Acrylamide

Evaluation of the carcinogenicity and genotoxicity

Dutch Expert Committee on Occuaptional Standards,

a committee of the Health Council of the Netherlands

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies over acrylamide
Uw kenmerk : DGV/MBO/U-932542
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Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr DGV/MBO/U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid de Gezondheidsraad om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In dat kader bied ik u hierbij een advies aan over de kankerverwekkende eigenschappen van acrylamide. Het is opgesteld door de Commissie WGD van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volksgezondheid, Welzijn en Sport, de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer en de Minister van Sociale Zaken en Werkgelegenheid.

Hoogachtend,

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

to

the Minister and State Secretary of Social Affairs and Employment

Nr 2002/02OSH, The Hague, 16 April 2002

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..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. 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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Contents

Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. In het voorliggende rapport neemt de Commissie WGD van de Raad, die deze beoordelingen verricht, acrylamide onder de loep. De commissie heeft haar oordeel gegoten in door de Europese Unie aangegeven termen.

De commissie concludeert dat acrylamide beschouwd moet worden als kankerverwekkend voor de mens (vergelijkbaar met EU categorie 2). Acrylamide is genotoxisch.

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the carcinogenic properties of substances at the workplace and proposes a classification with reference to the EU-directive. This evaluation is performed by the Dutch Expert Committee on Occupational Standards. The present report contains an evaluation by the committee on the carcinogenicity of acrylamide.

The committee is of the opinion that acrylamide should be regarded as carcinogenic to humans (comparable with EU category 2). Acrylamide is genotoxic.

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. The Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to study the carcinogenic properties of substances and to propose a classification with reference to an EU-directive (annex A and F). This task is carried out by the Council's Dutch Expert Committee on Occupational Standards, hereafter called the committee.

The evaluation of the carcinogenicity of a substance is, if possible, based on IARC* evaluations. The original publications are not reviewed and evaluated in the text of the report, but the overall conclusion of the IARC on the carcinogenic properties is included (annex D).

In addition to classifying substances with respect to their possible carcinogenicity according to the EU Guidelines, the committee also assesses the genotoxic properties of the substances in question. The committee expresses its conclusions in the form of standard sentences (annex E).

* International Agency for Research on Cancer

1.2 Committee and procedures

The present report contains evaluations by the committee of the carcinogenicity of acrylamide. The members of the committee are listed in annex B. The first draft of this report was prepared by H Stouten, from the TNO Nutrition and Food Research in Zeist, by contract with the Ministry of Social Affairs and Employment.

In 2000 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 C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation of the carcinogenicity of acrylamide has been based on an IARC evaluation (IARC94). Where relevant, the original publications were reviewed and evaluated in the text.

Additional literature for the evaluation of acrylamide was retrieved from CD ROMs of MEDline and TOXline+ covering the period 1985 to May 2001.

Chapter **2**

Acrylamide

2.1 Introduction

Name	:	acrylamide
CAS-no	:	79-06-1
CAS-name	:	2-propenamide
Description	:	white crystalline solid
Occurrence	:	Occurrence: not known to occur as a natural product; in occupational air, but not in ambient air close to six acrylamide-producing plants in the US; residues in potable water; in effluents of polyacrylamide using factories; in groundwater and wells in the vicinity of local grouting operations
Use	:	as a component of photopolymerisation systems, in adhesives and grouts, in cross-linking agents in vinyl polymers; as polymer, amongst others, in (waste) water treatment, in pulp and paper production, in oil drilling, in mineral processing
Mol weight	:	71.08
Chem formula	:	C_3H_5NO
Chem structure	:	$H_2C = CH - \overset{\overset{O}{\|}}{C} - NH_2$
EU classification	:	C < 6% T: toxic R: 45-46-24/25-48/23/24/25-43
EU carcinogenicity category		2 (substances which should be regarded as if they are carcinogenic to man)

2.2 IARC conclusion

In 1994, IARC concluded that concerning the carcinogenicity of acrylamide there was inadequate evidence in humans and sufficient evidence in experimental animals; acrylamide is probably carcinogenic to humans (Group 2A).

2.3 Human data

2.3.1 IARC data

IARC reported of two cohort mortality studies, which had been conducted among workers exposed to acrylamide. The first showed no significant excess of cancer (but suffered from small size, short duration of exposure and short latency). In the other study, with 2,300 men in one Dutch and three US plants, a non-significant increase was seen in deaths from pancreatic cancer, but there was no trend with increasing exposure (IARC94).

2.3.2 Additional data

Since 1994, no further data were registered in de literature-databases consulted.

2.4 Animal data

2.4.1 IARC data

Acrylamide was tested for carcinogenicity in one experiment in rats by oral administration. It increased the incidence of peritoneal mesotheliomas found in the region of the testis and of the follicular adenomas of the thyroid in males and of thyroid follicular tumours, mammary tumours, glial tumours of the central nervous system, oral cavity papillomas, uterine adenocarcinomas and clitoral gland adenomas in females. In screening bioassays, acrylamide, given either orally or interperitoneally, increased both the incidence and multiplicity of lung tumours in strain A mice.

Acrylamide was also tested as an initiating agent for skin carcinogenesis after oral, intraperitoneal and topical administration to mice of one strain and after administration to mice of another strain, followed by topical treatment with 12-*O*-tetradecanoylphorbol 13-acetate. It induced a dose-related increase in the incidence of squamous-cell papillomas and carcinomas of the skin in all four experiments.

2.4.2 *Additional data*

No further data were registered in de literature-databases consulted.

2.5 Mutagenicity and genotoxicity

2.5.1 *IARC data*

In the overall evaluation, IARC took the following supporting evidence for mutagenicity and genotoxicity into consideration: Acrylamide and its metabolite glycidamide form covalent adducts with DNA in rats and mice, as well as covalent haemoglobin adducts in exposed humans and rats, acrylamide induces gene mutations in germ cells of mouse and chromosomal aberrations in germ cells of mice and rats and forms covalent adducts with protamines in murine germ cells (*in vivo)*, acrylamide induces chromosomal aberrations in somatic cells in rodents *in vivo*, acrylamide induces chromosomal aberrations *in vitro*, and cell transformation in murine cell lines (IARC94).

2.5.2 *Additional data*

When tested in *Drosophila melanogaster*, marginally positive results without an exposure-response relationship were obtained in somatic cell assays (eye mosaic assay, wing spot test; larvae fed 350 to 5600 mg/L) (Bat94; Bat95; Vog93), while acrylamide tested negative in the sex-linked recessive lethal mutation assay (feeding or injecting larvae with 50 or 2,500 mg/L, respectively) (Fou94).

Using the “suspension method”, a statistically significant increase in micronucleus frequencies was detected in pre-leptotene spermatocytes of Lewis rats treated ip with a single dose of 100 mg/kg bw (sampling at days 18 and 20 after treatment) or with four daily doses of 50 mg/kg bw (sampling at day 19). The repeated treatment also caused a significant effect in zygotene spermatocytes sampled fifteen days after treatment. A single injection of 50 mg/kg bw did not cause an increase in frequencies of micronuclei (Xia94).

Using the “dissection” method and Sprague-Dawley rats, increases in frequencies in micronuclei were detected in pre-leptotene spermatocytes and late spermatogonial stages following a fractionated ip treatment with 4 x 50 mg/kg bw, but not after single ip doses of 50 or 100 mg/kg bw (Läh94).

Single intraperitoneal doses of 50 and 100 mg/kg bw and four repeated daily doses of 50 mg/kg bw caused increases in micronuclei in germ cells of male BALB/c mice.

The single doses also induced sister chromatid exchanges in germ cells and micronuclei in peripheral blood reticulocytes (repeated regime not tested) (Rus94).

Statistically significant increases of chromosomal aberrations were seen at cytogenetic analysis of first cleavage zygotes from untreated female mice mated with B6C3F1 mice seven days after ip injection of single doses of 75 and 125 mg/kg bw or four daily doses of 50 mg/kg bw. The single high dose did not cause an increase in aberrations when females were mated 28 days following treatment (Pac94).

Statistically significant increases in translocation frequencies (0.6% and 2.7%, respectively, versus 0.04% in controls) were obtained in a heritable translocation test in which male C3H/E1 mice were treated with single ip doses of 50 and 100 mg/kg bw and mated with untreated female 102/E1 mice at a mating ratio of 1:2 seven to sixteen days after treatment (Adl94).

Acrylamide produced no marked increase in converted spermatids of transgenic mice treated ip with daily doses of 50 mg/kg bw, for five days. However, the results may have been compromised by a possible meiotic arrest being induced by the high levels of acrylamide administered (Mur94).

2.6 Evaluation

No evidence for increased mortality from cancer was found in a study among approximately 2,300 men (from three plants in the US and one plant in The Netherlands) exposed to acrylamide.

The committee is of the opinion that there is sufficient evidence for carcinogenicity in animals. Oral administration of acrylamide increased the incidences of thyroid gland tumours and peritoneal mesotheliomas in the region of the testis in male rats and of tumours of the mammary gland, central nervous system, thyroid, oral cavity, uterus, and clitoral gland in female rats. In screening bioassays using strain A mice, it increased both the incidence and multiplicity of lung tumours following both oral and intraperitoneal administration. When tested as an initiating agent for skin carcinogenicity in mice, it increased the incidence of squamous-cell papillomas and carcinomas of the skin following oral, intraperitoneal, and topical application. This was dose-related. The opinion of the committee is in line with that of the European Union, which classified acrylamide in carcinogenicity category 2.

Acrylamide induced gene mutations, chromosomal aberrations, Sister chromatid exchanges, and mitotic disturbances in *in vitro* mammalian cell systems. *In vivo*, in rodents, it appeared genotoxic in somatic and germ cells. It caused somatic mutations in the spot test, heritable translocation, specific locus mutation, and dominant lethal mutations. It was positive in tests with *Drosophila*.

2.7 Recommendation for classification

The committee is of the opinion that acrylamide should be regarded as carcinogenic to humans (comparable with EU class 2). It is a genotoxic carcinogen.

References

Adl94 Adler ID, Reitmeir P, Schmöller R, *et al.* Dose response for heritable translocations induced by acrylamide in spermatids of mice. Mutat Res 1994; 309: 285-91.

Bat94 Batiste-Alentorn M, Xamena N, Creus A, *et al.* Further studies with the somatic white-ivory system of *Drosophila melanogaster*: genotoxicity testing of ten carcinogens. Environ Mol Mutagen 1994; 24: 143-7.

Bat95 Batiste-Alentorn M, Xamena N, Creus A, *et al.* Genotoxic evaluation of ten carcinogens in the *Drosophila melanogaster* wing spot test. Experientia 1995; 51: 73-6.

Fou94 Foureman P, Mason JM, Valencia R, *et al.* Chemical mutagenesis testing in *Drosophil*a. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ Mol Mutagen 1994; 23: 208-27.

IARC94 International Agency for Research on Cancer (IARC). Acrylamide. In: Some industrial chemicals. Lyon, France: IARC, 1994: 389-433, 502, 527 (IARC monographs on the evaluation of carcinogenic risks to humans; Vol 60).

Läh94 Lähdetie J, Suutari A, Sjöblom T. The spermatid micronucleus test with the dissection technique detects the germ cell mutagenicity of acrylamide in rat meiotic cells. Mutat Res 1994; 309: 255-62.

Mur94 Murti JR, Schimenti KJ, Schimenti JC. A recombination-based transgenic mouse system for genotoxicity testing. Mutat Res 1994; 307: 583-95.

Pac95 Pacchierotti F, Tiveron C, D'Archivio M, *et al.* Acrylamide-induced chromosomal damage in male mouse germ cells detected by cytogenetic analysis of one-cell zygotes. Mutat Res 1994; 309: 273-84.

Rus94 Russo A, Gabbani G, Simoncini B. Weak genotoxicity of acrylamide on premeiotic and somatic cells of the mouse. Mutat Res 1994; 309: 263-72.

Vog93 Vogel EW, Nivard MJM. Performance of 181 chemicals in a *Drosophila* assay predominantly monitoring interchromosomal mitotic recombination. Mutagenesis 1993; 8: 57-81.

Xia94 Xiao Y, Tates AD. Increased frequencies of micronuclei in early spermatids of rats following exposure of young primary spermatocytes to acrylamide. Mutat Res 1994; 309: 245-54.

A Request for advice

B The committee

C Comments on the public review draft

D IARC Monograph

E Classification of substances with respect to carcinogenicity

F Guideline 93/21/EEG of the European Union

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the

case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committee

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- P Boogaard
  toxicologist; Shell International Petroleum Company, The Hague
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  epidemiologist; Utrecht University, Utrecht
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- TM Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam
- IM Rietjens
  professor of toxicology; Wageningen University, Wageningen
- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague

- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- RA Woutsersen
  toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- P Wulp
  occupational physician; Labour Inspectorate, Groningen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague
- JM Rijnkels, *scientific secretary*
  Health Council of the Netherlands, The Hague

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

Secretarial assistance: Ms A van der Klugt.
Lay-out: J van Kan.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2000 for public review. The following organisations and persons have commented on the draft document:

- A Aalto, Ministry of Social Affairs and Health, Finland.

Annex **D**

IARC Monograph

See next pages.

Annex **E**

Classification of substances with respect to carcinogenicity

See next page.

The committee expresses its conclusions in the form of standard phrases:

Judgement of the committee	*Comparable with EU class*
This compound is known to be carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	1
This compound should be regarded as carcinogenic to humans ▪ It is genotoxic ▪ It is non-genotoxic ▪ Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether it is genotoxic	2
This compound is a suspected human carcinogen.	3
This compound has been extensively investigated. Although there is insufficient evidence of a carcinogenic effect to warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is cause for concern.	(A)
This compound has been insufficiently investigated. While the available data do not warrant a classification as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', they indicate that there is a cause for concern.	(B)
This compound cannot be classified	not classifiable

Annex **F**

Guideline 93/21/EEG of the European Union

4.2 Criteria for classification, indication of danger, choice of risk phrases

4.2.1 Carcinogenic substances

For the purpose of classification and labelling, and having regard to the current state of knowledge, such substances are divided into three categories:

Category 1:

Substances known to be carcinogenic to man.

There is sufficient evidence to establish a causal association between human exposure to a substance and the development of cancer.

Category 2:

Substances which should be regarded as if they are carcinogenic to man.

There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development of cancer, generally on the basis of:

- appropriate long-term animal studies
- other relevant information.

Category 3:

Substances which cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

There is some evidence from appropriate animal studies, but this is insufficient to place the substance in Category 2.

4.2.1.1 The following symbols and specific risk phrases apply:

Category 1 and 2:

T; R45 May cause cancer

However for substances and preparations which present a carcinogenic risk only when inhaled, for example, as dust, vapour or fumes, (other routes of exposure e.g. by swallowing or in contact with skin do not present any carcinogenic risk), the following symbol and specific risk phrase should be used:

T; R49 May cause cancer by inhalation

Category 3:

Xn; R40 Limited evidence of a carcinogenic effect

4.2.1.2 Comments regarding the categorisation of carcinogenic substances

The placing of a substance into Category 1 is done on the basis of epidemiological data; placing into Categories 2 and 3 is based primarily on animal experiments.

For classification as a Category 2 carcinogen either positive results in two animal species should be available or clear positive evidence in one species; together with supporting evidence such as genotoxicity data, metabolic or biochemical studies, induction of benign tumours, structural relationship with other known carcinogens, or data from epidemiological studies suggesting an association.

Category 3 actually comprises 2 sub-categories:

a substances which are well investigated but for which the evidence of a tumour-inducing effect is insufficient for classification in Category 2. Additional experiments would not be expected to yield further relevant information with respect to classification.

b substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

For a distinction between Categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in Category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high levels exceeding the 'maximal tolerated dose'. The maximal tolerated dose is characterized by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10% retardation in weight gain;
- appearance of tumours, especially at high dose levels, only in particular organs of certain species is known to be susceptible to a high spontaneous tumour formation;
- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds); if the particular target is not relevant to man;
- lack of genotoxicity in short-term tests *in vivo* and *in vitro*;
- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g. hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation;
- existence of a species - specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.

For a distinction between Category 3 and no classification arguments are relevant which exclude a concern for man:

- a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man;
- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories;
- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.