Health Council of the Netherlands

Ethylene

Evaluation of the carcinogenicity and genotoxicity

2013/24

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Ethylene*
Uw kenmerk : DGV/BMO-U-932542
Ons kenmerk : U-7909/BV/fs/246-X18
Bijlagen : 1
Datum : 18 oktober 2013

Geachte minister,

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

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 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

Ethylene

Evaluation of the carcinogenicity and genotoxicity

to:

the Minister of Social Affairs and Employment

No. 2013/24, The Hague, October 18, 2013

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

This report can be downloaded from www.healthcouncil.nl.

Preferred citation:
Health Council of the Netherlands. Ethylene - Evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2013; publication no. 2013/24.

ISBN: 978-90-5549-970-0

Contents

Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens het uitoefenen van hun beroep kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de Subcommissie Classificatie van carcinogene stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie ethyleen onder de loep.

Ethyleen wordt geproduceerd door het stoomkraken van koolwaterstoffen en wordt vooral gebruikt als chemisch intermediair in de productie van polymeren en andere industriële chemicaliën zoals ethyleenoxide, ethyleendichloride en ethylbenzeen; kleine hoeveelheden worden gebruikt om het rijpen van fruit en groente te bevorderen, en voor het lassen en snijden van metaal.

Op basis van de beschikbare gegevens over ethyleen meent de commissie dat deze niet voldoende zijn om de kankerverwekkende eigenschappen van deze stof 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 the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter called the Committee. In this report the Committee evaluated ethylene.

Ethylene is produced by steam-cracking of hydrocarbons and mainly used as a chemical intermediate in the production of polymers and other industrial chemicals such as polyethylene, ethylene oxide, ethylene dichloride and ethylbenzene; minor amounts are used to promote the ripening of fruits and vegetables, and for welding and cutting metals.

The Committee is of the opinion that the available data are insufficient to evaluate the carcinogenic properties of ethylene (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 the proposal for classification are expressed in the form of standard sentences (see Annex G).

This report contains the evaluation of the carcinogenicity of ethylene.

1.2 Committee and procedure

The evaluation is performed by the Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee. The members of the Committee are listed in Annex B. The submission letter (in English) to the Minister can be found in Annex C.

In June 2013 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 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 ethylene, such an IARC-monograph is available, of which the summary and conclusion are inserted in Annex E.

More recently published data were retrieved from the DIMDI database Medline and XToxline, and Chemical Abstracts. The last updated online search was in August 2013. The new relevant data were included in this report.

Chapter 2

General information

2.1 Identity and physico-chemical properties

Ethylene, the petrochemical manufactured in largest volume worldwide, is produced primarily by steam-cracking of hydrocarbons. It is used mainly as a chemical intermediate in the production of polymers and other industrial chemicals such as polyethylene, ethylene oxide, ethylene dichloride and ethylbenzene; minor amounts are used to promote the ripening of fruits and vegetables, and for welding and cutting metals. Ethylene is introduced into the environment from both natural and man-made sources with estimated total emissions of 74 and 26%, respectively, and includes emissions from vegetation, as a product of burning of organic material (such as cigarettes) and of incomplete combustion of fossil fuels, and in its production and use. In addition, ethylene is endogenously produced in mammals and humans (and also in plants). Possible endogenous sources of ethylene are lipid peroxidation, oxidation of free methionine, oxidation of haemin in haemoglobin and metabolism of intestinal bacteria.[1,2]

Background levels of ethylene in ambient air at rural and remote sites range from <1-15 µg/m^3, and in urban and indoor air contaminated with combustion products from a few to over 1,000 µg/m^3.[1-3] Ethylene in cigarette smoke amounts to 1-2 mg ethylene per cigarette, i.e. levels of 56-110 µg/m^3 were measured in smoky tavern air. Human endogenous production may yield atmospheric concentrations up to around 100 µg/m^3 from exhalation in a closed system.[4] Few

data are available on levels of occupational exposure. Monitoring of ethylene levels during use for ripening of bananas showed air concentrations to be in the range of 0.02-3.35 ppm (0.02-3.85 mg/m^3). In a petrochemical plant the mean exposure level was 4.5 mg/m^3. In a study on exposure of firefighters, samples taken during the "knockdown" phase of a fire showed a concentration of 46 ppm (53 mg/m^3) ethylene.[1,2]

The identity of ethylene and some of its physicochemical properties are given below.[1]

Chemical name	:	ethylene
CAS registry number	:	74-85-1
EC/EINECS number	:	200-815-3
Synonyms	:	ethene, acetene, bicarburetted hydrogen, elayl, olefiant gas
Colour and physical state	:	colourless gas
Molecular weight	:	28.05
Molecular formula	:	C2H4
Structure	:	H2C=CH2
Melting/boiling point	:	-169/-103.7 °C
Relative vapour density (air = 1)	:	0.9686
Vapour pressure	:	4,270 kPa at 0 °C
Stability: Lower explosive limit (in air)	:	2.75 vol% or 34.6 g/m^3 at 100 kPa and 20°C
Solubility	:	very slightly soluble in water (0.26% vol/vol; 131 mg/L at 20 °C); slightly soluble in acetone, benzene and ethanol; soluble in diethyl ether
Conversion factors (25 °C, 760 mm Hg)	:	1 ppm = 1.15 x mg/m^3 1 mg/m^3 = 0.87 x ppm
EU Classification	:	Flam. Gas 1: H220 (Extremely flammable gas) Press. Gas: H336 (May cause drowsiness or dizziness) STOT SE 3

2.2 IARC conclusion

In 1994, IARC[1] concluded that there is *inadequate evidence* for the carcinogenicity of ethylene in experimental animals and humans. Therefore, according to the IARC guidelines, ethylene was considered to be not classifiable as to its carcinogenicity to humans (Group 3).

Chapter 3

Carcinogenicity

3.1 Observations in humans

Two nested case-control studies are available. The first study (Leffingwel et al., 1983)[5] investigated the elevated standardised mortality ratios (>200) found at a Texas petrochemical plant for neoplasms of the brain (17 cases; 102 controls). Possible associations between gliomas of the brain and job title, departmental employment history, chemical exposure history, geographic location within the plant, dates of employment, and residence were examined. For ethylene odds ratios (OR) of 1.17-4.03 were found for the time intervals of 0-14, >15 years or ever possibly exposed to ethylene with or without maintenance men included in the pool of cases and controls; no dependence on duration of exposure was seen and confidence intervals were wide and included 1 (confounding factor of smoking was not investigated). Moreover, workers had been exposed to multiple chemicals and exposure levels are not known. Therefore, the uncertainty of this study is large as indicated by the wide confidence intervals including 1 and no conclusion can be drawn from this study.

In a nested case-control study (Bond et al., 1986)[6] among male workers (cases 308; controls 588) at Dow Chemical's Texas Operations, a potential association between frequency of occupational exposure to a number of agents and lung cancer deaths between 1940 and 1981 was investigated. Potential confounding factors such as smoking were taken into account. Ethylene and ethylene oxide showed no relation with lung cancer without or with a latency

period (no latency period: ethylene: OR 0.99 (95% CI 0.62-1.58); cases 31; ethylene oxide: OR 0.90 (95%CI 0.47-1.72); cases 14). Since all workers were exposed to multiple agents, including known carcinogenic agents, no conclusion concerning pulmonary carcinogenic potential of ethylene can be drawn from this study.

3.2 Carcinogenicity studies in animals

3.2.1 Carcinogenicity studies

Fischer-344 rats (n=120/dose/sex) were exposed to 0, 300, 1,000 or 3,000 ppm (0, 345, 1,150 or 3,450 mg/m^3) of ethylene (>99.9% pure) for 6 h/day, 5 days/ week for 2 years.[1,2,7] The highest concentration was chosen as to avoid explosion hazard. Necropsies were conducted after 6 and 12 months (5/dose/sex), and after 18 (19-20/dose/sex) and 24 months (all survivors) of exposure. Body weight was determined. Haematological, urinalysis and clinical chemistry parameters were measured in interim sacrificed animals and at 24 months. Tissues from animals at 0 and 3,000 ppm were examined histologically. There was no significant difference in survival between control and treated groups. No treatment-related toxicity or increased incidence in neoplasms was reported. Rostron (1985)[8] remarked that in the above study no discussion on the incidence of mononuclear cell leukaemia was present. The number of animals affected (out of 90) rose from 12 and 8 in the male and female control group to 21 and 11, in males and females at 3,000 ppm respectively. As ethylene oxide is known to induce this type of cancer in F344 rats[9] and ethylene oxide is one of the major metabolites of ethylene, it may be responsible for this type of cancer observed here. Unfortunately, no animals at intermediate dose levels were examined histologically. However, the OECD SIDS report[2] and the National Institute for Public Health and the Environment (RIVM)[10] state that mononuclear cell leukemia may occur in F344 rats at high background levels. Moreover, these incidences were within the historical incidence for control rats of this strain reported in NTP studies (Haseman et al., 1990)[11].Therefore, chemically induced mononuclear cell leukemia in F344 rats are considered to be not relevant for carcinogenicity in humans.

3.2.2 Tumour-initiation-promotion study

Ethylene was shown to have no initiating capacity in the following test. Sprague-Dawley male and female rats (3-5 days old) were exposed to 0 or 11,500 mg/m^3

of ethylene (saturated metabolism; purity not given) by inhalation for 8 h/day, 5 days/week for 3 weeks.[12] One week later, the rats were given 10 mg/kg bw Clophen A 50 (a mixture of polychlorinated biphenyls) by gavage twice a week for 8 weeks and livers were screened for foci with a deficiency in ATPase, a biomarker for initiating carcinogenic capacity. The number of ATPase-deficient foci in ethylene-exposed rats did not exceed the control values. In the same experiment, ethylene oxide, administered at 99 mg/m^3 and 180 mg/m^3 as a positive control, produced a significant increase in the incidence of ATPase-deficient foci in females.

3.3 Summary of the carcinogenicity studies

Only two nested case-control studies were available. Leffingwell et al. (1983)[5] found a relation between gliomas of the brain and ethylene exposure, but in this study the number of cases was very limited, and workers had been additionally exposed to unknown levels of multiple chemicals leading to a large uncertaintly as indicated by the wide confidence intervals. Bond et al. (1986)[6] found no relation with lung cancer exposure. Also in this study all workers were exposed to multiple agents and exposure levels were not known. Both human studies did not allow conclusions on the carcinogenicity of ethylene. In a 2-year study in F344 rats[7] no increased incidence of neoplasms related to ethylene was observed. [The Committee observed mononuclear cell leukemia in this study but this was considered animal specific and not related to the chemical exposure.] An initiation-promotion study in rats did not indicate ethylene as an initiator of carcinogenicity.[12]

Chapter 4

Genotoxicity

In vitro and in vivo genotoxicity data of ethylene are summarized below and are presented in a table in Annex F.

4.1 Gene mutation assays

4.1.1 In vitro

Ethylene (99.5% pure) did not induce gene mutations in *Salmonella typhimurium* TA100 exposed for 7 hours to 0.5-20% ethylene in air with or without metabolic activation.[13] The NTP showed that *Salmonella typhimurium* TA97, TA98, TA100 and TA1535 did not induce gene mutations when exposed to ethylene vapour (concentration not specified) with or without metabolic activation (10-30% S9-mix).[14] Exposure to 20,000 or 100,000 ppm ethylene vapour using *Salmonella typhimurium* TA 98, TA100, TA1535 and TA1537 did also not induce mutations, with and without metabolic activation, compared to the controls (only summary available).[7]

4.1.2 In vivo

Walker et al. (2000)[15] showed that repeated inhalation exposure to ethylene (up to 3,450 mg/m^3) did not increase *hprt* mutant frequencies in splenic T cells of exposed rats and mice compared with control animals.

4.2 Cytogenetic assays

4.2.1 In vitro

In a cytogenetic assay using CHO cells no increase in chromosomal aberrations was noted on exposure to ethylene up to 25 vol% in nitrogen (3 hours) with or without metabolic activation.[2]

4.2.2 In vivo

Exposure of F344 rats and B6C3F1 mice to ethylene up to 3,450 mg/m^3 for 6 h/day, 5 days/week for 4 weeks gave no increased frequency of micronuclei in the bone marrow cells.[16] Exposure of F344 rats to ethylene up to 11,473 mg/m^3 ethylene for 6 h/day, 5 days/week did not induce any increase in the frequencies of micronucleated peripheral blood reticulocytes, or any increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes.[17]

4.3 Summary of the genotoxicity studies

Ethylene did not induce gene mutations in *Salmonella typhimurium* strains or chromosomal aberrations in CHO cells with and without metabolic activation. No increased frequency in micronuclei was found in bone marrow from rat and mouse in vivo studies.

4.4 Role of ethylene oxide

Only 5.6% of ethylene inhaled by humans is absorbed and becomes systemically available. Ethylene is also endogenously produced in mammals and humans. Both the ethylene absorbed and endogenously formed are partly metabolised to ethylene oxide[1]. Ethylene oxide binds to macromolecules, forming hydroxyethyl adducts with haemoglobin and DNA. Moreover ethylene oxide was found to be mutagenic in vitro and in vivo and carcinogenic in experimental animals (IARC,1994)[1,18].

Ethylene oxide is currently classified by the IARC as a known human carcinogen (Group 1). The general conclusion from IARC (1994)[1,18] is that the actual tissue burden of ethylene oxide resulting from ethylene exposure was too low to lead to an increased tumour incidence. Data on ethylene oxide directly relevant to the

formation of ethylene oxide from ethylene will be discussed below. [Further information on ethylene oxide and its mechanism of carcinogenicity is outside the scope of this report and can be found in the IARC monographs[9,19].]

4.4.1 *Quantification of ethylene oxide*

The ethylene oxide blood levels in ethylene-exposed mice and humans are not reported yet. Only in rats, exposed to ethylene concentrations of between 300 and 1000 ppm, ethylene oxide-blood concentrations were quantified directly by gas chromatography with flame ionization detection.[20]

The formation of ethylene oxide can be quantified indirectly by measurement of its hydroxyethyl adduct to the N-terminal valine of haemoglobin (N-(2-hydroxy-ethyl)valine (HOEtVal) (mostly investigated in humans) and its adduct at the N^7-position of guanine in DNA, 7-hydroxyethylguanine (7-HEG; animals only).[1] At low exposure levels, both HOEtVal and 7-HEG increase linearly with exposure[21], but the exact relationship between 7-HEG and HOEtVal formation may vary with length of exposure, interval since exposure, species and tissue or even with cell type, due to differences in formation, persistence, repair, and chemical depurination of 7-HEG and toxicokinetic effects on erythrocytes.[15,22-27]

4.4.2 *Estimation of the ethylene oxide level upon ethylene inhalation*

In several studies ethylene oxide levels have been estimated measuring HOEtVal in humans exposed to ethylene. Non-smoking ethylene-exposed fruit store workers showed a statistically significant increase in HOEtVal levels in haemoglobin compared to non-smoking non ethylene-exposed referents. It was calculated that 1 ppm of ethylene in air would give rise to the same haemoglobin adduct level as 0.0345 mg/m^3 (0.03 ppm) of ethylene oxide which would correspond to a conversion rate of approximately 3% (1-10%).[28] A comparable value was found for alveolar retention of 2 ± 0.8% for ethylene by Filser et al.[4] They also determined that the metabolism of ethylene followed first-order kinetics up to 57.5 mg/m^3 exposure and they expect at higher concentrations a saturation of ethylene metabolism similar to observations in rats[1,4].

Granath et al.[29] performed two studies in Swedish workers in the plastics industry. in the first study, exposure to ethylene was estimated in 8 workers to be 4 mg/m^3 (95% CI 0.5-7.5) for high exposure using relevant (1988-1990) measurements available from the plant's hygienic surveillance programme. The

mean HOEtVal adduct level measured was 101 pmol/g (range 56-200). The mean HOEtVal level in the control group (n=9) was 15 pmol/g (range 9-32); the mean background levels in human nonsmokers range from 13 to 60 pmol/g Hb (Table 8 in Csanady[18]). The fraction of ethylene metabolised to ethylene oxide for 7 non-smokers was estimated to amount to 0.5% (95% CI 0.31-0.83).[29] For comparison, smoking contributes between 7 and 11 pmol/g Hb of HOEt Val per cigarette smoked per day.[30]

In the second study, airborne exposure to ethylene was monitored in 4 non-smoking men during 5 consecutive workdays and blood samples were collected at 3 times (5 days apart) for determination of HOEtVal. The average exposure level was 4.36 mg/m^3 and from the measured HOEtVal levels it was estimated that on average 0.47 ± 0.09% of the ethylene was converted to ethylene oxide. Comparing the abovementioned HOEtVal adduct level for ethylene (minus background) of 85 pmol/g Hb after exposure for 40 h per week at 4 mg/m^3 (3.6 ppm) and the adduct level of 2,400 pmol/g Hb after exposure to ethylene oxide for 40 h per week at 1.8 mg/m^3 (1 ppm), suggests that the adduct levels induced by exposure to ethylene is about 100 times lower than those induced by ethylene oxide at the same molar exposure level, suggesting that approximately 0.5 % of the ethylene becomes bioavailable as ethylene oxide.[29]

Csanady et al.[18] developed a physiological toxicokinetic model to describe the uptake and elimination of ethylene, and the endogenous production of ethylene/ethylene oxide in rats, mice and humans. The predictions for humans reflected actually measured haemoglobin adducts. The ethylene oxide concentration in blood from endogenous ethylene was predicted to be 0.04 nmol/L, assuming the formed ethylene oxide is 100% bioavailable. Exposure to about 0.063 mg/m^3 (0.055 ppm) ethylene (8 h/day, 5 days/week) leads according to the model to an average ethylene concentration in blood of about twice that of the endogenous value. Li et al. (2011)[14,31] determined kinetic parameters of ethylene and ethylene oxide in vitro in subcellular liver fractions of mice, rats and humans, for comparison with in vivo kinetic data. The results showed agreement between in vitro and in vivo data to a certain degree and may be used to improve the toxicokinetic model by Csanady et al.[18]

4.4.3 *Adduct formation*

The presence of 7-HEG itself in DNA does not induce mutagenicity, but labile alkylated DNA adducts, such as 7-HEG and 3-hydroxyethyladenine, may be depurinated by base excision repair and form mutagenic apurinic/apyrimidynic

sites.[22,32] However, Rusyn et al.[32] found no increase in the number of aldehydic DNA lesions, an indicator of apurinic/apyrimidynic sites, in brain, liver or spleen of rats exposed to ethylene (up to 3,450 mg/m^3) or ethylene oxide (115 mg/m^3) for 1,3 or 20 days by inhalation. The existence of labile DNA adducts was confirmed for ethylene oxide exposure by an increase in heat-induced AP sites compared to controls. Several genes for base excision DNA repair were not statistically significantly upregulated (or even downregulated) in the liver and target organs brain and spleen – only polymerase β and AP endonuclease in the brain were significantly upregulated – on exposure to ethylene (3,450 mg/m^3), while for ethylene oxide (115 mg/m^3) polymerase β was significantly upregulated in the spleen as well as a number of genes in the liver. This suggests that DNA damage induced by ethylene or ethylene oxide exposure is repaired without accumulation of AP sites and is associated with biologically insignificant changes in base excision DNA repair gene expression in target organs. The authors concluded that accumulation of AP sites is not likely to be a primary mechanism for mutagenicity and carcinogenicity of ethylene oxide.[32]

In rats, ethylene metabolism follows first-order kinetics up to about 92 mg/m^3 and a saturation of ethylene metabolism occurs at about 1,150 mg/m^3.[1,4,18,33,34] Rusyn et al.[32], Bolt et al.[35] and Walker et al.[15] explained the lack of mutagenicity/carcinogenicity of ethylene in rodents by the fact that not sufficient ethylene oxide can be formed due to saturation of metabolic activation at concentrations >1,150 mg/m^3. Moreover, Walker et al.[15] showed that repeated inhalation exposure to ethylene (up to 3,450 mg/m^3) did not increase *hprt* mutant frequencies in splenic T cells of exposed rats and mice compared with control animals, while exposure to 360 mg/m^3 ethylene oxide for 4 weeks did induce a 5- to 6-fold increase compared to control.

4.4.4 *Conclusion regarding the role of ethylene oxide*

Ethylene can be converted to ethylene oxide which gives rise to adduct formation. However, it seems likely that not enough ethylene oxide is formed to induce carcinogenicity in view of the following:

- no carcinogenic effects were found in rats after exposure up to 3,450 mg/m^3
- the absence of tumour initiating capacity in rats after exposure to ethylene at a 100 times higher concentration than an effective concentration of ethylene oxide
- exposure of animals to ethylene concentrations of a factor 15-30 higher than ethylene oxide concentrations showed a smaller amount of adduct formation

- the amount of ethylene oxide formed as estimated from HOEtVal concentration in humans is only 0.5-3% of ethylene exposure and the measured adduct levels induced by ethylene are about 100 times lower than the measured adduct level of ethylene oxide at equimolar exposure.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

No reliable data on the carcinogenicity in humans were available. In a 2-year study in rats no increased incidence of neoplasms related to ethylene was observed.

Ethylene did not induce C-G gene mutations in *Salmonella typhimurium* strains or chromosomal aberrations in CHO cells with and without metabolic activation. No increased frequency in micronuclei was found in bone marrow from rat and mouse in vivo studies.

No evidence is available to suggest that ethylene is genotoxic and/or carcinogenic by inhalation. Less than 10% of ethylene is taken up by the lungs and converted to ethylene oxide, a known genotoxic carcinogen. Although ethylene oxide is genotoxic and carcinogenic, the amount of ethylene oxide formed seems to be not sufficient to induce genotoxic or carcinogenic effects The potential damage to DNA induced by the ethylene oxide formed, seems to be adequately repaired by the mechanism present for the endogenously produced ethylene/ethylene oxide in rats up to 3450 mg/m^3. In humans, the level of ethylene oxide formed was estimated from HOEtVal adducts to be 3 or 0.5% of ethylene exposure after exposure for 8 h/day, 5 days/week to 0.063 mg/m^3 or 4 mg/m^3 of ethylene, respectively.

5.2 Recommendation for classification

The Committee is of the opinion that the available data are insufficient to evaluate the carcinogenic properties of ethylene (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	Carcinogenic classification of substances by the Committee

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 Quality of Life, Zeist, and 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 Chemical NV, Terneuzen *(until April 1, 2013);*
  Exponent, Menlo Park, United States *(from August 15, 2013)*
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- G.B. van der Voet, *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 *Ethylene*
Your Reference	: DGV/MBO/U-932342
Our reference	: U-7909/BV/fs/246-X18
Enclosed	: 1
Date	: October 18, 2013

Dear Minister,

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

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 (DECOS). The advisory 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 June 2013 for public review. The following organisations and persons have commented on the draft document:

- National Institute for Occupational Safety and Health (NIOSH), Cincinncati, USA
- Lower Olefins Sector Group (LOSG), European Chemical Industry Council (CEFIC), Brussels, Belgium
- Prof. D. Coggon, University of Southampton, Southampton, UK.

Annex **E**

IARC Monograph

D.1 Volume 19, 1979 (Excerpt from Ethylene and polyethylene, pp. 157-161)

Summary of Data Reported and Evaluation*.

Experimental data

No data on the carcinogenicity or mutagenicity of ethylene were available to the Working Group.

Human data

The massive production of ethylene (and polyethylene) and the general use of the polymer over the past several decades indicate that exposure of workers and the general population is common. No epidemiological studies relating to the carcinogenicity of ethylene were available to the Working Group

* only conclusions with regard to ethylene – not polyethylene – are copied here

Evaluation

No information was available to the Working Group for evaluating the possible carcinogenic effects of ethylene in humans. Experimental carcinogenicity studies on ethylene are recommended.

D.2 Volume 60, 1994 (Excerpt from Ethylene, pp. 45-71)

Summary of Data Reported and Evaluation

Exposure data

Ethylene, the petrochemical manufactured in largest volume worldwide, is produced primarily by the steam-cracking of hydrocarbons. It is used mainly as a chemical intermediate in the production of polymers and other industrial chemicals; small amounts are used to promote the ripening of fruits and vegetables. Ethylene is introduced into the environment from both natural and man-made sources, including emissions from vegetation, as a product of burning of organic material (such as cigarettes) and of incomplete combustion of fossil fuels, and in its production and use. Few data are available on levels of occupational exposure.

Human carcinogenicity data

The available data did not allow the Working Group to evaluate the carcinogenicity of ethylene to humans.

Animal carcinogenicity data

Ethylene was tested for carcinogenicity in one experiment in rats exposed by inhalation. No increase in tumour incidence was reported.

Other relevant data

Endogenous but unidentified sources of ethylene exist in man and experimental animals. Steady-state alveolar retention of ethylene is less than 10% in both man and rat. The biological half-time of ethylene in humans is about 0.65 h. In rats and man, the processes of uptake, exhalation and metabolism are described by first-order kinetics, at least up to 50 ppm; in rats, ethylene metabolism follows

first-order kinetics up to about 80 ppm. The maximal rate of metabolism in rats is reached at about 1000 ppm, the initial metabolite being ethylene oxide; hydroxyethyl cysteine is a urinary metabolite in mice. Because ethylene metabolism can be saturated, the maximal possible concentration of ethylene oxide in rat tissues is about 0.34 nmol/ml (15 ng/g bw).

Exposure to ethylene results in the formation of adducts with proteins. In nonsmokers, the background concentrations of the hydroxyethyl valine adduct of haemoglobin were 12-188 pmol/g haemoglobin. Environmental ethylene contributes to these concentrations; the endogenous contribution was calculated to be about 12 pmol/g haemoglobin in nonsmoking control subjects. The increment of N-terminal hydroxyethyl valine formed during a 40-h work week has been estimated as 100-120 pmol/g haemoglobin per part per million of ethylene. Tobacco smoke contributes to formation of this adduct: smoking 10-30 cigarettes/day was reported to result in 600-690 pmol/g haemoglobin.

Background concentrations of 7-hydroxyethyl guanine were 8.5 nmol/g DNA in one study of human peripheral lymphocytes and ranged from 2 to 6 nmol/g DNA in various tissues of rats and mice. A single exposure of mice to 50 ppm ethylene for 1 h resulted in 0.1-0.2 nmol/g DNA.

No data were available on the genetic and related effects of ethylene in exposed humans. In a single study, no micronuclei were induced in bone-marrow cells of mice and rats exposed *in vivo*. Gene mutation was not induced in *Salmonella typhimurium*. Although the genetic effects of ethylene have not been well studied, its metabolite, ethylene oxide, is genotoxic in a broad range of assays.

Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of ethylene. There is *inadequate evidence* in experimental animals for the carcinogenicity of ethylene.

Overall evaluation

Ethylene is *not classifiable as to its carcinogenicity to humans (Group 3).*

Annex **F**

Genotoxicity data

Genotoxic as well as possibly related DNA effects of ethylene are shown in this Table.

Test system	Dose[a] (LED or HID)		Result[b]		Ref.
	atmospheric concentration	dose relative to body weigth animal/human	exogenous metabolic activation		
			without	with	
Salmonella typhimurium TA100, reverse mutation	20 vol% (225,000 mg/m^3)		–	–	13
Salmonella typhimurium TA97, TA98, TA100, TA1535, reverse mutation	not specified		–	–	14
Salmonella typhimurium TA98, TA100, TA1535, TA1537, reverse mutation	115,000 mg/m^3		–	–	7
Escherichia coli, forward mutation	not specified		–		36
Chinese Hamster Ovary cells, chromosome aberrations, in vitro	25 vol% in nitrogen[c] (10 mM solution)		–	–	2
Micronucleus test, mouse bone marrow cells, in vivo	3,450 mg/m^3	1,490 mg/kg bw/d (inhal. 6 h, 5d/wk, 4 wks, resp. volume 1.8 l/h, bw 25 g)	–		16
Micronucleus test, rat bone marrow cells, in vivo	3,450 mg/m^3	776 mg/kg bw/d (inhal. 6 h, 5d/wk, 4 wks, resp. volume 6 l/h, bw 160 g)	–		16

DNA adduct formation				
Binding (covalent) to mouse DNA in vivo	57.5 mg/m^3	3.96 mg/kg bw/d (inhal. 8 h, resp. volume 1.8 l/h, bw 25 g)	+	22
7-alkylguanine formation in rat in vivo	345 mg/m^3	blood: 0.3 µmol/kg (12 h/d, 3 d)	+	27
7-hydroxyethylguanine formation in rat in vivo	3,450 mg/m^3	310 mg/kg bw/d (inhal. 6 h, 20 d, resp volume 6 l/h, bw 400 g	+	32

[a] LED = lowest effective dose; HID = highest ineffective dose; in vitro tests: µg/ml; in vivo tests: mg/kg bw/d; doses were calculated by the author of this report as it was not clear how values in the IARC were calculated

[b] + = positive; – = negative

[c] due to the explosive properties of ethylene in air, nitrogen was used to be able to test a high enough concentration; with nitrogen only 3 hour exposure was possible

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.[37]

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 opinions 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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