Vinblastine 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 *Vinblastine sulphate*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-1481/JR/pg/246-V11
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Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van vinblastinesulfaat. 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. 2007/09OSH, 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 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 vinblastinesulfaat onder de loep. Vinblastinesulfaat is een cytostatisch geneesmiddel dat wordt gebruikt ter bestrijding van kanker.

De commissie meent dat vinblastinesulfaat 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 vinblastine sulphate. Vinblastine sulphate is an antineoplastic cytotoxic agent that is used in the treatment of cancer.

The committee concludes that vinblastine 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.

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 vinblastine 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 vinblastine 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.

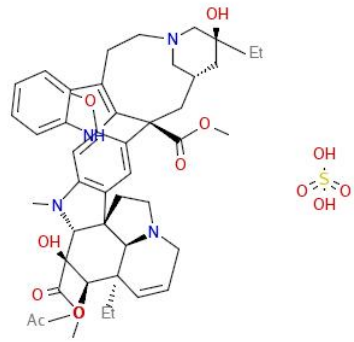
Chapter 2

General information

2.1 Identity and physico-chemical properties

Vinblastine 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] 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 physical and chemical properties.

Chemical name	:	vincaleukoblastine, sulfate (1:1) (salt)
CAS registry no.	:	143-67-9
Synonyms	:	vinblastine; vincaleukoblastine sulfate (1:1) (salt); vincaleucoblastine sulphate; vincaleukoblastine sulphate; Exal; 29060-LE; LE 29060; NSC49842; Velban; Velbe; VLB sulphate.
Description	:	white to slightly yellow, odourless, very hygroscopic, amorphous or crystalline powder.
Occurrence	:	vinblastine 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	:	$C_{46}H_{59}N_4O_9 \bullet H_2SO_4$

Molecular structure	:	
Molecular weight	:	909.1
Melting point	:	284-285°C (monohydrate)
Solubility	:	soluble in water (1 in 10), ethanol (1 in 12,200), chloroform (1 in 50) 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 vinblastine sulphate in humans. Overall, there is no evidence currently available to indicate that vinblastine sulphate is carcinogenic to humans, but the compound has not been extensively investigated. As a consequence, IARC concluded that vinblastine 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, vinblastine sulphate is given together with certain other types of agents, such as bleomycin and dacarbazine, in combination with ionizing radiation therapy.[2] These medicines 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 vinblastine sulphate application alone. Therefore, it is difficult to assess from observational data whether vinblastine 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.

In the seventies of the previous age, various cases were reported on patients with Hodgkin's disease who developed acute non-lymphocytic leukemia after therapy, including application of a mixture of medicines that contained vinblastine sulphate.[1,2] The database also included two cases of patients who were given radiotherapy followed by chemotherapy with vinblastine sulphate alone. Overall, case reports do not allow a conclusion on the association between treatment and secondary tumour development.

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.[3-5] One of these treatments concerned chemotherapy with ABVD (mixture of adriamycine, bleomycin, vinblastine and dacarbazine), with or without radiotherapy. The median follow-up time was 10 years. However, during this follow-up period, none of the ABVD-treated patients developed acute leukemia, nor were their signs of other neoplasms that could be related to former ABVD-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 vinblastine sulphate.[6] Animals (n=25/group/sex/species) were given intraperitoneal injections at maximum or half maximum tolerated doses of vinblastine 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 0.1-0.2 mg/kg bw, the survival time ranged from 18 to 100% (males), and 18 to 100% (females), compared to the survival times of non-treated control animals. The number of tumour-bearing male animals was 11/21. The principal malignant tumours found were 2 lymphosarcomas, 2 pituitary tumours, 1 peritoneal sarcoma, 1 reticulum cell sarcoma, and, 1 testis tumour. The tumour incidence in treated males was considered to be 1.5-2 times greater than that in controls. In female rats, eighteen of them developed tumours (18/25), of which three were malignant. The principal tumours were 11 breast tumours, 7 pituitary tumours, 2 adrenal tumours, and 2 sarcomas. The tumour incidence in vinblastine sulphate treated female animals was slightly greater or comparable to that in controls.

In male and female mice receiving 0.09-0.18 mg/kg bw, the survival time ranged from 41 to 42% (males), and 74 to 98% (females), compared to the survival time in non-treated mice. Only one benign bladder tumour was observed in one male mouse (1/19). In female mice, four animals developed tumours (4/14; 2 lung tumours, 1 tumour in the spleen and 1 in the uterus), of which one was malignant. 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.

To study the carcinogenic effects of vinblastine sulphate, Schmähl and Osswald (1970) divided male BR46 rats in three groups.[7] The first group (n=48) were given intravenous injections at a dose of 0.14 mg/kg bw, once per week for 52 weeks; the second group (n=96) received injections at a dose of 0.33 mg/kg bw, once per two weeks for 10 weeks; and, the third group (n=89) served as control group with no treatment. The survival rates at the time of appearance of first tumour were: 25/48 (first group), 31/96 (second group), and 65/89 (control). The percentage of tumour-bearing animals that died due to the cancer was: 4% (first group, one animal with benign thymoma, which died 18 months after starting the experiment); 3 and 9% (second group, malignant and benign tumours, respectively); and, 5 and 6% (control, malignant and benign tumours, respectively). The median latency period in control group was 23 months.

No other animal carcinogenicity data were available to the committee.

Chapter 4

Mutagenicity and genotoxicity

Vinblastine 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 reviewed in the next sections.

4.1 *In vitro* assays

Vinblastine sulphate did not induce reverse mutations in *Salmonella typhimurium* strains TA98 or TA100, in the presence or absence of a metabolic activation system.[1,2] Using the *hprt* test, no treatment-related mutations were observed in Chinese hamster lung cells (V79 cells).

Using a Comet assay, no significant DNA-damage was observed in L5178Y cells, which were exposed to vinblastine sulphate.[10]

Concerning its aneugenic and clastogenic potential, vinblastine sulphate increased the frequencies of chromosomal aberrations in Chinese hamster Don lung cells. It also increased the number of micronuclei in: V79 cells; human hepatoma (Hep G2) cells (in a dose-dependent matter); human peripheral blood lymphocytes; and, in a skin-based genotoxicity assay, using human skin *in vitro*.[11-14] Overall, based on the current knowledge, vinblastine sulphate is considered an aneugen, indicating that it induces numerical chromosomal changes

rather than chromosome breakage.[15] In somatic cells aneuploidy is associated with the development of several cancers, although the exact mechanism of action of aneugenic agents is not completely understood.

4.2 *In vivo* assays

No data were available to the committee on the mutagenic potential of vinblastine sulphate in humans. In ICR/Ha Swiss mice, which were given a single dose of 4.5 mg vinblastine sulphate/kg bw, the agent did not induce dominant lethal mutations.[16]

Regarding its aneugenic and clastogenic potential, increased presence of micronuclei was found in binucleated peripheral blood lymphocytes of patients with testicular carcinoma, who were treated with curative chemotherapy, compared to untreated cancer patients.[17] However, the significance of these findings is questionable, since the chemotherapy not only contained vinblastine sulphate, but also other drugs.

In a single bone marrow micronucleus study in mice, vinblastine sulphate at a dose of 0.5 mg/kg bw (administration route not given) induced a small increase in micronuclei.[2]

CD-1 mice were treated on day 14 and 15 of gestation with 0.5, 1 and 2 mg/kg bw vinblastine sulphate by intraperitoneal injection at 24 hr intervals, and sacrificed 40 hours after the first injection.[18] Erythrocyte precursor cells in maternal bone marrow and foetal livers from each pregnant mouse were used for micronucleus and sister chromatid exchange analyses. Vinblastine sulphate induced micronuclei in maternal bone marrow (19.8 -fold increase over control value). It also induced micronuclei in foetal liver cells (1.96-fold increase over control value). However, no treatment-related sister chromatid exchanges were observed.

Increased frequencies of micronuclei due to treatment with vinblastine sulphate were also reported by other investigators, such as: Heddle and Bruce (1977; mice)[19] Jenssen and Ramel (1980; mice)[20]; Russo and Pacchietotti (1988; mice)[21]; Salassidis *et al*. (1992; mice)[22]; Satya-Prakash *et al*. (1986; mice)[23]; and, Udroiu *et al*. (2006; new born rats).[24]

Tibura *et al*. (2002) used the wing somatic mutation and recombination test of *Drosophila melanogaster*, to test for genotoxicity of vinblastine sulphate.[25] 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 aberra-

tions, and/or by mitotic recombinations. In balancer-heterozygous flies, at the highest exposure levels, vinblastine sulphate did not show clear increases in total number of spots. In this fly, the presence of spots can be caused by somatic point mutations and/or chromosome aberrations, but not by mitotic recombinations.

Earlier, using the same kind of test, Graf *et al*. (1984) reported that vinblastine sulphate increased the number of large single spots in trans-heterozygous *Drosophila* flies.[26] These single spots may have been caused by point mutations, chromosome aberrations, and /or loss of chromosomes.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

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

Currently, there is inadequate evidence that vinblastine sulphate is carcinogenic to humans. Also, no clear evidence was found that vinblastine 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 committee agrees with IARCs' conclusion that the possible carcinogenicity of vinblastine sulphate has not been extensively investigated.

Vinblastine sulphate did not induce gene mutations in bacteria or mammalian cells, but it did induce micronuclei *in vivo* and *in vitro* in various test systems. In addition, based on the available genotoxicity data and the current understanding of the mechanism of action, many investigators consider the agent as an aneugen, which shows minor ability to induce clastogenic events.

5.2 Recommendation for classification

The committee concludes that vinblastine 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. Vinblastine sulphate. IARC Monogr Eval Carcinog Risk Chem Hum 1981; 26: 349-363.

2 IARC. Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl 1987;(7): 371-372.

3 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.

4 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.

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

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

7 Schmahl D, Osswald H. [Experimental studies on the carcinogenic effects of anticancer chemotherapeutics and immunosuppressive agents]. Arzneimittelforschung 1970; 20(10): 1461-1467.

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 Lee M, Kwon J, Chung MK. Enhanced prediction of potential rodent carcinogenicity by utilizing comet assay and apoptotic assay in combination. Mutat Res 2003; 541(1-2): 9-19.

11 Curren RD, Mun GC, Gibson DP, Aardema MJ. Development of a method for assessing micronucleus induction in a 3D human skin model (EpiDerm). Mutat Res 2006; 607(2): 192-204.

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 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.

14 Liu YG, Wu ZL, Chen JK. Differential effects of aneugens and clastogens on incidences of multinucleated cells and of micronucleate cells in Chinese hamster lung (V79) cell line in vitro. Mutat Res 1998; 413(1): 39-45.

15 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.

16 Epstein SS, Arnold E, Andrea J, Bass W, Bishop Y. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol Appl Pharmacol 1972; 23(2): 288-325.

17 Osanto S, Thijssen JC, Woldering VM, van Rijn JL, Natarajan AT, Tates AD. Increased frequency of chromosomal damage in peripheral blood lymphocytes up to nine years following curative chemotherapy of patients with testicular carcinoma. Environ Mol Mutagen 1991; 17(2): 71-78.

18 Xing SG, Shi X, Wu ZL, Chen JK, Wallace W, Whong WZ *et al*. Transplacental genotoxicity of triethylenemelamine, benzene, and vinblastine in mice. Teratog Carcinog Mutagen 1992; 12(5): 223-230.

19 Heddle JA, Bruce WR. Comparison of tests for mutagenicity or carcinogenicity using assays for sperm abnormalities, formation of micronuclei, and mutations in Salmonella. In: Hiatt HH, Watson JD, Winsten JA, editors. Origins of Human Cancer: Book C. Human Risk Assessment. N.Y.: Cold Spring Harbor Laboratory; 1977: 1549-1557.

20 Jenssen D, Ramel C. The micronucleus test as part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents tested. Mutat Res 1980; 75(2): 191-202.

21 Russo A, Pacchierotti F. Meiotic arrest and aneuploidy induced by vinblastine in mouse oocytes. Mutat Res 1988; 202(1): 215-221.

22 Salassidis K, Huber R, Zitzelsberger H, Bauchinger M. Centromere detection in vinblastine- and radiation-induced micronuclei of cytokinesis-blocked mouse cells by using in situ hybridization with a mouse gamma (major) satellite DNA probe. Environ Mol Mutagen 1992; 19(1): 1-6.

23 Satya-Prakash KL, Liang JC, Hsu TC, Johnston DA. Chromosome aberrations in mouse bone marrow cells following treatment in vivo with vinblastine and Colcemid. Environ Mutagen 1986; 8(2): 273-282.

24 Udroiu I, Ieradi LA, Cristaldi M, Tanzarella C. Detection of clastogenic and aneugenic damage in newborn rats. Environ Mol Mutagen 2006; 47(5): 320-324.

25 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.

26 Graf U, Wurgler FE, Katz AJ, Frei H, Juon H, Hall CB *et al.* Somatic mutation and recombination test in Drosophila melanogaster. Environ Mutagen 1984; 6(2): 153-188.

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 93/31/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. 349)[1]

Summary of Data Reported and Evaluation

Experimental data

Vinblastine sulphate was tested in three studies, two by intraperitoneal injection in mice and rats, and one by intravenous injection in rats. No evidence of carcinogenicity was found, but vinblastine sulphate has not been adequately tested at high doses.

Vinblastine sulphate can induce teratogenic effects in several animal species and embryolethality at doses nontoxic to the mother. On the basis of the available data, this compound cannot be considered to be mutagenic.

Human data

Vinblastine sulphate has been widely used since the early 1960s, almost always in combination with other cytotoxic agents, in the treatment of neoplastic diseases, particularly lymphoma.

The available data are insufficient to evaluate its teratogenic effects in humans. No data on the mutagenicity or chromosomal effects of vinblastine sulphate in humans were available.

Vinblastine sulphate, mainly in combination therapy, has been associated in case reports with the subsequent development of leukaemias. The only epidemiological study was small and of short duration and showed no excess of subsequent neoplasms in patients treated with a regimen including vinblastine sulphate, adriamycin, bleomycin and dacarbazine.

Evaluation

There is no evidence of carcinogenicity in rats or mice on the basis of the available data. The data from studies in man are inadequate to evaluate the carcinogenicity of vinblastine sulphate in humans.

There is no evidence currently available to indicate that vinblastine sulphate is carcinogenic to humans, but the compound has not been extensively investigated.

D.2 Supplement 7: (1987) (p. 371)[2]

CAS No.: 143-67-9
Chem. Abstr. Name: Vincaleukoblastine, sulfate (1:1) (salt)

Evidence for carcinogenicity to humans (inadequate)

No epidemiological study of vinblastine sulphate as a single agent was available to the Working Group. Occasional case reports of exposure to vinblastine sulphate, especially in the presence of concurrent therapy with other putative carcinogens, such as ionizing radiation, alkylating agents and other potent oncotherapeutic drugs, do not constitute evidence of carcinogenesis [ref: 1].

In a large systematic follow-up of patients with Hodgkin's disease treated with an intensive chemotherapeutic combination including vinblastine (plus adriamycin, bleomycin and dacarbazine) but no alkylating agent, preliminary evidence suggests no excess of acute nonlymphocytic leukaemia in the first decade after therapy [ref: 2,3].

Evidence for carcinogenicity to animals (inadequate)

No evidence of carcinogenicity was found after intraperitoneal administration of vinblastine sulphate to mice and rats or after its intravenous administration to rats, but it has not been adequately tested at high doses [ref: 1].

Other relevant data

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

Vinblastine sulphate weakly induced micronuclei in a single study using low doses, but it did not induce dominant lethal mutations in mice treated *in vivo*. It induced chromosomal aberrations but not mutation in Chinese hamster cells *in vitro* and was not mutagenic to bacteria [ref: 4].

Overall evaluation

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

References

1 IARC Monographs, 26, 349-363, 1981

2 Santoro, A., Viviani, S., Villarreal, C.J.R., Bonfante, V., Delfino, A., Valagussa, P. & Bonadonna, G. (1986) Salvage chemotherapy in Hodgkin's disease irradiation failures: superiority of doxorubicin containing regimens over MOPP. Cancer Treat. Rep., 70, 343-348

3 Valagussa, P., Santoro, A., Fossati Bellani, F., Franchi, F., Banfi, A. & Bonadonna, G. (1982) Absence of treatment-induced second neoplasms after ABVD in Hodgkin's disease. Blood, 59, 488-494

4 IARC Monographs, Suppl. 6, 561-562, 1987

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.	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 **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.