Health Council of the Netherlands

Naphthalene

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Naphthalene*
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Geachte minister,

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

Dit advies maakt deel uit van een uitgebreide reeks waarin kankerverwekkende stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. Het advies is getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb het 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

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Evaluation of the carcinogenicity and genotoxicity

Subcommittee on the Classification of Carcinogenic Substances
of the Dutch Expert Committee on Occupational Safety (DECOS),
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2012/30, The Hague, December 7, 2012

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).

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

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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INAHTA

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de Subcommissie Classificatie van carcinogene stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de Commissie naftaleen onder de loep. Naftaleen is een stof die onder andere wordt gebruikt als uitgangsmateriaal bij de productie van ftalaatanhydride, azokleurstoffen, naftaleensulfonzuren en in de synthese van diverse geneesmiddelen.

De commissie is van mening dat de gegevens over naftaleen niet voldoende zijn om de kankerverwekkende eigenschappen te evalueren (categorie 3).*

* Volgens het classificatiesysteem van de Gezondheidsraad (zie bijlage G).

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the Subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the Committee. In this report, the Committee evaluates naphthalene. Naphthalene is among others used as a feedstock in the manufacture of phthalic anhydride, in the production of azo dyes and naphthalene sulphonic acids, and in the synthesis of a number of miscellaneous chemicals and pharmaceuticals.

The Committee concludes that the available data are insufficient to evaluate the carcinogenic properties of naphthalene (category 3).*

* According to the classification system of the Health Council (see Annex G).

Chapter 1

Scope

1.1 Background

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

This report contains the evaluation of the carcinogenicity of naphthalene.

1.2 Committee and procedures

The evaluation is performed by the Subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter the Committee. The members of the Committee are listed in Annex B. The submission letter can be found in Annex C.

In 2012, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are

listed in Annex D. The Committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

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

More recently published data were retrieved from the CD ROMs of Chemical Abstracts, Medline, and the internet database Toxline, covering the period from 2002 to March 2012. The relevant data were included in this report.

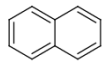
Chapter 2

General information

2.1 Identity and physico-chemical properties

The data have been retrieved from the European Union Risk Assessment report on naphthalene[1], the IARC evaluation of naphthalene[2] and the European Substance Information System (ESIS, which can be accessed via the ECB-site: http://ecb.jrc.it).

Naphthalene is used as a feedstock in the manufacture of phthalic anhydride, in the production of azo dyes and naphthalene sulphonic acids, as a feed stock in the synthesis of a number of miscellaneous chemicals and pharmaceuticals, in the manufacture of mothballs, in special effects for the film industry and as an artificial pore former in the manufacture of grinding wheels to give a high porosity product.

Chemical name	:	Naphthalene
CAS registry number	:	91-20-3
EINECS number	:	202-049-5
Synonyms	:	antimite, naphthalin, naphthene, tar camphor
Appearance	:	A colourless to brown solid, depending on manufacture and purity with a characteristic, readily detectable odour
Chemical formula	:	$C_{10}H_8$
Chemical structure	:	

Molecular weight	:	128.18
Boiling point	:	217.9-218˚C at 1013 hPa
Melting point	:	80.2-80.3˚C
Vapour pressure	:	Ca. 0.01 kPa
Vapour density (air = 1)	:	4.42
Solubility	:	Naphthalene is very slightly soluble in water (0.03 g/L), but is appreciably soluble in many organic solvents (alcohol, benzene, ether)
Conversion factor	:	1 ppm = 5.24 mg/m^3; 1 mg/m^3 = 0.19 ppm
GHS Classification	:	Carcinogenicity Category 2
	:	Acute toxicity Category 4
	:	Acute aquatic toxicity Category 1
		Chronic aquatic toxicity Category 1
Hazard statement(s)	:	H302; Harmful if swallowed.
		H351; Suspected of causing cancer.
		H410; Very toxic to aquatic life with long lasting effects.

2.2 IARC classification

Based on the evaluated data, IARC classified naphthalene in 2002 as *possibly carcinogenic to humans* (Group 2B: there is inadequate evidence in humans for the carcinogenicity of naphthalene; there is sufficient evidence in experimental animals for the carcinogenicity of naphthalene).[2]

Chapter 3

Carcinogenicity

3.1 Observations in humans

Data on workers exposed solely to naphthalene are lacking.[3] The available human data are limited to a few cases reports.

One case report involved a cluster of cancer cases among the employees of a naphthalene purification plant in former East Germany (original publications in German; cited in IARC).[2] A total of fifteen employees operated in the unit of this plant during the preceding 20-30 years between 1917 and 1968. Seven employees were diagnosed with cancer, including four cases of laryngeal cancer (IARC reported a background incidence for laryngeal cancer in the former East Germany in 1970 of 6.3 per 100,000). These four workers (all of them being smokers) were exposed for 7 to 31 years, whereas the limit value for exposure to naphthalene at that time was 20 mg/m^3, with peak values of 50 mg/m^3. The concomitant exposure to other possible carcinogens, e.g. various tar products, was noted by IARC.

Ajao et al. reported on 23 consecutive cases of colorectal carcinoma admitted during June 1982 and May 1984, to a university college hospital in Nigeria (Ajao et al., 1988; cited in IARC).[4] Of these patients, eleven were 30 years old or younger at the moment of diagnosis. Based on family history, gastrointestinal investigations, and autopsy, no indication of familial polyposis among these cases was ascertained. Half of the patients mentioned a history of taking *Kafura*,

a local indigenous treatment for anorectal problems, which contains naphthalene. However, the other half did not know whether they had been given the same drug during early childhood or not.

3.2 Carcinogenicity studies in animals

3.2.1 Inhalation

In an inhalation study by the National Toxicology Program (NTP) using Fischer 344/N rats, groups of 49 males or 49 females were exposed in inhalation chambers to 0, 10, 30 or 60 ppm (0, 52, 157 or 314 mg/m^3) naphthalene (>99% pure) for 6 hours per day, 5 days per week, for a total of 105 weeks.[5,6] Mean body weights of all exposed groups of male rats were less than that of the chamber control group throughout the study. Survival rates in all exposed groups were similar to that of the chamber controls.

An increase in neuroblastoma of the olfactory epithelium was observed, both in male and in female rats. These olfactory neuroblastomas had not been observed in the historical controls. Also adenomas of the nasal respiratory epithelium were observed, most notably in males. In addition to the nasal neoplasms, a variety of non-neoplastic lesions of the nasal tract in both male and female rats were observed in naphthalene-exposed animals compared with controls.

The incidences of (both carcinogenic and non-carcinogenic) respiratory lesions reported by the NTP are summarised in Table 1.

In another study of the NTP, groups of male or female B6C3F1 mice were exposed to 0, 10 ppm (52 mg/m^3) or 30 ppm (157 mg/m^3) naphthalene (>99% pure) for 6 hours per day, 5 days per week, for 104 weeks (75 animal per group for the control group and middle dose; 150 animals per group for the high dose).[7] Mean body weights of exposed mice were slightly lower than that of the controls throughout the study. Survival rates at the end of the study were significantly lower in control male mice compared to exposed males, according to the authors due to wound trauma and secondary infection related to fighting.

Exposed male mice showed increased incidences of bronchiolo-alveolar adenomas and carcinomas but these were not statistically significant. There was a statistically significant increase in the incidence of bronchiolo-alveolar

Table 1 Data reported by the NTP on respiratory and olfactory non-neoplastic and neoplastic lesions observed in rats.[5]

	0 ppm (0 mg/m^3)	10 ppm (52 mg/m^3)	30 ppm (157 mg/m^3)	60 ppm (314 mg/m^3)
Males				
Neoplastic lesions (nose)				
Respiratory epithelium, adenoma	0/49 (0%)	6/49 (12%)#	8/48 (17%)#	15/48 (31%)#
Olfactory epithelium neuroblastoma	0/49 (0%)	0/49 (0%)	4/48 (8%)	3/48 (6%)
Non-neoplastic lesions (nose)				
Olfactory epithelium, hyperplasia, atypical	0/49 (0%)	48/49 (98%)#	45/48 (94%)#	46/48 (96%)#
Olfactory epithelium, atrophy	3/49 (6%)	49/49 (100%)#	48/48 (100%)#	47/48 (98%)#
Olfactory epithelium, chronic Inflammation	0/49 (0%)	49/49 (100%)#	48/48 (100%)#	48/48 (100%)#
Olfactory epithelium, degeneration, hyaline	3/49 (6%)	46/49 (94%)#	40/48 (83%)#	43/48 (90%)#
Respiratory epithelium, hyperplasia	3/49 (6%)	21/49 (43%)#	29/48 (60%)#	29/48 (60%)#
Respiratory epithelium, squamous metaplasia	0/49 (0%)	15/49 (31%)#	23/48 (48%)#	18/48 (38%)#
Respiratory epithelium, degeneration, hyaline	0/49 (0%)	20/49 (41%)#	19/48 (40%)#	19/48 (40%)#
Respiratory epithelium, hyperplasia, goblet cell	0/49 (0%)	25/49 (51%)#	29/48 (60%)#	26/48 (54%)#
Glands, hyperplasia	1/49 (2%)	49/49 (100%)#	48/48 (100%)#	48/48 (100%)#
Glands, metaplasia, squamous	0/49 (0%)	3/49 (6%)	14/48 (29%)#	26/48 (54%)#
Females				
Neoplastic lesions (nose)				
Respiratory epithelium, adenoma	0/49 (0%)	0/49 (0%)	4/49 (8%)	2/49 (4%)
Olfactory epithelium neuroblastoma	0/49 (0%)	2/49 (4%)	3/49 (6%)	12/49 (24%)#
Non-neoplastic lesions (nose)				
Olfactory epithelium, hyperplasia, atypical	0/49 (0%)	48/49 (98%)#	48/49 (98%)#	43/49 (88%)#
Olfactory epithelium, atrophy	0/49 (0%)	49/49 (100%)#	49/49 (100%)#	47/49 (96%)#
Olfactory epithelium, chronic Inflammation	0/49 (0%)	47/49 (96%)#	47/49 (96%)#	45/49 (92%)#
Olfactory epithelium, degeneration, hyaline	13/49 (27%)	46/49 (94%)#	49/49 (100%)#	45/49 (92%)#
Respiratory epithelium, hyperplasia	0/49 (0%)	18/49 (37%)#	22/49 (45%)#	23/49 (47%)#
Respiratory epithelium, squamous metaplasia	0/49 (0%)	21/49 (43%)#	17/49 (35%)#	15/49 (31%)#
Respiratory epithelium, degeneration, hyaline	8/49 (16%)	33/49 (67%)#	34/49 (69%)#	28/49 (57%)#
Respiratory epithelium, hyperplasia, goblet cell	0/49 (0%)	16/49 (33%)#	29/49 (59%)#	20/49 (41%)#
Glands, hyperplasia	0/49 (0%)	48/49 (98%)#	48/49 (98%)#	42/49 (86%)#
Glands, metaplasia, squamous	0/49 (0%)	2/49 (4%)	20/49 (41%)#	20/49 (41%)#

# $P < 0.05$ (corresponding dose group compared to controls using Poly-3 test)
Source: NTP (2000).[5]

adenomas in high-dose females. Also, one bronchiolo-alveolar carcinoma was noted in a high-dose female. Non-neoplastic changes were seen only in the lungs and nose. A dose-related increase in bronchiolo-alveolar inflammation was seen both in males and females. Virtually all exposed animals but none of the controls had chronic nasal inflammation, respiratory epithelial hyperplasia and metaplasia of the olfactory epithelium.

The incidences of (both carcinogenic and non-carcinogenic) lung and nasal lesions reported by the NTP are summarised in Table 2.

Table 2 Data reported by the NTP on lung and nasal non-neoplastic and neoplastic lesions observed in mice.[7]

	0 ppm (0 mg/m^3)	10 ppm (52 mg/m^3)	30 ppm (157 mg/m^3)
Males			
Neoplastic lesions (lung)			
Alveolar/bronchiolar adenoma	7/70 (10%)	15/69 (22%)	27/135 (20%)
Adjusted rate[a]	25.7%	28.8%	22.7%
Alveolar/bronchiolar carcinoma	0/70 (0%)	3/69 (4%)	7/135 (5%)
Adjusted rate[a]	0.0%	5.5%	5.9%
Combined	7/70 (10%)	17/69 (25%)	31/135 (23%)
Adjusted rate[a]	25.7%	31.9%	26.0%
Non-neoplastic lesions (lung)			
Lymphocyte infiltration	3/70 (4%)	0/69 (0%)	8/135 (6%)
Histiocyte infiltration	1/70 (1%)	12/69 (17%)$^{\#}$	16/135 (12%)$^{\#}$
Inflammation	0/70 (0%)	21/69 (30%)$^{\#}$	56/135 (41%)$^{\#}$
Inflammation, granulomatous	0/70 (0%)	19/69 (28%)$^{\#}$	15/135 (11%)$^{\#}$
Hyperplasia alveolar epithelium	2/70 (3%)	7/69 (10%)	12/135 (9%)
Inflammation glands	7/70 (10%)	14/69 (20%)	22/135 (16%)
Non-neoplastic lesions (nose)			
Inflammation	0/70 (0%)	67/69 (97%)$^{\#}$	133/135 (99%)$^{\#}$
Metaplasia olfactory epithelium	0/70 (0%)	66/69 (96%)$^{\#}$	134/135 (99%)$^{\#}$
Hyperplasia respiratory epithelium	0/70 (0%)	66/69 (96%)$^{\#}$	134/135 (99%)$^{\#}$
Females			
Neoplastic lesions (lung)			
Alveolar/bronchiolar adenoma	5/69 (7%)	2/65 (3%)	28/135 (21%)$^{\#}$
Alveolar/bronchiolar carcinoma	0/69 (0%)	0/65 (0%)	1/135 (1%)
Non-neoplastic lesions (lung)			
Lymphocyte infiltration	11/69 (16%)	21/65 (33%)$^{\#}$	46/135 (34%)$^{\#}$
Histiocyte infiltration	1/69 (1%)	5/65 (8%)	4/135 (3%)
Inflammation	3/69 (4%)	13/65 (20%)$^{\#}$	52/135 (39%)$^{\#}$
Inflammation, granulomatous	0/69 (0%)	38/65 (58%)$^{\#}$	42/135 (31%)$^{\#}$
Hyperplasia alveolar epithelium	3/69 (4%)	6/65 (9%)	12/135 (9%)
Inflammation glands	1/69 (1%)	3/65 (5%)	15/135 (11%)$^{\#}$
Non-neoplastic lesions (nose)			
Inflammation	1/69 (1%)	65/65 (100%)$^{\#}$	135/135 (100%)$^{\#}$
Metaplasia olfactory epithelium	0/69 (0%)	65/65 (100%)$^{\#}$	135/135 (100%)$^{\#}$
Hyperplasia respiratory epithelium	0/69 (0%)	65/65 (100%)$^{\#}$	135/135 (100%)$^{\#}$

[a] In view of the high intercurrent mortality in male controls, adjusted rates of neoplasms are also specified; $^{\#}p < 0.05$ (pairwise comparison between the controls and corresponding dose group). Source: NTP (1992).[7]

Furthermore, a screening assay for lung tumours in highly susceptible A/J mice has been conducted with naphthalene.[8] Groups of 30 female mice were exposed in inhalation chambers to 0, 10 or 30 ppm (0, 52 or 157 mg/m^3) naphthalene (purity 98-99%) for 6 hours per day, 5 days per week, for 6 months. Survival was unaffected by treatment. Exposure to 10 or 30 ppm resulted in a non-significant increase in the incidence of lung adenomas (expressed as the number of tumours per animal) compared with controls. The number of tumours per lung of tumour-bearing animals, however, was reported as significantly different when compared to controls.

3.2.2 Oral exposure

In an oral administration study, a group of 28 rats (both strain BD I and BD III; further allocation and use of control animals not specified) were fed a diet containing spectrographically pure naphthalene in oil at a dose of 10-20 mg (not further specified in IARC) per day, on 6 days per week for 100 weeks (Schmähl, 1955; cited in IARC).[2] Animals were kept under observation until death. The average longevity was 800 days. All animals were subjected to necropsy with histopathological examination of abnormal tissues only. No tumours were found in any of the examined rats. The small number of animals and incomplete reporting of the study were noted by IARC.

3.2.3 Other routes

In a study by La Voie et al. (1988), a group of 31 male and a group of 16 female CD-1 mice received intraperitoneal injections of 0.05 M solution of naphthalene (unspecified purity) in dimethyl sulfoxide on days 1, 8 and 15 after birth.[9] The total dose received was reported as 1.75 µmol (0.22 mg) per mouse. Mice were weaned at 21 days, separated by gender, and maintained until termination at 52 weeks, at which time they were necropsied, and gross lesions as well as liver sections were examined histo-pathologically. There was no increase in the incidence of tumours in the naphthalene-treated mice compared to the vehicle controls.

IARC has also evaluated a series of studies in rats using intraperitoneal or subcutaneous administration (Schmähl, 1955; cited in IARC).[2] BD I and BD III rats (sex and age not specified) received weekly intraperitoneal or subcutaneous injections of 20 mg naphthalene (spectrographically pure) as 2% solution in “specially purified oil” for 40 weeks.[2] Average life span was reported as being

similar to survival of controls (without further details). All animals were necropsied with histopathological examination of abnormal tissues only. No tumours were found in any of the rats examined. Due to limitations in study design (e.g., small number of animals) and reporting, no conclusions can be drawn from this study.

Finally, IARC reports on a study in which groups of 38 white inbred rats (age, strain and sex unspecified) received seven subcutaneous injections of 0 or 50 mg/kg bw naphthalene (purified by chromatography) as a 15% solution in sesame oil, at intervals of around 14 days, extending over 3.5 months (Knake, 1956; cited in IARC).[2] In the test group, a total of five sarcomas (one uterine and four lymphosarcomas) and a single mammary fibroadenoma developed and, in the control group, a single sarcoma and a single mammary fibroadenoma. However, due to a high mortality rate during study, in both treated animals and controls, no conclusions can be drawn from this study.

3.3 Cell transformation assays

Naphthalene tested negative in cell transformation assays with BALB/c 3T3 cells and Rauscher leukemia virus (RLV)-infected Fischer rat embryo cells, in absence of an exogenous metabolic system.[2]

3.4 Conclusion

The Committee considers the available human data not sufficient to draw a conclusion on the carcinogenicity of naphthalene in humans.

Based on the available animal data, the Committee considers naphthalene carcinogenic in animals.

Chapter 4

Genotoxicity

The genotoxicity data are summarised in Annex F.

4.1 Gene mutation assays

In vitro assays

Naphthalene was inactive in several types of bacterial mutagenicity assays, under standard conditions*, either with or without metabolic activation.[10-24]

No increase in mutation frequency was observed at the *tk* or *htrp* locus in human MCL-5B-lymphoblastoid cells.[25]

In vivo assays

No results from mammalian *in vivo* gene mutation assays with naphthalene are available to the Committee.

* In the presence of nitrogen-containing reagents under photo-oxidising or photolytic conditions, mutagenic properties of naphthalene have been observed.[2]

4.2 Cytogenetic assays

In vitro

Naphthalene induced sister chromatid exchange (either in the presence or absence of S9) in Chinese hamster ovary cells, but not in human lymphocytes.[7,26] Naphthalene treatment resulted in chromosomal aberrations (only in the presence of S9) in Chinese hamster ovary cells.[7]

An increase in the frequency of CREST-negative micronuclei was reported in human MCL-5B lymphoblastoid cells, and a weak positive response has been reported in a micronucleus assay conducted with newt larvae erythrocytes.[25,27]

In isolated rat hepatocytes, no increase in single strand DNA breaks as measured by alkaline elution was observed after exposure to naphthalene for 3 h up to 3 mM (380 µg/mL).[28]

In vivo

Two in vivo micronucleus assays are available. In the first, groups of five male ICR Swiss mice were given a single oral dose (50, 250, or 500 mg/kg bw).[29] At 25 hours after administration, femoral bone marrow cells were harvested and scored for the presence of micronuclei. The second micronucleus test, conducted under OECD-guideline, involved CD-1 mice receiving a single intraperitoneal dose of 250 mg/kg bw (reported to be a sub-lethal dose).[23] Femoral bone marrow was harvested at 30, 48 and 72 hours after dosing. In both studies, no increase in micronucleated bone marrow cells was observed.

Rats received single oral doses of 359 mg naphthalene/kg bw, 21 hours and 4 hours before sacrifice.[30] No increase of DNA damage in isolated hepatocytes, as measured by alkaline elution, was observed.

In the *Drosophila melanogaster* wing-spot test following larval feeding, naphthalene induced predominantly homologous recombination, whereas gene mutations may also contribute to the induction of wing spots.[31]

4.3 Miscellaneous

In vitro

No increase in unscheduled DNA synthesis was detected in cultures of isolated rat hepatocytes exposed to naphthalene concentrations of 0.16-16 µg/mL.[23]

In vivo

In two independent experiments (performed according to OECD guidelines), animals were sacrificed at two or fourteen hours after exposure, livers were perfused and hepatocytes harvested.[23] Naphthalene did not cause unscheduled DNA synthesis in hepatocytes from rats exposed to a single oral dose as high as 1,600 mg/kg bw.

However, DNA fragmentation has been observed in brain and liver tissue from rats exposed to 110 mg/kg bw/day (0.05 LD_{50} according to the authors) for up to 120 days.[32] This was accompanied by increased lipid peroxidation, suggesting the involvement of oxidative stress. Similar results have been obtained in mice (particularly p53 knock-out) given single doses of 22, 220 or 1,100 mg/kg bw (0.01, 0.1 or 0.5 LD_{50}, respectively, according to the authors).[33,34]

4.4 Conclusion

Based on the negative results in in vitro gene mutation assays and in vivo cytogenicity assays, the Committee concludes that naphthalene acts by a non-genotoxic mode of action.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

The number of available human studies on naphthalene is very limited, and does not provide any specific information on naphthalene. Therefore, the Committee considers the human data to be inadequate for drawing conclusion on the carcinogenic properties of naphthalene.

Two NTP inhalation studies, one in rats and one in mice, have reported local tumours in the respiratory tract upon exposure to naphthalene. In both male en female rats, increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose have been observed. The Committee notes that both types of tumours are relatively rare in rats, and considers them related to the exposure to naphthalene.

The study in male mice has been compromised by a high intercurrent mortality. In female mice an increase in lung tumours – a tumour with a relatively high background incidence in mice – is reported only at the highest exposure group. Therefore, the Committee considers the outcome equivocal.

Overall, data on animal studies indicate that naphthalene is carcinogenic in animals.

The genotoxicity data indicate that naphthalene acts by a non-genotoxic mechanism.

The carcinogenic effects that have been observed in rats and mice after exposure to naphthalene occur at specific sites and in specific tissues (i.e. neuroblastoma of the olfactory epithelium in rats and alveolar/bronchiolar adenomas and carcinomas in mice). Concurrently, pronounced inflammatory responses were present at these sites. The Committee considers it likely that the carcinogenic effects are a consequence of chronic tissue damage and repair.

The metabolism and bioactivation of naphthalene have been identified as key determinants in naphthalene toxicity, and have been studied extensively.[35-37] Multiple competing pathways exist in which cytochrome p450s play a critical role in the formation of several reactive metabolites (e.g., 1,2-naphthalene oxide,1,2-naphthoquinone, and 1,4-naphthoquinone), leading to an array of conjugated and non-conjugated metabolites that are excreted predominantly in the urine.[35] The metabolic processes vary considerably between different species and between different tissues. This explains, at least partly, the reported differences in sensitivity towards naphthalene and the selective induction of tumours.

The human relevance of respiratory tumours observed in rodents has been questioned due to qualitative and quantitative metabolic differences that exist between species, and the relatively high exposure levels applied in animal studies.[36,38-41] The Committee also questions whether the exposure conditions at which the carcinogenic effects occurred in rats are relevant for humans, but notes that exposure conditions are not taken into account in the Committee's evaluation. With respect to the mode of action, the Committee notes particularly an analysis of Rhomberg et al.[41] which consists of a detailed weight-of-evidence approach to assess the carcinogenicity data on naphthalene. Rhomberg et al.[41] discussed each identified key event in the development of cancer in rats and mice after naphthalene exposure, i.e. metabolism, cytotoxicity, inflammation, genotoxicity and ultimately, tumour formation, in the context of the available data.

Rhomberg et al.[41] reasoned that the mouse lung tumours and rat nasal tumours developed after naphthalene exposure by a common mode of action, involving local cytotoxicity and subsequent genotoxicity and carcinogeniticy. These cytotoxic responses result from the generation of reactive metabolites (most likely by CYP2F), under conditions at which glutathione levels are depleted. In a thorough analysis, Rhomberg et al. evaluated the available data and addressed the (in)consistencies, and the subsequent uncertainties concerning the carcinogenic hazard for humans. Based on the data available, the authors considered it most plausible that humans have insufficient metabolic capacity to generate levels of reactive metabolites that deplete glutathione and produce

cytotoxicity when exposed to naphthalene. They concluded therefore that it is not likely that the mode of action that leads to carcinogenicity in rats and mice, will operate in humans (for details see Rhomberg et al.[41]).

The Committee agrees with a weight of evidence approach, and values the approach that has been proposed for naphthalene. The Committee concurs with the line of argument set forth by Rhomberg et al. and considers the available information sufficient to conclude that it is unlikely that upon naphthalene exposure, reactive metabolites are formed in humans to a degree that leads to cytotoxicity and subsequent carcinogenicity.

Therefore, the Committee considers the mode of carcinogenic action of naphthalene in rodents not relevant for humans.

5.2 Recommendation for classification

The Committee concludes that the available data are insufficient to evaluate the carcinogenic properties of naphthalene, and proposes to classify the compound in category 3.*

* According to the classification system of the Health Council (see Annex G).

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A	Request for advice
B	The Committee
C	The submission letter
D	Comments on the public review draft
E	IARC Monograph
F	Genotoxicity data
G	Classification of substances with respect to carcinogenicity

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, 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
- Ms M.J.M. Nivard
  Molecular Biologist and Genetic Toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  Epidemiologist, Dow Chemicals NV, Terneuzen
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- S.R. Vink, *scientific secretary*
  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

Subject	: Submission of the advisory report *Naphthalene*
Your Reference	: DGV/MBO/U-932342
Our reference	: U-7476/BvdV/fs/T17
Enclosed	: 1
Date	: December 7, 2012

Dear Minister,

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

This advisory report is part of an extensive series in which carcinogenic substances are classified in accordance with European Union guidelines. This involves substances to which people can be exposed while pursuing their occupation.

The advisory report was prepared by the Subcommittee on the Classification of Carcinogenic Substances, a permanent subcommittee of the Health Council's Dutch Expert Committee on Occupational Safety. The advisory report as 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 June 2012 for public review. The following organisations and persons have commented on the draft document:

- Mr. W. Cremers, Coal Chemicals Sector Group, Brussels, Belgium
- Ms. A. LeHuray, The Naphthalene Council, Inc., Alexandria, USA.

Annex **E**

IARC Monograph

Volume 82, 2002 (excerpt from Naphthalene, pp 367), CAS no. 91-20-3

Summary of Data Reported and Evaluation.

1 Exposure data

Naphthalene is a commercially important aromatic hydrocarbon which is produced from coal tar and petroleum. It is used mainly as an intermediate in the production of phthalic anhydride, naphthalene sulfonates and dyes and to a lesser extent as a moth-repellent. Human exposure to naphthalene can occur during its production, in creosote treatment of wood, in coal coking operations, during its use as an industrial intermediate, as a result of its use as a moth-repellent, and as a result of cigarette smoking.

2 Human carcinogenicity data

The only data available to the Working Group were two case series. No inference on the carcinogenicity of naphthalene could be drawn from these.

3 Animal carcinogenicity data

Naphthalene was tested for carcinogenicity by oral administration in one study in rats, by inhalation in one study in mice and one in rats and in one screening assay in mice, by intraperitoneal administration in newborn mice and in rats, and by subcutaneous administration in two studies in rats. Exposure of rats by inhalation was associated with induction of neuroblastomas of the olfactory epithelium and adenomas of the nasal respiratory epithelium in males and females. Both of these tumours were considered to be rare in untreated rats. In the screening assay study by inhalation using only female mice, there was an increase in lung adenomas per tumour-bearing mouse. In the inhalation study in mice, there was an increase in the incidence of bronchioloalveolar adenomas in female mice. An apparent increase in the incidence of these tumours in male mice was not statistically significant.

The studies by oral administration in rats, intraperitoneal administration in mice and subcutaneous administration in rats were too limited for an evaluation of the carcinogenicity of naphthalene.

4 Other relevant data

Animal studies suggest that naphthalene is readily absorbed following oral or inhalation exposure. Although no data are available from human studies on absorption of naphthalene, the determination of metabolites in the urine of workers indicates that absorption does occur, and there is a good correlation between exposure to naphthalene and the amount of 1-naphthol excreted in the urine. A number of metabolites, including quinones, naphthols and conjugates (glucuronides, sulfates, glutathione) are derived from the 1,2-epoxide either directly or through multiple metabolic steps.

Naphthalene causes cataracts in humans, rats, rabbits and mice. Humans accidentally exposed to naphthalene by ingestion develop haemolytic anaemia, but there is no evidence of haemolytic anaemia in rodents. Cases of haemolytic anaemia have been reported in children and infants after oral or inhalation exposure to naphthalene or after maternal exposure during pregnancy.

Naphthalene causes lung toxicity in mice, but not rats, following either intraperitoneal injection or inhalation exposure. In mice, the injury is dose-dependent and Clara cell-specific. After repeated administration of naphthalene, mouse Clara cells become tolerant to the naphthalene-induced injury that occurs

following a single dose of naphthalene. Acute and chronic exposure to naphthalene caused nasal toxicity in both mice and rats.

In isolated mouse Clara cells, 1,4-naphthoquinone and naphthalene 1,2-oxide were more toxic than naphthalene. Injury to Clara cells in perfused lungs occurred at lower concentrations of naphthalene 1,2-oxide compared with naphthalene or its other metabolites.

There is some evidence of developmental toxicity in rats and mice at dose levels that caused clear maternal toxicity. Clara cells of neonatal mice are more sensitive than those of adult mice to the cytotoxic effects of naphthalene.

There is little evidence for induction of gene mutations by naphthalene. In contrast, positive results were obtained in assays for micronucleus formation, chromosomal aberrations and chromosomal recombinations in vitro, which are consistent with a clastogenic potential.

Overall, the proposed mechanism of carcinogenic action is that the higher rates of metabolism of naphthalene in mice lead to cytotoxic metabolites in the lung, causing increased cell turnover and tumours. The absence of lung tumours in rats is entirely consistent with this mechanism. The maximal rates of metabolism measured in human lung microsomes are about 10-100 times lower than those in mice.

5 Evaluation

There is inadequate evidence in humans for the carcinogenicity of naphthalene.

There is sufficient evidence in experimental animals for the carcinogenicity of naphthalene.

Overall evaluation

Naphthalene is possibly carcinogenic to humans (Category 2B).

For definition of the italicized terms, see Preamble Evaluation.

Synonyms

- Naphthalin
- Naphthene
- Tar camphor
- White tar.

Last updated: 4 December 2002

Annex **F**

Genotoxicity data

Modified from IARC[2]

In vitro test system	Result		HID or LED[a]	Reference
	- S9	+S9		
Bacterial gene mutation assay				
E. coli K12 *envA- uvrB-*, prophage induction	NT	-	500	Ho & Ho (1981)[14]
E. coli GY5027 *envA- uvrB-*, GY40415 *amp*^{R}, prophage induction	NT	-	2,000 µg/plate	Mamber et al. (1984)[16]
E. coli PQ37, SOS induction (chromotest)	-	NT	NR	Mersch-Sundermann et al. (1993)[42]
S. typhimurium TA1535/pSK1002, *umu* gene expression (SOS-inducing activity)	-	-	83 µg/mL	Nakamura et al. (1987)[19]
E. coli WP2/WP100 *uvr*A- *rec*A- assay, differential toxicity	NT	-	2,000 µg/plate	Mamber et al. (1984)[16]
S.typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	NT	-	100 µg/plate	McCann et al.(1975)[17]
S. typhimurium TA100, TA98, reverse mutation	-	-	384 µg/plate	Florin et al. (1980)[11]
S. typhimurium TA100, TA98, UHT8413, UHT8414, reverse mutation	-	-	2,000 µg/plate	Connor et al. (1985)[10]
S. typhimurium TA100, TA98, TA2637, reverse mutation	-	-	500 µg/plate	Nohmi et al. (1985)[21]
S. typhimurium TA100, TA98, TA97, reverse mutation	-	-	50 µg/plate	Sakai et al. (1985)[22]
S. typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	-	-	33 µg/plate	Mortelmans et al. (1986)[18]
S. typhimurium TA1535, reverse mutation	NT	-	1,000 µg/plate	Narbonne et al. (1987)[20]
S. typhimurium TA1537, reverse mutation	NT	-	100 µg/plate	Gatehouse (1980)[12]
S. typhimurium TA1537, reverse mutation	NT	-	200 µg/plate	Seixas et al. (1982)[43]

S. typhimurium TA1538, reverse mutation	NT	-	500 µg/plate	Gatehouse (1980)[12]
S. typhimurium TA98, reverse mutation	NT	-	500 µg/plate	Ho et al. (1981)[13]
S. typhimurium TA98, reverse mutation	NT	-	100 µg/plate	Narbonne et al. (1987)[20]
S. typhimurium TM677, reverse mutation	NT	-	256 µg/plate	Kaden et al. (1979)[15]
S.typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	-	-	300 µg/plate	Schreiner (2003)[23]
S. typhimurium TA102, reverse mutation	-	-	NR	Yan et al. (2004)[24]
Mammalian cell genotoxicity test				
DNA single strand breaks, rat hepatocytes, (alkaline elution)	-	NT	38	Sina et al. (1983)[28]
Micronucleus formation, newt larvae (*Pleurodeles waltl*) erythrocytes	(+)		0.25 ppm	Djomo et al. (1995)[27]
Sister chromatid exchange, Chinese hamster ovary cells	+	+	27	National Toxicology Program (1992)[7]
Chromosomal aberrations, Chinese hamster ovary cells	-	+	30	National Toxicology Program (1992)[7]
DNA fragmentation, macrophage J774A.1 cells, centrifugation	+	NT	26	Bagchi et al. (1998)[44]
Gene mutation, human MCL-5B-lymphoblastoid cells, *TK* and *HPRT* loci	-	NT	40	Sasaki et al. (1997)[25]
Sister chromatid exchange, human lymphocytes	-	-	13	Tingle et al. (1993)[26]
Micronucleus formation (CREST-), human MCL-5B-lymphoblastoid cells	+	NT	30	Sasaki et al. (1997)[25]
Unschedules DNA synthesis, isolated rat hepatocytes	-	NT	16	Schreiner (2003)[23]

In vivo test system	Result	Dose	Reference
Somatic mutation; recombination in *Drosophila melanogaster*	+	1, 5, 10 mM (feeding larvae)	Delgado-Rodriguez et al. (1995)[31]
Micronucleus assay in ICR Swiss mice	-	500 mg/kg bw by gavage	Harper et al. (1984)[29]
Micronucleus assay in CD-1 mice	-	250 mg/kg bw p.i.	Schreiner (2003)[23]
Unscheduled DNA synthesis in rats	-	0, 600, 1000 and 1,600 mg/kg bw by gavage	Schreiner (2003)[23]
DNA damage (alkaline elution)	-	2x359 mg/kg bw by gavage	Kitchin et al. (1992)[30]
DNA fragmentation in rats, liver; brain	+	110 mg/kg, daily for 120 days	Delgado-Rodriguez et al. (1995)[31] Bagchi et al. (1998)[32]
DNA fragmentation in mice, liver; brain (wildtype and p53 knock-out)	+	single dose of 22, 220 or 1,100 mg/kg bw	Bagchi et al. (2000)[33] Bagchi et al (2002)[34]

[a] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests (in µg/ml unless otherwise specified).

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	
		(before 16 December 2008)	(as from 16 December 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 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.[45]