1,2-Catechol (pyrocatechol)

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Pyrocatechol*
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Geachte staatssecretaris,

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

Dit 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.

Het advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. Het advies is getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. L.J. Gunning-Schepers,
voorzitter

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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. 2011/05OSH, The Hague, February 25, 2011

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld. In het voorliggende advies neemt de subcommissie Classificatie van Carcinogene Stoffen van de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Raad, die deze evaluatie en beoordeling verricht, 1,2-catechol (pyrocatechol) onder de loep. Pyrocatechol is een natuurlijke voorkomende stof die als grondproduct wordt gebruikt voor diverse doeleinden.

Op basis van de beschikbare gegevens leidt de commissie af dat 1,2-catechol beschouwd moet worden als kankerverwekkend voor de mens en beveelt zij aan de stof te classificeren in categorie 1B*.

Pyrocatechol kan werken via een stochastisch genotoxisch proces. Vanuit mechanistisch oogpunt verwacht de commissie dat pyrocatechol pas boven een bepaalde blootstelling een duidelijke meetbare bijdrage aan DNA-schade en dus aan het kankerrisico zal leveren. Op grond van de beschikbare gegevens kan echter geen blootstellingsniveau worden aangewezen waarop die bijdrage zichtbaar wordt.

* Volgens het nieuwe classificatiesysteem van de Gezondheidsraad (zie bijlage F). Dit system is gebaseerd op richtlijn 1272/2008 van de Europese Unie, die op 20 Januari 2009 van kracht werd.

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 Safety of the Health Council. In this report, the Committee evaluated 1,2-catechol (pyrocatechol). Pyrocatechol is a natural occurring substance that is used for various purposes.

Based on the available information, the Committee is of the opinion that 1,2-catechol is presumed to be carcinogenic to man, and recommends classifying the substance in category 1B*.

Pyrocatechol is able to act by a stochastic genotoxic process. In view of the mechanism the Committee expects that pyrocatechol can contribute significantly to the DNA-damage, and thus to cancer risk, only above a certain exposure level. However, on the basis of the available data no exposure level can be determined at which the contribution becomes noticeable.

* According to the new classification system of the Health Council (see Annex F), which is based on regulation 1272/2008 of the European Union. This regulation entered into force on 20 January 2009.

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). The assessment and the proposal for a classification are expressed in the form of standard sentences (see Annex F). The criteria used for classification are partly based on an EU-directive (see Annex G). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question.

This report contains the evaluation of the carcinogenicity of 1,2-catechol (pyrocatechol).

1.2 Committee and procedures

The evaluation is performed by the subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee. The members of the Committee are listed in Annex B.

In 2010 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 and recommendation of the Committee is standardly based on scientific data, which are publicly available. The starting point of the Committees' report is, 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 pyrocatechol, such an IARC-monograph is available, of which the summary and conclusion of IARC is inserted in annex D.

More recently published data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in May 2010. The new relevant data were included in this report.

Chapter 2

General information

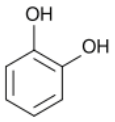
2.1 Identity and physico-chemical properties

Pyrocatechol is a natural occurring plant polyphenol that is used as an ingredient for the production of insecticides, perfumes, and drugs.[26] It is furthermore used in photography, dyestuffs, electroplating, specialty inks, antioxidants and light stabilizers, and in organic synthesis. Other purposes are as a polymerization inhibitor, and as an antiseptic. It is also a metabolite of benzene.

Occupational exposure may occur during manufacturing or packaging, or during use of the final products. The compound is present in fruits and vegetables as a natural compound. Therefore, regular consumption of low amounts of pyrocatechol is likely.

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

Name	:	pyrocatechol
CAS no	:	120-80-9 (CAS name, 1,2-benzenediol)
EINECS no	:	204-427-5
EEC no	:	604-016-00-4
Synonyms	:	catechol; 1,2-dihydroxybenzene; *o*-dihydroxybenzene; catechine; pyrocatechine
Description	:	colourless monoclinic crystals
Occurrence	:	naturally in fruits and vegetables such as onions, apples, and crude beet sugar, and in trees such as pine, oak, and willow; it is present in cigarette smoke (100-360 µg/cigarette)
Chemical formula	:	$C_6H_6O_2$

Chemical structure	:	
Molecular weight	:	110.11
Boiling point (101.3 kPa)	:	245 °C
Melting point (101.3 kPa)	:	105 °C
Relative density (20°/4°C)	:	1.344
Vapour pressure (20°C)	:	4 Pa
Solubility	:	very soluble in water and aqueous alkalis; very soluble in benzene, chloroform, diethyl ether, ethanol, pyridine
Log $P_{oct/water}$	:	0.88
Conversion factors (101.3 kPa; 20°C)	:	1 ppm = 4.5 mg/m^3 1 mg/m^3 = 0.22 ppm
EU classification		H312: harmful in contact with skin. H302: Harmful if swallowed. H319: Causes serious eye irritation. H315: Causes skin irritation. (Based on Regulation (EC) No 1272/2008 of the European Parliament and of the Council on Classification, labelling and packaging of substances and mixtures; 16 December 2008)

2.2 IARC classification

In 1999, IARC concluded that there is sufficient evidence for the carcinogenicity of pyrocatechol in experimental animals, but that there were no carcinogenicity data available from studies in humans.[26] Therefore, according to the IARC guidelines, it classified pyrocatechol in Group 2B, which means that the agent is possibly carcinogenic to humans.

Chapter 3

Carcinogenicity

3.1 Observations in humans

No data were available to evaluate the carcinogenicity of pyrocatechol in humans.

3.2 Carcinogenicity studies in animals

In the IARC monograph, various animal studies were evaluated, of which a summary is given below.[26]

Groups of Fischer rats and MRC-Wistar rats (n=30/group; males only) were given pyrocatechol in their drinking water at doses of 0 or 0.5% for 78 weeks, or were given the agent in their diet at doses of 0 or 2 mg/kg bw for up to 15 months (≈ 65 weeks).[30,37] In none of the studies pyrocatechol induced neoplasms. IARC noted the short durations of the studies.[26]

In two animal studies, performed independently from each other, male and female Fischer rats (n=30/group/sex), and Wistar, WKY, Lewis, and SD rats (n=20-30/group/strain; males only), were given pyrocatechol in their diet at a dose of 0.8% (w/w; final percentage in the diet) for 104 weeks (Hirose *et al*. 1990, 1993; Tanaka *et al*. 1995).[21,22,26,50] In both studies and in all rat strains, consumption of pyrocatechol-enriched food caused a statistically significant increase in number of tumour-bearing animals compared to control animals receiving a normal diet. In all rat strains, adenomas, adenomatous hyperplasia, or

adenocarcinomas were found in the glandular stomach, while no such tumours were found in control animals. No other treatment-related effects were observed in any of the animals.

Pathological effects in the glandular stomach were also reported in male and female $B6C3F_1$ mice (n=30/group/sex), which were fed a pyrocatechol-enriched diet (w/w; final concentration in diet, 0.8%) for 96 weeks (Hirose *et al*. 1990, 1993).[21,22,26] The type of effects concerned adenomatous hyperplasia of both sexes, but no adenocarcinomas. Also, in the forestomach of both sexes, hyperplasia and papillomas were observed. No such pathological lesions were found in control animals receiving a normal diet. Furthermore, no other treatment-related effects were detected.

More recently, Hagiwara *et al.* (2001) presented the results of a carcinogenicity study on Fischer rats (males only), which were given pyrocatechol in their diet.[16] Groups of thirty animals received dietary levels of 0, 0.1, 0.2, 0.4, and 0.8% (w/w) pyrocatechol for 104 weeks. The average pyrocatechol intake was estimated to be 33, 65, 141, and 318 mg/kg bw/day. Five animals per group were sacrificed at week 34. Gross pathology at week 34 revealed slight thickening of the pyloric region of the stomach in the 0.4 and 0.8% groups, but not in the other groups. At termination of the study, the thickening was marked to moderate in the 0.2, 0.4 and the 0.8% groups. Concerning glandular stomachs, pyrocatechol statistically significantly increased the number of animals with submucosal hyperplasia (all groups), and adenomas (all groups, except 0.1%). It also induced a non-significant increase in adenocarcinomas (0.4 and 0.8%). Furthermore, a dose-dependent increase in ulcerations was found. No neoplasms were found in control animals receiving normal diet. Neoplasms (squamous cell hyperplasia, papillomas) were also found in the forestomach, which reached statistical significance at 0.4 and 0.8% for squamous cell hyperplasia. In the lymph nodes surrounding the stomach, a statistically significant increase in cystic sinus dilatation was observed in 0.4 and 0.8% groups compared to control animals. Furthermore, in the 0.8% group a significant increase in number of animals with acinar cell adenomas in the pancreas were noted. The investigators did not find other treatment-related types of neoplasms.

Van Duuren *et al*. (1986) and Melikian *et al*. (1989) topically applied pyrocatechol on the skin of female SEN and Crl:DC-1 (1CR) BR mice (n=30/group), respectively.[26,36,53] The SEN mice received pyrocatechol at a dose of 0 or 2,000 µg/animal, three times per week for 490-560 days (≈ 64-80 weeks); the other mice strain received a dose of 0 or 250 µg/animal, five times per week for 48

weeks. At the end of the experiments, no skin tumours were observed in any of the animals.

IARC reported on several initiation-promotion studies to test pyrocatechol for its promotional potency.[26] In the majority of the studies Fischer F344 rats were used, which were given pyrocatechol-enriched diets (0.8 or 1.5% w/w) after a short initiation period with known carcinogens (*i.e*., nitrosoamines), but also other study designs were reported. In short, pyrocatechol enhanced tumour development in the stomach after tumour initiation (Hirose *et al*. 1987; Yamaguchi *et al*. 1989; Hagiwara *et al*. 1993; Hirose *et al*. 1993; Kawabe *et al*. 1994).[17,24,25,27,57] Kobayashi *et al*. (1999) observed that administration of pyrocatechol in the diet at low doses (4, 20, 100 and 500 ppm), did only enhance preneoplastic lesions, but not neoplastic lesions in the stomach of BALB/c mice, which received a tumour initiator earlier.[28] It also enhanced papillomas of the tongue, and carcinomas of the oesophagus (Hirose *et al*. 1987)[24] However, no tumour enhancement was observed regarding bladder tumours (Miyata *et al*. 1985; Kurata *et al.* 1990; Fukushima *et al*. 1991)[13,29,38], liver tumours (Stenius *et al*. 1989; Okazaki *et al*. 1993),[44,48] kidney tumours (Okazaki *et al*. 1993)[44], and pancreatic tumours (Maruyama *et al*. 1994),[34] compared to treatments with the initiators alone, or with pyrocatechol alone.

Chapter 4

Genotoxicity

The outcomes of the individual studies are given in Annex E.

4.1 *In vitro* assays

In bacterial reverse mutation assays, pyrocatechol did not induce gene mutations in various *Salmonella typhimurium* strains, in the presence or absence of a metabolic activation system.[11,18,19,26] No increased frequencies of mutations were observed in IC204, IC206, and IC208 *E. coli* strains, which are used to detect the induction of mutagens generated from 8-oxoguanine lesions. In the *WP2 Mutoxitest*, a test system to detect oxidative mutagenicity, pyrocatechol did induce reverse mutations in IC203 *Escherichia coli* strain, but only in the absence of a metabolic activation system (Martínez *et al*. 2000).[33] In the *Mut-Test*, a test system to detect substances, which prevent spontaneous mutations due to oxidative damage, pyrocatechol showed a dose-related antimutagenic activity in *E. coli mutT* mutants.[58] Regarding DNA damage and repair, pyrocatechol exposure did not induce DNA repair in *S. typhimurium* bacteria, in the presence or absence of a metabolic activation system.

No gene conversions or homozygosis were found in the yeast *Saccharomyces cerevisiae*, in the absence of a metabolic activation system, while in the same yeast pyrocatechol induced forward mutations.[26] Sommers and Schiestl (2006) showed that pyrocatechol induced intrachromosomal recombinations in *S. Cere-*

visiae strains RS112 and RS177, which are also used to detect DNA strand breaks.[47]

Using a ^{32}P-postlabelling technique, Oikawa *et al*. (2001) showed that pyrocatechol dose-dependently increased DNA damage in calf thymus DNA.[43] Exposure of mammalian cells to pyrocatechol resulted in gene mutations at the *tk* locus of mouse L5178Y lymphoma cells, and at the *hprt* and Na+/K+ ATPase loci in Syrian hamster embryo cells.[26] No unscheduled DNA synthesis was observed in primary rat hepatocytes, which were exposed to pyrocatechol at a concentration of 0.5 to 1000 µg/mL for approximately 24 hours.[9] Contradictory, unscheduled DNA synthesis was reported by Tsutsui *et al*. (1997) in Syrian hamster embryo cells.[51]

In the presence or absence of a metabolic activation system, in primary rat hepatocytes, and in human peripheral blood lymphocytes, exposure to pyrocatechol caused a weak increase of DNA strand breaks.[26] However, test results with mouse lymphoma cells were equivocal.[12,26] The Committee noted that the test results might have been influenced by differences in the composition of the incubation media. For instance, Fabiani *et al.* (2001) reported on DNA damage induced by pyrocatechol in human peripheral blood lymphocytes, which were incubated in four different media.[7] Only in the so-called PBS media in the absence of foetal calf's serum, DNA damage was statistically significantly increased compared to non-treated cells, while in media (PBS and RPMI) in the presence of serum no treatment-related DNA damage could be observed. Pyrocatechol also induced chromosomal aberrations in Syrian hamster embryo cells, and micronuclei and chromosomal loss in human lymphocytes. However, in at least one study, no micronuclei could be detected in the latter type of cells (Robertson *et al*. 1991).[46] Do Céu Silva *et al*. (2003) reported on a pH-dependent increase in frequencies of chromosomal aberrations in Chinese hamster V79 fibroblasts.[5] Using a human lymphoid cell line, Stillman *et al*. (1999) could detect aneuploidy, when pyrocatechol was given in combination with hydroquinone, but not when the substances were given alone.[49] Pyrocatechol exposure increased frequencies of sister chromatid exchanges in Syrian hamster embryo cells, and in human lymphocytes.[26]

De Oliveira *et al.* (2010) showed that pyrocatechol induced apoptosis in human glioblastoma GL-15 cells (Comet assay; exposure up to 600 µM for 48 hours).[4]

4.2 *In vivo* assays

A day after a single dose of 25 mg pyrocatechol/mL was given (by means of a glass filter paper, saturated with a solution of the compound), no increased frequencies of sex-linked recessive lethal mutations in male *Drosophila melanogaster* flies were observed.[2] Pyrocatechol did induce DNA repair in *E. Coli* (host-mediated assay) in mice. In the glandular stomach epithelium of rats, which were given pyrocatechol in their diet (0.8%) for two weeks, no clear increase in DNA adducts was observed.[23] In somatic cells, no (gene) mutations were observed in a mouse spot test.[26]

Regarding clastogenicity and aneuploidy, IARC reported in its monograph of a few studies, in which pyrocatechol induced micronuclei in CD-1 mice after a single oral or intraperitoneal administration, but not in NMRI mice after repeated subcutaneous administration.[26] In bone marrow cells of male Sprague-Dawley rats (n=15/group), which were given a single dose of 0, 10, 30, or 100 mg pyrocatechol/kg bw by gavage, no differences in frequencies of chromosomal aberration, mean chromosomal numbers, and mean mitotic indices were found compared to non-treated animals.[9] Furthermore, a single oral administration of pyrocatechol to Fischer F344 rats did not result in DNA strand breaks or cross links in the pyloric mucosa of the stomach.[26]

4.3 Carcinogenic mechanism of action

The mechanism through which pyrocatechol may show carcinogenic potential is still not fully understood. Both stochastic genotoxic as well as non-genotoxic mechanisms are likely to play a role.

A common hypothesis is that pyrocatechol induces oxidative DNA damage. It is for instance speculated that in aqueous environment (pH around or above neutrality) pyrocatechol undergoes Cu^{2+}-mediated autoxidation to generate Cu^{+} and semiquinone radicals.[5,43] Binding of Cu^{+} to oxygen generates reactive oxygen species, but also reduction of semiquinone radicals into 1,2-benzoquinone may do so.[26,31] These reactive oxygen species may ultimately lead to DNA damage.

About the type of reactive oxygen species and type of oxidative DNA damage, these are of the same type as generated by cells during normal cellular processes. Under normal conditions, the antioxidant defense system within the cell can easily handle the free oxygen radicals produced. Based on this knowledge,

the Committee expects that reactive oxygen species, which are generated by pyrocatechol at the lowest exposure levels, disappear in the pool of endogenous oxygen species, and are removed efficiently by the oxidant defense system. However, at increasing exposure a breaking point will be reached, at which the defense system gets exhausted. From that moment on, the Committee expects that the pyrocatechol-generated reactive oxygen species will contribute greatly to the oxidative DNA damage, and thus to the risk of cancer development. In terms of quantitative risk analysis, this would indicate that a threshold level exists, at or below which the risk of cancer is expected not to exceed the background risk.

Yet, formation of reactive oxygen species might not explain all positive outcomes of the genotoxicity studies *in vitro*. For instance, The presence of antioxidant enzymes, such as superoxide dismutase and catalase, should remove reactive oxygen species resulting in reduced DNA damage, but so far these enzymes did not clearly influence pyrocatechol-induced DNA damage *in vitro*.[5,43] Further research is needed to clarify these findings.

The Committee cannot exclude that pyrocatechol may exert its carcinogenic effect by its irritating potency, a nongenotoxic mechanism. Pyrocatechol was found to irritate the skin and eyes, the respiratory and gastrointestinal tract of humans; and, the skin and eyes of guinea pigs.[31] Chronic exposure to irritants may induce continuous cell proliferation, making the cells prone to DNA damage. However, so far known, no evidence is available that can confirm the validity of this hypothesis.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenesis and genotoxicity

No data on the genotoxicity and carcinogenicity of pyrocatechol in humans were available. In several animal carcinogenicity studies, chronic administration of pyrocatechol in the diet caused an increased number of tumour-bearing male and female animals with neoplasms in the glandular stomach (and forestomach). However, no tumours were found in other organs than the stomach when pyrocatechol was given alone, or after a tumour-initiation period with known carcinogenic initiators. The findings of the animal studies give sufficient evidence that exposure to pyrocatechol can result in cancer development, at least in animals.

The results of the available genotoxicity tests indicate that pyrocatechol can be considered as an *in vitro* genotoxic compound, inducing predominantly clastogenic effects. Studies also show that pyrocatechol is able to induce oxidative DNA damage by a stochastic genotoxic mechanism. The Committee further noted that except for one mouse study, all other *in vivo* studies were negative; therefore the Committee considers that *in vivo* results as ambiguous at worst.

Pyrocatechol is metabolized efficiently by conjugation (sulfation and glucuronidation) at its phenol groups. At higher concentrations, redox cycling may generate reactive oxygen species at an appreciable extent. These reactive oxygen species are of the same type as those generated by normal cellular processes. Therefore, they are expected to enter the pool of reactive oxygen species generated by endogenous metabolism and to be handled accordingly by the natural

antioxidant defense system. At (very) low exposure levels of pyrocatechol, no significant contribution to the endogenous pool of reactive oxygen species is expected. In addition, at those low exposure levels, DNA damage induced by pyrocatechol-generated reactive oxygen species will not noticeably increase DNA damage already caused by endogenous reactive oxygen species. This implies that only above a certain exposure level pyrocatechol contributes significantly to the DNA damage, and thus to cancer risk. However, on the basis of the available data on pyrocatechol no exposure level ('threshold') can be determined at which the contribution becomes noticeable.

The Committee is of the opinion that the observations in animals, and the proposed carcinogenic mechanism are relevant also to humans.

5.2 Recommendation for classification

The Committee is of the opinion that 1,2-catechol (pyrocatechol) is presumed to be carcinogenic to man, and recommends to classify the substance in category 1B*.

Pyrocatechol is able to act by a stochastic genotoxic mechanism. In view of the mechanism the Committee expects that pyrocatechol can contribute significantly to the DNA-damage, and thus to cancer risk, only above a certain exposure level. However, on the basis of the available data no exposure level can be determined at which the contribution becomes noticeable.

* According to the new classification system of the Health Council (see Annex F), which is based on regulation 1272/2008 of the European Union (see Annex G). This regulation entered into force on 20 January 2009.

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E	Mutagenicity and genotoxicity data
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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 Safety (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

- G.J. Mulder, *chairman*
  Emeritus Professor of Toxicology, Leiden University, Leiden
- J. van Benthem
  Genetic toxicologist, National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist, SHELL International BV, The Hague
- Ms M.J.M. Nivard
  Molecular biologist and genetic toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  Epidemiologist, Dow Chemicals NV, Terneuzen
- R.A. Woutersen
  Toxicologic pathologist, TNO Nutrition and Food Research, Zeist; Professor of Translational toxicology, Wageningen University and Research Centre, Wageningen
- A.A. van Zeeland
  Emeritus Professor of Molecular radiation dosimetry and radiation mutagenesis, Leiden University Medical Center, Leiden
- E.J.J. van Zoelen
  Professor of Cell biology, Radboud University Nijmegen, Nijmegen

- J.M. Rijnkels, *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 President 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 establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2010 for public review. The following organisations and persons have commented on the draft document:

- Ms V. Gálvez Pérez, Instituto Nacional de Seguridad e Higiene en el Trabajo, Madrid, Spain.
- Mr R.D. Zumwalde, National Institute of Occupational Health and Safety, Cincinnati, the USA.

Annex **D**

IARC evaluation and conclusion

Vol.: 71 (1999) (p. 433)[26]
CAS No.: 120-80-9
Chem. Abstr. Name: 1,2-Benzenediol

Summary of Data Reported and Evaluation

Exposure data

Exposure to catechol may occur in its production, in the production of insecticides, perfumes and drugs, in metal plating and in coal processing. Catechol occurs naturally in fruits and vegetables. It is present in cigarette smoke and has been detected at low levels in ambient air and water.

Human carcinogenicity data

No data were available to the Working Group.

Animal carcinogenicity data

Catechol was tested for carcinogenicity by oral administration in one study in mice and in two studies in rats. No increase in the incidence of malignant tumours was found in mice. In rats, it induced adenocarcinomas in the glandular

stomach in several strains. In one study in mice by skin application, no skin tumour was observed. In several experiments in rats involving administration with known carcinogens, catechol enhanced the incidence of papillomas of the tongue, carcinomas of the oesophagus, squamous-cell carcinomas of the forestomach and adenocarcinomas of the glandular stomach.

Other relevant data

Catechol is oxidized by peroxidases to the reactive intermediate benzo-1,2-quinone, which binds to protein. The acute toxicity of catechol is relatively low. In humans, the irritant action of catechol can lead to dermatitis and other dermal lesions. Chronic oral treatment of rodents causes hyperplasia of the forestomach and pyloric mucosa.

Catechol was shown to cause gene mutations in mammalian cells *in vitro*. Chromosomal aberrations and sister chromatid exchanges were reported in mammalian cells in culture. After application to mice, catechol was negative in one and positive in three studies of micronucleus formation in bone marrow.

Evaluation

No epidemiological data relevant to the carcinogenicity of catechol were available.

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

Overall evaluation

Catechol is possibly carcinogenic to humans (Group 2B).

Previous evaluations: Vol. 15 (1977); Suppl. 7 (1987).

Synonyms: Catechin, 1,2-Dihydroxybenzene, Pyrocatechol.

Annex **E**

Mutagenicity and genotoxicity data

Table E.1 Mutagenicity and genotoxicity of pyrocatechol in in vitro assays.

Test system	Dose range	Result - negative + positive	Reference
Mutagenicity			
Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537, TA1538; with and without metabolic activation	500-5,000 µg/plate	-	Farrow and McCArroll 1984[11]; Haworth *et al*. 1983[18,19]; Nazar *et al*. 1981[42]; Yoshida and Fukuhara 1983[59]
Gene mutations in *S. cerevisiae* MP1; no metabolic activation	2,500 µg/plate	+	Fahrig 1984[8]
Gene conversions in *S. cerevisiae* MP1; no metabolic activation	2,500 µg/plate	-	Fahrig 1984[8]
WP2 Mutoxitest, *E.coli strains* IC188, IC203, and IC203 plus metabolic activation (+S9)	1,000-3,000 µg/plate	+ (IC203) - (IC188, IC203+S9)	Martínez *et al*. 2000[33]
WP2 Mutoxitest, *E.coli* strains IC204 (*mut*+), IC206 (*mutY*), and IC208 (*mutY oxyR*)	2,000 µg/plate	-	Martínez *et al*. 2000[33]
Mut-Test, *E. coli WP2 mutT* strain; no metabolic activation	0-10 mM	Dose-dependent decrease in mutagenicity	Yonezawa *et al*. 2001[58]
Gene mutations *tk* locus of mouse lymphoma L5178Y cells; with and without metabolic activation	1.1-2.5 µg/mL	+	Farrow and Draus 1983[10]; McGregor *et al*. 1988[35]; Wangenheim and Bolcsfoldi 1988[55]
Gene mutation (*hprt* locus) in Syrian hamster embryo cells; no metabolic activation	0.33 µg/mL	+	Tsutsui *et al*. 1997[51]

Gene mutation (Na+/K+ATPase locus) in Syrian hamster embryo cells; no metabolic activation	1.1 µg/mL	+	Tsutsui *et al*. 1997[51]
Chromosome aberrations			
Syrian hamster embryo cells; no metabolic activation	0.33 µg/mL	+	Tsutsui *et al*. 1997[51]
Chinese hamster V79 cells; with and without metabolic activation and antioxidant enzymes		+ (pH-dependent; no stimulating effect by metabolic activation or antioxidant enzymes)	Do Céu Silva *et al*. 2003[5]
Sister chromatid exchange			
Syrian hamster embryo cells; no metabolic activation	1.1 µg/mL	+	Tsutsui *et al*. 1997[51]
Human peripheral blood lymphocytes; no metabolic activation	4-33 µg/mL	+	Erexson *et al*. 1985[6]; Morimoto 1983[39]; Morimoto and Wolff 1980[40]
Micronuclei			
Human peripheral blood lymphocytes; no metabolic activation	22 µg/mL	+ (Higher percentage stained kinetochore positive controls)	Yager *et al*. 1990[56]
Human peripheral blood lymphocytes; no metabolic activation	8.3 µg/mL	-	Robertson *et al*. 1991[46]
Aneuploidy			
Syrian hamster embryo cells; no metabolic activation	3.3 µg/mL	+	Tsutsui *et al*. 1997[51]
In chromosomes 5, 7 and 8 of human lymphoblastoid GM09948 cells; no metabolic activation. These chromosomes are considered to play a role in benzene-induced acute myelogenous leukemia and myelodysplastic syndrome. One of the metabolites of benzene is pyrocatechol.	16.6 µg/mL	-	Stillman *et al*. 1999[49]
DNA repair and damage			
DNA damage, calf thymus DNA	0-100 µM	+ (dose dependent)	Oikawa *et al*. 2001[43]
DNA repair *umu* assay using *S. typhimurium* pSK1002 (SOS system); with and without metabolic activation	3,300 µg/plate	-	Nakamura *et al*. 1987[41]
DNA strand breaks (intrachromosomal recombinations) in *S cerevisiae* strains RS112 and RS177 (RS112 rad2); no metabolic activation	0-5 mg/mL	+	Sommers and Schiestl 2006[47]
DNA strand breaks (alkali-labile sites) in primary rat hepatocytes	330 µg/mL	+/-	Wallis 1992[54]

DNA strand breaks in mouse lymphoma L5178Y cells; no metabolic activation	110 µg/mL	-	Pellack-Waller and Blumer 1986[45]
DNA strand breaks and cross links in mouse lymphoma cells; with and without metabolic activation	55 µg/mL	+	Garberg *et al*. 1988[15]
DNA strand breaks (alkali-labile sites; Comet assay) in human lymphocytes; with and without metabolic activation	11 µg/mL	+ (Higher percentage stained kinetochore positive controls)	Anderson *et al*. 1995[1]
DNA damage in human peripheral blood lymphocytes; incubation in different media	200-600 µM	+ (PBS) - (PBS + serum; RPMI; RPMI + serum)	Fabiani *et al*. 2001[7]
Unscheduled DNA synthesis			
Primary rat hepatocytes	1,000 µg/mL	-	Farrow and Draus 1983[10]
Homozygosis			
S. cerevisiae MP1; no metabolic activation	2,500 µg/plate	-	Fahrig 1984[8]

Table E.2 Mutagenicity and genotoxicity of pyrocatechol in in vivo test systems.

Test system	Dose range	Result - negative + positive	Reference
Mutagenicity			
Sex-linked recessives lethal test, using *Drosophila melanogaster* flies	25 µg/mL	-	Benson and Myhr 1984[2]
Spot test using C579BL x T mice embryos; single intraperitoneal injection	22 mg/kg bw	-	Fahrig 1984[8]
Chromosome aberrations			
Male Sprague-Dawley and CD rats; single oral administration	100 mg/kg bw	-	Farrow *et al.* 1984[9]
Micronuclei (micronucleus test assays)			
Bone marrow and fetal liver cells in pregnant CD-1 mice; single oral administration	40 mg/kg bw	+	Ciranni *et al.* 1988[3]
Bone marrow cells in male CD-1 mice; single oral administration	40 mg/kg bw	+	Ciranni *et al.* 1988[3]
Bone marrow cells in male CD-1 mice; single intraperitoneal injection	10 mg/kg bw	+	Marrazzini *et al.* 1994[32]
Bone marrow cells in male NMRI mice; subcutaneous injections, 6 times repeated	42 mg/kg bw	-	Tunek *et al.* 1982[52]
DNA repair and DNA damage			
Host-mediated assay; DNA repair activity in blood, liver, lung, kidney, testis cells of male NMRI mice (host); *E. coli* K-12 *uvr/rec* A; a single oral administration	200 mg/kg bw	-	Hellmér and Bolcsfoldi 1992[20]
Spot test (DNA strand breaks) using C579BL x T mouse embryos; single intraperitoneal injection	22 mg/kg bw	- (not clear which tissues were tested)	Fahrig 1984[8]
DNA strand breaks and cross links in pyloric mucosa of stomach of Fischer F344 rats; single oral administration	90 mg/kg bw	-	Furihata *et al.* 1989[14]
DNA adducts			
Glandular stomach of rats (strain not specified); repeated oral administration	Final concentration in the diet, 0.8%	- (no detailed data presented)	Hirose *et al.* 1999[23]

Annex **F**

Carcinogenic classification of substances by the Committee

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

Category	Judgement of the Committee (GRGHS)	Comparable with EU Category	
		67/584/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to man. • 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, the mechanism of action is not known.	1	1A
1B	The compound is presumed to be carcinogenic to man. • 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, the mechanism of action is not known.	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

Annex **G**

Regulation (EC) No 1272/2008

of the European Parliament and of the Council on classification, labelling, and packaging of substances and mixtures

3.6 Carcinogenicity

3.6.1 Definition

Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

3.6.2 Classification criteria for substances

See Table on the next page.

3.6.2.1 For the purpose of classification for carcinogenicity, substances are allocated to one of two categories based on strength of evidence and additional considerations (weight of evidence). In certain instances, route-specific classification may be warranted, if it can be conclusively proved that no other route of exposure exhibits the hazard.

3.6.2.2 Specific considerations for classification of substances as carcinogens.

3.6.2.2.1 Classification as a carcinogen is made on the basis of evidence from reliable and acceptable studies and is intended to be used for substances which have an intrinsic property to cause can-

Table 3.6.1 Hazard categories for carcinogens.

Categories	Criteria
Category 1:	Known or presumed human carcinogens. A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A:	Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B:	Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.
Category 2:	Suspected human carcinogens. The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

(1) Note: See 3.6.2.2.4.

cer. The evaluations shall be based on all existing data, peer-reviewed published studies and additional acceptable data.

3.6.2.2.2 Classification of a substance as a carcinogen is a process that involves two interrelated determinations: evaluations of strength of evidence and consideration of all other relevant information to place substances with human cancer potential into hazard categories.

3.6.2.2.3 Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms ‘sufficient’ and ‘limited’ have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;
- limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

- sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;
- limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, *e.g.* (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

3.6.2.2.4 Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here.

3.6.2.2.5 The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner.

3.6.2.2.6 Some important factors which may be taken into consideration, when assessing the overall level of concern are:

a tumour type and background incidence;
b multi-site responses;
c progression of lesions to malignancy;
d reduced tumour latency;
e whether responses are in single or both sexes;
f whether responses are in a single species or several species;
g structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
h routes of exposure;
i comparison of absorption, distribution, metabolism and excretion between test animals and humans;
j the possibility of a confounding effect of excessive toxicity at test doses;
k mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.

3.6.2.2.7 A substance that has not been tested for carcinogenicity may in certain instances be classified in Category 1A, Category 1B or Category 2 based on tumour data from a structural analogue together with substantial support from consideration of other important factors such as formation of common significant metabolites, *e.g.* for benzidine congener dyes.

3.6.2.2.8 The classification shall take into consideration whether or not the substance is absorbed by a given route(s); or whether there are only local tumours at the site of administration for the tested route(s), and adequate testing by other major route(s) show lack of carcinogenicity.

3.6.2.2.9 It is important that whatever is known of the physico-chemical, toxicokinetic and toxicodynamic properties of the substances, as well as any available relevant information on chemical analogues, i.e. structure activity relationship, is taken into consideration when undertaking classification.

3.6.4 Hazard communication

3.6.4.1 Classification for carcinogenicity:

Category 1A or Category 1B:

Hazard statement H350: May cause cancer <*state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard*>.

Category 2:

Hazard statement H351: Suspected of causing cancer <*state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard*>.