Health Council of the Netherlands

Ethyl acrylate

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Ethyl acrylate*
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Geachte staatssecretaris,

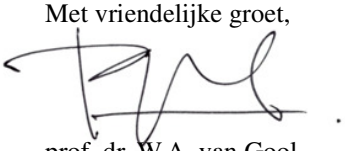
Graag bied ik u hierbij het advies aan over de gevolgen van beroepsmatige blootstelling aan ethylacrylaat.

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

Bezoekadres
Parnassusplein 5
2511 VX 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

Ethyl acrylate

Evaluation of the carcinogenicity and genotoxicity

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

to:

the State Secretary of Social Affairs and Employment

No. 2012/19, The Hague, November 13, 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, Agriculture & Innovation, 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 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 rapport neemt de commissie ethylacrylaat onder de loep. Ethylacrylaat wordt gebruikt als monomeer in acryl harsen. Ethylacrylaat kan tijdens de productie en gebruik via lekkage, schoorsteen emissie of afvalwater in het milieu terecht komen. Het is in lage concentraties aangetoond in afvalwater monsters. Daarnaast is het een vluchtige component in ananas en Beaufort kaas en wordt het gebruikt als chemische smaakstof in voeding.

Op basis van de beschikbare gegevens is de commissie van mening dat de gegevens over ethylacrylaat niet voldoende zijn om de kankerverwekkende eigenschappen te evalueren (categorie 3).*

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

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 Safety of the Health Council, hereafter called the Committee. In this report the Committee evaluated ethyl acrylate. Ethyl acrylate is used as a monomer in acrylic resins. Ethyl acrylate may be released into the environment in escape or stack emissions or in wastewater during its production and use. It has been detected at low levels in wastewater samples. It is also a volatile component of pineapple and Beaufort cheese and used as a chemically defined food flavouring substance.

The Committee is of the opinion that the available data are insufficient to evaluate the carcinogenic properties of ethyl acrylate (category 3).*

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

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 I).

This report contains the evaluation of the carcinogenicity of ethyl acrylate.

1.2 Committee and procedures

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 State Secretary can be found in Annex C.

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

More recently published data were retrieved from the online databases Toxline, Medline and Chemical Abstracts, covering the period 1990 to 2012. The last updated online search was in September 2012, using ethyl acrylate and CAS no 140-88-5 as key words in combination with key words representative for carcinogenesis and mutagenesis. The new relevant data were included in this report.

Chapter 2

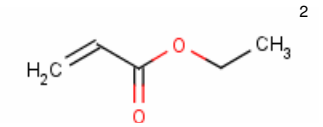
General information

2.1 Identity and physicochemical properties

Ethyl acrylate is used as a monomer in acrylic resins. In 1993, the production of ethyl acrylate in the United States was reported to be 160 345 tonnes.[1] In Europe producers and importers have been identified in France, Germany, the Netherlands and Belgium.[2] The production of ethyl acrylate in Europe is estimated to be between 100,000 and 500,000 tonnes per year.[3] Ethyl acrylate may be released into the environment in escape or stack emissions or in wastewater during its production and use. It has been detected at low levels in wastewater samples.[1] It is also a volatile component of pineapple and Beaufort cheese (a type manufactured in a small area of the French Alps).[1] It is also a food flavouring substance.[4,5]

Below is given the identity and some of its physical and chemical properties.

Chemical name	:	Ethyl acrylate
CAS registry number	:	140-88-5
EINECS number.	:	205-438-8[2]
Synonyms	:	2-propenoic acid ethyl ester, acrylic acid ethyl ester, ethyl propenoate, ethoxycarbonylethylene, ethyl 2-propenoate[1,6]
Appearance	:	clear liquid with an acrid, penetrating odour[6]
Chemical formula	:	$C_5H_8O_2$[1]

Structure	:	 [2]
Molecular weight	:	100.12 g/mol[6]
Boiling point	:	99.4 °C[6]
Melting point	:	-71.2 °C[1,6]
Vapour pressure	:	3.9 kPa at 20 °C, 38.6 mm Hg at 25 °C
Relative vapour density	:	3.45[6] (air = 1)
Solubility	:	slightly soluble in water; soluble in chloroform; miscible with dietyl ether and ethanol[1]
Conversion factor	:	1 mg/m^3
EU Classification (100% solution)	:	Flam. Liq. 2: H225 (Highly flammable liquid and vapour)[2] Acute Tox. 4: H332 (Harmful if inhaled) Acute Tox. 4: H312 (Harmful in contact with skin) Acute Tox. 4: H302 (Harmful if swallowed) Eye Irrit. 2: H319 (Causes serious eye irratation) STOT SE 3: H335 (May cause respiratory irritation) Skin Irrit. 2: H315 (Causes skin irritation) Skin Sens. 1: H317 (May cause an allergic skin reaction)

2.2 IARC classification

In 1999, IARC concluded that no epidemiological data relevant to the carcinogenicity of ethyl acrylate were available. They concluded that there was sufficient evidence in experimental animals for the carcinogenicity of ethyl acrylate. The overall conclusion of IARC was that ethyl acrylate is possibly carcinogenic to humans (Group 2B).[1]

2.3 EU classification

Although ethyl acrylate was characterised as “possibly carcinogenic to humans” (2B) by IARC[1,7], the substance was not classified for carcinogenicity in the Annex I to Directive 67/548/EEC in the 19th ATP in 1993. This classification was based on the available data up till approximately 1991 or 1992. Since 1991 few additional studies with respect to the carcinogenicity of ethyl acrylate have been published.

Chapter 3

Carcinogenicity

In addition to the IARC report, a recent review of the available human, animal, and mechanistic studies was available to the Committee (Williams and Iatropoulos, 2009).[8] To conclude on the carcinogenic classification of ethyl acrylate the Committee summarized and evaluated the original human and animal studies.

3.1 Observations in humans

In a cohort study, Walker et al. (1991)[9] evaluated the mortality from cancer of the colon or rectum among workers exposed to ethyl acrylate and methyl methacrylate. Three cohorts were assembled consisting of white male workers employed at two plants manufacturing and polymerizing acrylate monomers in the United States. In the earliest cohort, excess colon cancer seemed restricted to men employed extensively in the early 1940s in jobs entailing the highest exposures to vapour-phase ethyl acrylate and methyl methacrylate monomer, and volatile by-products of the ethyl acrylate/methyl methacrylate polymerization process. The excess mortality appeared some 20 years after the equivalent of three years work in jobs with the most intense exposures. A smaller elevation in colon cancer mortality appeared in a low-exposure group of this cohort. Rectal cancer mortality was elevated in the same categories that showed excess rates of colon cancer death; however, due to lower rates, the rectal cancer results are less precise.

The ethyl acrylate/methyl methacrylate exposures of members of the three cohorts were estimated on the basis of job histories and job-specific exposure rating scales. Monitoring data for ethyl acrylate/methyl methacrylate were available only from one of the plants beginning in 1972; earlier levels of exposure to ethyl acrylate/methyl methacrylate were reconstructed from production records and interviews with plant personnel. The resulting exposure scales were semi-quantitative, pertained to vapour exposure only, did not distinguish between ethyl acrylate and methyl methacrylate, relied on the recollection of long-term employees, were not verifiable, were not mutually comparable across all three cohorts, and did not take into account the presence of other substances in the workplace. These other substances included some which have subsequently been considered as either probable or possible carcinogens by the IARC (e.g. lead, ethylene dichloride, methylene chloride, and acrylonitrile).[9-11]

3.2 Carcinogenicity studies in animals

Oral administration

Groups of 25 male and 25 female albino Wistar rats were administered 0, 6-7, 60-70 or 2,000 mg/L ethyl acrylate [purity unspecified] in the drinking-water for two years.[12] From tests for losses of ethyl acrylate in the water near the end of the tube of the drinking bottles it was apparent that there was essentially no loss of ethyl acrylate due to volatilisation. Based upon average body weight and water consumption, the highest dose (2,000 ppm in drinking water) corresponded to approximately 170 or 120 mg ethyl acrylate per kilogram body weight per day for males or females, respectively.[5] Decreased body weights were reported in male and female rats receiving ethyl acrylate in the highest dose group. Haematological evaluations (i.e. erythrocyte volume fraction, haemoglobin, total white and differential white cell counts) and urine analysis (i.e. protein) conducted at 3-month intervals showed normal ranges for the parameters studied in all treated animals throughout the study.[5] At termination, histopathology (of the heart, lung, liver, kidney, urinary bladder, spleen, gastrointestinal tract, skeletal muscle, bone marrow, skin, brain, thyroid, adrenal, pancras, pituitary and gonads) revealed no compound-related neoplastic or non-neoplastic lesions in treated animals of either sex.[12] After two years of treatment, survival was: males 52%, 48%, 60% and 72%; females, 64%, 72%, 36% and 60% in the control, low-mid- and high-dose groups, respectively.

Groups of 50 male and 50 female Fischer 344/N rats, seven weeks of age, received 100 or 200 mg/kg bw ethyl acrylate (purity, 99-99.5% stabilized with 15

mg/kg bw of the monomethyl ether of hydroquinone) in 5 ml/kg bw corn oil by gavage five times per week for 103 weeks. Similar groups of rats received corn oil only and served as vehicle controls. The experiment was terminated 104-105 weeks after the beginning of the treatment. Survival in the control, low-dose and high-dose groups was: males, 82%, 64%, and 68% females, 72%, 72% and 84%, respectively. Histopathology was performed on all organ systems; neoplastic activity was seen in pancreas (acinar cell tumors), hematopoietic system (mononuclear cell leukemia) and especially in the forestomach. The incidence of squamous-cell papillomas and carcinomas of the forestomach are shown in Table 1. Dose related increases were observed in the incidence of non-neoplastic lesions (hyperkeratosis, hyperplasia and inflammation) in the forestomach in animals of each sex.[13]

Table 1 Incidence of forestomach tumours in rats after oral exposure to ethyl acrylate.[10]

	squamous cell papilloma			squamous cell carcinoma			squamous cell papilloma & carcinoma		
dose	control	low	high	control	low	high	control	low	high
males	1/50	15/50	29/50	0/50	5/50	12/50	1/50	18/50	36/50
females	1/50	6/50	9/50	0/50	0/50	2/50	1/50	6/50	11/50

Three groups of 25 male Fischer 344 rats, two months of age, were treated with 200 mg/kg bw ethyl acrylate (purity 99%) by gavage in corn oil on five days per week for six or 12 months. Control rats received 5 mL corn oil/kg bw per day on five days per week for 12 months. Five rats from each treatment group were killed 24 h after the last dose. The remaining rats were killed at 24 months of age. All animals were examined for gross lesions and the stomachs were collected and fixed in formalin. Microscopic examination was restricted to three or four sections of the stomach. No treatment-related neoplastic lesions were observed in the forestomach of rats exposed to ethyl acrylate for six months and autopsied at 24 months of age. After 12 months of ethyl acrylate administration, all rats showed hyperplastic lesions but no neoplastic lesions were detected. However, when rats received ethyl acrylate for 12 months and were killed after nine months of recovery, they developed squamous-cell carcinomas (3/13) and papillomas (1/13).[14] [The IARC Working Group noted that histopathological evaluation was limited to the stomach].

Groups of 50 male and 50 female $B6C3F_1$ mice, seven weeks of age, received 100 or 200 mg/kg bw ethyl acrylate (purity, 99-99.5% stabilised with 15 mg/kg of the monomethyl ether of hydroquinone) in 10 ml/kg bw corn oil by gavage five times per week for 103 weeks. Similar groups of mice received corn oil only and served as vehicle controls. The experiment was terminated 104-106

weeks after the beginning of the treatment. Survival in the control, low-dose and high-dose groups was: males, 56%, 72%, and 60% females, 54%, 70% and 52%, respectively. Histopathology was performed on all organ systems; no increased neoplastic activity was seen except in the forestomach. The incidence of squamous-cell papillomas and carcinomas of the forestomach are shown in Table 2. Dose related increases were observed in the incidence of non-neoplastic lesions (hyperkeratosis, hyperplasia and inflammation) in the forestomach in animals of each sex.[13]

Table 2 Incidence of forestomach tumours in mice after oral exposure of ethyl acrylate.[10]

	squamous cell papilloma			squamous cell carcinoma			squamous cell papilloma & carcinoma		
dose	control	low	high	control	low	high	control	low	high
males	1/48	4/47	9/50	0/48	2/47	5/50	0/48	5/47	12/50
females	1/50	4/49	5/48	0/50	1/49	2/48	1/50	5/49	7/48

Groups of two male and two female pure-bred beagle dogs were given corn oil gelatin capsules containing ethyl acrylate at a 'dietary equivalent' concentration of 0, 10, 100 or 1,000 mg/kg for 2 years.[12] This corresponds to average daily intakes of 0.75, 7.5 and 75 mg/kg bw, respectively.[5] Preliminary tests indicated that the amount of ethyl acrylate disappearing from the corn oil at room temperature over a 5 day period was no more that 5%. Dogs receiving the highest dose on the first day all vomited. After the dose was lowered to 300 mg/kg and gradually raised to 1,000 mg/kg bw over the first 16 weeks, no further difficulty was encountered. Reduced body-weight gains reported in dogs receiving ethyl acrylate at a dose of 75 mg/kg bw per day were correlated with decreased food consumption. Haematological evaluations (i.e. erythrocyte volume fraction, haemoglobin, total and differential leukocyte counts) and urine analysis (i.e. protein) conducted at 3-month intervals showed normal ranges for the parameters studied in all treated animals throughout the study. At termination, histopathology (of the heart, lung, liver, kidney, urinary bladder, spleen, gastrointestinal tract, skeletal muscle, bone marrow, skin, brain, thyroid, adrenal, pancreas, pituitary and gonads) revealed no compound-related neoplastic or non-neoplastic lesions in either sex of treated animals.

Skin application

A group of 40 male C3H/HeJ mice, 74-79 days of age, received thrice-weekly skin applications of 25 µl undiluted ethyl acrylate (purity, >99%) on the skin for life (approximately 23 mg per application; total dose, approximately 770 mg/kg

bw). Control groups were treated either with acetone (negative control) or with 0.1% 3-methylcholanthrene in acetone (positive control). The mean survival time of animals in the ethyl acrylate-treated group (408 days) did not differ significantly from that in the acetone controls (484 days). Complete necropsies were performed, the animals were examined for gross lesions and dorsal skin was examined histopathologically. No treatment-related tumours were observed in either ethyl acrylate- or acetone-treated mice. Skin tumours (mainly squamous-cell carcinomas) were observed in 39/40 mice treated with 3-methylcholanthrene.[15] [The IARC Working Group noted that no mention was made of control for possible losses of the parent compound by volatilization or polymerization].

Ethyl acrylate was tested in a transgenic mouse model. When applied to the shaved dorsal skin of female Tg.AC mice (three times per week for 20 weeks), ethyl acrylate did not cause the development of papillomatous lesions. The Tg.AC mouse is believed to respond to dermal applications of either genotoxic or non-genotoxic carcinogens with a rapid production of papillomas in the site of repeated applications.[16,17]

In another study, the dermal application of 60, 300, and 600 μmoles ethyl acrylate per mouse to the shaved dorsal skin of female Tg.AC mice (three times per week for 20 weeks) did not cause the development of papillomatous lesions.[18]

Inhalation exposure

Groups of 105 female and 105 male B6C3F1 mice, seven to nine weeks of age, were exposed to vapours of ethyl acrylate (purity, >99.5%) at concentrations of 100, 310 or 920 mg/m^3 [25, 75 or 225 ppm] for 6 h per day on five days per week. The treatment with the low and medium doses lasted 27 months, whereas high-dose treatment was discontinued after six months due to a significant decrease in body-weight gain. These animals were followed without further treatment for up to 27 months. Two concurrent control groups, each of 84 female and 84 male untreated mice, were used. Interim sacrifices of small groups of exposed and control animals were made at six, 12 and 18 months, such that groups of approximately 75 animals per sex in the exposed group and 60 animals per sex in the control groups were available for the full study. The mean body weight gains of both male and female mice in the mid- and high-dose groups were significantly lower than for the control groups throughout the study. Survival in all groups was adequate for evaluation of late-appearing tumours. No

treatment-related increase in the incidence of tumours was observed, with the exception of thyroid follicular adenomas, which were increased in high-dose male mice when compared to concurrent but not when compared to historical controls (2/121 in concurrent controls; 16% in historical controls; and 7/69 in high-dose males). Dose-related increases were observed in the incidence of non-neoplastic lesions of the olfactory mucosa (glandular hyperplasia and metaplasia) in animals of each sex.[19]

Groups of 115 female and 115 male Fisher 344 rats, seven to nine weeks of age, were exposed to vapours of ethyl acrylate (purity, >99.5%) at concentrations of 100, 310 or 920 mg/m^3 [25, 75 or 225 ppm] for 6 h per day on five days per week. The treatment with the low and medium doses lasted 27 months, whereas high-dose treatment was discontinued after six months due to a significant decrease in body-weight gain. The high-dosed animals were followed without further treatment for up to 27 months. Two concurrent control groups, each of 92 female and 92 male untreated rats, were used. Interim sacrifices of small groups of exposed and control animals were made at three, six, 12 and 18 months, such that groups of approximately 75 animals per sex in the exposed groups and 60 animals per sex in the control groups were available for the full study. The mean body weight gains of both male and female mice in the mid- and high-dose groups were significantly lower than for the control groups throughout the study. Survival in all groups was adequate for evaluation of late-appearing tumours. No treatment-related increase in the incidence of tumours was observed at any dose level. Dose-related increases were observed in the incidence of non-neoplastic lesions of the olfactory mucosa (glandular and basal-cell hyperplasia and metaplasia) in animals of each sex.[19]

Chapter 4

Mode of action

4.1 Genotoxic mode of action

4.1.1 Gene mutation assays

In vitro

Ethyl acrylate was not mutagenic in bacteria.[20,21] Ethyl acrylate did induce mitotic recombination in *Saccaromyces cerevisiae*.[22]

In mammalian cells it induced an increase in the mutant frequency at the *tk* locus in mouse L5178Y lymphoma cells, in the absence of exogenous metabolic activation[23-25], but not at the *hprt* locus in Chinese hamster ovary CHO cells.[25,26]

Ciaccio et al. investigated the relationship between ethyl acrylate induced cytotoxicity and the mutant frequency in the mouse lymphoma assay (MLA). They found a concentration-dependent increase in the mutant frequency at the *tk* locus. While it was observed that ethyl acrylate was negative for direct genotoxic generation of single-strand breaks, it induced apoptosis, and double-strand breaks indicative of necrosis, at the higher concentrations only. Pulsed field gel electrophoresis of directly loaded high dose cell preparations revealed both high- and low-molecular-weight DNA double strand breaks, but only at the highest concentrations. These observations indicated that ethyl acrylate induced mutagenic response correlated best with cellular cytotoxicity.[27]

In vivo

No results from mammalian in vivo gene mutation assays with ethyl acrylate were available to the Committee.

4.1.2 *Cytogenetic assays*

In vitro

Ethyl acrylate induced an increase in cells with chromosomal aberrations in mouse L5178Y lymphoma cells[25,28], Chinese hamster ovary CHO[25] and Chinese hamster lung CHL cells in vitro[29].

In vivo

In a study by Pzybojewska et al., a dose-related increase in the number of micronucleated polychromatic erythrocytes was observed. In this study, groups of four male Balb/c mice were given two intraperitoneal injections (24 hours apart) of ethyl acrylate (total dose, 225-1,800 mg/kg bw), and the bone marrow cells were examined six hours after the second injection.[30] However, the purity of the material tested was not reported. Furthermore, 2 out of 4 mice of the highest dose group died and the ratio of polychromatic to normochromatic erythrocytes was decreased in all tested doses, except for the lowest dose. This provided evidence of a toxic effect of ethyl acrylate on bone marrow cells. However, a toxicity-mediated positive response is not supported by the activity observed in the lowest dose level.

In a repeat of this experiment by Ashby et al., using groups of ten mice of strains Balb/c and C57BL/6 and two intraperitoneal doses, each up to 738-812 mg/kg (purity 98.5%), no increase in the number of bone marrow cells with micronuclei was found.[31] In this study dose-levels up to 80% of the median lethal dose were used.

In a study by Kligerman et al., groups of five male C57BL/6 mice were given a single intraperitoneal injection of ethyl acrylate at 125, 250, 500, or 1,000 mg/kg bw. In this study a small but statistically significant increase in splenocytes with micronuclei was found at the highest dose. This was, however, apparently due to an elevated frequency in a single animal.[32]

Ethyl acrylate failed to induce an increase in mouse splenocytes with sister chromatid exchanges[32] or chromosomal aberrations in mouse splenocytes in vivo[32].

It did not induce DNA strand breaks in peripheral white blood cells of mice[17] or in the forestomach of rats treated in vivo.[33]

No increases in cells with micronuclei were observed in the bone marrow of groups of six male BDF1 mice given a single intraperitoneal injection of ethyl acrylate at 375, 500, 750, or 1,000 mg/kg. In addition, no positive effects were seen when doses of 188, 375, 750, or 1,000 mg/kg were delivered by stomach tube.[34]

To evaluate the systemic genotoxicity ethyl acrylate was dermally applied to Tg.AC mice (3 times a week for 20 weeks). Peripheral blood leukocytes were evaluated for DNA damage (single-strand breaks, alkali labile sites, DNA cross linking) at weeks 4, 8, 12, 16 and 20. Peripheral blood polychromatic erythrocytes (PCE) and normachromatic erythrocytes (NCE) were evaluated for the presence of micronuclei at week 20. The extent of DNA migration in leukocytes and the frequency of micronucleated erythrocytes were not significantly altered by treatment with ethyl acrylate. The absence of genotoxicity in these two cell populations may suggest that ethyl acrylate is not genotoxic or not systemically available when applied dermally.[17]

4.1.3 Miscellaneous

In vitro

Ethyl acrylate did not bind to dexyribonucleosides in vitro.[35] Treatment of mouse fibroblast NCTC 929 cells with ethyl acrylate caused increases in cellular p53 protein levels. This protein is critical for cell cycle control and prevention of uncontrolled cell proliferation that can lead to cancer. Previous studies have shown that cells respond to DNA damage by increasing their levels of p53, which then acts to prevent replication of damaged DNA.[36]

In vivo

Ethyl acrylate failed to induce DNA binding in forestomach or liver of rats when given by gavage at doses up to 400 mg/kg[37] [The IARC Working Group noted the inadequate method for determining DNA binding].

4.2 Non-genotoxic mode of action

In vitro

No relevant in vitro assays with ethyl acrylate were available to the Committee.

In vivo

Single oral administration of 100, 200 or 400 mg/kg bw ethyl acrylate by gavage to F344 male rats, caused dose- and time-related mucosal and submucosal oedema, vacuolization of the *tunica muscularis* of the forestomach and mild submucosal oedema in the glandular stomach.[38] Equivalent subcutaneous or intraperitoneal dosing did not produce similar gastric lesions.[39] The absence of systemic toxicity and the dependency of gastric lesions on the gavage route of administration suggests that a localized response to an injurious agent at the site of application mediates the proliferative response.[10]

After repeated oral administration of 20-200 mg/kg/bw ethyl acrylate by gavage for two weeks to F344/N rats, dose-dependent irritation of the forestomach was observed. Repeated oral administration of ethyl acrylate in the drinking water led to a much lower incidence of forestomach irritation and less severe lesions at corresponding dose levels. Following 2 weeks of gavage dosing with ethyl acrylate a reduction in non-protein sulfhydryl content in the forestomach, but not in the glandular stomach or the liver was observed.[40] Interestingly, sulfhydryl-containing agents (cysteine and cysteamine) enhanced ethyl acrylate induced oedema of the forestomach, whereas depletion of the sulfhydryl content by fasting or pre-treatment with diethyl maleate was protective.[41]

After repeated oral administration of ethyl acrylate by gavage, the glandular part of the rat stomach becomes refractory to the local toxicity produced by the chemical. Glandular portions of stomach appeared normal after two weeks of repeated administrations of 100 mg/kg. Adaptation of the forestomach, however, was proliferative in nature and featured papillomatous thickening. Cessation of ethyl acrylate administration for two weeks after two weeks of administration of 100 mg/kg resulted in normalization of the forestomach epithelium.[10,42,43]

After 13 weeks of oral administration of 100 or 200 mg/kg ethyl acrylate by gavage, forestomachs of rats either returned to normal or showed (reversible) mucosal hyperplasia, depending on dose and time.[44,45] Another study provided evidence that a certain time of sustained hyperplasia of the forestomach is

required for effective tumorigenesis of ethyl acrylate in the forestomach of rats.[14,46]

Exposure of Fischer 344 rats and $B6C3F_1$ mice to 0, 0.1 or 0.31 mg/L ethyl acrylate vapour for 6 hours per day on five days per week for 27 months resulted in dose-dependent occurrence of basal-cell hyperplasia, an increase in intra-epithelial glands, metaplasia of respiratory epithelium and diffuse atrophy of the olfactory epithelium in rats and in hyperplasia of submucosal glands and metaplasia of olfactory epithelium in mice.[19] Inhalation exposure of Wistar rats to 1000 mg/m^3 ethyl acrylate for 6 hours led to a significant increase in urinary thioether excretion.[47] The average concentrations of ethyl acrylate in inhaled air that caused 50% depletion of non-protein sulfhydryl groups were estimated at 41.7 mmol/m^3 for blood, 50.4 mmol/m^3 for liver, 63.8 mmol/m^3 for lung and 81.5 mmol/m^3 for brain.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

In order to reach a conclusion on the classification of ethyl acrylate the studies summarized by IARC in 1986 and 1999 were used as a starting point. This information was supplemented with more recent data available in the public literature.

Only one human study with three cohorts of workers from two plants manufacturing and polymerizing acrylate monomers was available, which was not included in the IARC evaluation. Only the earliest cohort showed an excess mortality from colon and rectum cancer. The two cohorts with later dates of employment showed no excess mortality. Since this study did not take into account the presence of other substances in the workplace (including possible carcinogens), this study can neither establish nor rule out a causal relationship of ethyl acrylate with cancer.

In rats and mice dose-related increases in the incidence of squamous-cell papillomas and carcinomas of the forestomach were observed after oral dosing by gavage. Ethyl acrylate did not induce tumours after oral exposure of rats via drinking water, after inhalatory exposure of mice and rats and after dermal exposure of male mice. The forestomach neoplasia observed after gavage is correlated to extensive and sustained forestomach mucosal hyperplasia and cell proliferation.

Ethyl acrylate has been found positive in the mouse lymphomatest in vitro.[8] This positive finding is probably due to a clastogenic effect, since it was an increase in small colonies only. In most tests, ethyl acrylate was non-genotoxic in vivo. Ethyl acrylate does not induce tumours in experimental animals after subcutaneous or inhalation exposure. After long term oral exposure via gavage at high doses, rats and mice show chronic inflammatory changes in the forestomach and forestomach tumours. No tumours were seen in any other organ or tissue. In a drinking water study which delivered equivalent doses, neither irritation nor forestomach tumours were produced. Based on the available data, the carcinogenicity in the forestomach can be ascribed to a non-genotoxic mode of action.

The most likely explanation for tumour formation in rats may be site-specific tissue irritation due to chronic and prolonged exposure, which results in hyperplasia and subsequent tumour development. In rats hyperplasia and squamous cell carcinoma may occur in the forestomach during prolonged oral exposure because the forestomach functions as a reservoir in which retention time for the compound may be significant, whereas other parts of the upper gastro-intestinal tract (i.e. above the stomach) function mainly as conduction organs with negligible retention times. Humans do not have a homologue for the forestomach. Therefore, forestomach tumours in rats are considered irrelevant for humans.

The Committee concludes that the carcinogenic potency of ethylacrylate which has no demonstrable genotoxicity and which is exclusively carcinogenic in the forestomach squamous epithelium in rodents after oral administration, is rodent-specific and of no relevance for humans.[48]

5.2 Recommendation for classification

The Committee is of the opinion that the available data are insufficient to evaluate the carcinogenic properties of ethyl acrylate (category 3).*

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

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A	Request for advice
B	The Committee
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E	IARC Monograph
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G	Animal data
H	Genotoxicity data
I	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 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
- 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 *Ethyl acrylate*
Our reference	: U-7413/BvdV/fs/246-D17
Your Reference	: DGV/MBO/U-932342
Enclosed	: 1
Date	: November 13, 2012

Dear State Secretary,

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

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 2012 for public review. The following organisations and persons have commented on the draft document:

- National Institute for Occupational Safety and Health (NIOSH), Cincinnati, USA.

Annex **E**

IARC Monograph

Volume 71, 1999 (excerpt from Ethylacrylate, pp 1447-1457)

2 Studies of Cancer in Humans

No data were available to the Working Group.

3 Studies of Cancer in Experimental Animals

Ethyl acrylate was tested for carcinogenicity by oral gavage in mice and rats. Dose related increases in the incidence of squamous-cell papillomas and carcinomas of the forestomach were observed in both species. Ethyl acrylate was tested by inhalation in the same strains of mice and rats; no treatment-related neoplastic lesion was observed. No treatment-related tumour was observed following skin application of ethyl acrylate for lifespan to male mice (IARC, 1986).

3.1 Oral administration

Rat: Three groups of 25 male Fischer 344 rats, two months of age, were treated with 200 mg/kg bw ethyl acrylate (purity, 99%) by gavage in corn oil on five days per week for six or 12 months. Control rats received 5 mL corn oil/kg bw per day on five days per week for 12 months. Five rats from each treatment

group were killed 24 h after the last dose. The remaining rats were killed at 24 months of age. All animals were examined for gross lesions and the stomachs were collected and fixed in formalin. Microscopic examination was restricted to three or four sections of the stomach. No treatment-related neoplastic lesions were observed in the forestomach of rats exposed to ethyl acrylate for six months and autopsied at 24 months of age. After 12 months of ethyl acrylate administration, all rats showed hyperplastic lesions but no neoplastic lesions were detected. However, when rats received ethyl acrylate for 12 months and were killed after nine months of recovery, they developed squamous-cell carcinomas (3/13) and papillomas (1/13) (Ghanayem et al., 1993). [The Working Group noted that histopathological evaluation was limited to the stomach.]

4 Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

De Bethizy et al. (1987) administered ethyl [2,3-14C]acrylate to rats orally by gavage at doses of 2, 20 and 200 mg/kg bw. The total recovery in specific tissues and excreta fell with increasing dose from 108% at 2 mg/kg bw to 73% at 200 mg/kg bw. The major metabolite was 14CO2, with 52-61% exhaled within 24 h. The proportion of radioactivity excreted in the urine fell with increasing dose, from 28% at 2 mg/kg bw to 8% at 200 mg/kg bw. Three metabolites were identified: 3-hydroxypropionic acid and two mercapturic acids. *N*-Acetyl-*S*-(2-carboxyethyl)cysteine arises by glutathione conjugation of acrylic acid, while *N*-acetyl-*S*-(2-carboxyethyl)cysteine ethyl ester derives from the conjugation of intact ethyl acrylate. The percentage of the dose excreted as these mercapturic acids falls with increasing dose, consistent with depletion of glutathione. Although ethyl acrylate does not reduce non-protein sulfhydryls in the liver, marked and dose-dependent depletion occurs in the forestomach and glandular stomach, which is enhanced by pretreatment of rats with the esterase inhibitor tri(*ortho*-cresyl)phosphate. These data are consistent with the hydrolysis of ethyl

acrylate being a systemic detoxication reaction, since acrylic acid has no effect on non-protein sulfhydryl levels.

Linhart et al. (1994) reported increases in urinary levels of 3-hydroxypropanoic, lactic and acetic acids after administration of ethyl acrylate to rats.

Potter and Tran (1992) showed that ethyl acrylate reacts spontaneously with glutathione and protein sulfhydryl groups in many tissues: in liver alone, conjugation with glutathione was catalysed by cytosolic glutathione *S*-transferase. Miller et al. (1981) showed a major role for the liver in the hydrolysis of ethyl acrylate, the order of activities among tissues being liver >> blood >> lung > kidney. The hydrolysis of ethyl acrylate in various regions of the nose and respiratory tract was region-dependent (Frederick et al., 1994): high activity was found in homogenates of the dorsal meatus and olfactory septum, with much lower activity in respiratory epithelium. This distribution of activity does not correlate well with the distribution of cytotoxicity of ethyl acrylate after inhalation exposure.

Stott and McKenna (1985) found that ethyl acrylate was hydrolysed in homogenates of mouse nasal epithelium.

4.2 Toxic effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Frederick et al. (1990) treated male Fischer 344/N rats with 0, 2, 20, 50, 100 and 200 mg/kg bw ethyl acrylate by daily gavage for two weeks. Another group of animals received 200, 1000, 2000 and 4000 ppm (mg/L) in the drinking-water for two weeks. In the 20–200 mg/kg bw dose range, dose-dependent irritation of the forestomach, but not of the glandular stomach, was observed. In the animals dosed with ethyl acrylate in the drinking-water, much lower effects were observed at corresponding dose levels. Dosage of 200 mg/kg bw led to a reduction of about 90% in non-protein sulfhydryl content in the forestomach, but not in the glandular stomach or the liver. Interestingly, Ghanayem et al. (1991a) found that sulfhydryl-containing agents (cysteine and cysteamine) enhanced ethyl acrylate-induced oedema of the forestomach, whereas depletion of the sulfhydryl content by fasting or pretreatment with diethyl maleate was

protective. In Fischer 344 rats of both sexes receiving a single dose of 100, 200 or 400 mg/kg bw ethyl acrylate, dose- and time-dependent occurrence of mucosal and submucosal oedema, vacuolization of the tunica muscularis of the forestomach and mild submucosal oedema in the glandular stomach were observed (Ghanayem et al., 1985a). Equivalent subcutaneous or intraperitoneal dosing did not produce similar gastric lesions. Profound gastric toxicity was also obtained with methyl or ethyl acrylate, while acrylic acid, *n*butyl acrylate, methyl and ethyl propionate and methacrylic acid esters were inactive (Ghanayem et al., 1985b). Depending on dose and time, forestomachs of rats either returned to normal or showed (reversible) mucosal hyperplasia (Ghanayem et al., 1991b; Gillette & Frederick, 1993), while submucosal fibrosis became more prevalent in highdose animals with time (Ghanayem et al., 1986a). Another study (Ghanayem et al., 1993) provided evidence that a certain time of sustained hyperplasia of the forestomach is required for effective tumorigenesis of ethyl acrylate in the forestomach of rats. Daily gavage doses of 100 and 200 mg/kg bw ethyl acrylate on five days per week for two weeks resulted in a dramatic increase in forestomach epithelial cell proliferation in male Fischer 344 rats (Ghanayem et al., 1986b). Exposure of male and female Fischer 344 rats and B6C3F1 mice to 0, 0.1 or 0.31 mg/L ethyl acrylate vapour for 6 h per day on five days per week for 27 months resulted in dose-dependent occurrence of basal-cell hyperplasia, an increase in intraepithelial glands, respiratory metaplasia and diffuse atrophy of the olfactory epithelium in rats, and in hyperplasia of submucosal glands and respiratory metaplasia of olfactory epithelium in mice (Miller et al., 1985). Inhalation exposure of male Wistar rats to 1000 mg/m3 ethyl acrylate for 6 h led to a significant increase in urinary thioether excretion (Vodicrka et al., 1990). The average concentrations of ethyl acrylate in inhaled air that caused 50% depletion of non-protein sulfhydryl groups were estimated at 41.7 mmol/m3 for blood, 50.4 mmol/m3 for liver, 63.8 mmol/m3 for lung and 81.5 mmol/m3 for brain.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 Humans

No data were available to the Working Group.

4.4.2 Experimental systems

In single studies, ethyl acrylate did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* but did induce mitotic recombination in *Saccharomyces cerevisiae.* It was not mutagenic to bacteria.

In mammalian cells treated in vitro, it induced mutation at the *tk* locus in mouse L5178Y lymphoma cells, in the absence of exogenous metabolic activation, but not at the *hprt* locus in Chinese hamster ovary CHO cells. It induced chromosomal aberrations in mouse L5178Y lymphoma cells, Chinese hamster ovary CHO and Chinese hamster lung CHL cells in vitro.

In a single study, ethyl acrylate failed to induce DNA binding in forestomach or liver of rats when given by gavage at doses up to 400 mg/kg (Ghanayem et al., 1987) [The Working Group noted the inadequate method for determining DNA binding.] It induced micronucleus formation in mouse bone marrow and weakly in mouse splenocytes; another study performed under the same conditions was negative. In single studies, ethyl acrylate failed to induce sister chromatid exchanges or chromosomal aberrations in mouse splenocytes in vivo. It did not induce DNA damage in peripheral white blood cells of mice or in the forestomach of rats treated in vivo.

4.4.3 Mechanistic considerations

Ethyl acrylate appears to be clastogenic to mammalian cells in vitro. The preferential induction of small colonies rather than large ones in the mouse lymphoma L5178Y *tk* mutagenicity assay is thought to indicate that mutations arise from chromosomal damagerather than by point mutation. The clastogenic activity of ethyl acrylate seen in vitro is not readily expressed in vivo. Ethyl acrylate did not bind to eoxyribonucleosides in vitro (McCarthy et al*.,* 1994).

5 Evaluation

No epidemiological data relevant to the carcinogenicity of ethyl acrylate were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethyl acrylate.

Overall evaluation

Ethyl acrylate is *possibly carcinogenic to humans (Group 2B).*

Annex **F**

Human data

Human studies with regard to carcinogenic effects.

Reference	Design and population	Exposure	Carcinogenic effects	Potential confounders	Remarks
Walker et al., 1991[9]	Cohort workers in acrylic sheet manufacturing	Cumulative: intensity score (0-5) x number of days employed	Increase in colon and rectal cancer in workers with highest exposure	Other carcinogens	Cumulative exposure without distinction between long term low exposure or short term high exposure

Annex **G**

Animal data

Experimental studies with regard to carcinogenic effects.

Animal species (sex, number)	Dose and route of exposure	Exposure duration	Carcinogenicity	Remarks	Reference	Review
Oral						
Fischer 344 rats (25 m)	0, 200 mg/kg bw gavage (5 d/w)	6 or 12 months	Squamous-cell papillomas and carcinomas of forestomach	Histopathological evaluation was limited to forestomach	Ghanayem et al., 1993[14]	IARC, 1999[1]
B6C3F$_1$ mice (50-m, 50 f)	0, 100 or 200 mg/kg bw/d gavage	103 weeks	Squamous-cell papillomas and carcinomas of forestomach	Increased carcinomas (m) increased carcinomas and papillomas (m+f) also dose-resonse of non-neoplastic lesions	NTP, 1986[13]	IARC, 1986[7]
Fischer 344/N rat (50-m, 50f)	0, 100, 200 mg/kg bw/d gavage	103 weeks	Squamous-cell carcinomas of forestomach	Increased carcinomas (m) increased carcinomas and papillomas (m+f) also d-r non-neoplastic lesions	NTP, 1986[13]	IARC, 1986[7]
Wister rats (25m, 25f)	0, 6-7, 60-70, 2,000 mg/l drinking water	2 years	No treatment-related lesions	Incomplete description of the findings. The highest dose (2,000 ppm in drinking water) corresponded to approximately 170 or 120 mg/kg bw/day for males or females, respectively	Borzelleca et al., 1964[12]	IARC, 1986[7]
Beagle dogs (2m, 2f)	0, 10, 100 or 1,000 mg/kg in corn oil gelatin capsules	2 years	No treatment-related lesions	No details on survival and pathological examinations were given. The doses correspond to average daily intakes of 0.75, 7.5 and 75 mg/kg bw, respectively	Borzelleca et al., 1964[12]	WHO, 2006[5]

Dermal						
C3H/HeJ mice (40m)	23 mg/kg bw/ treatment, 3 times a week dermal application	74-79 days	No treatment-related lesions		DePass et al., 1984[15]	IARC, 1986[7]
Tg.AC (v-Ha-ras) mice (f)	dermal application, 3 times a week	20 weeks	No papillomatous lesions	Tg.AC mice are believed to respond to dermal applications of carcinogens with a rapid production of papillomas in the site of repeated applications	Tennant et al., 1996[16]	-
Tg.AC (v-Ha-ras) mice (f)	60, 300, or 600 µmoles/mouse dermal application, 3 times a week	20 weeks	No papillomatous lesions	Tg.AC mice are believed to respond to dermal applications of carcinogens with a rapid production of papillomas in the site of repeated applications	Nylander and French, 1998[18]	-
Inhalation						
B6C3F mice (105m, 105f)	100, 310, 920 mg/m^3	27 months	Thyroid follicular adenomas	Increased in males but not compared to historical controls, according to the authors not treatment related	Miller et al., 1985[19]	IARC, 1986[7]
Fischer 344 rat (115m, 115f)	100, 310, 920 mg/m^3	27 months	No treatment related lesions		Miller et al., 1985[19]	IARC, 1986[7]

Experimental studies with regard to toxic effects.

Reference	Review	Animal species (sex, number)	Dose and route of exposure	Exposure duration	Toxicity	Remarks
Frederick et al., 1990[40]	IARC, 1999[1]	Fischer 344/ N rats (m, 14)	0, 2, 10, 20, 50, 100, and 200 mg/kg bw/d 5 days/week gavage 0, 23, 99, 197, 369 mg/ kg bw/d drinking water	2 weeks	Irritation of the forestomach (20-200 mg/kg by gavage and drinking water) non-protein sulfydryl (NPSH) depletion (200 mg/kg by gavage) no lesions in glandular stomach or liver	The irritation was less severe after administration through drinking water
Ghanayem et al., 1991a[41]	IARC, 1999[1]	Fischer 344 rats (m, 50)	100 and 200 mg/kg bw/ d 5 days/week by gavage	13 weeks	Epithelial hyperplasia of the forestomach which regressed after 8-19 weeks of recovery no lesions in glandular stomach or liver	SH-containing agents (e.g. cysteine) enhanced submucosal Oedema in the forestomach, while SH-depletion (by fasting) was protective
Ghanayem et al., 1991b[44]	IARC, 1999[1]	Fischer 344 rats (m, 20)	100, 200 and 400 mg/kg bw/d by gavage	1, 2, 4, 14 or 90 days	Concentration and exposure duration dependent mucosal and sub mucosal oedema profound epithelial cell proliferation of the forestomach after 90 days, reversible after 8 weeks to 19 months recovery	

Ghanayem et al., 1985a[38]	IARC, 1999[1]	Fischer 344 rats	100, 200 and 400 mg/kg bw/d by gavage	Single dose	Dose- and time-related mucosal and submucosal oedema, vacuolization of the tunica muscularis of the forestomach and mild submucosal oedema in the glandular stomach
Ghanayem et al., 1985b[39]	IARC, 1999[1]	Fischer 344 rats	100, 200 and 400 mg/kg bw/d subcutaneous or intraperitoneal	Single dose	No gastric lesions
Ghanayem et al., 1986a[43]	IARC, 1999[1]	Fischer 344 rats	100 and 200 mg/kg bw/d by gavage	14 days	Submucosal fibrosis and foreign body reaction became more prevalent in high-dose animals with time, after 2 weeks of recovery, the forestomach returned to normal.
Ghanayem et al., 1986b[42]	IARC, 1999[1]	Fischer 344 rats	100 and 200 mg/kg bw/d 5 d/w by gavage	2 weeks	Forestomach epithelial cell proliferation
Ghanayem et al., 1993; 1994[14,46]	IARC, 1999[1]	Fischer 344 rats (m, 50)	200 mg/kg bw/d 5 days/week by gavage	3, 6, or 12 months	Forestomach hyperplasia after 6 months of exposure, recovery after 15 months of recovery squamous cell papillomas and carcinomas after 12 months of exposure
Miller et al., 1985[19]	IARC, 1999[1]	Fischer 344 rats and B6C3F1 mice	0, 0.1, and 0.31 mg/L for 6 hours/day, 5 d/w by inhalation	27 months	Dose-dependent occurrence of basal-cell hyperplasia, an increase in intra-epithelial glands, respiratory metaplasia and diffuse atrophy of the olfactory epithelium in rats and in hyperplasia of submucosal glands and respiratory metaplasia of olfactory epithelium in mice
Vodicka et al., 1990[47]	IARC, 1999[1]	Wistar rats	1000 mg/m^3 6 h		Significant increase in urinary thioether excretion

Annex **H**

Genotoxicity data

Genotoxicity and mutagenicity studies.

Test system		Endpoint	Lowest effective dose or highest ineffective dose[a]	Result without metabolic activation[b]	Result with metabolic activation[b]	Reference	Review
Bacteria	*S. typhymurium* TA100, TA1535, TA1537, TA1538 and TA98	Reverse mutations	670 and 1,666 µg/ml	-	-	Haworth et al., 1983[20] Waegemaekers & Bensink, 1984[21]	IARC, 1999[1]
Mammalian cells in vitro	L5178Y Mouse lymphoma cells	Gene mutation	20 µg/mL	+ (+) +	NT NT (+)	Moore et al., 1988[28] McGregor et al., 1988[24] Dearfield et al., 1991[23]	IARC, 1999[1]
	Chinese hamster ovary (CHO) cells	Gene mutation	23 and 80 µg/mL	-	NT	Moore et al., 1989[25] Moore et al.,1991[26]	IARC, 1999[1]
	L5178Y Mouse lymphoma cells	Single and double strand DNA breaks	10-40 µg/mL	+	NT	Ciaccio et al., 1998[27]	-
	Mouse splenocytes	Sister chromatid exchages (SCE)	25 µg/mL	-	NT	Kligerman et al., 1991[32]	IARC, 1999[1]

	Mouse splenocytes	Chromosome aberrations (CA)	2 µg/mL	(+)	NT	Kligerman et al., 1991[32]	IARC, 1999[1]
	L5178Y Mouse lymphoma cells	CA	20 µg/mL	+	NT	Moore et al., 1988[28] Moore et al., 1989[25]	IARC, 1999[1]
	Chinese hamster ovary (CHO) cells	CA	21 µg/mL	+	NT	Moore et al., 1989[25]	IARC, 1999[1]
	Chinese hamster lung cells	CA	9.8 µg/mL	+	NT	Ishidate et al., 1981[29]	IARC, 1999[1]
	Deoxyribonucleosides from red blood cells of Sprague-Dawley rats	Binding to deoxyribo-nucleosides	0.5 mN	-		McCarthy et al., 1994[35]	IARC, 1999[1]
Yeasts	*S. cerevisiae* D61,M	Mitotic recombination or gene conversion	733 mg/mL	+	NT	Zimmerman and Mohr, 1992[22]	IARC, 1999[1]
Droso-phila	D. melanogaster	Sex-linked recessive lethal mutations	40,000 ppm feed	-		Valencia et al., 1985[49]	IARC, 1999[1]
Mammalian in vivo	Fischer 344 rats forestomach	DNA strand breaks	4% po x 1	-	NT	Morimoto et al., 1990[33]	IARC, 1999[1]
	TgAC mouse peripheral blood leukocytes	DNA strand breaks	12 ug/mouse skin x 3/wk 20 wk	-		Tice et al., 1997[17]	IARC, 1999[1]
	rats forestomach or liver	DNA binding	400 mg/kg	-		Ghanayem et al., 1987[37]	IARC, 1999[1]
	C57BL/6 mouse splenocytes	SCE	1,000 mg/kg bw/d ip x 1	-		Kligerman et al., 1991[32]	IARC, 1999[1]
	C57BL/6 mouse splenocytes	Micronucleus test	1,000 mg/kg bw/d ip x 1	(+)		Kligerman et al., 1991[32]	IARC, 1999[1]
	BALB/c mouse bone marrow	Micronucleus test	225 mg/kg bw/d ip x 2	+		Przybojewska et al., 1984[30]	IARC, 1999[1]
	BALB/c and C57BL/6J mouse bone marrow	Micronucleus test	812 mg/kg bw/d ip x 2	-		Ashby et al., 1989[31]	IARC, 1999[1]
	C57BL/6J mouse bone marrow	Micronucleus test	738 mg/kg bw/d ip x 1	-		Ashby et al., 1989[31]	IARC, 1999[1]
	Tg.AC mouse peripheral blood cells	Micronucleus test	12 ug/mouse skin x 3/wk 20 wk	-		Tice et al., 1997[17]	IARC, 1999[1]
	BDF_1 mouse (m)	Micronucleus test	375-1000 mg/kg ip x 1	-		Morita et al., 1997[34]	NTP, 1998[10]
	BDF_1 mouse (m)	Micronucleus test	188-1000 mg/kg po x 1	-		Morita et al., 1997[34]	NTP, 1998[10]
	C57BL/6J mouse splenocytes	CA	1,000 mg/kg bw/d ip x 1	-		Kligerman et al., 1991[32]	IARC, 1999[1]

[a] +: positive; (+): weak positive; -: negative; NT: not tested

[b] in vitro tests: µg/mL; in vitro tests: mg/kg bw/day; ip: intraperitoneal; po: oral; wk: week

Annex I

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 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.[50]