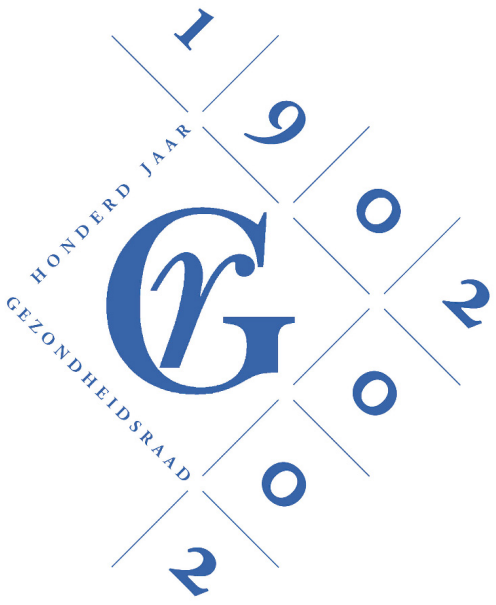
Hydrogen peroxide

Evaluation of the carcinogenicity and genotoxicity

Dutch Expert Committee on Occupational Standards,

a committee of the Health Council of the Netherlands



Aanbiedingsbrief

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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 2002/11OSH, 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, waterstofperoxide onder de loep. De commissie heeft haar oordeel gegoten in door de Europese Unie aangegeven termen.

De commissie is van mening dat er onvoldoende geschikte gegevens zijn over de kankerverwekkendheid van waterstofperoxide. Zij adviseert daarom waterstofperoxide niet te classificeren.

Executive summary

At the 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 hydrogen peroxide.

The committee concludes that there is a lack of appropriate data on the carcinogenic effects of hydrogen peroxide. The committee, therefore, recommends not to classify hydrogen peroxide.

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 annex I). 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 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 and E).

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 H).

* International Agency for Research on Cancer

1.2 Committee and procedures

The present report contains evaluations by the committee of the potential carcinogenicity of hydrogen peroxide. The members of the committee are listed in annex B. The first draft of this report was prepared by MI Willems, from 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 potential carcinogenicity of hydrogen peroxide has been based on IARC evaluations (IARC85, IARC87, IARC99). Where relevant, the original publications were reviewed and evaluated. In addition, the ECETOC review document has been used as starting point next to the IARC documents for evaluation of the genotoxicity of hydrogen peroxide: European Chemical Industry Ecology & Toxicology Centre (Hydrogen peroxide OEL Criteria Document, Cas nr 7722-84-1. Brussels, Belgium: ECETOC, 1996; Special report no. 10) (ECE96).

Furthermore, literature has been retrieved from the on-line data bases Cancerlit, Toxline, and Medline, covering the period 1983 to May 2001.

Chapter 2

Hydrogen peroxide

2.1 Introduction*

Chemical name	:	hydrogen peroxide
CAS registry number	:	7722-84-1
EEC number	:	008-003-00-9
EINECS number	:	231-765-0
IUPAC name	:	hydrogen peroxide
Synonyms	:	dihydrogen dioxide; hydrogen dioxide; hioxyl; inhibine; oxydol; albone
Description	:	colourless liquid
Application	:	A 90% solution is used in rocket propulsion. The compound is used as dough conditioner, maturing and bleaching agent in food, disinfectant, deodorant, antiseptic, hair bleaching agent and fabric bleaching agent
Molecular formula	:	H_2O_2
Structure	:	H-O-O-H
Molecular weight	:	34.0 g/mol

* data from IAR85, ACG91

Boiling point	:	152 °C (pure); 125 °C (70% w/w)
Melting point	:	-0.43 °C (pure); -40 °C (70% w/w)
Relative density (air=1; 25 °C)	:	1.4425 g/cm^3 (pure); 1.2839 (70% w/w)
Vapour pressure	:	3 hPa (25 °C, pure); 14.7 hPa (30 °C, 70% w/w)
Water solubility	:	miscible
Partition coefficient n-octanol/water (log P_{ow})	:	0.032 (calc.) at 20 °C (pure)
Solubility in organic solvents	:	soluble in diethyl ether
Conversion factors (20°C, air, 1,013 hPa)	:	1 ppm = 1.414 mg/m^3 1 mg/m^3 = 0.707 ppm
EEC-class. concentration ≥ 20%	:	C: corrosive R34: causes burns
5% ≤ concentration < 20%	:	Xi: irritant R 36/38: irritating to eyes and skin

2.2 IARC conclusion

In 1985, IARC concluded that there was no evidence for the carcinogenicity of hydrogen peroxide in humans and limited evidence in experimental animals (IARC85).

In 1999, IARC concluded that there was inadequate evidence in humans for the carcinogenicity of hydrogen peroxide. As in 1985, the IARC found hydrogen peroxide not classifiable as to its carcinogenicity to humans (Group 3) (IARC99).

2.3 Human data

2.3.1 IARC data

No adequate data on the carcinogenicity of hydrogen peroxide to humans were available to the Working Group (IARC99).

2.3.2 Additional data

From a literature search, no human carcinogenicity data were retrieved.

2.4 Animal data

2.4.1 IARC data

Hydrogen peroxide was studied for carcinogenicity in mice, by oral administration (drinking water), skin application and subcutaneous administration, and in Syrian golden hamsters by topical application to oral mucosa. In mice, adenomas and carcinomas of the duodenum were reported after oral administration. The other studies in mice and the one study in hamsters were inadequate for evaluation, due to a limited study design. One study by skin application in mice and one study by skin application in hamsters showed no promoting activity of hydrogen peroxide (IARC85, IARC99).

2.4.2 Additional data

Oral administration

The duodenal tumorigenesis of hydrogen peroxide was studied in three different mouse strains, B_6C_3 (C57BLxC3H) F_1, C3H/HeN, and C3H/C_s^b, having moderate, high and low catalase activities. Mice were administered hydrogen peroxide in distilled drinking water at a final concentration of 0.4% (4,000 ppm) throughout the experimental period of six months. The control mice received only distilled water. At the end of the exposure period, animals were sacrificed for histopathologic analysis of the proximal duodenum. Furthermore, blood samples were taken to determine catalase activity.

The incidence of duodenal tumours in mice treated with hydrogen peroxide were 9.5% (2/21), 31.8% (7/22) and 91.7% (22/24) in mice having high, moderate and low catalase activity, respectively. Their respective blood catalase levels were 5.5±0.3, not determined, and 2.7±0.2 x 10^{-4} mg/mg protein. None of the control mice developed duodenal tumours. The authors concluded that treatment with hydrogen peroxide resulted in an increased incidence of duodenal tumours, which was inversely related with catalase activity (Ito86).

The committee noted that in this study animals deficient in catalase, a key protective enzyme in the degradation of hydrogen peroxide, were used, which does not reflect the normal human situation. In a review of Desesso *et al*. (Des00), the results of this (Ito86) and comparable studies from Ito *et al*. are discussed. The authors speculate that greatly decreased water consumption and the resultant abrasion of the luminal lining on ingestion of pelleted dry rodent chow is the most likely cause of the observed duodenal lesions following hydrogen peroxide administration in drinking water in the study by Ito *et al*. (Ito86). Furthermore, contradictory results were reported in an

abstract, in which gastroduodenal tissues of Chinese hamsters were examined for histopathological changes after the animals (n=20/group) had been intubated with 70 mg/kg bw hydrogen peroxide on 5 days a week, for 15 weeks or six months. Histopathological findings of H_2O_2-treated animals did not differ from those of control animals intubated with water (Li93). Moreover, owing to the chemistry of dilute H_2O_2-solutions and the anatomy/physiology of the gastrointestinal tract, Desesso *et al*. found it unlikely that orally ingested hydrogen peroxide reaches the duodenum. Based on the arguments given by Desesso *et al*. and the use of catalase-deficient mouse strains, the committee concludes that the study by Ito *et al*. does not provide evidence that hydrogen peroxide is carcinogenic for genetically unaltered mice, that are normally used in carcinogenicity studies.

In a tumour-promotion test, four groups of male Fisher F344 rats were treated with hydrogen peroxide (1.5% in drinking water). Group 1 (n=8) received three intraperitoneal injections of 25 mg/kg bw methylazoxymethanol acetate (MAM), with an time interval of two weeks. At the injection times, treatment with hydrogen peroxide was stopped for two days. Following the last MAM injection, hydrogen peroxide treatment in drinking water continued until sacrifice in week 21. Group 2 (n=8) received the same treatment as group 1, except that, after the last MAM injection, hydrogen peroxide treatment was ended, and the animals received normal tap water during the rest of the experimental period. Group 3 (n=3) received only hydrogen peroxide in the drinking water for 21 weeks. Group 4 (n=3) was an untreated control group. In group 1, 8/8 rats developed adenocarcinomas in the duodenum (total number of carcinomas 21), 5/8 rats had adenocarcinomas in the jejunum (total number of carcinomas 7); no colon tumours were reported. In group 2, 2/8 rats developed adenocarcinomas in the duodenum (total number of carcinomas 2), 2/8 rats had adenocarcinomas in the jejunum (total number of carcinomas 2), and 1/8 rats had a carcinoma in the colon (total number of carcinomas 1). In hydrogen peroxide-treated and untreated control animals, no tumours were found, but hyperplastic epithelia of the duodenum and the upper jejunum occurred in 3/3 of the hydrogen peroxide-treated animals. The authors concluded that hydrogen peroxide had an enhancing effect upon intestinal tumorigenesis in animals treated with MAM (Hir81). The committee noted that no animals receiving MAM alone were included in the experiment.

Recently, a 13-week drinking water toxicity study has been performed under OECD GLP guidelines, including examination of possible reversibility of any possible effects during a 6-week recovery period. Catalase-deficient C57BL/6NCrlBR mice (n=15/sex/group) received 0, 100, 300, 1,000 or 3,000 ppm hydrogen peroxide in distilled drinking water for a treatment period of 91 days. Body weight, food and water consumption were recorded weekly. Immediately prior to sacrifice, blood was collected for haematological evaluations and clinical chemistry determinations. Tissues from all

body parts and gross lesions were collected and examined microscopically. Water consumption was significantly depressed among animals receiving 3,000 ppm hydrogen peroxide and intermittently among animals receiving 1,000 ppm. No biologically significant differences were noted in any haematological parameters. Males receiving 3,000 ppm hydrogen peroxide exhibited significant reductions in total protein and globulin, possibly attributable to reduced food consumption or reduced protein absorption. There were no treatment-related histopathological findings among treated animals other than mild lesions of the duodenum. Microscopically, no evidence for cellular atypia in the duodenum or architectural disruptions nor any indications of preneoplastic lesions were observed. The duodenal lesions noted at the end of the treatment period were reversible following the 6-week recovery period on distilled drinking water. The authors conclude, that based on the present study, hydrogen peroxide is not expected to present a significant health risk to humans in present occupational exposure scenarios (Wei00).

Dermal application

Kurokawa *et al*. examined the carcinogenicity and tumour promoting activity of hydrogen peroxide in a dermal study in female Sencar mice (Kur84). In the promotion test, hydrogen peroxide was applied twice a week (2 x 0.2 mL 5% hydrogen peroxide in acetone) for 51 weeks after initiation with dimethylbenzanthracene (DMBA, 1 x 20 nmol in 0.2 mL acetone). In the test for complete carcinogenic activity, hydrogen peroxide (2 x 0.2 mL 5% hydrogen peroxide in acetone) was topically applied once a week for 51 weeks. Negative vehicle controls and positive controls were included. Histopathology included the major organs (not indicated) and the skin. At study termination (week 51), epidermal hyperplasia was found in 1 out of 20 mice treated with hydrogen peroxide alone, compared with 0/15 in the control group (Kur84). In the test for tumour-promoting activity, epidermal hyperplasia and squamous cell carcinoma were seen in 9/20 and 1/20 of hydrogen peroxide-treated mice, respectively. The results are not considered indicative for either promoting or complete carcinogenic activity. However, a weak promoting activity cannot be excluded, as at the low test concentration epidermal hyperplasia was seen in 9 out of 20 animals (Kur84).

Female ICR Swiss mice (n=30) were initiated once with a solution of 0.125 mg dimethylbenzanthracene (DMBA) dissolved in 0.25 mL acetone applied dermally. After 20 days, animals were treated dermally with 0.25 mL of 3% H_2O_2 in acetone daily for 40 weeks. All mice were examined weekly and the number and distribution of tumours were noted. Positive control groups were present. No induction of tumours was seen (Sha72).

Miscellaneous, painting of the buccal pouch in hamsters

Dimethylbenzanthracene (DMBA) and/or hydrogen peroxide were applied onto the left buccal pouch of Syrian hamsters twice weekly for 19 or 22 weeks. Animals were treated either with 30% hydrogen peroxide alone (n=18), or with hydrogen peroxide (3% or 30%) plus the carcinogen DMBA (n=17 or n=15), or with DMBA alone (n=16). Controls were treated with the vehicle (mineral oil) or were untreated. Buccal pouches were studied histopathologically with multiple sections. In animals treated with 30% hydrogen peroxide alone, histopathology at week 22 revealed hyperkeratosis and hyperplasia in all animals (9/9) with hyperchromatic cells and mild dysplasia in 4/9 animals: no tumours were seen. In animals treated with DMBA alone, 3/7 (43%) developed an epidermoid carcinoma, while 6/11 (55%) of animals treated with DMBA plus 3% hydrogen peroxide and 5/5 (100%) of animals treated with DMBA plus 30% hydrogen peroxide developed carcinoma. The authors concluded that hydrogen peroxide can by itself induce histopathological changes associated with preneoplastic lesions and may augment carcinogenesis associated with the carcinogen DMBA (Wei86).

2.5 Mutagenicity and genotoxicity

2.5.1 IARC data

DNA damage has been demonstrated in bacteria and cultured mammalian cells. In addition, hydrogen peroxide induced mutations in bacteria, yeast and other fungi (IARC85), and there is some evidence that it can do so in Chinese hamster V79 cells and in mouse lymphoma L5178Y cells at the *hprt* locus (IARC99). Chromosomal aberrations and sister chromatid exchanges are induced in both human and other mammalian cells *in vitro*, but it did not induce chromosomal aberrations in the bone marrow cells of exposed rats (IARC 99).

2.5.2 Additional information

ECETOC critically reviewed the genotoxicity studies on hydrogen peroxide up to 1995 - 1996 as part of a critical assessment of the toxicology and ecotoxicology of hydrogen peroxide (ECE96). A copy of the section on genotoxicity from ECETOC is included in Annex G. Annex F summarises the genotoxicity tests and other tests considered being predictive for carcinogenicity, but which are not included in the ECETOC report or in the IARC evaluations.

Various publications show that hydrogen peroxide can induce gene mutations in mammalian cells *in vitro* (ECE96, IARC99, Kit96).

Hydrogen peroxide induced morphological cell transformation in cell assays with Syrian Hamster Embryo cells and C3H/10T1/2 cells (ECE96, Leb96).

Hydrogen peroxide exposure of MYP3 cells (an anchorage-dependent non tumourigenic rat bladder epithelial cell line) caused colony formation in soft agar. A marked increase in colony numbers was observed in the cells that were MNU-initiated and exposed to hydrogen peroxide ($p<0.01$). The transformants induced by MNU (methylnitroso urea) plus hydrogen peroxide or hydrogen peroxide alone formed high-grade transitional cell carcinomas when injected into nude mice (Oka96).

The committee noted that on *in vitro* base most mutagenicity and genotoxicity studies with positive results were carried out without exogenous metabolic activation. In the presence of such an activation system, the effects appear to be reduced or abolished. Furthermore, Desesso *et al*. indicate that mammalian cells *in vitro* express antioxidant enzymes, but that these enzymes are expressed at much higher levels *in vivo* (Des00). The committee agrees with the suggestion that mammalian cells *in vitro* may not be able to protect against hydrogen peroxide induced damage as well as mammalian cells *in vivo*.

Hydrogen peroxide was tested for possible local genotoxic effects *in vivo*. Application of 10, 100 and 200 µmol (in 200 µL of ethanol) hydrogen peroxide directly on the skin of female Sencar mice (n=10/group) twice weekly for four weeks, did not induce DNA damage, as measured by the formation of modified 8-hydroxy-2'-deoxyguanosine bases, or Ha-ras mutations in codon 60. The authors conclude that hydrogen peroxide was negative on all endpoints and therefore appear to be of no concern for carcinogenicity (Society for the Plastic Industry, SPI97).

2.6 Evaluation

No adequate data on the carcinogenic effects of hydrogen peroxide on humans are available.

The potential carcinogenicity of hydrogen peroxide has been examined in mice and rats following oral administration, in mice after dermal administration, and in hamsters following painting of buccal pouch epithelium. In most of these studies, hydrogen peroxide does not act as a carcinogen. The committee concludes that the results of the study with catalase-deficient mice, in which duodenal tumours were observed, should not be interpreted as evidence for a carcinogenic potential of hydrogen peroxide in humans. In addition, hydrogen peroxide treatment did not induce tumours in an oral study with rats, but the experimental period in this experiment was 21 weeks only, which is too short for detection of potential carcinogenicity. Furthermore, hydrogen

peroxide did not induce skin tumours in mice after dermal application (exposure periods 40-51 weeks).

Hydrogen peroxide induces DNA damage and mutations in bacteria, and DNA damage, gene mutations, sister chromatid exchanges, and chromosomal aberrations in mammalian cells *in vitro*. It did not induce recessive lethals in *Drosophila melanogaster*. Overall, most of the *in vitro* genotoxicity and mutagenicity studies with bacteria and mammalian cells are carried out in the absence of an exogenous metabolic activation system. A micronucleus assay in mice and a chromosomal aberration test in bone marrow of rats were reported to be negative. No detailed information on these latter two tests with mammals (*in vivo*) was available.

2.7 Recommendation for classification

Based on the available data, the committee is of the opinion that there is inadequate evidence for the carcinogenicity of hydrogen peroxide. Therefore, the committee recommends not to classify hydrogen peroxide.

References

ACG91 American Conference of Governmental Industrial Hygienists (ACGIH). In: Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Cincinnati OH, USA: ACGIH, 1991:782-3.

Bur96 Burdon R, Gill V, Boyd P, *et al*. Hydrogen peroxide and sequence-specific DNA damage in human cells. FEBS Lett 1996; 383: 150-4.

Des00 Desesso JM, Lavin AL, Hsia SM and Mavis RD. Assessment of the carcinogenicity associated with oral exposures to hydrogen peroxide. Food Chem Toxicol, 2000; 38: 1021-1041.

Día00 Díaz-Llera S, Podlutsky A, Österholm A-M, Hou S-M and Lambert B. Hydrogen peroxide induced mutations at the *HPRT* locus in primary human T-lymphocytes. Mut Res, 2000; 469: 51-61.

ECE96 European Chemical Industry Ecology & Toxicology Centre (ECETOC). Hydrogen peroxide OEL Criteria Document, Cas nr 7722-84-1. Brussels, Belgium: ECETOC, 1996; Special report no. 10.

Hir81 Hirota N, Yokoyama T. Enhancing effect of hydrogen peroxide upon duodenal and upper jejunal carcinogenesis in rats. Gann 1981; 72:811-2.

IARC85 International Agency for Research on Cancer (IARC). In: Allyl compounds, aldehydes, epoxides and peroxides. Lyon, France: IARC, 1985: 285-314 (IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans; Vol 36).

IARC87 International Agency for Research on Cancer (IARC). In: Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1 to 42. Lyon, France: IARC 1987: 64 (IARC monographs on the evaluation of carcinogenic risks to humans; Suppl 7).

IARC99 International Agency for Research on Cancer (IARC). In: Re-evaluation of some chemicals, hydrazine and hydrogen peroxide (part two). Lyon, France, 1999: 671-689 (IARC monographs on the evaluation of carcinogenic risks to humans; Vol 71).

Ili92 Iliakis G, Pantelas G, Okayasu R, *et al*. Induction of H_2O_2 of DNA and interphase chromosome damage in plateau-phase Chinese hamster ovary cells. Radiat Res 1992; 131: 192-203.

Ito86 Ito A, Watanabe H, Aoyama H, *et al*. Effect of 1,2-dimethylhydrazine and hydrogen peroxide for the duodenal tumorigenesis in relation to blood catalase activity in mice. Hiroshima J Med Sci 1986; 35: 197-200.

Kel92 Kelly J, Orner G, Hendricks J, *et al*. Dietary hydrogen peroxide enhances hepatocarcinogenesis in trout: correlation with 8-hydroxy-2'-deoxyguanosine levels in liver DNA. Carcinogenesis 1992; 13: 1639-1642.

Kit96 Kitahara J, Yamanaka K, Kato K, *et al*. Mutagenicity of cobalt and reactive oxygen producers. Mutat Res 1996; 370: 133-40.

Kur84 Kurokawa Y, Takamura N, Matsushima Y, *et al*. Studies on the promoting and complete carcinogenic activities of some oxidizing chemicals in skin carcinogenesis. Cancer Lett 1984; 24: 299-304.

LeB96 LeBoeuf R, Kerckaert G, Aardema M, *et al*. The pH 6.7 Syrian hamster embryo cell transformation assay for assessing the carcinogenic potential of chemicals. Mutat Res 1996; 356: 85-127.

LI93 Li Y, Noblitt T, Zhang A, Origel A, Kafrawi A and Stookey G. Effect of long-term exposure to a tooth whitener. J Dent Res, 1993; 72 (IADR Abstracts): no. 1162, page 248.

Mee99 WJ, Spencer JPE, Rannels DE, Welch DR, Knobbe ET and Ostrander GK. Hydrogen peroxide induces oxidative DNA damage in rat type II pulmonary epithelial cells. Environ Mol Mutagen, 1999; 33: 273-278.

Oka96 Okamoto M, Kawai K, Reznikoff C, *et al*. Transformation *in vitro* of a nontumorigenic rat urothelial cell line by hydrogen peroxide. Cancer Res 1996; 56: 4649-53.

Sha72 Shamberger RJ. Increase of peroxidation in carcinogenesis. J Natl Cancer Inst 1972; 48: 1491-6.

Spe96 Spencer J, Jenner Q, Aruoma O, *et al*. Oxidative DNA damage in the human respiratory tract epithelial cells. Time course in relation to DNA strand breakage. Biochem Biophys Res Commun 1996; 224: 17-22.

SPI97 Society for the Plastic Industry by Slaga T. Determination of the potential for organic peroxides to induce sustained skin hyperplasia and DNA damage. Unpublished Report SPI/SPRD Study #96-1 prepared for the Society for the Plastic Industry (has been submitted to the committee by the CEFIC and can be consulted at the Health Council of the Netherlands or at the CEFIC Peroxygens Sector Group in the CEFIC Brussels office), 1997.

Wei00 Weiner ML, Freeman C, Trochimowicz H, *et al*. 13-Week drinking water toxicity study of hydrogen peroxide with 6-week recovery period in catalase-deficient mice. Fd Chem Toxicol, 2000; 38:607-615.

Wei86 Weitzmann S, Weitberg A, Stossel T, *et al*. Effects of hydrogen peroxide on oral carcinogenesis in hamsters. J Peridontol 1986; 57: 685-8.

A	Request for advice
B	The committee
C	Comments on the public review draft
D	IARC monograph 1985
E	IARC monograph 1999
F	Additional genotoxicity tests not included in IARC99 or ECE96
G	ECETOC's Joint Assessment (ECE96)
H	Classification of substances with respect to carcinogenicity
I	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 base 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 base 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 Woutersen
  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 MI Willems, from the Department of Occupational Toxicology of the TNO Nutrition and Food Research, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance was provided by mrs 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 Lamse, Alcon Nederland BV, the Netherlands;
- F Carpanini, ECETOC, Belgium;
- L Le Doré, CEFIC hydrogen peroxide subgroup, Belgium;
- J Razenberg, Nederlandse Vereniging van Zeepfabrikanten, the Netherlands.

Annex **D**

IARC Monograph 1985

See next page.

Annex **E**

IARC Monograph 1999

See next page.

Annex **F**

Additional genotoxicity tests not included in IARC99 or ECE96

indicator cells/organisms	concentration/ exposure time	results (lowest positive response)	remarks	reference
mammalian cells				
HeLa cells, monolayer culture. Assay directed at detection of sequence-specific DNA damage	0 - 0.1- 1.0 - 10.0 mM for 20 hours	+ (1.0 mM)	DNA damage was not random, but associated with specific nucleotide sequences. Degradation, but no apoptosis was seen in DNA isolated from cells remaining attached to the monolayer at the highest doses tested	Bur96
Human bronchial epithelial cells: time course of oxidative DNA damage (GC-MS) in relation to that of DNA strand breaks	100 µM for 5, 15, 30, 45, 60 min	+	Effects were time dependent. The significance of the increase in number of oxidated bases depends on the base modifications under investigation	Spe96
Syrian hamster embryo cells cell transformation assay (pH 6.7)	0 - 1 - 1.25 - 2.5 - 5 or 10 µg/mL for 7 days	+	Hydrogen peroxide caused a statistically significant increase in morphologically transformed colonies at 2.5 µg/mL with a significant positive trend test	LeB96
V79 cells and the two *gpt*$^{+}$ transgenic cell lines G12 and G10, derived from Chinese hamster V79 cells	10 or 30 mM for 1 hour or 24 hours	+: G12 (10 mM) - : V79, V79-G10	hydrogen peroxide was only weakly mutagenic in G12 cells	Kit96

Continued...

indicator cells/organisms	concentration/ time	results (lowest positive response)	remarks	reference
Rat primary type II pulmonary epithelial cells: oxidative DNA damage by GC-MS	0 - 5 mM for 60 min	+	Hydrogen peroxide caused increased concentrations of 12 of 14 monitored DNA base modifications, suggesting oxidative damage.	Mee99
human primary T-lymphocytes: *HPRT* mutant frequency testing	0.34 - 1.35 mM for 60 min	+	Treatment caused a dose dependent increase of the *HPRT* mutant frequency	Día00
miscellaneous				
MYP3, anchorage dependent, non tumourigenic rat bladder epithelial cell line: colony formation	0 - 0.1 mM for 4 weeks, in triplicate	+ve (0.01 mM)	dose regimen based on cytotoxicity test (>0.1 mM significant decrease in viable cell count); daily exchange of H_2O_2-containing complete medium; dose-dependent and time-dependent increase in number of colonies formed;	Oka96
in vivo athymic BALB/e nude mic: tumour development	0 - 0.1 mM single subcutaneous injection of $5x10^6$ cells, in tiplicate	+ve (0.1mM)	cells exposed to 0.1 mM were injected into 6 mice; after 11-13 weeks, high grade transitional cell carcinomas were formed in 18/18 mice.	Oka96

Annex **G**

ECETOC's Special Report no.10 (ECE96)

See next pages.

Annex **H**

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 I

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 base 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 base 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.