Ifosfamide

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Ifosfamide*
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Geachte minister,

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

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Ifosfamide

Evaluation of the carcinogenicity and genotoxicity

Subcommittee on the classification of carcinogenic substances of the
Dutch Expert Committee on Occupational Standards,
a committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2008/06OSH, The Hague, April 1, 2008

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues...” (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature & Food Quality. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens het uitoefenen van hun beroep kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie ifosfamide onder de loep. Ifosfamide is een cytostaticum en een immuunsupressief middel dat wordt gebruikt ter behandeling van kanker.

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

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. In this report, the committee evaluated ifosfamide. Ifosfamide is used as an antineoplastic and immunosuppressive drug.

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

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Chapter 1

Scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification (see Annex A). The assessment and the proposal for a classification are expressed in the form of standard sentences (see Annex E). The criteria used for classification are partly based on an EU-directive (see Annex F). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question.

This report contains the evaluation of the carcinogenicity of ifosfamide.

1.2 Committee and procedures

The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the committee. The members of the committee are listed in Annex B. The first draft was prepared by I.A. van de Gevel and M.I. Willems, from the Department of Occupational Toxicology of the TNO Nutrition

and Food Research, by contract with the Ministry of Social Affairs and Employment.

In 2007 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

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

More recently published data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in June 2007. The new relevant data were included in this report.

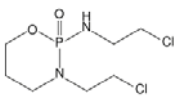
Chapter 2

General information

2.1 Identity and physico-chemical properties

Ifosfamide is used as an cytostatic and immunosuppressive drug to treat cancer, such as oatcell tumours in the lung, ovarian cancer, breast cancer, and non-Hodgkin's lymphomas.[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.[1]

Chemical name	:	2*H*-1,2,3-oxazaphophorin-2-amine; N,3-bis(2-chloroethyl)tetrahydro-,2-oxide
CAS registry no.	:	3778-73-2
EINECS no.	:	223-237-3
Synonyms	:	Isophosphamide; N,3-bis(2-chloroethyl)- 2-oxo-1-oxa-3-aza-2λ5-phosphacyclohexan-2-amin; Ifosfamide; IFO; Iphosphamide; NSC-109724; Ifex®; Holoxan®; Ifo-cell®
Description	:	White crystals
Molecular formula	:	$C_7H_{15}Cl_2N_2O_2P$
Structure	:	
Molecular weight	:	261.1
Melting point	:	48-50 °C
Vapour pressure	:	1.05 mPa (25 °C); 1.2 mPa (40 °C)

Solubility	:	Soluble in water and carbon disulphide
Stability	:	Very sensitive to hydrolysis, oxidation, and heat

2.2 IARC classification

In 1981, IARC concluded that there is limited evidence for the carcinogenicity of ifosfamide in mice and rats, and that in the absence of data on humans, no evaluation can be made of the carcinogenic risk of ifosfamide in man.[1] Therefore, in 1987 and according to the IARC guidelines, it classified ifosfamide in Group 3.

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

No data were available to evaluate the carcinogenicity of ifosfamide in humans.

3.2 Carcinogenicity studies in animals

The American National Cancer Institute performed a carcinogenicity study in rats and mice, of which the results were published in 1977.[2] Groups of 35 male and 35 female Sprague-Dawley rats and B6C3F1 mice received intraperitoneal injections of ifosfamide at doses of 6 or 12 mg/kg bw (rats), and 10 or 20 mg/kg bw (mice), three times per week for 52 weeks. The study also included untreated controls, and matched or pooled vehicle controls. After the end of exposure, the animals were observed for a further 31 weeks (rats) and 28 weeks (mice), before the study was terminated.

Concerning rats, from 25 weeks onwards, the mean body weight of the high-dose group was slightly lower than the low-dose and control groups. Also in the high-dose group, a significant higher rate of mortality in both males and females was observed compared to the low-dose group and the vehicle controls; at week 52 only 9/35 high-dose males, and 8/35 high-dose females were still alive (see Table 3.1).

Also in Table 3.1, a summary of the number of tumour-bearing animals is given.

Table 3.1 Summary of carcinogenicity data of Sprague-Dawley rats receiving intraperitoneal injections of ifosfamide for one year.[2]

	Control		Ifosfamide treatment	
	untreated	matched vehicle	6 mg/kg bw	12 mg/kg bw
Male rats				
Animals initially in study	10	10	35	35
Percentage of animals alive at termination	70	80	55	6
Animals examined histopathologically	8	9	32	34
No. of animals with primary tumours	4	5	17	12
No. of animals with malignant tumours	2	2	9	8
Female rats				
Animals initially in study	10	10	35	35
Percentage of animals alive at termination	100	70	42	3
Animals examined histopathologically	10	10	33	34
No. of animals with primary tumours	7	9	32	9
No. of animals with malignant tumours	2	6	23	6

In both treated and control male and female rats, a variety of tumours were found at various sites of the body. None of these tumours could be associated with exposure, except the occurrence of mesenchymal stromal tumours (*i.e.*, fibromas, fibrosarcomas, sarcomas, leiomyosarcomas); males and females, respectively: controls, 0/20 and 0/20; low-dose, 5/32 and 17/33; high-dose, 3/34 and 2/34. These types of tumours are not uncommon for the strain used. Regarding mammary fibroadenomas, in the low-dose group 28 of 33 animals developed such tumours, whereas in the high dose group the number of tumour-bearing animals was 6/34, and in controls 3/10. Furthermore, the controls showed a significant higher incidence of pituitary tumours compared to treated male and female rats.

Since in the high-dose group the mortality rates were very high, the investigators performed time-adjusted analyses, in which the mesenchymal stromal tumour incidence of treated groups were compared with matched or pooled vehicle controls. In treated male rats, no significant increases in tumour incidences were observed compared to controls. In low-dosed female rats, statistically significant increases in incidences of fibroadenomas in the mammary gland, and of leiomyosarcomas of the uterus were found. No such significant increases were found in the high-dosed group, but this could be explained by the low power of the test due to the small sample size of the vehicle and the high-dose treatment group.

Concerning mice, the mean body weight did not differ among the groups. Data on mortality are shown in Table 3.2. In treated mice, various types of

Table 3.2 Summary of carcinogenicity data of B6C3F1 mice receiving intraperitoneal injections of ifosfamide for one year.[2]

	Control		Ifosfamide treatment	
	untreated	matched vehicle	10 mg/kg bw	20 mg/kg bw
Male rats				
Animals initially in study	15	15	35	35
Percentage of animals alive at termination	90	7	31	31
Animals examined histopathologically	14	14	29	27
No. of animals with primary tumours	2	0	6	4
No. of animals with malignant tumours	0	0	1	1
Female rats				
Animals initially in study	15	15	35	35
Percentage of animals alive at termination	90	90	77	66
Animals examined histopathologically	15	14	32	34
No. of animals with primary tumours	0	0	7	15
No. of animals with malignant tumours	0	0	7	14

tumours were observed in different parts of the body, including the lungs, the liver, adrenal glands, skin (females only), and hemapoietic system (females only). In male mice, the tumour incidences were not significantly increased compared to pooled vehicle controls.

However, in female mice, a statistically significant dose-related increase in the number of animals with malignant lymphomas in the hematopoietic system was observed (controls, 0/14-15; low-dose, 3/32; high-dose, 13/34).

Overall, the committee noted the high mortality in most treated groups. This may have suppressed late tumour development, and, as the investigators also remarked, has lowered the power of the statistical analyses. The Working Group of IARC, which also evaluated this study, considered that insufficient matched control animals were available for evaluation, and that comparison with pooled control animals provides suggestive but inconclusive evidence for carcinogenicity.[1]

Groups of twenty male and female A/He mice, a strain known to be susceptible for developing lung tumours, were given intraperitoneal injections of ifosfamide at doses of 0 (vehicle control, sixty animals), 18.8 or 47 mg/kg bw, 3 times per week for 8 weeks.[3] An additional group received only five injections at a dose of 260 mg/kg bw. The study was terminated 24 weeks after the first administration. The survival rates were 100% (controls and low-dose group), 85% (high-dose group), and 60% (additional group). The lung tumour incidences were 26 and

36% (male and female controls, respectively), 60% (low-dose group), 76% (high-dose group), and 50% (additional group). The numbers of lung tumour-bearing animals in treated groups were statistically significantly increased compared to the control group.

Mitrou *et al*. (1979a/b) studied the effects of chronic use of immunosuppressive agents on the incidence of neoplasia, using female (NZB x NZW) mice.[4,5] The strain is used to investigate systemic lupus erythematosis, since the animals produce antinuclear antibodies and easily develop immune complex glomerulonephritis. The authors were interested in factors that may affect development of neoplasia, such as age, dose, and frequency of administration. The various treatments, median survival times, and tumour incidences are summarized in Table 3.3. Ifosfamide was given subcutaneously.

Overall, taken the 120 and 180 old animals together, the overall tumour incidences were 3% (controls), 13% (0.2 mg/mouse), 34.5% (0.4 mg/mouse), and 25.5% (2 mg/mouse). According to the authors the increase in tumour incidence in treated animals was significant. In the treated animals, a total of 23 lymphomas, 7 undifferentiated sarcomas, 1 fibrosarcoma, 6 adenocarcinomas of the lung, and 1 granulocytic leukaemia were found, whereas in control animals only 3 malignant lymphomas were located. No details were given as to what kinds of tumours were found in specific groups. The authors concluded that treatment with ifosfamide prolonged significantly survival time, but that it induced a dose-related increase in tumour frequency. The starting age did not affect tumour development. The Working Group of IARC noted that in the absence of age-adjusted comparisons, early death in (NZB x NZW) mice from autoimmune disease precluded direct comparison with treated mice.[1]

Table 3.3 Survival and tumour incidences in female (NZBxNZW) mice exposed to ifosfamide.[4,5]

	no. of animals	median survival time (days)	tumour incidence (%)	type of tumours
Control group (120 and 180 days old)				
0.0 mg/mouse, 5x/week, 14-16 months	96	292	3.0	n.r.
120 days (±17 weeks) of age				
0.2 mg/mouse, 5x/week, 14-16 months	34	417	12.0	n.r.
0.4 mg/mouse, 5x/week, 14-16 months	29	600	38.0	n.r.
2.0 mg/mouse, 1x/week, 14-16 months	15	600	26.5	n.r.
180 days (±27 weeks) of age				
0.2 mg/mouse, 5x/week, 14-16 months	27	435	15.0	n.r.
0.4 mg/mouse, 5x/week, 14-16 months	23	600	26.0	n.r.
2.0 mg/mouse, 1x/week, 14-16 months	20	600	13.5	n.r.
6, 7, 8, or 9 weeks of age				
0.2 mg/mouse, 5x/week, 7 or 8 months	9-10/age	n.r.	1 malignant lymphoma, age group not reported	

n.r., not reported.
0.2 mg/mouse = 6 mg/kg bw; 0.4 mg/mouse = 12 mg/kg bw; 2.0 mg/mouse = 60 mg/kg bw.

Chapter 4

Mutagenicity and genotoxicity

4.1 *In vitro* assays

Ifosfamide was tested on mutagenic activity in the *Salmonella typhimurium* bioassay. In the presence of an exogenous metabolic activation system, various authors reported on positive outcomes in strains TA 1535 and TA 100.[1,6-8] No mutagenic activity was observed in any of the tested strains in the absence of a metabolic activation system, in the presence of a naphthoflavone-metabolic system (TA100), and rat colon mucosa fractions (TA 100).[8] Addition of mixed-function oxidase inhibitors, such as naphthoflavone, metyrapone, and SKF 525-A, inhibited the metabolic activity systems, resulting in negative outcomes.[6]

Ifosfamide was also tested for mutagenicity in vapour phase at 23 °C and 37 °C in strain TA100.[9] In the presence of a metabolic activation system a significant increase in mutagenic frequency was observed at 37 °C, but not at 23 °C, indicating less or no vapourisation at room temperature. When tested in the standard plate incorporation assay at both temperatures, the compound scored positive.

Ifosfamide induced also forward and back mutations in *Escherichia coli* strain 343/113, but only in the presence of a metabolic activation system.[7]

Regarding clastogenic effects, the agent induced a dose-related increase in frequency of sister chromatid exchanges in Chinese hamster V79 cells.[10] This increase was only observed when a metabolic activation system was added.

4.2 *In vivo* assays

In *Drosophila melanogaster* flies, ifosfamide induced recessive lethal mutations.[11] However, no dominant lethal mutations or chromosome loss was observed.

Cavallo *et al*. (2005, 2007) and Ursini *et al*. (2006) of the same research group, investigated the presence of DNA-damage in circulating blood lymphocytes and/or exfoliated buccal cells obtained from a group of healthcare workers, who performed many drug administrations per week.[12-14] These drugs included various mixtures of antineoplastic agents, including ifosfamide. The authors found increased levels of DNA-damage (micronuclei, chromosomal aberrations) in exfoliated buccal cells, and to a much lesser extent in lymphocytes. In control individuals no excess in DNA damage was observed. Since exposure concerned mixed exposure and the study population was rather small, it is difficult to assess whether ifosfamide was responsible for the observed effects. Therefore, based on these data the subcommittee is not able to make a conclusion on the genotoxicity of this compound.

In addition, Burgaz *et al*. (2002) found increased frequencies of chromosomal aberrations in peripheral blood lymphocytes in healthcare workers, who were daily handling various antineoplastic drug in a hospital.[15] Also this study is of less value for genotoxicity evaluation of ifosfamide, because exposure included also other drug at the same day.

In the bone-marrow cells of Chinese hamsters, which were given a single intraperitoneal injection of ifosfamide, a dose-dependent increase in chromosomal aberrations was reported (Rohrborn and Basler, 1977; source IARC81).[1,16]

In another study, groups of NMRI mice (n= 4 mice/sex/group) were given two intraperitoneal injections of ifosfamide at doses of 0, 35, 70 or 140 mg/kg bw, once daily for two successive days. Six hours after the second injection the animals were killed and bone marrow smears prepared. At non-lethal and non-toxic levels, the agent induced a dose-related increase in frequency of micronucleated polychromatic erythrocytes.[17]

4.3 Carcinogenic mechanism

The mechanism(s) through which ifosfamide may exert its carcinogenic and genotoxic effects is not completely clarified. Yet, there is some information

available on the mechanism of its antineoplastic activity that might also explain its mutagenic potency.

Ifosfamide, an oxophosphorine, is known as a prodrug that requires metabolic activation before it can exert its antineoplastic and cytotoxic effects. In the liver, the agent undergoes cytochrome P450-catalyzed 4-hydroxylation that yields phosphoramide mustard, the therapeutically active DNA cross-linking metabolite, and acrolein. The prodrug may also undergo P450-catalyzed side chain oxidation that generates inactive *N*-dechloroethylated metabolites, and the neurotoxic and nephrotoxic byproduct chloroacetaldehyde.[1,18-20] Also chloroacetaldehyde is able to cross-link with DNA.[21]

More specifically, oxophosphorines are able to alkylate DNA by attaching the N-7 position of guanine with their reactive electrophilic groups, and to a lesser extent to the N-1 and N-3 positions of adenine, N-3 position of cytosine, and O-6 of guanine.[22,23] These cross-linking modifications may result in cytotoxicity and cell death, and for this reason ifosfamide is used as an antineoplastic agent[18-20] However, the modifications in the DNA structure may also result in mutagenicity and carcinogenicity. Further research on the action mechanism is needed to verify this possibility.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

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

Ifosfamide given by intraperitoneal injections or subcutaneously for a prolonged period, augmented exposure-related tumor incidence in one female rat strain and in various mice strains, and in various organs (*i.e.*, malignant lymphomas, lung tumours, and haematopoietic tumours). All of these studies had some limitations, such as short exposure duration, high early mortality, the use of vulnerable mice strains, and the correct use of control animals. However, overall, the whole set of data are indicative for the carcinogenic potency of ifosfamide.

Ifosfamide is a known DNA alkylating agent. It has been shown to be mutagenic in the standard bacterial mutagenicity bioassay. Furthermore, it showed to be clastogenic *in vitro* and *in vivo*. For these reasons, the committee is of the opinion that ifosfamide acts by a stochastic genotoxic mechanism.

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

5.2 Recommendation for classification

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

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Annexes

Annex **A**

Request for advice

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

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

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

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

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

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

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

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

Annex **B**

The committee

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

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

The Health Council and interests

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

Annex **C**

Comments on the public review draft

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

- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain;
- R.D. Zumwalde, National Institute for Occupational Safety and Health, the USA.

Annex **D**

IARC Monograph

Vol.: 26 (1981) (p. 237)
CAS No.: 3778-73-2
Chem. Abstr. Name: 2*H*-1,3,2-Oxazaphosphorin-2-amine, *N*,3-bis(2-chloroethyl)-tetrahydro-, 2-oxide

Summary of Data Reported and Evaluation

Experimental data

Isophosphamide was tested in four studies in mice and in one in rats by subcutaneous or intraperitoneal administration. In one study in mice with intraperitoneal injection it produced an increased incidence of lung adenomas. The other four studies, although indicating a carcinogenic effect, could not be evaluated.

Isophosphamide can induce teratogenic effects in mice and embryolethality at doses nontoxic to the mother. Isophosphamide is mutagenic in bacteria and produced chromosomal aberrations in Chinese hamster bone-marrow cells.

Human data

Isophosphamide has been used to a limited but increasing extent since the early 1970s as an antineoplastic and immunosuppressive drug. No data were available to evaluate the teratogenic or mutagenic potential or chromosomal effects of iso-

phosphamide in humans. No case report or epidemiological study on isophosphamide was available to the Working Group.

Evaluation

There is *limited evidence* for the carcinogenicity of isophosphamide in mice and rats. In the absence of data on humans, no evaluation can be made of the carcinogenic risk of isophosphamide to man.

Subsequent evaluation: Suppl. 7 (1987) (p. 65: Group 3)

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.