Health Council of the Netherlands

5-Nitroacenaphthene

Health-based calculated occupational cancer risk values

2014/17

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5-Nitroacenaphthene

Health-based calculated occupational cancer risk values

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *5-Nitroacenafteen*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-8165/BvdV/cn/459-F70
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Datum : 1 juli 2014

Geachte minister,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan 5-nitroacenafteen.

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.

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. W.A. van Gool,
voorzitter

Bezoekadres
Rijnstraat 50
2515 XP Den Haag
E-mail: b.v.d.voet@gr.nl
Telefoon (070) 340 74 47

Postadres
Postbus 16052
2500 BB Den Haag
www.gr.nl

5-Nitroacenaphthene

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. 2014/17, The Hague, July 1, 2014

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 samenhangen met een vooraf vastgesteld extra risico op sterfte aan kanker (4 per 1000 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 doet de commissie zo'n schatting voor 5-nitroacenafteen. 5-Nitroacenafteen wordt gebruikt bij de productie van kleurstoffen, plastics en pesticiden.

Naar schatting van de commissie is de concentratie van 5-nitroacenafteen in de lucht, die samenhangt met een extra kans op overlijden aan kanker van

- 4 per 1 000 ($4x10^{-3}$), bij 40 jaar beroepsmatige blootstelling, gelijk aan 1.5 mg/m^3
- 4 per 100 000 ($4x10^{-5}$), bij 40 jaar beroepsmatige blootstelling, gelijk aan 0.015 mg/m^3.

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.[2] In this report the Committee presents such estimates for 5-nitroacenaphtene. 5-Nitroacenaphtene is used in the production of dyes, plastics, and pesticides.

The Committee estimated that the concentration of 5-nitroacenaphtene in the air, which corresponds to an excess cancer mortality of

- 4 per 1,000 ($4x10^{-3}$), for 40 years of occupational exposure, equals to 1.5 mg/m^3
- 4 per 100,000 ($4x10^{-5}$), for 40 years of occupational exposure, equals to 0.015 mg/m^3.

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 genotoxic substances with a non-stochastic mechanism 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 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 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*.[2,3] 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 5-nitroacenaphthene (see Annex F and G). 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 January 2014, 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, 5-nitroacenaphthene and CAS registry number as key words. In addition, in preparing this report the following reviews were consulted:

- Review by the Commission of the European Communities[4]
- IARC Review.[5]

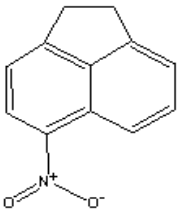
The last search was performed in May 2014 and covered the period 1997-2014 (a previous search was conducted in 1997 and covered the period 1985 to 19 February 1997).

Chapter 2

Identity, toxicity profile and classification

2.1 Identity and physical and chemical properties

5-Nitroacenaphthene is used in the production of dyes, plastics and pesticides. Physical and chemical data shown below are from IARC 1978[5], Richardson[6] and http://toxnet.nlm.nih.gov (HSDB and ChemIDplus data bases, accessed May 22, 2014).

Chemical name	:	5-nitroacenaphthene
CAS number	:	602-87-9
EINECS number	:	210-025-0
EEC number	:	609-037-00-2
IUPAC name	:	5-nitroacenaphthene
Synonyms	:	1,2-dihydro-5-nitro-acenaphtylene
Physical description and colour	:	solid, yellow
Molecular formula	:	$C_{12}H_9NO_2$
Structure	:	

Molecular weight	:	199.2
Melting point	:	102-103 °C
Boiling point (101.3 kPa)	:	279 °C
Density	:	no data
Solubility	:	0.91 mg/L at 25°C in water; soluble in ethanol, ethyl ether, ligroin
Octanol/water partition coefficient, Log $P_{oct/w}$	:	3.85
Vapour pressure (25°C)	:	2.65E-5 mm Hg
Relative vapour density (air = 1)	:	no data
Flash point	:	no data
Odour threshold	:	no data
Conversion factor (20 °C, 101.3 kPa)	:	no data
EU classification	:	H350 - may cause cancer

2.2 Classification as a carcinogenic substance

The European Union has classified 5-nitroacenaphtene as a category 1B carcinogen (may cause cancer). IARC has classified the compound as a 2B carcinogen (possibly carcinogenic to humans).[5] In this present evaluation, the Committee (DECOS) follows the recommendation of the DECOS Subcommittee on the Classification of Carcinogenic Substances and classified 5-nitroacenaphthene in category 1B (presumed to be carcinogenic to man) (see Annex F and G).

2.3 Genotoxicity

5-Nitroacenaphthene was mutagenic in the *Salmonella typhimurium* strains TA100 and TA98 without metabolic activation and, to a larger extent (2 or 5-fold increase, respectively) with metabolic activation.[7] Mutagenicity of 5-nitroacenaphthene in *Salmonella typhimurium* strains TA100 and TA98 with and without metabolic activation was confirmed by McCoy et al..[8] Further, 5-nitroacenaphthene was positive in an *umu* test system using *Salmonella typhimurium* strain NM3009.[9] In a DNA-repair test 5-nitroacenaphthene elicited a positive DNA-repair response in vitro in both rat and mouse hepatocytes.[10]

Since 5-nitroacenaphthene is positive for bacterial mutagenicity and for genotoxicity in the DNA-repair test, the Committee follows the recommendation of the DECOS Subcommittee on the Classification of Carcinogenic Substances (see Annex F) and concludes that 5-nitroacenaphthene is a genotoxic carcinogen with a stochastic mechanism.

2.4 Non-carcinogenic effects

Limited data on effects other than carcinogenicity are reported for 5-nitroacenaphthene. In the specific carcinogenicity studies reported in Chapter 3 a number of toxicological effects were observed and will be discussed there.

2.5 Existing occupational exposure limits

Worldwide, there are no occupational exposure limits for 5-nitroacenaphthene (http://www.ser.nl, accessed May 22, 2014).

Chapter 3

Carcinogenicity studies

Available data have been summarized and evaluated by working groups of IARC.[5] IARC concluded that there was sufficient evidence for carcinogenicity to animals and that no case reports or epidemiological studies were available to the working group. The Committee found a NCI/NTP long-term animal study not included in the IARC evaluation.[11] No epidemiological data on 5-nitroacenaphthene were found by the Committee.

3.1 Human studies

No human data were available.

3.2 Animal experiments

Table 1 (Annex E) summarizes the available carcinogenicity studies in experimental animals. These studies comprise an oral study in rats and hamsters by Takemura et al., an oral study in rats and mice by NCI/NTP and an intraperitoneal study in mice described by IARC.[5,11,12] No long-term inhalation or dermal studies were available. The design and carcinogenicity results of the oral studies are presented below. The results on non-cancer endpoints examined in these studies (mortality, body weight, non-neoplastic histopathological lesions) will also be discussed in this section.

In the NCI/NTP study, Fischer 344 rats (50/sex/dose) were given 5-nitroacenaphthene in their diet at concentrations of 0.12% (low dose) or 0.24% (high dose) for 78 weeks (after week 70, there were no surviving male rats in the high-dose group).[11] Following the treatment period, the animals were observed for an additional 22 weeks (low-dose) or 9 weeks (high-dose females). The study included two control groups kept on the basal diet. The daily intake of 5-nitroacenaphthene was calculated by the Committee to be 48 and 96 mg/kg bw/day for male rats, and 60 and 120 mg/kg bw/day for female rats (based on standard food intake values of 40 and 50 g/kg bw/day for male and female rats, respectively, as stated by Gold et al.[13]). 5-Nitroacenaphthene was carcinogenic to male and female rats. In both sexes the incidence of carcinomas in the region of the ear canal was increased markedly from none in controls to 49% in low-dose males, 43% in high-dose males, 55% in low-dose females and 73% in high-dose females. The incidences of alveolar/bronchiolar adenomas and carcinomas were also increased in both sexes. The incidences of these lung tumours were 1%, 17% and 6% in control, low- and high-dose animals (both sexes), respectively. The response was not dose-related, possibly because high-dose rats did not survive long enough to be at risk for these lung tumours. In addition, in female rats the incidences of mammary gland adenocarcinomas and of clitoral gland carcinomas were increased from 0% in controls to 10% (mammary gland tumours in both dosed groups, clitoral gland carcinomas in high-dose females) or 12% (clitoral gland carcinomas in low-dose females). The above tumours are considered to be relevant for humans.

As to the non carcinogenic effects: In male rats of both dose groups, severe body weight depression was evident after week 20 (up to 27% and 45% lower than controls in the low dose and high dose group respectively). In female rats body weight depression, although not as extreme, was also observed in both dose groups. Accelerated mortality was observed at both dose levels in male rats after week 45 and in female rats after week 33. Median survival of high-dose males was 61 weeks while low-dose males had a median survival of 83 weeks. The male control groups lived for at least 100 weeks. Median survival of female rats of the high-dose group was 56 weeks and 76 weeks low-dose females. Most control females lived for at least 100 weeks. Rats showed no treatment-related non-neoplastic lesions. The accelerated mortality in the treated rats may have been related to tumour development.

In the mouse study by NCI/NTP, B6C3F1 mice (50/sex/dose) received diet containing 0.06% (low dose) 5-nitroacenaphthene (in females the concentration was reduced to 0.03% after 51 weeks because of high mortality in high-dose females) or 0.12% (high dose) 5-nitroacenaphthene for 78 weeks.[11] Hereafter,

the mice were observed an additional 18 weeks. The study included a control group (50 mice/sex) kept on the basal diet. The daily intake of 5-nitroacenaphthene was calculated by the Committee to be 72 and 144 mg/kg bw/day for male mice, and 65 and 156 mg/kg bw/day for female mice (based on standard food intake values of 120 and 130 g/kg bw/day for male and female mice, respectively, as stated by Gold et al.[13]; for low-dose females, a time-weighted average dietary concentration of 0.05% was used in the calculation). 5-Nitroacenaphthene was carcinogenic to female mice, causing hepatocellular carcinomas (the incidences were 4%, 49% and 40% in control, low- and high-dose females, respectively) and ovarian tumours (the combined incidences of tubular cell adenomas, granulosa-cell tumours and luteomas were 10% at the low-dose and 18% at the high-dose versus 0% in controls). The induction of ovarian tumours is considered relevant for humans. An increase in the incidence of liver tumours in a mouse carcinogenicity study is generally considered to have little relevance to man.

At study termination in week 96, mean body weight of low-dose male mice was about 20% lower compared to controls and the mean weight of the few surviving high-dose males was about 30% lower. Accelerated mortality was observed in male mice at both dose levels and in female mice at the high-dose. Mortality of male high dose mice was very high (median survival was 20 weeks). The death of male high-dose mice was not due to cancer because no tumours were found in these decedents. Survival in male controls was 86%. In the high-dose group of female mice 24 deaths (48%) occurred in week 50, apparently due to toxicity. At scheduled termination, 36% of the high-dose, 76% of the low-dose and 72% of the control female mice were still alive. Treatment-related non-neoplastic lesions, namely fatty metamorphosis of the liver and calcification of the renal papilla, were observed in male mice at both dose levels and in female mice at the high-dose level.

Overall, though there are some deficiencies in the experimental design of the rat and mice study by NCI/NTP (see Table 1 in Annex E), the study is adequate for derivation of occupational cancer risk values. The other carcinogenicity studies summarized in Table 1 of Annex E are less adequate for this purpose but provide supportive evidence that 5-nitroacenaphthene is carcinogenic in experimental animals. A short description of these studies is given below (see Table 1 of Annex E for details).

In the study of Takemura et al. Wistar rats (30 females, 20 males) and Syrian hamsters (24 females, 10 males) were exposed for up to 6 months to 1% 5-nitroacenaphthene in the diet (see Table 1 in Annex E for daily substance intake per kg body weight).[12] Treatment of female rats was interrupted twice

because of toxicity. After the treatment period, regular diet was given until death (animals in moribund condition and those showing a grossly visible tumour were killed) or scheduled necropsy 500 days after initiation of treatment. Eleven treated female rats developed malignant tumours between days 280 and 500, namely: one rhabdomyosarcoma, two sebaceous, squamous cell carcinomas in the ear duct, five intraductal carcinomas in the breast, and ten adenocarcinomas in the small intestine. These tumours are considered to be relevant for humans. No tumours were observed in the 29 surviving control female rats or in the treated male rats. Seven of the 13 female hamsters which were still alive 270 days after initiation of treatment developed cholangiomas. As this type of tumour is benign (and was not accompanied by malignant tumours), it is not relevant for the determination of cancer risk values. No tumours were observed in the treated male hamsters and the controls.

Eighteen out of 30 treated female rats died, probably due to toxicity, within 200 days without developing a tumour. Weight gain of female rats was significantly impaired (no quantitative information reported) and their treatment had to be interrupted twice because of toxicity. Male rats grew normally and showed no toxic effects. Eleven out of 24 treated female hamsters and 3 out of 10 treated male hamsters died within 270 days. Female hamsters showed reduced body weight compared with the controls (body weight results of male hamsters were not reported).

A third study was described in the report of IARC.[5] In this study, 20 female mice were treated with 5-nitroacenaphthene by intraperitoneal injection (6 mg/kg bw) twice a week for 18 months. In the 15 surviving mice, myeloid leukaemia (4/15), reticulum-cell sarcoma (2/15), and mammary carcinoma (1/15) were observed.

3.3 Selection of the suitable study for risk estimation in the occupational situation

The study by NCI/NTP in rats and mice is considered to be the most suitable study available for estimation of the potential cancer risk in humans under occupational exposure conditions, since the exposure and experimental period covered the largest part of the lifespan of the experimental animals.[11] Moreover, adequate numbers of animals were used in this study. As the highest incidences of treatment-related malignant tumours were observed in female rats, the data from this species and strain were used as starting point. Since the report of the NCI/NTP study did not include individual animal data, the tumour incidences as reported by Gold et al.[13] (See Table 1 Annex E), who had access to the individual

animal pathology results of the NCI/NTP study, were used to calculate the health-based occupational cancer risk values.

The Committee considers the study of Takemura et al. not suitable for the derivation of the occupational cancer risk values because of the considerably shorter exposure time, the low numbers of animals used and several other limitations in experimental design and reporting.[12] The original report of the mouse study (using intraperitoneal injection) as described by IARC was not available, the study was reported only in an abstract.[5] Because of the lack of additional information on methods and results, and because the study is not published in a peer-reviewed journal, the Committee cannot evaluate the quality of this study and considers it not suitable for derivation of cancer risk values.

3.4 Calculation of the health-based occupational cancer risk values

3.4.1 Calculation of carcinogenic activity in experimental animals, life-time low-dose exposure

The Committee considered female rats particularly sensitive to the development of tumours in the region of the external ear canal. To calculate the carcinogenic activity expressed as the incidence per unit daily dose (mg per kg body weight per day) of 5-nitroacenaphtene, the number of female rats with carcinomas in the region of the external ear canal was used as starting point (0/99, 28/49 and 35/48 for control, low dose and high dose group respectively, see Table 1 in Annex E).[13]

Since the actual values for daily food or substance intake per kg body weight were not given in the available publications, the standard value for daily food intake per kg body weight (namely, 50 g/kg body weight/day for female rats, taken from Gold et al.[13]) was used to calculate the daily dose of the test substance.[3] Hence, the dietary concentration of 0.12% was calculated to provide a dose of 60 mg of 5-nitroacenaphthene/kg bw/day (while the dietary concentration of 0.24% resulted in a dose of 120 mg/kg bw/day).

Next, the Committee applied the benchmark dose (BMD) approach to describe mathematically the dose-response relationship and for deriving a reference dose (BMD10) for further cancer risk calculations (see Annex H for BMD analysis). The BMD method aims to describe the best possible dose-response relationship for a set of toxicity data using a set of different mathematical models. In the case of the ear canal tumours a log-logistic model provided the best fit on the data and

resulted in the lowest BMD10. Using this model a BMD10 equal to 4.85 mg/kg bw was chosen (see Annex H).

Then, the incidence per mg/kg bw/day was calculated as follows:

$$I_{dose} = \frac{BMR}{BMD \times (X_{po}/L) \times (X_{pe}/L) \times (\text{exposure hours per day}/24) \times (\text{exposure days per week}/7)} =$$

$$= \frac{0.10}{(4.85 \text{ mg/kg bw}) \times (546/1{,}000) \times (700/1{,}000)} =$$

$$= 5.4 \times 10^{-2} \text{ [mg/kg bw]}^{-1}$$

Where:

- I_{dose} is 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
- BMR is the benchmark response, expressed as 10% extra risk
- BMD is the benchmark dose, the dose corresponding to the BMR expressed in mg per kg body weight per day
- X_{po} and X_{pe} are the exposure and experimental periods, respectively
- L is the standard lifespan for the animals in question (L rat is assumed to be 1,000 days).

3.4.2 *Health risk to humans, life-time low-dose exposure*

To estimate the additional lifetime risk of cancer in humans under lifespan conditions on the basis of results in animal experiments, it was assumed that no difference exists between experimental animals and man with respect to toxicokinetics, mechanism of tumour 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. It is also assumed that biological availability of 5-nitroacenapthene is 100%.

3.4.3 *Health risk to workers, establishment of additional life-time cancer risk*

To estimate the additional lifetime risk of cancer in humans under workplace exposure conditions it was assumed that the average man is exposed for 40 years, 8 hours per day, 5 days per week, 48 weeks per year and inhales 10 m^3 air per 8-hour-working day.

Using as starting point the estimated incidence of 5.4 x 10^{-2} per mg/kg bw (I_{dose}), the additional life-time cancer risk per mg/m^3 under occupational exposure conditions (= HBC-OCRV) amounts to:

$$HBC\text{-}OCRV = I_{dose} \times \frac{40\ years}{75\ years} \times \frac{48\ weeks}{52\ weeks} \times \frac{5\ days}{7\ days} \times \frac{10\ m^3}{70\ kg} = 2.6 \times 0^{-3}[mg/m^3]^{-1}$$

The Committee estimated that the concentration of 5-nitroacenaphtene in the air, which corresponds to an excess cancer mortality of

- 4 per 1,000 ($4x10^{-3}$), for 40 years of occupational exposure, equals to 1.5 mg/m^3
- 4 per 100,000 ($4x10^{-5}$), for 40 years of occupational exposure, equals to 0.015 mg/m^3.

The toxicity data as summarized in this report are too limited to allow a conclusion with regard to risk of adverse effects other than carcinogenicity at the concentration levels associated with the referential cancer risk levels.

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A	Request for advice
B	The Committee
C	Submission letter (in English)
D	Comments on the public review draft
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G	Carcinogenic classification of substances by the Committee
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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 and 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, and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  Occupational Physician, Netherlands Center for Occupational Diseases, Amsterdam
- A.H. Piersma
  Professor of Reproductive and Developmental Toxicology, Utrecht University, and National Institute for Public Health and the Environment, Bilthoven

- H.P.J. te Riele
  Professor of Molecular Biology, VU University Amsterdam, and Netherlands Cancer Institute, Amsterdam
- I.M.C.M. Rietjens
  Professor of Toxicology, Wageningen University and Research Centre, Wageningen
- F. Russel
  Professor of Pharmacology and Toxicology, Radboud University Medical Centre, 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 *5-Nitroacenaphthene*
Your Reference : DGV/MBO/U-932342
Our reference : U-8165/BvdV/cn/459-F70
Enclosed : 1
Date : July 01, 2014

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to 5-nitroacenaphthene.

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

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

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 W.A. van Gool,
President

Annex **D**

Comments on the public review draft

A draft of the present report was released in January 2014 for public review. The following organization and person has commented on the draft document:

- Dr. T.J. Lentz, National Institute for Occupational Safety and Health (NIOSH), Cincinnati (OH), USA.

Annex **E**

Animal studies

Table 1 Carcinogenicity studies with 5-nitroacenaphthene.

Study design and animal species	Data on exposure and effect endpoints	Results	Remarks
Female DD mouse[5] 20 treated 20 control	Intraperitoneal injection, twice weekly, 6 mg/kg bw in arachis oil X_{po} = 18 months X_{pe} = unknown	Of 15 surviving treated mice, 4 developed myeloid leukaemia, 2 developed reticulum-cell sarcomas, and 1 developed mammary carcinoma. No tumours were observed in controls.	Klimisch score: 4 Only abstract available. Low number of animals, no data on effects other than those mentioned under results.
Fischer F344 rat[11] Low dose control: 50 rats/sex High dose control: 49 males and 50 females Treated: 50/sex/dose	0, 0.12% (low dose) and 0.24% (high dose) in the diet (48 and 96 mg/kg bw/day for male rats, 60 and 120 mg/kg bw/day for female rats)[a] X_{po} = Males 78 (low dose) or 70 (high dose) weeks. Females: 78 weeks (low and high dose) X_{pe} = 100 weeks (low dose males and females), 87 weeks (high-dose females), or 70 weeks (high dose males). Appropriate statistical analysis performed (for tumours: based solely on rats surviving 52 wk or,	Adverse effects: Body weight depression at both dose levels, severely in males after week 20, moderately in females. Mortality: accelerated at both dose levels in males from week 45 and in females from week 33 (dose-related response). Tumour incidences in control, low- and high-dose groups, respectively: Lung (alveolar/bronchiolar adenomas and carcinomas) : Male: 1/96, 7/41, 3/47; Female: 1/99, 8/48, 3/48; Carcinomas in the region of the external ear canal: Male: 0/96, 21/43, 20/47; Female: 0/99, 28/49, 35/48; Clitoral gland carcinomas: Female: 0/99, 6/49, 5/48 Mammary gland adenocarcinomas: Female: 0/99, 5/49, 5/48 Treatment-related tumour sites combined (from Gold et al.[13])	Klimisch score: 2 Adequate for carcinogenicity assessment. Deficiencies: some controls improperly matched, maximum tolerated dose exceeded, individual animal data not reported.

	when tumour of interest was seen earlier, at least as long as the time at which the first tumour of interest was seen.)	Male (carcinomas in the region of the ear canal, and/or alveolar/bronchiolar carcinomas and adenomas in the lungs): 1/99, 25/50, 21/50; Female (carcinomas in the region of the ear canal, in clitoral gland, mammary gland, and/or alveolar/bronchiolar carcinomas and adenomas in the lungs): 1/100, 37/50, 38/50	
B6C3F1 mouse[11] control: 50/sex treated: 50/sex/ dose	0, 0.06% (low dose) and 0.12% (high dose) in the diet (72 and 144 mg/kg bw/day for male mice, 65 and 156 mg/kg bw/day for female mice)[a] X_{po} = 78 weeks; low-dose females received 0.06% in the diet for 51 weeks and then 0.03% in the diet for 27 weeks (level lowered because of high mortality in week 50 at the high dose). X_{pe} = 96 weeks 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; high-dose males excluded because of high early mortality)	Adverse effects: Body weight depression in male mice of both dose groups. Mortality: accelerated in low- and high-dose males (dose-dependently) and in high-dose females. Non-neoplastic lesions: fatty metamorphosis of liver and calcification of renal papilla in males at both dose levels and in high-dose females. Tumours: Males: No treatment-related tumours observed at low-dose (survival at high-dose too low for carcinogenicity assessment). Females: Tumour incidences in control, low- and high-dose groups, respectively (no statistical analysis was conducted on these total incidences): Hepatocellular carcinomas: Female: 2/47, 23/47, 18/45; Ovaries (tubular cell adenomas, granulosa-cell tumours, luteomas): Female: 0/45, 4/41, 7/39; Treatment-related tumour sites combined (from Gold et al.[13]) Female (hepatocellular carcinomas and/or granulosa-cell tumours, luteomas, tubular adenomas in ovaries): 2/50, 23/50, 19/50	Klimisch score: 2 Adequate for carcinogenicity assessment, except in high-dose males (due to poor survival). Deficiencies: some controls improperly matched, maximum tolerated dose exceeded, individual animal data not reported.
Female Wistar rat[12] 30 treated, 30 control	1% in diet (500 mg/kg bw/day)[a] X_{po} = 4 months (with 2 interruptions of 3 weeks) X_{pe} = max 500 days (animals were left to die or killed when moribund or having a grossly visible tumour)	Adverse effects: Debilitation during treatment and impaired body weight gain. Mortality: 18 treated rats died within 200 days; one control died during the observation period. Tumours: 11 treated rats developed malignant tumours between days 280-500: 4 rats had adenocarcinoma in small intestine, 3 had intraductal carcinoma in the breast and adenocarcinoma in small intestine, 1 had intraductal carcinoma in the breast, 1 had squamous cell carcinoma in the ear duct, 1 had squamous cell carcinoma in the ear duct and adenocarcinoma in small intestine, and 1 had rhabdomyosarcoma, squamous cell carcinoma in the ear duct, and adenocarcinoma in small intestine. Controls: no malignant tumours observed	Klimisch score: 3 Supportive study. Only one dose tested; high mortality; no statistical analysis performed; exposure period only 4 months and interrupted because of toxicity; only macroscopically observed tumours were examined microscopically; effect on growth not quantified; no data on non-carcinogenic effects other than those mentioned under results.

Male Wistar rat[12] 20 treated	1% in diet (400 mg/kg bw/day)[a] X_{po} = 6 months X_{pe} = max 500 days (animals were left to die or killed when moribund or having a grossly visible tumour)	No malignant tumours observed. Growth not affected. No toxic effects seen during treatment (criteria for toxicity not specified).	Klimisch score: 3 Supportive study Only one dosed tested; exposure period only 6 months; only macroscopically observed tumours were examined microscopically; no data on survival; no information on control group; low number of animals; no data on non-cancer effects other than those mentioned under results.
Syrian Golden hamster[12] 24 treated females 10 treated males 20 control females	1% in diet (920 and 1045 mg/kg bw/day for males and females, resp.)[a] X_{po} = 6 months X_{pe} = max 500 days (animals were left to die or killed when moribund or having a grossly visible tumour)	Adverse effects: Body weight reduced in females. Mortality: 11 treated females and 3 treated males died within 270 days. Tumours: cholangiomas were observed histologically in 7 of 13 surviving treated females; no tumours were observed in control females and in the 7 surviving treated males.	Klimisch score: 3 Supportive study Only one dosed tested; high mortality in treated females; no data on mortality in control females; no statistical analysis performed; exposure period only 6 months; only macroscopically observed tumours were examined microscopically; low number of animals; no data on non-carcinogenic effects other than those mentioned under results.

[a] Calculated by the Committee using standard values for body weight and food intake from Gold et al. (g food/kg body weight/day: 40 in male and 50 in female rat, 120 in male and 130 in female mouse, 92 in male and 104.5 in female hamster).[13]

X_{po}= duration of exposure; X_{pe}= duration of the experiment.
Klimisch scores were based on Klimisch et al.[14]

Annex **F**

Evaluation by the Subcommittee on the Classification of carcinogenic substances

The European Union has classified 5-nitroacenaphthene as a category 1B carcinogen (may cause cancer). IARC (1978) has classified the compound as a 2B carcinogen (possibly carcinogenic to humans).[1]

In the present update (October 2013) the DECOS Subcommittee on the Classification of Carcinogenic Substances evaluated the existing and new information regarding human, animal and in vitro studies on carcinogicity and genotoxicity of 5-nitroacenaphthene.

Human studies

No human data were available to the Subcommittee.

Animal studies

These studies comprise an oral study in rats and hamsters by Takemura et al., an oral study in rats and mice by NCI/NTP and an intraperitoneal study in mice described by IARC.[1-3] No long-term inhalation or dermal studies were available.

In the NCI/NTP study, Fischer 344 rats (50/sex/dose) were given 5-nitroacenaphthene in their diet at concentrations of 0.12% (low dose) or 0.24% (high dose) for 78 weeks (after week 70, there were no surviving male rats in the high-dose group).[3] Following the treatment period, the animals were observed for an additional 22 weeks (low-dose) or 9 weeks (high-dose females). The study

included two control groups kept on the basal diet. The daily intake of 5-nitroacenaphthene was calculated by the Committee to be 48 and 96 mg/kg bw/day for male rats, and 60 and 120 mg/kg bw/day for female rats (based on standard food intake values of 40 and 50 g/kg bw/day for male and female rats, respectively, as stated by Gold et al.[4]). 5-Nitroacenaphthene was carcinogenic to male and female rats. In both sexes the incidence of carcinomas in the region of the ear canal was increased markedly from none in controls to 49% in low-dose males, 43% in high-dose males, 55% in low-dose females and 73% in high-dose females. The incidences of alveolar/bronchiolar adenomas and carcinomas were also increased in both sexes. The incidences of these lung tumours were 1%, 17% and 6% in control, low- and high-dose animals (both sexes), respectively. The response was not dose-related, possibly because high-dose rats did not survive long enough to be at risk for these lung tumours. In addition, in female rats the incidences of mammary gland adenocarcinomas and of clitoral gland carcinomas were increased from 0% in controls to 10% (mammary gland tumours in both dosed groups, clitoral gland carcinomas in high-dose females) or 12% (clitoral gland carcinomas in low-dose females). The above tumours are considered to be relevant for humans.

In the mouse study by NCI/NTP, B6C3F1 mice (50/sex/dose) received diet containing 0.06% (low dose) 5-nitroacenaphthene (in females the concentration was reduced to 0.03% after 51 weeks because of high mortality in high-dose females) or 0.12% (high dose) 5-nitroacenaphthene for 78 weeks.[3] Hereafter, the mice were observed an additional 18 weeks. The study included a control group (50 mice/sex) kept on the basal diet. The daily intake of 5-nitroacenaphthene was calculated by the committee to be 72 and 144 mg/kg bw/day for male mice, and 65 and 156 mg/kg bw/day for female mice (based on standard food intake values of 120 and 130 g/kg bw/day for male and female mice, respectively, as stated by Gold et al.[4]; for low-dose females, a time-weighted average dietary concentration of 0.05% was used in the calculation). 5-Nitroacenaphthene was carcinogenic to female mice, causing hepatocellular carcinomas (the incidences were 4%, 49% and 40% in control, low- and high-dose females, respectively) and ovarian tumours (the combined incidences of tubular cell adenomas, granulosa-cell tumours and luteomas were 10% at the low-dose and 18% at the high-dose versus 0% in controls). The induction of ovarian tumours is considered relevant for humans. An increase in the incidence of liver tumours in a mouse carcinogenicity study is generally considered to have little relevance to man. In the study of Takemura et al. Wistar rats (30 females, 20 males) and Syrian hamsters (24 females, 10 males) were exposed for up to 6 months to 1% 5-nitroacenaphthene in the diet (see Table 1 in annex D for daily substance intake

per kg body weight).[2] Treatment of female rats was interrupted twice because of toxicity. After the treatment period, regular diet was given until death (animals in moribund condition and those showing a grossly visible tumour were killed) or scheduled necropsy 500 days after initiation of treatment. Eleven treated female rats developed malignant tumours between days 280 and 500, namely: one rhabdomyosarcoma, two sebaceous, squamous cell carcinomas in the ear duct, five intraductal carcinomas in the breast, and ten adenocarcinomas in the small intestine. These tumours are considered to be relevant for humans. No tumours were observed in the 29 surviving control female rats or in the treated male rats. Seven of the 13 female hamsters which were still alive 270 days after initiation of treatment developed cholangiomas. As this type of tumour is benign (and was not accompanied by malignant tumours), it is not relevant for the determination of cancer risk values. No tumours were observed in the treated male hamsters and the controls.

A third study was described in the report of IARC.[1] In this study, 20 female mice were treated with 5-nitroacenaphthene by intraperitoneal injection (6 mg/kg bw) twice a week for 18 months. In the 15 surviving mice, myeloid leukaemia (4/15), reticulum-cell sarcoma (2/15), and mammary carcinoma (1/15) were observed.

The Subcommittee concludes that 5-nitroacenaphthene is carcinogenic to experimental animals.

Mechanism of genotoxicity (see Table 2)

5-Nitroacenaphthene was mutagenic in the *Salmonella typhimurium* strains TA100 and TA98 without metabolic activation and, to a larger extent (2 or 5-fold increase, respectively) with metabolic activation.[5] Mutagenicity of 5-nitroacenaphthene in *Salmonella typhimurium* strains TA100 and TA98 with and without metabolic activation was confirmed by McCoy et al.[6] Further, 5-nitroacenaphthene was positive in an umu test system using *Salmonella typhimurium* strain NM3009.[7] In a DNA-repair test 5-nitroacenaphthene elicited a positive DNA-repair response in vitro in both rat and mouse hepatocytes.[8]

Since 5-nitroacenaphthene is positive for bacterial mutagenicity and for genotoxicity in the DNA-repair test, the Subcommittee concludes that 5-nitroacenaphthene is a stochastic genotoxic carcinogen.

Table 2 Genotoxic effects of 5-nitroacenaphthene.

Test system	Concentration	Result[a]		Reference
		-S9	+S9	
Salmonella typhimurium, strains TA98, TA100, reverse mutation	0-100 µg/plate	+	++	Yahagi et al. 1975[5]
Salmonella typhimurium, strains TA98, TA100, reverse mutation	0-100 µg/plate	+	++	McCoy et al. 1983[6]
Salmonella typhimurium, strain NM3009, umu gene expression	Minimal concentration 25 ng/ml	+		Oda et al. 1993[7]
		Rat	Mouse	
DNA-repair tests with rat or mouse hepatocytes in primary culture	0.2 µg/ml- 0.2 mg/ml	+	+	Mori et al. 1987[8]

[a] + = positive; – = negative

Recommendation

Epidemological studies are not available regarding carcinogenicity of 5-nitroacenaphthene. However, sufficient evidence is available that 5-nitroacenaphthene is carcinogenic to animals. Therefore, the Subcommittee recommends to classify 5-nitroacenaphthene in category 1B (‘substance presumed to be carcinogenic to humans’). Moreover , the Subcommittee is of the opinion that a stochastic genotoxic mechanism may underly carcinogenicity. The Subcommittee recommends health-based calculated occupational cancer risk values (HBC-OCRVs) to be calculated for regulatory standard setting.

References

1 Some aromatic amines and related nitro compounds (hair dyes, colouring agents and miscellaneous industrial chemicals). IARC Monog Eval Carcinog Risk Chem Human 1978; 16: 319-323.

2 Takemura N, Hashida C, Terasawa M. Carcinogenic action of 5-nitroacenaphthene. Br J Cancer 1974; 30(5): 481-483.

3 National Toxicology Program. Bioassay of 5-nitroacenaphthene for possible carcinogenicity (CAS No. 602-87-9). Natl Cancer Inst Carcinog Tech Rep Ser 1978; 118: 1-129.

4 Gold LS, Sawyer CB, Magaw R, Backman GM, de VM, Levinson R et al. A carcinogenic potency database of the standardized results of animal bioassays. Environ Health Perspect 1984; 58: 9-319.

5 Yahagi T, Shimizu H, Nagao M, Takemura N, Sugimura T. Mutagenicity of 5- 15 nitroacenaphthene in salmonella. Gann 1975; 66(5): 581-582.

6 McCoy EC, De MG, Rosenkranz EJ, Anders M, Rosenkranz HS, Mermelstein R. 5-Nitroacenaphthene: a newly recognized role for the nitro function in mutagenicity. Environ Mutagen 1983; 5(1): 17-22.
7 Oda Y, Yamazaki H, Watanabe M, Nohmi T, Shimada T. Highly sensitive umu test system for the detection of mutagenic nitroarenes in Salmonella typhimurium NM3009 having high O-acetyltransferase and nitroreductase activities. Environ Mol Mutagen 1993; 21(4): 357-364.
8 Mori H, Sugie S, Yoshimi N, Kinouchi T, Ohnishi Y. Genotoxicity of a variety of nitroarenes and other nitro compounds in DNA-repair tests with rat and mouse hepatocytes. Mutat Res 1987; 190(2): 159-167.

The Subcommittee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Innovation for Life, Zeist; Professor of Translational
- Toxicology, Wageningen University and Research Centre, Wageningen J. van Benthem Genetic Toxicologist, National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- G.J. Mulder
  Emeritus Professor of Toxicology, Leiden University, Leiden
- M.J.M. Nivard
  Molecular Biologist and Genetic Toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  Epidemiologist, Dow Benelux NV, Terneuzen *(until April 1, 2013)*; Exponent, Menlo Park, USA *(from August 15, 2013)*
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- G.B. van der Voet, *scientific secretary*
  Toxicologist, Health Council of The Netherlands, The Hague

Date meeting: October 25, 2013

Annex **G**

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.[15]

Annex **H**

BMD-analysis: Diet study on carcinogenic effects by 5-nitroacenaphtene

Software	US EPA BMDS version 2.4.
Model type	Dichotomous, restricted models.
BMR, Risk type	10%, extra risk (default value for dichotomous (quantal) animal data.
BMDL	Lowest 95% confidence interval of the BMD.
Model fitting	Based on visual inspection of graphs, judgement on BMD-BMDL deviation (model accepted at a deviation of < factor 10), and calculated differences in log-likelihoods.
Data source	National Toxicology Program. Bioassay of 5-nitroacenaphthene for possible carcinogenicity (CAS No. 602-87-9). Natl Cancer Inst Carcinog Tech Rep Ser 1978; 118: 1-129 (a 2-year diet study).[11]
Animals	Female F344/N rats.
Exposure	0, 60 and 120 mg/kg bw/day (see Annex E for specific details)
Effects	Tumour incidence of ear canal, lung, mammary gland, clitoral gland (see Annex E for specific details).

Table 3 Results of a BMD-analysis using a loglogistic model.

	0 mg/kg bw/day	60 mg/kg bw/day	120 mg/kg bw/day	BMD10 (mg/kg bw)	BMDL10[a] (mg/kg bw)
Ear canal (squamous cell and ceruminous cell carcinomas)	0/99	28/49	35/48	4.85	3.36
Lung (alveolar/bronchial adenomas and carcinomas)	1/99	8/48	3/48	89.50	0.24
Mammary gland adenocarcinoma	0/99	5/49	5/48	85.69	51.12
Clitoral gland carcinoma	0/99	6/49	5/48	76.92	46.75

[a] BMDL alleen genoemd als deze minder dan een factor 10 afwijkt van de corresponderende BMD.

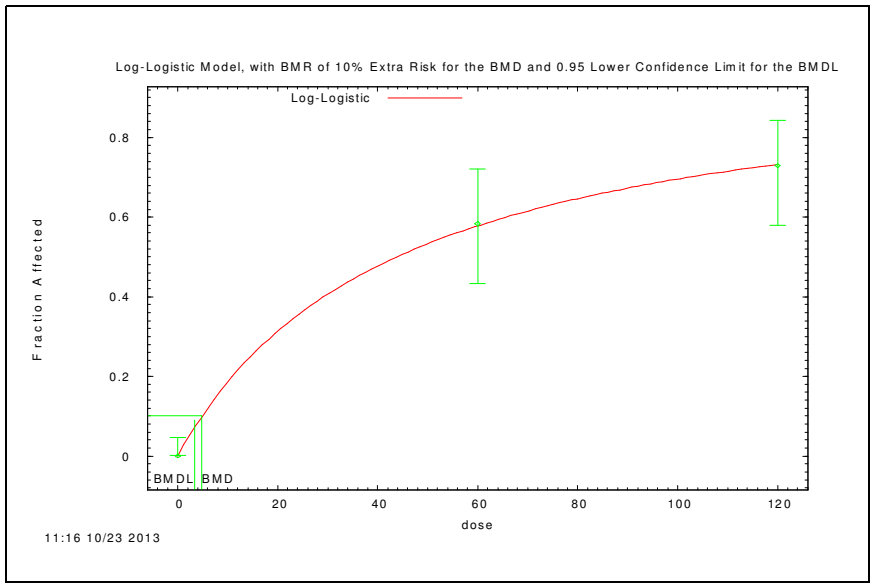

Figure 1 Graph BMD-analysis: Ear canal tumours.

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

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