Cyclosporin

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Cyclosporin*
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Geachte minister,

Graag bied ik u hierbij het advies aan over de kankerverwekkendheid van cyclosporine. 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. 2007/06OSH, 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 cyclosporine onder de loep. Cyclosporine verlaagt de immunologische afweer en wordt gebruikt als immunosuppressieve therapie voor onder andere patiënten die een orgaantransplantatie hebben ondergaan.

Op basis van de beschikbare gegevens leidt de commissie af dat cyclosporine kankerverwekkend is voor de mens. Dit komt overeen met een classificatie in categorie 1 volgens de richtlijnen van de Europese Unie. De commissie concludeert verder dat cyclosporine niet genotoxisch is.

10 Cyclosporin

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 cyclosporin. Cyclosporin is an immunosuppressive agent that is given to patients to prevent graft-versus-host reactions after organ transplantation, and to control certain diseases in which the immunological defense plays a role.

Based on the available information, the committee is of the opinion that cyclosporin should be classified as known to be carcinogenic to humans. This recommendation corresponds to the EU classification in category 1. The committee concludes furthermore that cyclosporin is not genotoxic.

12 Cyclosporin

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 cyclosporin.

1.2 Committee and procedure

The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. The members of the committee are listed in annex B. The first draft was prepared by MI Willems, from the Department of Occupational Toxicology of the TNO Nutrition and Food Research, by contract with the Ministry of Social Affairs and Employment.

In 2007 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are

listed in annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation and recommendation of the committee is standardly based on scientific data, which are publicly available. The starting points of the committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of cyclosporin, 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

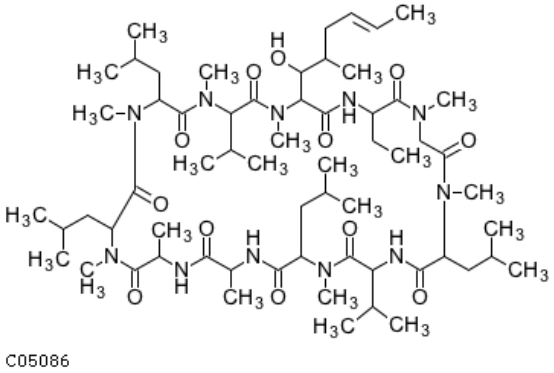
General information

2.1 Identity and physico-chemical properties

Cyclosporin is a fungal nonpolar oligopeptide, consisting of eleven amino acids, including a beta-hydroxy single unsaturated amino acid, which is isolated from the fungus *Tolypocladium inflatum* Grams1. It is the most commonly used immunosuppressive agent for organ transplantation.[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	:	{R-[R*,R*-(E)]}-L-alanyl-N-methyl-L-leucyl-N-methyl-leucyl-N-methyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-amino-6-octenoyl-L--aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl)
CAS registry number	:	79217-60-0 (cyclosporine A: 59865-13-3)
Synonyms	:	Cyclosporin A; ciclosporin, cyclosporin; OL-27-400; cyclo{[(E)-2S,3R,4R)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl}; cyclo{[4-(E)-but-2-enyl-N,4-dimethyl-L-threonyl]-L-homoalanyl(N-methyl-glycyl)(N-methyl-L-leucyl)-L-valyl(N-methyl-L-leucyl)-L-analyl-D-alanyl-(N-methyl-L-leucyl)(N-methyl-L-leucyl)(N-methyl-L-valyl); antibiotic S 7481F1; Ramihyphin A; S 7481F1; Sandimmun; Sandimmune
Description	:	White prismatic crystals from acetone

Molecular weight	:	1202.64
Boiling point	:	-
Melting point	:	148-151 °C
Vapour pressure	:	-
Solubility	:	Cyclosporin is insoluble in water and n-hexane and very soluble in all other organic solvents
Stability	:	Cyclosporin is stable in solution at temperature below 30 °C and is sensitive to light, cold and oxidization
Partition coefficient	:	-
Molecular formula	:	$C_{62}H_{111}N_{11}O_{12}$
Structure	:	

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2.2 IARC classification

In 1990, IARC concluded that there is sufficient evidence for the carcinogenicity of cyclosporin in humans, and that there is limited evidence for the carcinogenicity in experimental animals.[1] Therefore, according to the IARC guidelines, it classified cyclosporin in Group 1, which means that cyclosporin is carcinogenic to humans.

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

3.1.1 Case reports

In 1990, IARC reported in its monograph of numerous case reports of neoplasms in patients, who received long-term cyclosporin therapy alone after organ transplantation.[1] In general, the most reported neoplasms in these recipients were lymphomas, leukaemias and skin cancer (Kaposi's sarcomas). The lymphomas were predominantly found in the gastrointestinal tract.

In his reviews, Ryffel concluded that the incidence of lymphoproliferative disorders and other malignant diseases in cyclosporin-immunosuppressed patients was higher than in the general population, but comparable to patients who were treated with other immunosuppressive agents, such as azathioprine and prednisone.[2,3] Furthermore, data from the Collaborative Transplant Study have suggested that the combination therapy with cyclosporin and azathioprine was associated with an increased risk of non-Hodgkin's lymphoma.[4]

Cases are still reported and not only concern organ transplant recipients, but also psoriatic patients who suffered skin cancer after photochemotherapy. In most of these cases, cyclosporin was the only given immunosuppressive agent in combination with UV-irradiation.[5-11]

In a few case-reports regression of lymphomas were reported, following withdrawal of cyclosporin.[12]

Overall, case reports do not allow a conclusion on the association between cyclosporin treatment and the induction of cancer in humans. No cases were reported on occupational inhalation or dermal exposure in healthy workers.

3.1.2 *Epidemiological studies*

IARC reported on a number of cohort studies on organ transplant recipients.[1] Some of these cohorts concerned combined exposure with cyclosporin and other immunosuppressive agents or cytotoxic drugs, such as cyclophosphamide and azathioprine. Overall, IARC noted that there were a number of limitations in the cohort studies, such as: missing data on dose, survival and follow-up time; uncertainty whether cyclosporin was given in combination with other agents; and, the lack of comparison with the general population. From five cohort-studies, it was certain that only cyclosporin was given. From these studies, IARC estimated the upper limits for the expected values of Non-Hodgkin's lymphoma in organ transplant recipients, which are shown in table 3.1. Although IARC made a lot of assumptions, the incidence of lymphomas was remarkably high.

Table 3.1 Non-Hodgkin's lymphomas in organ transplant patients treated with cyclosporin (IARC 1990).

number of patients	maximal follow-up (years)	Non-Hodgkin's lymphomas		Reference
		expected[a]	observed	
28	1.5	0.02	2	Calne *et al*. (1979)[13]
498	4	1.0	11	Starzl *et al*. (1984)[14]
67	1.5	0.05	0	Bencini *et al*. (1986)[15]
120	5[b]	0.3	0	Sheil *et al*. (1987)[16]
160	5	0.4	0	Smith *et al*. (1989)[17]
873 (total)		1.8	13	

[a] as estimated by the IARC
[b] mean as given in paper

After the publication of the IARC monograph several other cohort studies were performed, of which a short description is given below.

Organ transplant recipients

Cockburn and Krupp reviewed the clinical situation of three different surveillance studies, concerning the occurrence of neoplasms in renal transplant recipients treated with cyclosporin.[18] Below only data of a post marketing surveillance

study are given. The surveillance started in 1982, and in 1989 already 5,000 recipients were followed, which comprised 4,040 renal transplant recipients. The overall incidence for both sexes of *de novo* neoplasms was 1.5%. The most frequent types of neoplasms were skin cancer (Kaposi's sarcomas and basal cell carcinomas), but also some lymphomas were diagnosed, whereas no leukaemia was found. The cyclosporin-treated recipients had a double risk for developing neoplasms compared to the normal non-exposed population. The two-fold increase in risk was comparable with the two- to four-fold increased risk that recipients had, who received other immunosuppressive agents. The authors concluded that the risk between the different types of immunosuppressive therapies did not differ, but that the latency period appeared to be shorter in patients treated with cyclosporin (about 10 months compared to about 3.5 years).

Baildam *et al*. (1996), performed a prospective cohort study on 39 women under the age 55 years, who had received a renal transplant at least 1 year earlier.[19] Of the 29 patients currently receiving cyclosporin or who had previously done so, 13 had fibroadenomas in their breast. None of the patients who received steroids or azathioprine developed fibroadenomas.

Dantal *et al*. (1998) reported of a randomized clinical trial study on renal transplant recipients who received a long-term combination therapy of cyclosporin and azathioprine.[20] The recipients were randomized one year after transplantation in two groups. The first group consisted of 115 recipients who received, after that first year, normal doses of cyclosporin (blood concentration, 150-250 ng/mL), the second group of 115 recipients received low doses of cyclosporin (blood concentration, 75-125 ng/mL). Both groups were followed for 66 months. A total of 60 recipients developed at least one cancer (normal- versus low-dose group, 37 versus 23). The difference between the groups was significant ($p<0.034$). Of the malignant tumours, 66% concerned skin cancer (normal- versus low-dose group, 26 versus 17). Overall, the authors observed that low-doses of cyclosporin reduced the number of malignant disorders, but increased the number of organ rejections. So far, only indirect evidence suggested that lowering the dose of cyclosporin could reduce cancer development.[21]

More recently, Kessler *et al*. (2006) reported on increased standard incidence rates (SIR) for various types of *de novo* cancer in a group of 488 renal transplant recipients, who received dual or triple therapy with cyclosporin, prednisone and azathioprine.[22] The recipients were followed up to ten years after transplantation. For all cancers the SIR was 2.2 (1.5-3.0) in males, and 3.0 (1.9-4.6) in females. More specifically, in males there was a significant excess of cancer for native kidneys, prostate cancer, and post-transplant lymphoproliferative disorder compared with the general population in France; in females it concerned excesses of

cervical cancer, native kidneys, and post-transplant lymphoproliferative disorder. Also the incidence of nonmelanoma skin cancer was high, but the authors could not calculate SIRs for this type of cancer, due to a lack of data in the general population.

Psoriasis patients

In a follow-up study, 1,223 patients with psoriasis who received cyclosporin were followed up for up to 60 months.[23] Also a control group of patients with high blood pressure not receiving such a treatment, and considered to have a same risk of developing cancer as the general population, was included. Of the cyclosporin-treated patients, 28 developed cancer. Almost 50% concerned skin cancer. The relative risk of all cancers in cyclosporin-treated patients versus the control group was 5.6 (95% confidence interval, 3.9-8.0); skin cancer, 12.4 (95% CI, 7.3-21.2); solid tumours, 3.2 (95% CI, 1.8-5.6); and, lymphomas, 5.1 (95% CI, 1.2-21.2). The authors discussed the complexity of their study results, in that several bias may have influenced the outcome. Therefore, it is difficult to prove the causality between cyclosporin-treatment and cancer. At least the study shows a trend that cyclosporin may increase cancer risk in psoriasis patients.

In 2004, Paul *et al.* reported on a prospective five-year cohort study to investigate the association between the incidence of malignancies in severe psoriasis patients and the treatment with cyclosporin.[24] A total of 1,252 patients from 10 different European countries, and Canada, participated in the study. Incidence rates for malignancy were compared to the standardized incidence ratios in the general population. On average the patients received cyclosporin-therapy for 1.9 years. In 3.8% of the patients malignancies were diagnosed, of which 49% had skin malignancies (mostly squamous cell carcinoma). The standardized incidence ratio was 2.1 as compared to the general population. The increased ratio was mainly ascribed to the higher incidence of skin malignancies; the overall incidence of nonskin malignancies was comparable with that of the general population. The authors also noted that a longer treatment period increased the risk for tumour development, and that previous photochemotherapy (psoralen and ultraviolet A) and previous treatment with other immunosuppressive agents contributed to the overall risk.

More recently, Behnam *et al.* (2005) performed a review of the literature in search for human data on dermatology patients and the adverse effects of cyclosporin.[25] Between the period 1979 and 2003, the authors found 26 English articles that provided some relevant data, of which the study by Paul *et al*. (2004) gave the most relevant information.[26] The reviewers commented that most scien-

tific papers had limitations, such as limitations in study design, and lack of comparisons with the general population. The reviewers, however, did not make clear what their final conclusion was on the appearance of malignancies in dermatology patients.

Other diseases

Over a thousand patients with rheumatoid arthritis (RA) were treated with cyclosporin (maximum daily dose of 5 mg/kg bw).[27] Seventeen of these patients developed a malignancy (skin cancer, 4 patients; B-cell lymphoma, 1 patient; solid tumours at various sites of the body, 12 patients). The time between first treatment and the development of the cancer varied greatly and ranged from 8 days to 46 months. Further analyses revealed that the overall relative risk for developing cancer in RA patients is 1.4 (95% CI, 1.4-1.5), which was lower than for cyclosporin-treated RA patients (3.6; 95% CI, 2.2-5.8). However, the relative risk of cyclosporin-treated patients was comparable with the risk of RA patients receiving a different treatment.

No observational studies were performed in an occupational setting in healthy workers, who produced or prepared cyclosporin.

3.2 Carcinogenicity studies in animals

IARC reported on several long-term animal studies.[1] No increased incidence of tumours was observed in OF1 mice (n=50/group/sex), which were given cyclosporin at a daily base in their diet (1, 4, or 16 mg/kg of diet) for 78 weeks, compared to non-treated animals. Also no increased incidence of tumours was observed in OFA rats (n=50/group/sex), which were fed cyclosporin-enriched food (0.5, 2 or 8 mg/kg of diet) for 95 weeks. In a screening assay based on the accelerated induction of leukaemia in a strain highly susceptible to develop this disease, 30 male AKR mice were fed cyclosporin at 150 mg/kg of diet for 17 up to 34 weeks. Not only the latency period was much shorter, but also the treated animals had a higher incidence of leukaemia than non-treated control animals.

IARC also reported on animal studies in which cyclosporin treatment was combined with other treatments.[1] Overall, cyclosporin enhanced the development of lymphomas induced in two strains of male mice by a single whole-body irradiation or by a single injection of N-methyl-N-nitrosourea. In grafted macaques, cyclosporin increased the incidence of lymphomas, a neoplasm that occurs extremely infrequently in this species of monkeys. When given in combination

with various other immunosuppressive regimens, cyclosporin induced a substantial increase in the incidence of lymphomas when compared to immunosuppressive regimens without cyclosporin. The substance also enhanced the incidence of intestinal adenocarcinomas in male rats, which were pre-treated with the genotoxic carcinogen N-methyl-N-nitrosourea.

After the publication of the IARC-monograph a few other animal studies were performed. These studies all concerned the promotion of cancer by cyclosporin, or the accelerated cancer development in genetically modified animals, which were made susceptible for certain diseases.

In 1993, Masuhara *et al*. reported a significant accelerated development of hepatocellular carcinomas in forty male F344 rats fed cyclosporin-enriched diet (amount in diet, 0.015%) for a maximum of 10 weeks, compared to a group of animals that did not receive cyclosporin.[28] This food regimen was followed after an initiation and promotion period of seven weeks, in which the animals were given a single intraperitoneal injection of the genotoxic carcinogen diethylnitrosamine and a choline-deficient diet.

In addition, a comparable animal study was performed by Yabu *et al*. (1991).[29] In that study male F-344 rats were given diethylnitrosamine (DEN) by intraperitoneal injections. Depending on the DEN regimen animals were given cyclosporin-enriched diets (0.011% or 0.015%), starting one or seven weeks after the last injection. A further eight or 14/15 weeks later, all animals were euthanized, after which the livers were examined on the number and size of enzyme-altered foci (glutathion-S-transferase changes, placental form). From the results obtained, the authors concluded that cyclosporin enhanced the growth of carcinogen-induced preneoplastic foci. Whether the enhancement could be ascribed by inhibition of the regression of the foci, or by stimulation of their growth is unclear. Also the authors could not determine whether the preneoplastic lesions observed in the animals could be converted in hepatocellular carcinomas by cyclosporine, because the study duration was too short for finding such an effect.

More recently, Morton *et al*. reported on the cancer promotion of cyclosporin in Eker rats.[30] These rats were genetically modified to study tuberous sclerosis, and are used as a model of dominantly inherited susceptibility to renal cell carcinomas and mesenchymal tumours in other organs. Twenty to twenty-five male animals were given cyclosporin orally by gavage at a dose of 30 mg/kg bw/day, on a daily base for 4 or 6 months. Tumour development was compared to concurrent untreated and vehicle controls. Both after 4 and 6 months, the cyclosporin-treated animals significantly showed more renal neoplasms (adenomas and carci-

nomas) than control groups (4 months: 80 % versus 26.7% (olive oil), respectively, $p<0.0001$; 6 months: 92% versus 55% (olive oil), respectively, $p<0.0001$). The cyclosporin treatment could not last longer, because of the high spontaneous renal neoplasia incidence at one year of age (almost 100%).

Koehl *et al.* (2006) used p53 knock-out mice ($p53^{-/-}$) to study de novo cancer prevention by several classes of immunosuppressive agents (rapamycin, mycophenolate mofetil, and cyclosporin).[31] Concerning cyclosporin, nine mice received cyclosporin in their diet at a daily base for a total of 20 weeks (blood levels of cyclosporin averaged 404±141 ng/mL). The treatment neither promoted, nor inhibited, de novo cancer development, as compared to the non-treated control group. However, the tumours found in cyclosporin-treated animals, showed a more systemic manifestation than in the tumours found in the control group. The authors showed that rapamycin did reduce spontaneous de novo cancer development in the knockout mice.

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

Cyclosporin did not induce reverse mutations in tests with various *Salmonella typhimurium* strains up to a dose of 3,000 µg/plate, nor did it induce mutations in the *hprt* locus of Chinese hamster V79 cells, in the presence or absence of a metabolic activation system.[1,32]

Herman *et al*. (2001) reported of a dose-dependent reduction in DNA repair in peripheral blood mononuclear cells, which were incubated *in vitro* with various concentrations of cyclosporin (up to 50 µg/mL cell suspension).[33] Cyclosporin was added to the cell cultures directly after stopping UV radiation to initiate DNA damage. The blood cells were obtained from healthy blood bank donors. Also in kidney transplant recipients, they found reduced DNA repair activity in blood cells *in vivo* during cyclosporin-treatment; however it is difficult to assess whether the reduction was due to cyclosporin alone, because the recipients received a combination therapy with azathioprine and prednisone.[34,35]

Concerning clastogenic activity, no chromosomal aberrations were found in human peripheral blood lymphocytes obtained from healthy volunteers, with or without a metabolic activation system, at cyclosporin doses up to 300 µg/mL.[36] However, in another study, sister chromatid exchanges (SCE) were observed in the same type of cells, in the absence of an activation system (cyclosporin doses

up to 4 μg/mL).[37] Later the same authors repeated the test and noted again that cyclosporin induced sister chromatid exchanges, but to a lesser extent than other immunosuppressive agents.[38] Zwanenburg (1988), who reviewed the results of this study, explained the lower SCE frequency by the low cell growth.[39] Overall, it is difficult to associate clastogenicity found in human cells to cyclosporin exposure, because other yet unknown biological factors may have influenced the outcome.

4.2 *In vivo* assays

Concerning mutagenicity, Fracasso *et al*. (1993) studied the urinary mutagenic activity in fifty kidney transplant recipients, using a liquid incubation assay with *E. Coli* WP2uvrA as the test strain.[40] This assay detects base pair substitutions in the presence or absence of a metabolic activation system. The recipients were divided into three groups: i) recipients (n=10) receiving an oral dose of cyclosporin (4.5 mg/kg bw); ii) recipients (n=20) receiving an oral dose of azathioprine (1.26 mg/kg bw); and, iii) recipients (n=20) receiving a combined oral dose of cyclosporin (3.89 mg/kg bw) and azathioprine (1.15 mg/kg bw). No mutagenic activity was detected in the group treated with cyclosporin alone. Contrary to this finding, 85% of the urines of recipients receiving azathioprine alone, and 40% of the urines of recipients receiving the combined therapy showed high mutagenic activity.

Using male CD-1 mice, Matter *et al.* (1982) could not demonstrate dominant lethal mutations after a single oral application of 100 and 1,000 mg cyclosporin/kg bw, nor did they observe unscheduled DNA synthesis.[41] In another study, although the study reporting was limited, also no unscheduled DNA synthesis was observed in blood lymphocytes of kidney transplant recipients, who underwent immunosuppressive therapy with cyclosporin.[1]

Ember and Kiss (1997) studied the expression of several oncogene and suppressor genes (c-myc, Ha-ras, p53) in CBA/Ca mice (n=6/group/sex), which received a single intraperitoneal dose of cyclosporin of 16 mg/kg bw, or cyclophosphamide (20 mg/kg bw).[42] The dose of cyclosporin corresponded to the therapeutic doses given to humans. As measured after 2 and 6 days, and 1, 6 and 12 months after the treatment, in cyclosporin-treated mice no significant induction of the gene expression was observed in the thymus, spleen, bone marrow, and mesenteric lymph nodes. On the contrary, in those organs, cyclophosphamide did increase the expression of all the measured genes.

A few studies were performed in organ transplant recipients to investigate cytogenetic or clastogenic endpoints. For instance, in the blood lymphocytes of thirty renal transplant recipients, the frequencies of sister chromatid exchanges were tested before and 3 months after the start of a cyclosporin therapy. As a result of the therapy, the SCE frequencies increased significantly compared to the pre-treatment period ($p<0.05$).[43]

IARC reported on a study, in which twenty-five kidney transplant recipients showed increased frequencies of chromosomal aberrations in their blood lymphocytes during cyclosporin therapy.[1] However, due to serious methodological shortcomings, the results of this study should be regarded as questionable.

Matter *et al*. (1982) applied cyclosporin (up to 1,500 mg/kg bw) orally to CD-1 mice and Chinese hamsters (4 animals/group).[44] However, no induction of micronucleated erythrocytes in bone marrow smears was observed. In addition, no chromosomal aberrations could be detected in Chinese hamsters (6 animals/group).

4.3 Carcinogenic mechanism

Overall, the precise carcinogenic mechanism(s) of cyclosporin are not completely understood by insufficient evidence. However, the weight of evidence from experimental data indicates a lack of genotoxicity. In addition, Rosenkranz and Klopman proposed that cyclosporin is devoid of mutagenicity, clastogenicity and DNA-modifying activity.[45,46] They made this conclusion by using a knowledge-based structure-activity relational expert system, called CASE.

Since cyclosporin is used as an immunosuppressive agent, and chemical immunosuppression carries the intrinsic risk of tumour growth, immunosuppression was, until recently, the most likely explanation of the tumour stimulatory effect of cyclosporin.[3,47,48]. Also certain other immunosuppressive agents, such as azathioprine and cyclophosphamide, have been associated with increased risk of cancer.[49,50]

As an immunosuppressive agent, cyclosporin affects both humoral and cellular immune defence systems. The exact mechanism of suppression is not completely understood, but it is clear that cyclosporin blocks the production of several lymphokines, such as interleukin-2, which are produced by T-lymphocytes.[1,51-53] This blockage prevents the stimulation of cells, which are involved in the regulation of immune responses. In addition, the impairment of immunological defence system may result in uncontrolled neoplastic cell growth after induction by a viral infection or other causes.[2,3,51,54,55]

In 2004, André *et al.* hypothesised that not only immunosuppressive properties of cyclosporin stimulate tumour growth, but also its ability to facilitate accumulation of DNA mutations, to diminish the clearance of altered cells, and to transform cancer cells into aggressive cancer cells.[56] They postulated their hypothesis after reviewing several scientific papers on the carcinogenic action mechanism of cyclosporin, which are published in the past years. In addition, five years earlier, Hojo *et al*. performed a series of *in vitro* experiments, and an *in vivo* animal study using immune-deficient SCID-*beige* mice, to study possible cancer progression of cyclosporin by mechanisms other than immunosuppression.[57] The investigators found that cyclosporin altered the characteristics of several cancerous cell lines *in vitro* and *in vivo*, into more aggressive and invasive cells. Furthermore, they found out that these effects were prevented by adding antibodies directed at transforming growth factor-β (TGF-β). The latter finding suggests that cyclosporin stimulates the production of TGF-β, a factor that not only stimulates cancer growth, but also suppresses immune responses.[58,59] Also Masuhara *et al*. (1993) postulated that cyclosporin may promote tumour development by stimulating specific growth factors, but he did not find evidence for this in an experimental animal model.[60]

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

Overall, data obtained from epidemiological studies on organ transplant recipients and psoriasis patients, who received cyclosporin therapy, produced sufficient evidence that cyclosporin acts as a systemic carcinogen in humans. The types of cancer included lymphomas, leukaemias and skin cancer.

Increased numbers of tumours were found in animals, in which cancer was initiated by genotoxic carcinogens or by irradiation, or in genetically modified animals, which are prone to cancer development. The types of cancer observed included lymphomas, leukaemia, intestinal adenocarcinomas, hepatocellular carcinomas, and renal cancer. No increased cancer development was observed in two animal studies, using normal animals without pre-treatment to initiate cancer.

Overall, the exact carcinogenic mechanisms are not completely unravelled, but since mutagenicity and genotoxicity tests were negative, the committee is of the opinion that cyclosporin is not mutagenic nor genotoxic. There are, however, strong indications that cyclosporin stimulates and promotes tumour development by suppressing the immunological defence system by inhibiting lymphokine production. More recently, it is also suggested that cyclosporin may stimulate cancer development by stimulating specific growth factors that are directly involved in cancer cell growth.

5.2 Recommendation for classification

Based in the available information, the committee is of the opinion that cyclosporin should be classified as known to be carcinogenic to humans. This recommendation corresponds to the EU classification in category 1. The committee, furthermore, concludes that cyclosporin is not genotoxic.

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10-4 and 10-6 per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council.

Annex **B**

The committee

- G..J. Mulder, *chairman*
  emeritus professor of toxicology, Leiden University, Leiden
- P.J. Boogaard
  toxicologist, SHELL International BV, The Hague
- Ms. M.J.M. Nivard
  Molecular biologist and genetic toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  epidemiologist, Dow Chemicals NV, Terneuzen
- R.A. Woutersen
  toxicologic pathologist, TNO Nutrition and Food Research, Zeist
- A.A. van Zeeland
  professor of molecular radiation dosimetry and radiation mutagenesis, University Medical Center, Leiden
- E.J.J. van Zoelen
  professor of cell biology, Radboud University Nijmegen, Nijmegen
- J.M. Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

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

The Health Council and interests

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

Annex **C**

Comments on the public review draft

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

- G. Jonkers, Vereniging van Verf en Drukinktfabrikanten, the Netherlands;
- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain;
- R.D. Zumwalde, National Institute for Occupational Safety and Health, the USA.

Annex **D**

IARC Monograph

VOL.: 50 (1990) (p. 77)1

CAS No.: 79217-60-0 (cyclosporin A)
CAS No.: 59865-13-3 (cyclosporine)
Chem. Abstr. Name: R-[R*,R*-(E)]}-L-Cyclic(L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-leucyl-N-methyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-amino-6-octenoyl-L- -aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl)

Summary of Data Reported and Evaluation

Exposure data

Ciclosporin has been used as an immunosuppressive agent since the mid-1980s.

Experimental carcinogenicity data

Ciclosporin was tested for carcinogenicity by oral administration in two studies in mice and in one study in rats. In one study in mice, it accelerated the development of leukaemias; tumours were not induced in a chronic bioassay. In rats, negative results were obtained in a study with limited sensitivity.

Ciclosporin enhanced the development of lymphomas induced in two strains of male mice by single whole-body irradiation or N-methyl-N-nitrosourea. In grafted macaques, ciclosporin increased the incidence of lymphomas, a neoplasm that occurs extremely infrequently in this species of monkeys. When given in combination with various other immunosuppressive regimens, ciclosporin induced a substantial increase in the incidence of lymphomas when compared to immunosuppressive regimens excluding ciclosporin. This drug also enhanced the incidence of intestinal adenocarcinomas induced in male rats by N-methyl-N-nitrosourea.

Human carcinogenicity data

In case reports, both lymphomas and Kaposi's sarcoma have been associated frequently with exposure to ciclosporin. Four cohort studies recorded a high incidence of lymphoma in organ transplant recipients; in two of these, ciclosporin was given without azathioprine or cytotoxic drugs. In several cases, there has been well-documented regression of lymphoma following withdrawal of the drug.

Other relevant data

Ciclosporin induced dose-dependent changes in reproductive organ weights in male rats and caused sterility at high doses. Fetal mortality was observed in rats and rabbits when the drug was administered during the second half of gestation at maternally toxic doses. No other sign of embryo- or fetotoxicity was noted.

Ciclosporin is rapidly absorbed and widely distributed in humans and in experimental animals. It is extensively metabolized by the cytochrome P450 system. Adverse effects include nephro- and hepatotoxicity. The compound is immunosuppressive, resulting in tolerance to tissue grafts; its main effect is on the early proliferation of T-cells.

In a single study, ciclosporin was reported to increase the incidence of chromosomal aberrations in the lymphocytes of kidney transplant patients.

Ciclosporin did not induce dominant lethal mutations in mice, chromosomal aberrations in the bone marrow of Chinese hamsters or micronuclei in the bone marrow of Chinese hamsters or mice in vivo. It induced sister chromatid exchange in human peripheral lymphocytes in vitro but did not induce gene

mutations in Chinese hamster cells. Ciclosporin did not induce mutations in Salmonella typhimurium.

Evaluation

There is sufficient evidence for the carcinogenicity of ciclosporin in humans.

There is limited evidence for the carcinogenicity of ciclosporin in experimental animals.

Overall evaluation

Ciclosporin is carcinogenic to humans (Group 1).

Annex **E**

Carcinogenic classification of substances by the committee

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

Judgment of the committee	*Comparable with EU class*
This compound is known to be carcinogenic to humans • It is stochastic or non-stochastic genotoxic • It is non-genotoxic • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	1
This compound should be regarded as carcinogenic to humans • It is stochastic or non-stochastic genotoxic • It is non-genotoxic • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	2
This compound is a suspected human carcinogen. • This compound has been extensively investigated. Although there is insufficient evidence for a carcinogenic effect to warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is cause for concern. • This compound has been insufficiently investigated. While the available data do not warrant a classification as ‘known to be carcinogenic to humans’ or as ‘should be regarded as carcinogenic to humans’, they indicate that there is a cause for concern.	3 (A) (B)
This compound cannot be classified • There is a lack of carcinogenicity and genotoxicity data. • Its carcinogenicity is extensively investigated. The data indicate sufficient evidence suggesting lack of carcinogenicity.	not classifiable

Annex **F**

Guideline 93/21/EEG of the European Union

4.2 Criteria for classification, indication of danger, choice of risk phrases

4.2.1 Carcinogenic substances

For the purpose of classification and labelling, and having regard to the current state of knowledge, such substances are divided into three categories:

Category 1:

Substances known to be carcinogenic to man.

There is sufficient evidence to establish a causal association between human exposure to a substance and the development of cancer.

Category 2:

Substances which should be regarded as if they are carcinogenic to man.

There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer, generally on the basis of:

- appropriate long-term animal studies
- other relevant information.

Category 3:

Substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

4.2.1.1 The following symbols and specific risk phrases apply:

Category 1 and 2:

T; R45 May cause cancer

However for substances and preparations which present a carcinogenic risk only when inhaled, for example, as dust, vapour or fumes, (other routes of exposure e.g. by swallowing or in contact with skin do not present any carcinogenic risk), the following symbol and specific risk phrase should be used:

T; R49 May cause cancer by inhalation

Category 3:

Xn; R40 Possible risk of irreversible effects

4.2.1.2 Comments regarding the categorisation of carcinogenic substances

The placing of a substance into Category 1 is done on the basis of epidemiological data; placing into Categories 2 and 3 is based primarily on animal experiments.

For classification as a Category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species; together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Category 3 actually comprises 2 sub-categories:

a substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.

b substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high levels exceeding the 'maximal tolerated dose'. The maximal tolerated dose is characterized by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain;
- appearance of tumours, especially at high dose levels, only in particular organs of certain species is known to be susceptible to a high spontaneous tumour formation;
- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds);
- if the particular target is not relevant to man;
- lack of genotoxicity in short-term tests *in vivo* and *in vitro*;
- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation;
- existence of a species - specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.

For a distinction between Category 3 and no classification arguments are relevant which exclude a concern for man:

- a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man;
- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories;
- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.