5-Azacytidine

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *5-Azacytidine*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-1476/JR/pg/246-Q11
Bijlagen : 1
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Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van 5-azacytidine. 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

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 340 66 31
E-mail: jolanda.rijnkels@gr.nl

Postadres
Postbus 16052
2500 BB Den Haag
Telefax (070) 340 75 23
www.gr.nl

5-Azacytidine

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. 2007/04OSH, The Hague, December 12, 2007

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.

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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 5-azacytidine (azacitidine) onder de loep. 5-Azacytidine is een cytostaticum dat wordt gebruikt ter behandeling van kanker.

Op basis van de beschikbare gegevens leidt de commissie af dat 5-azacytidine beschouwd moet worden als kankerverwekkend voor de mens. Dit komt overeen met een classificatie in categorie 2 volgens de richtlijnen van de Europese Unie. De commissie concludeert verder dat 5-azacytidine een stochastisch genotoxisch werkingsmechanisme heeft.

10 5-Azacytidine

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 5-azacytidine (azacitidine). 5-Azacytidine is a cytostatic agent that is used to treat cancer.

Based on the available information, the committee is of the opinion that 5-azacytidine should be considered as carcinogenic to humans. This recommendation corresponds to the EU classification in category 2. The committee concludes furthermore that 5-azacytidine acts by a stochastic genotoxic mechanism.

12 5-Azacytidine

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 with reference to an EU-directive (see annex A and F). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question. The assessment and the proposal for a classification are expressed in the form of standard sentences (see annex E). This report contains the evaluation of the carcinogenicity of 5-azacytidine.

1.2 Committee and procedure

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 MI 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 5-azacytidine, 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 March 2007. The new relevant data were included in this report.

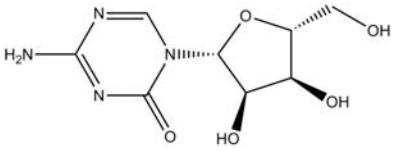
Chapter **2**

General information

2.1 Identity and physico-chemical properties

5-Azacytidine is a cytostatic agent that is used to treat cancer, such as myeloblastic anemia, acute lymphoblastic leukaemia, and myelodysplastic syndromes.[1] Occupational exposure may occur during manufacturing or packaging, or during the final preparation and administration to patients.

Below is given the identity and some of its physico-chemical properties.

Chemical name	:	1,3,5-Triazin-2(1*H*)-one, 4-amino-1-β-ribofuranosyl
CAS registry number	:	320-67-2
Synonyms	:	Antibiotic U 18496; azacitidine; ladakamycin; NSC 102816; U-18496; WR-183027
Description	:	White crystalline powder
Occurrence	:	5-Azacytidine is produced by the bacterium *Streptoverticillium ladakanus*
Molecular formula	:	$C_8H_{12}N_4O_5$
Structure	:	
Molecular weight	:	244.2
Melting point	:	235-237 °C (decomposes)

Solubility	:	Soluble in water, 0.1 N hydrochloric acid, 0.1 N sodium hydroxide, ethanol, acetone, chloroform, hexane, and dimethyl sulfoxide
Stability	:	Very unstable in aqueous media, rapid degradation to complex products occurring within hours of dissolution in intravenous solutions at room temperature

2.2 IARC classification

In 1990, IARC concluded that there is sufficient evidence for the carcinogenicity of 5-azacytidine in experimental animals, but that there were no carcinogenicity data available from studies in humans.[1] Therefore, according to the IARC guidelines, it classified 5-azacytidine in Group 2A, which means that 5-azacytidine is probably carcinogenic to humans. IARC also took notice of the possible genotoxic effects of 5-azacytidine.

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

No data were available to evaluate the carcinogenicity of 5-azacytidine in humans.

3.2 Carcinogenicity studies in animals

In 1990, IARC reported on six different animal carcinogenicity studies in which 5-azacytidine was given to animals by intraperitoneal injections.[1] In summary, Vesely and Cihák (1973) exposed AKR female mice, a strain highly susceptible to develop neoplasms, to 5-azacytidine.[2] They received six injections over 20 to 30 days. All the treated animals died of leukaemia by 60 days, whereas the non-treated animals survived the 120 day-observation period without any symptoms of disease.

Stoner *et al*. (1983) also used a strain highly susceptible to develop neoplasms, namely A/He mice.[3] Male and female mice received intraperitoneal injections of 5-azacytidine (33, 62, and 90 mg/kg bw), three times a week for eight weeks. All animals were killed 24 weeks after the first injection. The incidence of lung tumours was higher in treated mice than in untreated and vehicle-treated mice. The number of lung tumours per mouse in the highest dose group was statistically significantly higher than in the untreated and vehicle-treated

mice. In the other treated groups, the increase in tumour frequency per mouse did not reach statistical significance.

The American National Cancer Institute (1978) performed a carcinogenicity study on B6C3F1 mice.[4] The animals (n= 35/group/sex) received intraperitoneal injections of 5-azacytidine (2.2, and 4.4 mg/kg bw), three times a week for 52 weeks. Surviving animals were killed at 81 and 82 weeks. Survival rates were lowest for the high-dose group (high-dose, 0/35 (females), 7/35 (males); low-dose group, 17/35 (females), 13/35 (males); untreated versus vehicle controls, 25/30 versus 20/30). Increases in incidence of tumours were only observed in the low-dose female group: lymphocytic and granulocytic neoplasms of the haematopoietic system, 17/29 (low-dose), 0/14 (control), $p<0.001$; granulocytic tumours (sarcomas, leukaemia), 10/29 (low-dose), 0/14 (control).

Cavaliere *et al.* (1987) gave azacytidine (2.0 mg/kg bw) to BALB/c mice (n=50/group/sex), once a week for 50 weeks.[5] In both males and females the incidence of lymphoreticular neoplasms, and skin tumours were significantly increased compared to vehicle-controls ($p<0.001$-0.05). In males, also the incidence in lung adenomas was increased ($p<0.01$), and in females an increased number of animals with mammary tumours were found.

Carr *et al.* (1984 and 1988) performed two carcinogenicity studies on male Fischer rats.[6,7] In the first study, a limited number of animals were given intraperitoneal injections of 5-azacytidine (2.5, and 10 mg/kg bw), twice a week for nine months. Surviving animals were killed at eighteen months. In the non-treated animals no tumours occurred, whereas in the treated animals interstitial-cell testicular tumours (mainly in the low-dose group), and skin tumours (high-dose group only) were found. In the second study, animals received 5-azacytidine (0.025, 0.25, and 2.5 mg/kg bw), three times a week for 12 months. At 12 months the study was terminated. Again the authors found an increased number of highest dose treated animals with interstitional-cell testicular tumours compared to controls ($p<0.001$). In the highest dose group also four lymphomas, four renal tumours, one lung tumour, three skin tumours, two mesotheliomas, and two sarcomas were found. No such tumours were found in the control group. IARC noted the short duration of the experiment, and the small numbers of animals in some groups.[1]

IARC also reported on a study on pregnant NMRI mice (n=32-37/group), which were given intraperitoneal injections of 5-azacytidine (1, and 12 mg/kg bw), three times during gestation (Schmahl *et al.* 1985).[8] Concerning the progeny, increased numbers of tumour-bearing animals were observed. The types of tumours observed were leukaemia, lymphomas, lung and liver tumours, and soft-tissue sarcomas.

Luz and Murray (1988) reported on a sudden outbreak of leukaemia-like lesions in female CBA mice after repeated intraperitoneal injections of 5-azacytidine (1 mg/kg bw).[9] In the same experiment, also female BALB/c mice were treated identically, but in those animals no leukaemia-like or other tumour-like lesions were observed.

Hammond *et al.* (1990) used a lung cancer model to study the carcinogenic effects of 5-azacytidine.[10] Outbred Syrian golden hamsters (n=54) received intraperitoneal injections of 5-azacytidine at a dose of 5 mg/kg bw, twice a week until sacrifice. During the experiment, animals were killed at several time points, of which the last was carried out 70 weeks after the first injection. No untreated or vehicle-treated animals were included in the study, since, according to the authors, the incidence and variety of spontaneous tumours in these animals has been well documented. No treatment related non-neoplastic lesions were observed. Also, in none of the animals, grossly apparent tumours, and microscopic neoplasms in the various organs studied, was observed.

In the same study, the authors also studied the effect of 5-azacytidine on animals with bronchial implants that released benzo[a]pyrene (BaP), a known genotoxic carcinogen. These implants were used to induce preneoplastic mucosal changes, which progresses to bronchogenic cancer. The investigators observed that 5-azacytidine-treatment slowed down the neoplastic change from BaP in comparison with animals, which had only a BaP-implant. Furthermore, when 5-azacytidine was given early after the implantation, it inhibited only the late (promotional) phase of BaP-induced epidermoid cancer, while it inhibited both the early and late phase of BaP-induced nonsquamous cancer development. Comparable results were obtained with syngeneic (F_1D) hamsters with BaP-implants and 5-azacytidine treatment. This strain is highly susceptible for spontaneous lung neoplasms. Based on their results, the authors suggested that 5-azacytidine does not initiate de novo cancer, but rather affects the late tumour development phase, at least in hamsters.

Carr *et al*. (1984) reported on an initiation-promotion study on male Fischer rats, which were given a single dose of *N*-nitrosodiethylamine, eighteen hours before partial hepatectomy, alone or with two 5-azacytidine injections (2.5, and 10 mg/kg bw).[6] Lung tumours were found in two (2/10, low dose), and eight (8/10, high dose) 5-azacytidine-treated animals, whereas no tumours were found in hepatectomised-animals that only received *N*-nitrosodiethylamine. In the study, no animals were included that received 5-azacytidine alone.

Chapter 4

Mutagenicity and genotoxicity

5-Azacytidine is a nucleoside analogue of cytidine that specifically inhibits DNA methylation by incorporating into DNA, and trapping DNA methyltransferases.[11-14] DNA methylation results in the silencing or down regulating of genes, without a change in their coding sequence. As such, DNA methylation plays an important role in DNA repair and genome stability.[13,15] Concerning its antineoplastic effects, it is proposed that in cancer patients, and in combination with its cytotoxic effects, the inhibition of DNA methylation by 5-azacytidine results in reactivation of previously silenced genes, such as tumour-suppressor genes.[14] However, it is also hypothesized that hypomethylation may contribute to oncogenesis, for instance by activating oncogenes, or by stimulating chromosome instability.[13] The possible mutagenic and genotoxic properties of 5-azacytidine are further reviewed in the next sections.

4.1 *In vitro* assays

5-Azacytidine induced DNA mutations and damage in various bacterial strains (*Salmonella typhimurium*, *Escherichia coli*).[1] Doiron *et al*. (1999) observed that in *Escherichia coli* strains, 5-azacytidine did produce mainly C-to-G mutations in the *lacZ* gene in a methylase-independent manner.[16] It also induced mitotic gene conversions and reverse mutations in the yeast *Saccharomyces cerevisiae*.[1] Furthermore, increased mutation frequencies were observed in (transgenic) Chinese hamster cells (*hprt*, and *gpt* loci), mouse lymphoma L5178Y cells (*tk* locus),

and in human fibroblasts (*hprt* and *tk* loci).[1,17] IARC reported that 5-azacytidine induced DNA strand breaks in human HeLa cells. A few investigators reported that 5-azacytidine mainly caused G:C → C:G transitions.[1,16,18,19] In addition, Broday *et al*. (1999) showed that in transgenic Chinese hamster cells, 5-azacytidine was able to induce transgene-specific DNA methylation.[17]

On the other hand, 5-azacytidine did not induce gene mutations at the *hprt* locus in Syrian hamster BHK cells, primary rat tracheal epithelial cells, mouse lymphoma L5178Y cells, and in Chinese hamster V79 cells (one study).[1] Also, it did not induce ouabain-resistant mutations in various rodent cells.[1]

Regarding clastogenicity, contradictory results have been reported on the induction of sister chromatid exchanges (SCEs), and chromosomal aberrations. Positive outcomes were found in hamster cell lines, CHO cells, CHO-K1 and XRS-5 cells (both deficient for doubel-strand break rejoining), and in human peripheral lymphocytes; negative outcomes were found in human lymphocytes (another study), and in human lymphoblasts (chromosomal aberrations).[1,20,21]

Albanesi *et al.* (1999) demonstrated that 5-azacytidine did induce SCEs in Chinese hamster ovary cells, but only when the cells were tested at the second cell cycle after treatment.[22] Their findings support the idea that demethylation, which is a process that takes two cell cycles, increases the risk of errors. In addition, Perticone *et al*. (1990) observed in the same type of cells, that 5-azacytidine (single exposure for 12 hours) induced SCEs starting two cycles from treatment, that persisted for the entire 16 cycles, while the DNA methylation levels returned to normal after 10 cell cycles.[23]

5-Azacytidine induced micronuclei in Syrian hamster embryo cells, mouse L5178Y cells, and in human lymphocyte cells.[24-27]

The substance did not induce unscheduled DNA synthesis, but it induced morphological cell transformations in Syrian hamster embryo cells, and in mouse BALB/c-3T3 cells.[26,28]

4.2 *In vivo* assays

5-Azacytidine is positive in the wing spot and in the eye mosaic assay in the fruit fly *Drosophila melanogaster.*[1,29]

No dominant lethal mutations were found in male mice after they were given 5-azacytidine intraperitoneally at single doses of 5 and 10 mg/kg bw.[1,30]

Regarding clastogenicity, losses of sex chromosomes in DNA-repair-deficient *Drosophila melanogaster* flies were observed.[31]

4.3 Carcinogenic mechanism

The exact carcinogenic mechanism of 5-azacytidine is not well understood yet. Though data do suggest a genotoxic mechanism, it is also suggested that the agent may partly exert its effect by elicitating alterations in chromosome structure, due to hypomethylation of the DNA nucleotide cytidine, which results in gene and chromosomal instability and even in loss of specific chromosomes.[21,24,31,32]

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No data on the genotoxicity and carcinogenicity of 5-azacytidine in humans were available, nor were there any data available on inhalation exposure in animals. However, repeated intraperitoneal injections of 5-azacytidine in various (and sometimes susceptible) mouse strains, and in rats, induced a variety of tumours, including leukaemia, lymphomas, sarcomas and solid tumours in different organs. At least in experimental animals, these findings give sufficient evidence that exposure to 5-azacytidine can result in cancer development.

Data on the mutagenic and genotoxic properties of 5-azacytidine were mainly limited to *in vitro* test systems. Although these tests revealed contradictory outcomes, overall, data do support the idea that the agent possesses genotoxic and mutagenic properties. Therefore, the committee is of the opinion that 5-azacytidine should be considered as a genotoxic carcinogen that acts by a stochastic mechanism. Furthermore, 5-azacytidine has DNA-demethylating properties, in that hypomethylation may lead to gene and chromosomal instability.

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 5-azacytidine should be considered as carcinogenic to humans. This recommendation corresponds to the EU classification in category 2. The committee concludes furthermore that 5-azacytidine acts by a stochastic genotoxic mechanism.

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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
  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 Nutrition and Food Research, 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:

- G. Jonkers, Vereniging van Verf en Drukinktfabrikanten, the Netherlands;
- 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: 50 (1990) (p. 47)[1]

CAS No.: 320-67-2
Chem. Abstr. Name: 1,3,5-Triazin-2(1*H*)-one, 4-amino-1-β-ribofuranosyl

Summary of Data Reported and Evaluation

Exposure data

Azacitidine is a cytostatic agent that has been used since the 1970s for the treatment of acute leukaemia.

Experimental carcinogenicity data

Azacitidine was tested for carcinogenicity by intraperitoneal injection in four studies in mice and in two studies in rats and by transplacental exposure in one study in mice. In one study in mice, it accelerated the development of leukaemias; in the two long-term studies and in the transplacental study, it increased the incidence of lymphoid neoplasms. In one of the long-term studies, the incidence of lung adenomas was increased in male mice and that of skin tumours in mice of each sex. In the transplacental study in mice, it also increased the inci-

dences of lung and liver tumours. It accelerated the induction of lung tumours in mice. In rats, it increased the incidence of testicular tumours.

Intraperitoneal administration of azacitidine to rats enhanced the development of liver tumours induced by *N*-nitrosodiethylamine.

Human carcinogenicity data

No data were available to the Working Group.

Other relevant data

During the early stages of gestation, azacitidine induces embryomortality in mice; during the organogenesis period, multiple, gross structural malformations can be induced; and during later stages of gestation, mainly central nervous system defects have been induced in mice.

Azacitidine is readily deaminated to azauridine and further degraded. It is incorporated into DNA and alters gene expression. In humans, it causes leukopenia.

Azacitidine causes hypomethylation of DNA both *in vivo* and *in vitro*.

In one study, azacitidine did not induce dominant lethal mutations in mice. Contradictory results have been reported with respect to the induction of chromosomal aberrations and sister chromatid exchange in human cells. In single studies, azacitidine induced gene mutations and DNA strand breaks in human cells. It induced chromosomal aberrations in Chinese hamster cells, sister chromatid exchange in cloned Chinese hamster cells, gene mutations in Chinese hamster and mouse lymphoma cells and transformation in various cell lines. It induced mitotic recombination and mutations in *Drosophila*. Azacitidine induced chromosomal aberrations in *Vicia faba*. In *Saccharomyces cerevisiae*, it induced gene mutations and mitotic recombination but not chromosomal loss. It induced mutations and DNA damage in *Salmonella typhimurium* and *Escherichia coli*.

Evaluation

There is *sufficient evidence* for the carcinogenicity of azacitidine in experimental animals.

No data were available from studies in humans on the carcinogenicity of azacitidine.

In making the overall evaluation, the Working Group also took note of the following information. Azacitidine is active in a broad spectrum of assays for genetic and related effects, including those involving mammalian cells. Furthermore, azacitidine, a pyrimidine analogue, is incorporated into DNA, causing hypomethylation.

Overall evaluation

Azacitidine is *probably carcinogenic to humans (Group 2A).*

Previous evaluations: Vol. 26 (1981) (p. 37); Suppl. 7 (1987) (p. 57)

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.