Azathioprine

Health based calculated occupational cancer risk values

Aanbiedingsbrief

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

to

the Minister and State Secretary of Social Affairs and Employment

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid schat de Commissie WGD van de Gezondheidsraad het extra kankerrisico bij beroepsmatige blootstelling aan stoffen die door de Europese Unie of door de Commissie WGD als genotoxisch kankerverwekkend zijn aangemerkt. In dit rapport maakt zij zo'n schatting voor azathioprine. Zij heeft daarbij gebruik gemaakt van de methode die is beschreven in het rapport 'Berekening van het risico op kanker' (1995/06WGD) (Hea95).

Naar schatting van de commissie is de extra kans op kanker voor azathioprine:

- 4×10^{-5} bij 40 jaar beroepsmatige blootstelling aan 0.005 mg/m^3
- 4×10^{-3} bij 40 jaar beroepsmatige blootstelling aan 0.5 mg/m^3

Executive summary

On request of the Minister of Social Affairs and Employment the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, estimates the additional cancer risk associated with occupational exposure to substances that have been classified by the European Union or the DECOS as genotoxic carcinogen. In this report the committee presents such estimates for azathioprine. It has used the method described in the report 'Calculating cancer risks due to occupational exposure to genotoxic carcinogens' (1995/06WGD) (Hea95).

The committee estimated that the additional lifetime cancer risk for azathioprine amounts to:

- 4×10^{-5} for 40 years of occupational exposure to 0.005 mg/m^3
- 4×10^{-3} for 40 years of occupational exposure to 0.5 mg/m^3

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for 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 Standards (DECOS), a committee of the Health Council of the Netherlands, on request of the Minister of Social Affairs and Employment (annex A). This evaluation should lead to a health-based recommended exposure limit for the concentration of the substance in air. Such an exposure limit cannot be derived if the toxic action cannot be evaluated using a threshold model, as is the case for substances with genotoxic carcinogenic properties.

In this case an exposure-response relationship is recommended for use in regulatory standard setting, ie. 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 genotoxic carcinogens by the European Union or by the present committee.

For the establishment of the HBC-OCRV's the committee generally uses a linear extrapolation method, as described in the committee's report 'Calculating cancer risk due to occupational exposure to genotoxic carcinogens' (1995/06WGD). 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 derivation of HBC-OCRVs by the committee for azathioprine. The members of the committee are listed in Annex B. The first draft of this report was prepared by H Stouten and MI Willems, from the TNO Nutrition and Food Research Institute in Zeist, by contract with the Ministry of Social Affairs and Employment.

Chapter 2

Azathioprine

2.1 Introduction

Azathioprine (CAS-no: 446-86-8) has been classified as a genotoxic carcinogen by DECOS (DECOS95).

The carcinogenicity of azathioprine has been evaluated by IARC (IARC81; IARC87). IARC concludes that there is sufficient evidence for carcinogenicity to humans and limited evidence for carcinogenicity to animals. This evaluation of the carcinogenicity was based on reviews by IARC (IARC81; IARC87). In addition, literature was retrieved from online databases Medline, Toxline and Chemical Abstracts, covering the period 1966 to January 1996.

2.2 Carcinogenicity studies and selection of study suitable for risk estimation in the occupational situation

Main tumours in humans after treatment with azathioprine in renal transplant patients include non-Hodgkin's lymphoma, squamous cell cancers of the skin, hepatobiliary carcinomas and mesenchymal tumours. In other groups of patients who have received azathioprine as an immunosuppressant for treatment of diseases like rheumatoid arthritis, systemic lupus, inflammatory bowel disease etc. the same array of malignancies was found to be in excess, although to a lesser extent. For these patients, however, the picture is still not completely clear. Patients with rheumatoid arthritis constituted the largest category in the latter study, and some, but not all studies have

found that this disease conveys a risk for non-Hodgkin's lymphoma in the absence of treatment (IARC87). Therefore, it is concluded that the available epidemiological studies are not suitable for quantitative cancer risk estimation in workers, because it is not clear to what extent the health status of the examined groups of patients interferes with the carcinogenic activity of azathioprine.

Azathioprine was tested by intraperitoneal, subcutaneous and/or intramuscular administration in mice and by oral and intraperitoneal administration in rats. Suggestive evidence was obtained for the induction of lymphomas after intraperitoneal, subcutaneous or intramuscular injection in mice and for ear-duct carcinomas in rats after oral administration (IARC81; IARC87). Because of limitations in design and reporting, the results were considered to be inconclusive (IARC81, IARC87).

Table 1 summarizes the oral carcinogenicity studies with experimental animals (Annex D). Despite their limitations, these animal studies are used for quantitative risk assessment in workers, because the available epidemiological studies do not allow quantitative risk assessment in workers as pointed out above. In the absence of animal inhalation studies, oral study are used to calculate the carcinogenic activity in experimental animals.

2.3 Carcinogenic activity in experimental animals, lifetime low-dose exposure

Below the carcinogenic activity is calculated for the study of Frankel *et al* (Fra70) and for the two experiments reported by Cohen et al. (Coh83). The committee is of the opinion that the available data do not indicate that the use of the linear model is not appropriate.

Frankel *et al*., 1970

To calculate the carcinogenic activity, expressed as the incidence per mg azathioprine per kg bw/day, the incidence of male and female rats with a squamous cell carcinoma of the ear-duct was used as starting point. Assuming that the daily food intake amounts to 40 and 50 g/kg bw/day for males and females, respectively, the intake of the test substance is calculated to be 6.75 mg/kg bw/day, taken males and females together (Fra70).

The incidence of tumour bearing animals per mg/kg bw/day (lifespan conditions, assuming a linear dose response relationship), I_{dose}, is calculated as follows:

$$I^*_{dose} = \frac{I_e - I_c}{C \text{ x } (X_{po}/L) \text{ x } (X_{pe}/L) \text{ x exposure hours per day/24 x exposure days per week}} =$$

$$= \frac{6/42 - 0/20}{(6.75 \text{ mg/kg/d}) \text{ x } (364^d/1000^d) \text{ x } (364^d/1000^d) \text{ x } 24/24 \text{ x } 7/7} = 1.6*10^{-1} \text{ [mg/kg bw/d]}^{-1}$$

Cohen *et al.* 1983

The incidence of female rats with malignant tumours (squamous cell carcinoma of the ear-duct, thymic lymphomas) was used as starting point to calculate the incidence per mg/kg bw/day. Assuming a mean body weight of 275 g (see Coh83, Charts 1 and 2), the intake of the test substance is calculated to amount to 16.2 mg/kg bw/day (Xpo 336 days) and 17.3 mg/kg bw/day (Xpo = 294 days) for the first and second experiment, respectively (Coh83).

According to the above equation, the estimated incidence of tumour bearing animals per mg/kg bw/day (lifespan conditions) amounts to:

$$\frac{10/14 - 0/20}{(16.2 mg/kg/d) x (462^d/1000) x (336^d/1000) x 24/24 x 7/7} = 2.8 \text{ x } 10^{-1} \text{ in the first experiment, and to}$$

$$\frac{9/19 - 0/30}{(27.2 mg/kg/d) x (462^d/1000) x (294^d/1000) x 24/24 x 7/7} = 1.3 \text{ x } 10^{-1} \text{ in the second experiment.}$$

To calculate the additional risk of cancer at the workplace, the incidence of $1.6x10^{-1}$ calculated from the data of Frankel *et al*. (Fra70) was used as starting point, since the dose administered in the latter study was lower than the dose used in the experiments of Cohen and was clearly less toxic (see Table 1, remarks).

2.4 Health risk to humans

To estimate the additional lifetime risk of cancer in humans under lifespan conditions on the basis of results in animal experiments, it is assumed that no difference exists between experimental animals and man with respect to toxicokinetics, mechanism of tumour

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* I_{dose}= the carcinogenic activity attributable to the exposure to the substance per unit daily dose under lifespan conditions, assuming a linear dose response relationship, usually expressed per mg/m^3 or per mg/kg bw/day.
I_e and I_c = incidence of tumour bearing animals or tumours in exposed and control animals, respectively,
X_{po} = exposure period, X_{pe} = experimental period
L = standard lifespan for the animals in question (L rat is assumed to be 1000 days)

induction, target, susceptibility etc, unless specific information is available which justifies a different approach. Furthermore, it is assumed that the average man lives 75 years, weighs 70 kg, and is exposed 24 hours per day, 7 days per week, 52 weeks per year, for lifetime.

2.5 Health risk to workers, calculation of the HBC-OCRV

To estimate the additional lifetime risk of cancer in humans under workplace conditions, it is assumed that the average man lives 75 years, is exposed 8 hours per day, five days a week, 48 weeks a year, for 40 years, and inhales 10 m^3 air per 8 hour-working day.

Using as starting point the estimated incidence of 1.6 x 10^{-1} per mg/kg bw/day, the additional lifetime cancer risk per mg/m^3 under occupational conditions (=HBC-OCRV) amounts to:

$$\text{HBC-OCRV} = 1.6 \times 10^{-1} x \frac{40y}{75y} x \frac{48w}{52w} x \frac{5d}{7d} x \frac{10m^3}{70kg} = 8.0 \times 10^{-3} [mg/m^3]^{-1}$$

Based on the HBC-OCRV of 8.0 x 10^{-3} the additional lifetime cancer risks amount to:

- 4 x 10^{-5} for 40 years of exposure to 0.005 mg/m^3
- 4 x 10^{-3} for 40 years of exposure to 0.5 mg/m^3

2.6 Existing occupational exposure limits

The regulatory authorities of the Netherlands, Germany, United Kingdom, Denmark, Sweden, and the EU and the USA-ACGIH have not established occupational exposure limits for azathioprine (ISZW99; DFG99; HSE99; Arb96, NBO93; ACG99).

2.7 Toxicity profile of azathioprine

Human data (from IARC81; IARC87)

Azathioprine is an immunosuppressive agent. Dosage depends upon clinical and haematological responses of individual patients. Therapy is usually initiated at a level of 2 - 5 mg/kg bw per day, orally, with a long-term maintenance dose of 1 - 3 mg/kg bw per day (IARC87).

The major toxic effect of azathioprine is bone-marrow depression. Use of azathioprine during pregnancy may reduce birth weight significantly. The data were insufficient to evaluate the teratogenic potential of this drug to humans.

Animal data (from IARC81; IARC87)

The single oral LD_{50} for azathioprine was 2500 mg/kg bw in mice and 400 mg/kg bw in rats; and the ip LD_{50} was 650 mg/kg bw in mice and 310 mg/kg bw in rats. Maximum tolerated doses after chronic daily treatment were 20 mg/kg bw in mice; 2 mg/kg bw in dogs; 1 mg/kg bw in Patas monkeys; and about 20 mg/kg bw in male rats. The main toxic effect of azathioprine after, e.g., 45 mg/kg bw per day in rats or 4 mg/kg bw per day in dogs is bone-marrow depression. Azathioprine is embryolethal at doses nontoxic to the mother and can induce a variety of severe teratogenic effects irrespective of the route of administration in several animal species. A NOAEL for teratogenic effects is not reported, the lowest reported teratogenic dose range, orally, is 5 - 15 mg/kg bw per day in rabbits. Azathioprine is mutagenic in bacteria, yeast, *Drosophila melanogaster* and in mice *in vivo*. At high concentrations the drug is clastogenic to human lymphocytes *in vitro*.

In conclusion, a health-based occupational exposure limit for azathioprine derived from data other than on genotoxicity/carcinogenicity would in all likelihood be expected to be in between the concentration levels associated with the referential cancer risk levels.

The Hague, 20 December 1999,
for the committee

drASAM van der Burght,
scientific secretary

Prof. dr GJ Mulder,
chairman

References

ACG99 American Conference of Governmental Industrial Hygienists (ACGIH). 1999. TLVs® and BEIs®. Guide to Occupational Exposure Values, Cincinnati OH, USA: ACGIH, 1999: 126.

Arb96 Arbejdstilsynet. Exposure Limit value for substances and materials. Copenhagen, Danmark: Arbejdstilsynet, 1996:10.

DECOS95 Scientific documentation on the Dutch list of occupational Carcinogens (I). Dutch Expert Committee on OCcupational Standards; 1995; RA 1/95.

DFG99 Deutsche Forschungsgemeinschaft (DFG): Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe. MAK- und BAT-Werte-Liste 1999. MAK- und BAT-Werteliste 1999. Maximale Arbeitsplatzkonzentrationen und biologische Arbeitsstofftoleranzwerte. Weinheim, (Mitteilung 35).

Hea95 Health Council of the Netherlands, Dutch Expert Committee on Occupational Standards (DECOS). Calculating cancer risks, THe HAgue, The Netherlands. 1995; pub no 1995/06 WGD.

HSE99 Health and Safety Executive (HSE). Occupational exposure limits 1999. Sudbury (Suffolk), UK: HSE Books, 1999; Guidance note EH 40/96.

Hun97 Hunter WJ, Aresini G, Haigh R *et al.* Occupational exposure limits for chemicals in de European Union. Occup. Environ. Med, 1997; 54:217-22.

IARC81 International Agency for Research on Cancer (IARC). In: Some antineoplastic and immunosuppressive agents. Lyon, France: IARC, 1981: 47-78 (IARC monographs on the evaluation of carcinogenic risks to humans; Vol 26).

IARC87 International Agency for Research on Cancer (IARC). In: Overall evaluations of carcinogenicity: an updating of IARC monographs: Volumes 1 to 42. Lyon, France: IARC, 1987: 119-20 (IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans; Suppl 7).

ISZW99 Inspectiedienst van het Ministerie van Sociale Zaken en Werkgelegenheid (ISZW). De Nationale MAC-lijst 1999. The Hague, The Netherlands: Sdu Servicecentrum Uitgeverijen, 1999;

NBO93 Noational Board of Occupational Safety and Health (NBOSH). Occupational exposure limits. Solna, Sweden: NBOSH, 1993; Ordinance AFS 1993/9.

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

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- VJ Feron,
  professor of toxicology; TNO Nutrition and Food Research Institute, Zeist
- DJJ Heederik
  epidemiologist; Wageningen University, Wageningen
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- G de Jong
  occupational physician; Shell International Petroleum Maatschappij, The Hague
- J Molier-Bloot
  occupational physician; BMD Akers bv, Amsterdam
- IM Rietjens
  professor in Biochemical toxicology; Wageningen University, Wageningen.

- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, Den Haag
- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- HG Verschuuren
  toxicologist; DOW Europe, Horgen (Switzerland)
- AAE Wibowo
  toxicologist; Coronel Institute, Amsterdam
- F de Wit
  occupational physician; Labour Inspectorate, Arnhem
- CA Bouwman, *scientific secretary*
  Health Council of the Netherlands, Den Haag
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present advisory report was prepared by H Stouten and M Willems, from the Department of Occupational Toxicology of the TNO Nutritionand Food Research Institute, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: E Vandenbussche-Parméus.
Lay-out: J van Kan.

Annex **C**

Comments on the public draft

A draft of the present report was released in 1999 for public review. The following organizations and persons have commented on the draft document:

- mr P Ridgeway, Health and Safety Executive, United Kingdom
- mr A Aalto, Tampere, Finland

Annex **D**

Animal studies

See next page.

Table 1 Carcinogenicity studies with azathioprine[a].

authors	species	exposure characteristics	dose	exposure and experimental period	findings	remark
Fra70	rat[b]	diet	0, 150 mg/kg food (= 6.75 mg/kg bw/day)	Xpo=Xpe is 52 weeks	Squamous-cell ear-duct carcinomas: control: 0/10 , 0/10 ; treatment group: 3/17, 3/25.	The observed increase in incidence of carcinomas is not statistically significantly different from that in the control.
Coh83 Exp. 1	rat[c](females) control, N=20 test, N=36	diet	In total 1.5 g/rat In view of the toxicity, dose levels were changed during the experiment.	Xpo= 48 weeks Xpe= 66 weeks	Treatment group: 10/14 effective rats with tumours (lymphoma in thymus 6/14, squamous cell carcinoma in ear duct 4/14). Control group: 2/20 fibroadenomas of the mammary glands.	It is noted, that the treatment group started with 36 animals. The effective number of rats includes those that survived 10 weeks or more of the experiment. There were no deaths in the control group.
Exp. 2	rat[c] (females) control, N=30 test, N=28	diet	In total 2.2 g/rat In view of the toxicity, dose levels were changed during the experiment.	Xpo= 42 weeks Xpe= 66 weeks	Treatment group: 9/19 effective rats had tumour (lymphoma in thymus 7/19, squamous cell carcinoma in ear duct 2/19). Control group: 3/30 fibroadenomas of the mammary glands.	It is noted, that the treatment group started with 28 animals. The effective number of rats includes those that survived 10 weeks or more of the experiment. There were no deaths in the control group.

Xpo: exposure period;

Xpe: experimental period

[a] References with respect to subcutaneous and/or intramuscular administration (mouse) and intraperitoneal administration (mouse and rat) are not summarized. IARC (1981) considered the results to be inconclusive, because of limitations in design and reporting of the studies.

[b] Fischer 344 rats

[c] Sprague-Dawley rats