occupational Safety and Health, the USA.

Annex **D**

In vitro genotoxicity data

Test system	Exposure conditions	Result	Reference
Gene mutations			
Salmonella typhimurium strains:		with and without exogenous metabolic activation	
TA97	up to 6,666 μg/plate	- (without activation) - (with activation)	2,13-17
TA98	up to 6,666 μg/plate	+ (without activation) + (with activation)	2,15,18-21
	up to 1,000 μg/plate	- (without activation) - (with activation)	22
	up to 1,000 μg/plate	- (without activation) + (with activation)	12,23-25
TA98 FMN	0.1 – 10 μmol/plate	+ (with activation)	15
TA98 NR	up to 3,000 μg/plate	- (without activation) - (up to 50 μg/plate, with activation) + (with activation)	14,18
TA100	up to 6,666 μg/plate	- (without activation) - (with activation)	2,13,16-23,26
TA100 FMN	up to 10 μmol/plate	+ (with activation)	15
TA100 NR	up to 3,000 μg/plate	- (without activation) - (with activation)	18,26
TA1535	up to 6,666 μg/plate	- (without activation - (with activation)	2,16,19-22
TA1535	no data available	- (with activation, aroclor) + (with activation, phenobarbitone)	24,25

TA1537	up to 6,666 µg/plate	- (without activation) - (with activation)	2,16,19,21,22,26
TA1538	up to 6,666 µg/plate	- (without activation) - (with activation)	14,22
TA1538	up to 1,000 µg/plate	- (without activation) + (with activation)	26,27
TA1538	up to 10 µg/plate	+ (without activation)	21
TA1538 NR	up to 50 µg/plate	- (without activation) - (with activation)	14
G46	up to 1,000 µg/plate	- (without activation) - (with activation)	26
C3076	up to 1,000 µg/plate	- (without activation) - (with activation)	26
D3052	up to 1,000 µg/plate	- (without activation) + (with activation)	26
Photobacterium leiognathi SD 18 (forward mutations)	up to 100 µg/plate	+ (no data on metabolic activation)	28,29
Photobacterium phophoreum (forward mutations)	no data available	+ (no data on metabolic activation)	30
Escherichia coli WPuvrA	up to 1,000 µg/plate	- (without activation) - (with activation)	20,26,31
Bacillus subtilus	up to 10 mg/plate	+ (without activation)	21
L5178Y TK+/- mouse lymphoma cells	up to 1,000 µg/mL	+ (without activation) - and + (with activation)	2,32
Chinese hamster ovary cells; HGPRT mutations	up to 800 µg/mL	- (without activation) - (with activation)	33
Chromosomal abberations			
Chinese hamster ovary cells	up to 1,600 µg/mL	- and + (without exogenic metabolic activations)	2,18,34
Chinese hamster ovary cells	up to 5,000 µg/mL	+ (with metabolic activation)	2,34
human peripheral lymphocytes	up to 0.10 mmol/L	+ (without activation)	35
Sister chromatid exchanges			
Chinese hamster ovary cells	up to 200 µg/mL	- and equivocal (without metabolic activation)	2,34
Chinese hamster ovary cells	up to 5,000 µg/mL	- and + (with metabolic activation)	2,34
Unscheduled DNA synthesis			
primary rat liver cells	up to 1,000 nmol/mL (µg/well)	- and equivocal	26,36

Annex **E**

Carcinogenic classification of substances by the committee

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

Judgment of the committee	*Comparable with EU class*
This compound is known to be carcinogenic to humans • It is stochastic or non-stochastic 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 stochastic or non-stochastic 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. • This compound has been extensively investigated. Although there is insufficient evidence for 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. • 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.	3 (A) (B)
This compound cannot be classified • There is a lack of carcinogenicity and genotoxicity data. • Its carcinogenicity is extensively investigated. The data indicate sufficient evidence suggesting lack of carcinogenicity.	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 Possible risk of irreversible effects

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.