2-Nitroanisole

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *2-Nitroanisole*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-5135/JR/pg/246-H12
Bijlagen : 1
Datum : 1 april 2008

Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van 2-nitroanisole. 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,

prof. dr. J.A. Knottnerus

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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. 2008/02OSH, The Hague, April 1, 2008

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 22, 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 & Food Quality. 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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INAHTA

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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 2-nitroanisol onder de loep. 2-Nitroanisol wordt gebruikt bij de synthese van kleurstoffen en als intermediair bij productie van geneesmiddelen.

Op basis van de beschikbare gegevens leidt de commissie af dat 2-nitroanisol *beschouwd moet worden als kankerverwekkend voor de mens*. Dit is vergelijkbaar met een classificatie in categorie 2 volgens de richtlijnen van de Europese Unie. De commissie is verder van mening dat 2-nitroanisol een stochastisch genotoxisch werkingsmechanisme heeft.

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 2-nitroanisole. 2-Nitroanisole is used in the synthesis of azo dyes, and as an intermediate for the preparation of pharmaceuticals.

Based on the available information, the committee is of the opinion that 2-nitroanisole *should be considered as carcinogenic to humans*. This recommendation is comparable to the EU classification in category 2. The committee is furthermore of the opinion that 2-nitroanisole acts by a stochastic genotoxic mechanism.

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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 2-nitroanisole.

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 2-nitroanisole, such an IARC-monograph is available, of which the summary and conclusion of IARC is inserted in Annex D.

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

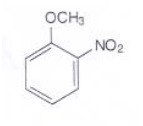
General information

2.1 Identity and physico-chemical properties

2-Nitroanisole is primarily used as a precursor of *o(rtho)*-anisidine by nitro-reduction.[1,2] As such, it is used for the synthesis of azo dyes, and as an intermediate for the preparation of pharmaceuticals.[1,2] Occupational exposure may occur during manufacturing of azo dyes and pharmaceuticals.

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

Chemical name	:	2-nitroanisole
CAS registry number	:	91-23-6
EEC number	:	609-047-00-7
EINECS number	:	202-052-1
IUPAC name	:	ortho-Nitroanisole
Synonyms	:	2-Methoxynitrobenzene; 2-methoxy-1-nitrobenzene; *ortho*-nitroanisole; *ortho*-nitrobenzene methyl ether; 2-nitromethoxy-benzene; *ortho*-nitromethoxybenzene; 1-nitro-2-methoxybenzene; *ortho*-nitrophenyl methyl ether
Description	:	colourless to yellowish liquid
Application	:	2-Nitroanisole is reduced (using a H2-catalyst or iron-formic acid) to ortho-anisidine or to ortho-dianisidine, both of which are important as dye intermediates. 2-Nitroanisole has also been used as an intermediate for various pharmaceuticals (IARC96)
Molecular formula	:	$C_7H_7NO_3$

Structure	:	
Molecular weight	:	153.13
Boiling point	:	277 °C
Melting point	:	10.5 °C
Relative density (air = 1)	:	1.254 at 20 °C/4 °C
Vapour pressure	:	4 Pa at 30 °C
Solubility	:	moderately soluble in warm water (1.69 g/L at 30 °C); soluble in ethanol and diethyl ether
Stability	:	Explosive reaction with sodium hydroxide and zinc
Log Pow	:	1.73
Conversion factors (20°C)	:	1 ppm = 6.35 mg/m³ air 1 mg/m³ = 0.15 ppm
Risk and safety phrases	:	R22: harmful if swallowed R45: may cause cancer S45: in case of accident or if you feel unwell, seek medical advice immediately (show label where possible) S53: avoid exposure – obtain special instructions before use
EC classification	:	Carcinogenic category 2

2.2 IARC classification

In 1996, IARC concluded that there is *inadequate evidence* in humans for the carcinogenicity of 2-nitroanisole, but that there is *sufficient evidence* in experimental animals.[1] Therefore, it classified the agent in group 2B, indicating that it is *possibly carcinogenic to humans*.

Chapter 3

Carcinogenicity

3.1 Observations in humans

No data were available to evaluate the carcinogenicity of 2-nitroanisole in humans.

3.2 Carcinogenicity studies in animals

Groups of 60 male and 60 female F344 rats were fed 2-nitroanisole-enriched diets for 2 years.[2,3] The diets contained concentrations of 2-nitroanisole of 0, 222, 666, or 2,000 ppm (mg/kg diet). Feed and water were freely available. Ten rats per group were killed at week 65 for interim evaluation. In males receiving 2,000 ppm in the diet, the survival rate and the mean body weights were lower than controls; for all other groups these were comparable with controls. No increases in incidences of neoplasms were observed in exposed animals. However, 2-nitroanisole was associated with nonneoplastic lesions of the kidneys and forestomach.

In a second experiment by the same research institute, groups of 60 male and 60 female F344 rats were given 2-nitroanisole-enriched diets for only 27 weeks, after which they received control diets for up to an additional 76 weeks (total study duration, 2 years).[2,3] The diets contained concentrations of 2-nitroanisole of 0, 6,000, or 18,000 ppm (mg/kg diet). During the experiment four interim evaluations were performed, leaving 20 animals per group per sex alive for the total

duration of the experiment. Survival rates, mean body weight and food consumption of exposed animals were significantly reduced compared to controls. Furthermore, increased incidences of neoplasms in the urinary bladder, kidneys, forestomach, and large intestines were found in animals fed 6,000 and 18,000 ppm 2-nitroanisole. Details on the type of lesions are shown in Table 3.1 on the next page.

The same research group used also male and female $B6C3F_1$ mice under the same experimental conditions as in the first two-year rat study.[2,3] The mice were given 2-nitroanisole in the diet at doses of 0, 666, 2,000, or 6,000 ppm. No treatment related changes in survival were observed. However, the body weights of animals receiving 2,000 and 6,000 ppm were significantly reduced compared to controls. Significantly increased incidences of nonneoplastic lesions of the liver were found in all exposed groups compared to controls. The only neoplastic lesions were found in the liver of male mice. It concerned hepatocellular adenomas and carcinomas in males that received 2,000 or 6,000 ppm in their diet (control, 21/50; 666 ppm, 33/50; 2,000 ppm, 46/50; 6,000 ppm, 34/50; adenomas, carcinomas and hepatoblastomas taken together). In female mice, no neoplastic lesions were observed.

No animal data were available on inhalation exposure or other routes of exposure.

Table 3.1 Neoplastic lesions in F344 rats, which were fed 2-nitroanisole enriched diets for 27 weeks. Animals were examined microscopically after an additional 76 weeks.[2,3]

Dose administered	0 ppm	6,000 ppm	12,000 ppm
Males			
Urinary bladder			
No. of animals examined	21	27	34
Transitional epithelium carcinoma	0	23**	33**
Transitional epithelium papilloma	0	3	1
Squamous cell carcinoma	0	0	5
Squamous cell papilloma	0	0	4
Sarcoma[a]	0	1	7**
The kidneys			
No. of animals examined	21	27	34
Transitional cell papilloma	0	0	3
Transitional cell carcinoma	0	1	6*
Large intestine			
No. of animals examined	21	27	34
Adenomatous polyp	0	21**	24**
Carcinoma	0	0	4

Table 3.1 Continued.

Dose administered	0 ppm	6,000 ppm	12,000 ppm
Females			
Urinary bladder			
No. of animals examined	20	20	34
Transitional epithelium carcinoma	0	18**	32**
Transitional epithelium papilloma	0	1	1
Squamous cell carcinoma	0	0	1
Squamous cell papilloma	0	0	4
Sarcoma	0	2	12**
The kidneys			
No. of animals examined	22	20	34
Transitional cell papilloma	0	0	1
Transitional cell carcinoma	0	0	1
Large intestine			
No. of animals examined	22	20	34
Adenomatous polyp	0	5*	17**
Carcinoma	0	0	2

[a] Includes a leiomyosarcoma in one 6,000 ppm and two 18,000 females, and a fibrosarcoma in one 18,000 ppm female.

* $p < 0.05$ versus controls; ** $p < 0.01$ versus controls.

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

2-Nitroanisole was tested in the *Salmonella* mutagenicity bioassay.[1,4,5] Variable responses were found depending on the type of strain used and doses applied. Overall, positive outcomes were observed using TA100 (with and without an exogenous metabolic system), and in some but not all tests using TA1535, TA1538, and TA98. Furthermore, 2-nitroanisole induced mutations in *Bacillus subtilis rec* H97 and M45 strains, in the absence of a metabolic system.[1,4] Mutations at the TK locus were also found of mouse lymphoma L1578Y cells.[1,4]

Using human hepatic cytosolic samples, the ^{32}P-post-labeling technique, and ^{3}H-labeled 2-nitroanisole, Stiborová *et al.* (2004) showed that 2-nitroanisole was activated to form DNA-adducts *in vitro*.[6]

Concerning the clastogenic potential, 2-nitroanisole increased the frequency of chromosomal aberrations and sister chromatid exchanges in Chinese hamster cells.[1,4,7]

4.2 *In vivo* assays

Hengstler *et al*. (1995) found increased levels of DNA single-strand breaks in mononuclear blood cells of 16 fire fighters, who were exposed to a mixture of 2-nitroanisole and other chemical agents.[8] Three months after the accident, levels of single-strand breaks were decreased to reference levels. Since during the fire

accident also other chemicals were released in the environment, it is difficult to assess whether 2-nitroanisole was the only agent that was responsible for the observed effects.

Stiborová *et al*. (2004) also investigated the capacity of 2-nitroanisole to form DNA adducts *in vivo*.[6] Six male Wistar rats were given intraperitoneal injection of 2-nitroanisole (0.15 mg/kg bw), once a day for five consecutive days. An additional two animals served as vehicle-controls. Animals were killed 24 hours after the last injection, and various organs were removed and prepared for further analysis. Using the ^{32}P-post-labeling technique, in exposed animals, the authors found the highest levels of treatment-related DNA-adducts in the urinary bladder, followed by the liver, kidney and spleen. The types of adducts found in animals were similar to those formed *in vitro*. No adducts were found in the lungs, brain and heart, nor were there adducts found in the organs of control animals.

Esmaeili *et al.* (2006) used a host-mediated *in vitro/in vivo* system to study 2-nitroanisole-induced transformations of peritoneal macrophages.[9] Groups of five male NMRI mice were given a single intraperitoneal injection of 0, 1.3, 65 or 130 mg/kg bw (0%, 0.1%, 5%, or 10% of LD_{50}, respectively). The transforming potential of the agent increased dose-dependently. Moreover, the investigators could establish an immortal cell line from the peritoneal macrophages, obtained from mice at the highest dose group. The immortal cell line was further tested for its tumour-inducing potency by subcutaneous injection of these cells on nude mice (nu/nu; number of animals not specified). This resulted in visible tumour formation, three weeks after the injection. Microscopic examination of the tumour tissue indicated typical signs of malignancy of mesenchymal origin.

The cell line was also tested on activity of the proto-oncogenes c-jun, c-myc, and c-fos. Dysfunction of these proto-oncogenes has been shown in diverse human tumours, like lymphomas and sarcomas. The transformed cells showed a significant down-regulation of c-myc and c-fos, and an overexpression of c-jun.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the genotoxicity and carcinogenicity of 2-nitroanisole in humans were available, nor were there any data available on inhalation exposure in animals.

Oral administration of 2-nitroanisole for up to two years increased the incidence of tumours in the urinary bladder (rats), large intestines (rats), the kidneys (rats), and the liver (mice) compared to nontreated animals. These findings give sufficient evidence that oral exposure to 2-nitroanisole results in tumour development.

The number of studies on the mutagenic and genotoxic potential of the agent is limited. Overall, 2-nitroanisole induced mutations in bacteria and in mammalian cells, and showed to be clastogenic *in vitro*. It was also able to transform normal peritoneal macrophages into immortal cells. These immortal cells had altered expression of proto-oncogenes and, furthermore, formed tumours at the injection site. Based on these mutagenicity and genotoxicity data, the committee considers 2-nitroanisole as a genotoxic carcinogen that acts by a stochastic mechanism.

The committee did not find indications that the observations in animals, and the proposed carcinogenic mechanism would not occur in humans.

5.2 Recommendation for classification

The committee is of the opinion that 2-nitroanisole should be considered as carcinogenic to humans. This recommendation is comparable to the EU classification in category 2. The committee is furthermore of the opinion that 2-nitroanisole acts by a stochastic genotoxic mechanism.

References

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A	Request for advice
B	The committee
C	Comments on the public review draft
D	IARC Monograph
E	Carcinogenic classification of substances by the committee
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.

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
  molecular biologist and genetic toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  epidemiologist, Dow Chemicals NV, Terneuzen
- R.A. Woutersen
  toxicologic pathologist, TNO Quality of Life, Zeist
- A.A. van Zeeland
  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**

IARC Monograph

Vol.: 65 (1996) (p. 369)[1]
CAS No.: 91-23-6
Chem. Abstr. Name: 1-Methoxy-2-nitrobenzene

Summary of Data Reported and Evaluation

Exposure data

2-Nitroanisole is produced by the reaction of methanolic sodium hydroxide with 2-chloronitrobenzene. It is mainly used in the production of the dye intermediates *ortho*-anisidine and *ortho*-dianisidine. Human exposure may occur during its production and use.

Human carcinogenicity data

No data on the carcinogenicity of 2-nitroanisole in humans were available to the Working Group.

Animal carcinogenicity studies

2-Nitroanisole was tested for carcinogenicity by oral administration in one study in mice and in two studies in rats. In mice, the incidence of hepatocellular ade-

nomas was increased in males and females, and that of hepatoblastomas was increased in males. In one study in rats, the incidence of mononuclear-cell leukaemia was increased in males and females. In the second study, which used a shorter duration of treatment but higher doses, increases were seen in the incidences of tumours of the urinary bladder, the large intestine and the kidney.

Other relevant data

No human data were available on the metabolism of 2-nitroanisole.

In rats, 2-nitroanisole is absorbed after oral administration, and the major route of its rapid elimination is the urine. The predominant metabolic pathway involves the formation of 2-nitrophenol, with its subsequent conjugation with sulfate and glucuronic acid. 2-Nitroanisole causes methaemoglobinaemia following dietary administration of high doses to rats and mice. Pathological lesions observed in rats occurred in the urinary bladder, spleen, kidney and liver. In mice, 2-nitroanisole causes hypertrophy in the liver.

2-Nitroanisole is mutagenic in bacteria. In single studies, it induced mutations, sister chromatid exchange and a low frequency of chromosomal aberrations in cultured mammalian cells.

Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 2-nitroanisole.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-nitroanisole.

Overall evaluation

2-Nitroanisole is *possibly carcinogenic to humans (Group 2B)*.

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.