Health Council of the Netherlands

(Di)benzoyl peroxide

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *(Di)benzoyl peroxide*
Uw kenmerk : DGV/MBO/U-932342
Ons kenmerk : U-7439/JR/fs/246-I17
Bijlagen : 1
Datum : 23 november 2012

Geachte staatssecretaris,

Graag bied ik u hierbij het advies aan over de gevolgen van beroepsmatige blootstelling aan (di)benzoylperoxide.

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: sr.vink@gr.nl
Telefoon (070) 340 55 08

Postadres
Postbus 16052
2500 BB Den Haag
www.gr.nl

(Di)benzoyl peroxide

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. 2012/24, The Hague, November 30, 2012

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

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

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

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

The Health Council of the Netherlands is a member of the International Network of Agencies for Health Technology Assessment (INAHTA), an international collaboration of organisations engaged with health technology assessment.

INAHTA

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

Preferred citation:
Health Council of the Netherlands. (Di)benzoyl peroxide. Evaluation of the carcinogenicity and genotoxicity. The Hague: Health Council of the Netherlands, 2012; publication no. 2012/24.

ISBN: 978-90-5549-937-3

Contents

Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige blootstelling aan stoffen van de raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie (di)benzoylperoxide onder de loep. (Di)benzoylperoxide wordt voornamelijk gebruikt als radicaalinitiator voor polymerisaties. Daarnaast wordt het ook gebruikt als bleekmiddel voor meel, vet, olie, was en melk; in verharding van rubber; in de eindbewerking van sommige acetaatgarens, en in geneesmiddelen voor acnebehandeling.

De commissie concludeert dat de gegevens over (di)benzoperoxide niet voldoende zijn om de kankerverwekkende eigenschappen te evalueren (categorie 3).*

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

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the Subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter the Committee. In this report the Committee evaluates (di)benzoyl peroxide. (Di)benzoyl peroxide is mainly used as a radical initiator for polymerisation. In addition, it is used as: a bleaching agent for flour, fats, oils, waxes and milk; in rubber curing; as a finishing agent for some acetate yarns, and in pharmaceuticals for topical treatment of acne.

The Committee concludes that the data on (di)benzoyl peroxide are insufficient to evaluate the carcinogenic properties (category 3). *

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

Chapter 1

Scope

1.1 Background

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

This report contains the evaluation of the carcinogenicity of (di)benzoyl peroxide, further referred to as benzoyl peroxide.

1.2 Committee and procedure

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

In 2012 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex D. The Committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

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

More recently published data were retrieved from the databases Medline and XToxline, and Chemical Abstracts. The last updated online search was in March 2012. The relevant new data were included in this report.

Chapter 2

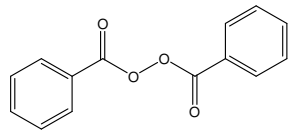
General information

2.1 Identity and physico-chemical properties

Benzoyl peroxide is predominantly used as a radical initiator for polymerization of acrylates (including dental cements and restoratives) and other polymers. In addition, it is used as: a bleaching agent for flour, fats, oils, waxes and milk; in the preparation of certain cheeses; in rubber curing; as a finishing agent for some acetate yarns; and, in pharmaceuticals for the topical treatment of acne.

Occupational exposure may occur during production, use in plastics, rubber and pharmaceutical industries, and in food processing.[1,2]

The identity of benzoyl peroxide and some of its physico-chemical properties are given below.[1,2]

Chemical name	:	benzoyl peroxide
CAS registry number	:	94-36-0
EC/EINECS number	:	202-327-6
Synonyms	:	dibenzoyl peroxide; benzoic acid, peroxide; benzoperoxide; benzoyl superoxide; diphenylglyoxal peroxide
Colour and physical	:	white granular crystalline solid with a faint odour of benzaldehyde
Molecular weight	:	242.22
Molecular formula	:	$C_{14}H_{10}O_4$
Structure	:	
Melting point	:	104-106 °C
Vapour pressure	:	<13 Pa at 20 °C
Relative density	:	1.33 g/cm^3
Solubility	:	slightly soluble in water (9.1 mg/L at 25 °C); soluble in acetone, diethyl ether, ethanol and most other organic solvents
Reactivity	:	highly flammable, explosive and oxidising
Conversion factors (25 °C, 760 mm Hg)	:	1 ppm = 0.101 x mg/m^3 1 mg/m^3 = 9.91 x ppm

2.2 IARC conclusion

In 1999, IARC considered benzoyl peroxide *not classifiable as to its carcinogenicity to humans* (Group 3). According to IARC, there was inadequate evidence in humans for the carcinogenicity of benzoyl peroxide, and there was limited evidence in experimental animals for the carcinogenicity of benzoyl peroxide.

Chapter 3

Carcinogenicity

3.1 Observations in humans

Limited human data are available, and only involve exposure to benzoyl peroxide via the dermal route.

In England, a pilot case-control study of malignant melanoma has been performed under patients who had used benzoyl peroxide for acne treatment (Cartwright et al.[3], cited in IARC).[1] This case control study involved 159 cases seen by their general practitioner between 1984 and 1986, who were compared to 213 controls matched for general practitioner, sex and age. No increased risk was found. The risk ratio between use of benzoyl peroxide and malignant melanoma was 0.5 (95% CI, 0.2-1.5).

In a Canadian population-based case-control study, the relationship between skin cancer of the head and neck, and use of benzoyl peroxide for acne treatment, was investigated. Cases (n= 964) selected from the Saskatchewan cancer registry were linked to four age- and sex-matched controls for each case (Hogan et al.[4], cited in IARC).[1] Interviews were conducted in 1989 among female residents aged 10-56 and male residents aged 10-51 years, benzoyl peroxide was marketed in Canada from 1966 onwards. The response rate was 91% for cases, and 80% for controls. Of the cases that responded 92.3% had basal-cell carcinoma, 4.8% squamous-cell carcinoma, and 2.9% malignant melanoma. Nine percent of the cases, and 10.1% of the controls, recalled use of preparations containing benzoyl

peroxide, for average periods of two and a half and two years, respectively. The odds ratio for use of benzoyl peroxide for all cases combined was 0.8 (95% CI, 0.5-1.3). There was no association found for the use of any specific preparation containing benzoyl peroxide.

The Committee concludes that the available human data, and the apparent lack of positive findings despite long term use, indicate that clinical dermal use of benzoyl peroxide does not represent a dermal carcinogenic hazard.

3.2 Carcinogenicity studies in animals

3.2.1 Carcinogenicity studies

Inhalation exposure

No inhalation studies are available.

Oral administration

Sharratt et al. conducted a study on the treatment of wholemeal flour with benzoyl peroxide.[5] Albino rats and albino mice (groups of 25 animals/sex/species; strain and age were unspecified) were fed a diet with increasing amount of Novadelox, a commercial powder containing 18% benzoyl peroxide (purity unspecified). Control groups received a diet lacking Novadelox. According to the authors, treatment groups received 10, 100 or 1,000 times the daily intake, for 120 weeks (rats) or 80 weeks (mice). The estimated doses of benzoyl peroxide as calculated by IARC amounted to 28, 280 and 2,800 mg/kg diet.

In parallel, groups of rats and mice (100 animals sex/species/group) were fed untreated bread crumbs (control), bread crumbs containing normal levels of Novadelox, or bread crumbs containing 10 times the normal amount of Novadelox.

Body weight gain was depressed in male and female rats consuming the diets containing the two highest levels of benzoyl peroxide. The authors stated that Novadex at the top dosage levels marginally reduced the concentration of one or several nutrients in the diet. There were some statistically significantly different mortality rates observed in rats between experimental groups and controls, and in mice in the middle dose group that suffered from a large number of accidental deaths. No evidence for benzoyl peroxide-related carcinogenicity was observed.

The Committee notes the limitations in the design and reporting of the study.

Dermal application

Sharrat et al. injected groups of 25 male and 25 female rats, and 25 male and 25 female mice subcutaneously with 120 mg (rats) or 50 mg (mice) benzoyl peroxide, as a 20% suspension in a starch solution.[5] Another group, consisting of 25 male and 25 female mice, were painted on the back of the neck on 6 days per week with approximately 50 mg of a 50% suspension of benzoyl peroxide paste. No significant difference between control groups and groups treated with benzyol peroxide, either subcutaneously or topically, in mortality or development of tumours was reported.

Additional data on the potential carcinogenic properties of benzoyl peroxide after dermal exposure can be derived from initiator-promotion studies that have included benzoyl peroxide as a control group. Below, only those studies are summarised in which animals were exposed to benzoyl peroxide alone.

In an extensive study by the National Toxicology Program (NTP), benzoyl peroxide was tested for promoting properties but was also applied as single agent to B6C3F1, Swiss (CD-1) and Sencar mice.[6] Two control groups (one as promotion control for DMBA, and one for MNNG) (30/sex/strain) were exposed to 20 mg benzoyl peroxide alone once a week for 52 weeks. Control animals received 0.1 mL acetone alone. Necropsies were conducted after 52 weeks. In the strains used, no skin tumours were observed.

Kurokawa et al. applied 0.2 mL of a 100 mg/mL solution of benzoyl peroxide in acetone, twice weekly for 51 weeks to a group to the shaved skin of 20 female SEN mice, four weeks of age (Kurokawa et al.[7], cited in IARC).[1] A group of 15 mice that served as controls received 0.2 mL acetone alone, There were no skin tumours among the control mice at the termination of the experiment, whereas eight tumours were noted in the benzoyl peroxide-treated mice ($p < 0.05$; five squamous-cell carcinomas). Kraus et al. has questioned the outcome reported by Kurokawa et al., due to potential methodological limitations, and the fact that an unusually high responsiveness of the model was observed.[8]

In a study by Spalding et al., groups of five male heterozygous TG:AC mice (carrying a v-Ha-ras gene) derived from the wild-type FVB/N strain were treated with 0, 1, 5 or 10 mg benzoyl peroxide in 0.2 mL acetone on the shaved dorsal skin, twice a week for 20 weeks (Spalding et al.[9], cited in IARC).[1] Groups of five male FVB/N mice were similarly treated. An increase in papillomas was noted in TG:AC mice at the two top dose groups (incidences of 0/5, 0/5, 3/5 and 3/4 in order of increasing doses). In addition, one papilloma-bearing mouse in the 10-

mg group died before the end of the experiment. No papillomas developed in the FVB/N mice.

In a promotion study in Syrian hamsters (age unknown), 20 animals received 160 mg benzoyl peroxide in 1 mL acetone to the shaved dorsal skin three times per week for 16 months (Schweizer et al.[10], cited in IARC).[1] The formation of a general and pronounced melanosis, coinciding with a usually moderate skin scaling, and a mild hyperplasia was reported. Melanotic tumours were not observed.

In addition to the studies noted in IARC, several other subcutaneous and/or dermal carcinogenicity studies with benzyol peroxide are reported in an extensive review by Kraus et al. (1995).[8] These involve mainly control groups in dermal promotion studies in mice, all with negative results.

3.2.2 Tumour promoting properties

Mancuso et al. showed that Car-S mice treated with DMBA and subsequent promotion twice weekly with benzoyl peroxide developed 11.0 (± 1.3) papillomas/mouse (a tumour incidence of 86%), whereas animals treated with DMBA alone had not yet developed papillomas.[11]

IARC and Kraus et al. (1995) further report several additional studies where benzoyl peroxide has been tested for initiating, and in particular, promoting properties.[1,8] Benzoyl peroxide did not display initiating properties in two studies in mice. Results of promotion studies have been both positive and negative, depending on the initiator, the vehicle and animal model used. Clear evidence of promoting activity of benzoyl peroxide has been reported for well-known initiating agents DMBA, MNNG and BaP – but not ultraviolet light - in several strains of mice, especially the responsive SENCAR strain.

The Committee concludes that the overall weight of the available initiating-promotion studies shows that benzoyl peroxide displays tumour promoting properties in various mouse models when applied dermally. This promoting activity of benzoyl peroxide has been associated with the generating ability of oxygen radicals.[1,12] The Committee considers these findings of low relevance for subsequent classification for carcinogenicity.

3.3 Cell transformation assays

Rivedal et al. did not observe an increased number of transformed colonies in the SHE cell transformation assay with benzoyl peroxide.[29]

Chapter 4

Mode of action

4.1 Genotoxic mode of action

The in vitro and in vivo genotoxicity data are summarised below and presented in Annex F.

4.1.1 Gene mutation assays

In vitro

Several bacterial mutagenicity assays have been conducted using benzoyl peroxide, consistently with negative results (references[13-20], cited by IARC and Kraus et al.).[1,8]

The only data on mammalian gene mutation assays are reported in an abstract, that describes positive results under unspecified conditions in T51B epithelial cells (Swierenga et al.[21], cited by Kraus et al.[8]).

In vivo

No data on mammalian in vivo gene mutation assays with benzoyl peroxide are available.

4.1.2 *Cytogenetic assays*

In vitro

Benzoyl peroxide did not cause chromosomal aberrations or aneuploidy in Chinese hamster lung cells without activation (Ishidate et al.[17], cited in IARC and Kraus et al.).[1,8]

However, benzoyl peroxide has been shown to induce DNA single-strand breaks in human bronchial epithelial cells (Saladino et al.[22] cited in IARC)[1] and sister chromatid exchange in CHO cells (Järventaus et al.[23] ,cited in Kraus et al.)[8] and V79 cells (Swierenga et al.[21], abstract cited in Kraus et al.).[8]

In vivo

No in vivo cytogenetic data from tests in somatic cells on benzoyl peroxide are available.

No significant increase in dominant lethal mutation rate was observed in mice following intraperitoneal injection of 54 or 62 mg/kg bw benzoyl peroxide (Epstein et al.[24], cited in IARC and Kraus et al.).[1,8]

4.1.3 *Miscellaneous*

In vitro

In human bronchial epithelial cells, DNA single-strand breaks and DNA-protein cross-links were observed in the absence of a metabolic activation system (Saladino et al.[22] cited in IARC).[1]

DNA strand breaks and subsequent DNA repair in primary hepatocytes were noted in an abstract of Swierenga et al. (Swierenga et al.[21], cited by Kraus et al.).[8]

Incubation of calf thymus DNA with benzoyl peroxide alone, or benzoyl peroxide and chelated iron, did not result in DNA single-strand breaks, as measured by S1-nuclease hydrolysis. Deoxyribose degradation, as measured by thiobarbituric acid product formation, was moderately (125% of control) affected by benzoyl peroxide, and significantly (175% of control) when combined with chelated iron.[25]

Hazlewood and Davies showed that benzoyl peroxide in the presence of Cu(I) resulted in benzoyloxyl and phenyl radicals.[12] Also DNA adduct formation occurred when the compound was added to DNA in solution.

Incubation of benzoyl peroxide with DNA fragments obtained from the human p53 tumour suppressor gene and c-Ha-ras-1 protooncogene, did not induce DNA damage in the presence of Cu(I).[26] When Cu(I) was added, an increase in DNA cleavage was noted in single and double stranded DNA.

Benzoyl peroxide induced the formation of 8-hydroxy-2'-deoxyguanosine in the DNA of murine keratinocytes.[27] Addition of a copper chelator blocked this formation. Depletion of intracellular glutathione resulted in increased levels of 8-OHdG formation, while addition of intracellular glutathione protected cells against DNA damaging.

In a unconventional in vitro test for the prediction of carcinogenicity, benzoyl peroxide increased the number of foci in bovine papillomavirus DNA-carrying C3H/10T1/2 cells compared to controls. This result was interpreted as positive by the authors.[28]

4.2 Non-genotoxic mode of action

No data are available.

4.3 Conclusion

The Committee is of the opinion that the available genotoxicity data do not allow any conclusions on the mode of action of benzoyl peroxide.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

Limited epidemiological data are available for benzoyl peroxide and only address the dermal route. Two case-control studies showed no evidence for carcinogenicity of benzoyl peroxide. The majority of animal carcinogenicity data consist of results obtained in mouse dermal initiator-promotion studies. The overall evidence from these studies suggest that benzoyl peroxide can act as a promoter.

For conclusions on the carcinogenic properties, insufficient data are available.

Benzoyl peroxide did not induce gene mutations in bacterial assays. Standard in vitro gene mutation assays in mammalian cells are lacking. One in vitro chromosomal aberration test conducted without metabolic activation was negative; whereas one sister chromatid exchange assay was positive in the presence of a metabolic activation system. Positive results have been reported in several in vitro DNA damage assays. In vivo genotoxicity data are lacking, with the exemption of a dominant lethal mutation assay in mice with negative results.

Overall, due to absence of several standard in vitro and particularly in vivo genotoxicity assays, the Committee cannot draw conclusions concerning the genotoxic properties of benzoyl peroxide.

5.2 Recommendation for classification

The Committee concludes that the data on benzoyl peroxide are insufficient to evaluate the carcinogenic properties (category 3).*

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

References

1 Benzoyl peroxide. IARC monographs on the evaluation of carcinogenic risks to humans. 1999: 71 Pt 2: 345-58.

2 Benzoyl Peroxide. SIDnitial Assessment Report For SIAM 15. 2002.

3 Cartwright RA, Hughes BR, Cunliffe WJ. Malignant melanoma, benzoyl peroxide and acne: a pilot epidemiological case-control investigation. Br J Dermatol 1988; 118(2): 239-242.

4 Hogan DJ, To T, Wilson ER, Miller AB, Robson D, Holfeld K e.a. A study of acne treatments as risk factors for skin cancer of the head and neck. Br J Dermatol 1991; 125(4): 343-348.

5 Sharratt M, Frazer AC, Forbes OC. Study of the biological effects of benzoyl peroxide. Food Cosmet Toxicol 1964; 527-538.

6 Comparative initiation/promotion skin paint studies of B6C3F1 mice, Swiss (CD-1) mice, and Sencar mice. 1996: Technical Report Series No. 441.

7 Kurokawa Y, Takamura N, Matsushima Y, Imazawa T, Hayashi Y. Studies on the promoting and complete carcinogenic activities of some oxidizing chemicals in skin carcinogenesis. Cancer Lett 1984; 24(3): 299-304.

8 Kraus AL, Munro IC, Orr JC, Binder RL, LeBoeuf RA, Williams GM. Benzoyl peroxide: an integrated human safety assessment for carcinogenicity. Regul Toxicol Pharmacol 1995; 21(1): 87-107.

9 Spalding JW, Momma J, Elwell MR, Tennant RW. Chemically induced skin carcinogenesis in a transgenic mouse line (TG.AC) carrying a v-Ha-ras gene. Carcinogenesis 1993; 14(7): 1335-1341.

10 Schweizer J, Loehrke H, Edler L, Goerttler K. Benzoyl peroxide promotes the formation of melanotic tumors in the skin of 7,12-dimethylbenz[a]anthracene-initiated Syrian golden hamsters. Carcinogenesis 1987; 8(3): 479-482.

11 Mancuso M, Pazzaglia S, Tanori M, Rebessi S, Di M, V, Covelli V e.a. Only a subset of 12-O-tetradecanoylphorbol-13-acetate-promoted mouse skin papillomas are promotable by benzoyl peroxide. Mutat Res 2004; 548(1-2): 35-45.

12 Hazlewood C, Davies MJ. Benzoyl peroxide-induced damage to DNA and its components: direct evidence for the generation of base adducts, sugar radicals, and strand breaks. Arch Biochem Biophys 1996; 332(1): 79-91.

13 Mutagenic evaluation of benzoyl peroxide. 1975: NTIS Pub. No. PB-245-494/AS.

14 De Flora S., Zanacchi P, Camoirano A, Bennicelli C, Badolati GS. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat Res 1984; 133(3): 161-198.

15 De Flora S., Camoirano A, Zanacchi P, Bennicelli C. Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds, negative with other Salmonella strains. Mutat Res 1984; 134(2-3): 159-165.

16 Dillon D, Combes R, Zeiger E. The effectiveness of Salmonella strains TA100, TA102 and TA104 for detecting mutagenicity of some aldehydes and peroxides. Mutagenesis 1998; 13(1): 19-26.

17 Ishidate M, Jr., Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M e.a. Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 1984; 22(8): 623-636.

18 Matula TL, Downie RH, Barret N. Mutagenicity studies of benzoyl peroxide in bacteria. Environ Mutat 1987; 9 (Suppl. 8): 69.

19 Yamaguchi T, Yamashita Y. Mutagenicity of hydroperoxides of fatty acids and some hydrocarbons. Agric Biol Chem 1980; 44: 1675-1678.

20 Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Mol Mutagen 1988; 11 Suppl 12: 1-157.

21 Swierenga SH, Hasnain SH, Lee FJ. An evaluation of cytotoxicity and genotoxicity of benzoyl peroxide (abstract). Environ Mutat 1987; 9 (Suppl. 8): 105.

22 Saladino AJ, Willey JC, Lechner JF, Grafstrom RC, LaVeck M, Harris CC. Effects of formaldehyde, acetaldehyde, benzoyl peroxide, and hydrogen peroxide on cultured normal human bronchial epithelial cells. Cancer Res 1985; 45(6): 2522-2526.

23 Järventaus H, Norppa J, Linnainmaa K, Sorsa M. SCE induction in CHO cells by peroxides used in the plastics industry. Mutat Res 1984; 130: 249.

24 Epstein SS, Arnold E, Andrea J, Bass W, Bishop Y. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol Appl Pharmacol 1972; 23(2): 288-325.

25 Iqbal M, Sharma SD, Mizote A, Fujisawa M, Okada S. Differential role of hydrogen peroxide and organic hydroperoxides in augmenting ferric nitrilotriacetate (Fe-NTA)-mediated DNA damage: implications for carcinogenesis. Teratog Carcinog Mutagen 2003; Suppl 1: 13-21.

26 Kawanishi S, Oikawa S, Murata M, Tsukitome H, Saito I. Site-specific oxidation at GG and GGG sequences in double-stranded DNA by benzoyl peroxide as a tumor promoter. Biochemistry 1999; 38(51): 16733-16739.

27 King JK, Egner PA, Kensler TW. Generation of DNA base modification following treatment of cultured murine keratinocytes with benzoyl peroxide. Carcinogenesis 1996; 17(2): 317-320.

28 Kowalski LA, Assi KP, Wee RK, Madden Z. In vitro prediction of carcinogenicity using a bovine papillomavirus DNA--carrying C3H/10T 1/2 cell line (T1). II: Results from the testing of 100 chemicals. Environ Mol Mutagen 2001; 37(3): 231-240.

29 Rivedal E, Mikalsen SO, Sanner T. Morphological transformation and effect on gap junction intercellular communication in Syrian hamster embryo cells as screening tests for carcinogens devoid of mutagenic activity. Toxicol In Vitro 2000; 14(2): 185-192.

30 Martinez A, Urios A, Blanco M. Mutagenicity of 80 chemicals in Escherichia coli tester strains IC203, deficient in OxyR, and its oxyR(+) parent WP2 uvrA/pKM101: detection of 31 oxidative mutagens. Mutat Res 2000; 467(1): 41-53.

31 Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands; 2010: publication no. A10/07E.

A	Request for advice
B	The Committee
C	The submission letter (in English)
D	Comments on the public review draft
E	IARC Monograph
F	Genotoxicity data
G	Carcinogenic classification of substances by the Committee

Annexes

Annex **A**

Request for advice

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

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

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

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

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10-4 and 10-6 per year.

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

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

Annex **B**

The Committee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Innovation for Life, Zeist; Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- J. van Benthem
  Genetic Toxicologist, National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist, SHELL International BV, The Hague
- G.J. Mulder
  Emeritus Professor of Toxicology, Leiden University, Leiden
- Ms M.J.M. Nivard
  Molecular Biologist and Genetic Toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  Epidemiologist, Dow Chemicals NV, Terneuzen
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- S.R. Vink, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter

Subject	: Submission of the advisory report *(di)benzoyl peroxide*
Your Reference	: DGV/MBO/U-932342
Our reference	: U-7439/JR/fs/246-I17
Enclosed	: 1
Date	: November 23, 2012

Dear State Secretary,

I hereby submit the advisory report on the effects of occupational exposure to *(di)benzoyl peroxide*.

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

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

- Mr. T.J.Lentz, National Institute for Occupational Safety and Health, USA.

Annex **E**

IARC Monograph

VOL: 36

CAS No.: 94-36-0

Summary of Data Reported and Evaluation

Exposure data

Benzoyl peroxide has been produced commercially since 1921. Major sources of exposure are its use in the plastics industry (principally for polyester resin production) and in acne medications. Another potential source is its use in bleaching foods.

Experimental data

Benzoyl peroxide was tested for carcinogenicity in mice and rats by oral administration in the diet and by subcutaneous administration, and in mice by skin application. In three studies by skin application in mice, benzoyl peroxide was tested for either initiating or promoting activity. All of the studies were inadequate for an evaluation of complete carcinogenicity; two studies indicated that benzoyl peroxide has promoting activity in mouse skin.

The available data are inadequate to evaluate the teratogenic potential of benzoyl peroxide in mammals.

Benzoyl peroxide was not mutagenic in bacteria. It did not induce chromosomal aberrations in mammalian cells *in vitro* and did not induce dominant lethal mutations in mice.

Human data

Among a small factory population, two cases of lung cancer were found in young men who were involved primarily in the production of benzoyl peroxide but were also exposed to benzoyl chloride and other chemicals.

Evaluation

There is *inadequate evidence* for the carcinogenicity of benzoyl peroxide to experimental animals. There is *inadequate evidence* for the carcinogenicity of benzoyl peroxide to humans. No evaluation could be made of the carcinogenicity to humans of benzoyl peroxide.

VOL: 71 (1999) (p.345)

Summary of Data Reported and Evaluation

Exposure data

Exposure to benzoyl peroxide may occur in its manufacture and use as an initiator in polymer production, food bleaching and rubber curing. Consumer exposure occurs from acne medications and dental products containing benzoyl peroxide.

Human carcinogenicity data

Two case-control studies have evaluated exposure to benzoyl peroxide among cases of malignant melanoma. One of these studies (the smallest) (among chemists) suggested a greater frequency of exposure among cases than controls. A third large population-based case-control study, designed specifically to evaluate the possible risk of benzoyl peroxide used as an acne medication among

young persons, included largely cases of basal-cell carcinoma of the skin. There was no association with use of benzoyl peroxide in this study.

Animal carcinogenicity data

Benzoyl peroxide was tested in two studies by skin application in strains of mice susceptible to the development of skin papillomas and in several skin-painting studies in mice and in one study in hamsters in combination with known carcinogens. In one study by skin application in mice, it induced benign and malignant skin tumours and, in the other study, benign tumours. Benzoyl peroxide was active as a skin tumour promoter in several strains of mice.

Other relevant data

Benzoyl peroxide forms radicals that are involved in its covalent binding to macromolecules. Its biological effects are inhibited by antioxidants.

Its genotoxic properties have received little attention. DNA damage has been observed in treated mammalian cells, but it is not mutagenic in bacteria and does not cause chromosomal damage in cultured mammalian cells or dominant lethal effects in mice.

Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of benzoyl peroxide. There is *limited evidence* in experimental animals for the carcinogenicity of benzoyl peroxide.

Overall evaluation

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

Annex **F**

Genotoxicity data

Results from genotoxicity assays conducted with benzoyl peroxide.

Test system	Result [a,b]		Reference
	exogenous metabolic activation		
	without	with	
Gene mutations			
In vitro			
Salmonella typhimurium	–	–	[13] (from Kraus et al.)
Salmonella typhimurium	–	–	[19] (from Kraus et al.)
Salmonella typhimurium	–	–	[14] (from Kraus et al.)
Salmonella typhimurium	–	–	[15] (from Kraus et al.)
Salmonella typhimurium	–	–	[17] (from IARC; Kraus et al.)
Salmonella typhimurium	+	–	[18] (from Kraus et al.)
Salmonella typhimurium	–	–	[20]
Salmonella typhimurium	–	–	[16]
Yeast gene conversion	–	–	[13] (from Kraus et al.)
T51B mutation	+[b]		[21] (from Kraus et al.)
in vivo			
Dominant lethal test, mice	NA	–	[24] (from IARC and Kraus et al.)
Chromosomal aberrations			
In vitro			
Sister Chromatide Exchange, CHO cells[b]	–	+	[23] (from Kraus et al.)
Chromosomal aberrations, Chinese hamster lung cells	–	NT	[17] (from IARC; Kraus et al.)

Sister Chromatide Exchange, V79 cells	+[b]		[21] (from Kraus et al.)
Aneuploidy, Chinese hamster lung cells	–	NT	[17] (from IARC)
DNA single-strand breaks	+	NT	[22] (from IARC)
Miscellaneous			
in vitro			
Escherichia coli DNA repair	+	–	[14] (from Kraus et al.)
Escherichia coli WP2 uvrA/pKM101 and IC203	–	NT	[30]
DNA-protein cross-links, human bronchial epithelial cells	+	NT	[22] (from IARC)
Hepatocyte DNA repair	+[b]		[21] (from Kraus et al.)
DNA strand breaks, calf thymus DNA	–	NT	[25]
Deoxyribose degradation, calf thymus DNA	± (+ with Fe)	NT	[25]
DNA adduct, calf thymus DNA	+	NT	[12]
Foci formation, bovine papillomavirus DNA-carrying C3H/10T1/2 cells	+	NT	[28]
DNA cleavage, ss- and ds-DNA fragments from human p53 and c-Ha-ras-1 gene, calf thymus DNA and human leukemia cells (HL-60)	– (+ with Cu)	NT	[26]
Oxidized guanine in DNA, murine keratinocytes	+	NT	[27]

[a] Based only on abstract; methodology not completely clear.
[b] Presumed to be performed in absence of S9.
NA = not applicable; NT = not tested.

Annex **G**

Carcinogenic classification of substances by the Committee

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

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category	
		67/584/EEC (before 12/16/2008)	EC No 1272/2008 (as from 2/16/2008)
1A	The compound is known to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	The compound is presumed to be as carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	Not applicable	Not applicable
(4)	The compound is probably not carcinogenic to man.	Not applicable	Not applicable

Source: Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council, 2010; publication no. A10/07E.[31]