Health Council of the Netherlands

Thiotepa

Health-based calculated occupational cancer risk values

2015/17

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Thiotepa*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-783200/BvdV/cn/459
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Geachte minister,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan thiotepa.

Dit advies maakt deel uit van een uitgebreide reeks, waarin concentratieniveaus in lucht worden afgeleid die samenhangen met een extra kans op (overlijden aan) kanker van 4 per 1.000 en 4 per 100.000 door beroepsmatige blootstelling. De conclusies van het genoemde advies zijn opgesteld door de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en omgeving.

In dit advies concludeert de commissie dat thiotepa een carcinogene stof is. De commissie is echter van mening dat wegens gebrek aan adequate humane en dierexperimentele gegevens het niet mogelijk is om de extra kans op kanker na blootstelling aan thiotepa te berekenen.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. J.L. Severens,
vicevoorzitter

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Health-based calculated occupational cancer risk values

Dutch Expert Committee on Occupational Safety,
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2015/17, The Hague, July 16, 2015

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 and health (services) research...” (Section 22, Health Act).

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid, leidt de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad, de concentraties van een stof in de lucht af die overeenkomen met een vooraf vastgesteld extra risico op sterfte aan kanker (4 per 1.000 en 4 per 100.000 individuen) door beroepsmatige blootstelling gedurende het arbeidzame leven. Het gaat om kankerverwekkende stoffen die door de Gezondheidsraad of de Europese Unie geclassificeerd zijn in categorie 1A of 1B en die kankerverwekkend zijn via een stochastisch genotoxisch mechanisme. Voor de schatting maakt de commissie gebruik van de *Leidraad Berekening risicogetallen voor carcinogene stoffen* van de Gezondheidsraad.[1] In dit advies onderzoekt de commissie de mogelijkheid om zo'n schatting voor thiotepa te maken. Thiotepa wordt als chemotherapeuticum aan patiënten toegediend. Beroepsmatige blootstelling komt voor bij werknemers die betrokken zijn bij de productie, bereiding en toediening van deze stof.

De commissie concludeert dat thiotepa een carcinogene stof is met een stochastisch genotoxisch werkingsmechanisme.

De commissie is van mening dat wegens gebrek aan adequate humane en dierexperimentele gegevens het niet mogelijk is om de extra kans op kanker na blootstelling aan thiotepa te berekenen.

Executive summary

At the request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Safety (DECOS), a Committee of the Health Council of the Netherlands, derives so-called health-based calculated occupational cancer risk values (HBC-OCRVs) associated with excess mortality levels of 4 per 1,000 and 4 per 100,000 as a result of working life exposure to substances. It concerns substances which are classified by the Health Council or the European Union in category 1A or 1B, and which are considered stochastic genotoxic carcinogens. For the estimation, the Committee uses the *Guideline for the calculation of occupational cancer risk values* of the Health Council.[1] In this report the Committee evaluates the possibility to establish such estimates for thiotepa. Thiotepa is administered to patients as a chemotherapeutic agent. Occupational exposure occurs in employees involved in the production, preparation and administration of this substance.

In this report, the Committee concludes that thiotepa is a carcinogenic substance with a stochastic genotoxic mechanism.

The Committee is of the opinion that due to a lack of adequate human and animal data, it is not possible to establish the health-based calculated occupational cancer risk values for thiotepa.

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for genotoxic chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Safety (DECOS), a Committee of the Health Council of the Netherlands, at request of the Minister of Social Affairs and Employment (Annex A). This evaluation should lead for thresholded genotoxic substances to a health-based recommended occupational exposure limit for the concentration of the substance in air. Such an exposure limit cannot be derived if the toxic action has no threshold, as is the case for substances with stochastic genotoxic carcinogenic properties. In that case, an exposure-response relationship is recommended for use in regulatory standard setting, i.e., the calculation of so-called health-based calculated occupational cancer risk values (HBC-OCRVs). The Committee calculates HBC-OCRVs for compounds, which are classified as stochastic genotoxic carcinogens by the European Union or by the Committee.

For the establishment of the HBC-OCRVs, the Committee generally uses a linear extrapolation method, as described in the Committee's reports *Calculating cancer risk* and *Guideline for the calculation of occupational cancer risk values*.[1,2] The linear model to calculate occupational cancer risk is used as a

default method, unless scientific data would indicate that using this model is not appropriate.

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister of Social Affairs and Employment on the feasibility of using the HBC-OCRVs as regulatory occupational exposure limits. In the final step of the procedure, the Minister sets the official occupational exposure limits.

1.2 Committee and procedure

The present document contains the evaluation of the DECOS, hereafter called the Committee. The members of the Committee are mentioned in Annex B. The Committee requested the DECOS Subcommittee on the classification of carcinogenic substances to evaluate the genotoxic mechanism of theotepa (see Annex G and H). The recommendations of the Subcommittee were used by DECOS to decide on the appropriate approach to risk assessment.

The submission letter (in English) to the Minister can be found in Annex C. In February 2015, the president of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft are listed in Annex D. The Committee has taken these comments into account in deciding on the final version of the advisory report. The received comments, and the replies by the Committee, can be found on the website of the Health Council.

1.3 Data

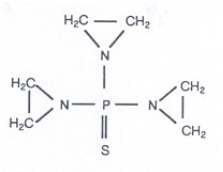
The Committee's recommendation has been based on scientific data, which are publicly available. Data were obtained from the online databases Toxline, Medline and Chemical Abstracts, using carcinogenic properties, carcino*, cancer, neoplastic, thiotepa and CAS registry number as key words. In addition, in preparing this report the following reviews were consulted: IARC reviews and the NTP Report on Carcinogens.[3-6] The last search covered the period 1997-May 2015 (a previous search was conducted in 1997 and covered the period 1965 to January 1997).

Chapter 2

Identity, toxicity profile and classification

2.1 Identity and physical and chemical properties

Thiotepa is used as chemotherapeutic agent. Physical and chemical data shown below are from http://toxnet.nlm.nih.gov (HSDB and ChemIDplus data bases, accessed May 1, 2015).

Chemical name	:	tris(1-aziridinyl)phosphine sulfide
CAS number	:	52-24-4
EINECS number	:	200-135-7
EEC number	:	--
IUPAC name	:	thiotepa
Synonyms	:	triethylenethiophosphoramide; *N,N"*-triethylenethiophosphamide; *N,N',N"*-tri-1,2-ethanediylphosphoro-thioictriamide; NSC-6396; aziridine, 1,1'1"-phosphinothioylidynetris; TSPA; WR-45312
Physical description and colour	:	Crystalline solid, white
Molecular formula	:	$C_6H_{12}N_3PS$
Structure	:	

Molecular weight	:	189.2
Melting point	:	51.5 °C
Boiling point (101.3 kPa)	:	no data
Density	:	no data
Solubility in water	:	190 g/L in water at 25 °C
Solubility in organic solvents	:	soluble in alcohol, benzene, ether, chloroform, diethyl ether
Octanol/water partition coefficient, Log $P_{oct/w}$	:	0.53
Vapour pressure (25 °C)	:	8.45E-3 mm Hg
Relative vapour density (air = 1)	:	no data
Flash point	:	no data
Odour threshold	:	no data
Conversion factors (25 °C, 101.3 kPa)	:	no data
EU classification	:	no classification

2.2 Classification as a carcinogenic substance

The European Union did not classify thiotepa. IARC concluded that there is sufficient evidence for the carcinogenicity of thiotepa in humans and in experimental animals and has classified the compound as a group 1 carcinogen (*carcinogenic to humans*).[5] In 2011 the 12the NTP Report on Carcinogens considers thiotepa as *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans.[6]

In the present evaluation the Committee (DECOS) follows the recommendation of the DECOS Subcommittee on the Classification of Carcinogenic Substances and classifies thiotepa in category 1A (*known to be carcinogenic to humans*) (see Annex G and H).

2.3 Genotoxicity

Thiotepa is a direct alkylating agent with potent genotoxic activity in a wide variety of prokaryotic, lower eukaryotic, and mammalian in vitro and in vivo test systems. Thiotepa causes DNA damage, mutations, micronucleus formation, and/or chromosomal aberrations in somatic and germ cells from exposed rodents, rabbits, and nonhuman primates and chromosomal aberrations in peripheral-blood lymphocytes from treated humans (NTP RoC 2011; IARC 1990; Chen et al., 1999; Casciano et al., 1999).[5-8]

The Committee (DECOS) follows the recommendation of the DECOS Subcommittee on the Classification of Carcinogenic Substances (see Annex G) and concludes that thiotepa is a stochastic genotoxic carcinogen.

2.4 Non-carcinogenic effects

Thiotepa is poorly absorbed from the gastrointestinal tract. No data for the dermal or inhalation route of exposure were found.

In rats, rabbits, dogs and humans tepa (N,N',N"- triethylenephosphoramide) was found to be the main metabolite of thiotepa. In the mouse, thiotepa is metabolized to inorganic phosphate as the only detectable product. The conversion of thiotepa to tepa is catalyzed by specific cytochrome isoenzymes. Excretion of thiotepa and tepa can be monitored in urine, but the urinary excretion of thiotepa and tepa differs per species and accounts only for a limited fraction of the administered dose. (Maanen et al. (2000)[9]; EMA website: http://www.ema.europa.eu/ema/, accessed May 1, 2015).

In humans, toxicity to the haemopoietic system (severe myelosuppression) was observed in 13 ovarian cancer patients treated with two intravenous bolus injections of thiotepa (60 and 80 mg, 4-week interval between doses).[10] Other side effects in this study were limited to transient nausea and vomiting in two patients on the day of treatment with 80 mg.

Myelosuppression was also observed in a study with 27 children with malignancies refractory to conventional therapy.[11] Nineteen children received an intravenous bolus of thiotepa at a starting dose of 25 mg/m^2 with escalations to 50, 65 or 75 mg/m^2 (only one escalation dose was allowed in an individual patient). Eight children received an 8-hour infusion at 50 or 65 mg/m^2. The maximum tolerated bolus dose was 65 mg/m^2, and the dose-limiting toxicity was myelosuppression, characterized by granulocytopenia and thrombocytopenia. Myelosuppression was the only clinically significant toxicity. Nausea and vomiting was uncommon (2 patients) up to the highest dose examined.

In a study by Lazarus et al., twenty-five patients with malignancies resistant to conventional chemoradiation therapy or for which no effective therapy is known were treated with intravenous escalating doses of thiotepa (135-1,215 mg/m^2 over 3 days).[12] Treatment was followed by reinfusion of previously cryopreserved autologous bone marrow (3 days after the last dose of thiotepa). The organs that were affected most by thiotepa were bone marrow, gastrointestinal tract and CNS. All patients experienced severe neutropenia and thrombocytopenia. The six patients treated with 135 or 270 mg/m^2 developed minimal extramedullary toxicity. Five of the 14 patients treated with ≥810 mg/m^2 experienced moderate to severe diarrhoea, stomatitis, esophagitis or other

mucosal injury. Five patients treated at ≥810 mg/m^2 experienced severe infection while neutropenic, four of which died. One patient of the 810 mg/m^2 group died due to intracranial haemorrhage. Damage to the CNS occurred in three patients, all treated at 1,005 mg/m^2.

Regarding experimental animals, the oral LD50 is 38 mg/kg bw in mice.[13] The LD50 in rats was approximately 9.5 mg/kg bw after intravenous injection and about 8.8 mg/kg bw after intra-arterial injection.[5]

Thiotepa is teratogenic in mice treated at 1 mg/kg bw (intraperitoneal; lowest single teratogenic dose), and induces developmental effects in rats at 4 mg/kg bw by intraperitoneal injection on gestation day 12.[5]

2.5 Occupational exposure and existing occupational exposure limits

Health-care professionals may be potentially exposed during the preparation and administration of the compound in cancer therapy.[14,15] Also workers involved in its formulation and packaging may be potentially exposed. The (American) National Occupational Exposure Survey (1981-1983) indicated that 11,452 workers, including 8,724 women, potentially were exposed to thiotepa (NIOSH 1990).[6,16] The Committee did not find reliable data with regard to the present size of the exposed population (Kauppinen et al. 2000).[17]

Worldwide, no occupational exposure limits for thiotepa are set at the present time (http://www.ser.nl, accessed May 1, 2015).

Chapter 3

Carcinogenicity studies

3.1 Observations in humans

Table 1 (Annex E) summarizes four main epidemiological studies with thiotepa.

Kaldor et al. (1990) assessed the risk of acute or non-lymphocytic leukaemia associated with exposure to thiotepa, in a multicentre nested case-control study that was imbedded in a cohort of 99,113 survivors of ovarian cancer, previously treated with chemotherapeutics.[18] In this study, 114 case patients who were diagnosed with leukaemia at least one year after the diagnosis with ovarian cancer were matched with control patients who survived free of a second cancer for at least as long as the interval between the diagnosis of ovarian cancer and leukaemia in the case patient (3 controls per case of leukaemia). The time between the diagnosis of ovarian cancer and leukaemia was between two and nine years in 74% of the cases, and acute or non-lymphocytic leukaemia's represented 89% of the total. To investigate the association of thiotepa exposure with the risk of leukaemia, the analysis was conducted on patients with ovarian cancer for which the only chemotherapy had been thiotepa (9 cases with leukemia and 11 controls). These patients were assigned to a low dose group (4 cases, 5 controls) and a high dose group (5 cases, 6 controls). To create these groups, the median dose in controls (30 and 600 mg thiotepa for the low and high dose, respectively) was used as cutoff point. No other data on the treatment were given in the report. The relative risk for acute or non-lymphocytic leukaemia compared to patients receiving only radiotherapy or surgery was 8.3 ($p<0.05$) for

the low dose and 9.7 (not statistically significantly different from 1.0) for the high dose. [The Committee considers this study well performed although it is aware of the small number of patients treated with thiotepa.]

In the three other studies no increased risk of secondary malignancies was found among patients with colorectal or breast cancer and treated with thiotepa. In a study by Boice et al. (1980) (randomized clinical trial), 470 male patients with colorectal cancer received surgical resection and low-dose chemotherapy with thiotepa and were compared with 867 male colorectal patients treated with surgery alone.[19] Thiotepa was administered at 0.8 mg/kg bw (4 doses of 0.2 mg/kg bw at and two days after surgery) and the patients were followed up for up to 19 years with an average survival of 6.8 years. Due to the small number of non-white patients, analysis was limited to white patients. No difference in the observed/expected ratio of second malignancies was observed among patients treated with thiotepa (0.9; 28 cases observed /30.7 expected) compared to patients who received surgery alone (1.0; 57 cases observed /55.4 expected). [The Committee considers this a well-performed study, but recognizes that the number of patients included is not large].

In a retrospective study of Chan et al. (1980) 633 breast cancer patients received simple or radical surgery (mastectomy) with or without postoperative radiotherapy and were then treated with 0.6 mg/kg bw thiotepa perisurgically followed by prophylactic therapy with 1 mg thiotepa/kg bw every three months for up to two years.[20] A group of 632 breast cancer patients treated with surgery only or with surgery followed by radiotherapy were used as historical controls. All patients were followed between 5 to 10 years. The total incidence of secondary breast carcinoma was the same in the thiotepa group and the control group (5.7%). The total incidence of second non-breast malignancies was 6.3% in the thiotepa group and 5.4% in the control group. In total 12.0% of the thiotepa treated patients developed a secondary tumour compared with 11.1% in the control group. The authors concluded that prolonged adjuvant thiotepa chemotherapy does not seem to increase the risk of second primary cancer. [The Committee recognizes that the number of patients included is not large. Further it is noted that the thiotepa group and the control group were not comparable with respect to the fraction of patients given radiotherapy after surgery (7% and 36% in the thiothepa and control group, respectively).]

In a prospective randomized clinical trial by Kardinal & Donegan (1980), 90 women treated with radical mastectomy for early cancer of the breast were subsequently treated with 0.8 mg/kg bw thiotepa perisurgically and then with 0.2 mg/kg bw thiotepa once weekly for one year.[21] Seventy-seven breast cancer patients treated with radical mastectomy only served as control group. The

average follow-up period was 64.6 months in the thiotepa group and 61.6 months in the control group. Excluding skin cancer (basal cell tumours), 11 patients developed second cancers: five (5.6%) in the thiotepa group and six (7.8%) in the control group. Using these numbers and total follow up values of 5,819 and 4,746 person years for the thiotepa group and the control group, respectively, the Committee calculated a relative risk of 0.68 for developing a second malignancy after thiotepa treatment. The authors stated that the intergroup differences in total or site-specific second cancers were not significant. [However from the report it is not clear whether any statistical analysis was performed. Further, the Committee recognizes that the number of participants is too low.]

[The Committee recognizes that these three latter studies were clinical trials and not designed specifically to analyze the long term side-effects of thiotepa. Moreover due to the low number of patients none of the studies had sufficient power to find any side effects.]

3.2 Carcinogenicity studies in animals

Table 2 (Annex F) summarizes the available carcinogenicity studies in experimental animals. Thiotepa was tested for carcinogenicity by intraperitoneal administration in male and female mice and rats and by intravenous administration in male rats. A short description of these studies is given below.

In a screening assay by Stoner et al. (1973), based on accelerated induction of lung tumours in a mouse strain (A/He) highly susceptible to development of this neoplasm, ten mice per sex per dose received 19, 47 or 94 mg/kg bw thiotepa (total doses) by intraperitoneal injection over a period of 4 weeks.[22] The experiment was terminated 24 weeks after the first injection. All thiotepa treated animals except one of the 47 mg/kg bw group survived. Survival in the control groups was 94% (untreated control) or 96% (vehicle control). The incidence of lung tumours was 11 (55%), 20 (100%) and 16 (80%) in the low, mid and high dose groups, respectively, compared to 19 (19%) and 38 (24%) in untreated and vehicle controls, respectively. The number of lung tumours per mouse at the mid and high dose was statistically significantly increased compared to controls. [The Committee noted that this study is limited (only one type of tumour investigated, small number of treated animals, short treatment and observation periods) and that a positive result in this pulmonary tumour screening assay should be confirmed by other test systems.]

The carcinogenicity of thiotepa was also determined in a study performed by the National Cancer Institute (NCI) of the US Department of Health, Education, and Welfare.[23] Male and female Sprague-Dawley rats (31-39/sex/dose) were treated intraperitoneally with thiotepa in phosphate-buffered saline at dose levels of 0.7, 1.4 or 2.8 mg/kg bw three times a week for a maximum period of 52 weeks followed by observation periods (length depending on the dose level) resulting in experimental periods of 82-87 weeks. Mean body weights of all dosed male rats, particularly those of the mid- and high-dose groups, were depressed throughout the study, when compared with either matched or vehicle controls; those of the dosed females were less markedly depressed. Thiotepa decreased survival in a dose-related manner. At the high dose, all males and females were dead by week 19 and 21, respectively. At the mid dose, all males were dead by week 78 and only 8.6% of the females survived till the end of study. At the low dose, survival was 15.4% in males and 42% in females. Survival in controls was 80-100%. The high dose of 2.8 mg/kg bw was too toxic for an evaluation of carcinogenic activity. Treatment with thiotepa was associated with increased incidences of squamous-cell carcinoma in the skin or ear canal, neuroepitheliomas and nasal carcinomas in each sex, haematopoietic tumours (lymphoma, lymphocytic leukaemia, or granulocytic leukaemia) in male rats, adenocarcinoma of the uterus, and adenocarcinoma of the mammary gland. These tumours are considered to be relevant for humans. [Although this study has limitations, such as pooled control groups to obtain significance (see Table 2 in Annex F), it allows the conclusion that thiotepa is carcinogenic in Sprague-Dawley rats.]

In the same study by NCI, male and female B6C3F1 mice (35/sex/dose) were treated ip with thiotepa in phosphate-buffered saline at dose levels of 1.15 or 2.3 mg/kg bw three times a week for a maximum period of 52 weeks followed by an observation period resulting in an experimental period of 86 weeks (or only 43 or 56 weeks in high-dose females and males, respectively, due to mortality).[23] Body weight depression was observed in high dose animals, particularly in females. All males and females of the high dose group were dead by week 56 and 43, respectively. At the end of the experimental period, 15 (43%) male and 17 (49%) female mice of the low dose group survived compared to 7 (46%) males and 12 (80%) females in the vehicle control group. Treatment with thiotepa was associated with increased incidences of squamous cell carcinoma (at skin, ear and preputial gland) in male mice and of haematopoietic tumours (lymphoma and lymphocytic leukaemia) in male and female mice. These tumours are considered to be relevant for humans. [Although this study has limitations (see

Table 2 in Annex F), it allows the conclusion that thiotepa is carcinogenic in B6C3F1 mice.]

In the study of Schmähl and Osswald (1970), 48 male R46 rats were treated with 1 mg/kg bw thiotepa by intravenous injection once a week for 52 weeks.[24] Malignant tumours were observed in 9 of the 30 rats that were still alive when the first tumour appeared. These tumours occurred at a variety of sites (see Table 1 in Annex E). The incidence of malignant tumours in controls was 4/65. The authors stated that the difference in total tumour incidence between treated rats and controls was statistically significant (no p value was reported). [The Committee noted that this study has several flaws (short exposure period, only one dose tested, only one sex used, no statistical analysis performed on individual tumour types, high early mortality in treated group).]

3.3 Risk assessment

Limited evidence is available that thiotepa is carcinogenic to humans. Sufficient evidence is available that thiotepa is carcinogenic to animals. The Committee classifies thiotepa in category 1A (*known to be carcinogenic to humans*) and concludes that a stochastic genotoxic mechanisms underlies carcinogenicity.

The logical approach to risk assessment would be the derivation of health-based calculated occupational cancer risk values (HBC-OCRVs). However, in view of the Committee none of the animal or human studies is sufficiently adequate for quantitative risk assessment. Therefore the Committee concludes that due to a lack of adequate human and animal data, it is not possible to establish the health-based calculated occupational cancer risk values for thiotepa.

3.4 Additional consideration

In spite of this conclusion above (paragraph 3.3) the Committee decided to process the data from the epidemiological studies from Kaldor et al.[18], Boice et al.[19], Chan et al.[20], Kardinal & Donegan[21] in a quantitative risk calculation in order to speculate on the exposure levels related to an additional life-time cancer risk. The results of this calculation are presented in Annex I. However, given the uncertainties as discussed above (paragraph 3.1) and in Annex I the Committee decided not to use this calculation.

The Committee emphasizes that thiotepa is a potent carcinogen. With the exception of cancer patients no individual should be exposed to this compound. Therefore, workers involved in handling the compound in patient-treatment, or in packaging etc., should avoid exposure.

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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. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Innovation for Life; Professor of Translational toxicology, Wageningen University and Research Centre, Wageningen
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- D.J.J. Heederik
  Professor of Risk Assessment in Occupational Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational Hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders (NECORD), Utrecht
- H. van Loveren
  Professor of Immunotoxicology, Maastricht University, Maastricht; National Institute for Public Health and the Environment, Bilthoven
- A.H. Piersma
  Professor of Reproductive Toxicology, National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  Professor of Molecular Biology, VU University Amsterdam; Netherlands Cancer Institute, Amsterdam

- I.M.C.M. Rietjens
  Professor of Toxicology, Wageningen University and Research Centre, Wageningen
- G.B.G.J. van Rooy
  Occupational Physician, Arbo Unie Expert Centre for Chemical Risk Management; Radboud UMC Outpatient Clinic for Occupational Clinical Toxicology, Nijmegen
- F.G.M. Russel
  Professor of Molecular Pharmacology and Toxicology, Radboud University, Nijmegen
- G.M.H. Swaen
  Epidemiologist, Maastricht University, Maastricht
- R.C.H. Vermeulen
  Epidemiologist, Institute for Risk Assessment Sciences, Utrecht
- P.B. Wulp
  Occupational Physician, Labour Inspectorate, Groningen
- B.P.F.D. Hendrikx, *advisor*
  Social and Economic Council, The Hague
- G.B. van der Voet, *scientific secretary*
  Toxicologist, Health Council of the Netherlands, The Hague

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 chairperson 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 inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter (in English)

Subject : Submission of the advisory report *Thiotepa*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-783200/BvdV/cn/459
Enclosed : 1
Date : July 16, 2015

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to thiotepa.

This advisory report is part of an extensive series in which carcinogenic substances are evaluated for the possibility to establish health-based occupational cancer risk values in accordance with European Union guidelines. This involves substances to which people can be exposed under working conditions.

The advisory report was prepared by the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council. The advisory report has been assessed by the Health Council's Standing Committee on Health and the Environment.

In this report, the Committee concludes that thiotepa is a carcinogenic substance.

The Committee is of the opinion that due to a lack of adequate data, it is not possible to estimate the additional lifetime cancer risk for thiotepa.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,
(signed)
Professor J.L. Severens
Vice President

Annex **D**

Comments on the public review draft

A draft of the present report was released in February 2015 for public review. The following organizations and persons have commented on the draft document:

- Coggon D. University of Southampton, Southampton, UK
- Lentz TJ, Ding M, Settle T. National Institute for Occupational Safety and Health (NIOSH), Cincinnati OH, USA.

Annex **E**

Epidemiological studies

Table 1 Thiotepa, epidemiological studies.

Study design and population	Data on exposure and health assessment	Results	Remarks
Kaldor et al.[18] Nested case-control study.8 Country: Canada, 7 European countries. Participants: 114 case patients with leukaemia and 342 matched controls selected from a cohort of 99,113 ovarian cancer patients. Thiotepa treated case patients with leukaemia: 9 (4 low dose, 5 high dose); thiotepa treated matched control patients free of a second cancer: 11 (5 low dose, 6 high dose).	Selected patients were divided into a low (30 mg) and high (600 mg) dose group; median dose in controls was used as cutoff point to create dose groups. The risk of leukaemia was determined relative to patients receiving surgery or radiotherapy. Appropriate statistical analysis performed.	Relative risk of leukaemia (acute or nonlymphocytic) after thiotepa treatment compared to surgery and radiotherapy is 8.3 in the low-dose group ($p<0.05$) and 9.7 (not statistically significantly different from 1.0) in the high-dose group.	Study used to calculate cancer risk value. Well performed with limitations. No details on treatment reported; small number of cases and controls.
Boice et al.[19] Randomized clinical trial.9 Country: USA Participants (enrolled in the study between 1958 and 1964): 470 male patients with colorectal cancer treated with thiotepa after surgery, followed for 3102 person-years; 867 non-exposed controls (colorectal cancer treated with surgery only). Follow-up period: up to 19 years (1977), mean survival 6.8 years.	Dose: 0.8 mg thiotepa/kg bw in total (4 doses of 0.2 mg/kg bw: 1 i.v. and 1 i.p. at surgery, 2 i.v on 1st and 2nd day after surgery). Appropriate statistical analysis performed.	Observed/Expected ratio of second malignancies among thiotepa-treated patients (0.9) not different from ratio in surgery-treated patients (1.0)	Well performed with limitations. Follow-up period (mean survival 6.8 years) may be too short; sample size of 470 patients is not large.

Chan et al.[20] Retrospective study.10 Country: USA Participants (treated with mastectomy from 1953-1967): Cases: 633 breast cancer patients treated with long-term adjuvant thiotepa therapy after mastectomy with or without radiotherapy. Historical controls: 632 breast cancer patients treated with mastectomy with or without radiotherapy. Follow-up period: 5-10 years.	Dose: 0.6 mg/kg bw perisurgical, followed by prophylactic therapy (1 mg/kg bw) 3 months after surgery and then every three months for maximally 2 years (total dose in each 3-month period did not exceed 60 mg).	No difference in incidence of second primary cancers (12% in thiotepa treated patients, 11% in controls).	Well performed with limitations. Follow-up period (5-10 years) may be too short; sample size of 633 patients is not large; treated and control group differed with respect to fraction of patients treated with radiotherapy (7% in control group, 36% in treated group); no statistical analysis performed.
Kardinal et al.[21] Prospective randomized clinical trial.11 Country: USA Participants (accrued from 1963-1972): breast cancer patients treated with radical mastectomy and then randomly assigned to long-term adjuvant thiotepa therapy (90 patients) or no further treatment (77 patients, control group). Follow-up period: 64.4 and 61.6 months (average) in thiotepa and control group, respectively.	Dose: 0.8 mg/kg bw i.v. perisurgical, and then 0.2 mg/kg bw once weekly for 1 year.	No difference in survival between the groups. Relative risk of second malignancies (skin basal cell tumours excluded) after thiotepa treatment is 0.68 (5/5819 person-year)/ (6/4746 person year). The authors stated that this difference was not significant.	Well performed with limitations. Follow-up period (about 5 years) may be too short; small number of participants; no information on statistical analysis.

i.p. = intraperitoneal; i.v. = intravenous

Annex **F**

Animal studies

Table 2 Thiotepa, animal studies.

Study design and animal species	Data on exposure and effect endpoints	Results	Remarks
Stoner et al.[22] Mouse (A/He) Treated: 10/sex/dose Control: 50/sex untreated and 80/sex vehicle control	Intraperitoneal injection, total doses of 19, 47, 94 mg/kg bw (12 injections in 0.1 mL tricaprylin, 3 times/week) X_{po} = 4 weeks X_{pe} = 24 weeks	*Survival:* 20/20, 19/20, 20/20 in low, mid, and high dose, resp., 94/100 and 154/160 in untreated and vehicle controls, resp. *Lung tumour incidence:* 11/20, 20/20, 16/20 in low, mid, high dose, resp., 19/100 and 38/160 untreated and vehicle controls, resp. Number of lung tumours per mouse was statistically significantly higher at the high dose (1.50; $p<0.001$) and mid dose (0.74; $p<0.05$) compared to vehicle controls (male 0.24, female 0.20).	Klimisch score: 3 Supportive study. Positive result in A mouse lung tumour screening assay should be confirmed by other test systems. Histopathology restricted to lungs and suspicious tissues seen at necropsy; gross examination limited to liver, kidneys, spleen, thymus, intestines, and salivary and endocrine glands. Short administration and observation period. Insufficient number of animals. Not clear whether decedents were included in statistical analysis.

NCI[23] Sprague Dawley rat 35 rats/sex at mid and high dose, 39 males and 31 females at low dose. Concurrent control: 10 untreated and 10 vehicle treated rats/sex; Pooled control: vehicle controls from different studies added to give a total of 30 rats/sex in each of 2 pooled vehicle control groups (one for the low dose, one for the mid dose).	Intraperitoneal injection, 0.7, 1.4, 2.8 mg/kg bw, 3 times/week X_{po} = 21 weeks (high dose), 34 weeks (mid dose), 52 weeks (low dose and controls) X_{pe} = high dose 21 weeks, mid dose 78 (males) or 81 (females) weeks, low dose and controls 82-87 weeks. Appropriate statistical analysis performed (for tumours: based solely on rats surviving 52 wk or, when tumour of interest was seen earlier, at least as long as the time at which the first tumour of interest was seen; high-dose animals excluded because of high early mortality).	*Survival:* High dose: no survivors by week 19 (males) or 21 (females). Mid dose: no male survivors by week 78, 8.6% of females survived till end of study. Low dose: 15.4 and 42% survival by the end of the study. Control: 80% (males) or 70% (females) survival in mid and high dose vehicle controls, 100% (males) or 90% (females) in low dose vehicle controls. *Adverse effects:* Decreased mean body weight in males (especially at the mid and high dose). Effect in females less pronounced. *Tumour incidences (relative to pooled controls):* *Haematopoietic* (lymphoma, lymphocytic leukaemia or granulocytic leukaemia) in males: 6/34* at low dose, 6/16* at mid dose, 0/29 low-dose control, 0/30 mid-dose control. *Squamous-cell carcinoma of skin or ear canal*: Males: 7/33* at low dose, 3/13* at mid dose, 0/29 low-dose control, 0/30 mid-dose control; Females: 8/21* at mid dose, 0/28 in mid-dose control. *Adenocarcinoma uterus*: 2/29 at low dose, 7/21* at mid dose, none in control. *Adenocarcinomas mammary gland*: 8/24* at mid dose, 1/28 in mid-dose control. *Neuroepitheliomas or nasal carcinomas*: 3 in low dose males, 2 in low dose females, 2 in mid dose females, 0 in controls. * Statistically significantly different from pooled control.	Klimisch score: 2 Well performed with limitations. Pooled control groups used to obtain statistical significance (too few concurrent controls); maximum tolerated dose exceeded in mid and high dose group; no microscopy conducted on high-dose rats (because of high mortality); short exposure period. To control respiratory disease (clinically evident in treated rats and controls), oxytetracycline was given in weeks 24-35 (in drinking water).
NCI[23] B6C3F1 mouse Treated: 35 mice/sex/dose. Concurrent control: 15 untreated and 15 vehicle treated mice/sex Pooled control: vehicle controls from different studies added to give a total of 30 mice/sex in each of 2 pooled vehicle control groups (one for the low dose, one for the high dose).	Intraperitoneal injection, 1.15 and 2.3 mg/kg bw, 3 times/week X_{po} = 43 weeks (females high dose), 52 weeks (other groups) X_{pe} = 43 weeks (females high dose), 56 weeks (males high dose), 86 weeks (low dose and controls). Appropriate statistical analysis performed (for tumours: based solely on mice surviving 52 wk or, when tumour of interest was seen earlier, at least as long as the time at which the first tumour of interest was seen).	*Survival:* All high dose males and females were dead by week 56 and 43, resp. Survivors at end experimental period: low dose 15/35 males and 17/35 females; vehicle control 7/15 males and 12/15 females. *Adverse effects:* Body weight depression high dose animals, in particular females. *Tumour incidences:* *Lymphoma and lymphocytic leukaemia combined*: Males: 2/24 at low dose, 26/28* at high dose, 1/8 vehicle control, 1/18 pooled control; Females: 5/26 at low dose, 32/32* at high dose, 0/14 vehicle control, 0/29 pooled control. *Squamous-cell carcinoma of skin, preputial gland and ear canal combined:* Males: 14/24* at low dose, 1/2 at high dose, 0/8 vehicle control, 0/18 pooled control. * Statistically significantly different from vehicle control and pooled control.	Klimisch score: 2 Well performed with limitations. Maximum tolerated dose exceeded in the high dose group; short exposure period.

Schmähl & Osswald[24] Male BR46 rat 48 treated, 89 untreated control	Intravenous,1 mg/kg bw, once a week X_{po} = 52 weeks X_{pe} = not indicated	*Survival:* 30/48 treated, 65/89 control. Mean survival time in treated group was about 7 months shorter compared to controls which survived on average about 2 years. Infection of respiratory or gastrointestinal tract predominant cause of death in treated animals. *Malignant tumours:* (number of tumour bearing animals relative to number of animals still alive when first tumour appeared): 9/30 treated (2 sarcomas of abdominal cavity; 1 lymphosarcoma; 1 myelosis; 1 seminoma, 1 fibrosarcoma and 1 haemangioendothelioma of salivary gland, 1 mammary sarcoma, 1 phaeochromocytoma); 4/65 control (3 mammary sarcomas, 1 phaeochromocytoma). Mean time of onset of tumours in treated animals was about 8 months shorter compared to controls in which the mean time of onset was about 23 months.	Klimisch score: 3 Supportive study. Short exposure period; only one dose tested; only one sex used; no statistical analysis on individual tumour types; high early mortality in treated group due to infection.

X_{po}= duration of exposure; X_{pe}= duration of the experiment.
Klimisch scores were based on Klimisch et al..[25]

Annex **G**

Evaluation of the Subcommittee on the Classification of Carcinogenic Substances

The European Union did not classify thiotepa (tris-(1-aziridinyl)phosphine sulphide). IARC (1990) concluded that there is sufficient evidence for the carcinogenicity of thiotepa in humans and in experimental animals and has classified the compound as a group 1 carcinogen (*carcinogenic to humans*).[1] [In 2011 the 12th NTP Report on Carcinogens considers thiotepa as *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans].

In the present evaluation (November 2013) the DECOS Subcommittee on the Classification of Carcinogenic Substances evaluated the existing and new information regarding human, animal and in vitro studies on carcinogenicity and genotoxicity of thiotepa.

Human studies (see Table 1 in Annex E)

Exposure to thiotepa is specifically associated with leukemia in humans (IARC 1975,1987,1990; NTP RoC 2011).[1-4] Adamson & Seiber (1981) summarized nine case reports from 1970 to 1978 of secondary development of nonlymphocytic leukemia in patients with primary cancer at other sites who had received only thiotepa as a therapeutic agent.[5] Additional evidence was provided by a case-control study by Kaldor et al. (1990).[6] Kaldor et al. (1990) assessed the risk of acute or non-lymphocytic leukaemia associated with exposure to thiotepa in a multicentre nested case-control study that was imbedded in a cohort of

99,113 survivors of ovarian cancer.[6] In this study, 114 case patients who were diagnosed with leukaemia at least one year after the diagnosis with ovarian cancer were matched with control patients who survived free of a second cancer for at least as long as the interval between the diagnosis of ovarian cancer and leukaemia in the case patient (3 controls per case of leukaemia). The time between the diagnosis of ovarian cancer and leukaemia was between two and nine years in 74% of the cases, and acute or no lymphocytic leukaemia's represented 89% of the total. To investigate the association of thiotepa exposure with the risk of leukaemia, the analysis was conducted on patients for which the only chemotherapy was thiotepa (9 cases and 11 controls). These patients were assigned to a low dose group (4 cases, 5 controls) and a high dose group (5 cases, 6 controls). To create these groups, the median dose in controls (30 and 600 mg thiotepa for the low and high dose, respectively) was used as cutoff point. No other data on the treatment were given in the report. The relative risk for acute or non-lymphocytic leukaemia compared to patients receiving radiotherapy or surgery was 8.3 ($p<0.05$) for the low dose and 9.7 (not statistically significantly different from 1.0) for the high dose. [The Subcommittee considers this study well performed although it is aware of the small number of patients treated with thiotepa.]

In three other studies no increased risk of secondary malignancies was found among patients with colorectal or breast cancer. In a study by Boice et al. (1980) (randomized clinical trial), 470 male patients with colorectal cancer received surgical resection and low-dose chemotherapy with thiotepa and were compared with 867 male colorectal patients treated with surgery alone.[7] Patients were followed up for up to 19 years. No difference in the observed/expected ratio of second malignancies was observed among patients treated with thiotepa (0.9) compared to patients who received surgery alone (1.0).

In a retrospective study of Chan et al. (1977) 633 breast cancer patients received simple or radical surgery (mastectomy) with or without postoperative radiotherapy and were then treated with thiotepa for up to two years.[8] A group of 632 breast cancer patients treated with surgery only or with surgery followed by radiotherapy were used as historical controls. All patients were followed between 5 to 10 years. In total 12.0% of the thiotepa treated patients developed a secondary tumour compared with 11.1% in the control group. The authors concluded that prolonged adjuvant thiotepa chemotherapy does not seem to increase the risk of second primary cancer.

In a prospective randomized clinical trial by Kardinal et al. (1980), 90 women treated with radical mastectomy for early cancer of the breast were subsequently treated with 0.8 mg/kg bw thiotepa perisurgically and then with 0.2

mg/kg bw thiotepa once weekly for one year.[9] Seventy-seven breast cancer patients treated with radical mastectomy only served as control group. The average follow-up period was approximately 60 months in both the thiotepa group and the control group. A relative risk of 0.68 was calculated for developing a second malignancy after thiotepa treatment.

[The Subcommittee recognizes that these three studies were clinical trials and not designed specifically to analyze the long term side-effects of thiotepa. Moreover the number of patients in all three studies was too low to find such an effect. The thiotepa group and the control group in the Chan et al. study were not comparable regarding radiotherapy.[8] From the Kardinal et al. study it is not clear whether any statistical analysis was performed.[9]]

The Subcommittee recognizes that only one reliable study exists (Kaldor et al.) showing the association of thiotepa with leukemia and agrees that limited evidence exists for the carcinogenicity of thiothepa to humans.[6]

Animal studies (see Table 2 in Annex F)

In the NTP-RoC report (2011) it was summarized that thiotepa administered by intraperitoneal injection caused lymphoma and/or leukemia (lymphocytic or granulocytic) in mice of both sexes and in male rats.[2] It also caused benign lung tumors in mice of both sexes, cancer of the mammary gland and uterus in female rats, cancer of the skin or ear canal (squamous-cell carcinoma) in rats of both sexes and in male mice, and cancer of the preputial gland (squamous cell carcinoma) in male mice (IARC 1975,1990; NCI 1978).[1,3,10] In male rats administered thiotepa by intravenous injection, cancer occurred at numerous tissue sites, including the abdominal cavity, mammary gland, blood vessels, bone marrow, lymphatic system, salivary glands, adrenal gland, and testis (IARC 1975,1987,1990).[1,3,4]

Table 2 (Annex F) summarizes the details of the carcinogenicity studies in experimental animals described below. In a screening assay by Stoner et al. (1973), based on accelerated induction of lung tumours in a mouse strain (A/He) highly susceptible to development of this neoplasm, ten mice per sex per dose received 19, 47 or 94 mg/kg bw thiotepa (total doses) by intraperitoneal injection over a period of 4 weeks.[11]

The experiment was terminated 24 weeks after the first injection. All thiotepa treated animals except one of the 47 mg/kg bw group survived. Survival in the control groups was 94% (untreated control) or 96% (vehicle control). The incidence of lung tumours was 11 (55%), 20 (100%) and 16 (80%) in the low, mid and high dose groups, respectively, compared to 19 (19%) and 38 (24%) in

untreated and vehicle controls, respectively. The number of lung tumours per mouse at the mid and high dose was statistically significantly increased compared to controls. The Subcommittee noted that this study is limited (only one type of tumour investigated, small number of treated animals, short treatment and observation periods) and that a positive result in this pulmonary tumour screening assay should be confirmed by other test systems.

The carcinogenicity of thiotepa was also determined in a study performed by the National Cancer Institute (NCI) of the US Department of Health, Education, and Welfare.[10] Male and female Sprague-Dawley rats (31-39/sex/dose) were treated with thiotepa in phosphate-buffered saline at dose levels of 0.7, 1.4 or 2.8 mg/kg bw three times a week for a maximum period of 52 weeks followed by observation periods (length depending on the dose level) resulting in experimental periods of 82-87 weeks. Mean body weights of all dosed male rats, particularly those of the mid- and high-dose groups, were depressed throughout the study, when compared with either matched or vehicle controls; those of the dosed females were less markedly depressed. Thiotepa decreased survival in a dose-related manner. At the high dose, all males and females were dead by week 19 and 21, respectively. At the mid dose, all males were dead by week 78 and only 8.6% of the females survived till the end of study. At the low dose, survival was 15.4% in males and 42% in females. Survival in controls was 80-100%. The high dose of 2.8 mg/kg bw was too toxic for an evaluation of carcinogenic activity. Treatment with thiotepa was associated with increased incidences of squamous-cell carcinoma in the skin or ear canal, neuroepitheliomas and nasal carcinomas in each sex, haematopoietic tumours (lymphoma, lymphocytic leukaemia, or granulocytic leukaemia) in male rats, adenocarcinoma of the uterus, and possibly adenocarcinoma of the mammary gland. These tumours are considered to be relevant for humans. Although this study has limitations (see Table 2 in Annex F), it allows the conclusion that thiotepa is carcinogenic in Sprague-Dawley rats.

In the same study by NCI, male and female B6C3F1 mice (35/sex/dose) were treated with thiotepa in phosphate-buffered saline at dose levels of 1.15 or 2.3 mg/kg bw three times a week for a maximum period of 52 weeks followed by an observation period resulting in an experimental period of 86 weeks (or only 43 or 56 weeks in high-dose females and males, respectively, due to mortality).[10] Body weight depression was observed in high dose animals, particularly in females. All males and females of the high dose group were dead by week 56 and 43, respectively. At the end of the experimental period, 15 (43%) male and 17 (49%) female mice of the low dose group survived compared to 7 (46%) males and 12 (80%) females in the vehicle control group. Treatment with thiotepa was

associated with increased incidences of squamous cell carcinoma (at skin, ear and preputial gland) in male mice and of haematopoietic tumours (lymphoma and lymphocytic leukaemia) in male and female mice.

These tumours are considered to be relevant for humans. Although this study has limitations (see Table 2 in Annex F), it allows the conclusion that thiotepa is carcinogenic in B6C3F1 mice.

In the study of Schmähl & Osswald (1970), 48 male R46 rats were treated with 1 mg/kg bw thiotepa by intravenous injection once a week for 52 weeks. Malignant tumours were observed in 9 of the 30 rats that were still alive when the first tumour appeared.[12] These tumours occurred at a variety of sites (see Table 2 in Annex F). The incidence of malignant tumours in controls was 4/65. The authors stated that the difference in total tumour incidence between treated rats and controls was statistically significant (no p value was reported). The Committee noted that this study has several flaws (short exposure period, only one dose tested, only one sex used, no statistical analysis performed on individual tumour types, high early mortality in treated group).

The Subcommittee did not retrieve animal studies of a more recent date and agrees with IARC and the NTP that thiotepa is carcinogenic to animals.[1,2]

Mechanism of genotoxicity

Thiotepa is a direct alkylating agent with potent genotoxic activity in a wide variety of prokaryotic, lower eukaryotic, and mammalian in vitro and in vivo test systems. Thiotepa causes DNA damage, mutations, micronucleus formation, and/or chromosomal aberrations in somatic and germ cells from exposed rodents, rabbits, and nonhuman primates and chromosomal aberrations in peripheral-blood lymphocytes from treated humans (NTP RoC 2011; IARC 1990; Chen et al., 1999[13]; Casciano et al., 1999; Dertinger el el., 2014; Labash et al., 2015).[1,2,13,14-16]

The Subcommittee agrees with IARC that thiotepa is a genotoxic carcinogen and concludes that a stochastic genotoxic mechanism underlies its carcinogenicity.

Recommendation

Limited evidence is available that thiotepa is carcinogenic to humans. Moreover, sufficient evidence is available that thiotepa is carcinogenic to animals. The Subcommttee recommends to classify thiotepa in category 1A (‘substance known to be carcinogenic to humans’) (see Annex H). Moreover, the

Subcommittee is of the opinion that a stochastic genotoxic mechanism underlies carcinogenicity. The Subcommittee recommends health-based calculated occupational cancer risk values (HBC-OCRVs) to be calculated for regulatory standard setting.

References (in Annex G)

1 IARC monographs on the evaluation of the carcinogenic risk of chemicals to man: Pharmaceutical drugs. IARC Monogr Eval Carcinog Risk Chem Man 1990; 50: 123-141.

2 NTP 12th Report on Carcinogens. Rep Carcinog 2011; 12: iii-499.

3 IARC monographs on the evaluation of the carcinogenic risk of chemicals to man: some aziridines, N-, S- & O-mustards and selenium. IARC Monogr Eval Carcinog Risk Chem Man 1975; 9: 85-94.

4 Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl 1987; 7: 368-369.

5 Adamson RH, Seiber SM. Chemically induced leukemia in humans. Environ Health Perspect 1981; 39: 93-103.

6 Kaldor JM, Day NE, Pettersson F, Clarke EA, Pedersen D, Mehnert W et al. Leukemia following chemotherapy for ovarian cancer. N Engl J Med 1990; 322(1): 1-6.

7 Boice JD, Greene MH, Keehn RJ, Higgins GA, Fraumeni JF, Jr. Late effects of low- dose adjuvant chemotherapy in colorectal cancer. J Natl Cancer Inst 1980; 64(3): 501- 511.

8 Chan P, Sadoff L, Winkley J. Second malignancies following first breast cancer in prolonged thiotepa adjuvant chemotherapy. In: SE Salmon, SE Jones, editors. Adjuvant therapy of cancer. Amsterdam: Elsevier; 1977: 597-607.

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Date meeting: November 11, 2013.

Annex **H**

Carcinogenic classification of substances by the Committee

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

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category	
		67/548/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	The compound is presumed to be as carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	not applicable	not applicable
(4)	The compound is probably not carcinogenic to man.	not applicable	not applicable

Source: Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.[26]

Annex I

Health-based occupational risk calculations

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Introduction

Currently, there are no occupational exposure limits for Thiotepa anywhere in the world. To derive these occupational exposure limits, four epidemiological studies, which included cancer patients who were treated for a primary tumor with Thiotepa, have been reviewed to calculate risk values.[1-4] No studies are available which describe health risks for occupationally exposed individuals.

The article with the most clearly increased risk after Thiotepa use is the study of Kaldor et al. (1990).[1] In this study patients received chemotherapy with alkylating agents for the treatment of ovarian cancer. A total of 9 patients only received Thiotepa as chemotherapeutic agent divided in a group of a median dose of 30 mg (4 patients and 5 controls) and a median dose of 600 mg (5 patients and 6 controls). Follow-up was more than 9 years. Kaldor et al. concluded that treatment with Thiotepa for ovarian cancer increased the risk of leukemia with a relative risk of 8.3 for the 30 mg dose group and 9.7 for the 600 mg group. Patients were compared with patients receiving only surgery and/or radiotherapy.

Three other studies found no significant increase in hematopoietic cancers after treatment with Thiotepa. In a study by Chan et al. (1977) 633 patients received Thiotepa and were compared with 632 patients who only received surgery and/or

radiotherapy.[2] They found 3 cancers cases in the hematopoietic system after treatment with Thiotepa and 4 cases in the control group. Also in a study by Boice et al. (1980) only 3 cases of leukemia were found after treatment with Thiotepa in a group of 470 treated patients.[3] The control group consisted of 595 patients were 4 cases of leukemia were found. In an even smaller study by Kardinal and Donegan (1980) just 1 case of leukemia was found in group of 90 patients who were treated with Thiotepa.[4] 77 patients were randomly assigned as a control group who were treated with surgery alone. None of those patients developed leukemia. Follow-up for all these studies was between 5 and 10 years.

Table 1 Number of patients with leukemia after Thiotepa-treatment.

	Thiotepa-treated patients			Control patients (treated with other therapies than Thiotepa)		
	Total	Leukemia	No Leukemia	Total	Leukemia	No Leukemia
Kaldor et al.[1] (30 mg)	9	4	5	208	21	187
Kaldor et al.[1] (600 mg)	11	5	6	208	21	187
Chan et al.[2] (388 mg)[a]	632	2	630	630	2	628
Boice et al.[3] (0.8*70 = 56 mg)	470	3	467	595	4	591
Kardinal & Donegan[4] (11.2*70 = 784 mg)	90	1	89	77	0	77

[a] different doses are given in the appendix.

Exposure-Response modelling

We estimate the dose-response curve for Thiotepa and the cumulative incidence of leukemia using a binomial regression model with a log link as follows:

$$E(\log(C_i / N_i)) = \mu_{s[i]} + \beta * dose_i$$

In this formula *i* indexes the different groups, and C = number of leukemia cases, N = total number of subjects, s = the study, and dose = administered dose of Thiotepa. $\mu_{s[i]}$ is the study-specific (log-transformed) baseline risk (i.e. risk for the unexposed), and is included to account for different background risks in the different study populations.

Under this model the Relative Risk (RR) or Cumulative Incidence Ratio (CIR) at a dose of 10 mg can be estimated as $\exp(\mu_{s[i]} + \beta*10)/ \exp(\mu_{s[i]} + \beta*0) = \exp(\beta*10)$. The estimated RR [95%CI] at a dose of 10 mg is 1.023 [1.008-1.034].

If we subtract the estimated study-specific baseline risks from the observed (empirical) risks, we can standardize results from the different studies and plot the estimated regression line along with study-specific data to asses model fit. In figure 1 the risks were standardized using the Kaldor study as the reference. From this figure it is clear that the risks for most groups cannot be estimated very precisely, but that the regression line is consistent with most of the individual data points, except for the 30 mg dose group in the Kaldor study. If this group is removed from the analysis, estimated RR [95%CI] at a dose of 10 mg slightly changes to 1.024 [1.009-1.036]. This result is due to a decrease in the estimated baseline risk for the Kaldor et al. study. Based on data from the Kaldor study alone, the estimated RRs [95%CI] at a dose of 10 mg are 1.024 [1.008-1.035] and 1.025 [1.010-1.037] including respectively excluding the 30 mg dose-group. Excluding all data from the Kaldor study results in a lower estimated slope of 1.012 [0.976-1.061], which is no longer statistically significant. The pooled results are very close to that obtained using only the Kaldor et al. data, which can be explained by the much greater precision of risk estimates at higher doses for that study.

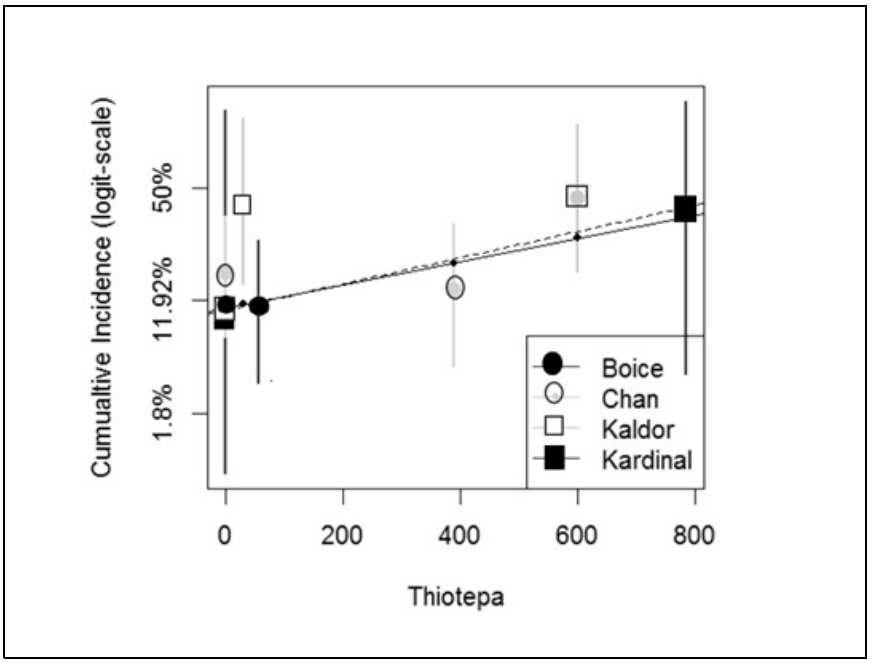

Figure 1 Regression plot of the relation between administered dose of Thiotepa and risk of leukemia (including (solid line) respectively excluding (dotted line) the 30 mg dose group in the Kaldor study).

Excess Risk calculations

Excess Risk (ER) calculations used information on incidence of leukemia (excluding lymphoma's) in men and women. For a scenario with uniform exposure from age 20-65, the ER at age 75 for an exposure of 0.061 mg is estimated to be $40*10^{-6}$. For a scenario with uniform exposure from age 20-65, the ER at age 75 for an exposure of 4.82 mg is estimated to be $40*10^{-4}$.

References (in Annex I)

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Appendix

Kaldor et al. (1990)[1]:

30 mg	Leukemia	No Leukemia
Thiotepa	4	5
No Thiotepa	21	187

600 mg	Leukemia	No Leukemia
Thiotepa	5	6
No Thiotepa	21	187

Chan et al. (1977)[2]:

	Leukemia	No Leukemia
Thiotepa	2	630
No Thiotepa	2	628

0,6 mg/kg (in first 3 days). After that cumulative doses are assigned to a number of patients:

65 patients □ 38 mg
71 patients □ 136 mg
65 patients □ 258 mg
66 patients □ 390 mg
366 patients □ 522 mg

Boice et al. (1980)[3]:

	Leukemia	No Leukemia
Thiotepa	3	467
No Thiotepa	4	591

0,8 mg/kg (in first 3 days). Assuming an average body weight of 70 kg amounts to 56 mg in total.

Kardinal & Donegan (1980)[4]:

	Leukemia	No Leukemia
Thiotepa	1	89
No Thiotepa	0	77

0,8 mg/kg (in first 3 days) + 0,2 mg/kg weekly for 1 year (total = 11,2 mg/kg). Assuming an average body weight of 70 kg amounts to 784 mg in total.

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