Vincristine sulphate

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Vincristine sulphate*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-1482/JR/pg/246-W11
Bijlagen : 1
Datum : 12 december 2007

Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van vincristinesulfaat. 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

Vincristine sulphate

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. 2007/10OSH, 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.

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.

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

The Health Council of the Netherlands is a member of the International Network of Agencies for Health Technology Assessment (INAHTA), an international collaboration of organisations engaged with *health technology assessment*.

INAHTA

This report can be downloaded from www.healthcouncil.nl.

Preferred citation:
Health Council of the Netherlands. Vincristine sulphate; Evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2007; publication no. 2007/10OSH.

ISBN: 978-90-5549-674-7

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 de beroepsmatige uitoefening 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 vincristinesulfaat onder de loep. Vincristinesulfaat is een cytostatisch geneesmiddel dat wordt gebruikt ter bestrijding van kanker.

De commissie meent dat vincristinesulfaat 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 de commissie van mening dat waakzaamheid geboden is. De commissie adviseert daarom vinblastinesulfaat te classificeren als *verdacht kankerverwekkend voor de mens*. Volgens de richtlijnen van de Europese Unie komt dit overeen met een classificatie in categorie 3. 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 vincristine sulphate. Vincristine sulphate is an antineoplastic cytotoxic agent that is used in the treatment of cancer.

The committee concludes that vincristine sulphate 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*. This recommendation corresponds to EU classification in category 3. This situation is, furthermore, comparable with subcategory b of this category.

12 Vincristine sulphate

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 G). 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 F). This report contains the evaluation of the carcinogenicity of vincristine sulphate.

1.2 committee and procedure

The evaluation is performed by the committee 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 IA van de Gevel and 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 vincristine sulphate, 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

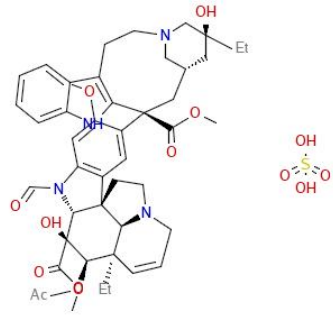
General information

2.1 Identity and physico-chemical properties

Vincristine sulphate is an antineoplastic cytotoxic agent that is mainly used to treat certain types of cancer, such as Hodgkin's disease, non-Hodgkin's lymphomas, and cancer of the breast or testicles.[1] It is also used in some non-malignant conditions, such as idiopathic thrombocytopenia. 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	:	Vincaleukoblastine, 22-oxo-, sulfate (1:1) (salt)
CAS registry no.	:	2068-78-2
EINECS no.	:	218-190-0
Synonyms	:	Leurocristine sulfate (1:1) (salt); Des-*Na*-methyl-*Na*-formylvinblastine sulphate; LCR sulphate; leurocristine sulphate; leurocristine, sulphate (1:1) (salt); VCR sulphate; NSC 67574, Oncovin; Onkovin; Vincristine; Vincrisul; 37231
Description	:	White to slightly yellow, odourless, very hygroscopic, amorphous or crystalline powder
Occurrence	:	Vincristine is a naturally occurring alkaloid, which has been isolated from several members of the plant genus *Catharanthus* (formerly called *Vinca*), a pantropical shrub.
Molecular formula	:	$C_{46}H_{56}N_4O_{10} \bullet H_2SO_4$

Structure :



Molecular weight : 923.0

Melting point : After recrystalization from absolute ethanol, 273-281°C

Solubility : Soluble in water (1 in 2), ethanol (1 in 600), chloroform (1 in 30) and methanol; insoluble in diethyl ether

Stability : Sensitive to hydrolysis, oxidation and heat

2.2 IARC classification

In 1981 and 1987, IARC concluded that there is no evidence of carcinogenicity in rats or mice on the basis of available data. The data from studies in man are inadequate to evaluate the carcinogenicity of vincristine sulphate in humans. Overall, there is no evidence currently available to indicate that vincristine sulphate is carcinogenic to humans, but the compound has not been extensively investigated. As a consequence, IARC concluded that vincristine sulphate was not classifiable as to its carcinogenicity to humans (Group 3).[1,2]

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

In general, vincristine sulphate is given together with certain other types of agents, such as bleomycin, procarbazine, nitrogen mustard, prednisone, or methotrexate, in combination with ionizing radiation therapy.[2] These agents and treatments have the potential to induce secondary cancers by themselves, and are as such suspected carcinogens. None of the published data on humans, which are available to the committee, concern vincristine sulphate application alone. Therefore, it is difficult to assess from observational data whether vincristine sulphate is the only responsible agent that may have caused secondary cancers. Below is given a short evaluation of some of the data on combination therapy.

Various case reports and epidemiological studies have been reported on patients with Hodgkins' disease, who were treated with combined chemotherapy, including vincristine sulphate, and who developed acute nonlymphocytic leukemia as a secondary cancer.[1].

Coleman *et al*. (1977) performed a follow-up study on 680 patients with Hodgkins' disease.[1,3] No excess risk of developing acute nonlymphocytic leukemia was observed in the group of patients receiving combination therapy. However, various other patient-cohort studies on patients with Hodgkins' disease reported on excess risk of acute nonlymphocytic leukemia by combination therapy compared to other forms of therapy or control groups.[1]

To assess an association between treatment of cancer patients and development of secondary cancers, a group of Italian investigators performed a series of randomized studies on more than 1,000 patients who have been cured of their Hodgkin's disease, and who were given various different treatments to control cancer.[4-6] One of these treatments concerned chemotherapy with MOPP (mixture of mechloretamine, vincristine sulphate, prodacarbazine, and prednisone), with or without radiotherapy. The median follow-up time was 10 years. Overall, the 15-year actuarial risk of developing leukemia was: 2.8% for patients treated with MOPP alone; 0.3% for patients having radiotherapy alone; and, 5.4% for combined modality therapy.

No data were available on occupational exposure to the agent.

3.2 Carcinogenicity studies in animals

Weisburger (1977) used Sprague-Dawley rats and Swiss mice to study the possible carcinogenicity of various agents, including vincristine sulphate.[7] Animals (n=25/group/sex/species) were given intraperitoneal injections at maximum or half maximum tolerated doses of vincristine sulphate, three times per week for 6 months. After those six months, animals were followed an additional year before the study was ended.

In male and female rats receiving 0.06 or 0.12 mg/kg bw, the survival time ranged from 19 to 100% (males), and 100% (females), compared to the survival times of non-treated control animals. The number of tumour-bearing male animals was 3/16. The types of tumours found were two pituitary tumours, and one testis tumour. None of these were malignant. The tumour incidence in treated males was lower than in controls (18% versus 34% in control animals (also tumours in pituitary gland and testis)). In female rats, twelve of them developed tumours (12/22), of which two had malignant tumours. The principal tumours were 10 breast tumours, 3 pituitary tumours, and one adrenal tumour. The tumour incidence in vincristine sulphate treated animals was comparable with that in controls (55% versus 58% in control animals (tumours in same organs as treated animals)).

In male and female mice receiving 0.075-0.15 mg/kg bw, the survival time ranged from 33 to 55% (males), and 97 to 100% (females), compared to the survival time in non-treated mice. Only one benign skin tumour was observed in one male mouse (1/15). In female mice, four animals developed tumours (4/13), of which 3 had malignant tumours (3 lung tumours, and one leukemia). Overall, the tumour incidences did not differ markedly from the 26% incidence seen in both male and female controls. In its monograph, IARC noted the incomplete

reporting of certain items, such as on survival times, the amalgation of various experimental groups and tumours types, as well as the lack of age-adjustment in the analyses.[1] For this reason a complete evaluation was not possible and no final conclusion could be given. The committee agrees with the comments of IARC.

Chapter 4

Mutagenicity and genotoxicity

Vincristine sulphate is a *Vinca* alkaloid, which is known to bind to the microtubular proteins of the mitotic spindle. This causes microtubule-destabilisation, which finally leads to mitotic arrest or cell death. It are these properties, which are related to the antineoplastic activity of the agent.[8,9] The possible mutagenic and genotoxic properties are summarized in the next sections. The outcomes of the individual studies are given in annex E.

4.1 *In vitro* assays

Vincristine sulphate did not induce reverse point mutations in *Salmonella typhymurium* strains TA98 or TA100.[2] It did induce gene mutations in mouse lymphoma cells, but not in other commercial cell lines.[2] No unscheduled DNA synthesis was observed in mammalian and human cells.[2,10]

Regarding its aneugenic and clastogenic potential, the agent did not induce a clear increase in chromosomal aberrations in various mammalian, and in human peripheral blood lymphocytes.[2,11] Vincristine sulphate induced sister chromatid exchanges in human lymphocytes in one study, but not in three other studies.[2]

A few micronucleus assays were performed to study the induction of micronuclei by vincristine sulphate in various mammalian cell types, and in human peripheral blood lymphocytes.[2,12-14] All scores were positive.

More recently, Kopjar and Garaj-Vrhovac (2000) used human peripheral blood lymphocytes from one donor, to assess aneugenic and clastogenic potency of vincristine sulphate.[15] Administration of 0.0875 µg vincristine/mL for up to 72 hours resulted in DNA damage and chromosome aberrations (chromatid breaks and acentric fragments). Also polyploid cells with numerous chromosomes were observed.

Furthermore, vincristine sulphate induced aneuploidy and cell transformations in Syrian hamster embryo cells, and in human fibroblasts.[16,17] However, no transformed cells were found in vincristine-treated C3H/10T½ cells.[18]

Overall, based on the current knowledge, vincristine sulphate is considered an aneugen. This indicates that it induces numerical chromosomal changes rather than chromosome breakage.[19] In somatic cells, aneuploidy is associated with the development of several cancers, although the exact mechanism of action of aneugenic agents is not yet completely understood.

4.2 *In vivo* assays

No data were available to the committee on the mutagenic potential of vincristine sulphate in humans and animals.

In various animal studies, a single intraperitoneal injection of vincristine sulphate increased the frequencies of micronuclei in bone marrow cells, in a dose-related matter, compared to non-treated controls.[20-24] The animals included mice, hamsters and rats. Based on the crescent shape and large size of the micronuclei, Tinwell and Ashby (1991) considered vincristine sulphate aneugenic.[24] In two separate animal studies, vincristine sulphate also increased the frequency of chromosomal aberrations.[25,26].

In two separate experiments, Sheu *et al*. (1990, 1992) treated male Chinese hamsters with vincristine sulphate to study aneuploidy.[27,28] By counting chromosomes in the metaphase, no aneuploidy (hyperploidy) was observed in bone marrow cells. Also no increased frequency in hyperploidy was observed in spermatogonial and germ cells in the meiotic I phase, but it did increase the frequency in germ cells in the meiotic II phase.

In *Drosophila melanogaster* flies the results were negative in the sex-linked recombination lethal test, but positive in the somatic mutation and recombination assay.[2,29,30] For instance, Tiburi *et al*. (2002) used the wing somatic mutation and recombination test of *Drosophila melanogaster*, to test for genotoxicity of vinc-

ristine sulphate.[29] In marker-heterozygous flies, the agent statistically significantly increased the frequencies of total spots, which were mainly related to small single spots. These single spots can be produced by somatic point mutations, chromosome aberrations, and/or by mitotic recombinations. In balancer-heterozygous flies, in the highest exposure level, vincristine sulphate clearly increased the total number of total spots. In this fly, the presence of spots can be caused by somatic point mutations and/or chromosome aberrations, but not by mitotic recombinations.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

Human carcinogenicity data on vincristine sulphate is limited to patients, who underwent curative therapy to cure a primary cancer. No epidemiological studies or case reports were available in which vincristine sulphate was the only agent used to cure these patients.

Currently, there is inadequate evidence that vincristine sulphate is carcinogenic to humans. Also, no clear evidence was found that vincristine sulphate is carcinogenic to animals, but the committee emphasizes that the number of animal studies was limited, and that the reporting of the available studies was incomplete. Overall, the carcinogenic properties of vincristine sulphate have been insufficiently investigated.

Vincristine sulphate did not induce gene mutations in bacteria or mammalian cells, but it did induce micronuclei and aneuploidy in various test systems *in vivo* and *in vitro*. Controversial findings were reported on chromosome aberrations and sister chromatid exchanges. In addition, based on the available genotoxicity data and the current understanding of the mechanism of action, the committee is of the opinion that vincristine sulphate is an aneugen.

5.2 Recommendation for classification

The committee concludes that vincristine sulphate 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*. This recommendation corresponds to EU classification in category 3. This situation is, furthermore, comparable with subcategory b of this category.

References

1 IARC. Vincristine sulphate. IARC Monogr Eval Carcinog Risk Chem Hum 1981; 26: 365-384.

2 IARC. Vincristine sulphate. IARC Monogr Eval Carcinog Risks Hum Suppl 1987; 26(Suppl.6): 563-565.

3 Coleman CN, Williams CJ, Flint A, Glatstein EJ, Rosenberg SA, Kaplan HS. Hematologic neoplasia in patients treated for Hodgkin's disease. N Engl J Med 1977; 297(23): 1249-1252.

4 Brusamolino E, Anselmo AP, Klersy C, Santoro M, Orlandi E, Pagnucco G *et al*. The risk of acute leukemia in patients treated for Hodgkin's disease is significantly higher aft [see bined modality programs than after chemotherapy alone and is correlated with the extent of radiotherapy and type and duration of chemotherapy: a case-control study. Haematologica 1998; 83(9): 812-823.

5 Brusamolino E, Baio A, Orlandi E, Arcaini L, Passamonti F, Griva V *et al*. Long-term events in adult patients with clinical stage IA-IIA nonbulky Hodgkin's lymphoma treated with four cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine and adjuvant radiotherapy: a single-institution 15-year follow-up. Clin Cancer Res 2006; 12(21): 6487-6493.

6 Valagussa P, Bonadonna G. Hodgkin's disease and the risk of acute leukemia in successfully treated patients. Haematologica 1998; 83(9): 769-770.

7 Weisburger EK. Bioassay program for carcinogenic hazards of cancer chemotherapeutic agents. Cancer 1977; 40(4 Suppl): 1935-1949.

8 Mukherjee AK, Basu S, Sarkar N, Ghosh AC. Advances in cancer therapy with plant based natural products. Curr Med Chem 2001; 8(12): 1467-1486.

9 Zhou XJ, Rahmani R. Preclinical and clinical pharmacology of vinca alkaloids. Drugs 1992; 44 Suppl 4: 1-16.

10 Benigni R, Calcagnile A, Dogliotti E, Falcone E, Giuliani A. DNA repair induction by cytostatic drugs in proliferating and quiescent MRC-5 cells. Teratog Carcinog Mutagen 1983; 3(6): 481-490.

11 Kucerová M, Polívková Z. Mutagenic effects of different mutagens on human chromosomes in vitro as detected by conventional and "harlequin" methods. Dev Toxicol Environ Sci 1977; 2: 319-325.

12 Darroudi F, Meijers CM, Hadjidekova V, Natarajan AT. Detection of aneugenic and clastogenic potential of X-rays, directly and indirectly acting chemicals in human hepatoma (Hep G2) and peripheral blood lymphocytes, using the micronucleus assay and fluorescent in situ hybridization with a DNA centromeric probe. Mutagenesis 1996; 11(5): 425-433.

13 Miyakoshi Y, Suzuki Y, Ooida M, Takahashi A, Tsukui M. Micronucleus test using cultured new born rat astrocytes. Ind Health 1999; 37(1): 95-102.

14 Vian L, Bichet N, Gouy D. The in vitro micronucleus test on isolated human lymphocytes. Mutat Res 1993; 291(1): 93-102.

15 Kopjar N, Garaj-Vrhovac V. Application of cytogenetic endpoints and Comet assay on human lymphocytes treated with vincristine in vitro. Neoplasma 2000; 47(3): 162-167.

16 Natarajan AT, Duivenvoorden WC, Meijers M, Zwanenburg TS. Induction of mitotic aneuploidy using Chinese hamster primary embryonic cells. Test results of 10 chemicals. Mutat Res 1993; 287(1): 47-56.

17 Tsutsui T, Suzuki N, Maizumi H, Barrett JC. Aneuploidy induction in human fibroblasts: comparison with results in Syrian hamster fibroblasts. Mutat Res 1990; 240(4): 241-249.

18 Benedict WF, Banerjee A, Gardner A, Jones PA. Induction of morphological transformation in mouse C3H/10T1/2 clone 8 cells and chromosomal damage in hamster A(T1)C1-3 cells by cancer chemotherapeutic agents. Cancer Res 1977; 37(7 Pt 1): 2202-2208.

19 Aardema MJ, Albertini S, Arni P, Henderson LM, Kirsch-Volders M, Mackay JM *et al*. Aneuploidy: a report of an ECETOC task force. Mutat Res 1998; 410(1): 3-79.

20 Grawe J, Abramsson-Zetterberg L, Eriksson L, Zetterberg G. The relationship between DNA content and centromere content in micronucleated mouse bone marrow erythrocytes analysed by flow cytometry and fluorescent in situ hybridization. Mutagenesis 1994; 9(1): 31-38.

21 Krishna G, Fiedler R, Theiss JC. Simultaneous evaluation of clastogenicity, aneugenicity and toxicity in the mouse micronucleus assay using immunofluorescence. Mutat Res 1992; 282(3): 159-167.

22 Krishna G, Urda G, Theiss JC. Comparative mouse micronucleus evaluation in bone marrow and spleen using immunofluorescence and Wright's Giemsa. Mutat Res 1994; 323(1-2): 11-20.

23 Shi XC, Krishna G, Ong TM. Induction of micronuclei in rat bone marrow by four model compounds. Teratog Carcinog Mutagen 1991; 11(5): 251-258.

24 Tinwell H, Ashby J. Micronucleus morphology as a means to distinguish aneugens and clastogens in the mouse bone marrow micronucleus assay. Mutagenesis 1991; 6(3): 193-198.

25 Choudhury RC, Das B, Misra S, Jagdale MB. Spermatogonial cytogenetic toxicity of vincristine and its transmission in the germline cells of Swiss mice. J Environ Pathol Toxicol Oncol 2002; 21(3): 249-257.

26 Sieber SM, Whang-Peng J, Botkin C, Knutsen T. Teratogenic and cytogenetic effects of some plant-derived antitumor agents (vincristine, colchicine, maytansine, VP-16-213 and VM-26) in mice. Teratology 1978; 18(1): 31-47.

27 Sheu CW, Lee JK, Arras CA, Jones RL, Lavappa KS. The feasibility of using Chinese hamsters as an animal model for aneuploidy. Environ Mol Mutagen 1990; 16(4): 320-323.

28 Sheu CW, Lee JK, Arras CA, Jones RL, Lavappa KS. Detection of vincristine-induced hyperploidy in meiotic II metaphases of male Chinese hamsters. Mutat Res 1992; 280(3): 181-186.

29 Tiburi M, Reguly ML, Schwartsmann G, Cunha KS, Lehmann M, Rodrigues de Andrade HH. Comparative genotoxic effect of vincristine, vinblastine, and vinorelbine in somatic cells of Drosophila melanogaster. Mutat Res 2002; 519(1-2): 141-149.

30 Vogel EW, Nivard MJ. Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination. Mutagenesis 1993; 8(1): 57-81.

31 Seino Y, Nagao M, Yahagi T, Hoshi A, Kawachi T, Sugimura T. Mutagenicity of several classes of antitumor agents to Salmonella typhimurium TA98, TA100, and TA92. Cancer Res 1978; 38(7): 2148-2156.

32 Suter W, Brennand J, McMillan S, Fox M. Relative mutagenicity of antineoplastic drugs and other alkylating agents in V79 Chinese hamster cells, independence of cytotoxic and mutagenic responses. Mutat Res 1980; 73(1): 171-181.

33 Matheson D, Brusick D, Carrano R. Comparison of the relative mutagenic activity for eight antineoplastic drugs in the Ames Salmonella/microsome and TK+/- mouse lymphoma assays. Drug Chem Tox 1978; 1: 277-304.

34 Tsutsui T, Suzuki N, Maizumi H, Barrett JC. Vincristine sulfate-induced cell transformation, mitotic inhibition and aneuploidy in cultured Syrian hamster embryo cells. Carcinogenesis 1986; 7(1): 131-135.

35 Au WW, Johnston DA, Collie-Bruyere C, Hsu TC. Short-term cytogenetic assays of nine cancer chemotherapeutic drugs with metabolic activation. Environ Mutagen 1980; 2(4): 455-464.

36 Maier P, Schmid W. Ten model mutagens evaluated by the micronucleus test. Mutat Res 1976; 40(4): 325-337.

37 Morgan WF, Crossen PE. Mitotic spindle inhibitors and sister-chromatid exchange in human chromosomes. Mutat Res 1980; 77(3): 283-286.

38 Raposa T. Sister chromatid exchange studies for monitoring DNA damage and repair capacity after cytostatics in vitro and in lymphocytes of leukaemic patients under cytostatic therapy. Mutat Res 1978; 57(2): 241-251.

39 Banerjee A, Benedict WF. Production of sister chromatid exchanges by various cancer chemotherapeutic agents. Cancer Res 1979; 39(3): 797-799.

40 Todd GC, Gibson WR, Morton DM. Toxicology of vindesine (desacetyl vinblastine amide) in mice, rats, and dogs. J Toxicol Environ Health 1976; 1(5): 843-850.

41 Yamamoto KI, Kikuchi Y. Studies on micronuclei time response and on the effects of multiple treatments of mutagens on induction of micronuclei. Mutat Res 1981; 90(2): 163-173.

42 Norppa H, Penttila M, Sorsa M, Hintikka EL, Ilus T. Mycotoxin T-2 of Fusarium tricinctum and chromosome changes in Chinese hamster bone marrow. Hereditas 1980; 93(2): 329-332.

---

30 Vincristine sulphate

A	Request for advice
B	The committee
C	Comments on the public review draft
D	IARC Monograph
E	Mutagenicity and genotoxicity data
F	Carcinogenic classification of substances by the committee
G	Guideline 93/31/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 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

D.1 VOL.: 26 (1981) (p. 365)1

Summary of Data Reported and Evaluation

Experimental data

Vincristine sulphate was tested in mice and rats by intraperitoneal injection. In these limited studies no evidence of carcinogenicity was found.

Vincristine sulphate can induce teratogenic effects in several animal species, and it induced embryolethality at doses nontoxic to the mother. There is no evidence to suggest that this compound is mutagenic.

Human data

Vincristine sulphate has been used since the early 1960s for treatment of acute leukaemia in children, often in combination with other antineoplastic agents. It is also frequently a part of combination chemotherapeutic regimens for Hodgkin's disease, non-Hodgkin's lymphoma, chronic lymphocytic leukaemia and other adult neoplasms.

The available data are insufficient to evaluate the teratogenicity of this drug in humans. No data were available on its mutagenic or chromosomal effects.

Both case reports and epidemiological studies indicate that acute nonlymphocytic leukaemia is produced in patients with Hodgkin's disease treated with combined therapeutic regimens which include vincristine sulphate, alkylating agents and procarbazine hydrochloride, often in conjunction with radiotherapy. No data were available on vincristine sulphate alone.

Evaluation

The available data in experimental animals were insufficient for evaluation. There is *sufficient evidence* for the carcinogenicity in humans of intensive chemotherapeutic regimens that include alkylating agents, vincristine sulphate, procarbazine hydrochloride and prednisone. There is *inadequate evidence* for the carcinogenicity of vincristine sulphate itself.

On the basis of the available data, no conclusion could be drawn as to the carcinogenicity of vincristine sulphate.

D.2 Supplement 7: (1987) (p. 372)[2]

CAS No.: 2068-78-2
Chem. Abstr. Name: Vincaleukoblastine, 22-oxo, sulfate (1:1) (salt)

A Evidence for carcinogenicity to humans (inadequate)

No epidemiological study of vincristine sulphate as a single agent was available to the Working Group. Intensive combination chemotherapy with regimens including vincristine has been shown to result in increased risks for acute nonlymphatic leukaemia (ANLL). (See also the summary of data on MOPP and other combined chemotherapy including alkylating agents) Such combinations usually include procarbazine together with an alkylating agent such as nitrogen mustard (see p. 269), both of which are potent animal carcinogens, suggesting more plausible explanations for the association between combination chemotherapy and ANLL. In the presence of concurrent therapy with other putative carcinogens, including ionizing radiation and other potent drugs, occasional case reports of exposure to vincristine sulphate do not constitute evidence of carcinogenesis [ref: 1].

B Evidence for carcinogenicity to animals (inadequate)

In limited studies in mice and rats, no evidence of carcinogenicity was found after intraperitoneal administration of vincristine sulphate [ref: 1].

C Other relevant data

No data were available on the genetic and related effects of vincristine sulphate in humans.

Vincristine sulphate induced micronuclei in bone-marrow cells of mice and hamsters treated *in vivo*. Conflicting results were obtained for induction of sister chromatid exchanges in human lymphocytes *in vitro*. It induced aneuploidy in and transformation of Syrian hamster embryo cells, but it did not transform mouse C3H 10T1/2 cells. It did not induce chromosomal aberrations, sister chromatid exchanges or unscheduled DNA synthesis in rodent cells *in vitro*. It induced mutation in mouse lymphoma cells but not in other rodent cells. It did not induce sex-linked recessive lethal mutations in *Drosophila* and was not mutagenic to bacteria [ref: 2].

Overall evaluation

Vincristine sulphate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

References

1 IARC Monographs, 26, 365-384, 1981

2 IARC Monographs, Suppl. 6, 563-565, 1987

Annex **E**

Mutagenicity and genotoxicity data

Table E.1 Mutagenicity and genotoxicity of vincristine sulphate in *in vitro* test systems.

test system	dose range	Result - negative + positive	remarks	reference
Mutagenicity				
Ames *Salmonella* assay; TA98 and TA100; with and without metabolic activation	250 µg/mL	-	No further details given	Seino *et al*. 1978[31]
Hprt locus; Chinese hamster lung V79 cells; no metabolic activation	0.3 µg/mL	-	No further details given	Suter *et al*. 1980[32]
TK locus ; mouse lymphoma L5178Y cells; with and without metabolic activation	> 5 to 40 g/mL	+ (>1 g/mL)	No further details given	Matheson *et al*. 1978[33]
Other animal cells	0.03 µg/mL	-	No further details given	Tsutsui *et al*. 1986[34]
Chromosome aberrations				
Chinese hamster cells; with and without metabolic activation	10 µg/mL	-	No further details given	Au *et al*. 1980[35]
Chinese hamster cells; no metabolic activation	0.1 µg/mL	-	No further details given	Maier *et al*. 1976[36]
Syrian hamster cells; no metabolic activation	0.03 µg/mL	-	No further details given	Tsutsui *et al*. 1986[34]
Transformed cells; no metabolic activation	0.05 µg/mL	-	No further details given	Benedict *et al*. 1977[18]

Human peripheral blood lymphocytes; no metabolic activation	0.1 – 1,000 ng/mL	-	No further details given. The author considers the increase in numbers of SCE/cell to be of doubtful if any significance, since it concerned only one observation. In addition the number of SCE/cell observed was in the range of the control limits of 8 donors.	Kučerová and Polívková 1977[11]
Human peripheral blood lymphocytes from one donor	Dose applied was 0.0875 µg/mL for up to 72 hours; negative control was included	+	Chromatid breaks and acentric fragments were observed. Authors also reported of DNA damage in the same cells, as measured by Comet assay, and of polyploidy.	Kopjar and Garaj-Vrhovac 2000[15]
Sister chromatid exchange				
Human peripheral blood lymphocytes; no metabolic activation	0.1 – 10 ng/mL	-	No further details given	Kučerová and Polívková 1977[11]
Human lymphocytes; no metabolic activation	1.0 µg/mL	-	No further details given	Morgan *et al*. 1980[37]
Human lymphocytes; no metabolic activation	0.1 µg/mL	+	No further details given	Raposa *et al.* 1978[38]
Transformed cells; no metabolic activation	1.0 µg/mL	-	No further details given	Banerjee *et al*. 1979[39]
Micronuclei				
Human peripheral blood lymphocytes; no metabolic activation	1 - 5 pM	+ (>3 pM)	Dose-related increase in micronucleated cells	Vian *et al*. 1993[14]
Human peripheral blood lymphocytes (CB-FISH micronucleus assay) ; no metabolic activation	3 – 1.2 nM	+ (>3 nM)	Dose-related increase in micronucleated cells; 87-89% of micronuclei centromere positive; 50% of controls were centromere positive	Darroudi *et al*. 1996[12]
Primary rat astrocytes; no metabolic activation	8.5 – 135 nM	+ (>8.5 nM)	Dose- and time-related increase	Miyakoshi *et al*. 1999[13]
Aneuploidy				
Syrian hamster embryo cells (chromosome counting in metaphases)	1-30 ng/mL	+ (>3 ng/mL)		Tsutsui *et al*. 1986[34]
Human JHU-1 fibroblasts (chromosome counting in metaphases)	1 – 10 ng/mL	+ (10 ng/mL)		Tsutsui *et al*. 1986[34]
Chinese hamster embryo cells	0.005 – 0.50 µg/mL	+	Increases concern both anauploidy and polyploidy	Natarajan *et al*. 1993[16]
Cell transformation				
C3H/10T½ cells; no metabolic activation	0.075 µg/mL	-	No further details given	Benedict *et al*. 1977[18]
Syrian hamster embryo cells, clonal assay; no metabolic activation	1.0 ng/mL	+	No further details given	Tsutsui *et al*.1986[34]
Unscheduled DNA synthesis				
Human fibroblast MRC-5 cells; no metabolic activation	0.0025 - 25 µg/mL	-		Benigni *et al*. 1983[10]
Other animal cells; no metabolic activation	0.10 µg/mL	-	No further details given	Tsutsui *et al*.1986[34]

Table E.2 Mutagenicity and genotoxicity of vincristine sulphate in *in vivo* test systems.

test system	dose range	Result - negative + positive	remarks	reference
Mutagenicity				
Sex-linked recessives lethal test, using *Drosophila melanogaster* flies	15 µg/mL	-	No further details given	Todd *et al*. 1983[40]
Eye mosaic assay, using *Drosophila melanogaster* flies	0 – 0.2 mM	+	The clone induction was relatively low, and was associated with indications of reduced survival and cytotoxicity	Vogel *et al*. 1993[30]
Somatic mutation and recombination test, using *Drosophila melanogaster* flies	0 – 0.05 mM	+		Tiburi *et al.* 2002[29]
Chromosome aberrations				
Pregnant mice, bone marrow and embryonic tissue	single intraperitoneal injection of 0.3 mg/kg bw, on day 6 of pregnancy	+	Increase concerned numerical and structural chromosome aberrations	Sieber *et al.* 1978[26]
Male Swiss mice, spermatogonia	single intraperitoneal injection of 0.25, 0.5 or 1.0 mg/kg bw. Animals were followed for 24 hours, 4 weeks, and 8 weeks	+	Also a statistically significantly increased frequency of aberrant spermatogonial metaphases was observed.	Choudhury *et al.* 2002[25]
Micronuclei (micronucleus test assays)				
Mice, bone marrow	0.05 mg/kg bw	+	No further details given	Maier *et al*. 1976[36]
Mice, bone marrow	0.125 mg/kg bw	+	No further details given	Yamamoto *et al*. 1981[41]
Hamsters	0.2 mg/kg bw	+	No further details given	Norppa *et al*. 1980[42]
CBA mice, bone marrow	single intraperitoneal injection of 0.001 – 0.25 mg/kg bw	+ (>0.025 mg/kg bw)	Dose-related increase in micronucleated polychromatic erythrocytes. Microscopic examination revealed an increased incidence of crescent-shaped and large-sized next to normal-sized micromuclei	Tinwell and Ashby 1991[24]
CD-1 mice, bone marrow and spleen	single intraperitoneal injection of 0.1-0.2 mg/kg bw	+	Dose-related increase in micronucleated polychromatic erythrocytes in bone marrow and spleen in both sexes. In males 87-89% of micronuclei K+, in females 75 - 82%.	Krishna *et al*. 1992, 1994[21,22]
CBA mice, males bone marrow	Single intraperitoneal injection of 0.125 mg/kg bw	+	Percentage centromere positive: 76% in treated animals versus 20% in controls	Grawe *et al.* 1994[20]
Sprague-Dawley rats, males, bone marrow	Single intraperitoneal injection of 0.05-0.2 mg/kg bw	+	Dose- and time-related increase in micronucleated polychromatic erythrocytes	Shi *et al.* 1992[23]

Aneuploidy

Chinese hamsters, bone marrow (chromosome counting in metaphase)	single intraperitoneal injection of 0.25-0.75 mg/kg bw	-	No treatment-related increase in numbers of chromosomes per metaphase was observed	Sheu *et al.* 1990[27]
Chinese hamsters, germ cells: spermatogonia, meiotic I and meiotic II	Single intraperitoneal injection of 0.25 – 0.75 mg/kg bw	+ (hyperploidy)	Treatment-related increased frequency of hyperploid meiotic II metaphase cells at 6, 24 and 48 h but not 72 and 96 h after treatment	Sheu *et al.* 1992[28]

Annex **F**

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.	3
• 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.	(A)
• This compound has been insufficiently investigated. While the available data do not warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is a cause for concern.	(B)
This compound cannot be classified • 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 **G**

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.