Health Council of the Netherlands

Hydroquinone and benzoquinone

Health-based recommended occupational exposure limit

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies over *Hydroquinone and benzoquinone*
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Geachte minister,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan hydrochinon en benzochinon.

Dit advies maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige adieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Het genoemde advies is opgesteld door de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en omgeving.

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. W.A. van Gool,
voorzitter

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

to:

the Minister of Social Affairs and Employment

No. 2012/26, The Hague, December 3, 2012

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Samenvatting en advieswaarde

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in lucht waaraan mensen beroepsmatig blootgesteld kunnen worden. Deze advieswaarden vormen vervolgens de basis voor grenswaarden – vast te stellen door de minister – waarmee de gezondheid van werknemers beschermd kan worden.

In dit advies bespreekt de commissie de gevolgen van blootstelling aan hydrochinon en benzochinon en stelt zij een gezondheidskundige advieswaarde vast. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór oktober 2012 zijn verschenen.

Fysische en chemische eigenschappen

Hydrochinon (CAS nr. 123-31-9) is een wateroplosbare kristallijne stof die in grote hoeveelheden wordt geproduceerd, maar in kleine hoeveelheden ook van nature in sommige planten aanwezig is. De stof heeft een molecuulmassa van 110,11 Dalton; het smeltpunt is 172 ºC en het kookpunt is 286 ºC.

In waterige oplossing is hydrochinon gevoelig voor zowel zuur-base omzettingen als redox-cycling, wat leidt tot de vorming van benzochinon en

semichinon, alsmede verschillende reactieve vormen van zuurstof (ROS: reactive oxygen species).

Hydrochinon wordt vrijwel uitsluitend gebruikt als industriële stof: als antioxidant in de productie van rubbers, als remmer in de stabilisatie van monomeren, in fotografische en lithografische bedrijven, als stabilisator voor onder andere verven, lakken en vernisssen, en als antioxidant voor industriële vetten en oliën.

Benzochinon (CAS nr. 106-51-4) is een slecht wateroplosbare kristallijne stof, die in grote hoeveelheden wordt geproduceerd. Het is in kleine hoeveelheden echter ook in de natuur aanwezig: het wordt bijvoorbeeld door vele insecten gesynthetiseerd en uitgescheiden. De stof heeft een molecuulmassa van 108,09 Dalton en een smeltpunt van 116 ºC; bij deze temperatuur sublimeert de stof.

In waterige oplossing is benzochinon gevoelig voor zowel redox-cycling als zuur-base transformaties, leidend tot de vorming van hydrochinon en semichinon, alsmede verschillende reactieve vormen van zuurstof.

Benzochinon wordt voornamelijk gebruikt voor de productie van hydrochinon, maar wordt ook gebruikt als polymerisatieremmer in de productie van plastics, en als intermediair in de productie van verschillende chemicaliën, zoals rubber-acceleratoren en oxidatiemiddelen. Daarnaast vindt het toepassing in de fotografie.

Monitoring

Voor monitoring van hydrochinon in lucht zijn methoden beschikbaar voor concentraties tussen 0,7 en 25 mg hydrochinon per m^3. Naast deze milieumonitoring is ook biologische monitoring van aan meer dan 2 mg/m^3 hydrochinon blootgestelde werknemers mogelijk, namelijk door analyse van hydrochinon in de urine en door analyse van adducten van hydrochinon en albumine in het bloed.

Voor benzochinon zijn methoden voor milieu- en biologische monitoring beschikbaar die gebaseerd zijn op albumine-adducten. In urine is benzochinon niet aantoonbaar.

Grenswaarden

Nederland heeft momenteel geen grenswaarden voor beroepsmatige blootstelling aan hydrochinon en benzochinon. De American Conference of Governmental Industrial Hygienists (ACGIH) heeft in 2008 voor hydrochinon een TLV

(threshold limit value) van 1 mg/m^3 vastgesteld als 8-uurs tijdgewogen gemiddelde (TWA), en in 2001 voor benzochinon een TLV-TWA van 0,44 mg/m^3. Het Verenigd Koninkrijk heeft voor hydrochinon een beroepsmatige blootstellingslimiet van 0,5 mg/m^3 vastgesteld; zij hebben benzochinon niet geëvalueerd. Duitsland heeft geen beroepsmatige blootstellingslimieten voor hydrochinon en benzochinon.

Kinetiek

In proefdieronderzoek met ratten zijn de absorptie, distributie, metabolisme en excretie van hydrochinon onderzocht, daarnaast is er een huidabsorptie-studie met humane huid in vitro beschikbaar. Er is geen informatie beschikbaar over de kinetiek na inademing van hydrochinon (inhalatoire blootstelling). De absorptie via de huid werd geclassificeerd als “langzaam”. Na toediening via het maag-darmkanaal (orale toediening) wordt hydrochinon snel en goed geabsorbeerd. De distributie na orale toediening, intraveneuze injectie of inbrengen in de luchtpijp is nagenoeg identiek.

Hydrochinon wordt enzymatisch via semichinon geoxideerd tot benzochinon; deze reactie kan ook spontaan verlopen. De reductie van benzochinon tot hydrochinon wordt eveneens enzymatisch gekatalyseerd, maar ook deze kan spontaan verlopen. Hydrochinon kan conjugeren met glucuronide en sulfaat; de gevormde conjugaten worden via de urine uitgescheiden. Benzochinon (en semichinon) kunnen conjugeren met glutathion, resulterend in mono-, di- en triglutathionconjugaten, die in de gal worden teruggevonden. De glutathionconjugaten kunnen verder worden omgezet tot cysteine-conjugaten en mercaptuurzuren.

De belangrijkste uitscheidingsroute is via de urine (> 85%), als wateroplosbare metabolieten, waarvan glucuronides (45-56%) en sulfaten (19-43%) de belangrijkste zijn. Mercaptuurzuren zijn in kleinere hoeveelheden aanwezig (< 5%). Slechts een klein deel (1-3%) wordt onveranderd in de urine uitgescheiden.

Voor benzochinon is geen informatie over de kinetiek na inademing beschikbaar. De stof wordt vanuit het maag-darmkanaal en via de huid snel geabsorbeerd. Het wordt gedeeltelijk onveranderd en gedeeltelijk als hydrochinon uitgescheiden; van de laatste het grootste deel als conjugaten.

Effecten

Hydrochinon

Mens

Uit de beschikbare humaan-epidemiologische studies met hydrochinon konden geen conclusies met betrekking tot de kankerverwekkende eigenschappen worden getrokken.

Bij beroepsmatige blootstelling induceert hydrochinon depigmentatie van de huid, pigmentatie van het hoornvlies, en sensibilisering.

Dier

In proefdieronderzoek werden oog- en huidirritatie, depigmentatie en sensibilisering waargenomen. In de acute toxiciteitsstudies werden neurologische effecten zoals overmatige opwinding, rillingen, toevallen en coma gerapporteerd.

Er zijn subacute studies met toedieningen via de huid (dermaal) en via het maag-darmkanaal (oraal) uitgevoerd. Na dermale toediening gedurende 14 dagen aan ratten werd verminderd lichaamsgewicht vastgesteld. Na orale toediening gedurende 13 weken aan ratten en muizen werden veranderde nier- en levergewichten gevonden, bij ratten werden ook milde, tijdelijke rillingen en een verminderde kooi-activiteit waargenomen.

Er zijn orale carcinogeniteitsstudies uitgevoerd (toediening via maagsonde en via het voer); studies via de dermale route of via de ademhaling zijn niet beschikbaar.

Bij 50 mg/kg lichaamsgewicht/dag oraal toegediend aan ratten gedurende 104 weken werden verminderde lichaamsgewichten gevonden, alsmede renale tubulaire adenomen (goedaardige gezwellen in de niertubulus) in mannetjes en mononucleaire celleukemieën (een bepaalde vorm van kanker van de witte bloedcellen) in vrouwtjes. In de dieetstudie werden eveneens renale renale tubulaire adenomen geïnduceerd. In de rattenstudies werd tevens chronische progressieve nefropathie (chronische en voortschrijdende nierschade)

waargenomen. Alleen uit de sonde-studie kon een NOAEL* van 25 mg/kg lichaamsgewicht/dag worden afgeleid, uit de dieetstudie kon dat niet.

In muizen induceerde 50 mg/kg lichaamsgewicht/dag gedurende 104 weken toegenomen relatieve levergewichten in mannetjes en hepatocellulaire adenomen (goedaardige gezwellen in de lever) in vrouwtjes. In de dieet-studie werden eveneens hepatocellulaire adenomen gevonden, maar alleen in mannetjes. Ook in deze studies konden geen NOAEL's worden afgeleid.

Hydrochinon kan het DNA en de chromosomen beschadigen.

Op basis van de reproductietoxiciteitstudies met hydrochinon concludeert de commissie dat de stof niet toxisch is voor de vruchtbaarheid en het nageslacht. De NOAEL voor ontwikkelingstoxiciteit was 75 mg/kg lichaamsgewicht/dag, voor maternale toxiciteit was de NOAEL 25 mg/kg lichaamsgewicht/dag.

De beschikbare gegevens over neurotoxiciteit tonen milde en tijdelijke tremoren en een verminderde kooi-activiteit; de NOAEL voor neurotoxiciteit was 20 mg/kg lichaamsgewicht/dag.

Benzochinon

Mens

Ten aanzien van de carcinogeniteit van benzochinon zijn geen relevante epidemiologische studies beschikbaar.

Blootstelling van mensen aan hoge luchtconcentraties benzochinon kan leiden tot oogirritatie, terwijl dermale blootstelling kan resulteren in ernstige huidirritatie en dermatitis. In oudere onderzoekingen werden na beroepsmatige blootstellingen tot 5 jaar aan 0,44 mg/m^3 benzochinon geen systemische effecten gezien.

Dier

Op basis van diverse dierstudies lijkt benzochinon huid-sensibiliserend te zijn. De stof induceerde schade aan de huid en irritatie na onderhuidse injectie in cavia's.

In acute toxiciteitsstudies in proefdieren werden verschillende neurologische effecten waargenomen, waaronder verlies van reflexen, tremoren, en verlamming van de achterpoten. Er zijn geen acute dermale of inhalatoire studies beschikbaar.

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* No observed adverse effect level (NOAEL): het hoogste niveau waarbij nog juist geen toxische effecten optreden.

Met benzochinon zijn subacute studies uitgevoerd na inhalatoire blootstelling en na intraperitoneale en subcutane injecties. De kritische effecten waren hematologisch van aard: vermindering van aantal bloedcellen, methemoglobinevorming (een vergrote hoeveelheid van een geoxideerde vorm van hemoglobine in het bloed, leidend tot een verminderde zuurstofcapaciteit) en thrombopenie (een verminderde hoeveelheid bloedplaatjes). Een inhalatoire NOAEL kon niet worden vastgesteld.

Afgezien van twee zeer beperkte carcinogeniteitsstudies (een orale muizen- en een dermale rattenstudie) zijn er geen chronische studies met benzochinon beschikbaar. In de dermale studie werden na subcutane injectie enkele locale fibrosarcomen (kwaadaardige bindweefselgezwellen) waargenomen; in de orale studie werden tumoren in de lever en de milt gevonden. Getuige de hoge sterfte nog vóór de inductie van tumoren was er in beide studies sprake van een substantiële toxiciteit. Daardoor en door de lage kwaliteit van de studies kunnen geen conclusies worden getrokken met betrekking tot de carcinogene potentie van benzochinon.

Benzochinon kan het DNA en de chromosomen beschadigen.

Er zijn geen studies betreffende reproductietoxiciteit beschikbaar.

Evaluatie en advies

Benzochinon en hydrochinon zijn metabolieten van elkaar. De toxiciteit van de een is daarom ook relevant voor de ander, hoewel er een verschil in toxische potentie kan bestaan.

Er zijn geen bruikbare humane gegevens op basis waarvan gezondheidskundige advieswaarden voor hydrochinon en benzochinon afgeleid kunnen worden.

Als de beschikbare proefdierstudies in ogenschouw worden genomen, hebben de toxiciteitsprofielen van hydrochinon en benzochinon veel gemeen: beiden zijn irriterend voor huid en ogen, werken sensibiliserend op de huid en hebben een vergelijkbaar genotoxiciteitsprofiel. De meer complete gegevens voor hydrochinon kunnen mogelijk als indicatie voor de potentiële effecten van benzochinon dienen.

Hydrochinon

Subchronische en chronische blootstelling van proefdieren aan hydrochinon leidt tot veranderingen in lichaams-, lever- en niergewichten, en tot tijdelijke tremoren en veranderingen in kooi-activiteit. In dieet- en sondestudies met muizen en

ratten werd tumorinductie in levers en nieren geconstateerd, voorafgegaan door toenemende lever- en niergewichten en histopathologische veranderingen, in de nier met hyperplasie (vergroting van een bepaald orgaan of weefsel als gevolg van een abnormaal hoge celdeling), in de lever met hypertrofie (toenemende grootte van weefsels of organen door vergroting van het volume van de afzonderlijke cellen). In de rat werd tevens nefropathie waargenomen; terwijl in de muis schildklier-effecten (hyperplasie, en adenomen en carcinomen) werden gezien. De waargenomen nefropathie bij ratten is soortspecifiek en niet relevant voor de mens. De Subcommissie Classificatie van carcinogene stoffen is van oordeel dat de resultaten betreffende de waargenomen tumoren inconsistent waren, en tumoren betroffen die niet relevant voor de mens worden geacht. Op basis van de beschikbare gegevens is de Subcommissie Classificatie van carcinogene stoffen van mening dat de gegevens over hydrochinon onvoldoende zijn om de kankerverwekkende eigenschappen te beoordelen (categorie 3). Verder concludeerde de subcommissie dat hydrochinon mutaties kan induceren, doch uitsluitend bij hoge blootstellingen, waarschijnlijk via reactieve vormen van zuurstof. Zolang blootstellingen aan hydrochinon onder de concentraties blijven die locale cytotoxiciteit (en derhalve hyperplasie) veroorzaken, worden de risico's op carcinogene effecten verwaarloosbaar geacht.

Op basis van deze conclusies en de overweging van de Subcommissie Classificatie van carcinogene stoffen dat het mechanisme van de carcinogeniteit van hydrochinon in proefdieren naar alle waarschijnlijkheid berust op een niet-stochastisch genotoxisch werkingsmechanisme (zoals het genereren van ROS, maar mogelijk speelt ook remming van topo-isomerase II een rol) kiest de commissie dan ook voor de drempelwaarde-benadering om een gezondheids-kundige advieswaarde voor hydrochinon af te leiden.

Als startpunt voor de afleiding van de gezondheidskundige advieswaarde voor hydrochinon neemt de commissie de chronische muizenstudie met sonde-toediening, met schildklierhyperplasie als kritisch effect. In deze studie vertonen de vrouwtjes een ongeveer drie maal hogere gevoeligheid dan de mannetjes, zodat de vrouwtjes als uitgangspunt zijn genomen. De commissie prefereert de BMD* methode, echter, een analyse van de onderzoeksgegevens toonde aan dat die van de vrouwtjes niet geschikt zijn voor een BMD analyse; de data van de mannetjes zijn dat wel, maar de daaruit afgeleide BMDL moet dan gecorrigeerd worden voor de grotere gevoeligheid van de vrouwtjes. Uit de gegevens van de mannetjes wordt een BMDL voor 10% extra risico afgeleid van 15,7 mg/kg lichaamsgewicht/dag. Aangezien vrouwtjes ongeveer drie maal gevoeliger zijn

* BMD: benchmark dosis; BMDL: benchmark dosis bij de onderste 95% betrouwbaarheidsgrens.

dan mannetjes voor dit effect wordt een correctiefactor van 3 op deze waarde toegepast, hetgeen resulteert in een gecorrigeerde BMDL van 5,2 mg per kg lichaamsgewicht per dag. Om te extrapoleren van de muis naar de mens wordt een factor van 3 toegepast, wat leidt tot een waarde van 1,7 mg per kg lichaamsgewicht per dag. Route-to-route extrapolatie (van orale naar inhalatoire blootstelling; 70 kg lichaamsgewicht, 10 m^3 ademvolume per 8-urige werkdag) leidt vervolgens tot een waarde voor dagelijkse blootstelling van 12 mg/m^3. Om te corrigeren voor de verschillen in gevoeligheid tussen mensen onderling wordt een factor van 3 toegepast. Daaruit volgt dan een gezondheidskundige advieswaarde van 4 mg/m^3.

Hoewel er slechts beperkte gegevens beschikbaar zijn, verwacht de commissie dat bij deze concentratie de risico's voor oog- en huidirritatie en sensibilisering te verwaarlozen zijn.

Benzochinon

De commissie volgt dezelfde lijn van argumentatie voor benzochinon, met inachtneming van het vermoeden dat deze stof de hoogste intrinsieke toxiciteit van de twee heeft. Omdat geschikte gegevens voor benzochinon zelf ontbreken heeft de commissie geprobeerd om de gezondheidskundige advieswaarde voor hydrochinon te gebruiken voor de afleiding van een gezondheidskundige advieswaarde voor benzochinon. De commissie concludeert echter dat de beschikbare gegevens over de relatieve toxiciteit van benzochinon ten opzichte van hydrochinon van onvoldoende kwaliteit zijn om op basis daarvan een betrouwbare gezondheidskundige advieswaarde voor benzochinon af te leiden. Op basis van de beschikbare gegevens is de Subcommissie Classificatie van carcinogene stoffen van mening dat de gegevens over benzochinon onvoldoende zijn om de kankerverwekkende eigenschappen te beoordelen (categorie 3). In hoofdstuk 9.4 geeft de commissie enkele nadere overwegingen met betrekking tot een gezondheidskundige advieswaarde voor beroepsmatige blootstelling aan benzochinon.

Om de verschillen in toxische potentie van hydrochinon en benzochinon te kunnen vergelijken beveelt de commissie voorts aan om de inhalatoire toxiciteit van beide stoffen te onderzoeken, bij voorkeur na chronische blootstelling,

Gezondheidskundige advieswaarden

De commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen van de Gezondheidsraad stelt bij beroepsmatige blootstelling aan hydrochinon een

gezondheidskundige advieswaarde voor van 4 mg/m^3, als 8 uurs tijdgewogen gemiddelde.

De commissie is van mening dat de beschikbare gegevens over de toxiciteit van benzochinon van onvoldoende kwaliteit zijn om op basis daarvan een betrouwbare gezondheidskundige advieswaarde voor benzochinon af te leiden.

Executive summary

Scope

At request of the Minister of Social Affairs and Employment (Annex A), the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations on the toxicity of existing substances that are used in the workplace. The purpose of the evaluations is to recommend a health-based recommended occupational exposure limit (HBROEL) for concentrations in the air at the workplace, provided the database allows derivation of such a value. In the Netherlands, these recommendations serve as a basis in setting public occupational exposure limits by the minister.

In this report, the Committee discusses the consequences of occupational exposure to hydroquinone and benzoquinone, and recommends a HBROEL. The Committee's conclusions are based on scientific papers published prior to October 2012.

Physical and chemical properties

Hydroquinone (CAS no. 123-31-9) is a water soluble crystalline substance, that is produced in large amounts, but also occurs in small amounts naturally in some plants. Hydroquinone has a molecular weight of 110.11, a melting point of 172 °C, and a boiling point of 286 °C.

When present in aqueous solution, hydroquinone is susceptible to both redox cycling and acid-base transformations, leading to the formation of benzoquinone and semiquinone, and also various reactive oxygen species (ROS).

Hydroquinone is used almost exclusively as an industrial chemical, i.e., as antioxidant in rubber manufacturing, as inhibitor in stabilization of monomers, in photographic industry and lithography, as stabilizer for paints and varnishes, and as antioxidant for industrially used fats and oils.

Benzoquinone (CAS no. 106-51-4) is a poorly water soluble crystalline substance, that is produced in large amounts, but also occurs in small amounts naturally, i.e., in a variety of arthropods: the substance is synthesized and excreted by many insects. Benzoquinone has a molecular weight of 108.09, and a melting point of 116 °C (sublimation).

When present in aqueous solution, benzoquinone is susceptible to both redox and acid-base transformations, leading to the formation of hydroquinone, semiquinone, and also ROS.

Benzoquinone's major use is in hydroquinone production, but it is also used as a polymerization inhibitor, photographic chemical, tanning agent, and as an intermediate in the production of a variety of substances, including rubber accelerators and oxidizing agents.

Monitoring

Methods for air monitoring of hydroquinone have a working range of 0.7 to 25 mg per m^3.

Biological monitoring of workers exposed to hydroquinone is possible by the analysis of hydroquinone in urine, at or above an airborne concentration of 2 mg/m^3. Where urinary levels of hydroquinone give exposure on a daily basis, adducts of hydroquinone to albumin might reflect exposures over weeks to months prior to collection of a blood sample: serum albumin typically has a half life of 21 days in humans.

For benzoquinone methods for environmental monitoring and biomonitoring based on albumin adducts are available. Benzoquinone is not detectable in urine.

Exposure limits

Currently The Netherlands do not have limit values for occupational exposure to hydroquinone and benzoquinone. The American Conference of Governmental Industrial Hygienists (ACGIH) established in 2008 a threshold limit value (TLV) for exposure to hydroquinone of 1.0 mg/m^3 as 8 hr time weighted average

(TWA). For benzoquinone ACGIH established a TLV-TWA of 0.44 mg/m^3 in 2001. The United Kingdom has set an occupational exposure limit (8 hr TWA) for hydroquinone of 0.5 mg/m^3, based on its (suspected) genotoxicity. No short term exposure level (STEL) was proposed. In Germany no Maximal Acceptable Concentration (MAK) value was derived; hydroquinone was classified in category 2 for carcinogenicity and category 3A for germ cell mutagenicity. The United Kingdom has not evaluated benzoquinone. In Germany no MAK value was derived, and benzoquinone was classified in category 3B for carcinogenicity and category 3B for germ cell mutagenicity.

Kinetics

Various ADME (absorption, distribution, metabolisme and excretion) studies with hydroquinone in rats are available, as well as an in vitro percutaneous absorption study using human skin. No information is available on kinetics of hydroquinone after inhalation. Hydroquinone absorption via the skin was determined to be "slow" (0.5-1 µg/cm^2/hr), but may be more rapid with vehicles such as alcohols. After oral administration or intratracheal instillation, hydroquinone was rapidly and extensively absorbed. Distribution was similar for administration via gavage, intravenous injection and intratracheal instillation.

Hydroquinone can be oxidised via semiquinone to benzoquinone by various enzymes, and also spontaneously (auto-oxidation). The reverse reaction can also occur both spontaneously and enzyme mediated. Hydroquinone can be conjugated with sulphate and glucuronide resulting in the respective conjugates which are excreted via the urine. Benzoquinone can be conjugated with glutathione resulting in mono-, di-, and tri-glutathione conjugates which are detectable in the bile. The glutathione conjugates can be further metabolised to cysteine conjugates and mercapturic acids.

The primary route of elimination is via the urine (> 85%) in the form of water soluble metabolites. The major urinary metabolites are glucuronide conjugates (45-56%) and sulphate conjugates (19-43%). Mercapturic acids are present at lower levels (< 5%). Only a small fraction (about 1-3%) is excreted unchanged in the urine.

For benzoquinone no information is available on kinetics after inhalation. Benzoquinone is reported to be readily absorbed from the gastrointestinal tract and subcutaneous tissue. It is excreted partly unchanged and partly as hydroquinone, the major proportion of which is eliminated as conjugates. No quantitative information was available.

Effects

Hydroquinone

Hydroquinone was reported to induce skin depigmentation, cornea pigmentation and sensitization in humans after occupational exposure. Eye irritation, skin irritation, depigmentation and sensitization were also observed in animal studies. The results from the studies on sensitization show “weak” to “extreme” sensitization, depending on the methods or vehicle used.

In the acute toxicity studies in animals, various neurological symptoms were observed, including hyperexcitability, tremors, convulsions and coma.

Sub-acute toxicity studies have been performed via dermal and oral (gavage) exposure. Reduced body weight was observed after topical application to rats for 14 days. After oral repeated dose administration, hydroquinone induced changes in kidney and liver weights of rats and mice for 13 weeks; with rats also mild and transient tremors and reduced home-cage activity were observed.

Only oral carcinogenicity studies (diet studies and gavage application) were performed; carcinogenicity studies via dermal or inhalation exposure were not available. In F344 rats administered 50 mg hydroquinone per kg bw per day by gavage for 104 weeks, reduced body weights were observed, and renal tubular adenomas in males and mononuclear cell leukaemia in females were found. In the diet study also renal tubular adenomas were induced. In both studies chronic progressive nephropathy was observed. Only for the gavage study a NOAEL* of 25 mg/kg bw/day was derived; the diet study did not allow the derivation of a NOAEL.

In B6C3F1 mice, 50 mg/kg bw/day hydroquinone for 104 weeks induced increased relative liver weights in males and hepatocellular adenomas in females. In the diet study induction of hepatocellular adenomas was also observed, but in this study only in males. Also in these studies no NOAELs were derived.

No conclusion regarding the carcinogenic properties of hydroquinone could be drawn from the human epidemiological studies with the substance.

Hydroquinone was negative in Salmonella strains TA 97, 98, 100, 1535, and 1537, with or without metabolic activation, but positive in strains TA102 and 104, both sensitive for oxidative mutagens. Hydroquinone was able to induce DNA strandbreaks, SCEs (sister chromatid exchanges), gene mutations, chromosomal aberrations and micronuclei in mammalian cells and cell lines in

* no observed adverse effect level.

vitro. Numerous in vivo studies were performed with hydroquinone, investigating the induction of micronuclei, chromosomal aberrations or polyploidy in bone marrow, and chromosomal aberrations or hyperploidy in germ cells. All but one of these studies used intraperitoneal administration and most of them were positive. One oral study gave a weak positive result. The positive responses may well be explained by the postulated underlying mechanisms, demonstrated to occur under in vitro conditions: redox-cycling with ROS generation, inhibition of topoisomerase II, and direct covalent binding to macromolecules (either directly or following conjugation with glutathione). Although this latter capability may underlie the in vitro observed genotoxicity, several attempts to demonstrate in vivo formation of hydroquinone-derived DNA-adducts failed so far.

Based on the studies on reproduction, it is concluded that hydroquinone is not a reproductive toxicant. The NOAEL for developmental toxicity was 75 mg/kg bw/day, for maternal toxicity it was 25 mg/kg bw/day.

The available data on neurotoxicity showed mild and transient tremors and reduced home-cage activity at 50 and 64 mg/kg bw/day. The NOAEL for neurotoxicity was 20 mg/kg bw/day.

Benzoquinone

In humans, exposure to high air levels of benzoquinone may result in irritation of the eyes. Dermal exposure of humans may result in dermatitis and severe irritation. Older studies reported that no systemic effects were observed following prolonged occupational exposure to benzoquinone vapour at a level of 0.44 mg/m^3 for periods up to 5 years.

Benzoquinone appeared to be a skin sensitizer based on results of various animal studies. It induced skin lesions and irritation when injected subcutaneously in guinea pigs.

In acute toxicity studies in animals, various neurological symptoms were observed, including loss of reflexes, writhing and paralysis of the hind limbs. No acute dermal or inhalation studies are available.

Subacute toxicity studies with benzoquinone have been performed via inhalation exposure, and intraperitonal and subcutanous injections. The critical effects were all of a haematological character and included decreases in blood cells, methaemoglobin formation and thrombopenia. A NOAEL by inhalation could not be established.

There are no chronic toxicity studies on benzoquinone except two very limited carcinogenicity studies: one dermal study (via subcutanous injections)

with rats and one oral study with mice. After dermal exposure a few local fibrosarcomas were found, whereas after oral exposure liver and spleen tumours were observed. In both studies toxicity by benzoquinone was quite substantial, as evidenced by a high mortality before tumour occurrence. Due to this and because of the poor quality of the studies no conclusions can be drawn about the potential of benzoquinone to induce carcinogenic effects.

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

Benzoquinone was negative in Salmonella strains TA 98, 1535, and 1537, with or without metabolic activation, and positive in strain TA 100 without metabolic activation. Salmonella strains TA102 and 104, both sensitive for oxidative mutagens and responding positive towards hydroquinone, were not tested. Benzoquinone was able to induce DNA strandbreaks, SCEs, gene mutations, and micronuclei in mammalian cells, and cell lines in vitro. In vivo micronuclei induction studies – via the oral route – were weakly positive, while one dominant lethal test with C3H mice was negative.

These positive responses may well be explained by the postulated underlying mechanisms, demonstrated to occur under in vitro conditions: redox-cycling with ROS generation, inhibition of topoisomerase II, and covalent binding to macromolecules (either directly or after conjugation with glutathione). Although this latter capability may underlie the in vitro observed genotoxicity, several attempts to demonstrate in vivo formation of benzoquinone-derived DNA-adducts failed so far.

No studies on reproduction and developmental toxicity of benzoquinone were available.

Evaluation and recommendation

Since benzoquinone and hydroquinone are metabolites of each other (interconverted at reaction rates dependent on prevailing local conditions), the toxicity observed with one of the two is also relevant for the other, although there may be a difference in potency.

There are no suitable data from human studies that can be used for deriving a HBROEL for either hydroquinone or benzoquinone.

As far as available animal data permit, toxicity profiles of hydroquinone and benzoquinone have indeed a lot in common: both substances are irritating to eyes and skin, act as skin sensitizers, and have a comparable genotoxicity profile. The database on hydroquinone, however, is more complete: i.e., for benzoquinone adequate data on (sub)chronic toxicity, carcinogenicity, and reproductive toxicity

are not available. Thus, for these data gaps for benzoquinone, available data on hydroquinone could be used to indicate its potential effects.

Hydroquinone

Subchronic or chronic exposure of experimental animals to hydroquinone leads to changes in body weight, changes in liver and kidney weight, and in transient tremors and changes in home-cage activity. Hydroquinone is not a reproductive toxicant.

In diet and gavage carcinogenicity studies with mice and rats, tumour induction was observed in kidney and liver, preceded by increased kidney and liver weights and histopathological changes: in the kidney with hyperplasia, and in liver with hypertrophy. In the rat also nephropathy was seen, while in the mouse the thyroid was affected (hyperplasie and adenomas and carcinomas). The nephropathy observed in rats is species-specific and not relevant for humans. The Subcommittee on the Classification of carcinogenic substances is of the opinion that the results regarding tumour induction were inconsistent and concerned tumour types that are considered not relevant for humans. Based on the available information, the subcommittee is of the opinion that the data are as yet insufficient to evaluate the carcinogenic properties of hydroquinone (category 3).

Further, the Subcommittee concluded that hydroquinone may induce mutations under high exposure conditions, probably due to the formation of ROS. But as long as exposure levels to hydroquinone are below levels inducing local cytotoxicity (and consequently hyperplasia), the risk for carcinogenic effects were considered to be negligible.

Based on these conclusions and the consideration of the Subcommittee on Classification of carcinogenic substances that the carcinogenic mechanism of hydroquinone in animal studies is in all probability a non-stochastic genotoxic mechanism (due to ROS-generation, but inhibition of topoisomerase II may also play a role) the Committee adopts a threshold approach for deriving a HBROEL for hydroquinone.

As starting point for deriving a HBROEL for hydroquinone, the Committee selects the chronic mouse gavage study as the pivotal one, with thyroid hyperplasia as the critical effect. In this study the females were approximately three times as sensitive compared to the males, and thus the effect in the females should be taken as the starting point. However, analysis of the study data of the females showed that these are not suited for a BMD* analysis. Only with the data

* BMD: benchmark dose; BMDL: benchmark dose at lower 95% confidence limit.

of the males a BMD analysis is possible, but then the resulting BMDL needs to be corrected for the higher susceptibility of the females. From the data of the males a BMDL for 10% extra risk of 15.7 mg/kg bw/day is derived. In view of the higher susceptibility of the females a correction factor of 3 is applied, resulting in an adjusted BMDL of 5.2 mg/kg bw/day. To extrapolate from mice to men a factor of 3 is applied, leading to a value of 1.7 mg/kg bw/day. Extrapolation to man, including route-to-route extrapolation (from oral to inhalation exposure; 70 kg bw, breathing volume 10 mg/m^3 during a 8-h working day), results in a value for daily exposure of 12 mg/m^3. To correct for potential differences in interindividual susceptibility, a factor of 3 is applied. Applying this factor results in a HBROEL of 4 mg/m^3. Although only limited data are available, the Committee expects that at this level the risk for eye and skin irritation and sensitization is negligible.

Benzoquinone

The Committee adopts a similar line of reasoning for benzoquinone, recognizing that this chemical intrinsically may be the more potent of the two toxicants. As suitable data for benzoquinone itself are lacking, the Committee tried to use the established HBROEL for hydroquinone for deriving a HBROEL for benzoquinone. However, the Committee concludes that the available data on the relative toxicity of hydroquinone and benzoquinone are of insufficient quality for deriving a solid HBROEL for benzoquinone. The Subcommittee on the Classification of carcinogenic substances is of the opinion that the data are as yet insufficient to evaluate the carcinogenic properties of benzoquinone (category 3). In Section 9.4 the Committee presents some further considerations with respect to a health-based limit value for occupational exposure to benzoquinone.

Health-based recommended occupational exposure limits

The Dutch Expert Committee on Occupational Safety recommends a health-based recommended occupational exposure limit for hydroquinone of 4 mg/m^3, as 8 hr time weighted average.

The Committee concludes that the available data on the toxicity of benzoquinone are of insufficient quality to derive a health-based recommended occupational exposure limit for benzoquinone.

Chapter 1

Scope

1.1 Background

At request of the Minister of Social Affairs and Employment (Annex A), the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations on the toxicity of existing substances that are used in the workplace. The purpose of the evaluations is to recommend a health-based occupational exposure limit (HBROEL) for concentrations in the air at the workplace, provided the database allows derivation of such a value. In the Netherlands, these recommendations serve as a basis in setting public occupational exposure limits by the minister.

1.2 Committee and procedure

This document contains the assessment by DECOS (hereafter called the Committee) of the health hazard of hydroquinone and benzoquinone. The members of the Committee are listed in Annex B. The submission letter (in English) to the Minister 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 Committee's recommendations on the health-based occupational exposure limit of hydroquinone and benzoquinone have been based on scientific data, which are publicly available. Data were obtained from on-line databases, Toxline, Medline, Current Contents, and Chemical Abstracts and TSCATS. The names and CAS numbers of hydroquinone (123-31-9) and benzoquinone (106-51-4) were used in combination with the following key words: adverse effects, occupation exposure, kinetics, human, animal and in vitro. Literature references containing the term therapeutic were excluded. The literature from this search was selected based on titles and abstract. The final search was performed in October, 2012.

Chapter 2

Identity, properties and monitoring

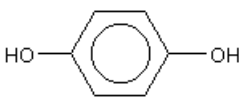
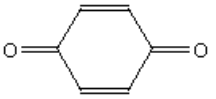
2.1 Chemical identity

Table 1 Chemical identity of hydroquinone and benzoquinone

Chemical name	Hydroquinone	Benzoquinone
IUPAC name	benzene-1,4-diol	cyclohexa-2,5-diene-1,4-dione
Synonyms	1,4-benzenediol; p-dihydroxybenzene; p-hydroxyphenol; 1,4-dihydroxybenzene; p-benzenediol; benzohydroquinone; benzoquinol; p-dioxobenzene; p-dioxybenzene; hydroquinol; hydroquinole; alpha-hydroquinone; p-hydroquinone; ß-quinol; quinol	1,4-benzoquinone, p-benzoquinone; p-quinone; quinone
CAS-number	123-31-9	106-51-4
EINEC-number	204-617-8	203-405-2
EEC-number	604-005-00-4	606-013-00-3
RTECS-number	MX 3500 000	DK2625000

2.2 Physical and chemical properties

Table 2 Physical and chemical properties of hydroquinone and benzoquinone[1-13]

	Hydroquinone	Benzoquinone
Physical description	White crystalline	Yellow crystalline
Molecular formula	$C_6H_4(OH)_2$	$C_6H_4O_2$
Structural formula		
Molecular weight	110.11	108.09
Melting point (°C)	172.3	115.7
Boiling point (°C)	286	sublimes
Vapour pressure (Pa)	2.4×10^{-3} (at 25 °C)	12 (at 20 °C)
Specific gravity (g/cm^3)	1.332 (at 15 °C)	1.318 (at 20 °C)
Auto ignition temperature (°C)	515 °C	560 °C
Log $P_{octanol/water}$	0.59	0.2
Solubility	Highly soluble in water (70 g/L at 25 °C); highly soluble in alcohol and ether; slightly soluble in benzene	Slightly soluble in water; soluble in alcohol, ether, and alkaline solutions
Odour threshold (mg/m^3)	Odourless	0.084 ppm
Conversion factors (at 25 °C and 760 torr)	1 ppm = 4.50 mg/m^3; 1 mg/m^3 = 0.222 ppm	1 ppm = 4.42 mg/m^3; 1 mg/m^3 = 0.226 ppm
Relative vapour density (air=1)	3.81	3.7

Oxidation-reduction equilibrium

Hydroquinone is a water-soluble, crystalline solid. When present in aqueous solution, hydroquinone is susceptible to both redox cycling and acid-base transformations (Figure 1). The products of these reactions include benzoquinone and semiquinone, and various reactive oxygen species (ROS). Hydroquinone, semiquinone and benzoquinone are the most stable species under aqueous physiological conditions. While all possible conversions are indicated in Figure 1, in vivo interconversions between hydroquinone, semiquinone and benzoquinone likely proceed via simultaneous electron-proton (i.e. hydrogen atom) transfers (diagonal arrows).[14]

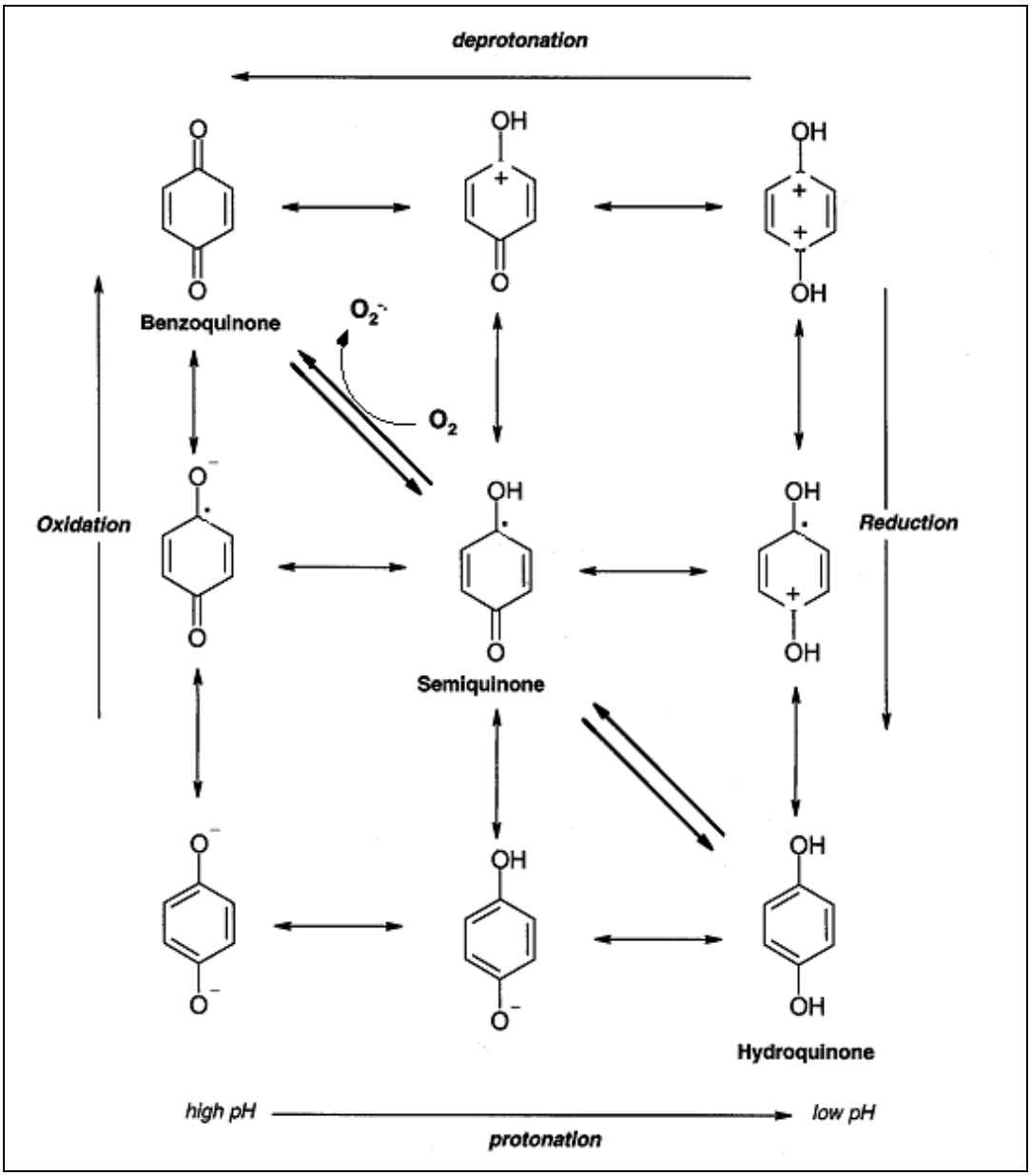


Figure 1 Acid-base and oxidation-reduction scheme for hydroquinone, semiquinone and benzoquinone.[14] Electron and proton transfer reactions are shown on vertical and horizontal axes, respectively. For the biotransformations of hydroquinone and benzoquinone, see Section 5.2.

The rate of hydroquinone autooxidation in an aqueous medium is pH dependent, occurring rapidly under alkaline, but slowly under acidic conditions.[13] At pH above neutrality, hydroquinone can oxidize spontaneously to yield semiquinone and benzoquinone. This may give rise to the formation of superoxide anion ($O_2^{-\bullet}$)[15] and subsequently to H_2O_2.[16] Both H_2O_2 and $O_2^{-\bullet}$ can be converted to $OH^{\bullet}$ which (in contrast to H_2O_2, and $O_2^{-\bullet}$) can directly induce DNA breaks.[17] These ROS are also capable of inducing cytotoxicity and cell damage.

The differences in physical and chemical properties between hydroquinone and benzoquinone clearly have their impact on both exposure conditions and toxicokinetics. But considering the possible interconversion, observations made for one of the compounds may also be relevant for the other. The likelihood that toxicity observed after exposure to one of the compounds will also occur after exposure to the other will depend on both exposure levels and local physiological conditions.

2.3 EU Classification and labelling

Table 3 Classification of hydro- and benzoquinone.

Compound	Classification	Reference
Hydroquinone (CAS no.123-31-9)	Carc. Cat. 3; R40 Muta. Cat. 3; R68 Xn; R22 Xi; R41-43 N; R50	http://www.inchem.org/documents/icsc/icsc/eics0166.htm (December 13, 2011)
Hazard and precaution phrases	H302, H317, H318, H341, H351, H400 P273, P280. P305, P351, P338	EG 1271/2008
Benzoquinone (CAS no.106-51-4)	T; R23/25 Xi; R36/37/38 N; R50	http://www.inchem.org/documents/icsc/icsc/eics0799.htm (December 13, 2011)
Hazard and precaution phrases	H301, H315, H319, H331, H335, H400 P261, P273, P301, P310, P305, P351, P338, P311	EG 1271/2008

2.4 Validated analytical methods

2.4.1 Environmental monitoring

hydroquinone

Hydroquinone in air is present both as vapour and in particulate form. The proportion of total hydroquinone captured by particle filters is strongly

temperature-dependent in the range of 10-30ºC, so measurement methods must take the vapour fraction into account.[18]

NIOSH (1994) developed a fully validated method (method 5004) for air monitoring of hydroquinone with a working range of 0.7 to 8 mg/m^3 for a 90-L air sample or 2 to 25 mg/m^3 for a 30-L air sample. Samples are collected by drawing a known volume of air through a 0.8 µm cellulose ester membrane. Samples are immediately desorbed with 1% acetic acid and analyzed by HPLC-UV (290 nm) using an ID Partisil 10-ODS m-Bondapack C18 column.[19]

OSHA described a partially evaluated method for air monitoring of hydroquinone with a target concentration of 2 mg/m³ (TWA) (method no. PV2094). Samples are collected by drawing a known volume of air through an XAD-7 tube coated with 10% phosphoric acid (maximum air volume: 20 litres and sampling rate: 0.2 L/min). Samples are desorbed with methanol and analyzed by gas chromatography with a flame ionization detector (GC-FID) using capillary column. Liquid chromatography with an ultraviolet detector (LC-UV) can be used for better sensitivity. The status of method is "Stopgap method" and is presented for information and trial use.[20]

The UK Health and Safety Executive (HSE)[21] describes a method that has been validated to demonstrate that it complies with BS EN 482. The method covers the concentration range 0.05 mg/m^3 to 3.0 mg/m^3 for 15 minute or 8 hour sampling. Samples are taken using a glass fibre filter containing in a personal inhalable dust sampler which is backed up with a Tenax sorbent tube. After sampling, the samplers are desorbed into acetonitrile and analysed by HPLC. Separation is achieved using a Zorbax CN column or equivalent and separated species are detected using a photodiode array detector.[21]

In 1999, a method for measuring hydroquinone in air, both in the laboratory and at the workplace, was evaluated. The method involved sampling the inhalable fraction onto a filter contained in a multi-holed sampler with a back-up of Tenax TA, followed by desorption into acetonitrile and analysis by High Performance Liquid Chromatography. It was shown to be effective at measuring hydroquinone over the range 0.1 to 2 times a concentration of 0.5 mg/m^3 for 8 h.[22]

benzoquinone

For the monitoring of benzoquinone, Clayton and Clayton (1981) referred to a method described in 1947. Samples are collected in a midget impinger containing isopropanol. After reaction with phloroglucinol the absorption is measured spectrophotometrically at 520 nm.[23]

OSHA describes a method which is based on a modified method of NIOSH, method S18.1.[24,25] The method is fully validated and has a working range of 0.17-0.75 mg/m³. Samples are collected by drawing a known volume of air through a XAD-2 tube #SKC ST226-30-04 (100/50 mg sections, 20/50 mesh; maximum air volume: 24 litres and sampling rate: 0.2 L/min). Samples are desorbed with isopropanol/hexane (20/80) and analyzed by HPLC-UV (HPLC/UL68).[24,25]

2.4.2 Biological monitoring

hydroquinone in urine

Biological monitoring of workers exposed to hydroquinone is possible by the analysis of hydroquinone in urine. Biological monitoring may be able to detect occupational exposure to hydroquinone at or above an airborne concentration of 2 mg/m^3. However, the background levels of hydroquinone in urine produced from dietary and environmental sources may make detection and interpretation of low levels of exposure difficult.[18] The mean amount of hydroquinone detected in human urine was reported to be 53 ± 37 µg/day (n=7; Niwa et al., 1981). Inoue et al. (1988) found a mean concentration of 4.2 ± 4.7 mg/L (n=131). In a study of Waidyanatha et al. (2004) hydroquinone levels in urine of control workers (i.e. not (occupationally) exposed to benzene or hydroquinone) were between 0.066 and 2.1 mg/L (mean 0.45 mg/L).[26] It has also been determined that urinary hydroquinone excretion is positively correlated with cigarette smoking.[27] There are no data in the literature on which to base a biological monitoring guidance value.[18]

Analytical methods for the determination of hydroquinone in urine are based on the acid-hydrolysis of glucuronide and sulphate conjugates followed by solvent extraction. Chromatography can be via HPLC-UV, HPLC with fluorescence detection, GC of the trimethylsilyl derivates with flame ionization detection, GCMS detection of GC of the heptofluorobutyryl derivatives with electron capture detection. The most sensitive is the HPLC-fluorescence method, with a detection limit of 0.03 mg/L and a coefficient of variation of 3% within day and 6% day to day. There are no international quality assurance schemes for urinary hydroquinone at present.[18]

Nikolic et al. (2004) described a salting out extraction of hydroquinone from aqueous solutions (such as urine). The extraction was performed with diisopropyl ether and NaCl-saturated samples. The extracts were analysed by UV-VIS spectrophotometry.[28]

benzoquinone in urine

No biomonitoring method of benzoquinone in urine exists. Probably benzoquinone is not detectable in urine, as the acidity of urine will result in the conversion of benzoquinone to hydroquinone.

hydroquinone and benzoquinone adducts

Since hydroquinone and benzoquinone are metabolites of benzene, monitoring tools used for benzene might also be suitable for hydroquinone and benzoquinone.

Urinary metabolites of benzene are eliminated rapidly and as a result can only serve as biomarkers for approximately 24 hours following exposure. On the contrary, serum albumin typically has a half life of 21 days in humans. Therefore, measurement of its adducts might reflect exposures over weeks to months prior to collection of a blood sample.[29]

In studies of Rappaport et al. (2002 and 2005), benzene exposure over longer periods of time was monitored by measuring of adducts of benzoquinone with serum albumin. To determine levels of benzoquinone-albumin (BQ-Alb) adducts in blood serum, cysteinyl groups in the protein were selectively cleaved resulting in volatile derivatives, which are suitable for GC-MS analysis.[30,31] The authors reported a half life of 13.5 days for these adducts.[30,31]

Identical to the situation of urinary monitoring, high background levels of benzoquinone adducts to albumin and haemoglobin were determined in unexposed mice, rats and humans[27] making detection and interpretation of low levels of exposure difficult.

In the study of Rappaport, mean BQ-Alb levels in non-smokers and smokers were 2.1 ± 1.1 nmol/g and 3.5 ± 2.0 nmol/g, respectively. The lowest level of BQ-Alb detected in unexposed workers was 0.9 nmol/g.[27]

Chapter 3

Sources

3.1 Natural occurrence

hydroquinone and benzoquinone

There are no natural sources of benzoquinone or hydroquinone at large scale.

No data on benzo- and hydroquinone concentrations in air, soil or water have been found.

Due to its physicochemical properties, hydroquinone will be distributed mainly to the water compartment when released into the environment. It degrades both as a result of photochemical and biological processes; consequently, it does not persist in the environment. No bioaccumulation is observed.[13]

At small scale, benzoquinone occurs in a variety of arthropods. It is excreted and synthesized by many insects.[32] Hydroquinone occurs in various plant products both as free, unbound hydroquinone and as a β-D-glucopyranoside conjugate, called arbutin (also see Section 4.1).

3.2 Man-made sources

3.2.1 Production

hydroquinone

In 1996, the world capacity for the production of hydroquinone was estimated to be 40,000 - 45,000 tonnes per year.[33] More recent information is not available.[34]

There are three current manufacturing processes for hydroquinone: oxidative cleavage of diisopropylbenzene, hydroxylation of phenol, and oxidation of aniline. For oxidative cleavage of diisopropylbenzene, the para-isomer is isolated and oxidized with oxygen to produce the corresponding dihydroperoxide, which is treated with sulphuric acid to produce acetone and hydroquinone. In the phenol hydroxylation process, hydrogen peroxide is used as a hydroxylation agent. Strong mineral acids or ferrous or cobaltous salts are used as catalysts. In the aniline oxidation process, aniline is oxidized with manganese dioxide and sulphuric acid to benzoquinone, which is subsequently reduced to hydroquinone with iron dust and water, or by catalytic hydrogenation.[13,33]

benzoquinone

Benzoquinone was first produced commercially in 1919, and has since been manufactured in several European countries, Japan and the United States.[9] Large scale preparations involve oxidation of aniline or phenol. Benzoquinone is subsequently steam-distilled, chilled and obtained in high purity and yield.[35]

3.2.2 Use

hydroquinone

Virtually all the uses of hydroquinone are industrial. In the USA, approximately 25% of the hydroquinone manufactured is used as an intermediate for chemical conversion to hydroquinone-based rubber antioxidants and antiozonants. Another 25% is used as an intermediate for chemical conversion to inhibitors used to stabilize monomers. An additional 33% is used in the photographic industry including black and white photographic film, lithography, and hospital x-ray film. Other uses (11-12%) include chemical conversion to stabilizers for paints, varnishes, motor oils, and fuels, and for antioxidants in the industrial use

of fats and oils.[33] A small, but not quantified, amount of the hydroquinone is used by the photo-hobbyist, and about 0.05% is used in non-prescription drugs such as skin bleaching creams. Both are considered consumer use.[33] In recent years the demand in photographic processing has stabilized because conversion to digital photography is largely complete and film usage has leveled off.[36]

benzoquinone

The major use of benzoquinone is in hydroquinone production[9], but it is also used as a polymerization inhibitor, photographic chemical, tanning agent, and as an intermediate in the production of a variety of substances, including rubber accelerators and oxidizing agents.[6,9]

Chapter 4

Exposure

4.1 General population

Tobacco smoke

hydroquinone and benzoquinone

Inhalation exposure to benzoquinone and hydroquinone may occur from tobacco smoke.[37] Hydroquinone was measured in mainstream smoke from non-filter cigarettes at amounts ranging from 110 to 300 µg per cigarette.[38,39] Levels of benzoquinone have not been measured, but semiquinone has been found in cigarette smoke.[40]

Diet

hydroquinone

Significant exposure to hydroquinone can occur through dietary sources. Hydroquinone occurs in nature as the β-D-glucopyranoside conjugate (arbutin) and as free, unbound hydroquinone. The concentrations of free and total hydroquinone have been measured in a variety of foods and beverages by Hill et al. (1993)[41], summarized in Table 4. Also in the study of Deisinger et al. (1996), significant amounts of arbutin were detected in wheat products (1-10 mg/kg),

pears (4-15 mg/kg), and coffee and tea (0.1 mg/kg). Free hydroquinone was found in coffee (0.2 mg/kg), red wine (0.5 mg/kg), wheat cereals (0.2-0.4 mg/kg), and broccoli (0.1 mg/kg).[42]

In most of the samples derived from plant sources, the levels of arbutin are considerably higher than those of free hydroquinone. However, arbutin is hydrolysed readily by dilute acids yielding hydroquinone and glucose. Therefore, both free hydroquinone and arbutin may contribute to hydroquinone exposure from natural sources.[13]

Table 4 Concentrations of free and total hydroquinone in various foods and beverages (Hill et al. 1993).[41]

Food sample	Concentrations hydroquinone (mg/kg ± SD)	
	Free (unbound)	Total (bound+unbound)
Wheat germ, toasted	0.59	8.4
Whole wheat bread (100% whole wheat)	0.6 ± 0.2	0.9 ± 0.5
Whole wheat cereal (commercially available)	0.21 ± 0.02	0.99 ± 0.16
Dip-brewed coffee (pre-ground)	0.29 ± 0.003	0.39 ± 0.02
Diet cola	0.036	0.029
Pear skin (D'Anjou, fresh)	-[a]	38
Pear flesh (D'Anjou, fresh)	-[a]	1.3

[a] Background levels comparable to that observed in control blanks

benzoquinone

Exposure to benzoquinone via the diet can occur due to the excretion of benzoquinone by insects. Benzoquinone occurs in a variety of arthropods, and many insects synthesize and excrete a mixture of quinones including benzoquinone.[32]

Deisinger et al. (1996) investigated dietary and other potential sources of hydroquinone and their contribution to hydroquinone concentrations in the plasma and urine of human volunteers. Low concentrations of hydroquinone were detected in the urine and plasma of humans with no occupational or other known exposure to hydroquinone. After consuming a meal including arbutin- and hydroquinone-containing foods, volunteers showed significant increases in plasma and urinary levels of hydroquinone and its conjugated metabolites (total hydroquinone). Mean plasma concentrations of total hydroquinone peaked at 5 times background levels at 2 h after the completion of the meal, and mean urinary excretion rates of total hydroquinone peaked at 12 times background at 2-3 h after the meal. Immediately after smoking four cigarettes in approximately 30 min, mean plasma concentrations of total hydroquinone were maximally 1.5 times background levels; mean urinary excretion rates of total hydroquinone peaked at 2.5 times background at 1-3 h after smoking. These data indicate that

considerable human exposure to hydroquinone can result from plant-derived dietary sources and, to a lesser extent, from cigarette smoke.[42]

With a meal of 200 grams of bread and a pear of 300 grams, a person might consume at least 0.6 mg hydroquinone per meal. A moderate smoker (10 cigarettes per day) may inhale 3 mg hydroquinone/day due to smoking alone.

Other routes of exposure

hydroquinone and benzoquinone

Since benzene and p-phenylenediamine can be metabolized enzymatically and via air oxidation, respectively, to benzoquinone, exposure to these substances may result in exposure to benzoquinone (and hydroquinone).[43]

hydroquinone

Photo-hobbyists can be exposed to hydroquinone dermally or by inhalation. In 1980, the number of photo-hobbyists was estimated to be about 2.2 million in the USA; more recent data, or data on exposure levels are not available.[13,34]

Dermal exposure may also result from the use of cosmetic and medical products containing hydroquinone, such as skin lighteners. In the USA, hydroquinone has been used in cosmetics. Both over-the-counter and prescription drugs are used to lighten areas of hyper-pigmented skin. Over-the-counter skin lighteners may contain up to 2% hydroquinone. Concentrations up to 4% may be found in prescription drugs. In some countries even higher concentrations may be found in skin lighteners.[13]

In the Cosmetic Ingredient Review (CIR), the Environmental Working Group (EWG) determined that hydroquinone should not be used in non-drug cosmetic products that are left on the skin and not immediately rinsed off.[44] The panel drew this conclusion based on studies linking (orally administered) hydroquinone to cancer and immune system damage that targets bone marrow. EWG identified hydroquinone on the ingredients list of 18 products left on the skin, most designed as depigmenters or skin lighteners.

No data on hydroquinone concentrations in air, soil or water have been found (see Section 3.1).[13]

Based on various calculations, in 1995 the US Environmental Protection Agency (EPA) considered hydroquinone exposure through drinking water and fish consumption negligible for processor/user sites.

benzoquinone

No specific data available.

4.2 Working population

hydroquinone

Hydroquinone can be encountered in a solid form or in solution during its production and use.[10] It has a very low vapour pressure, but can be oxidized in the presence of moisture to form benzoquinone, which is more volatile. The saturated concentration in air for hydroquinone vapour under standard conditions is estimated to be 0.108 mg/m^3 (approximately 0.024 ppm at 25°C).[10]

Occupations in which hydroquinone exposure may occur are: hydroquinone manufacturing workers, antioxidant makers, drug makers, hair dressers and cosmetologists, paint makers, photographic developer makers, photo processors, organic chemical synthesizers, plastic stabilizer workers, and rubber coating workers.[10,13]

Hydroquinone manufacture is a heavily automated procedure, consequently only 81 employees in the USA are potentially exposed during manufacture, materials handling, maintenance, quality control sampling and analysis. Employee protective clothing and engineering controls, such as local exhaust ventilation systems, are used to further prevent exposure at points in the process where exposure can occur.[33] No information on the number of employees exposed during manufacturing in Europe was found.

The average airborne hydroquinone concentration (~8,000 breathing zone and area samples) in one hydroquinone manufacturing plant in the USA has decreased from 6.0 mg/m^3 in the 1950s to 0.1 mg/m^3 in 1990.[45]

Inhalation exposure levels in two US manufacturing plants were estimated and calculated dose rates are shown in Table 5. These estimated exposure levels assume that no personal protective devices were used. However, producers in the USA have reported the use of engineering controls (local exhaust and dust collectors) and personal protective devices (e.g. gloves, uniforms, goggles, respirators, boots) to control exposure. Therefore, the calculated dose rates may well exceed actual expected dose rates.[33]

Very rough worst-case estimates of total dermal contact with hydroquinone (650-18,200 mg/day) were calculated using models which assume that employees regularly immerse their hands in hydroquinone for the entire work day without protective equipment. All US manufacturers of hydroquinone report that

engineering controls and personal protective equipment are used to minimize exposure, and thus the bounding estimates are likely to overestimate the potential contact that employees can have with hydroquinone dust.[33]

The number of employees working in US industries which use hydroquinone has been estimated at 350,000 to 560,000 at 16,000 to 66,000 facilities. The amount of exposure to hydroquinone at these work sites was not available and it was assumed that the exposures were equivalent to that of hydroquinone manufacturing employees (Table 5).[33] There are, however, several reasons to believe that this assumption will overestimate occupational exposures at processor/user sites. The first is that many operations which use or process hydroquinone are batch operations in which hydroquinone is added to hoppers, reactors, or mixing vessels which are then closed. In these types of operations, bags or drums are opened and their contents emptied out during periods of 10-60 minutes. Automation also reduces exposure to hydroquinone. For example, commercial photo processors are heavily automated and emissions from machines are typically below detectable levels.[33]

A study of the US National Institute of Occupational Safety and Health (NIOSH) did not detect hydroquinone in the atmosphere of rooms were films were being developed. Monitoring data were also available from the US Occupational and Safety Administration (OSHA) Compliance Information System on airborne concentrations of hydroquinone in processor/user facilities. The data base included 78 air samples from facilities in 16 different standard industrial code categories. All samples, except of one, were less than the analytical limit of detection or less than 1 mg/m^3. The majority of the samples were below detectable levels.[33]

Table 5 Estimated occupational exposure associated with the manufacture of hydroquinone in the USA[33]

Type of worker	No. of workers; h/day; days/year	Airborne concentrations (n=29) (mg/m^3)		Potential inhalation dose rate[a] (mg/kg bw/day)	
		Average	Maximum	Average	Maximum
Operator/sampler	34; 1-8; 100-250	0.116	0.300	0.0166	0.0429
Loader/packager	34; 1-8; 100-250	0.254	1.870	0.0363	0.2670
Maintenance personnel/ housekeeper	13; 1-8; 1-250	0.135	0.295	0.0193	0.0421

[a] Dose rates assume: the medium work inhalation rate of 1.25 m^3 air/h; the maximum number of hours in a range; no use of personal protective equipment; the chemical is 100% concentrated, and 70 kg bw.

In the manufacturing of photographic developers, monitoring data are commonly collected as total dust in the breathing zone. Exposure levels recorded by manufacturers indicated the 8-hr TWA values were below 1 mg/m^3 with average exposures at ~0.15 mg/m^3.[33] Measurements for hydroquinone among users of photographic developers indicate hydroquinone levels in air below the analytical limit of detection (0.01 mg/m^3).[33] However, spills of photo developers in the workplace could result in exposure to hydroquinone.[33]

It has been estimated by the UK Health and Safety Executive (HSE) that in the UK 3,000-5,000 people are exposed to hydroquinone at work. The main route for work-related potential exposure is by inhalation (UK-HSE 1993, cited in OECD 1996).[33] Estimated occupational exposure associated with the processing of hydroquinone in the UK in different industries is presented in Table 6.

The potential dose rates shown in Table 6 assume that no personal protective equipment is worn. Available information indicated that hydroquinone users in the UK rely on personal protective equipment for control of exposure. Current control measures in the UK maintain exposure levels below the TLV of 2 mg/m^3 and in many cases below 1 mg/m^3. A combination of local ventilation and appropriate handling procedures should be adequate to control exposure below 0.5 mg/m^3 8-hr TWA.[33]

Biological monitoring of darkroom workers did not indicate an increase in work-related hydroquinone exposure as urinary levels were less than control worker values.[33] However, as is already mentioned in Section 2.4.2, the background levels of hydroquinone in urine produced form dietary and environmental sources may make detection and interpretation of low levels of exposure difficult.[18]

Table 6 Estimated occupational exposure associated with the processing of hydroquinone in the UK.[33]

Industry category	Average airborne concentration[a] (mg/m^3)	Potential inhalation dose rate for typical exposure periods (mg/kg)[b]	
	average (maximum)	10 min average (maximum)	60 min average (maximum)
Food	0.42	1.2×10^{-3}	7.5×10^{-3}
Rubber	4.3 (10)	1.2×10^{-2} (2.9×10^{-2})	7.7×10^{-2} (1.8×10^{-1})
Agrochemical	0.03 (0.1)	8.6×10^{-5} (2.9×10^{-4})	5.4×10^{-4} (1.8×10^{-3})

[a] Sampling periods were 5-120 min.

[b] Assumes 1.25 m^3 of air inhaled in 1 h, 0.2 m^3 of air inhaled in 10 min, 70 kg bw, and that chemical hoppers or reaction vessels are filled once per 8-h work shift.

benzoquinone

Occupational exposure to benzoquinone may occur in the dye -, textile -, chemical -, tanning -, and cosmetic industries.[6] No detailed information on occupational exposure to benzoquinone was available.

Chapter 5

Kinetics

5.1 Absorption and distribution

hydroquinone

For an overview of studies performed on absorption see Annex F-1.

In the study of Barber et al. (1995)[46], the rate of percutaneous absorption of a 5% aqueous hydroquinone solution through human stratum corneum and whole rat skin was measured in vitro. The absorption rate through human skin was determined to be 0.522 µg/cm^2/h; the absorption rate through rat skin was 1.09 µg/cm^2/h. These absorption rates were considered to be "slow" based on published definitions of absorption (OECD 1996).[33]

In the study of Stenius (1989)[47], a solution of 4% hydroquinone in oil was applied to human and rat skin in vitro (40 mg/cm^2). After 24 h 1.68% of the dose had been absorbed by the rat skin and 0.28% by the human skin.

The actual amount of hydroquinone absorbed following dermal exposure depends on the exposure concentration, the time of exposure, and the vehicle, as well as other factors. Bucks et al. (1988) applied a solution of ^{14}C-hydroquinone to human foreskin at a concentration of 125 µg/cm^2. Percutaneous absorption was estimated by measurement of radioactivity in the urine. Peak elimination was observed within the first 12 h, and elimination was completed within 5 days. Average absorption per hour was 1.6, 2.3 and 2.5% of the dose in the first,

second and third 4 h period after application, respectively (Bucks et al. in WHO 1994).[13]

In the study of DiVincenzo et al. (1984), groups of male Sprague-Dawley rats were orally administered ^{14}C-labelled hydroquinone at single doses of 3, 30, or 200 mg/kg bw by gavage. In addition, one group of rats was given 4 daily doses of 200 mg/kg bw unlabelled hydroquinone followed by a single dose of 200 mg/kg bw ^{14}C-labelled hydroquinone by gavage. Radioactivity was widely distributed throughout the tissues with higher concentrations in the liver and kidneys. Less than 2% remained in the carcass after 96 h. The distribution did not change with repeated dosing.[48]

Fox et al. (1986, cited in OECD 1996[33]) administered ^{14}C-labelled hydroquinone to groups of F344 rats (males) at a single dose of 50 mg/kg bw by oral gavage, or by intravenous injection, or by intratracheal instillation. Of some groups, blood was collected for up to 8 h and other groups were euthanized at 10, 20, 40, 60, 120, and 480 h. Absorption was rapid ($t_{1/2}$ after gavage administration was approximately 1 min). Distribution was similar for the three routes. Initially, radioactivity in the blood was associated with the plasma, but these levels declined rapidly in the first hour. After 4 h, 64% of the radioactivity was associated with the erythrocytes.[31]

English et al. (1988, cited in OECD 1996[33]; English and Deisinger 2005[49]), administered ^{14}C-labelled hydroquinone to groups of F344 rats (males and females) at single oral doses of 25 or 350 mg/kg bw by gavage, or after 14 repeated oral doses of unlabelled hydroquinone. In addition, some groups of animals were dermally treated (occlusive) with ^{14}C-labelled hydroquinone at levels of 25 or 150 mg/kg bw for 24 h. After oral administration, hydroquinone was rapidly absorbed. Less than 1% remained in the tissues. The liver and kidneys contained the highest concentrations, with females generally having higher concentrations than males. After dermal application 61-71% of the dose was recovered from the application site immediately after dosing. Blood concentrations after topical application were generally below the limit of detection. After 168 h, the skin at the application site contained 0.1-2% of the total radioactivity. Approximately 2-13% of the total radioactivity was associated with the tissues and carcass.[33]

benzoquinone

Few data on absorption and distribution of benzoquinone are available. Benzoquinone is reported to be readily absorbed from the gastrointestinal tract and

after subcutaneous injection (species not specified).[9,50] However, no quantitative information was available.

5.2 Biotransformation

Since hydroquinone and benzoquinone can be converted into each other, the description of biotransformation of these compounds is combined.

hydroquinone and benzoquinone

In Figure 2, a comprehensive metabolic scheme for hydroquinone and benzoquinone is given. The Figure shows the enzyme-mediated conversions between hydroquinone and benzoquinone and the intermediate semiquinone. Furthermore, the conjugation reactions, the process of redox cycling resulting in formation of ROS, and the covalent binding to macromolecules such as proteins (haemoglobin) and DNA are depicted.

Oxidation and reduction reactions

The oxidation of hydroquinone into semiquinone and subsequently into benzoquinone can be exerted by cytochrome P450 (CYP) and various peroxidases (e.g., prostaglandin H synthase and myeloperoxidase).[51,52]

In turn, benzoquinone can be reduced to hydroquinone via a one-electron reduction by enzymes such as CYP, cytochrome P450 reductase, ubiquinone oxidoreductase, xanthine oxidoreductase, and cytochrome b_5 reductase. Additionally, reduction of benzoquinone to hydroquinone via a two-electron reduction can be catalysed by the flavoproteins NAD(P)H-quinone oxidoreductases (NQO1 and NQO2). NQO1 and NQO2 are cytosolic proteins that catalyze metabolic reduction of quinones and their derivatives to protect cells against redox-cycling and oxidative stress[53], as demonstrated by the observation that over-expression of NQO1 in CHO cells protects cells from the toxicity of benzoquinone.[54]

Conjugation reactions

Hydroquinone can be conjugated with sulphate or glucuronic acid. Quantitatively these conjugations are the most important reactions: after single oral administration of hydroquinone to rats, approximately 65-85% was excreted in the urine as glucuronide- and sulfate conjugates within 8 h (see Section 5.3).

Semiquinone and benzoquinone can be conjugated with glutathione (GSH), both spontaneously as well as mediated by glutathione S-transferases (GSTs) to form mono-, di-, or tri-glutathione-conjugates (depicted as $Q\text{-}SG_{1,2,3}$ in Figure 2, in which Q is the quinone moiety, SG is the conjugated glutathione, and 1,2,3 indicates the number of conjugated glutathione moieties). The glutathione conjugates can be further metabolized by γ-glutamyl transpeptidase (γ-GT) and subsequently by dipeptidase to cysteine (cys-) conjugates; finally, N-acetylation yields the corresponding mercapturic acids.

5.3 Elimination

hydroquinone

For an overview of studies performed on elimination see Annex F-1.

In the study of Fox et al. (1986, cited in OECD 1996[33]) with F344 rats, elimination of hydroquinone was rapid. Analysis of plasma indicated rapid metabolism of hydroquinone; only 1% of the total radioactivity in plasma ultrafiltrate was unaltered hydroquinone. Glucuronide and glutathione conjugates of hydroquinone were detected 40 min after oral gavage administration.[33]

In the study of DiVincenzo et al. (1984; see Section 5.1) with male rats administered ^{14}C-labelled hydroquinone, the primary route of elimination was the urine (92-99%) with 87% of the total radioactivity recovered excreted in the first 24 h. The faeces contained 2-4% of the total radioactivity recovered. Less than 1% of the dose was expired as CO_2. Only 1% of the urinary metabolites was unchanged hydroquinone; the majority was identified as glucuronide conjugates with a smaller portion as sulphate conjugates. After repeated oral dosing 72% was glucuronide and 23% sulphate conjugate, while after a single oral dose this was 56% and 43%, respectively.[48]

In the study of English et al. (1988, cited in OECD 1996[33]; English and Deisinger 2005[49]; see Section 5.1), elimination followed a biphasic character and occurred within the first 8 hrs after oral administration to F344 rats. Dose-related differences were observed, suggesting that elimination processes are saturated at high-dose levels. Hydroquinone was excreted mainly (> 86%) in the form of water soluble metabolites via the urine; 1-2% was excreted in the faeces. Approximately 45-53% of the urinary metabolites consisted of glucuronide conjugates and 19-33% of sulphate conjugates. A mercapturic acid conjugate was also identified at levels of 4.7% and 2.3% of the dose in females and in males, respectively. Less than 3% of the dose was excreted as the parent

compound. After dermal application only 15-18% of the total radioactivity was recovered in the urine with 2-4% in the feces, 168 h after dosing.[33]

In the study of Hill et al. (1993) hydroquinone (1.8 mmol/kg, ip) was administered to acivicin*-pretreated male Sprague-Dawley rats. Five S-conjugates of hydroquinone were identified in bile, and one S-conjugate was identified in urine. The major biliary S-conjugate identified was 2-(GS-S-yl)HQ** (18 µmol/4 h). Additional biliary metabolites were, 2,5-bis(GS-S-yl)HQ (2 µmol/4 h), 2,6-bis(GS-S-yl)HQ (0.7 µmol/4 h), and 2,3,5-tris(GS-S-yl)HQ (1.2 µmol/4 h). 2-(N-acetylcystein-S-yl)HQ was the only urinary thioether metabolite identified (11.4 µmol/4 h). The quantity of S-conjugates excreted in urine and bile within 4 h of hydroquinone administration was 34.3 ± 4.5 µmol/4 hr (4.3 ± 1.1% of dose).[41]

In the study of Lau et al. (1996), male F344 rats were administered ^{14}C-labelled hydroquinone either 1.8 mmol/kg bw by gavage alone or after 14 daily doses of unlabelled hydroquinone. The metabolites detected in urine were hydroquinone-glucuronide, hydroquinone-sulphate, hydroquinone-mercapturate, 2-(GS-S-yl)HQ, 2,5-bis(GS-S-yl)HQ, 2,6-bis(GS-S-yl)HQ, and 2,3,5-tris(GS-S-yl)HQ. Subchronic administration of hydroquinone appeared to increase the rate and extent by which hydroquinone was metabolized to its thioethers.[55]

benzoquinone

Few data on elimination of benzoquinone are available. Benzoquinone is reported to be excreted partly unchanged and partly as hydroquinone, the major proportion of which is eliminated as conjugates.[9] However, no quantitative information was available.

5.4 Physiologically based pharmacokinetic models

hydroquinone

A physiologically based pharmacokinetic (PBPK) model for hydroquinone was described by Corley et al. (2000)[56] and refined by Poet et al. (2010)[57] to include a description of dermal exposure.

* Acivicin is an irreverible inhibitor of γ-glutamyltranspeptidase.
** GS: conjugated glutathione; HQ: hydroquinone.

benzoquinone

PBPK models for benzoquinone are not available.

5.5 Summary

hydroquinone

Various absorption, distribution, metabolism and excretion studies on hydroquinone were performed in rats. No information is available on kinetics of hydroquinone after inhalation. Hydroquinone absorption via the skin was determined and classified as “slow” (0.5-1 µg/cm^2/h) but may be more rapid with vehicles such as alcohols. After oral administration or intratracheal instillation, hydroquinone was rapidly and extensively absorbed. Distribution was similar for administration via gavage, intravenous injection and intratracheal instillation.

Hydroquinone can be oxidized via semiquinone to benzoquinone by various enzymes. The reverse reaction can also occur both spontaneously and enzymatically mediated.

Hydroquinone can be conjugated with sulphate and glucuronic acid resulting in the respective conjugates, which are excreted via the urine. Benzoquinone and semiquinone can also be conjugated with glutathione, resulting in mono-, di-, and tri-glutathione conjugates which are detectable in the bile. The glutathione conjugates can be further metabolised to cysteine conjugates and mercapturic acids.

The primary route of elimination is via the urine (> 85%) in the form of water soluble metabolites. The major urinary metabolites are glucuronide conjugates (45-56%) and sulphate conjugates (19-43%). Mercapturic acids are present at lower levels (< 5%). Only a small fraction (about 1-3%) of the urinary metabolites consists of the parent compound.

benzoquinone

No information is available on kinetics of benzoquinone after inhalation. Benzoquinone is reported to be readily absorbed from the gastrointestinal tract and subcutaneous tissue. It is excreted partly unchanged and partly as hydroquinone, the major proportion of which is eliminated as conjugates. However, no quantitative information was available.

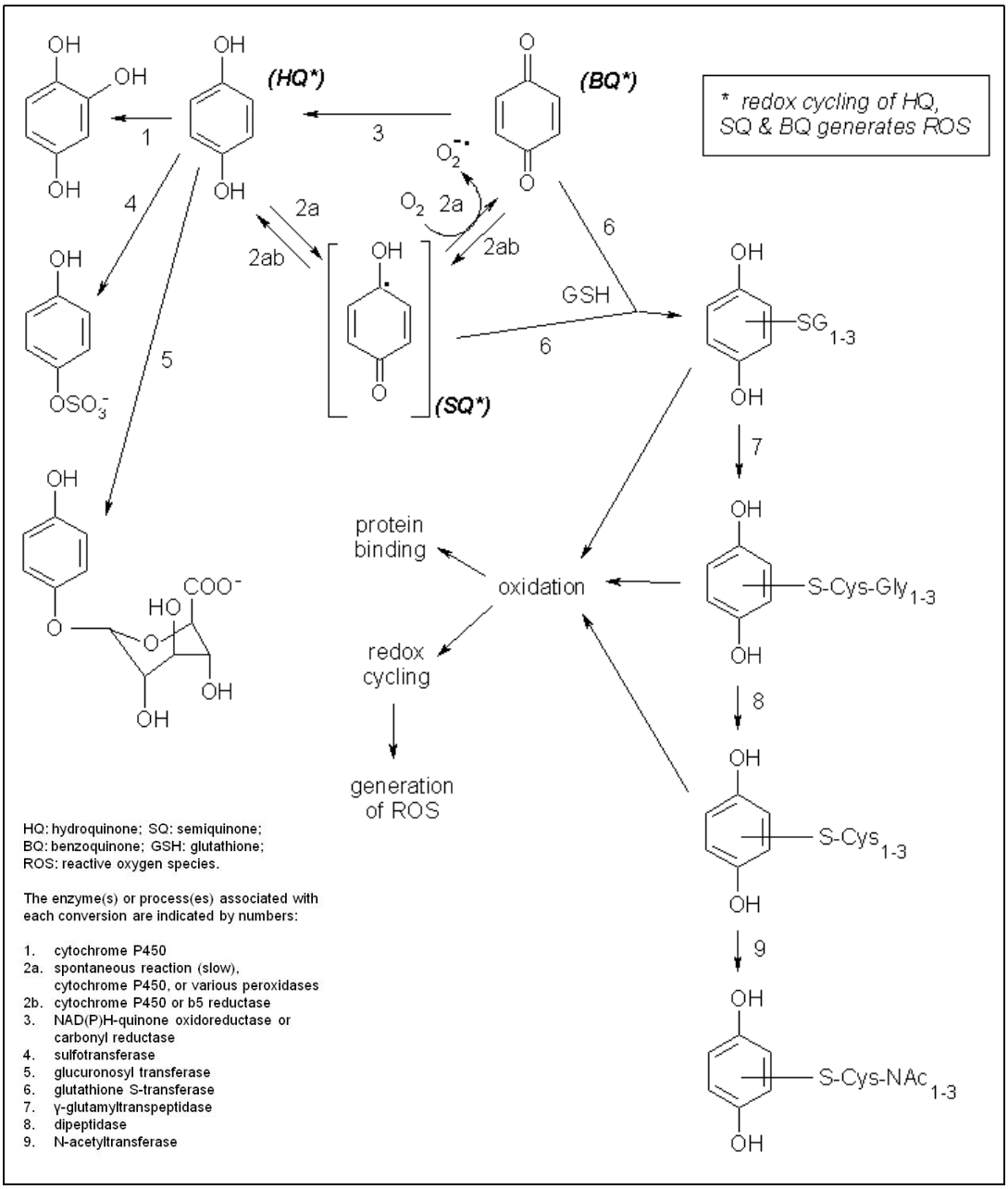


Figure 2 Biotransformation of hydroquinone and benzoquinone (modified from DeCaprio 1999[14] and English et al. 1994b[58]).

Chapter 6

Mechanisms of action

The molecular mechanisms of quinone toxicity are well described by O'Brien (1991).[59] Specific mechanistic studies are summarized in Annex F-8.

6.1 Bioactivation

Potentially several pathways may be involved in toxicity induced by occupational exposure to hydroquinone and benzoquinone:

- Production of reactive oxygen species (ROS)
- Inhibition of topoisomerase II
- Macromolecular binding of benzoquinone and semiquinone.

ROS production

As described in Section 5.2, the conversions between benzoquinone, hydroquinone and the semiquinone (SQ) can occur both spontaneously and enzymatically (see Figure 2). Once the semiquinone is formed, redox cycling can occur in the presence of molecular oxygen, resulting in ROS, including superoxide anion ($O_2^{-\bullet}$) and H_2O_2.[60]

ROS potentially may cause lipid peroxidation and membrane damage, cytotoxicity, DNA damage, mutagenicity, and carcinogenicity.[60] Benzoquinone and hydroquinone have been demonstrated to induce double strand breaks when incubated with naked DNA in a cell free system. The absence or presence of

various protective compounds (glutathione, trolox*, etc.) and enzymes (catalase, superoxide dismutase) in these studies resulted in clear differences in effects which indicated that free radicals may play a role.[17,61]

Also the glutathione conjugates of benzoquinone, particularly 2,6-bis (GS-S-yl)HQ, and 2,3,5-tris(GS-S-yl)HQ, are capable of redox cycling and have been demonstrated to be efficient generators of ROS.[62] The latter has been shown to induce a mutation spectrum in isolated DNA that was consistent with a $OH^{\cdot}$-induced mutation spectrum.[14,60]

Inhibition of topoisomerase II

The enzyme topoisomerasse II is essential for the maintenance of proper chromosome structure and segregation. Several studies have shown that hydroquinone and benzoquinone inhibit in vitro the funtionality of topoisomerase II and enhance DNA cleavage (Lindsey et al. 2005[63]; Smith 2010[64]). In vitro, the presence of glutathione protected topoisomerase II from inhibition (Chen and Eastmond 1995[65]). Bioactivation of hydroquinone by peroxidase to benzoquinone enhanced topoisomerase II inhibion (Eastmond et al. 2005).[66] In a cell-free system benzoquinone is a more potent inhibitor of topoisomerase II than hydroquinone is (Hutt and Kalf 1996[67]; Baker et al. 2001[68]).

Macromolecular binding

Unlike hydroquinone, the reactive benzoquinone and the unstable semiquinone are capable of direct covalent binding to macromolecules to form DNA- and protein adducts in vitro.[16,60] It was observed that hydroquinone in the presence of prostaglandin H synthase was converted to reactive metabolite(s) that irreversibly bind to DNA and sulphydryl groups. Additionally, benzoquinone appeared to have been formed[61], implying that it might have been one of the reactive metabolites.

Benzoquinone has been demonstrated to alkylate isolated, naked thymus DNA to form exocyclic DNA adducts.[69] The adducts formed have been identified as (3'-hydroxy)-3,N4-benzetheno-2'-deoxycytidine-3'-phosphate, (3'-hydroxy)-1,N6-benzetheno-2'-deoxyadenosine-3'-phosphate, and (3'-hydroxy)-

* 'Trolox' is the trade name for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble derivative of vitamin E. Like vitamin E it is an antioxidant, and is used in biological or biochemical applications to reduce oxidative stress or damage.

1,N2-benzetheno-2'-deoxyguanosine-3'-phosphate.[70] This type of adduct can be excised by DNA repair mechanisms, as was proven in in vitro studies by using HeLa cell extracts.[69] However, the DNA adduct formed in HL-60 cells and human bone marrow in vitro did not correspond to any of the principal adducts formed in naked DNA reacted with benzoquinone. This suggests that cellular environment modifies the DNA adduct formation by benzoquinone. The same DNA adducts were detected if HL-60 cells and human bone marrow in vitro were exposed to hydroquinone.[70,71]

After oxidation to the corresponding quinones the glutathione conjugates are also capable of direct covalent binding to macromolecules.[16,60]

Potentially, macromolecular binding may also cause membrane damage, lipid peroxidation, cytotoxicity, DNA damage, mutagenicity, and carcinogenicity.[60,72] Benzoquinone can bind to critical thiol groups of tubulin resulting in inhibition of microtubule formation. As a consequence, the formation of a functional spindle apparatus in the mitotic cell may be disturbed, leading to abnormal chromosome segregation and aneuploidy.[73]

DNA alkylation by benzoquinone (and hydroquinone upon its oxidation to benzoquinone) has been demonstrated in vitro only. The existence of such DNA-adducts in vivo has not yet been proven[74,75], although several attempts have been undertaken to demonstrate these.

Mechanistic studies on hydroquinone-induced kidney toxicity

Prostaglandin H synthase is selectively located in the kidney medulla, and γ-GT activity is found in the brush border membrane of proximal tubular cells.[76] In Section 5.2 (and also above) it has already been mentioned that prostaglandin H synthase is capable of activating hydroquinone to form reactive metabolites that might be benzoquinone and/or semiquinone.

γ-GT is involved in the further metabolism of the glutathione conjugates of hydro- and benzoquinone.

The role of γ-GT in the hydroquinone-induced kidney toxicity was investiged in the study of Peters et al. (1997) (see Annex F-8). The study showed that hydroquinone was selectively toxic to the proximal tubular cells of the male rat kidney, and causes injury at the junction of the medullary rays and outer strips of the outer medulla.[77] In these regions, prostaglandin H synthase and γ-GT activities are high. They also showed that inhibition of γ-GT resulted in a significant reduction of hydroquinone-induced nephrotoxicity[77], indicating that γ-GT plays a role in the hydroquinone-induced nephrotoxicity.

Administration of 2,3,5-tris(GS-S-yl)HQ to rats caused hyperplasia in the necrotic proximal tubular cells in kidneys of F344 rats (Peters et al. 1997)[77] and of Eker rats (Lau et al. 2001)[78], corresponding to the γ-GT region specificity in the kidney. The conjugate was about 600 times more potent in inducing nephrotoxicity than hydroquinone[77], indicating that this might be a major part of the mechanism of the hydroquinone-mediated nephrotoxicity.

In the study of Hill et al. (1994) the potency of inducing nephrotoxicity of 2,3,5-tris(GS-S-yl)HQ and 2-(GS-S-yl)HQ were compared in the in situ perfused rat kidney. The compound 2,3,5-tris(GS-S-yl)HQ was considerably more potent than 2-(GS-S-yl)HQ, probably due to the fact that the metabolites derived from the former are more reactive.[79] Apparently the damage to the kidneys is selectively induced in the areas where enzymes are located involved in the activation of hydroquinone. Key enzymes in the hydroquinone-induced nephrotoxicity are likely to be γ-GT and prostaglandin H synthase.

In order to study the mechanism of induction of kidney neoplasms by hydroquinone, English et al. (1994a,b) studied both hydroquinone-induced covalent DNA binding and cell proliferation in kidneys of F344 rats administered hydroquinone by gavage at levels of 2.5, 25, 50 mg/kg bw, 5 days/week, for 6 weeks. No DNA-adducts were observed[80], but a dose level of 50 mg/kg bw hydroquinone resulted in cell proliferation in the kidney, and excretion of urinary enzymes indicative of proximal tubule damage, though only in males.[58] They also observed that this latter effect did not occur in F344 females and in Sprague-Dawley rats. Therefore, a F344 rat-specific nongenotoxic etiology of tumours in the kidney was suggested, with hydroquinone-induced cell proliferation secondary to cytotoxicity.

In the study of Peters et al. (1997)[77], hydroquinone was found to induce hyperplasia in the necrotic proximal tubular cells in kidneys of F344 rat (see above). The hyperplasic regions were at the same location at which tumours ultimately developed (Jeong et al. 1999).[60] Thus the hydroquinone-induced nephrocarcinogenesis appears to be linked to the increased cell proliferation as a response to the hydroquinone-induced nephrotoxicity and cell necrosis.

6.2 Detoxification

Detoxicification of ROS formed due to redox cycling between hydro- and benzoquinone and their glutathione conjugates can be accomplished by the cell's cytoprotective mechanisms consisting of antioxidants, scavenging enzymes and repair processes.

Reduction of the reactive benzoquinone to the less reactive hydroquinone via two-electron reduction is catalysed by NQO1 and NQO2 (see Section 5.2).[54] Further detoxification of hydroquinone may proceed via conjugation with sulphate or glucuronide (see Figure 2).

6.3 Summary

Hydroquinone can be detoxified by sulphation and glucuronidation. Benzoquinone can be converted to hydroquinone via two-electron reduction by NQO1 and NQO2.

Redox cycling between hydro- and benzoquinone as well as of their glutathione conjugates can lead to the generation of ROS, which potentially causes lipid peroxidation, membrane damage, cytotoxicity, DNA damage, mutagenicity, and carcinogenicity. The cytoprotective mechanisms of the cell (antioxidants, scavenging enzymes, repair processes) counteract the effects of ROS production. The net effect of free radicals on cellular function thus depends on the balance between radical production and the capacity of these cytoprotective systems.

Benzoquinone, and to a lesser extent hydroquinone, are in vitro inhibitors of topoisomerase II, an enzyme responsible for proper chromosome structure and segregation.

The reactive benzoquinone, the unstable semiquinone and the quinones of their glutathione conjugates are capable of direct covalent binding to macromolecules such as proteins and DNA. This results in protein- and DNA-adducts, and may consequently result in cytotoxicity and/or genotoxicity. The binding of benzoquinone to tubulin may result in the inhibition of microtubule formation.

DNA-alkylation by benzoquinone (and hydroquinone upon its oxidation to benzoquinone) has been demonstrated in vitro only; despite several attempts, the existence of such DNA-adducts in vivo has not yet been proven, probably because of extensive biotransformation.

Mechanistic studies regarding the hydroquinone-induced kidney toxicity showed that the induction of kidney adenomas by hydroquinone is not accompanied by any covalent binding to DNA, but is apparently associated with local cytotoxicity and necrosis in proximal tubular cells, indicating that hydroquinone induced nephrocarcinogenesis is likely linked to increased cell proliferation.

Overall, the balance of the various metabolic pathways involved, as well as other local physiological conditions (e.g., pH) determine the toxicological outcome of exposure to either hydroquinone or benzoquinone.

Chapter 7

Effects

The mechanism of action of hydroquinone and benzoquinone, with the semiquinone as intermediate, is rather complex (see Chapter 6), resulting in a number of target sites/organs. Various enzymes are influencing the ultimate toxicity of hydro- and benzoquinone, both by increasing and decreasing the reactivity/toxic potential. Consequently, the vulnerability of a particular tissue for hydro- and benzoquinone depends on the levels of these enzymes.

In this Chapter, hydroquinone and benzoquinone are described separately. However, due to the possible interconversion between them, observations made for one compound might also be relevant for the other. The toxicological properties of both compounds may be similar in a qualitative sense (i.e. inducing the same toxicological endpoint via the same mechanism of action). Whether toxicity induced by one of both will also be induced by the other, will be dependent on aspects as exposure and local physiological conditions, such as pH and enzyme levels.

7.1 Observations in humans

The studies on effects of hydro- and benzoquinone in humans are summarized in Annex E.

7.1.1 *Irritation and sensitization*

hydroquinone

Sterner et al. (1947; cited in ACGIH 2008)[1] reported that occupational exposure to hydroquinone dust and benzoquinone vapour caused eye irritation, photophobia, lacrimation, and corneal ulceration, with no serious cases appearing from exposures of duration shorter than five years. Benzoquinone was believed to be the chief causative agent, although hydroquinone dust was suspected as a contributory cause. Hydroquinone dust concentrations and benzoquinone vapour concentrations were reported to vary between 1 and 55 mg/m^3, and 0.04 and 14 mg/m^3, respectively.

Anderson and Oglesby (1958, cited in ACGIH 2008)[1] found corneal changes consisting of changes in the curvature of the lens, long after (occupational) exposure had ceased and after the staining and pigmentation of the cornea had disappeared. They concluded that that the corneal changes observed were caused by benzoquinone vapour or hydroquinone dust. However, exposure levels were not reported.

Hydroquinone can be considered as a common contact allergen, and sources of non-occupational exposure are for example rubber products in which hydroquinone is present as preservative.[81] Allergic contact dermatitis represents a delayed (type IV) hypersensitivity reaction (which is distinguished from irritant contact dermatitis).

Cross-reactions between chemicals may occur if they share similar functional groups critical to the formation of complete allergens (hapten + carrier protein). A cross-reactor of hydroquinone is resorcinol and hydroquinone is a cross-reactor of methyl hydroxybenzoate and phenol.[81]

benzoquinone

Local cutaneous effects of exposure to benzoquinone include discolouration, severe irritation, erythema, swelling, and the formation of papules and vesicles.[6] Prolonged contact can lead to necrosis. Vapour condensing on the eyes can produce serious disturbance in vision (Anderson and Oglesby 1958, Fasset 1960, cited in ACGIH 2001a).[2] Sterner et al. (1947) reported that the vapour of benzoquinone and the dust of hydroquinone, arising in the manufacture of hydroquinone, produced characteristic ocular injuries in workers. The injuries developed gradually over a period of years with no serious cases appearing from

exposures of durations shorter than 5 years. Benzoquinone was believed to be the chief causative agent, although hydroquinone dust was suspected as a contributory cause. Hydroquinone dust concentrations and benzoquinone vapour concentrations were reported to vary between 1 and 55 mg/m^3, and 0.04 and 14 mg/m^3, respectively (Sterner et al. 1947, cited in ACGIH 2001a, 2008).[1,2]

7.1.2 *Acute and short-term toxicity*

hydroquinone

No information on effects on humans after acute or short-term exposure to hydroquinone was found, other than the effects mentioned in Section 7.1.1.

benzoquinone

No information on effects on humans after acute or short-term exposure to benzoquinone was found, other than the effects mentioned in Section 7.1.1.

7.1.3 *Long-term toxicity*

hydroquinone

Friedlander et al. (1982)[82] reported a cohort mortality and cancer incidence study of 478 workers (7162 person-years of follow-up) engaged in colour printing and processing at nine laboratories in the continental USA during the period 1964-1976. Six job activities were combined within the processing definition: chemical mix, analytical laboratory, film processing, film take-off, print processing and print take-off, but in only one of these (film processing) was hydroquinone identified as an occupational exposure. A single industrial hygiene measurement of hydroquinone indicated a concentration range and annual mean time-weighed average of < 0.01 mg/m^3 air. The control populations were (1) two separate groups of employees with the same company not defined as processors for mortality, and (2) the up-state New York population for cancer incidence. There were 36 deaths (12 from malignancies) observed, giving standardized mortality ratios (SMRs) well below 1.0 for all mortalities and about 1.0 for most malignancies. Overall, there were 7 cases of cancer and usually the standardized incidence ratios (SIRs) were either below or about 1.0. The only exception was central nervous system tumours, but this was based on just two cases. Given the mixed population observed, only some of whom appear to have been exposed to

hydroquinone, and the level of characterisation of exposure to this chemical, it is considered that this study is not informative with regard to the carcinogenicity of hydroquinone.[67]

Pifer et al. (1995)[45] reported a cohort mortality study of 879 workers (22,895 person-years of follow-up) at a Tennessee (USA) plant in which hydroquinone was manufactured and used over several decades. Job history records were linked to extensive industrial hygiene data and expertise to estimate cumulative exposure to hydroquinone. Average hydroquinone dust levels ranged from 0.1 to 6.0 mg/m^3, with levels over 2 mg/m^3 for most of the period of operation of the plant. Mean employment duration was 13.7 years and mean follow-up from first exposure was 26.8 years. Relative risk estimates (SMRs) for this cohort were derived by comparison with the general population of Tennessee as well as with an occupational cohort not exposed to hydroquinone (a plant of the same company, located in New York State). The SMR for all causes of death combined (n=168) was significantly below 1.0, as was the SMR for all cancers combined (n=33). Only two sites, colon (n=5) and lung (n=14) had more than three observed cases. Most site-specific SMRs were well below 1.0. The results were similar for both comparison populations. The dose-response analyses of selected cancer sites did not reveal any meaningful trend of heterogeneity.[45] The International Agency for Research on Cancer (IARC) noted in 1999 that the numbers of individual cancer sites were small and the power to detect effects was weak, and that this cohort had systematically lower SMRs than the comparison industrial cohort (IARC 1999a).[8]

Nielsen et al. (1996)[83] carried out a cohort incidence study among 837 Danish lithographers born between 1933 and 1942 and registered with the Danish Union of Lithographers in 1947 or later. Questionnaires were sent to cohort members in 1989 to obtain information on job exposures; usable responses were received from 620 workers. About one-quarter of the cohort members reported working regularly with hydroquinone for photographic development. The entire cohort was traced in the Danish Cancer Registry from 1947 to 1989. Relative risk estimates (SIRs) for this cohort were derived by comparison with the general population of Denmark. There were a total of 24 cancers registered, giving an SIR of 0.9. For no site except skin were there more than three cases. Five cases of malignant melanoma occurred, with 1.5 expected (SIR 3.4, 95% confidence interval (CI) 1.2-7.5). Among these five, two had reportedly been exposed to photochemicals (such as hydroquinone). In the study it was not possible to distinguish between the carcinogenic effects of the exposure to pigments, dyes, and organic solvents.[83] It must be noted that the power to detect effects was weak considering the co-exposure with other

photochemicals and the unestablished exposure to hydroquinone of lithographers suffering from malignant melanomas.

Fryzek et al. (2005)[84] reported a retrospective cohort mortality study of 2,624 workers engaged in motion picture film processing at a California (USA) laboratory that is the oldest continuously operating laboratory of this type in the world. All workers were employed for at least 3 months between January 1960 and December 2000. There were 54,462 person-years of follow up with 666 observed deaths (hourly workers, 44,019 person-years with 561 deaths; administrative workers, 10,444 person-years with 105 deaths. Thirty three percent of hourly workers and 19% of administrative workers had worked 10 years or more. The SMR ± 95% CI for all causes of death combined among hourly and administrative workers, respectively, were 1.1 (1.0-1.2) and 1.1 (0.9-1.3), and for all malignancies combined, 1.0 (0.9-1.2) based on 135 deaths, and 1.2 (0.8-1.7) based on 30 deaths. In most instances the SMRs for individual malignancies were < 1.0. Borderline significant excesses of non-Hodgkin lymphoma were observed among hourly workers, SMR = 2.2 (1.0-4.0) based on 10 cases and borderline significant excesses of malignancies of the respiratory system were observed among all workers combined, SMR = 1.3 (1.0-1.6) based on 62 cases. However, there was no exposure-relationship for these malignancies if duration of employment was used as an indicator of exposure. Also no information on cigarette smoking habits of personal was available, so no adjustment for this potential confounder was possible. In the case of non-Hodgkin lymphoma, there was no simple relationship with either duration of employment or year of first employment for hourly workers as a group. As a minimum, 75 chemicals were identified as being used on film production since 1960, including hydroquinone which was used in film development. Only three air sampling data were collected for hydroquinone. One sample was taken before 1981 and was below the detection limit. Two samples taken after 1981 contained 0.014 and 0.052 mg/m^3, respectively.[84]

benzoquinone

Chronic (long-term) inhalation exposure to benzoquinone (exposure levels not reported) of humans resulted in visual disturbances; chronic dermal contact caused skin ulceration.[6]

No systemic effects were observed in the study of Sterner et al. (1947, cited in ACGIH 2001a, 2008).[1,2] Since this publication, a large number of clinical and environmental studies were performed on workers in plants where benzoquinone (and hydroquinone) were produced. These studies confirmed the findings of

Sterner et al. that no systemic effects arose at a level of 0.44 mg/m^3 (0.1 ppm) benzoquinone vapour during a period of 5 years (Fasset 1960, cited in ACGIH 2001a).[2]

No information is available on the reproductive, developmental, or carcinogenic effects of benzoquinone in humans. No epidemiological data of benzoquinone were available.

7.2 Animal experiments

7.2.1 Irritation and sensitization

The studies on irritation and sensitization of hydroquinone and benzoquinone in animals are summarized in Annex F-2. Relevant studies are described in more detail below.

hydroquinone

Eye irritation

There are several data on eye irritation of hydroquinone. Hydroquinone in aqueous solution, e.g., in tears, is oxidized by air, forming a brown coloured substance partly due to conversion to benzoquinone.[13]

In a study performed by the Eastman Kodak Company (1971) several crystals of hydroquinone powder were placed into the tight eye of two rabbits. The treated eye of one animal was washed, while the treated eye of the other animal was unwashed. Irritation was scored at 1, 24, 48 h and 14 days after treatment. Slight erythrema of the palpebra developed after 1 h in both washed and unwashed eyes. Eythrema of the nictitating membrane was also evident in the unwashed eye at 1 h. By 24 h, the washed eye appeared normal, but the unwashed eye continued to demonstrate slight erythrema of the palpebra, orbital, and nictitating membranes. Erythrema of the nictitating membranes persisted to 48 h after instillation but was not observed 14 days after treatment (Eastman Kodak Company 1971, cited in OECD 1996).[33]

In the study of Ferraris de Gaspare (1949), the effect of light on hydroquinone induced eye irritation was studied in rabbits. Hydroquinone (pure substance) was applied daily, for 2-4 months, to the eyes of rabbits which were, respectively, kept in the dark, in sunlight, in normal light, irradiated with UV light, or pre-sensitized with haematoporphyrin and then kept under either reduced light or sunlight. Most rabbits developed pigmentation, first in the

conjunctiva and then in the cornea. Degenerative alterations of the corneal parenchyma were also observed. Pigment formation appeared earlier in animals exposed to light. Older animals seemed more prone to develop pigment than younger ones. Pigment was deposited in albino rabbit eyes as well as in those of rabbits with normal pigmentation (Ferraris de Gaspare 1949, cited in WHO 1994).[13]

Following an injection of 0.1 ml of a solution of hydroquinone (0.012-0.05 mol/L) into the cornea of rabbits, the resultant reaction was graded 5 out of the possible maximum of 100 (Hughes 1948, cited in WHO 1994).[13]

In guinea pigs, hydroquinone (1-3 mg pure substance instilled into the eyes twice daily for 9 weeks) caused immediate but transient irritation. During the second day of application a slight corneal opacity was observed in some animals, and on the third day opacity to varying degrees occurred in most of the animals. Ulcers appeared in two animals. The eyes had fully recovered 3 days after cessation of treatment.[13]

In dogs, hydroquinone (2-5 mg pure substance) instilled twice daily (5 days per week for 9 weeks) into the eyes caused immediate but transient irritation and lacrimation. Opacity of the cornea, lacrimation and redness of the conjunctiva were produced within 4 days, but ulcers were not observed. The eyes returned to normal within two days after cessation of treatment (Dreyer 1940, cited in WHO 1994).[13]

Skin irritation

Hydroquinone in concentrations up to 0.1% in water was not found to be irritant when administered intracutaneously to female guinea pigs.[85]

Bleehen et al. (1968)[86] observed skin irritation in black guinea pigs after non-occlusive, topical applications of creams containing 5% or 10% hydroquinone, 5 days/week for one month (surface treated not indicated). No irritation was seen at levels of 3% hydroquinone.[86]

In the study of Maibach and Patrick (1989)[13] male and female black guinea pigs were administered hydroquinone in a hydrophilic ointment at concentrations of 0.1, 1.0, and 5.0% via non-occlusive, topical application (surface treated not indicated) for 5 days/week for 13 weeks. The lowest concentration caused marginal irritation, and the medium concentration resulted in a slight to marginal irritation in 30% of the animals (mainly females). Moderate to severe irritation and severe ulcerated inflammatory responses occurred at the highest concentration (Maibach and Patrick 1989, cited in WHO 1994).[13]

Depigmentation

In consistence with its application in skin bleaching creams (see Section 3.2.2) and its mechanism of action of hypo-pigmentation (see Section 6.1), depigmentation of the skin was observed in different skin irritation studies. Bleehen et al. (1968)[86] reported weak to moderate depigmentation of the skin of black guinea pigs after topical, dermal application of creams containing 1-10% hydroquinone, once daily, five times per week for one month.[86] In the study of Jimbow et al. (1974)[87], depigmentation in the epilated skin of 24 black guinea pigs (males and females) after topical applications of hydroquinone was observed. Creams containing 2 or 5% hydroquinone in an oil-water emulsion were applied daily, 6 days per week, for 3 weeks. The depigmentation was first seen within 8-10 days and was greatest between 14 and 20 days. It was more marked at the higher concentration. Inflammatory changes and thickening of the epidermis were also reported. When hydroquinone was applied topically for three weeks, biopsy specimens showed that it had caused a marked reduction both in the numbers of melanised melanosomes in the cells and the number of actively functioning melanocytes.[73]

Depigmentation of the skin was also observed in the study of Maibach and Patrick (1989[13]; study details are outlined in the Section on skin irritation above). At the highest concentration, approximately 40% of the animals dosed showed moderate depigmentation of the skin (females only). At the medium concentration, weak depigmentation was observed in the females (not in males), and at the lowest concentration no depigmentation effects were seen. At the highest concentration, hypopigmentation was noticed in 80-100% of the animals in all dose groups (Maibach and Patrick 1989, cited in WHO 1994).[13]

Sensitization

Several sensitization studies on guinea pigs with hydroquinone have been reported. The methods and results are summarized in Annex F-2.

In the sensitization study of Rajka and Blohm (1970)[85], the induction and challenge were performed by injecting 0.1 ml 0.001% hydroquinone solution. At challenge 4/18 animals had a positive sensitization reaction, which was considered a “weak” sensitivity reaction. A challenge with 0.001% p-phenylenediamine or benzoquinone after a hydroquinone induction resulted in a positive reaction in 6/18 and 9/18 animals, respectively. After an induction with 0.001% p-phenylenediamine and a challenge with hydroquinone, 4/20 animals were sensitized.[85]

Goodwin et al. (1981)[88], compared three guinea pig sensitization procedures. Sensitization induced by hydroquinone was “strong” when assayed by the Magnusson and Kligman maximization test, “moderate” by the single injection adjuvant test and “weak” by the modified Draize procedure.[88]

Hydroquinone was found to be a “moderate” sensitizer in female guinea pigs in both the guinea pig maximization test and Freund's complete adjuvant test as performed by Van der Walle et al. (1982a,b).[89,90] Hydroquinone produced identical sensitization potentials in the Freund's complete adjuvant test using induction concentrations of 0.5 mol/litre and 0.45 µmol/litre.

In 1988, Basketter and Goodwin[91] used three sensitization test methods representing both topical and intradermal routes of application. Groups of 10 guinea pigs were sensitized by using the guinea pig maximization test, a modified single injection adjuvant test, and a cumulative contact enhancement test. The sensitization potential of hydroquinone was assessed as “strong”, “weak”, and “moderate”, respectively, in these three tests. Subsequent cross-challenges with *p*-phenylenediamine, sulfanilic acid, and benzoquinone gave only “restricted evidence” of cross-reactions.[90]

In the maximization test of Basketter and Scholes (1992)[92], the induction injections were performed with 2.0% hydroquinone by injection and 1.0% by patch, followed by a challenge with 0.5% by patch. All guinea pigs (number not indicated) were sensitized and therefore the compound was classified as “extreme” sensitizer. In the local lymph node assay (LLNA) hydroquinone was positive for sensitisation.[92] Roberts et al. (2007)[93] reported a murine LLNA threshold (EC3*) of 0.11% for hydroquinone, classifying it as a strong sensitizer.

The data in the above cited studies clearly indicate that hydroquinone is a strong dermal sensitizer.

benzoquinone

Eye irritation

No data on eye irritation were available.

* EC3 value: the effective concentration for stimulation of a three-fold increase in lymph node cell proliferation.

Skin irritation

In the study of Rajka and Blohm (1970)[85], intracutaneous injections of 0.1% benzoquinone solutions in female guinea pigs gave necrotic reactions. At a concentration of 0.01%, redness and slight infiltration was observed. Injecting a 0.001% solution did not result in effects of irritancy.[85]

Sensitization

In the study of Rajka and Blohm (1970)[85], the induction and challenge were performed by injecting 0.1 ml 0.001% benzoquinone solution. At challenge, 19/20 animals had a positive sensitization reaction. A challenge with 0.001% p-phenylenediamine or hydroquinone after a benzoquinone induction resulted in a positive reaction in 5/20 and 1/20 animals, respectively. After an induction with 0.001% p-phenylenediamine and a challenge with benzoquinone, 16/20 animals were sensitised.[85]

In a guinea pig maximization test performed by Möllgaard et al. (1990)[94], the cross-reactivity between p-phenylenediamine and benzoquinone has also been established. However, no data are available on concentrations used and the time schedule for challenge and induction.[94]

In the study of Basketter and Scholes (1992)[92], the local lymph node assay was compared with the guinea pig maximization test using various compounds among which benzoquinone and hydroquinone. In the maximization test the induction injections were performed with benzoquinone dissolved in 0.09% NaCl aided by acetone if required. For the induction and challenge patch benzoquinone was dissolved in acetone with 30% polyethyleneglycol. The concentrations were 0.005 (injection induction), 10 (induction patch) and 2.5% (challenge patch). All guinea pigs (number not indicated) were sensitized and therefore benzoquinone was classified as “extreme” sensitizer.[92] In the local lymph node assay, groups of 4 mice were treated with benzoquinone by a daily topical application of 25 µL of a series of concentrations, from 0.5 to 2.5% dissolved in acetone with 20% olive oil on the dorsal surface of each ear for 3 consecutive days. Four to five days after the first topical application, all mice were injected intraveneously with phosphate buffered saline containing ^{3}H-methylthymidine. After 5 h the mice were killed and the amount of ^{3}H-methyl-thymidine incorporation in the auricular lymph nodes was assayed. Benzo-quinone was positive in this assay.[92] Roberts et al. (2007)[93] reported a murine LLNA threshold (EC3) of 0.01% for benzoquinone, classifying it as an extreme dermal sensitizer.

The data in the above cited studies clearly indicate that benzoquinone is an extreme dermal sensitizer.

7.2.2 *Acute toxicity*

The acute toxicity studies on hydroquinone and benzoquinone are summarized in Annex F-3.

hydroquinone

Inhalation LC_{50} values are not available.

The dermal LD_{50} value was determined to exceed 1,000 mg/kg bw in guinea pigs.[33] The HSG (Health and Safety Guide; IPCS INCHEM) estimated that the dermal LD_{50} value for hydroquinone may exceed 3,800 mg/kg bw in rodents.[95]

Oral LD_{50} values for several animal species ranged between 300 and 1,300 mg/kg bw[33,94] (see also Annex F-3). Acute high-level exposure to hydroquinone caused severe effects on the central nervous system (CNS) including hyper-excitability, tremor, convulsions, coma, and death. At sublethal doses, these effects were reversible. According to the OECD hydroquinone is moderately acute toxic via the oral route.[33]

benzoquinone

Inhalation LC_{50} and dermal LD_{50} values were not available.

In the study of Omaye et al. (1980)[32] in rats, the oral toxicity of benzoquinone was primarily caused by respiratory impairment. The secondary symptoms appeared within 6 hours after dosing. These include ataxia, some loss of righting reflex, loss of corneal reflex, depressed respiration and hypothermia. Mild to extreme skin blanching was frequently observed and cyanosis was seen occasionally. At autopsy, petechial haemorrhages on the lungs, reddened lungs, dark livers, darkened spleens and green to black coloured intestines were observed.[32] In the experiment of Serif and Seymour (1963)[97] with mice intraperitoneally administered hydroquinone, the animals experienced writhing, paralysis of the hind limbs and cyanosis due to benzoquinone administration.

In conclusion, oral LD_{50} values for benzoquinone for rats ranged between 130 and 165 mg/kg bw (see also Annex F-3). Acute high-level exposure to benzoquinone caused severe effects on the central nervous system (CNS) including loss of reflexes, depressed respiration and paralysis. Intraperitoneal LD_{50}s were 8.5 mg/kg bw for mice[97], and 25 mg/kg bw for rats.[2]

7.2.3 *Sub-acute/sub-chronic toxicity*

The short-term toxicity studies on hydroquinone and benzoquinone are summarized in Annex F-4.

hydroquinone

No inhalation repeated dose toxicity studies are available. Values of other repeated dose studies are presented in Table 7.

Table 7 Subacute/subchronic toxicity of hydroquinone.

Species	Exposure duration	NOAEL	LOAEL Critical effects	Reference
Dermal				
Rat (F344)	14 days	1,920 mg/kg bw/day	3,840 mg/kg bw/day Reduced body weight (m).	NTP 1989[98]
Rat (F344)	13 weeks	~74[a] mg/kg bw/day	> 74 mg/kg bw/day No (overt) toxicity.	David et al. 1998[99]
Mouse (B6C3F1)	14 days	4,800 mg/kg bw/day	> 4,800 mg/kg bw/day	NTP 1989[98]
Oral (all gavage)				
Rat (Sprague-Dawley)	13 weeks	20 mg/kg bw/day	64 mg/kg bw/day Mild, transient tremors and reduced home-cage activity.	Bernard 1988, cited in OECD 1996[33]
Rat (F344)	14 days	250 mg/kg bw/day	500 mg/kg bw/day Mortality, tremors, reduced final mean body weight.	NTP 1989[98]; Kari et al. 1992[100]
Rat (F344)	13 weeks	< 25 mg/kg bw/day	25 mg/kg bw/day Decreased absolute and relative liver weights (m). Increased absolute and relative liver weights (f) at doses ≥ 100 mg/kg bw/day.	NTP 1989[98]; Kari et al. 1992[100]
Mouse (B6C3F1)	14 days	125 mg/kg bw/day	250 mg/kg bw/day Mortality, tremors, decreased final mean body weight.	NTP 1989[98]; Kari et al. 1992[100]
Mouse (B6C3F1)	13 weeks	< 25 mg/kg bw/day	25 mg/kg bw/day Increased absolute and relative liver weights (m).	NTP 1989[98]; Kari et al. 1992[100]

[a] Actual dosing was 5.0% oil-in-water emulsion (equivalent to ~74 mg/kg bw/day).

Dermal exposure

A fourteen-day dermal study in F344 rats and B6C3F1 mice was conducted in the National Toxicology Program (NTP 1989)[98]. During 14 days, 12 doses were dermally administered, ranging between 240 and 3,840 mg/kg bw/day to rat and between 300 and 4,800 mg/kg bw/day to mice. The final mean body weight of male rats that received 3,840 mg/kg bw/day was 6% lower than that of the vehicle controls. Body weights of female rats at this dose level and of mice at 4,800 mg/kg bw/day were comparable to controls. The final mean body weights of mice at all dose groups did not deviate from the vehicle controls. There were no compound-related clinical signs of toxicity in either species.[98]

In the study of David (1998)[99], prolonged dermal dosing of F344 rats for 5 days/week during 13 weeks with 2.0, 3.5 or 5.0% hydroquinone in an oil-in-water emulsion cream resulted in minimal to minor dermal irritation, but no overt toxicity. No adverse effects or compound related effects occurred in organ weight, clinical pathology, or histopathology.[99]

Oral exposure

In the programme of NTP (1989)[98], 14-days gavage studies were conducted by administering hydroquinone in corn oil to groups of 5 F344/N rats of each sexe in doses ranging from 63 to 1,000 mg/kg bw/day, and to groups of 5 B6C3F1 mice of each sexe in doses ranging from 31 to 500 mg/kg bw/day. All rats receiving 1,000 mg/kg bw/day and 1/5 male and 4/5 female rats receiving 500 mg/kg bw/day died before the end of the 14 days. The final mean body weight of rats receiving 500 mg/kg bw/day were 9% lower than that of the vehicle controls for male and 18% lower for females. Compound-related clinical signs in rats included tremors lasting up to 30 minutes after each dosing at 500 and 1,000 mg/kg bw/day. In the 14-day study with mice, 4/5 male mice and 5/5 female mice receiving 500 mg/kg bw/day and 3/5 males receiving 250 mg/kg bw/day died before the end of the study. The final mean body weights of male mice that received 250 or 125 mg/kg bw/day were 8% or 4% lower than those of the vehicle controls. Tremors followed by convulsions were seen at 250 and 500 mg/kg bw/day.[98]

In 13-weeks gavage studies conducted by NTP (1989)[98], doses for F344 rats and B6C3F1 mice (groups of 10 males and 10 females each) ranged from 25 to 400 mg/kg bw/daty. All rats receiving 400 mg/kg bw and 3/10 female rats receiving 200 mg/kg bw/day died before the end of the study. The mean body

weight at necropsy of male rats administered 100 or 200 mg/kg bw was about 8-9% lower than that of vehicle controls. Mean body weights of vehicle control and dosed female rats at necropsy were similar. The liver weight to body weight ratios for the dosed male rats were lower than those for vehicle controls. These ratios for the three highest dose groups of female rats were significantly greater than those for the vehicle controls. Tremors and convulsions were observed after dosing in most rats receiving 400 mg/kg bw/day and in several female rats receiving 200 mg/kg bw/day. Inflammation and/or epithelial hyperplasia (acanthosis) of the forestomach were seen in 4/10 male rats and 1/10 female rats receiving 200 mg/kg bw/day. Toxic nephropathy, characterized by tubular cell degeneration in the renal cortex, was seen in 7/10 male and 6/10 female rats receiving 200 mg/kg bw/day and in 1/10 females receiving 100 mg/kg bw/day.[98] In the studies with mice, 8/10 males and 8/10 females receiving 400 mg/kg bw/day and 2/10 male mice receiving 200 mg/kg bw/day died early. Mean body weights of dosed and vehicle control mice at necropsy were similar. Liver weight to body weight ratios for dosed male mice were significantly greater than those for vehicle controls. Ulceration and inflammation, or epithelial hyperplasia of the forestomach was found in 3/10 male and 2/10 female mice receiving 400 mg/kg bw/day and 1/10 females receiving 200 mg/kg bw/day.[98]

No adverse effects on the kidney were reported in Sprague-Dawley rats treated by gavage with 0, 20, 64 or 200 mg/kg bw/day hydroquinone in distilled water, 5 days per week for 13 weeks (Bernard 1988, cited in OECD 1996; Topping et al. 2007).[33,101] Neurohistopathology was conducted on various brain areas. The kidneys of the high-dose and control male rats were also processed for histopathology. No mortality occurred. Mild, transient tremors and reduced home-cage activity were observed in the mid- and high-dose groups immediately after dosing, with the incidence increased in a dose-dependent manner (unfortunately no more detailed quantitative data were reported). No differences in body weight, feed consumption, and brain or kidney weight were noted. Morphologic lesions associated with the treatment were not observed.

benzoquinone

No oral and dermal repeated dose toxicity studies are available. Other repeated dose studies are presented in Table 8.

The studies performed before 1970 were evaluated by the German MAK Committee in 1970. Two evaluated studies were summarized in the MAK document of 2000.[102] No references to the original studies or publications were given.

Table 8 Subacute/subchronic toxicity of benzoquinone.

Species	Exposure duration	NOAEL	LOAEL Critical effects	Reference
Inhalation				
Rat	4 months, 4 h/day	< 0.27-0.36 mg/m^3	0.27-0.36 mg/m^3 Thrombopenia	Anonymous 2, no date (cited in MAK 2000)[100]
Subcutaneous injection				
Rat	2.5-5 months, 2 times/week	< 7 [a] mg/kg bw/day	7 mg/kg bw/day Anaemia, methaemoglobinemia, decreases in serum albumin and serum cholinesterase activity	Anonymous 1, no date (cited in MAK 2000)[100]
Intraperitoneal injection				
Mice (Swiss)	6 weeks, 6 days/week	< 2 mg/kg bw/day	2 mg/kg bw/day Decreases in red blood cells and bone marrow cell counts, and haemoglobin content	Rao et al. 1988[101]

[a] Actual dosing scheme was 25 mg/kg bw, twice weekly (equivalent to 7 mg/kg bw/day).

In the first study, rats were exposed to benzoquinone at levels of 0.27-0.36 or 2.7-3.6 mg/m^3 for 4 h per day, during 4 months by inhalation. All animals survived. However, in the high concentration group, weight loss, easy tiredness, transient anaemia and thrombopenia was observed. In the low concentration group, 2 out of 8 rats suffered from trombopenia.[81] Since the original document can not be retrieved, the relevance and accuracy of these data can not be assessed.

In the second study described, 25 mg/kg bw benzoquinone was administered twice weekly to rats by subcutaneous injection during 2.5-5 months. This resulted in anaemia, methaemoglobinemia, decrease in serum albumin and serum cholinesterase activity. Furthermore, changes in heart and liver were observed.[102]

The most recent study on short-term toxicity of benzoquinone was performed in 1988 by Rao et al.[103] In this study benzoquinone (2 mg/kg bw/day) was administered intraperitoneally to Swiss mice 6 days per week for 6 weeks. The animals were killed after the last exposure. Benzoquinone produced significant decreases in red blood cells and bone marrow cell counts, and haemoglobin content, together with relative changes in organ weights. In addition, benzoquinone elicited histopathological injuries in liver, thymus, spleen, kidneys and peripheral lymph nodes.[103]

7.2.4 *Chronic toxicity and carcinogenicity*

The long-term toxicity and carcinogenicity studies on benzoquinone and hydroquinone are summarized in Annex F-5.

hydroquinone

Dermal and inhalation carcinogenicity studies are not available. The neoplastic and non-neoplastic effects observed in the oral carcinogenicity studies are presented in Table 9. In the following, firstly the rat studies, and secondly the mouse studies are described.

Table 9 Long-term toxicity of hydroquinone.

Species	Exposure duration	NOAEL	LOAEL Critical effects	Reference
Rat (F344) oral (with diet)	104 weeks	< 350 [a] mg/kg bw/day	350 mg/kg bw/day Reduced body weight gain (m, f). Higher absolute and relative liver and kidney weights (m). Higher relative kidney weight (f). Chronic nephropathy (m, f). Renal tubular adenomas (m)	Shibata et al. 1991[104]
Rat (F344) oral (gavage)	104 weeks, 5 days/week	25 mg/kg bw/day	50 mg/kg bw/day Lower mean body weight (m). Renal tubular adenomas (m).	NTP 1989[98]; Kari et al. 1992[100]
Mouse (B6C3F1) oral (with diet)	96 weeks	< 1,050 [a] mg/kg bw/day	1,050 mg/kg bw/day Reduced body weight gain (m). Higher relative liver and kidney weight (f). Hepatocellular adenomas (m).	Shibata et al. 1991[104]
Mouse (B6C3F1) oral (gavage)	104 weeks, 5 days/week	< 50 mg/kg bw/day	50 mg/kg bw/day Increased relative liver weights (m). Hepatocellular adenomas (f).	NTP 1989[98]; Kari et al. 1992[100]

[a] rounded figures from 0.8% w/w in diet.

Rat studies

In the study of Shibata et al. (1991)[104], groups of 28-30 male and 28-30 female Fischer 344/N rats were given hydroquinone in the diet at concentrations of 0 or 0.8% for 104 weeks (equivalent to 351 and 368 mg/kg bw/day for males and females, respectively). Body weight gain was decreased in both exposed males and females. Absolute and relative liver and kidney weight were increased in exposed males. Relative kidney weights were increased in females. The neoplastic lesions observed are presented in Table 10. Chronic nephropathy was more severe in males than in female. In the kidneys of exposed males, the incidence of tubule hyperplasia was 30/30 and that of adenomas was 14/30 (p = 0.01), compared with 1/30 and 0/30, respectively, in unexposed controls. No other tumour type was increased by exposure.[8,104]

Table 10 Histopathological lesions in the 2-year rat diet study with hydroquinone by Shibata et al. (1991).[104]

Rat (F344)	No. of animals with lesions			
	males		females	
Dose (mg/kg bw/day)	Control (30)[a]	350 [b] (30)	Control (30)	370 [b] (30)
Kidneys				
Chronic nephropathy+ [c]	17	10	1	8
++	0	9 **	0	0 **
+++	0	5	0	0
Hyperplasia, papilla	2	11*	0	0
Hyperplasia, tubular	1	30**	0	2
Adenoma	0	14**	0	0

[a] in parentheses no. of animals studied.
[b] rounded figures from 0.8% w/w in diet.
[c] +, ++ and +++ denote severity of effect.
* and ** denote significant differences from control values (*p<0.05, **p<0.01); for severity for chronic nephropathy by the two-sided Fischer's exact test, for severity of non-neoplastic lesions by the Mann-Whitney test.

In the study conducted by NTP[98], groups of 65 F344 rats of each sex were administered 0, 25, or 50 mg/kg bw hydroquinone in deionised water by gavage, 5 days per week for 104 weeks. Ten rats from each group were killed after 15 months for an interim evaluation.[84] In the rats killed at 15 months, the relative kidney weight of high dose male rats was greater than that for vehicle controls. The haematocrit value, haemoglobin concentration, and erythrocyte count for high dose female rats were decreased. Compound-related increased severity of nephropathy was observed in male rats.[98]

In the 2-year study, mean body weight of high dose males was 5-13% lower than that of vehicle controls after week 73, and that of low dose males was 5-9%

lower than that of vehicle controls after week 89. Mean body weight of dosed females was similar to that of vehicle controls throughout the study. The relative kidney and liver weights for high dose males were higher than those for vehicle controls. No significant differences in survival were observed between any groups of rats of either sex after 2 years (male rats: vehicle control, 27/55; low dose, 18/55; high dose, 18/55; female rats: 40/55; 27/55; 32/55, respect-ively).[98,103] The neoplastic lesions observed are presented in Table 11. Renal tubular cell adenomas developed in 4/55 low-dose (p=0.069) and 8/55 high-dose (p=0.003) males, compared with 0/55 controls.[83] In exposed females, mono-nuclear cell leukaemia developed in 15/55 of the low-dose group (p=0.048) and 22/55 of the high-dose group (p=0.003), compared with 9/55 controls. The historical incidence of leukaemia for water/vehicle control females was 25±15%.[98]

Table 11 Histopathological lesions in the 2-year rat gavage study with hydroquinone by NTP (1989).[98]

Rat (F344)		No. of animals with lesions					
		males (55)[a]			females (55)		
Dose (mg/kg bw/day)		Control	25	50	Control	25	50
Kidneys							
• Chronic nephropathy	none	2	3	0			
	minimaal	3	1	3	No treatment related		
	mild	12	12	5 *	effects		
	noderate	26	31	15			
	marked	12	8	32			
• Hyperplasia		0	0	2			
• Adenoma		0	4	8*			
Lymphoid system							
• Mononuclear cell leukaemia		No treatment related effects			9	15	22*

[a] in parentheses no. of animals studied.

* denotes significant differences (p<0.01); for severity of chronic nephropathy by the two-sided Fischer's exact test, for severity of non-neoplastic lesions by the Mann-Whitney test.

Hard et al. (1997)[105] re-evaluated the renal histopathology of the NTP study in rats. They reviewed the grade of chronic progressive nephropathy (CPN) and presence of atypical tubular hyperplasia and adenomas; see Table 12. Hydro-quinone exposure in males at 50 mg/kg bw/day produced a statistically significant increase in the grade of CPN. There was no statistically significant difference between low-dose males and controls regarding the CPN grade (p= 0.63). At 0, 25 and 50 mg/kg bw/day, 0/44, 4/49 and 15/51 male rats had either atypical tubular hyperplasias or adenomas. All were within areas of severe or end-stage CPN and were statistically significantly associated with CPN grade. Additionally, there was a dose-related increase in profiles believed to represent new tubule proliferation within areas of advanced CPN, as well as an apparent

expansion of these into unusual complex tubule profiles in end-stage kidneys of the high-dose male group. Based on their re-evaluation the authors suggested a mechanism for hydroquinone-related adenoma formation that includes enhancement of the severity of CPN coupled with stimulation of tubular proliferation.

Hard and Khan reviewed CPN in 2004.[106] They argued that CPN is a spontaneous age-related disease that occurs in high incidence in F344 and Sprague-Dawley rats, exhibiting a male predisposition. The disease generally starts at about 2 months of age, when some rats develop basophilic renal tubules with a thickened basement membrane. Progression involves an increase in number of tubules affected, tubular degeneration and atrophy, and an ongoing tubule-cell proliferation. By the time that end-stage is reached, there are virtually no normal tubules remaining and death from renal failure is highly probable. The authors stated that this degenerative and regenerative disease is not the result of any chemical treatment, and it is necessary to distinguish its regenerative aspects from preneoplasia (atypical hyperplasia) from which adenomas develop.

Furthermore they expressed the opinion that, although the precise etiology of CPN and the mechanisms underlying its pathogenesis remain unknown, evidence is emerging that advanced CPN is a risk factor for a marginal increase in the background incidence of renal tubular tumours. Reviewing the pathological entities associated with chronic renal failure in man, the authors finally concluded that this rodent CPN has no strict human counterpart.[106]

McGregor (2007)[107] reviewed the human risks of hydroquinone from its carcinogenic and mutagenic properties including the pathology re-evaluation by Hard et al (1997).[105] His evaluation showed that all renal tubular adenomas and all cases of renal tubular atypical hyperplasia occurred in areas of severe or end-stage CPN and that the neoplasms were not otherwise confined to any particular part of the kidney. The author proposed and evaluated a non-genotoxic mode of action involving exacerbation of CPN, considering CPN to be a spontaneously occurring rodent renal disease process.

Mouse studies

In the study of Shibata et al. (1991)[104], groups of 28-30 male and 28-30 female B6C3F1 mice were given hydroquinone in the diet at concentrations of 0 or 0.8% for 96 weeks (equivalent to 1,046 and 1,486 mg/kg bw/day for males and females, respectively). Statistically significant reduction in body weight gain was noted in males. The neoplastic lesions observed are presented in Table 13. No increase in tumour incidence was found in females. In males the combined incidence of hepatocellular adenomas and carcinomas was increased to 20/30 in

exposed animals ($p<0.05$) compared with 13/28 in controls. This increase was solely due to an increased number of adenomas: from 6/28 in controls to 14/30 in exposed animals.

Table 12 Re-evaluation (Hard et al. 1997)[105] of the microscopic slides for chronic progressive nephropathy of the 2-year rat gavage study (NTP 1989).[98]

Rat (F344)	No. of animals with lesions					
	males			females		
Dose (mg/kg bw/day)	Control (44)[a]	25 (49)	50 (51)	Control (53)	25 -	50 (46)
Kidneys						
Chronic progressive nephropathy						
minimal	0	2	0	4	-	2
mild	0	2	2	6	-	7
low moderate	2	2	2 *	15	-	18
high moderate	20	20	11	27	-	15
severe	20	21	16	1	-	1
end-stage	2	2	20	0	-	3
Atypical.tubule hyperplasia/adenoma	0	4	15*	-	-	-

[a] in parentheses no. of animals graded for chronic progressive nephropathy (CPN).

* denotes significant differences (for CPN regarding grade of severity): $p<0.001$ by X^2 test.

Table 13 Histopathological lesions in the chronic mouse diet study with hydroquinone by Shibata et al. (1991).[104]

Mouse (B6C3F1)	No. of animals with lesions			
	males		females	
Dose (mg/kg bw/day)[a]	Control (28) [b]	1,050 (30)	Control (29)	1,490 (30)
Kidneys				
Hyperplasia, tubular	0	9**	0	0
Adenoma	0	3	0	0
Liver				
Hypertrophy	0	26**	0	3
Foci of cellular alteration	4	14*	0	2
Hepatocellular adenoma	6	14*	0	1
Hepatocellular carcinoma	7	6	1	0
Forestomach				
Hyperplasia	1	11**	3	14**
Squamous cell carcinoma	0	1	0	1

[a] rounded figures from 0.8% w/w in diet.

[b] in parentheses no. of animals studied.

* and ** denote significant differences from control values (*$p<0.05$, **$p<0.01$); for severity of neoplastic lesions by the two-sided Fischer's exact test, for severity of non-neoplastic lesions by the Mann-Whitney test.

While no other tumour type was significantly increased, 9/28 and 3/28 males showed tubular hyperplasia, and adenomas in the kidney, respectively, against no

such lesions in controls; this incidence of hyperplasia was significant ($p<0.01$). In addition, significantly increased incidences of hyperplasia in the forestomach were observed in both sexes.[8,105]

In the study conducted by the NTP[98], groups of 65 B6C3F1 mice of each sex were administered 0, 50, or 100 mg/kg by gavage, 5 days per week for 104 weeks. Ten mice from each group were killed after 15 months for an interim evaluation.[98]

In mice killed at 15 months, the relative liver weights for high dose male and female mice were significantly greater than those for vehicle controls. Lesions seen in the liver of male mice included increased syncytial cells and diffuse cytomegaly.[98]

In the 2-years study, mean body weights of high dose male mice were 5-8% lower than those of vehicle controls after week 93, and those of high dose female mice were 5-14% lower after week 20. Relative liver weights were increased for all dosed male and high dose female mice. No significant differences in survival were observed between any groups of mice of either sex after 2 years (male mice: vehicle control 33/55; low dose 37/54; high dose 36/55; female mice: 37/55; 39/55; 36/55). The neoplastic lesions observed are presented in Table 14. No increase in tumours was found in exposed males. In females,

Table 14 Histopathological lesions in the 2-year mouse gavage study with hydroquinone by NTP (1989).[98]

Mouse (B6C3F1)	No. of animals with lesions					
	males (55)[a]			females (55)		
Dose (mg/kg bw/day)	Control	50 [b]	100 [c]	Control	50	100
Liver						
Basophilic focus	2	5	11	2	6	3
Hepatocellular adenoma	9	21	20	2	15*	12*
Hepatocellular carcinoma	13	11	7	1	2	2
Adenoma or carcinoma	20	29	25	3	16*	13*
Thyroid						
Hyperplasia	5	15*	19*	13	47*	45*
Adenoma	2	1	2	3	5	6
Carcinoma	0	0	0	0	0	1
Adenoma or carcinoma	2	1	2	3	5	7

[a] in parentheses no. of animals studied.
[b] 53 animals in this group.
[c] 54 animals in this group.

* denotes significant differences from control values ($p<0.01$); for severity of neoplastic lesions by the by two-sided Fischer's exact test, for severity of non-neoplastic lesions by the Mann-Whitney test.

combined incidences of hepatocellular adenomas and carcinomas found were 3/55 in controls, 17/55 in the low-dose group ($p=0.001$) and 14/55 in the high-

dose group (p=0.005), i.e. entirely due to an increase in the incidence of adenomas (2/55 controls, 15/55 low-dose group (p=0.001) and 12/55 high-dose group (p=0.005)), and without a clear dose-response relationship.[8,98] In addition, significantly increased incidences of hyperplasia in the thyroid were observed in both sexes.[98,100]

NTP concluded from their observations in the carcinogenicity studies in rats and mice that there was hydroquinone-related carcinogenicity in male rats, as indicated by increased incidences of tubular adenomas of the kidney, in female rats as shown by increases in mononuclear cell leukaemia, and in female mice based on increases in hepatocellular neoplasms, mainly adenomas. There was no evidence of carcinogenicity in male mice.[98]

IARC (1999a) noted that the incidences of leukaemia in the exposed female rats were within the historical control range.[8] Additionally, it is noted that the increase in kidney tumours entirely concerns adenomas, i.e., in none of these studies adenocarcinomas were observed. Also remarkable is the finding that with the same B6C3F1 mouse strain NTP (1989)[98] found an increased incidence of liver tumours in females without any clear effects in males, while Shibata et al. (1991)[104] found an increased effect in males without any effect in females.

IARC (1999a) considered these studies and concluded that they provided limited evidence for experimental carcinogenicity by hydroquinone.[8] EPA has not classified hydroquinone for carcinogenicity (EPA 1999).[5]

Initiation-promotion studies

Whysner et al. (1995)[108] gave a good overview of initiation-promotion studies investigating the potential of hydroquinone to promote carcinogenesis in various rat organs (i.e. after administration of organ-specific initiators), such as bladder (Miyata et al. 1985, cited in IARC 1999a[8]; Kurata et al. 1990[109]), stomach (Hirose et al. 1989, cited in IARC 1999a[8]), liver (Stenius et al. 1989[47], Okazaki et al. 1993[110]), upper digestive tract (Yamaguchi et al. 1989[111], Hasegawa et al. 1990[112]), kidney (Okazaki et al. 1993[110]), and pancreas in hamsters (Maruyama et al. 1991[113]) (see Annex F-8). In some of the studies, the final body weights of hydroquinone-treated rats were reduced as compared to controls. Under the applied exposure regimens, hydroquinone was unable to increase the incidence or multiplicity of tumours, when either given alone or after organ-specific initiators.

Stenius et al. (1989) performed a similar kind of initiation-promotion study with lesions considered as preneoplastic, i.e., enzyme-altered (γ-GT) foci (GGT-foci) in male Sprague-Dawley rats. Groups of 7-10 rats were given hydroquinone

in dietary concentrations of 0, 100 or 200 mg/kg (equivalent with 0 or approximately 5 or 10 mg/kg bw/day) for six weeks, beginning one week after partial hepatectomy (PH) and i.p. injection of 300 mg/kg bw N- nitrosodiethylamine (DEN) to initiate liver carcinogenesis. In the group submitted to PH and fed the high dose of hydroquinone no GGT-foci were induced. Hydroquinone after initiation (PH and DEN) increased the multiplicity of foci in the low-dose group and, though less marked, in the high-dose group. In a second experiment in which groups of 10 rats received 0 or 1 mg/kg bw hydroquinone (by oral gavage, five days per week for seven weeks) after initiation of liver carcinogenesis, no increase in multiplicity of enzyme-altered foci was found, but an increase of their area ($p<0.05$), and volume ($p<0.001$). According to the authors the results indicate that GSH depletion may act to develop enzyme-altered foci.[47]

In a study of Okazaki et al. (1993), groups of 15 or 20 male Wistar/Crj rats were given hydroquinone at concentrations of 0 or 0.8% in the diet (equivalent to 350 mg/kg bw/day) for 36 weeks with or without a preceding exposure to 0.1% N-ethyl-N-hydroxyethylnitrosamine (EHEN) via the dinking water for three weeks to initiate liver and kidney carcinogenesis. The final body weights of rats given hydroquinone were lower than the concurrent controls. The relative liver and kidney weights of rats receiving hydroquinone were higher than those of the basal diet group. Hydroquinone alone did not induce preneoplastic or neoplastic liver or kidney lesions. In the kidney, hydroquinone exposure after initiation increased the multiplicity of renal cell tumours (from 2.58 after initiation to 5.22 per rat; $p<0.01$) and of microadenomas (from 0.94 after initiation to 2.77 per rat; $p<0.05$). The authors concluded that hydroquinone potentially enhances the second stage of EHEN-induced renal carcinogenesis.[110]

Summary

Long term oral exposure to hydroquinone resulted exclusively in renal tubular adenomas in male F344 rats (and in males of one of the mouse studies), and an increased incidence of hepatocellular tumours in B6C3F1 mice, i.e., in one study in males, in the other in females: in both cases, however, the increase consisted entirely of an increase of adenomas only. In addition, significantly increased incidences of hyperplasia in the thyroids of both sexes were observed in the 2-years mouse gavage study.

benzoquinone

No long term inhalation toxicity and carcinogenicity studies are available. Some information on long-term non-neoplastic toxicity effects of benzoquinone could be derived from the dermal and oral carcinogenicity studies described below.

Dermal exposure

In a study of Umeda (1957), 15 Wistar and 9 hybrid strain rats were administered benzoquinone dissolved in propylene glycol and injected subcutaneous on the back once weekly. The first 53 days, the rats received 0.5 ml of a 10 g/L solution, from day 53 to 173 0.5 ml of 2 g/L and from day 173 to 394 0.5 ml of 4 g/L. Seventeen rats (about 70%) survived the injection period of 394 days. Three fibrosarcomas developed in two rats at the site of injection. No other tumours were found. In the control group (18 animals of different strains receiving only the vehicle) no tumours were found (Umeda 1957).[114] Due to the low number of treated animals and the use of different strains, limited conclusions can be drawn.

Oral exposure

El-Mofty et al. (1992) investigated the carcinogenic effect of benzoquinone using Swiss albino mice. Male (69) and female (65) animals were administered 2 mg benzoquinone by gavage, twice weekly for 13 months. Within 7 months, 36% of the males and 43% of the females died. The tumour incidence was 33.6%; the tumours were characterised as lympholeukaemias* and were located in the liver and spleen.[115] At the time the first tumours appeared (7-9 months after start of exposure), mortality was 36% in males, and 43% in females. Because of this high mortality and the absence of any histopathological information no conclusion can be drawn whether benzoquinone induced tumours via a secondary effect, such as chronic cell and/or organ damage, or via a direct genotoxic mechanism.[115]

* The authors described these lympholeukaemias as follows: `The liver tumors appeared as white nodules on the outer surface. Microscopically, the liver tissue was infiltrated with typically lymphocytic cells and was diagnosed as lympholeukemia. The nuclei of the neoplastic cells were hyperchromatic, and mitotic figures were usually not numerous. The spleen in most cases was much larger than normal, and histologically, spleen tumors were diagnosed as lympholeukemia.`

Information on other endpoints than mortality and carcinogenicity was not given in the oral and dermal carcinogenicity studies on benzoquinone described above.

Initiation-promotion studies

In the study of Gwynn and Salaman (1953), groups of 19 albino "S" mice received a topical, dermal application of a 0.15% 7,12-dimethylbenzanthracene solution in acetone as an initiation treatment. Three weeks later benzoquinone at a concentration of 3.6 g/L in acetone was applied weekly for 27 weeks. At the end of the study no tumours or hyperplasia of the epidermis were observed.[116]

In the study of Monks et al. (1990), groups of 25 female Sencar mice received a dermal application of 25 nmol 7,12-dimethylbenzanthracene. Two weeks later benzoquinone was applied weekly on a shaved skin area at doses of 0, 440, 880 or 1,760 nmol/mouse. After 31 weeks of application of benzoquinone, no signs of possible promoter capacity had been observed.[117]

IARC considered that the available data did not allow a conclusion on the carcinogenicity of benzoquinone in animals (IARC 1999b).[9] EPA considered the results of available animal studies insufficient to evaluate the carcinogenicity of benzoquinone (EPA 1992, revised in 2000).[6]

Summary

Benzoquinone was reported to induce lympholeukaemias in liver and spleen in mice after gavage administration for 13 months. Due to the high mortality and the absence of any histopathological information, this study is considered of too limited value to draw conclusions on the carcinogenic potential of benzoquinone. The initiation-promotion studies available did not indicate any sign of promoter capacity of benzoquinone.

7.2.5 Mutagenicity and genotoxicity

Mutagenicity and genotoxicity of hydroquinone and benzoquinone were extensively and well described by IARC in 1999a,b.[8,9] The reviews generated by IARC are presented in Annexes H-1 (hydroquinone) and H-2 (benzoquinone). Below, IARC data and additional studies found in the literature are summarised.

hydroquinone

DNA strand breaks / SCEs

Hydroquinone induced SCEs in vitro in V79 Chinese hamster cells[118], in CHO cells either with or without exogenous metabolic activation[98], and in human lymphocytes in the absence of metabolic activation. Hydroquinone tested negative in an in vivo SCE test using bone marrow cells.[8]

DNA adduct formation

Covalent binding of hydroquinone to DNA was observed in vitro in various cell types.[8] However, covalent DNA binding of hydroquinone could not be demonstrated in vivo.

Clastogenic effects

Hydroquinone tested positive in the in vitro micronuclei test in the absence of metabolic activation using embryonic human liver cells, human lymphocytes and V79, IEC-17 and 18 cells.[8,118] Chromosome aberrations were induced by hydroquinone in vitro in human lymphocytes in the absence of metabolic activation.[8,98]

The effect of polymorphism of the glutathione S-transferases GST-M1, GST-T1 and GST-P1 on induction of micronuclei (MN) and SCEs was studied by in vitro hydroquinone treatment of human lymphocytes isolated from healthy volunteers. Hydroquinone induced a significant higher frequency of MN in lymphocytes with the GST-M1 null genotype than with GST-M1 present, while this effect was not seen for SCEs. Additionally, the other polymorphisms did not significantly affect the frequency of MN or SCEs. This suggests that GST-M1 is involved in the metabolic fate of hydroquinone and that polymorphisms in GST-M1 could be related to inter-individual differences in DNA damage arising from the exposure to this compound.[74]

Hydroquinone was in vivo weakly positive in the micronucleus test in mouse bone-marrow cells and in mouse liver cells *in utero*.[8] Chromosome aberrations were induced by hydroquinone in vivo in mouse bone marrow and spermatocytes/spermatogonia.[8]

DNA mutation

Hydroquinone was negative in Salmonella strains TA 97, 98, 100, 1535, and 1537, with or without metabolic activation.[8,98] Hydroquinone was mutagenic in Salmonella strains TA102 and TA104 (strains sensitive for oxidative mutagens).[8] Hydroquinone tested positive for genotoxicity in *S. cerevisiae* without exogenous metabolic activation, and induced gene mutations in mouse lymphoma and Syrian hamster embryo cells in the absence of metabolic activation system.[8] Hydroquinone induced gene mutations in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation.[98] Hydroquinone was a potent inducer of gene mutations (6-thioguanine resistance) in V79 cells.[118]

No information on in vivo induction of gene mutations by hydroquinone was available.

Aneuploidy induction

Aneuploidy was induced in vitro by hydroquinone in Chinese hamster cells, Syrian hamster embryo cells and human lymphocytes, in the absence of metabolic activation.[8]

Hydroquinone clearly increased the frequencies of hyperploid secondary spermatocytes, which indicated non-disjunction induction during the first meiotic division. Concomitantly, hydroquinone induced meiotic delay in primary and/or secondary spermatocytes.[119]

Aneuploidy was also induced in vivo in mouse bone marrow and spermatocytes.[8]

Overall, the observations reported above may indicate the potency of hydroquinone to (1) induce DNA strand breaks and SCEs, (2) bind covalently to DNA, (3) induce clastogenic effects both in vitro and in vivo, (4) induce DNA mutations in vitro, and (5) induce aneuploidy both in vitro and in vivo. It should be noted, however, that generally effects in the in vitro tests were seen only at relatively high doses.

benzoquinone

DNA strand breaks and SCEs

Benzoquinone was demonstrated to induce double strand breaks in an in vitro cell-free system, incubated with λ-DNA in the absence and presence of various

anti-oxidants (GSH, trolox, etc.) and anti-oxidant enzymes (catalase, superoxide dismutase).[17] Benzoquinone induced DNA strand breaks in mouse lymphoma cells in vitro in the absence of metabolic activation, and in human lymphocytes in the presence of metabolic activation.[9] No in vivo data on DNA strand break induction by benzoquinone are available. Benzoquinone tested positive in an in vitro SCE test using human lymphocytes.[9]

DNA adduct formation

In the study of Gut et al. (1996) it was demonstrated that benzoquinone reacted spontaneously with DNA in vitro to form DNA-adducts.[16] No additional studies were found that show the potency of benzoquinone to form DNA-adducts.

Clastogenic effects

Benzoquinone increased the frequency of micronuclei formation in V79 cells.[118] Benzoquinone also tested positive in various micronucleus tests in vitro in the absence of metabolic activation, and weakly positive in vivo.[9]

DNA mutation

Benzoquinone tested negative for genotoxicity in *S. typhimurium*.[118] The IARC data on DNA mutations induced in *S. typhimurium* by benzoquinone were inconclusive.[9] Benzoquinone was a potent inducer of gene mutations in V79 cells[9], as it produced an approximately 100-fold increase in the frequency of 6-thioguanine-resistant cells at a concentration of 1 µmol/L.[118] Benzoquinone was positive in vivo in the TK-assay.[9,120]

Aneuploidy induction

No information on aneuploidy induction by benzoquinone was available.

Overall, it can be concluded that benzoquinone has the potency of inducing DNA strand breaks in vitro, may induce clastogenic effects both in vitro and in vivo, and has the potency to induce DNA mutations. In one study it was demonstrated that benzoquinone was able to form DNA-adducts in vitro. It should be noted, however, that generally effects in the in vitro tests were seen only at relatively high doses.

7.2.6 *Evaluation of the carcinogenicity and genotoxicity data*

In this Chapter, the evaluation of the Subcommittee on the Classification of carcinogenic substances is summarized. The full evaluation of the Subcommittee is reproduced in Annex I.

hydroquinone

Only oral carcinogenicity studies with hydroquinone were performed (NTP 1989[98], Kari et al. 1992[100], Shibata et al. 1991[104]); no carcinogenicity studies via dermal or inhalation exposure were located. Carcinogenicity studies (via diet and gavage) with B6C3F1 mice and F344 rats on hydroquinone are available for evaluation of this endpoint. The following tumour responses were observed:

In gavage studies with two test doses:
- Kidney adenomas in male rats
- Mononuclear cell leukaemia in female rats
- Hepatocellular adenomas in female mice.

In diet studies with a single test dose:
- Kidney adenomas in male rats
- Hepatocellular adenomas in male mice.

As to the hepatocellular adenomas, the Subcommittee noted that they were not consistently seen, and did not progress to malignant carcinomas. Because of this inconsistent picture and the susceptibility of the $B6C3F_1$ mouse to develop liver tumours, the Subcommittee considered that the induction of these hepatocellular adenomas is not relevant for humans.

The renal tubular adenomas were found in both the diet and the gavage study, but in male rats only. These benign tumours did not progress to malignant ones. Severe kidney damage appeared to be a prerequisite for the development of the adenomas. This chronic progressive nephropathy is a spontaneous disease in, particularly, ageing male rats. The Subcommittee was of the opinion that hydroquinone plays an indirect role in the development of the adenomas by exacerbating already existing chronic progressive nephropathy and enhancing proliferative aspects of this disease process. Because of this indirect role involving exacerbation of an already existing spontaneous disease which is further common in rats but without a human counterpart, the Subcommittee was

of the opinion that the induction of these renal tubular adenomas in male rats is not relevant for humans.

The mononuclear cell leukaemias were found at statistically significantly increased incidences in the high-dose female rats in only one study. The Subcommittee noted that especially F344 rats show high background incidences of this tumour type while the rate in controls is very variable and depends upon several factors such as e.g., sex, vehicle and diet. In view of the susceptibility of these rats to develop monuclear cell leukaemias, the very variable background incidences, and the fact these leukaemias were seen in one sex in one study only, the Subcommittee considered that the induction of these mononuclear cell leukaemias in female F344 rats is not relevant for humans.

The Subcommittee concluded that hydroquinone is a genotoxic compound. In vitro, it induced amongst others, gene mutations, micronuclei, chromosomal aberrations, aneuploidy, sister chromatid exchanges, DNA single strand breaks and oxidative DNA damage in mammalian cell systems. In in vivo tests, mainly performed in mice using intraperitoneal injection, hydroquinone produced increases in the incidences of micronuclei and chromosomal aberrations in bone marrow and of chromosomal aberrations in spermatogonia. In vitro, hydroquinone is an inhibitor of topoisomerase II. Redox-cycling of hydroquinone/benzoquinone generates ROS. Based on the literature it is quite likely that the mode of action of hydroquinone's genotoxicity is by a non-stochastic mechanism.

benzoquinone

One dermal (Umeda1957) and one oral study (El-Mofty et al. 1992) addressed the (potential) carcinogenicity of benzoquinone. The Subcommittee was of the opinion that because of several serious flaws in design and reporting, these studies cannot be used to evaluate the carcinogenicity of benzoquinone.

Recommendation for classification

hydroquinone

The Subcommittee concluded that there are two valid carcinogenicity studies in which hydroquinone was orally administered to rats and mice. The findings were inconsistent and concerned tumour types that were considered not relevant for humans. Based on the available information, the Subcommittee was of the

opinion that the data are as yet insufficient to evaluate the carcinogenic properties of hydroquinone (Health Council category 3; see Annex J).

benzoquinone

The Subcommittee concluded that there are no valid carcinogenicity studies, Based on the available information, the Subcommittee was of the opinion that the data are as yet insufficient to evaluate the carcinogenic properties of benzoquinone (Health Council category 3; see Annex J).

Approach for deriving health-based occupational exposure limits

Overall, the Committee adopts the conclusions and recommendation of the Subcommittee on the Classification of carcinogenic substances. Since a non-stochastic genotoxic mechanism is considered responsible for the carcinogenicity of hydroquinone in animals (probably ROS-generation, while also inhibition of topoisomerase II may play a role), the Committee concludes that with respect to hydroquinone a threshold approach is appropriate for deriving a health-based occupational exposure limit for hydroquinone.

Furthermore the Committee is of the opinion that the line of reasoning described above for hydroquinone is in principle also valid for benzoquinone.

7.2.7 Reproduction toxicity (fertility and development)

hydroquinone

Two 2-generation studies were conducted in rats. The results are summarized in Annex F-7 and in Table 15.

Table 15 Reproductive toxicity studies with hydroquinone.

Species	NOEL (mg/kg bw/day)		LOEL (mg/kg bw/day) / Critical effects		Reference
Rat (Sprague-Dawley)	15 150 150	General toxicity Parental reprotoxicity Toxicity F1	50 >150	General toxicity F0 and F1: mild, transient tremors and decreased body weight Reproduction and fertility: no treatment related effects	Blacker et al. 1993[121]
Rat (Sprague-Dawley)	15 150 150	General toxicity Parental reprotoxicity Reprotoxicity F1	50	General toxicity F0 and F1: transient tremors	Schroeder 1989 (unpublished), cited in OECD 1996[33]

In the study of Blacker et al. (1993), performed according to guideline OECD 416, hydroquinone was administered in an aqueous solution by gavage at doses of 0, 15, 50, or 150 mg/kg bw/day. F0 and F1 parental animals were dosed daily for at least 10 weeks prior to cohabitation, during cohabitation, and until scheduled termination. At all dose levels tested, no adverse effects were observed on feed consumption, survival, or reproductive parameters for the F0 or F1 parental animals. Mild, transient tremors were observed shortly after dosing at 150 mg/kg bw/day in several F0 and F1 parental animals and in a single F0 male at 50 mg/kg bw/day. These tremors occurred infrequently and were considered to be due to an acute stimulatory effect of hydroquinone on the nervous system. Body weights for F0 and F1 parental females were similar between all dose groups throughout the study. Body weights for F0 parental males were also comparable to those of control throughout the study. Statistically significant differences in body weights were noted for the F1 parental males in the 50 and 150 mg/kg bw/day dose groups at several intervals during the pre-mating, mating, and post-mating periods. No treatment-related effects on pup weight, sex distribution, or survival were noted for pups of either generation. Upon post-mortem examination, no treatment-related gross lesions were observed in either the F0 or F1 parental animals or their weanlings. Histopathologic examination of reproductive tissues and pituitary glands from high-dose F0 and F1 parental animals did not reveal any changes related to treatment with hydroquinone. Thus no adverse effects on reproduction or fertility were observed in either generation at any dose level, and the results of the present study indicate that hydroquinone is not a selective reproductive toxicant. The NOAELs for general and reproductive toxicity were 15 and 150 mg/kg bw/day, respectively.[121]

In the study of Schroeder (1989; guideline OECD 416), Sprague-Dawley rats were treated with 0, 15, 50 or 150 mg/kg bw/day hydroquinone in distilled water by gavage. In the maternal and paternal generation, no adverse effects of treatment were evident from mortality, body weight, or feed consumption at the 15 and 50 mg/kg bw/day dose levels. Mild and transient tremors were observed in one male at 50 mg/kg bw/day, and in several males and females in the 150 mg/kg bw dose groups. Concerning reproductive toxicity evaluated both in parental animals (fertility gestation, reproductive organ toxicity, etc) and in offspring (weights of litter, post-natal growth, viability, etc.), no adverse effects were seen in body weight, sex distribution, survival, or gross pathology of pups delivered (Schroeder 1989, cited in OECD 1996).[33]

Teratogenicity/developmental studies were conducted in rats and rabbits. The results are summarized in Annex F-7 and Table 16.

In the study of Krasavage et al. (1992; guideline OECD 414), pregnant rats (COBS-CD-BR) were given 0, 30, 100, or 300 mg/kg bw/day hydroquinone by gavage on gestation days (GD) 6 to 16. Maternal effects included a slight, but significant ($p \leq 0.05$) reduction in body weight gain and feed consumption for the 300 mg/kg bw/day dams. Reproductive indices, i.e., pregnancy rate, numbers of corpora lutea, implantation sites, viable foetuses, and early and late resorptions, foetal sex ratio, pre- and post-implantation losses, and gravid uterine weights, were not affected by treatment. A slightly reduced ($p \leq 0.05$) mean foetal body weight seen at the 300 mg/kg bw/day dose level was associated with the slightly reduced body weight gain seen for the dams at this dose level. Gross external, internal soft tissue, and skeletal examinations of the foetuses revealed no treatment-related malformations. The incidences of gross external variations (small hematomas) and internal soft tissue variations (dilated renal pelvis, hydronephrosis, and hydrourether) in the treated litters were not statistically different from the control incidences. Skeletal variations (delayed ossification of membranous skull bones, hyoid bone, thoracic centre 1-3, sacral arches 3 and 4, and bilobed thoracic centre 9-13) were seen with similar frequency in the control and treated groups. A statistically significant increase in the incidence of total common vertebral variations seen at the 300 mg/kg bw/day dose level was not considered toxicologically significant (according to the authors, analyses of individual skeletal veriations, including individual vertebral variations, and statistical analysis of the the total number of fetuses with a skeletal alteration indicated no significant effects on skeletal development in the treated groups compared with the control group). The authors concluded that hydroquinone was not selectively toxic to the developing rat conceptus and, thus, appears not to have the properties of a developmental toxicant. The NOAEL for both maternal and developmental toxicity was 100 mg/kg bw/day, whereas 300 mg/kg bw/day was the LOAEL (maternal and developmental).[122]

In the study of Murphy et al. (1992; guideline OECD 414), hydroquinone was administered to pregnant New Zealand White rabbits (18 mated per dose group) in aqueous solution (0, 25, 75, or 150 mg/kg bw/day) by gavage on GDs 6 to 18. Caesarean sections were performed on GD 30. Doses of 75 and 150 mg/kg bw/day adversely affected feed consumption and/or body weight of dams during the treatment period. At these doses, however, treatment-related effects were not evident from physical observations, liver and kidney weights, premature delivery incidence, and caesarean sectioning data. The NOAEL for maternal toxicity was 25 mg/kg bw/day. In the 150 mg/kg bw/day dose group, total incidences of external, visceral, and skeletal findings for fetuses did not differ statistically from controls. Slight but statistically insignificant increases were found, however, in

the incidences of ocular and minor skeletal malformations (micro-ophthalmia, vertebral/rib defects, angulated hyoid arch) on both a per foetus basis and a per litter basis. Under the conditions of this study, the authors concluded that hydroquinone at 150 mg/kg bw/day produced minimal developmental alterations in the presence of maternal toxicity. The NOAEL for developmental toxicity was 75 mg/kg bw/day.[123]

Table 16 Developmental toxicity studies with hydroquinone.

Species	NOAEL (mg/kg bw/day)		LOAEL (mg/kg bw/day) / Critical effects		Reference
Rat (COBS-CD-BR)	100	General toxicity	300	Maternal: decreased food consumption and body weight gain	Krasavage et al. 1992[122]
	300	Pregnancy/litter			
	100	Fetal data	300	Fetuses: decreased body weight	
Rabbit (NZW)	25	General toxicity	75	Maternal: decreased food consumption	Murphy et al. 1992[123]
	150	Pregnancy/litter			
	75	Fetal data	150	Developmental: minimal developmental alterations	

benzoquinone

No data were available.

7.2.8 *Immunotoxicological and haematological effects*

hydroquinone

The cytotoxic as well as immunosuppressive potential of hydroquinone was demonstrated in in vivo (ip injection in mice) and in vitro studies that showed a reduced cellularity of bone marrow and spleen, and an inhibition of maturation of B-lymphocytes.

Wierda and Irons (1982) investigated the in vivo toxicity of hydroquinone toward the development of polyclonal, plaque-forming cells (PC-PFC) from progenitor B lymphocytes. Dextran sulfate (DxS), lipopolysaccharide (LPS), or the two mitogens combined (DxS + LPS) were used to induce proliferation and maturation of these progenitors to PC-PFC. Groups of 4 C57BL/6 mice were exposed to 2 daily doses of hydroquinone (100 mg/kg bw), administered either iv or ip for 3 consecutive days. Spleen and marrow cells were harvested for culture one day later. Hydroquinone (100 mg/kg bw) was cytotoxic to spleen cells and reduced bone marrow cellularity. It reduced the frequency of PC-PFC developed from the spleens and bone marrows of treated mice. These experiments demonstrate the immunotoxic potential of hydroquinone in vivo through the

reduction of progenitor B lymphocytes[124], though via routes irrelevant for the occupational situation.

Several studies have been performed on the effects of hydroquinone on various haemopoietic cells aiming at unravelling the mechanism of myelotoxicity induced by benzene, of which hydroquinone and benzoquinone are metabolites. Most of the studies performed were on haemopoietic cells in vitro. However, the relevance for the in vivo situation is difficult to estimate. Observations were:

- Macrophage peroxidase catalyzed the metabolic oxidation of hydroquinone to benzoquinone, and benzoquinone and/or its semiquinone intermediate did bind to protein and cysteine (Schlosser 1989).[125]
- Hydroquinone may exert many of its haematopoietic effects via synergism with granulocyte-macrophage colony stimulating factor (GM-CSF) in TF-1 erythroleukaemia cells, in human CD34+ bone marrow cells, and in granulocyte-macrophage colonies from mouse lineage restricted marrow cells[117] (Irons et al. 1992, cited in Klaassen 1996).[79]
- Hydroquinone inhibited mitogen-stimulated activation of both T and B lymphocytes (Guy et al. 1991).[128]
- Hydroquinone inhibited the production of many cytokines including IL-1, IL-2, IFN, and TNF (Kin et al. 1989; Post et al. 1985; Cheung et al., cited in Pyatt et al. 2000).[126]
- Hydroquinone directly inhibited T cells by blocking the activity of the transcription factor nuclear factor kB (NFkB) (Pyatt et al. 2000).[126]
- Hydroquinone disrupted normal NFkB activity in B cells, which accounted for the observed loss in B cell function and maturation, and thus may play a role in mediating the immunosuppressive effects of hydroquinone on B cells (Patt et al. 1988, cited in Pyatt et al. 2000).[126]
- Hydroquinone induced an increase in total granulocyte-macrophage colony forming cells (GM-CFC) in the bone marrow of mice in vivo (and in vitro; see for the in vitro part Annex G-3) (Henschler et al. 1996).[127]
- Hydroquinone was demonstrated to decrease ^{59}Fe uptake in erythrocytes after administration to Swiss albino mice. Hydroquinone was 25-100 times less potent than benzoquinone (Guy et al. 1991).[128]
- Hydroquinone appeared to activate circulating neutrophils of mice in vivo, thereby impairing further stimulatory responses (Ribeiro et al. 2011).[129]
- Hydroquinone induced apoptosis of neutrophils and eosinophils isolated from the blood of healthy humans, through the caspase 9/3-dependent pathway and the increased ROS production (Yang et al. 2011).[130]

Myelotoxic effects were not observed in a long-term bioassay on rodents and are, therefore, not considered to be critical effects of hydroquinone-induced toxicity.

benzoquinone

Reactions of benzoquinone with whole blood of both F344 rats and humans resulted in linear formation of adducts with haemoglobin and albumin[131] (see also Section 7.2.3).

Benzoquinone has been reported to decrease ^{59}Fe uptake in erythrocytes after administration to Swiss albino mice[128], demonstrating its erythrotoxic potential.

Benzoquinone has been shown to inhibit Fc receptor-mediated phagocytosis in cultured murine peritoneal macrophages. This is likely to be due to disruption of filamentous actin via an effect other than the direct alkylation of actin by benzoquinone.[132]

7.2.9 Neurological effects

hydroquinone

Acute high-level exposure to hydroquinone causes hyper-excitability tremor, convulsions and coma (see Sections 7.2.2 and 7.2.3).

A 90-day study including neurological examinations was performed by Bernard 1988, cited in OECD 1996[33]) and Topping et al. 2007.[101] Sprague-Dawley rats were administered hydroquinone at dose levels of 0, 20, 64, or 200 mg/kg bw/day by gavage, 5 days per week for 13 weeks. A functional-observational battery was performed. Mild and transient tremors and reduced home-cage activity were observed in mid- and high-dose groups immediately after dosing, with the incidence increasing in a dose dependent manner, but unfortunately no more detailed quantitative data were reported. The results of neuropathological examinations were negative. The NOAEL in this study was 20 mg/kg bw/day. Note that the NOAEL for parental toxicity in the 2-generation reproductive study with rats (Blacker et al. 1993[121]) was 15 mg/kg bw/day, based on mild, transient tremors, and decreased body weight; see Section 7.2.7).

benzoquinone

Neurological effects were observed in the acute toxicity test described in Section 7.2.2. Rats treated with benzoquinone experienced ataxia, loss of righting reflex and corneal reflex. Mice treated with benzoquinone suffered from writhing and

paralysis of the hind limbs. No long-term neurological studies with benzoquinone have been reported.

7.3 Summary and evaluation

hydroquinone

No conclusion regarding the carcinogenic properties of hydroquinone could be drawn from the epidemiological studies with the substance.

Hydroquinone was reported to induce skin depigmentation, cornea pigmentation and sensitization in humans after occupational exposure. Eye irritation, skin irritation, depigmentation and sensitization was also observed in animal studies. According to the authors, the results from the studies on sensitization showed “weak” to “extreme” sensitization, depending on the methods or vehicle used. Cross-reactivity for allergic contact dermatitis exists between p-phenylenediamine, hydroquinone and benzoquinone.

In the acute toxicity studies in animals, various neurological symptoms were observed, including hyperexcitability, tremors, convulsions, and coma. The lowest oral LD_{50} was about 300 mg/kg bw in rat. The dermal LD_{50} may be well above 1,000 mg/kg bw. No acute inhalation studies were available.

Sub-acute toxicity studies with hydroquinone have been performed via dermal and oral (gavage) exposure. Reduced body weight was observed after dermal application of 3,840 mg/kg bw/day for 14 days. No adverse effects were observed at dermal application of 74 mg/kg bw/day for 13 weeks. After oral repeated dose administration, hydroquinone induced changes in kidney and liver weights of F344 rats and B6C3F1 mice at a level of 25 mg/kg bw/day for 13 weeks. Toxic nephropathy, characterised by tubular cell degeneration in the renal cortex, was seen in 7/10 male and 6/10 female rats receiving of 200 mg/kg bw/day and 1 female receiving 100 mg/kg bw/day. However, because the Committee does not consider changes in liver and kidney weights to be a adverse effect, a NOAEL for toxic nephropathy of 25 mg/kg bw/day can be derived. In Sprague Dawley rats, no effects were observed at 20 mg/kg bw/day for 13 weeks. At 64 mg/kg bw/day, mild, transient tremors and reduced home-cage activity were observed.

With hydroquinone only oral carcinogenicity studies were performed; carcinogenicity studies via dermal or inhalation exposure are not available. At levels of 50 mg/kg bw/day administered to F344 rats for 104 weeks, reduced body weights were observed, and renal tubular adenomas in males and mononuclear cell leukaemias in females were found. The Committee does not

consider these tumours as relevant (see Section 7.2.6). The NOAEL was set at 25 mg/kg bw/day.

In B6C3F1 mice, 50 mg/kg bw/day for 104 weeks induced increased relative liver weights in males and hepatocellular adenomas in males and females. Based on the considerations of the Subcommittee on the Classification of carcinogenic substances, the Committee does not consider these tumours as relevant (see Section 7.2.6). No NOAEL was derived in this study.

In vitro, hydroquinone is an inhibitor of topoisomerase II, an enzyme responsible for the maintenance of proper chromosome structure and segregation.

Hydroquinone was negative in Salmonella strains TA 97, 98, 100, 1535, and 1537, with or without metabolic activation, but positive in strains TA102 and TA104, both sensitive for oxidative mutagens. Hydroquinone was able to induce DNA strandbreaks, SCEs, gene mutations, chromosomal aberrations and micronuclei in mammalian cells, and cell lines in vitro. Numerous in vivo studies were performed with hydroquinone, investigating induction of micronuclei, chromosomal aberrations or polyploidy in the bone marrow, and chromosomal aberrations or hyperploidy in germ cells. All but one of these studies used ip administration and most of them were positive. One oral study gave a weakly positive result.

These positive responses may well be explained by the postulated underlying mechanisms, demonstrated to occur under in vitro conditions: redox-cycling with ROS generation, inhibition of topoisomerase II, and direct covalent binding to macromolecules (either directly or after conjugation with glutathione). Although this latter capability may underlie the in vitro observed genotoxicity, several attempts to demonstrate the in vivo formation of hydroquinone-derived DNA-adducts failed.

Based on the studies on reproduction, the Committee concludes that hydroquinone is not a reproductive toxicant. In one of the developmental studies, slight (statistically insignificant) malformations were observed. The NOEL for developmental toxicity was set at 75 mg/kg bw/day.

The available data on neurotoxicity showed mild, transient tremors and reduced home-cage activity at 64 mg/kg bw/day; the NOAEL for neurotoxicity was 20 mg/kg bw/day. The tremors observed in the 2-generation rat reproductive study with a NOAEL of 15 and a LOAEL of 50 mg/kg bw/day were also only mild and transient.

Following the Subcommittee on the Classification of carcinogenic substances the Committee concludes that there are two valid carcinogenicity studies in which hydroquinone was orally administered to rats and mice. The

findings were inconsistent and concerned tumour types that were considered not relevant for humans. Based on the available information, the Committee is of the opinion that the data are as yet insufficient to evaluate the carcinogenic properties of hydroquinone (category 3; see Annex J).

The Committee concludes that a threshold approach is suitable for deriving a health-based occupational exposure limit for hydroquinone.

benzoquinone

Human epidemiological studies with benzoquinone are not available.

In humans, exposure to high air levels of benzoquinone may result in irritation of the eyes. Dermal exposure of humans may result in dermatitis and severe irritation. No systemic effects arose upon occupational exposure of humans at a level of 0.44 mg/m^3 benzoquinone vapour during a period of 5 years. Based on the results of various animal studies, benzoquinone appeared to be a skin sensitizer. It induced skin lesion and irritation when injected subcutaneously in guinea pigs. Cross-reactivity for allergic contact dermatitis exists between p-phenylenediamine, hydroquinone and benzoquinone.

In the acute toxicity studies in animals, various neurological symptoms were observed, including loss of reflexes, writhing and paralysis of the hind limbs. The lowest oral LD_{50} in rats was 130-160 mg/kg bw. No acute dermal or inhalation studies are available.

Sub-acute toxicity studies with benzoquinone have been performed via inhalation exposure, and ip and sc injection. The critical effects were all of a haematological character and included decreases in blood cells, methaemoglobinaemia and trombopenia. A NOAEL by inhalation in rats was not established, since effects were already observed at the lowest dose of 0.27-0.36 mg/m^3. Since the original document of this 4 months study could not be retrieved, the relevance and accuracy of these data can not be assessed.

There are only two very limited carcinogenicity studies. Because of the poor quality of the studies and the high mortality, no conclusions can be drawn regarding the potential of benzoquinone to induce carcinogenic effects at maximum tolerated levels. The initiation-promotion studies indicated no signs of promoter capacity of benzoquinone. Epidemiological data relevant to the carcinogenicity of benzoquinone were not available.

In vitro, benzoquinone is an inhibitor of topoisomerase II, an enzyme responsible for the maintenance of proper chromosome structure and segregation.

Benzoquinone was negative in Salmonella strains TA 98, 1535, and 1537, with or without metabolic activation. Benzoquinone was able to induce DNA strand breaks, SCEs, gene mutations, and micronuclei in mammalian cells, and cell lines in vitro. In vivo micronuclei induction studies – via the oral route – were weakly positive, while one dominant lethal test with C3H mice was negative.

Studies on reproduction and developmental toxicity with benzoquinone were not available.

Overall (based on the information available) a NOAEL for benzoquinone could not be derived.

In line with the Subcommittee on the Classification of carcinogenic substances the Committee concludes that too little information on carcinogenicity of benzoquinone is available, and thus it is as yet not possible to evaluate its carcinogenic properties (category 3; see Annex J).

As is clarified in Sections 2.2 and 6, the mechanism of action of benzoquinone and hydroquinone may be similar due to the fact that they are metabolites of each other and because conversions between them are part of the mechanism of action. Therefore, the Committee adopts the opinion that the line of reasoning described above for hydroquinone is also valid for benzoquinone.

Chapter 8

Existing guidelines, standards and evaluations

8.1 General population

Over-the-counter skin lighteners may contain up to 2% hydroquinone, and prescription drugs may contain higher concentrations. A 2% upper limit on hydroquinone concentration was set by the South African government in 1980 and followed by the United Kingdom and USA.[13]

In 2002, the European Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) concluded that due to very low exposure to the consumer hydroquinone is safe for use, within certain restrictions and conditions, as technical aids in artificial nail systems. Hydroquinone is listed in the “restrictive list”, Annex III - Part 1 (substances which cosmetic products must not contain except subject to restrictions and conditions) of the European Cosmetics Directive (76/768 as amended). This Directive has also been amended in order to allow the use of this substance as a technical aid in artificial nail systems. Hydroquinone is allowed at a maximum level of 0.02% (after mixing for use). The following warnings must be printed on the label: “For professional use only”, “Avoid skin contact”, “Read directions for use carefully”. Details are laid down in Directive 2003/83/EC of 24 September 2003, which was published in issue no. L238/23 of the Official Journal of the European Union.

In 2003 the Cosmetic Ingredient Review (CIR) Expert Panel of the EU determined that hydroquinone should not be used in non-drug cosmetic products

that are left on the skin and not immediately rinsed off. The panel drew this conclusion based on studies linking hydroquinone to cancer and immune system damage that targets bone marrow (EWG 2003[44]).

No international guideline for hydroquinone in drinking water has been established (WHO 1993, cited in IARC 1999a[8]).

8.2 Working population

hydroquinone

Occupational exposure limits

See Table 17 for occupational exposure limits for hydroquinone previously derived by national and international organisations.

The American Conference of Governmental Industrial Hygienists (ACGIH) recommended in 2008 a 8-h TWA (time weighted average) TLV (threshold limit value) of 1 mg/m^3 stating that this exposure limit will minimize the potential risk to workers from eye irritation and eye damage associated with occupational exposure to hydroquinone (Sterner et al. 1947; Oglesby et al. 1947, Anderson et al. 1958[133], cited in ACGIH 2008[1]). Insufficient data were considered available to elaborate a TLV-STEL (short-term exposure limit, generally for a period of 15 min).[1]

EPA concluded in 1992 (revised in 2000) that no information is available on the reproductive or developmental effects of hydroquinone in humans. A slight reduction in maternal body weight gain, decreased foetal weight, increased resorption rate, and reduced fertility in males have been observed in rats orally exposed to hydroquinone via gavage or in the diet. Exposure of rabbits to hydroquinone via gavage produced negligible developmental alterations. EPA has not established a Reference Concentration (RfC) for hydroquinone. EPA has calculated a provisional Reference Dose (RfD) of 0.04 mg/kg bw/day for hydroquinone based on haematological effects in humans.[5] The provisional RfD is a value that has had some form of Agency review but is not in the Integrated Risk Information System (IRIS). The provisional RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime. It is not a direct estimator of risk but rather a reference point to gauge the potential effects. At exposures increasingly greater than the RfD, the potential for adverse health effects increases. Lifetime exposure above the RfD does not imply that an adverse health effect would necessarily occur.

Table 17 Established limits for occupational exposure to hydroquinon in various countries.

Country Organisation	Occupational exposure limit (mg/m^3)	Time-weighted average [a]	Note [b]	Reference
Denmark	2.0	8 h	OEL	2007
Finland	0.5	8 h	-	2005
	2.0	15 min	STEL	
Germany				
AGS [c]	-	-	-	2007
DFG [d]	-	-	-	
Norway	0.5	8 h	-	2007
Sweden	0.5	8 h	-	2005
	1.5	15 min	STEL	
UK				
HSE [e]	0.5	8 h	MEL	2007
USA				
ACGIH [f]	1.0	8 h	TLV	2008
NIOSH [g]	2.0	15 min	REL	2004
OSHA [h]	2.0	8 h	PEL	2004
Iceland	0.5	8 h	-	1999
European Union				
SCOEL [i]	-	-	-	-

[a] Time-weighted average (TWA): a maximum mean exposure limit based generally over the period of a working day.
[b] Abbreviations - OEL: occupational exposure limit; STEL: short-term exposure limit; MEL: maximum exposure limit; TLV: threshold limit value; REL: recommended exposure limit; PEL: permissible exposure limit.
[c] Ausschuss für Gefahrstoffe.
[d] Deutsche Forschungsgemeinschaft.
[e] Health and Safety Executive.
[f] American Conference of Governmental Industrial Hygienists.
[g] National Institute for Occupational Safety and Health.
[h] Occupational Safety and Health Administration.
[i] Scientific Committee on Occupational Exposure Limit Values.

In the UK, the current 8-h TWA MEL (maximum exposure limit) at 0.5 mg/m^3 is based on a HSE review (2001)[18] concluding that the key health effect of concern is genotoxicity. A level of exposure below which there would be no concerns for this endpoint would not be identified, and therefore the criteria for establishing an OES (occupational exposure standard) were not met. Hence, in view of the serious health concerns associated with genotoxicity, a MEL was established. A STEL was not proposed.

In Germany, no MAK (Maximale Arbeitsplatz-Konzentration) value has been derived (2005).[102]

Up to January 1, 2007, the limit value for occupational exposure to hydroquinone in The Netherlands was 2.0 mg/m^3; this value has been withdrawn due to the introduction of a new system of legal limit values. The value had been adopted from the ACGIH 8-h TWA TLV of 2001.[134] ACGIH stated that this exposure limit will minimize the potential risk to workers from eye injury, dermatitis, central nervous system effects, and other systemic effects potentially associated with occupational exposure to hydroquinone (ACGIH 2001b[134]). Insufficient data were considered available to recommend skin, sensitization, or carcinogenicity notations.

Classification and labelling

See Table 18 for classification and labelling of hydroquinone derived by national and international organisations.

In Germany, hydroquinone was labelled for sensitization; carcinogen category 2; germ cell mutagen category 3A (last German update in 2003[136]; Greim 1998[135]). The argumentation was the following: Hydroquinone is genotoxic. In mammalian cells in vitro and in vivo it induces micronuclei, chromosomal aberrations, DNA single strand breaks, oxidative damage to DNA and in vitro also gene mutations and SCEs. In addition it has C-mitotic effects. DNA-adducts were detected in vitro, adducts in cellular macromolecules also in vivo. In two well-documented carcinogenicity studies with oral administration of hydroquinone an increase was found in the incidence of hyperplasia and adenomas of the kidneys in male rats and mice; liver adenomas were also detected in the mice. In one study thyroid gland hyperplasia and adenomas, and forestomach hyperplasia occurred in mice, and in another study mononuclear leukaemia was found in female rats. Pathological changes in the blood count and damage to the bone marrow were also variously reported in studies with repeated administration. A cohort study, which, however, is only of limited meaning due

Table 18 Classification and labelling of hydroquinone in various countries

Country	Classification/labelling	Reference
Germany	H (skin absorption) Sh (skin sensitization) Group 2 (carcinogenicity) Group 3A (germ cell mutagen)	Greim 1998[135]; MAK-Werten 1994, 2000[136]
USA	Group A3 (carcinogenicity)	ACGIH 2008[1]
USA	Not classified for carcinogenicity	EPA 1992 (revised 1999)[5]
UK	Not classified for carcinogenicity	HSE 2000[18]
IARC	Group 3 (carcinogenicity)	IARC 1999a[8]

to the small number of participants, yielded no evidence of hydroquinone-induced tumours in man. Because of its genotoxicity and the results of carcinogenicity studies in animals hydroquinone is classified in Category IIIA2 in the "List of MAK and BAT Values". Hydroquinone induces chromosomal aberration and hyperploidy in germ cells of male mice and is therefore classified in Germ cell mutagens group 3. Allergological investigations in man and animals have shown hydroquinone to have sensitizing effects. However, despite the numerous opportunities for exposure, sensitization is rarely observed at the usual maximum concentrations used of 2 %. For this reason the substance has provisionally not been designated with an "S".[135]

ACGIH concluded that the incidence of bladder carcinomas in mice bladder-implanted with cholesterol pellets containing 20% hydroquinone was significantly greater than in mice receiving a cholesterol-only pellet implant (Boyland et al. 1964, cited in ACGIH 2008).[1] Equivocal evidence of carcinogenicity expressed as hepatocellular neoplasm in rats treated with hydroquinone via water-gavage has also been reported.[1,98] Accordingly, an A3 (Confirmed Animal Carcinogen with Unknown Relevance to Humans) notation was assigned to hydroquinone. Insufficient data were considered available to recommend labelling for skin irritation or sensitization.[1]

EPA has not classified hydroquinone for carcinogenicity.[5]

HSE considered the available data on the mechanism(s) of tumour formation in experimental animals not clearly identified and consequently found it difficult to judge the relevance for humans. But in view of some positive genotoxicity findings in vitro, HSE had also difficulties in deciding to a "non-genotoxic" mechanism involved in the tumour production.[18]

IARC concluded in 1999 that there is inadequate evidence in humans for the carcinogenicity of hydroquinone. There is limited evidence in experimental animals for the carcinogenicity of hydroquinone. The conclusion was that hydroquinone is not classifiable as to its carcinogenicity to humans (Group 3). (IARC 1999a[8]; see also the section on benzoquinone in IARC 1999b).[9]

benzoquinone

Occupational exposure limits

See Table 19 for occupational exposure limits for benzoquinone previously derived by national and international organisations.

Table 19 Established limits for occupational exposure to benzoquinone in various countries.

Country Organisation	Occupational exposure limit (mg/m^3)	Time-weighted average [a]	Note [b]	Reference
Denmark	0.4	8 h	OEL	2007
Finland	0.45	8 h	-	2005
	1.3	15 min	STEL	
Germany				
AGS [c]	-	-	-	2007
DFG [d]	-	-	-	
Norway	0.4	8 h	-	2007
Sweden	0.4	8 h	-	2005
	1.3	15 min	STEL	
UK			-	
HSE [e]	-	8 h	MEL	2007
USA				
ACGIH [f]	0.44	8 h	TLV	2001
NIOSH [g]	0.4	8 h	REL	2004
OSHA [h]	0.4	8 h	PEL	2004
Iceland	0.4	8 h	-	1999
European Union				
SCOEL [i]	-	-	-	-

[a] Time-weighted average (TWA): a maximum mean exposure limit based generally over the period of a working day.
[b] Abbreviations - OEL: occupational exposure limit; STEL: short-term exposure limit; MEL: maximum exposure limit; TLV: threshold limit value; REL: recommended exposure limit; PEL: permissible exposure limit.
[c] Ausschuss für Gefahrstoffe.
[d] Deutsche Forschungsgemeinschaft.
[e] Health and Safety Executive.
[f] American Conference of Governmental Industrial Hygienists.
[g] National Institute for Occupational Safety and Health.
[h] Occupational Safety and Health Administration.
[i] Scientific Committee on Occupational Exposure Limit Values.

Up to January 1, 2007, the limit value for occupational exposure to benzoquinone in The Netherlands was 0.4 mg/m^3; this value has been withdrawn due to the introduction of a new system of legal limit values. The value had been adopted from the ACGIH 8-h TWA TLV, which is based on industrial experience that showed mild, transient ocular irritation at air concentrations of 0.44 mg/m^3 (0.1 ppm) or greater, whereas no systemic effects have been observed at this concentration (0.44 mg/m^3). Insufficient data were considered available to recommend skin, sensitization, or carcinogenicity notations (ACGIH 2001a).[2]

Classification and labelling

In Germany, benzoquinone was labelled for skin sensitization; carcinogen category 3B (substances for which in vitro tests or animal studies have yielded evidence for carcinogenic effect that is not sufficient for classification in one other categories); germ cell mutagen category 3B[136] (last German update in 2000).[102]

ACGIH was of the opinion that insufficient data were available to recommend skin, sensitization, or carcinogenicity notations, or a TLV-STEL.[2]

EPA concluded in 1992 (revised in 2000) that the available data were insufficient to evaluate the carcinogenicity of the compound. EPA has not classified benzoquinone for carcinogenicity.[6]

IARC concluded in 1999 that the available data on benzoquinone did not allow an evaluation of its carcinogenicity. No epidemiological data relevant to the carcinogenicity of benzoquinone were available. There is inadequate evidence in experimental animals for the carcinogenicity of benzoquinone. The overall evaluation was that benzoquinone is not classifiable as to its carcinogenicity to humans (Group 3) (IARC 1999b[9]; see also the section on hydroquinone in IARC 1999a).[8]

Chapter 9

Hazard assessment

9.1 Assessment of the health risk

hydroquinone

There are no human data on hydroquinone that might justify their use for deriving a health-based occupational exposure limit: studies lack exposure data, exposure is not exclusively to hydroquinone, or the group size is too small.

Hydroquinone was reported to induce skin depigmentation, cornea pigmentation and sensitization in humans after occupational exposure. Eye irritation, skin irritation, depigmentation and sensitization were also observed in animal studies. In a local lymph node assay hydroquinone was classified as a strong sensitizer with an EC3 of 0.11%. The EC3 can be used as a point of departure for deriving a limit value for dermal sensitization (IPCS 2012), but there is as yet no scientifically reliable method to extrapolate dermal sensitization to respiratory sensitization (IPCS 2012).[137] Hence the derivation of a health-based limit value for inhalation on the basis of dermal sensitization is not applicable.

Although ACGIH derived in 2008[1] a TLV of 1 mg/m^3 for hydroquinone based upon its eye irritating properties, the Committee is of the opinion that ACGIH appears to have evaluated it in a rather worst-case approach. Firstly the human occupational studies which formed the basis of its evaluation all date from over four decades ago, with exposure data that are not very reliable in view

of the then available chemical analytical power, and secondly there were co-exposures to benzoquinone and aniline which are likely to have influenced negatively the eye and skin irritations observed.

A number of animals studies on hydroquinone toxicity is available. The effects observed in the relevant studies are summarised in Table 20, arranged in order of increasing NOAELs.

The lowest NOAELs were observed in the 13- and 104-week oral exposure studies with F344 rats and B6C3F1 mice, and in the two generation reproduction studies.

In the 13-week oral exposure study of hydroquinone with F344 rats and B6C3F1 mice,[100,102] gavage administration at a level of 25 mg/kg bw/day induced changes in liver weights. Toxic nephropathy, characterised by tubular cell degeneration in the renal cortex, was seen in 7/10 male and 6/10 female rat at 200 mg/kg bw/day and in 1/10 females receiving 100 mg/kg bw/day. The Committee does not consider the changes in liver and kidney weights as relevant effects, and set the NOAEL for toxic nephropathy at 25 mg/kg bw/day.

In the 104-week oral carcinogenicity rat study, reduced body weights and renal tubular adenomas in males were seen at 50 mg/kg bw/day. The NOAEL in this study was 25 mg/kg bw/day. In the mouse study, increased relative liver weights in males, hepatocellular adenomas in females, and thyroid hyperplasia in males and females were observed at 50 mg/kg bw/day for 104 weeks. No NOAEL was derived in this study. The Committee considers these liver and kidney adenomas not to be relevant for humans.

The available data on reproduction showed that hydroquinone is not a reproductive toxicant. In one of the developmental studies, slight but statistically insignificant malformations were observed, when expressed per foetus or on a litter basis, resulting in a NOAEL for developmental toxicity of 75 mg/kg bw/day.

The available data on neurotoxicity showed mild, transient tremors and reduced home-cage activity at 64 mg/kg bw/day in a 13-week rat study; the NOAEL for neurotoxicity was 20 mg/kg bw/day. Also in a 2-generation rat study mild and transient tremors were seen at 50 mg/kg bw/day, with a NOAEL of 15 mg/kg bw/day.

Table 20 Effects induced by hydroquinone at different exposure levels and time frames (including NOAELs/LOAELs as reported by the authors)

NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Duration	Species	Effects	Reference
Eye irritation					
-	-	Eye instillation	Rabbit, dog	Eye irritation (no or only semi-quantitative data)	WHO 1994[13], OECD 1996[33]
Dermal (skin itrritation and sensitization)					
-	-	Up to 13 weeks	Guinea pig	Skin irritation and depigmentation (no or only semi-quantitative data)	WHO 1994[13], OECD 1996[33]
-	-	Induction and challenge	Guinea pig, mouse	Skin sensitization (no or only semi-quantitative data)	WHO 1994[13], OECD 1996[33]
-	EC3: 0.11%	-	Local lymph node assay	Strong sensitizer	Roberts 2007[93]
Dermal (acute and short term toxicity)					
-	> 1,000	single exposure	Guinea pig	None	OECD 1996[33]
1,900	3,840	14 days	Rat	Reduced body weight	NTP 1989[98]
4,800	> 4,800	14 days	Mouse	None	NTP 1989[98]
Oral toxicity					
15	50	2 generations	Rat (gavage)	Mild, transient tremors and decreased body weight (F0 and F1)	Blacker 1993[121]
20	64	13 weeks	Rat (gavage)	Mild, transient tremors and reduced home-cage activity	Bernard 1988, in OECD 1996[33]; Topping 2007[101]
< 25	25	13 weeks	Rat (gavage)	Decreased absolute and relative liver weights (m). Increased absolute and relative liver weights (f) at doses ≥ 100 mg/kg bw/day. No toxic nephropathy.	NTP 1989[98]; Kari 1992[100]
< 25	25	13 weeks	Mouse (gavage)	Increased absolute and relative liver weights (m).	NTP 1989[98]; Kari 1992[100]
25	50	104 weeks, 5 days/week	Rat (gavage)	Lower mean body weights (m). Renal tubular adenomas (m).	NTP 1989[98]; Kari 1992[100]
25	75	2 generations	Rabbit (gavage)	Decreased food consumption (maternal)	Murphy 1992[123]
25	150	2 generations	Rabbit (gavage)	Minimal developmental alterations (developmental)	Murphy 1992[123]
< 50	50	104 weeks, 5 days/week	Mouse (gavage)	Increased relative liver weights (m). Hepatocellular adenomas (f).	NTP 1989[98]; Kari 1992[100]
100	300	2 generations	Rat (gavage)	Decreased food consumption and body weight (maternal and development).	Krasavage 1992[122]
125	250	14 days	Mouse (gavage)	Mortality, tremors, decreased final mean body weight.	NTP 1989[98]; Kari 1992[100]

< 350	350	104 weeks	Rat (via diet)	Reduced body weight gain (m, f). Higher absolute and relative liver and kidney weights (m). Higher relative kidney weight (f). Chronic nephropathy (m, f). Renal tubular adenomas (m)	Shibata 1991[104]
-	298 - 390	single exposure	Rat (gavage)	LD_{50}	OECD 1996[33]; Carlson 1953[96]
-	390 - 680	single exposure	Mouse (gavage)	LD_{50}	OECD 1996[33]
250	500	14 days	Rat (gavage)	Mortality, tremors, reduced final mean body weight.	NTP 1989[98]; Kari 1992[100]
< 1,050	1,050	96 weeks	Mouse (via diet)	Reduced body weight gain (m). Higher relative liver and kidney weights (f). Hepatocellular adenomas (m).	Shibata 1991[104]

Furthermore the Subcommittee on the Classification of carcinogenic substances concluded that hydroquinone may induce mutations under high exposure conditions, probably due to the formation of ROS. But as long as exposure levels to hydroquinone are below levels inducing local cytotoxicity (and consequently hyperplasia), the risk for carcinogenic effects was considered to be negligible. Based on the available information, the subcommittee was of the opinion that the data are as yet insufficient to evaluate the carcinogenic properties of hydroquinone.

Based on these conclusions, and the consideration of the Subcommittee on Classification of carcinogenic substances that the carcinogenic mechanism of hydroquinone in animal studies is in all probability a non-stochastic genotoxic mechanism (due to ROS-generation, while inhibition of topoisomerase II may also play a role), the Committee adopts a threshold approach for deriving a HBROEL for hydroquinone.

benzoquinone

In humans, exposure to high air levels of benzoquinone may result in irritation of the eyes. Dermal exposure of humans may result in dermatitis and severe irritation. Older studies reported that no systemic effects were observed following prolonged occupational exposure to benzoquinone vapour at a level of 0.44 mg/m^3 for periods up to 5 years.

Only a limited number of animal studies is available. In a local lymph node assay benzoquinone was classified as an extreme sensitizer with an EC3 of 0.01%. The EC3 can be used as a point of departure for deriving a limit value for

dermal sensitization (IPCS 2012), but there is as yet no scientifically reliable method to extrapolate dermal sensitization to respiratory sensitization (IPCS 2012).[137] Hence the derivation of a health-based limit value for inhalation on the basis of dermal sensitization is not applicable.

Sub-acute toxicity studies on benzoquinone have been performed via inhalation exposure and intraperitoneal and subcutaneous injections. The critical effects were all of a haematological character and included decreases in blood cells, methaemoglobinemia and trombopenia. A LOAEL of 0.27-0.36 mg/m^3 was derived in 4 months inhalation study in rats. However, as the original document could not be retrieved, the relevance and accuracy of these data can not be assessed.

There are only two very limited carcinogenicity studies on benzoquinone. Due to their poor quality and high mortality rates, no NO(A)ELs or LO(A)ELs could be derived from these.

With regard to the current standard on scientific reliability there are no human data on benzoquinone that might justify their use for deriving a HBROEL. Also the data from experimental animal studies provide too little information to derive a HBROEL.

In Section 9.4 some additional considerations regarding the toxic potency of benzoquinone are presented.

9.2 Recommendation of the health-based occupational exposure limit

hydroquinone

With regard to the current standard on scientific reliability there are no human data on hydroquinone that might justify their use for deriving a HBROEL, and hence the Committee used experimental animal data as the starting point in deriving a HBROEL. The Committee selected the 2-year mouse carcinogenicity study by NTP as the pivotal one (NTP 1989[98]; Kari et al. 1992[100]). In this study a statistically significant increase of the incidence of thyroid hyperplasia was observed in both sexes at 50 and 100 mg/kg bw/day, albeit with a serious difference in susceptibility for this effect between males and females. The Committee also took into account the neurotoxic effects with NOAELs of 20 and 15 mg/kg bw/day (tremors and reduced home-cage activity at 64 mg/kg bw/day in the 13-week rat study of Bernard 1988 (cited in OECD 1996[33]) and Topping 2007[101], and tremors and decreased body weight at 50 mg/kg bw/day in the 2-generation study of Blacker 1993[121], respectively). However, these neurotoxic

effects were mild and of a transient character. Moreover, a BMD* analysis of the study by Blacker (the only one with quantitative data) showed the lowest BMDL for 10% extra risk to be 48 mg/kg bw/day, which is much higher than the BMDL derived from the thyroid effects (see below).

The 2-years NTP mouse gavage study (NTP 1989[98], Kari et al. 1992[100]) with the data on thyroid hyperplasia (Table 21; see also Table 14) was thus selected as the pivotal study for deriving the HBROEL. With these data BMD analyses were done.

However, analysis of the study data using the software programme PROAST (a software package developed for analysing dose-response data of toxicity studies and calculating BMDs; Slob 2002[138], 2009[139]; EFSA 2011[140]) showed that the data of the males and the females can not be combined. The incidences of the lesions in the males differ too much from the incidences in the females, both in the controls and in the dosed groups. In addition, a BMD analysis of the results with the females is not possible: the incidences flattens off already at the low dose, and since there are only two dose groups it is not possible to estimate a BMD for e.g. 10% extra risk (Slob, personal communication). Only for the results with the males a BMD analysis is possible, and consequently an adjustment factor will be needed to correct for the difference in susceptibility between males and females. The BMD analysis of the data of the males, using US-EPA's BMD software[141], is outlined in Annex K. Only a BMD/BMDL for 10% extra risk could be estimated, the data were of insufficient quality to derive a reliable BMD/BMDL for 5% extra risk.

The lowest BMD and BMDL for 10% extra risk are produced by the log-logistic model, with a BMD of 25.1 and BMDL of 15.7 mg/kg bw/day. The other models resulted in BMDs varying from 28.5 - 43.5 mg/kg bw/day and BMDLs varying from 19.1 - 34.0 mg/kg bw/day. The Committee considers the BMDL of 15.7 mg/kg bw/day the appropriate starting point for the derivation of the HBROEL.

Since the exposure levels inducing effects in the acute and short term exposure studies are significantly higher than those at in the sub-acute and

Table 21 Thyroid hyperplasia in mice (2-year gavage study; NTP 1989[98], Kari et al. 1992[100]).

Thyroid hyperplasia in mice (B6C3F1)	Number of animals with lesions / total number of animals per dose group					
	males			females		
Dose (mg/kg bw/day)	Control	50	100	Control	50	100
Thyroid hyperplasia	5/55	15/53	19/54	13/55	47/55	45/55

* BMD: benchmark dose; BMDL: benchmark dose at lower 95% confidence limit.

chronic studies, no elaboration of a Short Term Exposure Limit (STEL) or ceiling value for hydroquinone is considered necessary.

In order to convert this animal BMDL into a HBROEL the following corrections are made:

- Correction for the difference in susceptibility for thyroid hyperplasia between male and female mice;
- Differences in sensitivity between mice and men;
- Route-to-route extrapolation, i.e., from the oral to the inhalation route of exposure.

From the data shown in Table 21 it appears that females are approximately three times as sensitive as males for the thyroid hyperplasia resulting from exposure to hydroquinone. Hence an adjustment factor of 3 is considered appropriate to correct for this difference in susceptibility (there are no data that would justify another factor). The observed BMDL of 15.7 mg/kg bw/day is thus adjusted to 5.2 mg/kg bw/day.

With regard to the possible difference in sensitivity between mice and man (i.e., both kinetic and dynamic differences) an uncertainty factor of 3 will be used. Applying this results in a value of 1.7 mg/kg bw/day.

Based on the available data it is concluded that oral absorption is nearly complete, and similar for mice and man. It is also assumed, from a precautionary principle view, that absorption by inhalation is 100%. Consequently, an external burden of 1.7 mg/kg bw/day will result in an equal internal daily human body burden of (1.7 mg/kg bw x 70 kg =) 119 mg. When this internal dose is divided by the total volume of air inhaled by a human during a working day of 8 hours (10 m^3) the adjusted mouse BMDL is converted to an inhalation BMDL in humans of 12 mg/m^3.

For intraspecies differences, i.e., to compensate for possible differences in sensitivity among workers, an uncertainty factor of 3 will be used. Taking this last adjustment into account results in a HBROEL for hydroquinone of 12/3 mg/m^3 = 4 mg/m^3.

Most occupational exposure limits for hydroquinone of other countries are 2 mg/m^3 or lower. In general these are based on its eye and skin irritation properties. But the quantitative data of these properties date from over four decades ago, with exposure data that are not very reliable in view of the then available chemical analytical power. Moreover, there were co-exposures to benzoquinone and aniline which are likely to have influenced negatively the eye and skin irritations observed. Although only limited data are available, the

Committee expects that at the level of 4 mg/m^3 the risk for eye and skin irritation and sensitization is negligible (see also Sections 7.1.1 and 8.2).

Skin notation

Absorption of hydroquinone through the human skin averaged 1.6, 2.3 and 2.5% per hour of the dose in the first, second and third 4 hours after application of 125 μg/cm^2 (see Section 5.1). When 2,000 cm^2 human skin would be exposed for 1 hr, the quantity absorbed would be at least 2,000 x 125 x 0.016 µg = 4,000 µg. In view of the exposure level advised, dermal absorption can add considerably (more than 10%) of the amount taken up via the inhalatory route. For benzoquinone the same way of reasoning is assumed, in absence of experimental data. Therefore the Committee recommends a skin notation for hydroquinone and benzoquinone.

benzoquinone

The available data do not allow the derivation of a HBROEL for benzoquinone (see also additional considerations in Section 9.4).

9.3 Groups at extra risk

No groups at extra risk have been identified.

9.4 Additional considerations - benzoquinone

Since benzoquinone and hydroquinone are metabolites of each other (converted at reaction rates dependent on prevailing local conditions), it is clear that toxicity observed with one of the two will be also relevant for the other, though there may be a difference in potency.

Indeed, as far as available data permit, their toxicity profiles have a lot in common: both substances are irritating to eyes and skin, act as skin sensitizers, and have a comparable genotoxicity profile. The database on hydroquinone, however, is more complete: i.e., for benzoquinone there are no adequate data on (sub)chronic toxicity, carcinogenicity, and reproductive toxicity. Thus, for these data gaps for benzoquinone, available data on hydroquinone could be used to indicate its potential effects.

As suitable data for benzoquinone itself are lacking, the Committee tries to use the HBROEL established for hydroquinone to derive a HBROEL for

benzoquinone, addressing the difference in potency between the two toxicants. The Committee is of the opinion that a threshold approach is suitable for benzoquinone because the reasoning followed for hydroquinone (see Section 7.2.6) is also considered valid for benzoquinone.

The data that allow a comparison on differences in toxic potency between hydroquinone and benzoquinone are summarized in Table 22. In addition to a few quantitative data on skin irritation and dermal sensitization, only some comparable data for acute oral toxicity are available. Already from these limited data it appears that the relative toxic potency of benzoquinone as compared to that of hydroquinone varies with the endpoint considered. Therefore the Committee concludes that this is not a sufficient basis for deriving a HBROEL for benzoquinone.

The value of 0.4 mg/m^3 (8 hr TWA) that was valid in the Netherlands until January 1, 2007 (due to the introduction of the new system of legal limit values; see Table 19) was adopted from the ACGIH (2001a).[2] It was based on industrial experience that showed ocular irritation at air concentrations of 0.44 mg/m^3, whereas no systemic effects have been observed during approximately 5 years of occupational exposure at this concentration. Likely this limit value protects against adverse systemic effects of exposure to benzoquinone. ACGIH (2001)[2] was of the opinion that insufficient data were available to recommend skin, sensitization, or carcinogenicity notations.

Table 22 Comparative toxicity of hydroquinone and benzoquinone.

Toxicity parameter	Hydroquinone	Benzoquinone	Reference
Oral LD_{50} rat (mg/kg bw)	300-390	130-165	Tables E-3A and E-3B
NOAEL for irritation in female guinea pig (% solution in water; sc injection)	0.1%	0.001%	Rajka and Blohm 1970[86]
EC3 for sensitization in a murine local lymph node assay	0.11%	0.01%	Roberts et al. 2007[93]
Internal daily dose inducing systemic toxicity (mg/kg bw/day) in the rat:			
• rat (oral, gavage, 3 months)	< 25[a]		NTP 1989[98]; Kari et al. 1992[100]
• rat (inhalation, 4 h/day, 4 months)		< 0.6[a]	Anonymous, cited in MAK 2000[102]

[a] derived assuming 100% absorption, default body weight 300 g, and default respiration rate 200 ml/min.

9.5 Health-based recommended occupational exposure limits

The Dutch Expert Committee on Occupational Safety recommends a health-based recommended occupational exposure limit for hydroquinone of 4 mg/m^3, as 8 hour time weighted average.

The Committee concludes that the available data on the toxicity of benzoquinone are of insufficient quality to derive a health-based recommended occupational exposure limit for benzoquinone.

Chapter 10

Recommendation for research

It is recommended to investigate the toxicity of hydroquinone and benzoquinone after inhalation, preferably in a chronic exposure experiment, to compare the difference in toxic potential between benzoquinone and hydroquinone.

In addition, it is recommended to investigate the irritating properties of hydroquinone and benzoquinone in studies according to OECD guidelines, in order to get an accurate insight at which concentrations occupationally exposed people might start to develop local toxic effects. If possible, human studies should be used to support any animal data.

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H	Mutagenicity studies
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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, an '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
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- D.J.J. Heederik
  Professor of Risk Assessment in Occupational Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational Hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders, Utrecht
- H. van Loveren
  Professor of Immunotoxicology, Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  Occupational Physician, Netherlands Centre for Occupational Diseases, University of Amsterdam, Amsterdam
- A.H. Piersma
  Professor of Reproductive Toxicology, Utrecht University, Utrecht, and National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  Professor of Molecular Biology, VU University Amsterdam, and Netherlands Cancer Institute, Amsterdam

- I.M.C.M. Rietjens
  Professor of Toxicology, Wageningen University and Research Centre, Wageningen
- G.M.H. Swaen
  Epidemiologist, Dow Benelux NV, Terneuzen
- R.C.H. Vermeulen
  Epidemiologist, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R.A. Woutersen
  Toxicologic Pathologist, TNO Quality of Life, Zeist, and Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- P.B. Wulp
  Occupational Physician, Labour Inspectorate, Groningen
- B.P.F.D. Hendrikx, *advisor*
  Social and Economic Council, The Hague
- A.J. Baars, *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**

Letter of submission

Subject	: Submission of the advisory report *Hydroquinone and benzoquinone*
Your Reference	: DGV/MBO/U-932342
Our reference	: U-7449/BJB/fs/459-S67
Enclosed	: 1
Date	: December 3, 2012

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to *hydroquinone and benzoquinone*.

This advisory report is part of an extensive series in which health-based recommended exposure limits are derived for the concentrations of various substances in the workplace. The advisory report in question was prepared by the Health Council's Dutch Expert Committee on Occupational Safety (DECOS) and assessed by the 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 2012 for public review. The following organisation has commented on the draft document:

- Th.J. Lentz, National Institute for Occupational Safety and Health, Cincinnati (OH), USA.

Annex **E**

Human data

Human studies, hydroquinone.

	Compound	Exposure through	Subjects, sample size	Exposure duration	Exposure concentration	Effects	Reference
Oral							
Acute poisoning	Hydroquinone	Accidental ingestion	-	-	80-200 mg/kg bw	Death	WHO 1994[13]
Controlled study	Hydroquinone	Ingestion	2 males, volunteers	5 months	500 mg/day	No effects in blood and urine	EC 2000[7], WHO 1994[13]
			17 males + females, volunteers	3-5 months	300-500 mg/day (~6 mg/kg bw/day)		
Dermal							
Cohort	Hydroquinone	Occupational exposure Controls: general population of Denmark	Danish lithographers registered with the Danish Union of Lithographers in 1947 or later (n=837)	About one-quarter of the cohort members reported working regularly with hydroquinone for photographic development.	No information	The SIR[a] for cancers was 0.9. For no site except skin there were more than three cases. Five cases of malignant melanoma occurred, of which two had reportedly been exposed to hydroquinone.	Nielsen et al. 2003[83]
Epidemio-logical	Hydroquino-lone, among other active compounds	Skin lightening cosmetics, bleaching creams	Traders from the Lagos metropolis, Selected (ad random): 130 males, 320 females Exposed: 96 males, 252 females	Modal: 1-3 years 8.3% < 6 months 12.6%: 5 years	Crèmes contain maximal 2% hydroquino-lone	Ochronosis[b] (stated to be associated with hydroquinolone exposure)[c]	Adebajo 2002[142]
Cross sectional	Hydroquinone, among other active compounds	Skin lightening cosmetics	Hospitalized women Exposed: 47 Not exposed 100	-	-	Ochronosis[c]	Raynaud et al. 2001[143]
Case study	Hydroquinone	Skin lightening cosmetics	Two patients experiencing effects Exposed 2/2	-	Crèmes containing 2% hydroquinone	Ochronosis-like pigmentation	Hoshaw et al. 1995[144]

Case study	Hydroquinone	Occupational exposure	Three workers in a hydroquinone producing factory experiencing effects Exposed 3/3	Prolonged period (about 10 years)	No information	Corneal damage; conjunctival pigmentation and degenerative changes	Naumann 1966[145]
Cohort study	Hydroquinone	Occupational exposure	105 people experiencing evidence of exposure (out of 201 persons that might have received significant exposure) were followed for 10 years	No information	No information	Altered curvature of the cornea, which may become apparent long after exposure	Anderson et al. 1958[133]
Follow up after modernization, interviews, dermatological examinations, patch testing	Hydroquinone	Working in a film laboratory, study period 1983-1986	78 employees of which 54 chemically exposed.	Various	No information	34/54 exposed subjects had dermatoses; 12/54 were contact allergic to film chemicals. Reduced chemical exposure reduced the occurrence and severity of skin problems	Lidén 1989[146]
Inhalation							
Case study	Hydroquinone	Occupational exposure	Two female medical radiation technologists in same primary case center	16 yr	No information	Acute monocytic leukaemia; promyelo-genous leukaemia	Makropoulos & Alexopoulos 2006[147]
Cohort	Hydroquinone	Occupational exposure	Workers in motion picture film processing in California n=2646	At least 3 months between 1960-2000	Three measurements: before 1981: below detection level; after 1981: < 0.014 mg/m^3 and 0.052 mg/m^3	SMR[d] most malignancies below 1.0; Non Hodgkin lymphoma SMR 2.2 (1.0-4.0). Respiratory mailignancies SMR 1.9 (1.1-3.0) n=18	Fryzek et al. 2005[84]

Cohort, death causes	Hydroquinone	Occupational exposure Controls: general population and occupational controls	Workers at a Tennessee chemical plant (USA), study period 1972-1982, n= 9,000	No information	No information	Mortality was lower than that in the population at large and consistent with the occupational controls	Pifer et al. 1986[148]
Cohort	Hydroquinone	Occupational exposure Controls: general population and occupational controls	Workers at a Tennessee chemical plant (USA), study period 1942-1990 n=879	Mean employment duration 13.7 years; mean follow-up from exposure year 26.8 year	Average dust levels from 0.1-6.0 mg/m^3, with levels > 2 mg/m^3 for most of the period of operation	The SMR for all causes of death as well as for cancers combined was significantly below 1.0 for both comparison populations.	Pifer et al. 1995[45]
Epidemiologic investigations, mortality, cancer incidence, sickness-absence	Hydroquinone, quinines and other chemicals	Occupational exposure; study period 1964-1979	Workers in nine Eastman Kodak Color Print and Processing laboratories in the US n=478 Plant controls	-	Hydroquinone < 0.01 mg/cm^3 (1 sample) Quinone vapours 0.2-0.9 ppm (8 samples)	No significant excess of mortality, sickness-absence or cancer incidence	Friedlander et al. 1982[82]
	Benzene	Occupational exposure, quantified by using batches and measuring phenol in urine	Workers in Shanghai, China Controls 44 Exposed 43	Medium 31 ppm 8-hr time weighted average	# 31 ppm (n= 21) > 31 ppm (n= 22)	Trisomy of chromosome 9 statistically significant increased in the > 31 ppm group	Zhang et al. 1996[149]
	Hydroquinone, trimethyl-hydroquinone, retinene-hydroquinone	Occupational exposure	Workers in a large chemical plant engaged in the synthesis of methionine and vitamins Exposed 33 Not exposed 55	-	-	Increased prevalence of allergic symptoms (dyspnoca and reversible obstruction)	Choudat et al. 1988[150]

[a] SIR:standardised incidence ratio.

[b] Ochronosis: a brown, blue, or black pigmentation that develops in the skin, cartilage, and various organs of patients; the condition is often associated with alkaptonuria.[143]

[c] Skin lightening cosmetics based on different active ingredients have been studied. The side effects experienced are presented without splitting up. Therefore no conclusions with respect to hydroquinolone can be drawn.

[d] SMR:standardised mortality ratio.

Annex **F**

Animal data (in vivo)

Table F-1 Absorption, distribution, metabolism, and excretion.

Animal species	Test conditions	Results	Reference
Dermal absorption			
Rat (F344) skin in vitro	Hydroquinone 5% aqueous solution	Absorption rate through rat skin is 1.09 µg/cm²/h	Barber et al. 1995[46]
Human abdominal skin in vitro	Hydroquinone 5% aqueous solution	Absorption rate through human skin is 0.522 µg/cm²/h	Barber et al. 1995[46]
Toxicokinetics			
Rat (Sprague-Dawley), males	[^{14}C-]Hydroquinone 3, 30, or 200 mg/kg bw, single dose, by gavage (3 groups of animals) Or Hydroquinone (unlabelled) 200 mg/kg bw, 4 daily doses, by gavage, followed by [^{14}C-]hydroquinone, 200 mg/kg bw, single dose, by gavage (1 group of animals)	The primary route of elimination was the urine. Of the tissues examined highest concentrations in the liver and kidneys. The majority of the urinary metabolites was identified as glucuronide conjugates with a smaller portion as sulphate conjugates; only 1% was unchanged hydroquinone. After repeated dose there was a shift to glucuronide conjugates at the expense of sulphate conjugates.	DiVincenzo et al. 1984[48]
Rat (F344), males	[U-^{14}C-]Hydroquinone 50 mg/kg bw, single dose, gavage, iv, or it instillation (4 animals per treatment). Of some groups, blood was collected for up to 8 h and other groups were euthanized at 10, 20, 40, 60, 120, and 480 h.	Absorption and elimination were rapid; distribution was similar for the three routes. Radioactivity in the blood was associated initially with the plasma, but by 4 h most of the radioactivity is associated with the red blood cells. Only 1% of the total radioactivity in plasma ultrafiltrate was unaltered hydroquinone. Glucuronide and GSH conjugates of hydroquinone were detected 40 min after gavage administration.	Fox et al. 1986 (unpublished), cited in OECD 1996[33]
Rat (F344), males and females	[^{14}C-]Hydroquinone 25 or 350 mg/kg bw, po, single dose, or after 14 repeated doses of unlabelled hydroquinone or [^{14}C-]hydroquinone, 25 or 150 mg/kg bw, dermal application, 24 h occlusive. (OECD 417)	After oral administration, absorption was rapid. Blood concentrations after dermal application were generally below the limit of detection. Elimination followed a biphasic character and occurred within the first 8 h after oral administration. The primary route of elimination was the urine. The liver and kidneys contained the highest concentrations. Approximately 45-53% of the urinary metabolites after an oral dose was the glucuronide conjugate and 19-33% was the sulphate conjugate. The parent compound was less than 3% of the dose.	English et al. 1988 (unpublished), cited in OECD 1996[33]; English & Deisinger 2005[49]

Rat (F344), male	[^{14}C]-Hydroquinone 1.8 mmol/kg, po, or 1.8 mmol/kg po, at day 15 after 14 daily doses of unlabelled hydroquinone.	Metabolites found in urine: hydroquinone-glucuronide, hydroquinone-sulphate, hydroquinone-mercapturates [2-(GS-S-yl)HQ, 2,5-bis(GS-S-yl)HQ, 2,6-bis(GS-S-yl)HQ, 2,3,5-tris(GS-S-yl)HQ]. Subchronic administration of hydroquinone increases the rate and extant by which hydroquinone is metabolized to its nephrotoxic thioethers	Lau et al. 1996[55]
Rat (Sprague-Dawley), male	[^{14}C]-Hydroquinone, 1.8 mmol/kg, ip, after acivicin* pre-treatment	Metabolites detected in bile: 2-(GS-S-yl)HQ (18.9 ± 2.7 µmol), 2,5-bis(GS-S-yl)HQ (2.2 ± 0.6 µmol), 2,6-bis(GS-S-yl)HQ (0.7 ± 0.3 µmol), 2,3,5-tris(GS-S-yl)HQ (1.2 ± 0.1 µmol), and 2-(cystein-S-ylglycyl)hydroquinone. 2-(N-Acetylcystein-S-yl)HQ was the only urinary thioether metabolite (11.4 ± 3.6 µmol) identified. The quantity of S-conjugates excreted in urine and bile within 4 h of hydroquinone administration was 34.3 ± 4.5 µmol (4.3 ± 1.1% of dose).	Hill et al. 1993[41]
Dog (Beagle), male	[^{14}C-]Hydroquinone, 25 mL of a 4.5 g/L solution in water, dermal application, 60 min. The solution was held in contact with the shaved skin for 60 min using absorption cells.	No measurable radioactivity was found in blood. Urinary excretion of radioactivity was low with the peak between 24 and 48 h. The dermal absorption rate was calculated to be 1.1/µg/cm^2/h	Hamilton 1985 (unpublished), cited in OECD 1996[33]

HQ: hydroquinone
GS: glutathione moiety
ip: intraperitoneal
iv: intraveneous
it: intratracheal
po: per os
* acivicin is an irreversible inhibitor of γ-glutamyltransferase

Table F-2 Irritation and sensitisation studies.

Animal species	Test conditions	Result	Reference
Skin irritation / Depigmentation			
Guinea pig (strain ns), female	Hydroquinone; intracutaneous injections, 0.1 ml, 0.001%, 0.01%, and 0.1% in water, 10 days	Not primarily irritating	Rajka & Blohm 1970[85]
Guinea-pig (black)	Hydroquinone; dermal application, creams containing 0%, 1%, 3%, 5%, 7% or 10%, 5 days/week for one month.	Skin irritation occurred with creams containing 5% or 10% hydroquinone. No irritation was seen at levels of 3% hydroquinone. Weak to moderate depigmentation of the skin at all dose levels.	Bleehen et al. 1968[86]
Guinea-pig (black), male and female	Hydroquinone; dermal administration in a hydrophilic ointment at concentrations of 0.1%, 1.0%, and 5.0%, 5 days/ week for 13 weeks.	The 0.1% dose caused marginal irritation; the 1.0% dose resulted in a slight to marginal irritation in 30% of the animals (mainly females); the 5.0% dose induced moderate to severe irritation and severe ulcerated inflammatory responses. At the 5% dose, approximately 40% of the animals dosed showed moderate depigmentation of the skin (females only)	Maibach & Patrick 1989, cited in WHO, 1994[13]
Guinea-pig (black), male and female	Hydroquinone; dermal application, creams containing 2% or 5%, in an oil-water emulsion, daily, 6 days/week for 3 weeks (n=24).	Depigmentation, inflammatory changes and thickening of the epidermis. A marked reduction both in the numbers of melanized melanosomes in the cells and the number of actively functioning melanocytes	Jimbow et al. 1974[87]
Guinea pig (strain ns), female	Benzoquinone; intracutaneous injections, 0.1 ml, 0.001%, 0.01%, and 0.1% in water, 10 days	0.1%: necrotic reactions 0.01%: redness and slight infiltration 0.001%: not primarily irritating	Rajka & Blohm 1970[85]
Eye irritation			
Rabbit (strain ns), sex ns	Hydroquinone crystals were placed into the right eye (n=2).	Eyrthrema of the nictitating membrane of the unwashed eye persisted to 48 h after instillation but was not observed 14 days after treatment	Toxicity report Kodak 1971, cited in OECD 1996[33]
Rabbit (strain ns), sex ns	Hydroquinone powder was instilled into the eyes daily, for 2-4 months, to the eyes of rabbits which were kept in the dark, in sunlight, in normal light, irradiated with UV light, or pre-sensitized with haematoporphyrin and then kept under either reduced light or sunlight, respectively.	Most rabbits developed pigmentation, first in the conjunctiva and then in the cornea. Pigment formation appeared earlier in animals exposed to light. Older animals seemed more prone to develop pigment than younger ones. Pigment was deposited in albino rabbit eyes as well as in those of rabbits with normal pigmentation.	Ferraris de Gaspare 1949, cited in WHO 1994[13]
Rabbit (strain ns), sex ns	Hydroquinone injections into the cornea, 0.1 ml, 0.012-0.05 mol/L	The resultant reaction was graded 5 out of the possible maximum of 100	Hughes 1948, cited in WHO 1994[13]

Guinea-pig (strain ns)	Hydroquinone; 1-3 mg powder was instilled into the eyes, twice daily for 9 weeks	Immediate but transient irritation. During the second day of application a slight corneal opacity was observed in some animals, and on the third day opacity of varying degrees occurred in most of the animals. Ulcers appeared in two animals. The eyes had fully recovered 3 days after cessation of treatment	WHO 1994[13]
Dog (strain ns)	Hydroquinone; 2-5 mg powder was instilled into the eyes, twice daily, 5 days/week for 9 weeks	Immediate but transient irritation and lacrimation. Opacity of the cornea, lacrimation and redness of the conjunctiva were produced within 4 days, but no ulcers were seen. The eye returned to normal within 2 days after cessation of treatment	Dreyer, 1940, cited in WHO 1994[13]
Skin sensitisation			
Guinea pig (strain ns)	Hydroquinone; induction with 0.1 ml 0.001% by injection; challenge with 0.001% by injection (n=18) (Intracutaneous sensitization)	Number of animals with skin reaction at challenge: 4/18. Cross-reactivity with p-phenylenediamine and benzoquinone.	Rajka & Blohm 1970[85]
Guinea pig (strain ns)	Hydroquinone; induction with 0.1 ml 2.0% by injection, or 10% by patch; challenge with 5.0% by patch (Maximization test in compliance with OECD 406)	Number of animals with skin reaction at challenge: 7/10; "strong" sensitizer	Goodwin (1981)[88]
Guinea pig (strain ns)	Hydroquinone; induction with 2.5%, by injection; challenge with 1.0% by injection or 30% by topical application. (Draize test in compliance with OECD 406)	Number of animals with skin reaction at challenge: 3/10; "weak" sensitizer	Goodwin 1981[88]
Guinea pig (strain ns)	Hydroquinone; induction with 0.5 mol/L (day 0) and 1 mol/L (day 7) by patch; challenge with 0.125 mol/L (day 21) and 0.250 mol/L (day 35). (Maximization test)	Number of animals with skin reaction at challenge: 5/10 (day 21) and 5/10 (day 35)	van der Walle et al. 1982[90]
Guinea pig (strain ns)	Hydroquinone; induction with 5 x 0.1 ml 0.45 µmol/L and 5 x 0.5 mol/L by injection; challenge with 0.115 µmol/L and 0.125 mol/L by patch. (Freund's complete adjuvant test)	Number of animals with skin reaction at challenge: 3/8 (day 21) and 4/8 (day 35) 4/8 (day 21) and 4/8 (day 35)	van der Walle et al. 1982[90]
Guinea pig (strain ns)	Hydroquinone; induction with 0.1 ml 2.0% by injection, or 1% by patch; challenge with 5.0% by patch. N=10. (Maximization test)	"Strong" sensitizer	Basketter & Goodwin 1988[91]

Guinea pig (strain ns)	Hydroquinone; induction with 0.1 ml 2.0% by injection; challenge with 10% by patch. N=10. (Modified single injection adjuvant test)	"Weak" sensitizer	Basketter & Goodwin 1988[91]
Guinea pig (strain ns)	Hydroquinone; induction with 1.0% by patch; challenge with 20% by patch. N=10. (Cumulative contact enhancement test)	"Moderate" sensitizer	Basketter & Goodwin 1988[91]
Guinea pig (strain ns)	Hydroquinone; induction with 2.0% by injection, or 1.0% by patch; challenge with 0.5% by patch. N=ns. (Maximization test),	"Extreme" sensitizer	Basketter & Scholes 1992[92]
Mouse (strain ns)	Hydroquinone; daily topical applications on the dorsal surface of each ear, 0.5 to 2.5% during 3 days. At day 4-5, i.v. injection of [^{3}H] methyl-thymidine. Determination of ^{3}H-incorporation in lymph nodes 5 h later. N=4. (Local lymph node assay)	Positive	Basketter & Scholes 1992[92]
Guinea pig (strain ns)	Benzoquinone; induction with 0.1 ml, 0.001% by injection; challenge with 0.001% by injection (n=20) (Intracutaneous sensitization)	Number of animals with skin reaction at challenge: 19/20. Cross-reactivity with p-phenylenediamine.	Rajka & Blohm 1970[85]
Guinea pig (strain ns)	Benzoquinone; Guinea Pig Maximization test, no data on concentrations used and the time schedule for challenge and induction	Cross-reactivity between p-phenylenediamine and benzoquinone.	Möllgaard et al. 1990[94]
Guinea pig (strain ns)	Benzoquinone; induction with 0.005% by injection, or 10% by patch; challenge with 2.5% by patch. (Maximization test)	"Extreme" sensitizer	Basketter & Scholes 1992[92]
Mouse (strain ns)	Benzoquinone; daily topical applications on the dorsal surface of each ear, 0.5 to 2.5% during 3 days. At day 4-5, iv injection of [^{3}H] methyl-thymidine. Determination of ^{3}H-incorporation in lymph nodes 5 h later (n=4). (Local lymph node assay)	Positive	Basketter & Scholes 1992[92]
Local lymph node assay (murine)	-	Hydroquinone EC3: 0.11% Benzoquinone EC3: 0.01%	Roberts et al. 2007[93]

ns: not specified

Table F-3A Acute toxicity studies, hydroquinone.

Animal species	Test conditions	LD_{50} (mg/kg bw)	Reference
Oral			
Rat (strain ns)	Fasted / water * (similar to OECD 401)	390	Hodge et al. 1949 (unpublished report), cited in OECD 1996[33]
Mouse (strain ns)		680	
Rat (Priestly), sex ns	Unfasted / glycerin (similar to OECD 401)	1,295	Carlson & Brewer 1953[96]
Rat (Prague-Dawley), sex ns	Unfasted / propylene glycol	1,090	Carlson & Brewer 1953[96]
	Unfasted / water	1,182	
	Unfasted / glycerine	1,081	
	Fasted / propylene glycol (similar to OECD 401)	323	
Rat (Wistar), sex ns	Unfasted / propylene glycol	731	
	Fasted / propylene glycol (similar to OECD 401)	298	
Rat (Osborne-Mendel), male and female	Fasted / water * (similar to OECD 401)	302	Woodard 1951, cited in OECD 1996[33]
Mouse (Swiss), male and female	Unfasted / water *	390	Woodard 1951, cited in OECD 1996[33]
Dermal			
Guinea pig (strain ns)	24 h, occlusive (similar to OECD 402)	> 1,000	Toxicity report Kodak 1971, cited in OECD 1996[33]

* : carrier used
ns: not specified

Table F-3B Acute toxicity studies, benzoquinone.

Animal species	Test conditions	LD_{50} (mg/kg bw)	Reference
Oral			
Rat (strain ns)	ns	130	Woodward et al. 1949, cited in ACGIH 2001a[2]
Gastric intubation			
Rat (Sprague-Dawley), male	ns	165	Omaye et al. 1980[32]
Injection iv			
Rat (strain ns)	ns	25	Woodward et al. 1949, cited in ACGIH 2001a[2]
Injection ip			
Mouse, white (Beyers)	ns	8.5	Serif & Seymour 1963[97]

ns: not specified
ip: intraperitoneal
iv: intraveneous

Table F-4 Short-term and subchronic toxicity studies.

Animal species	Test conditions	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) / Critical effects	Reference
Oral				
Rat (Sprague-Dawley)	Hydroquinone 0, 20, 64, 200 mg/kg bw/day, by gavage, 13 weeks, 5 days/week	20	64 Tremors and reduced home-cage activity	Bernard 1988 (unpublished), cited in OECD 1996[33]
Rat (F344)	Hydroquinone 0, 63, 125, 250, 500, 1,000 mg/kg bw/day, by gavage (n=5/sex/dose), 14 days, 5 days/week	250	500 Tremors and decreased final mean body weight	NTP 1989[98], Kari et al. 1992[100]
Mouse (B6C3F1)	Hydroquinone 0, 31, 63, 125, 250, 500 mg/kg bw/day, by gavage (n=5/sex/dose), 14 days, 5 days/week	125	250 Mortality, tremors, and decreased final mean body weight	NTP 1989[98], Kari et al. 1992[100]
Rat (F344)	Hydroquinone 0, 25, 50, 100, 200, 400 mg/kg bw/day, by gavage (n=10/sex/dose), 13 weeks, 5 days/week (similar to OECD 408)	< 25	25 Decreased absolute and relative liver weights in males (at all dose levels). Increased absolute and relative liver weights in females at ≥ 100 mg/kg bw/day.	NTP 1989[98], Kari et al. 1992[100]
Mouse (B6C3F1)	Hydroquinone 0, 25, 50, 100, 200, 400 mg/kg bw/day, by gavage (n=10/sex/dose), 13 weeks, 5 days/week (similar toe OECD 408)	< 25	25 Increased absolute and relative liver weights in males (at all dose levels).	NTP 1989[98], Kari et al. 1992[100]
Dermal				
Rat (F344)	Hydroquinone 0, 240, 480, 960, 1,920, 3,840 mg/kg bw/day in ethanol, 14 days, 12 doses	1,920	3,840 Reduced body weight	NTP 1989[98], Kari et al. 1992[100]
Mouse (B6C3F1)	Hydroquinone 0, 300, 600, 1,200, 2,400, 4,800 mg/kg bw/day in ethanol, 14 days, 12 doses	4,800	> 4,800	NTP 1989[98], Kari et al. 1992[100]
Rat (F344), male and female	Hydroquinone 0, 2.0, 3.5, 5.0% in oil-in-water emulsion, 13 weeks, 5 days/week (similar to OECD 411)	5.0% (~74 mg per kg bw/day)	> 5.0% No overt toxicity (based on standard tox parameters, BrdU-incorporation and histopathology of the kidney)	David et al. 1998[99]

Injection ip				
Mouse (Swiss)	Benzoquinone 2 mg/kg bw/day, ip, 6 weeks, 6 days/week	< 2	2 Relative changes in organ weights. Significant decreases in red blood cells and bone marrow cell counts and haemoglobin content. Histological injuries in liver, thymus, spleen, kidney and peripheral lymph nodes.	Rao et al. 1988[103]
Injection sc				
Rat	Benzoquinone 25 mg/kg bw twice weekly, sc, 2.5-5 months (equivalent to 7 mg/kg bw/day)	< 7	7 Anaemia, methaemoglobinemia, decrease in serum albumin and serum cholinesterase activity	Rao et al. 1988[103]
Inhalation				
Rat	Benzoquinone 0.27-0.36 mg/m^3 or 2.7-3.6 mg/m^3, 4 months, 4 h/day	< 0.27-0.36 mg/m^3	0.27-0.36 mg/m^3 Thrombopenia. At 2.7-3.6 mg/m^3 weight lost, easy tiredness, transient anaemia, thrombopenia	Anonymous 2 (not traceable, cited in MAK 2000[102])

ip: intraperitoneal
sc: subcutaneous
BrdU: bromodeoxyuridine

Table F-5 Long-term toxicity and carcinogenicity studies.

Animal species	Test conditions	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) / Critical effects	Reference
Oral				
Rat (F344)	Hydroquinone 0.8% in the diet [351 and 368 mg/kg bw/day, m and f] (n=30/sex/dose), 104 weeks	< 351	351 (0.8% in diet) Reduced body weight gain (males and females). Higher absolute and relative liver and kidney weight in males. Higher relative kidney weight in females. Chronic nephropathy was more severe in males. In the kidneys of exposed male rats, the incidence of tubule hyperplasia was 30/30 and that of adenomas was 14/30 ($p< 0.01$), compared with 1/30 and 0/30, respectively, in controls. Incidence of other tumour types was not increased by exposure	Shibata et al. 1991[104]
Mouse (B6C3F1)	Hydroquinone 0.8% in the diet [1,046 and 1,486 mg/kg bw/day, m and f] (n=30/sex/dose), 94 weeks	< 1,046	1,046 (0.8% in diet) Reduced body weight gain (males). The incidence of hepatocellular adenoma was increased to 14/30 in exposed male ($p<0.05$) compared with 6/28 in controls. Incidence of other tumour type was not significantly increased by exposure of males, although three renal adenomas occurred. No increase in tumour incidence was found in females.	Shibata et al. 1991[104]
Rat (F344)	Hydroquinone 0, 25, 50 mg/kg bw/day, by gavage (n=65/sex/dose), 2 year, 5 days/week (similar to OECD 451)	< 25	25 Mean body weights of both low and high dosed male rats were decreased. The relative kidney and liver weights of high dosed male rats were higher than those of controls. Relative liver weights were increased for dosed males and high dosed females. In exposed males, renal tubule cell adenomas developed in 4/55 low-dose group ($p = 0.069$) and 8/55 high-dose group ($p = 0.003$) compared with 0/55 controls. In exposed females, mononuclear cell leukaemia developed in 15/55 low-dose group ($p = 0.048$) and 22/55 high-dose group ($p = 0.003$) compared with 9/55 controls.	NTP 1989[98], Kari et al. 1992[100]

Mouse (B6C3F1)	Hydroquinone 0, 50, 100 mg/kg bw/day, by gavage (n=65/sex/dose), 2 year, 5 days/week (similar to OECD 451)	< 50	50 Mean body weights of high dosed males and females were decreased. Relative liver weights were increased for dosed males and high dosed females. In males and females, increased thyroid hyperplasia was found in 5/55 male and 13/55 female controls, 15/53 male and 47/55 female low-dose group ($p<0.01$), and 19/54 male and 45/55 female high-dose groups ($p<0.01$). In females, hepatocellular adenomas were found in 2/55 controls, 15/55 low-dose group ($p<0.01$) and 12/55 high-dose group ($p<0.01$). No increase in tumours was found in exposed males.	NTP 1989[98], Kari et al. 1992[100]
Rat (F344)	Histopathological examination of kidney material from the study of NTP 1989[98]	< 25	25 Hydroquinone-related adenoma formation may include enhancement of the severity of chronic progressive nephropathy coupled with stimulation of tubule proliferation	Hard et al. 1997[105]
Mouse (Swiss albino)	Benzoquinone, gavage 2 mg; 13 weeks, 2 days/week.	<2	2 Lympholeukaemias in liver and spleen	El-Mofty et al. 1992[115]
Injection sc				
Rat (Wistar)	Benzoquinone, injection sc 0.5 ml of a 10 g/L solution (first 53 days), 0.5 ml of 2 g/L (days 53-173) and 0.5 ml of 4 g/L (days 173-294) (n=15+9)	-	Seventeen rats survived the injection period of 394 days. Three fibrosarcomas developed in two rats at the site of injection. No other tumours were found.	Umeda 1957[114]

sc: subcutaneous.

Table F-6 Neurotoxicity.

Animal species	Test conditions	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) / Critical effects	Reference
Oral				
Rat (Sprague-Dawley)	Hydroquinone 0, 20, 64, 200 mg/kg bw/day by gavage, 13 weeks, 5 days/ week. Full battery of neurological tests	20	64 Tremors and reduced home-cage activity were observed in mid-and high-dose groups immediately after dosing with the incidence increased in a dose dependent manner.	Bernard 1988 (unpublished), cited in OECD 1996[33]

Table F-7 Reproduction toxicity (fertility and development).

Animal species	Test conditions	NOAEL (mg/kg bw /day)	LOAEL (mg/kg bw/day) / Critical effects	Ref Reference
2 generation repro				
Rat (CD Sprague-Dawley)	Hydroquinone 0, 15, 50, 150 mg/kg bw/day by gavage, until scheduled termination (OECD 416)	General: 15 Reproductive: 150	General F0 and F1: 50 Mild, transient tremors (F0: 0/58, 0/58, 1/58, 24/58; F1: 0/58, 0/58, 0/58, 21/58) and decreased body weight. Reproduction and fertility: > 150 No treatment related effects	Blacker et al. 1993[121]
Rat (CD Sprague-Dawley)	Hydroquinone 0, 15, 50, 150 mg/kg bw/day by gavage, until scheduled termination	P generation: 15 F1 generation: 150 F2 generation: 150	General F0 and F1: 50 Transient tremors (no quantitative data) Reproduction and fertility: > 150 No treatment related effects	Schroeder 1989 (unpublished), cited in OECD 1996[33]
Developmental				
Rabbit (New Zealand White)	Hydroquinone 0, 25, 75, 150 mg/kg bw/day by gavage (18 mated/dose), GD 6-18, section on GD 30 (OECD 414)	Maternal: 25 Development: 75	Maternal: 75 Decreased food consumption Development: 150 Minimal developmental alterations	Murphy et al. 1992[123]
Rat (COBS-CD-BR)	Hydroquinone 0, 30, 100, 300 mg/kg bw/day by gavage, GD 6-15 (OECD 414)	Maternal: 100 Development: 100	Maternal: 300 Decreased food consumption and body weight gain Development: 300 Decreased body weight	Krasavage et al. 1992[122]

Table F-8 Mechanistic studies in vivo.

Animal species	Test conditions	Results / Conclusions	Reference
Carcinogenesis, benzoquinone after initiation			
Mouse (albino "S"), female	Benzoquinone 0, 3.6 g/L, dermal application, weekly for 27 weeks, three weeks after initiation with 0.15% 7,12-dimethylbenzanthracene (n=19)	No tumours or hyperplasia of the epidermis	Gwynn & Salaman 1953[116]
Mouse (Sencar), female	Benzoquinone, 0, 444, 880 or 1,760 nmol/mouse, dermal application, weekly for 31 weeks, two weeks after initiation with 25 nmol 7,12-dimethylbenzanthracene (n=25)	No signs of possible promoter capacity	Monks et al. 1990[117]
Carcinogenesis, hydroquinone after initiation			
Rat (Fischer 344), male	Hydroquinone, 0 or 0.2% in the diet for 22 weeks, after two weeks 0 or 0.05% N-nitrosobutyl-N-(4-hydroxybutyl)amine in the drinking-water followed by uteric ligation one week later. Animals were killed at week 24 (n=15)	Hydroquinone alone induced no bladder lesions. When hydroquinone was given after initiation, no increase in bladder lesions was observed	Miyata et al. 1985, cited in IARC 1999a[8]
Rat (Fischer 344), male	Hydroquinone, 0 or 0.8% in the diet for 51 weeks, one week after exposure to 0 or 150 mg/kg bw N-methyl-N'-nitro-N-nitrosoguanidine by oral gavage (n=10-16)	The body weights of rates given hydroquinone after initiation were lower than those given initiator only. Hydroquinone alone did not induce forestomach lesions, nor did it enhance the incidence of forestomach or glandular stomach lesions induced by the initiator	Hirose et al. 1989, cited in IARC 1999a[8]
Rat (Sprague-Dawley), male First experiment	Hydroquinone, 0, 100 or 200 mg/kg in the diet for six weeks one week after partial hepatectomy and ip injection of 300 mg/kg N-nitrosodiethylamine One group underwent only partial hepatectomy and was fed the high dose of hydroquinone (n=7-10)	In the hepatectomized group exposed only to hydroquinone, no liver enzyme-altered (γ-GT) foci were induced. Hydroquinone after initiation increased the multiplicity of foci from 0.08 per cm^2 to 0.68 in the low-dose group and to 0.34 in the high-dose group	Stenius et al. 1989[47]
Rat (Sprague-Dawley), male Second experiment	Hydroquinone, 0 or 1 mg/kg bw by oral gavage, five days per week for seven weeks after the regimen to initiate liver carcinogenesis (n=10)	Hydroquinone did not increase the multiplicity of enzyme-altered foci, but their area was increased from a mean of $1.00x10^{-4}$ cm^2 to $1.30x10^{-4}$ cm^2 ($p<0.05$) and their volume from $1.49x10^{-4}$ cm^3 to $3.12x10^{-4}$ cm^3 ($p<0.001$)	Stenius et al. 1989[47]

Rat (Fischer 344), male	Hydroquinone, 0 or 0.8% in the diet for 49 weeks either alone or starting one week after six intraperitoneal injections of 25 mg/kg bw N-nitrosomethyl-n-amylamine (n=12-15)	Hydroquinone alone reduced weight gain. In animals given hydroquinone after carcinogen, the incidence of oesophageal carcinoma was 4/12 rats (not significant) compared with 0/11 in the group given initiator only, and the multiplicity was increased to 0.33 tumours per rats ($p<0.05$) compared with 0 in the controls	Yamaguchi et al. 1989[111]
Rat (Fischer 344/Du Crj), male	Hydroquinone, 0 or 0.8% in the diet for 30 weeks either alone or after exposure to 0.1% N-nitroso-bis(2-hydroxypropyl)amine in the drinking water for two weeks (n=10 or 20). No unexposed controls were included.	Body weight was reduced by hydroquinone given after the initiator and liver weight was increased compared with the group given initiator only. Hydroquinone alone induced no lung or thyroid tumours. Rats given initiator developed low incidences of tumours in the thyroid, lung, urinary bladder and kidney. None of these incidences was increased by hydroquinone	Hasegawa et al. 1990[112]
Rat (Fischer 344), male	Hydroquinone, 0 or 0.8% in the diet for 36 weeks either alone or after exposure to 0.05% N-nitrosobutyl-N-(4-hydroxybutyl)amine in the drinking water for four weeks (n=10 or 20)	Hydroquinone alone did not affect body weight or bladder weight. Hydroquinone exposure alone did not induce bladder tumours and feeding of hydroquinone after initiator did not increase the incidence or multiplicity of bladder neoplasms induced by the initiator alone	Kurate et al. 1990, cited in IARC 1999a[8]
Rat (Wistar/Crj), male	Hydroquinone, 0 or 0.8% in the diet for 36 weeks alone or one week after exposure to 0.1% N-nitrosoethyl-N-hydroxyethylamine in the dinking water for three weeks (n=15 or 20)	Hydroquinone alone resulted in decreased final body weights compared to controls (basal diet and initiator) and increased relative liver and kidney weights compared to the basal diet group. Hydroquinone alone did not induce preneoplastic or neoplastic liver or kidney lesions. In the kidney, hydroquinone exposure after initiation increased the multiplicity of renal cell tumours to 5.22 per rat ($p<0.01$) compared with 2.58 after initiation only, and increased the multiplicity of microadenomas to 2.77 ($p<0.05$) compared with 0.94 after initiation only	Okazaki et al. 1993, cited in IARC 1999a[8]

Hamster (Syrian golden), female	Hydroquinone, 0 or 1.5% in the diet for 16 weeks either alone or after two subcutaneous injections of 50 mg/kg bw N-nitrosobis (2-oxopropyl)amine (n=10-20)	Hydroquinone alone did not affect body weights or liver or pancreas weights compared with untreated controls. Given after initiation, hydroquinone did not affect body weight or liver weight, but reduced pancreas weight compared with animals given initiator only. Hydroquinone alone did not induce neoplastic lesions in the pancreas or liver. In animals given hydroquinone after initiation, the multiplicity of pancreatic lesions was reduced	Maruyama et al. 1991[113]
Nephrotoxicity and carcinogenicity			
Rat (F344, Sprague-Dawley)	Hydroquinone, 200 or 400 mg/kg bw, single dose by gavage (n=4-6)	Nephrotoxicity and renal tubule cell proliferation in F344 rats at ≥ 200 mg/kg bw. Females were more sensitive than males. Sprague-Dawley rats and mice were not/hardly susceptible	Boatman 1996[151]
Mouse (B6C3F1)	Hydroquinone, 350 mg/kg bw, single dose by gavage (n=4-6)		
Rat (F344), male	Hydroquinone, single dose, po in corn oil 1.8 mmol/kg (n=3) 4.5 mmol/kg (n=3) 4.5 mmol/kg, 1 h after 10 mg/kg acivicin ip (n=3) Controls (corn oil; n=3)	Effects were seen at ≥ 1.8 mmol/kg Tremors after dosing. Nephrotoxicity in some rats accompanied with elevation of γ-GT, ALP, GST, and glucose. Selectively toxic to the proximal tubular cells of the kidney and injury at the junction of the medullary rays and the OSOM. Increased cell proliferation. Steep dose response curve. γ-GT inhibition by acivicin strongly inhibited the hydroquinone-mediated kidney toxicity	Peters et al. 1997[77]
Rat (F344), male	2,3,5-tris(GS-S-yl)HQ, 7.5 µmol/kg, single dose, iv (n=3)	Nephrotoxicity evidenced by the urinary excretion of γ-GT and ALP (brush-border enzymes), GST (indicator of the loss of cell membrane integrity) and decreased glucose excretion (indicating loss of renal function). The primary site was the OSOM. Single cell and tubular necrosis and necrosis at the junctions of the medullary rays and the OSOM. Increased cell proliferation	Peters et al. 1997[77]

Eker rat, mutant Tsc-2$^{Ek/+}$	2,3,5-tris(GS-S-yl)HQ, 2.5 µmol/kg bw/day ip for 4 months followed by 3.5 µmol/kg bw/day ip for 6 month, 5 days/week. Treated n=10, controls n=20	Effects after 4 months: increased cell proliferation (determined by BrdU-incorporation) in the kidney. Numerous toxic tubular dysplasias. Increased number of basophilic dysplasias. After 12 months: significant increase in the number of renal tumours. Loss of the normal Tsc-2 allele in 12/12 tumours investigated	Lau et al. 2001[78]
Rat (F344), male and female	Hydroquinone, 0, 2.5, 25, 50 mg/kg bw/day by gavage, 5 days per week, 6 weeks	Increased NAG excretion in males of the 50 mg/kg bw/day dosed group indicating mild kidney toxicity. Hydroquinone does not produce covalent DNA adducts in the kidneys, suggesting a nongenotoxic aetiology of tumours in the kidney.	English et al. 1994b[80]
Rat (F344), male and female	Hydroquinone, 0, 2.5, 25, 50 mg/kg bw/day by gavage, 5 days per week, 1, 3 or 6 weeks (n=5/sex/dose),	Hydroquinone induces cell proliferation (determined by BrdU-incorporation) and nephrotoxicity (determined by urinalysis and histopathology) in male F344 rats (50 mg/kg), but not in female F344 or male SD rats. Hydroquinone induced cell proliferation might be secondary to toxicity.	English et al. 1994a[58]
Rat (Sprague-Dawley), male	Hydroquinone, 0, 50 mg/kg bw, by gavage,5 days/week, 6 weeks (n=5/dose)		
Heamatotoxicity			
Rat (Sprague-Dawley), male Mouse (DBA/2), male	Hydroquinone, 0.9 mmol/kg bw + phenol, 1.1 mmol/kg bw, ip, single dose (co-administration)	2-(GS-S-yl)HQ, 2,3,5-tris(G-S-yl)HQ, 2,5-bis(G-S-yl)HQ and 2,6-bis(GS-S-yl)HQ were detected in bone marrow, reaching maximum values at 15 minutes. The γ-GT metabolite 2-(Cys-Gly)HQ, the dipeptidase metabolite 2-(Cys)HQ, and the mercapturic acid metabolite 2-(NAC)HQ reached maximum levels at 30 min	Bratton et al. 1997[62]
Rat (Sprague-Dawley), male	2,3,5-tris(GS-S-yl)HQ, 17µmol/kg bw, single dose iv 2,6-bis(GS-S-yl)HQ, 50 µmol/kg bw, single dose iv	Effects 2,3,5-tris(GS-S-yl)HQ at 17µmol/kg bw and of 2,6-bis(GS-S-yl)HQ at 50 µmol/kg bw: Significant (45 and 28 %, respectively) reducing of ^{59}Fe incorporation into immature erythrocytes	Bratton et al. 1997[62]

Mouse (Swiss, albino) female	Hydroquinone, 25, 50, 75, 100 mg/kg bw ip, 3 doses, at t = 0, 16, 24 h[59] Fe was administered at t = 64 h	^{59}Fe uptake is significantly decreased (25-100 times less potent than benzoquinone)	Guy et al. 1991[128]
Mouse (Swiss, albino) female	Benzoquinone, 0.5, 1.0, 2.0, 3.0, 4.0 mg/kg bw ip, 3 doses, at t = 0, 16, 24 h.[59] Fe was administered at t = 64 h	^{59}Fe uptake is 46% decreased at 3.0 mg/kg	
Proliferation/differentiation disturbing of heamapoetic cells			
Mouse (C57BL6)	Hydroquinone, 75 mg/kg bw, twice daily for 11 days (n=6)	Doubling of GM-CFC per femur, hydroquinone induce an increase in total GM-CFC in the bone marrow of mice in vivo (and in vitro; see Table F-3)	Henschler et al. 1996[127]

2,3,5-tris(GS-S-yl)HQ:	2,3,5-tris(glutathione-S-yl)hydroquinone
2,5-bis(GS-S-yl)HQ:	2,5-bis(glutathione-S-yl)hydroquinone
2,6-bis(GS-S-yl)HQ:	2,6-bis(glutathione-S-yl)hydroquinone
2-(GS-S-yl)HQ:	2-(glutathione-S-yl)hydroquinone
Acivicin:	γ–GT inhibitor
ALP:	alkaline phosphatase
ALT:	alanine transaminase
AST:	aspartate transaminase
BrdU:	bromodeoxyuridine
BUN:	blood urea nitrogen
GM-CFC:	granulocyte-macrophage colony forming cells
GST:	glutathione S-transferase
γ–GT:	γ-glutamyl transpeptidase
ip:	intraperitoneal
iv:	intravenous
NAG:	N-acetyl-β-D-glucosaminidase
po:	per os
OSOM:	outer stripe of the outer medulla
Tsc-2:	tuberous sclerosis-2

Annex **G**

In vitro data

Proliferation/differentiation disturbing of heamapoetic cells.

System / cell line	Test conditions	Results / conclusions	Reference
FDCP-mix cells	Hydroquinone, 10^{-12} – 10^{-5} M	Almost a doubling of GM-CFC between 10^{-9} -10^{-6} M, hydroquinone induced an increase in the number of GM-colonies in vitro	Henschler et al. 1996[127]
In situ perfused rat kidney	2,3,5-(TriGSyl)HQ, 2-(GSyl)HQ, infusion into the right renal artery	5 and 10 µmol 2,3,5-(triGSyl)HQ and 40 µmol 2-(GSyl)HQ caused a time-dependent elevation in γ-GT. Metabolites of 2,3,5-(triGSyl)HQ (10 µmol) were observed in urine and bile only within the first 30 min of perfusion. At the lower dose (5 µmol) neither parent compound nor metabolites were found. After 2-(GSyl)HQ perfusion, 2-(N-acetyl-cystein-S-yl)HQ (9.2 ± 0.5 µmol). 2-(cystein-S-ylglycine)HQ (0.8 ± 0.3µmol), and 2-(cystein-S-yl)HQ (1.3 ± 0.3 µmol) were detected in urine and bile. Unchanged 2-(GSyl)HQ was also detected (0.8 ± 0.1 µmol). A greater fraction of the dose was retained by the kidney following treatment with 10 µmol 2,3,5-(triGSyl)[^{14}C]HQ than following treatment with 40µmol 2-(GSyl)[^{14}C]HQ (36 and 11%, respectively).	Hill et al. 1994[79]

2,3,5-(triGSyl)HQ: 2,3,5-(triglutathione-S-yl)hydroquinone
2-(GSyl)HQ: 2-(glutathione-S-yl)hydroquinone
FDCP: factor-dependent cells Paterson
GM-CFC: granolocyte-macrophage colony forming cells
γ-GT: γ-glutamyl transpeptidase

Annex **H**

Mutagenicity studies

Table H-1 Genetic and related effects of hydroquinone (adapted from IARC, 1999a[8]).

Test system	Results		Dose (LED or HID)	Reference
	Exogenous metabolic system			
	without	with		
DNA strand breaks / SCEs				
Mammalian cells, in vitro				
DIA, DNA strand breaks, cross-links or related damage, LYS mouse lymphoma cells, alkaline elution	-	n.t.	11	Pellack-Walker & Blumer 1986
DIA, DNA strand breaks, isolated rat hepatocytes cells, alkaline elution	+	n.t.	33	Walles 1992
DIH, DNA strand breaks, cross-links or related damage, human lymphocytes (comet assay)	?	+	11	Anderson et al. 1995
DIH, DNA strand breaks, human promyelocytic HL60 cells, pulse field electrophoresis	+	n.t.	1.1	Hiraku & Kawanishi 1996
DNA single-strand breaks on supercoiled Bluescript plasmid DNA	-	+	11	Schlosser et al. 1990
SIC, SCE, CHO cells	+	+	0.5	Galloway et al. 1987
SIS, SCE, Syrian hamster embryo cells	+	n.t.	0.11	Tsutsui et al. 1997
SHL, SCE, human lymphocytes	+	n.t.	4.4	Morimoto & Wolff 1980
SHL, SCE, human lymphocytes	+	+	110	Morimoto et al. 1983
SHL, SCE, human lymphocytes	+	n.t.	6	Erexson et al. 1985
SHL, SCE, human lymphocytes	? [a]	n.t.	4.4	Knadle 1985
Mammalian cells, in vivo				
SVA, SCE, (C57BL/Cnc x C3H/Cn3)F1 mouse bone marrow	-		120 ip x 1	Pacchierotti et al. 1991
DNA adduct formation				
Mammalian cells, in vitro				
BID, binding (covalent) to DNA, mouse P388D_1 cells	+	n.t.	5.5	Kalf et al. 1990
BID, binding (covalent) to calf thymus DNA	+	n.t.	5.5	Leanderson & Tagesson 1990
BID, binding (covalent) to DNA, cultured rat Zymbal glands	+	n.t.	750	Reddy et al. 1990
BID, binding (covalent) to calf thymus DNA	-	+ [b]	11	Schlosser et al. 1990
BID, binding (covalent) to DNA, human promyelocytic HL-60 cells	+	n.t.	5.5	Lévay et al. 1991
BID, binding (covalent) to DNA, male B6C3F_1 mouse bone-marrow cells	+	n.t.	11	Lévay et al. 1993
BID, binding (covalent) to DNA, human bone-marrow macrophages	+	n.t.	11	Lévay et al. 1993
BID, binding (covalent) to DNA, human promyelocytic HL-60 cells	+	n.t.	27.5	Pathak et al. 1995
BID, binding (covalent) to DNA, B6CeF$_1$ mouse bone marrow	+	n.t.	27.5	Pathak et al. 1995
BID, binding (covalent) to DNA, human promyelocytic HL-60 cells	+	n.t.	5.5	Lévay & Bodell 1996

Binding (covalent) to porcine brain tubulin [porcine brain tubulin assembly assay]	-	n.t.	2,750	Brunner et al. 1991
Mammalian cells, in vivo				
BVD, binding (covalent) to DNA, Sprague-Dawley rat Zymbal gland, liver or spleen	-		150 po x 4	Reddy et al. 1990
BVD, binding (covalent) to DNA, Fischer 344 rat kidneys	-		50 po, 5 d/wk, 6 wk	English et al. 1994b
Clastogenic effects				
Mammalian cells, in vitro				
MIA, micronucleus test, CL-1 cells	+ [c]	n.t.	1	Antoccia et al. 1991
MIA, micronucleus test, V79 cells	(+)	n.t.	31.6	Seelbach et al. 1993
MIA, micronucleus test, V79 cells	+	n.t.	2.8	Ellard & Parry 1993
MIA, micronucleus test, XEM2 cells	+	n.t.	2.8	Ellard & Parry 1993
MIA, micronucleus test, SD1 cells	+	n.t.	2.8	Ellard & Parry 1993
MIA, micronucleus test, V79 cells	NT	+ [d]	11.5	Dobo & Eastmond 1994
CIC, chrom. ab., CHO cells	-	+	450	Galloway et al. 1987
CIS, chrom. ab., Syrian hamster embryo cells	+	n.t.	3.3	Tsutsui et al. 1997
MIH, micronucleus test, human lymphocytes (kinetochore-positive)	+	n.t.	2.8	Yager et al. 1990
MIH, micronucleus test, human lymphocytes (kinetochore-positive)	+	n.t.	8.2	Robertson et al. 1991
MIH, micronucleus test, human lymphocytes	?	?	1	Van Hummelen & Kirsch-Volders 1992
MIH, micronucleus test, human lymphocytes	(+) [e]	n.t.	20	Ferguson et al. 1993
MIH, micronucleus test, human lymphocytes	-	+	50	Vian et al. 1995
CHL, chrom. abb. human lymphocytes (FISH)	+	n.t.	11	Eastmond et al. 1994
Mammalian cells, in vivo				
MVM, micronucleus test, NMRI mouse bone marrow	+		50 sc x 6	Tunek et al. 1982
MVM, micronucleus test, S mouse bone marrow	+		80 ip x 1	Ciranni et al. 1988
MVM, micronucleus test, S mouse bone marrow	(+)		80 po x 1	Ciranni et al. 1988
MVM, micronucleus test, (101/E1 x C3H/E1)F_1 mouse bone marrow	+		50 ip x 1	Adler & Kliesch 1990
MVM, micronucleus test, (101/E1 x C3H/E1)F_1, mouse bone marrow	+		15 ip x 3	Adler & Kliesch 1990
MVM, micronucleus test, Swiss CD-1 mouse bone marrow	(+)		60 ip x 1	Barale et al. 1990
MVM, micronucleus test, (102/E1 x C3H/E1)F_1, mouse bone marrow	+		50 ip x 1	Adler et al. 1991
MVM, micronucleus test, (102/E1 x C3H/E1)F_1, mouse bone marrow	+ [c]		100 ip x 1	Miller et al. 1991
MVM, micronucleus test, (C57BL/Cnc x C3H/Cne)F_1 mouse bone marrow	+		40 ip x 1	Pacchierotti et al. (1991)
MVM, micronucleus test, Swiss CD-1 mouse bone marrow	+		20 ip x 1	Marrazinni et al. 1994a
MVM, micronucleus test, Swiss CD-1 mouse bone marrow	+		80 ip x 1	Marrazinni et al. 1994b

MVM, micronucleus test, CD-1 mouse bone marrow	+		60 ip x 3	Chen & Eastmond 1995a
CBA, chrom. ab., (102/E1 x C3H/E1)F_1 mouse bone marrow	+		75 ip x 1	Xu & Adler 1990
CBA, chrom. ab., Swiss CD-1 mouse bone marrow	+		80 ip x 1	Marrazinni et al. 1994b
CCC, chrom. ab. (102/E1 x C3H/E1)F_1 mouse spermatocytes treated	+		40 ip x 1	Ciranni & Adler 1991[152]
CGG, chrom. ab. (102/E1 x C3H/E1)F_1 mouse spermatogonia treated	+		40 ip x 1	Ciranni & Adler 1991[152]
DNA mutation				
Bacteria				
PRB, SOS repair activity, *S. typhimurium* TA1535/ pSK1002, umu test	-	-	3,300	Nakamura et al. 1987
SA0, *S. typhimurium* TA100, rev. mut.	-	-	333	Haword et al. 1983
SA0, *S. typhimurium* TA100, rev. mut.	-	-	125	Sakai et al. 1985
SA5, *S. typhimurium* TA1535, rev. mut.	-	-	333	Haword et al. 1983
SA7, *S. typhimurium* TA1537, rev. mut.	-	-	333	Haword et al. 1983
SA9, *S. typhimurium* TA98, rev. mut.	-	-	333	Haword et al. 1983
SA9, *S. typhimurium* TA98, rev. mut.	-	-	125	Sakai et al. 1985
SAS, *S. typhimurium* TA97, rev. mut.	-	-	125	Sakai et al. 1985
SA2, *S. typhimurium* TA102, rev. mut.	+	n.t.	NG	Hakura et al. 1996
SA4, *S. typhimurium* TA104, rev. mut.	+	n.t.	25	Hakura et al. 1996
SCG, *S. cerevisiae* MP1 gene conversion	+	n.t.	1,320	Fahrig 1984
SCH, *S. cerevisiae* MP1 homozygosis by mitotic recombination or gene conversion	-	n.t.	1,320	Fahrig 1984
SCF, *S. cerevisiae* MP1, for. mut.	+	n.t.		Fahrig 1984
Insect cells				
DMX, *D. melanogaster*, sex-linked rec. lethal mut.	?		28,000 ppm feed	Foureman et al. 1994
DMX, *D. melanogaster*, sex-linked rec. lethal mut.	-		1,500 ppm inj x 1	Foureman et al. 1994
Mammalian cells, in vitro				
G5T, Gene mutation, mouse lymphoma L5178Y cells, tk locus	+	n.t.	2.5	McGregor et al. 1988a,b
GIA, Gene mutation, Syrian hamster embryo cells, hprt locus	+	n.t.	1.1	Tsutsui et al. 1997
GIA, Gene mutation, Syrian hamster embryo cells, ouabain resistance	+	n.t.	1.1	Tsutsui et al. 1997
Aneuploidy induction				
Mammalian cells, in vitro				
AIA, aneupoidy, DON:Wg3h Chinese hamster cells, dislocating metaphase chromosomes	+	n.t.	10	Warr et al. 1993
AIA, aneupoidy, LUC2 Chinese hamster cells	-	n.t.	5	Warr et al. 1993
AIA, aneupoidy, Syrian hamster embryo cells	-	n.t.	3.3	Tsutsui et al. 1997
TCS, cell transformation, Syrian hamster embryo cells, clonal assay	+	n.t.	0.33	Tsutsui et al. 1997
AIA, aneupoidy, human lymphocytes MN multicolour chrom. staining (FISH)	+	n.t.	8.3	Eastmond et al. 1994

ICR, Inhibition of intercellular communication, V79MZ Chinese hamster cells	+	n.t.	0.055	Vang et al. 1993
Inhibition of assembly of bovine microtubules	(+)	n.t.	110	Wallin & Hartley-Hasp 1993
Mammalian cells, in vivo				
AVA, aneuploidy, (102/E1xC3H/Ea)F_1 mouse bone marrow polyploidy	-		100 ip x 1	Xu & Adler 1990
AVA, aneuploidy, (C57BL/Cnc x C3H/Cne)F_1 mouse bone marrow hyperploidy	+		80 ip x 1	Pacchierotti et al. 1991
AVA, aneuploidy, (C57BL/Cnc x C3H/Cne)F_1 mouse bone marrow polyploidy	-		120 ip x 1	Pacchierotti et al. 1991
AVA, aneuploidy, (C57BL/Cnc x C3H/Cne)F_1 mouse spermatocytes hyperploidy	+		80 ip x 1	Leopardi et al. 1993[152]
AVA, aneuploidy, Swiss CD-1 mouse bone marrow polyploidy	-		80 ip x 1	Marrazinni et al. 1994b
AVA, aneuploidy, Swiss CD-1 mouse bone marrow hyperploidy	+		80 ip x 1	Marrazinni et al. 1994b
AVA, aneuploidy, CD-1 mouse bone marrow, MN multicolour chromosome staining (FISH)	+		60 ip x 3	Chen & Eastmond 1995a

+; (+); -; ?:	positive; weakly positive; negative; inconclusive
LED; HID:	lowest effective dose; highest ineffective dose (in vitro tests: µg/mL; in vivo tests: mg/kg bw/day)
n.t.; n.g.:	not tested; not given
inj.; ip; sc; po:	injection; intraperitoneral; subcutaneous; oral
rev. mut.; for. mut.:	reverse mutation; forward mutation
SCE:	sister chromatid exhange
V79 cells:	Chinese hamster lung V79 cells
CHO cells:	Chinese hamster ovary cells
CL-1 cells :	Chinese hamster embryonic lung CL-1 cells
XEM2 cells :	Chinese hamster V79 exp CYP1A1 cells
SD1:	Chinese hamster V79 exp CYP2B1 cells

[a] Positive if glutathione was depleted with diethyl maleate

[b] With prostaglandin H synthetase for oxidation

[c] No increase in % kinetochore-positive micronuclei compared with controls.

[d] Supplemented with arachidonic acid; increase in both kinetochore-positive and -negative micronucleated cells compared with controls (CREST-labelling procedure).

[e] Size ratio of micronuclei to nucleus was not significantly different from controls.

Table H-2 Genetic and related effects of benzoquinone (adapted from IARC, 1999b[9]).

Test system	Results		Dose (LED or HID)	Reference
	Exogenous metabolic system			
	without	with		
DNA strand breaks / SCEs				
Mammalian cells, in vitro				
DIA, DNA strand breaks, mouse lymphoma L5178YS cells	+	n.t.	0.11	Pellack-Walker & Blumer 1986
DIH, DNA strand breaks, cross-links or related damage, human lymphocytes (comet assay)	-	+	11	Anderson et al. 1995
SIC, SCE, V79 cells	-	n.t.	11	Ludewig et al. 1989[154]
SHL, SCE, human lymphocytes	+	n.t.	0.55	Erexson et al. 1985
DNA adduct formation				
No IARC data available				
Clastogenic effects				
Mammalian cells, in vitro				
MIA, micronucleus test, V79 cells	+	n.t.	5.4	Ludewig et al. 1989[154]
MIA, micronucleus test, V79, IEC-17 and 18 cells	+	n.t.	0.01	Glatt et al. 1990
MIH, micronucleus test, HuFoe-15 embryonal human liver cells	+	n.t.	0.01	Glatt et al. 1990
MIH, micronucleus test, human lymphocytes	+	n.t.	0.275	Yager et al. 1990
Mammalian cells, in vivo				
MVM, micronucleus test, pregnant CD-1 mouse bone-marrow cells	(+)		20 po x 1	Ciranni et al. 1988a
MVM, micronucleus test, foetal CD-1 mouse liver cells *in utero*	(+)		20 (to dam) x 1	Ciranni et al. 1988a
MVM, micronucleus test, CD-1 mouse	(+)		20 po x 1	Ciranni et al. 1988b
DLM, dominant lethal test, male C3H and (C3Hx101)F1 mice	-		6.25 ip x 1	Rohrborn & Vogel 1967
DNA mutation				
Bacteria				
SA0, *S. typhimurium* TA100, rev. mut.	+	n.t.	5.0	Nazar et al. 1981
SA0, *S. typhimurium* TA100, rev. mut.	-	-	16.5	Mortelmans et al. 1986
SA5, *S. typhimurium* TA1535, rev. mut.	-	-	16.5	Mortelmans et al. 1986
SA7, *S. typhimurium* TA1537, rev. mut.	-	-	16.5	Mortelmans et al. 1986
SA9, *S. typhimurium* TA98, rev. mut.	-	-	16.5	Mortelmans et al. 1986
NCR, *N. crassa*, rev. mut. to arg+	-	n.t.	n.g.	Reissig 1963
NCF, *N. crassa*, for. mut. to pyrimidine dependence	-	n.t.	n.g.	Reissig 1963

Mammalian cells, in vitro				
G9H, gene mut., V79 cells, hprt locus	+	n.t.	0.54	Ludewig et al. 1989[154]

Aneuploidy induction

No IARC data available

+; (+); -:	positive; weakly positive; negative
LED; HID:	lowest effective dose; highest ineffective dose (in vitro tests: µg/mL; in vivo tests: mg/kg bw/day)
n.t.; n.g.:	not tested; not given
rev. mut.; for. mut.:	reverse mutation; forwards mutation
po; ip:	oral; intraperitoneal
SCE:	sister chromatid exchange
V79 cells:	Chinese hamster lung V79 cells

Annex I

Evaluation of the Subcommittee on the Classification of carcinogenic substances

I.1 Scope

On request of the Dutch Expert Committee on Occupational Safety of the Health Council, the Subcommittee on the Classification of Carcinogenic Substances evaluated the carcinogenic properties of hydroquinone and benzoquinone.

I.2 Carcinogenicity of hydroquinone

The International Agency for Research on Cancer (IARC) classified hydroquinone as *not classifiable as to its carcinogenicity to humans (Group 3)*, based on *inadequate evidence* in humans for the carcinogenicity of hydroquinone, and *limited evidence* in experimental animals for the carcinogenicity of hydroquinone (IARC 1999).

For the evaluation of the carcinogenicity of hydroquinone, two oral studies were available. In the first study (study I), performed and comprehensively reported by the US National Toxicology Program (NTP), F344 rats and $B6C3F_1$ mice (n=55/species/sex/group) were exposed by gavage to concentrations of hydroquinone (in deionised water) of 25 and 50 mg/kg bw/day and of 50 and 100 mg/kg bw/day, respectively, 5 days/week, for 104 weeks (Kari et al. 1992, NTP 1989). In the second study (study II), performed and limitedly reported by Shibata et al. 1991, hydroquinone was administered in the diet to F344 rats (n=30/sex/group) at doses of ca. 350 and 370 mg/kg bw/day for males and

females, respectively, and to B6C3F$_1$ mice (n=30/sex/group) at doses of ca. 1,050 and 1,490 mg/kg bw/day for males and females, respectively.

In these studies, administration of hydroquinone induced increased tumour incidences (control, low dose, high dose) as summarized in Table I.1.

As to the hepatocellular adenomas, the Subcommittee notes that they were not consistently seen, viz., in males in (diet) study II and in females in (gavage) study I; that they were benign and did not progress to malignant carcinomas; and that there was a relatively small increase in (diet) study I and no dose response in (gavage) study II. Because of this inconsistent picture and the susceptibility of the B6C3F$_1$ mouse to develop liver tumours, the Subcommittee considers that the induction of hepatocellular adenomas is not relevant for humans.

The renal tubular adenomas were found in both studies, but in male rats only. These benign tumours did not progress to malignant ones. Severe kidney damage appeared to be a prerequisite for the development of the adenomas. This chronic progressive nephropathy is a spontaneous disease in, particularly, ageing male rats. The Subcommittee is of the opinion that hydroquinone plays an indirect role in the development of the adenomas by exacerbating already existing chronic progressive nephropathy and enhancing proliferative aspects of this disease process. This view of an indirect role is supported by the findings that increased cell proliferation but no DNA adducts were detected in the F344 male rat kidney following repeated oral administration of nephrotoxic doses.

Because of this indirect role involving exacerbation of an already existing spontaneous disease which is further common in rats but without a human counterpart, the Subcommittee is of the opinion that the induction of renal tubular adenomas in male rats is not relevant for humans.

Table I.1 Increased tumour incidences in chronic rat and mouse studies (study I: NTP 1989, Kari et al. 1992; study II: Shibata et al. 1991).

Species; study	Dose	Control	Low dose	High dose
Rat, male; study I renal tubular adenomas	0, 25, 50 mg/kg bw/day	0 / 55	4 / 55 (7%, p=0.059)	8 / 55 (14%, p=0.003)
Rat, male; study I re-evaluated (Hard et al. 1997) renal tubular adenomas	0, 25, 50 mg/kg bw/day	0 / 44	4 / 49 (8%)	15 / 51 (29%, p<0.01
Rat, male; study II renal tubular adenomas	0, 350 mg/kg bw/day	0 / 30	-	14 / 30 (47%, p<0.01)
Rat, female; study I mononucleaer cell leukaemia	0, 25, 50 mg/kg bw/day	9 / 55	15 / 55 (27%.p=0.124)	22 / 55 (40%, p=0.005)
Mouse, male; study II hepatocellular adenomas	0, 1050 mg/kg bw/day	6 / 28 (22%)	-	14 / 30 (47%, p<0.05)
Mouse, female; study I hepatocellular adenomas	0, 50, 100 mg/kg bw/day	2 / 55 (4%)	15 / 55 (27%, p=0.001)	12 / 55 (22%, p=0.004)

The mononuclear cell leukaemias were found at statistically significantly increased incidences in the high-dose female rats in study I only. The Subcommittee notes that especially F344 and Wistar-Furth rats show high background incidences of this tumour type while they are low (i.e., $\leq 6\%$) in other rat strains and species. The rate in control F344 rats is very variable and depends upon several factors such as e.g., sex, vehicle and diet. In addition, the rates recorded in the laboratories conducting NTP studies increased in the course of time. In the period 1980-1984, in which the hydroquinone study was performed, the average incidences of mononuclear cell leukaemia in water-treated (gavage) control F344 rats were 43.8±12.6 and 26.2±10.6% in males and females, respectively.

In view of the unique susceptibility of F344 rats to develop monuclear cell leukaemias, the very variable background incidences, and the fact these leukaemias were seen in one sex in one study only, the Subcommittee considers that the induction of mononuclear cell leukaemias in female F344 rats is not relevant for humans.

Mode of action

The Subcommittee concludes that hydroquinone is a genotoxic compound. In vitro, amongst others, it induced gene mutations, micronuclei, chromosomal aberrations, aneuploidy, sister chromatid exchanges, DNA single strand breaks, and oxidative DNA damage in mammalian cell systems. In in vivo tests, mainly performed in mice using intraperitoneal injection, hydroquinone produced increases in the incidences of micronuclei and chromosomal aberrations in bone marrow and of chromosomal aberrations in spermatogonia. Based on the literature it is quite likely that the mode of action of hydroquinone's genotoxicity is by a non-stochastic mechanism.

I.3 Carcinogenicity of benzoquinone

One dermal (Umeda 1957) and one oral study (El-Mofty et al. 1992) addressed the (potential) carcinogenicity of benzoquinone. The Subcommittee is of the opinion that because of several serious flaws in design and reporting these studies cannot be used to evaluate the carcinogenicity of benzoquinone.

I.4 Recommendation for classification

Hydroquinone

The Subcommittee concludes that there are two valid carcinogenicity studies in which hydroquinone was orally administered to rats and mice. The findings are inconsistent and concern tumour types that are considered not relevant for humans.

The Subcommittee is of the opinion that the available data are insufficient to evaluate the carcinogenic properties of hydroquinone (category 3).

Benzoquinone

The Subcommittee concludes that there are no valid carcinogenicity studies.

The Subcommittee is of the opinion that the available data are insufficient to evaluate the carcinogenic properties of benzoquinone (category 3).

I.5 References

El-Mofty MM, Khudoley VV, Sakr SA, Fathala NG. Flour infested with Tribolium castaneum, biscuits made of this flour, and 1,4-benzoquinone induce neoplastic lesions in Swiss albino mice. Nutr Cancer 1992; 17(1): 97-104.

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Shibata MA, Hirose M, Tanaka H, Asakawa E, Shirai T, Ito N. Induction of renal cell tumors in rats and mice, and enhancement of hepatocellular tumor development in mice after long-term hydroquinone treatment. Jpn J Cancer Res 1991; 82(11): 1211-1219.

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Annex **J**

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 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

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

Annex **K**

BMD analysis of thyroid hyperplasia in mice exposed to hydroquinone

The results of the NTP study with hydroquinone administered orally to mice (NTP 1989; Kari et al. 1992), resulting (among others, see Section 7.2.4) in hyperplasia of the thyroid, are shown in Table K-1.

Analysis of these study data using the software programme PROAST (a software package developed for analysing dose-response data of toxicity studies and calculating BMDs [Slob 2002, 2009; EFSA 2011]) showed that the data of the males and the females can not be combined. The incidences of the lesions in the males differ too much from the incidences in the females, both in the controls and in the dosed groups.

In addition, a BMD analysis of the results with the females is also not possible: there are only two dose groups and the incidences flattens off already at the low dose, resulting in the impossibility to estimate a BMD for e.g. 10% extra risk (Slob, personal communication).

Only for the results with the males a BMD analysis is possible. This analysis, using the BMD software program of US-EPA (US-EPA 2011) is shown in Table K-2 and Figure K-1.

Table K-1 Thyroid hyperplasia in mice (2-year gavage study; NTP 1989, Kari et al. 1992).

Thyroid hyperplasia in mice (B6C3F1)	Number of animals with lesions / total number of animals per dose group					
	males			females		
Dose (mg/kg bw/day)	Control	50	100	Control	50	100
Thyroid hyperplasia	5/55	15/53	19/54	13/55	47/55	45/55

The lowest BMD and BMDL for 10% extra risk (25.1 and 15.7 mg/kg bw/day, respectively) are produced by the log-logistic model. Other BMDs and BMDLs vary from 28.5 - 43.5 and 19.1 - 34.0 mg/kg bw/day, respectively.

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Table K-2 BMD analysis for 10% extra risk of thyroid hyperplasia in males using US-EPA's BMD software.

Model	BMD	BMDL	Log-likelihood fitted vs. full model	Degrees of freedom	Model accepted
Gamma	28.5	19.1	0.236	1	yes
Logistic	43.5	34.0	0.827	1	yes
LogLogistic	25.1	15.7	0.134	1	yes
Multistage	28.5	19.1	0.236	1	yes
Multistage Cancer	28.5	19.1	0.236	1	yes
Probit	41.3	32.1	0.730	1	yes
Weibull	28.5	19.1	0.236	1	yes
Quantal Linear	28.5	19.1	0.236	1	yes

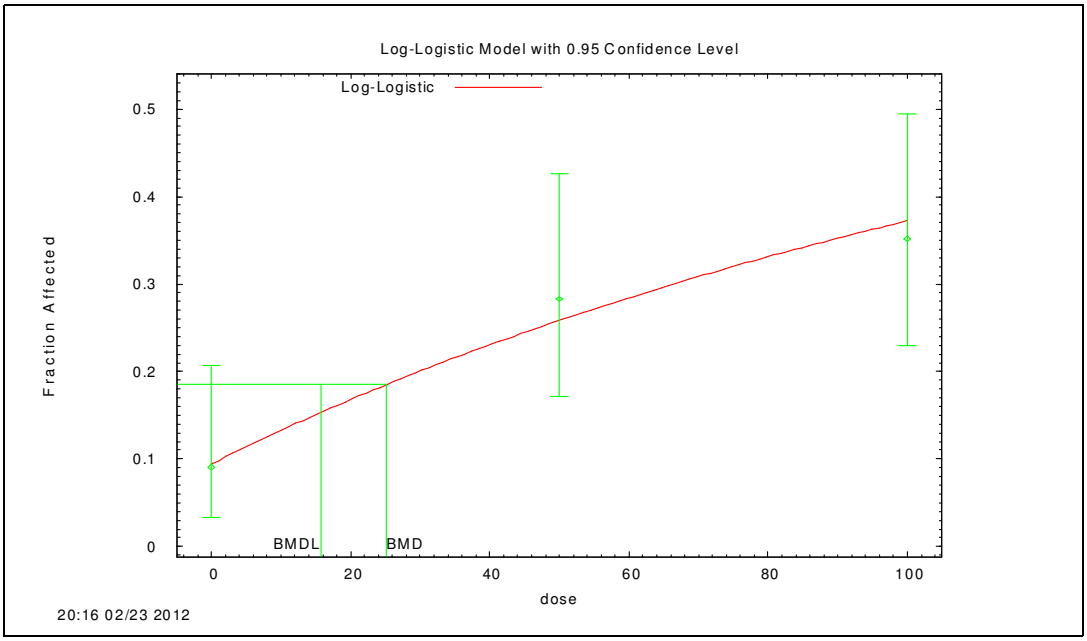

Figure K-1 Log-logistic model.