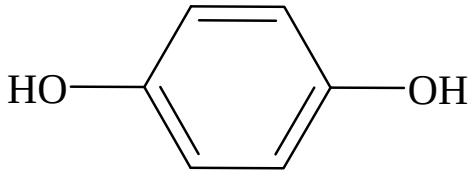
Nomination Profile

Hydroquinone
[CAS 123-31-9]

Supporting Information for Toxicological Evaluation
by the
National Toxicology Program

21 May 2009

Prepared by:
U.S. Food & Drug Administration
Department of Health and Human Services



Hydroquinone

Summary of Nomination:

Hydroquinone has a number of uses due to its properties as a water-soluble reducing agent. It is used in medicine (up to 5% in concentration) to treat dyschromias (*e.g.* melasma, an acquired hypermelanosis) and in cosmetics (up to 2%) as a depigmenting agent in a number of topical skin creams. Hydroquinone lightens skin through melanocyte toxicity and inhibition of melanogenesis. Hydroquinone is absorbed through the skin and metabolized primarily to sulfate and glucuronide conjugates, which are excreted in the urine. Additional metabolites include benzoquinone, multiple glutathione conjugates, and *N*-acetyl cysteine conjugates. At high doses, hydroquinone taken orally transiently affects the central nervous system. Although most genotoxicity and mutagenicity tests are negative, nephrotoxicity and "some evidence" of hydroquinone carcinogenicity in rat kidneys has been reported (NTP TR-366). Cases of skin irritation and allergic reaction associated with hydroquinone are rare. Hydroquinone use has been associated with contact vitiligo (areas of complete lack of skin color) and with exogenous ochronosis (hyperpigmentation of skin). Studies involving the effects of hydroquinone on fertility and reproduction have produced conflicting results. The data in the areas of fertility and reproduction toxicity, dermal absorption, carcinogenicity and occurrence of exogenous ochronosis are insufficient to make a final determination on the toxicological hazard of hydroquinone. The FDA recommends a dermal carcinogenicity study in an appropriate model using over-the-counter concentrations of hydroquinone, as well as additional reproductive toxicity studies to characterize the reproductive hazard (fertility) of hydroquinone use.

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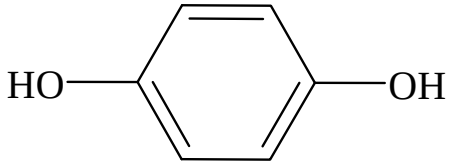


A. Chemical Information

Molecular Identification

Chemical Name:	Hydroquinone
Chemical Abstracts Service (CAS) Registry Number:	123-31-9
Synonyms:	1,4-Benzenediol *p*-Benzenediol Quinol Arctuvin Benzohydroquinone Benzoquinol *p*-Dihydroxybenzene Eldoquin Hydroquinol Pyrogentistic acid
Formula:	$C_6H_6O_2$
Molecular Weight:	110.11

Physical Chemical Properties

Physical State of Pure Material:	Colorless, hexagonal prisms or crystals
Melting Point:	170-171 ^{o}C
Boiling Point:	285-287 ^{o}C
Vapor Pressure:	$6.7x10^{-4}$ mm Hg at 25 ^{o}C (extrapolated) 1 mm Hg at 132.4 ^{o}C
Flash Point:	165 ^{o}C, closed cup
Solubility:	water, alcohol, ether, acetone, carbon tetrachloride, and slightly soluble in benzene
Density/Specific Gravity:	1.332
pK_a:	9.96
Log K_{ow}:	0.59

B. Exposure Potential

Production

Hydroquinone may be produced by the oxidation of aniline or phenol, by the reduction of quinone, or from a reaction of acetylene and carbon monoxide (Merck, 2006).

In 1985 the total hydroquinone production capacity of the U.S. was approximately 15,422 metric tons (approx. 34 million pounds) (Santodonato *et al.*, 1985). The U.S. production of hydroquinone in 1992 was approximately 16,000 metric tons and world-wide production was approximately 35,000 metric tons (IARC, 1999). It is unknown whether U.S. production has remained at the same levels (~16,000 metric tons) since 1992.

Use

Hydroquinone has a number of uses, primarily as a result of its properties as a water-soluble reducing agent. It is used as a reducing agent in most photographic developing solutions. It is also used in the manufacture of rubber antioxidants, other antioxidants, and dyes. Hydroquinone acts as a polymerization inhibitor for some chemicals, such as acrylic acid and methyl methacrylate. It is used as a stabilizer in paints, varnishes, motor fuels, and oils. In 1984, 46% of hydroquinone consumption was in photographic chemicals, 35% in rubber and other antioxidants, 11% in polymerization inhibition, and the remaining 8% in "other" (HSDB, 2005d). Hydroquinone (or its derivatives) is used at less than 0.1% in a range of products, including plastics, feed additives for cattle, and acrylic dental prostheses (Camarasa and Serra-Baldrich, 1994). The use of hydroquinone in wet photographic development may have decreased in recent years due to the increased popularity of digital photography and laser jet-based printing.

Hydroquinone is used in medicine (up to 5% in concentration) to treat dyschromias (*e.g.* melasma, an acquired hypermelanosis). To date, only one prescription product containing 4% hydroquinone and two other active ingredients has been approved by the FDA. Hydroquinone is used in cosmetics (up to 2%) as a depigmenting agent in a number of topical skin creams. It is also found in other cosmetics, such as hair dyes and products for coating finger nails.

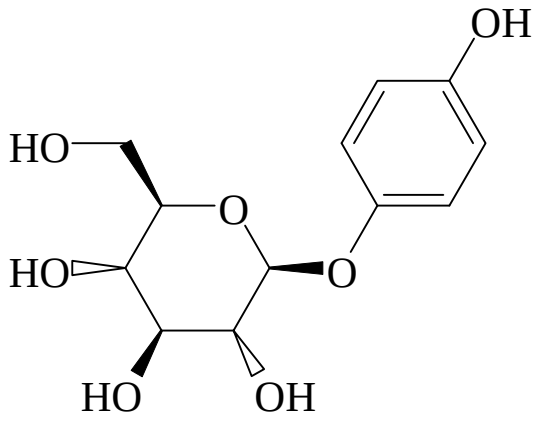
Human Exposure

Regulatory Exposure Limits:

The Occupational Safety and Health Administration (OSHA) has set permissible hydroquinone exposure limits for general industry, construction industry, and maritime at 2 mg/m^3 for an 8 hr time weighted average (Sylvain, 1993; OSHA, 2007). The National Institute for Occupational Safety and Health (NIOSH) has set a recommended exposure limit to hydroquinone at 2 mg/m^3 for 15 min and an immediately dangerous to life or health concentration at 50 mg/m^3 (OSHA, 2007).

Consumer Exposure:

Hydroquinone occurs in some plants as free hydroquinone or as arbutin (hydroquinone β-D-glucopyranoside) and therefore may be found in many consumer products, such as vegetables, fruits, grains, coffee, tea, beer, and wine (DeCaprio, 1999). The concentration of hydroquinone may exceed 1% in some food preparations (Gillner *et al.*, 1994).



Arbutin

Hydroquinone is approved for use in skin-lightening creams, which may contain up to 2% hydroquinone as over-the-counter (OTC) products, and at higher concentrations in prescription-only products. In 2006, the FDA's Drug Listing System identified 206 products marketed by approximately 65 different manufacturers containing 0.4–5.0% hydroquinone (21 CFR Part 310, FDA, 2006). Currently, only one prescription product, which contains 4% hydroquinone along with two additional active ingredients, has been approved by the FDA.

Occupational Exposure:

Occupational exposure to hydroquinone may occur by inhalation or dermal contact, especially in those who develop black and white film, since hydroquinone is a common component of developing solutions (HSDB, 2005d). During photographic development, ambient air levels of hydroquinone are lower than 0.01 mg/m^3, however, during preparation of the developing solutions from dry hydroquinone, the levels are likely higher (DeCaprio, 1999). Darkroom workers do not have increased urinary levels of

hydroquinone as compared to unexposed controls, suggesting that hydroquinone uptake in that industry is minimal (DeCaprio, 1999).

A 1985 report by the Center for Chemical Hazard Assessment (CCHA) found that airborne concentrations of hydroquinone in production facilities typically ranged up to 4.8 mg/m^3, with the highest concentrations in packaging areas, and that concentrations reached up to 35 mg/m^3 where no local exhaust ventilation was available (Santodonato *et al.*, 1985). A cohort study involving workers employed in the manufacture and use of hydroquinone for at least 6 months at the Tennessee Eastman Division (TED) of Eastman Chemical Company between 1930 and 1990 found no significant increase in kidney cancer, liver cancer or leukemia when compared to the general population of Tennessee or to employees of Eastman Kodak Company in Rochester, NY (Pifer *et al.*, 1995). Using air monitoring data from routine surveys at TED since the late 1940s, hydroquinone exposure levels were estimated to be: 5.0 mg/m^3 (1930-1945); 2.5 mg/m^3 (1946-1951); 6.0 mg/m^3 (1952-1956); 2.0 mg/m^3 (1957-1966); and 0.4 mg/m^3 (1967-1990) (Pifer *et al.*, 1995).

The National Occupational Exposure Survey (NOES), conducted from 1981 to 1983 by NIOSH, estimated that a total of 442,749 employees in 36 different industries were potentially exposed to hydroquinone (http://www.cdc.gov/noes). Among these, 19.4% and 12.4% of potentially exposed employees were in the printing/publishing and the health services industries, respectively. Among the remaining industries, each employed less than 10% of the potentially exposed workers. The NOES database does not include information about the extent of exposure to chemicals that were evaluated.

Environmental Exposure:

Hydroquinone occurs in nature in the leaves, bark, and fruit of several plants, especially certain berry-bearing shrubs (HSDB, 2005d). Hydroquinone also may be an important factor in the defense mechanisms of some insects, most notably the bombardier beetle (HSDB, 2005d).

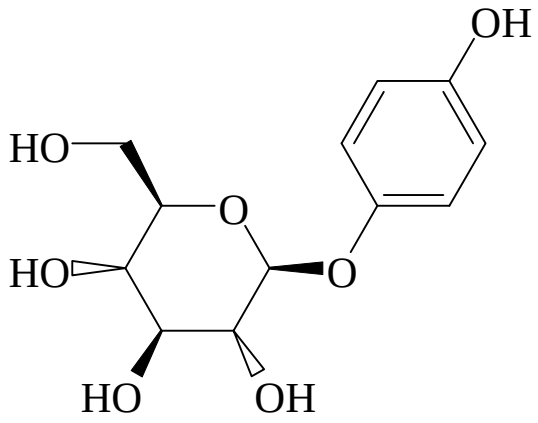
Hydroquinone may be released into the environment during its production, formulation, and use as a chemical intermediate, a photographic chemical, or as a stabilizer in paints, varnishes, motor fuels, and oils (HSDB, 2005d). Hydroquinone has been detected in cigarette smoke (mainstream and sidestream) from non-filter cigarettes, with a range of 110-300 μg per cigarette in mainstream smoke (Devillers *et al.*, 1990; Gillner *et al.*, 1994).

Among states that reported environmental releases of hydroquinone in 2002, Texas reported the highest at 394,966 lbs of hydroquinone in total

environmental releases, followed by Louisiana at 89,238 lbs, and Tennessee at 15,351 lbs (Scorecard, 2002). The remaining reporting states, Ohio, Mississippi, New York, West Virginia, the commonwealth of Puerto Rico, Illinois, Nebraska, Georgia, and Wisconsin, released a combined total of 13,356 lbs (Scorecard, 2002). In 2002, 65 companies nationwide reported environmental releases of hydroquinone, including off-site waste transfers, totaling 1,329,253 lbs (TRI, 2005).

Exposure from other compounds:

Hydrolysis of Arbutin



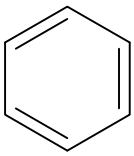
Arbutin

Arbutin (hydroquinone β-D-glucopyranoside) is found in the leaves and fruits of a number of plants that are used as foods. Arbutin is used in some skin-lightening cosmetic products as an alternative to hydroquinone. In addition, arbutin is the active ingredient found in bearberry leaves and leaf extracts, which are used in dietary supplements as a diuretic and for the treatment of urinary tract infection, cystits, and kidney stones.

Arbutin hydrolysis to hydroquinone occurs enzymatically in mammals and some bacteria. In humans, arbutin is rapidly metabolized and excreted as hydroquinone, hydroquinone glucuronide, and hydroquinone sulfate.

As a result, subsets of the population may be exposed to hydroquinone through consumption of arbutin in the diet followed by metabolic hydrolysis, and topical application or industrial exposure to hydroquinone.

Oxidation of Benzene



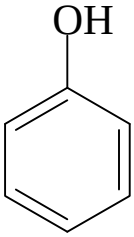
Benzene

Benzene is used as a solvent and is an intermediate in the production of a number of chemicals. Its use as a solvent has declined due to concerns about its carcinogenic effects and is generally found only in industrial settings. Risk of excess benzene exposure exists among those working in industries involving benzene production (*e.g*. petrochemical industry), in rubber film manufacturing, in transport or storage of benzene or benzene-containing products, and in gas stations (HSDB, 2005b).

In 2002, total environmental releases of benzene, including off-site waste transfers, were reported at 13,359,547 lbs (TRI, 2005).

In the liver, benzene is oxidized to benzene oxide, which spontaneously forms phenol, which may be hydroxylated to form hydroquinone (Kari, 1989).

Oxidation of Phenol



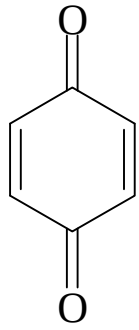
Phenol

Phenol is used as a disinfectant and antiseptic, and as a chemical intermediate. It is also released by combustion processes, including wood stoves and vehicle exhaust, and is produced in decaying organic matter (HSDB, 2005c). Exposure to phenol in industrial settings typically occurs through accidental skin contact or inhalation.

In 2002, reported environmental releases of phenol, including off-site waste transfers, totaled 21,817,499 pounds (TRI, 2005).

Hydroquinone is produced by oxidation of phenol. Metabolites from phenol (oral administration to humans) include trace amounts (>0.1%) of sulfate and glucuronide conjugates of hydroquinone (HSDB, 2005c).

Reduction of Benzoquinone



Benzoquinone

Benzoquinone is found in tobacco smoke and as a waste product from the coal industry. It is an intermediate in the production of hydroquinone, is used as an oxidizing agent, is used in insecticides and fungicides, and is used in the production of cortisone (HSDB, 2005a).

In 2002, reported environmental releases of 1,4-benzoquinone, including off-site waste transfers, totaled 413,717 pounds (TRI, 2005).

Hydroquinone may be produced by reduction of 1,4-benzoquinone.

C. Absorption, Distribution, Metabolism, Elimination (ADME)

Absorption

Oral

Oral administration of hydroquinone leads to high absorption rates. Peak concentration of plasma hydroquinone (5 times background) and peak urinary excretion of hydroquinone (12 times background) were reported in humans 2-3 h following a meal high in hydroquinone-containing foods (Deisinger *et al.*, 1996).

A toxicology review of hydroquinone noted several reports indicating relatively rapid absorption of hydroquinone via the oral route, including a study involving rats that ingested 3% hydroquinone in developer solution (DeCaprio, 1999). In addition, in CD and F344 rats dosed with 350 mg/kg, >90% absorption was measured in blood levels, with peak levels observed within 1 h (DeCaprio, 1999). Gavage administration of hydroquinone to F344 rats resulted in peak blood concentration within 20 min, with elimination means of 87-94% in urine and cage rinses, and 1-3% in feces by 48 hr (English and Deisinger, 2005).

Dermal

Human absorption of hydroquinone upon topical application is less efficient than with oral administration. When absorption was measured as elimination

of hydroquinone via urine following application (2.0% in alcohol) to the foreheads of human volunteers (6 males per preparation) for 24 hr, the average percutaneous absorption reported was 57% (SD = 11%) with peak elimination within 12 hr and complete elimination by 5 days (Bucks *et al.*, 1988). The addition of a sunscreen (3.0% Escalol 507) significantly decreased the absorption (26%, SD = 14%), and the addition of a penetration enhancer (0.5% Azone) did not significantly increase absorption in the presence or absence of the sunscreen (35%, SD = 17% and 66%, SD = 13%, respectively) (Bucks *et al.*, 1988).

When 2% [^{14}C]-hydroquinone was administered to human forearms (n = 4 males) in an unspecified cream, hydroquinone moved rapidly and continuously into the stratum corneum and radiolabel was detected in plasma samples within 0.5 hr (Wester *et al.*, 1998). Over an 8 hr plasma sampling period, hydroquinone levels peaked at 4 hr (0.04 μg-equivalents/ml) (Wester *et al.*, 1998). Following application of the 2% cream on the foreheads of 6 male volunteers for 24 hr, the recovery of hydroquinone in urine was 45.3% (SD = 11.2%) (Wester *et al.*, 1998).

Aqueous solutions containing hydroquinone permeated mouse and rat skin *in vitro* with permeability constants (K_p) of 28 x 10^{-6} and 23 x 10^{-6} cm/hr, respectively, and *in vitro* human skin K_p values of 4 x 10^{-6} and 9.3 x 10^{-6} cm/h have been reported (Barber *et al.*, 1995; DeCaprio, 1999). *In vitro* human skin absorbed 43.3% of hydroquinone from a 2% cream at 2.85 μg/cm^2/h (Wester *et al.*, 1998). Inhibition of metabolic enzymes with sodium azide did not affect *in vitro* absorption (Wester *et al.*, 1998), suggesting that the penetration was by passive and not active transport.

Following dermal administration of 4 or 40 mg in 95% ethanol to male F344/N rats and B6C3F$_1$ mice, hydroquinone (at least partially as glucuronide and sulfate conjugates) was qualitatively detected in the urine by 2 hr, and continued to be detected up to 72 hr (Kari, 1989). Dermal application of 5.4% hydroquinone in an aqueous solution for 24 hr on the skin of F344 rats produced low absorption with 61-71% recovered from the surface and 0.65 μg hydroquinone equivalents/g in the blood of females and not quantifiable in the blood of males (English and Deisinger, 2005).

Distribution

Following intravenous (i.v.) administration of radiolabeled hydroquinone, radioactivity (either hydroquinone or a metabolite) was detected within 2 hr in bone marrow and thymus of rats given 1.2–12 mg/kg (DeCaprio, 1999). Radioactivity was also detected in the liver and bone marrow of these rats up to 24 h. Whether given in single or repeated oral doses, radioactivity was

found in various rat tissues, with the highest concentrations in the liver and kidneys. Following i.v. administration of radiolabeled hydroquinone in dogs, radioactivity was found in the skin, liver, and intestine. When mice were administered 75 mg/kg radiolabeled hydroquinone by intraperitoneal (i.p.) injection, radioactivity was detected covalently bound to proteins in the liver, kidneys, blood, and bone marrow, with 10-fold higher specific activity in the liver than in the bone marrow (DeCaprio, 1999).

Metabolism and Elimination

Hydroquinone elimination occurs rapidly, primarily via urine. A review found that in F344 rats, the blood elimination half-life of hydroquinone administered i.v. was 18.7 min and that with oral administration was 14.8 min (DeCaprio, 1999). With i.p. administration to $B6C3F_1$ mice, the hydroquinone half-life was 9 min (DeCaprio, 1999).

Biotransformation of hydroquinone to its detoxified urinary glucuronide and sulfate conjugated metabolites has been recognized for decades (Figure 1). A toxicological review of hydroquinone indicated that in rats, oral administration of [^{14}C]-hydroquinone resulted in <1% of the detected radiolabel in blood found associated with the parent compound, indicating extensive, rapid metabolism (DeCaprio, 1999). The review also noted that oral dosing of experimental animals with up to 350 mg/kg hydroquinone leads to 90% recovery in the form of these metabolites, with roughly two thirds as the glucuronide and one third as the sulfate (DeCaprio, 1999). Additional minor elimination products included unchanged hydroquinone, benzoquinone (likely formed *in situ* by autoxidation of hydroquinone), and the detoxified metabolite *N*-acetyl-(*L*-cystein-*S*-yl)hydroquinone (Figure 1) (DeCaprio, 1999).

Glucuronate and ethereal sulfate conjugates, but not free hydroquinone, were found in the composite 24 hr urine of a single human volunteer who received 3 mg/kg hydroquinone orally (CIR, 1986).

Only glucuronide conjugates were found as urinary metabolites following human dermal exposure to a 2% hydroquinone cream for 24 hr (Wester *et al.*, 1998). In addition to hydroquinone, benzoquinone was detected in receptor fluid during *in vitro* skin experiments with the same cream. Treatment with azide decreased the benzoquinone levels, without affecting absorption (Wester *et al.*, 1998).

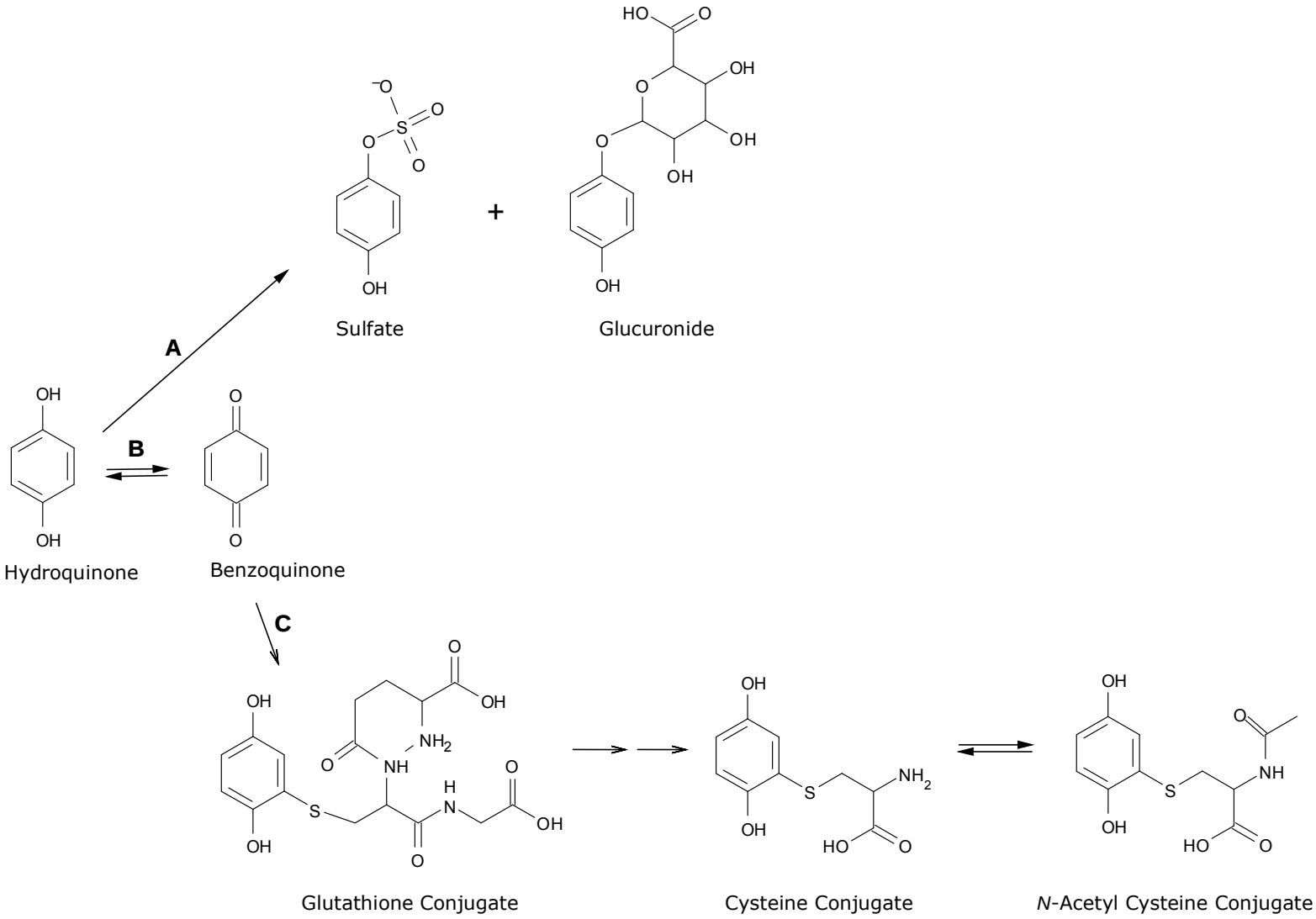


Figure 1. Hydroquinone metabolic pathways. A) The glucuronide and sulfate metabolites comprise approximately 90% of hydroquinone metabolic products in urine (at a ratio of 2 glucuronide to 1 sulfate). The reactions are performed by glucuronyl transferase and sulfotransferase, respectively. B) Hydroquinone forms benzoquinone in a reversible reaction that may be spontaneous, or may be performed by cytochrome P450. C) Glutathione-*S*-transferase adds 1-4 glutathion-*S*-yl group(s), which may then undergo cleavage by γ-glutamyl transpeptidase (γGT) and a dipeptidase to form the cysteine conjugate(s). *N*-acetyltransferase performs the final step in this pathway, forming the mercapturate(s), or *N*-acetyl cysteine conjugate(s).

Hydroquinone compounds recovered in urine after i.v. (20 mg/kg) administration to cats included approximately 87% sulfate and 3% glucuronide conjugates, and 10% unchanged hydroquinone (CIR, 1986). In his review, DeCaprio found that although parenteral administration of hydroquinone produces high levels of urinary glucuronide and sulfate conjugates, additional conjugates and oxidized products are also found, likely because the hepatic portal and intestinal sulfate and glucuronide conjugation pathways are bypassed (DeCaprio, 1999). Following i.p. administration of 50 mg/kg hydroquinone to rats and rabbits, the oxidation product 1,2,4-benzenetriol was recovered in urine (DeCaprio, 1999). Additional conjugation products following i.p. administration included *N*-acetyl-(*L*-cystein-*S*-yl)hydroquinone in rat urine, and 2-(*L*-cystein-*S*-yl)hydroquinone and a number of glutathione conjugates in rat bile (Figure 1) (DeCaprio, 1999).

Following i.p. administration of 1.8 mmol/kg hydroquinone in SD rats, the sum of *S*-conjugates excreted into the bile and urine totaled 4.3% (± 1.1%) of the dose (Hill *et al.*, 1993). In the bile, the major *S*-conjugate metabolite was 2-(glutathion-*S*-yl)hydroquinone [2-(GSyl)HQ] (18.9 ± 2.7 μmol) (Hill *et al.*, 1993). Additional biliary metabolites in the rats included 2,5-(diGSyl)HQ (2.2 ± 0.6 μmol); 2,6-(diGSyl)HQ (0.7 ± 0.3 μmol); 2,3,5-(triGSyl)HQ (1.2 ± 0.1 μmol); and 2-(CysSylGlycyl)HQ (no quantity reported). A single urinary thioether metabolite, 2-(*N*-AcetylCysSyl)HQ (11.4 ± 3.6 μmol), was also identified (Hill *et al.*, 1993).

Using metabolic rate constants for the metabolism and ultimate detoxification of hydroquinone via mercapturic acid formation in cultured human and rat (F344) hepatocytes, a computer model suggested that human hepatocytes have a greater capacity to metabolize hydroquinone and its mono-glutathione conjugate than rat hepatocytes (Poet *et al.*, 2004). When applied to an existing physiologically based pharmacokinetic model, the model predicted that bioactivation of hydroquinone predominated in the rat, while deactivation predominated in humans (Poet *et al.*, 2004).

In rabbits given hydroquinone orally, the concentration of hydroquinone in the body affected the rate of glucuronide conjugation, but not that of organic sulfate formation (CIR, 1986).

Mechanism/Enzyme Induction and Inhibition

Melanins are a class of compounds derived from tyrosine that serve predominantly as pigments. Eumelanins (brown or black pigments) and pheomelanins (yellow or red pigments) are responsible for the pigmentation of human skin and hair. Melanin is produced (melanogenesis) in melanocytes

through tyrosinase catalyzed-pathways including: 1) the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine (L-dopa) and 2) the oxidation of L-dopa to 4-(2-aminoethyl)-1,2-benzoquinone (dopaquinone) (DeCaprio, 1999; Parvez *et al.*, 2006).

Hydroquinone lightens skin through melanocytotoxicity and inhibition of melanogenesis. Hydroquinone toxicity is specific for melanocytes as a result of their tyrosinase activity (Penney *et al.*, 1984; Curto *et al.*, 1999; DeCaprio, 1999; Parvez *et al.*, 2006; Draelos, 2007). Hydroquinone inhibits DNA and RNA synthesis and alters melanosome formation (Penney *et al.*, 1984; Briganti *et al.*, 2003; Draelos, 2007). Hydroquinone also inhibits tyrosinase, thereby inhibiting melanogenesis (Penney *et al.*, 1984; Chakraborty *et al.*, 1993; DeCaprio, 1999; Gupta *et al.*, 2006; Parvez *et al.*, 2006; Draelos, 2007). Hydroquinone competes with tyrosine as a substrate for tyrosinase, especially in the presence of L-dopa (Gupta *et al.*, 2006). Tyrosinase oxidizes hydroquinone, producing toxic benzoquinones (Penney *et al.*, 1984; Si *et al.*, 1987).

Glutathione-*S*-transferase (GST) catalyzes the conjugation of glutathione to quinones to detoxify the compounds. Inhibition of glutathione (GSH) synthesis or depletion of GSH potentiates hydroquinone inhibition of tyrosinase, reducing melanin production (Bolognia *et al.*, 1995; Briganti *et al.*, 2003; Kasraee *et al.*, 2003). The reduced GSH available for conjugation with the toxic quinone metabolites of hydroquinone likely contributes to the increase in depigmentation (DeCaprio, 1999). All-*trans*-retinoic acid (TRA), which also potentiates hydroquinone depigmentation, is an effective inhibitor of GST (Kasraee *et al.*, 2003).

D. Acute, Subchronic, and Chronic Toxicity

Acute Toxicity

Rodents

Reviews of hydroquinone toxicology listed hydroquinone acute oral LD_{50} values of: 245 mg/kg in the mouse, 320 mg/kg in the rat, 200 mg/kg in the dog, 70 mg/kg in the cat, and 550 mg/kg in the guinea pig (Devillers *et al.*, 1990; DeCaprio, 1999). The acute oral LD_{50} value in the Sprague-Dawley (SD) rat was >375 mg/kg (Topping *et al.*, 2007).

Among F344/N rats that received hydroquinone orally in corn oil 12 times over 14 days, 1 of 5 males and 4 of 5 females given 500 mg/kg and all rats given 1,000 mg/kg died during the study (National Toxicology Program [NTP] TR-366, Kari, 1989). Body weights of males and females were 9% and

18% lower, respectively, than controls, and clinical signs included tremors for up to 30 min immediately following dosing.

Daily administration of hydroquinone in corn oil to B6C3F$_1$ mice resulted in the deaths of 3 of 5 males at 250 mg/kg and of 4 of 5 males and 5 of 5 females at 500 mg/kg within 3 days (Kari, 1989). Signs of acute toxicity included decreased body weights in male mice receiving 250 or 500 mg/kg and tremors followed by either convulsions and death, or by recovery, in mice that survived the 2-week (12 doses) study (Kari, 1989).

The acute dermal toxicity of hydroquinone in F344/N rats and B6C3F$_1$ mice is >3840 and >4800 mg/kg, respectively, as all animals survived 12 doses in 95% ethanol at these levels over 14 days with no clinical signs, except male rat body weight was 94% that of controls (Kari, 1989). No mortality or neurobehavioral effects were observed in rabbits dermally exposed to 2000 mg/kg hydroquinone under an occlusive wrap for 24 hr (Topping *et al.*, 2007).

Subcutaneous (s.c.) injection of hydroquinone in mice resulted in LD$_{50}$ values of 182 and 190 mg/kg, while the value for i.p. injection was 100 mg/kg (Devillers *et al.*, 1990; DeCaprio, 1999). LD$_{50}$ values in rats for i.p. injection were reported as 160 and 170 mg/kg, and that for i.v. injections was 115 mg/kg (Devillers *et al.*, 1990; DeCaprio, 1999). In rabbits, i.p. injection resulted in a LD$_{50}$ of 125 mg/kg (Devillers *et al.*, 1990).

DeCaprio (1999) suggested that acute effects produced by exposure to high levels of hydroquinone primarily affect the central nervous system (CNS), with signs including tremor, salivation, hyperexcitability, incoordination, convulsions, respiratory failure, coma, and death (DeCaprio, 1999). Topping *et al*. (2007) found that tremors occurred within 1 hr of oral administration of 64 or 200 mg/kg of hydroquinone in SD rats, but had resolved by 6 hr. The 1989 NTP study noted tremors for up to 30 min in F344/N rats immediately following oral dosing with 500 or 1,000 mg/kg hydroquinone and tremors, convulsions, and death in some male B6C3F$_1$ mice that received 250 or 500 mg/kg (Kari, 1989). Blacker *et al.* (1993) also noted infrequent, mild, transient tremors in several (21 to 24 of 56 to 60 per group) CD SD rats shortly after dosing with 150 mg/kg/day, and in a single male dosed at 50 mg/kg/day.

Following large, sublethal doses of hydroquinone, recovery from CNS symptoms is rapid and complete (DeCaprio, 1999). Chronic exposure to lower doses (64 or 200 mg/kg/day) given to SD rats 5 days a week for 13 weeks resulted in only transient tremors and decreased motor activity without neuropathological changes (Topping *et al.*, 2007).

Humans

At least one case report has suggested that dermal application of hydroquinone might cause peripheral neuropathy (Karamagi *et al.*, 2001). The case involved a 30-year old woman who had used two skin bleaching creams containing hydroquinone for approximately 4 years. The woman had weakness, a burning sensation, loss of deep tendon reflexes, and impairment of deep sensation in her lower extremities, along with very low blood pressure. After 4 months of no skin bleaching cream use, symptoms disappeared (Karamagi *et al.*, 2001).

Humans who have accidentally or deliberately ingested ≥ 1 g of hydroquinone have also experienced CNS signs such as tremor, dizziness, muscular twitching, headache, and delirium, in addition to tinnitus, nausea, respiratory difficulty, convulsions, and unconsciousness (Devillers *et al.*, 1990; DeCaprio, 1999). When 544 men aboard a U.S. Navy ship accidentally consumed water contaminated with photographic developer, they experienced acute gastroenteritis (DeCaprio, 1999; HSDB, 2005d).

The 1985 CCHA report indicated that exposure to airborne hydroquinone, usually during production and packaging, caused noticeable eye irritation at 2.2 mg/m^3 and more severe irritation at 13.6 mg/m^3, although systemic toxic effects were not produced by repeated exposure to 20-30 mg/m^3 (Santodonato *et al.*, 1985). The concern that hydroquinone dust concentrations above 2 mg/m^3 or vapor levels over 0.1 ppm might result in irreversible eye damage led to more stringent controls over exposure during the manufacture of hydroquinone (Santodonato *et al.*, 1985).

Subchronic Toxicity

Rodents

Hydroquinone administered by gavage at 200 mg/kg in corn oil 5 days per week resulted in the deaths in 3 of 10 female F344/N rats after 11 weeks (Kari, 1989). Administration of a higher dose of hydroquinone (gavage, 400 mg/kg) resulted in the deaths of all 10 male and 10 female F344/N rats after approximately 7 weeks (Kari, 1989). Surviving males given 200 mg/kg were lethargic after 10 weeks, and females exhibited tremors and some convulsions, but doses of 100 mg/kg and lower did not produce clinical signs. Upon necropsy, nephrotoxicity consisting of tubular cell degeneration and regeneration in the renal cortex, was noted in 7 of 10 male and 6 of 10 female rats at 200 mg/kg and in 1 of 10 female rats at 100 mg/kg (Kari, 1989). Lesions in the kidneys of male rats were described as more severe than those in the females.

In a 13-week gavage study involving dosing with hydroquinone in corn oil 5 days per week, 16 of 20 B6C3F_1 mice receiving 400 mg/kg died (1 death was due to error) and 2 of 10 male mice receiving 200 mg/kg died during the study (Kari, 1989). At both doses mice were lethargic and at the high dose tremors were observed, often followed by convulsions. Male mice also exhibited tremors at 200 mg/kg and significantly higher liver to body weights at all doses, as compared to controls. Additionally, 5 of 20 mice at 400 mg/kg and 1 of 20 mice at 200 mg/kg exhibited ulceration, inflammation, or epithelial hyperplasia of the forestomach (Kari, 1989).

In another study with lower doses, male F344 rats receiving approximately 25 or 100 mg/kg/d hydroquinone *ad libitum* in the diet for 13 weeks exhibited no toxicity, measured as body and liver weights and microscopic evaluation of liver samples (Williams *et al.*, 2007).

Although many of its metabolites are detoxification products, at least one minor intermediate product, 2,3,5-triglutathion-*S*-yl-hydroquinone (TGHQ), is more toxic than hydroquinone (Lau *et al.*, 2001). When administered i.p. (2.5 μmol/kg for 4 months followed by 3.5 μmol/kg for 6 months), TGHQ caused numerous toxic tubular dysplasias with subsequent adenomas, basophilic dysplasias and renal cell carcinomas in Eker rats (Lau *et al.*, 2001). These rats carry a mutation inactivating one of the alleles of the tuberous sclerosis 2 (*tsc-2*) suppressor gene, making them highly susceptible to renal tumor development.

Humans

When human subjects ingested 300-500 mg hydroquinone in 3 divided doses with meals daily for 3-5 months, no abnormal results were noted in percent hemoglobin, hematocrit, red blood cell count, differential white blood cell count, sedimentation rate, platelet count, coagulation time, and icteric index in blood samples and no abnormal levels were noted in urinary albumin, reducing sugars, white and red cell counts, casts, and urobilinogen (Carlson and Brewer, 1953). The authors suggested that the lack of toxic response may have been due to the division of dose throughout the day at meals, which may have decreased peak blood levels.

Renal Toxicity

The major products of hydroquinone metabolism are the detoxified sulfate and glucuronide metabolites, which together account for approximately 90% of hydroquinone metabolites and are excreted in urine (Figure 1) (Kari, 1989; DeCaprio, 1999). The glutathione conjugates are minor hydroquinone metabolic intermediates (Figure 1), exhibit increasing nephrotoxicity with

increasing glutathione substitution, with the exception of the tetra-substituted intermediate (Lau *et al.*, 1988; Lau *et al.*, 1995; Peters *et al.*, 1997; Lau *et al.*, 2001; Boatman *et al.*, 2004). Using 24 hr blood urea nitrogen (BUN) concentrations to measure nephrotoxicity, the mono- and tetra-glutathione conjugates, 2-(glutathion-*S*-yl)hydroquinone [2-(GSyl)HQ] and 2,3,5,6-(tetraGSyl)HQ, were not nephrotoxic in male SD rats when injected i.v., 2-(GSyl)HQ required a dose of 250 μmol/kg to elicit an increase in BUN, and 2,3,5,6-(tetraGSyl)HQ did not increase BUN at doses of 10-50 μmol/kg (Lau *et al.*, 1988). The di-glutathione-hydroquinone conjugates (2,3-, 2,5- and 2,6-) exhibited similar levels of nephrotoxicity, eliciting increased BUN at doses of 30-50 μmol/kg. The most nephrotoxic among the glutathione metabolites, 2,3,5-(triGSyl)HQ, produced a steep dose-response curve, with elevation of BUN beginning at 20 μmol/kg (Lau *et al.*, 1988).

The glutathione-hydroquinone conjugates form protein adducts in the blood, liver, kidney, and spleen in a dose-dependent manner (Boatman *et al.*, 2000). When administered hydroquinone orally, female F344 rats had higher levels of adducts in the blood, liver, and kidney than did their male counterparts, and male SD rats had the lowest levels among the 3 groups (Boatman *et al.*, 2000). Liver adducts were predominantly those of 2-(GSyl)HQ (>72% of liver total), while adducts in the kidney were predominantly those of 2,3,5-(tri-) and 2,3,5,6-(tetraGSyl)HQ (combined, 60% of kidney total) (Boatman *et al.*, 2000). When hydroquinone was administered i.p., protein adduct levels increased in each tissue type, especially blood and spleen (Boatman *et al.*, 2000). While the concentration of the most nephrotoxic metabolite [2,3,5-(triGSyl)HQ] in the kidney likely contributes to nephrotoxicity, the authors suggest that it is not the only factor (Boatman *et al.*, 2000). Other factors are indeed likely involved, especially since male F344 rats, rather than females, are generally more sensitive.

The toxicity and carcinogenicity of hydroquinone and its metabolites in the rat kidney have been concentrated in the outer stripe of the outer medulla (OSOM), particularly in the renal proximal tubules (Figure 2) (Lau *et al.*, 1988; Hard *et al.*, 1997; Peters *et al.*, 1997; Lau *et al.*, 2001; Boatman *et al.*, 2004). Distinctive hypotheses have been suggested to explain the focus of nephrotoxicity in this area of the kidney. Literature reviews and an independent reevaluation of tissue sections from the 1989 NTP study (TR-366, Kari, 1989) have suggested that hydroquinone exacerbates chronic progressive nephropathy (CPN), a spontaneous, age-related disease occurring primarily in male rats with no known counterpart in humans (Whysner *et al.*, 1995; Hard *et al.*, 1997; Hard, 1998; DeCaprio, 1999). Others have postulated that the high concentration of γ-glutamyl transpeptidase (γGT) on the surface of the renal proximal tubule cells may

promote reabsorption of the glutathione-conjugated metabolites of hydroquinone (Lau *et al.*, 1988; Peters *et al.*, 1997; Lau *et al.*, 2001).

Chronic Progressive Nephropathy and Cell Proliferation:

Reevaluation of tissues from the 1989 NTP gavage study (also see section F of this document) found that the degree of CPN in male F344 rats increased with hydroquinone treatment (Hard *et al.*, 1997). Atypical tubule hyperplasias or adenomas were found only in areas of severe or end-stage CPN and their association with the degree of CPN was statistically significant (Hard *et al.*, 1997). A 2-year feeding study (0.8% hydroquinone in diet) also found renal tubular hyperplasia and adenomas associated with CPN in male F344 rats (Shibata *et al.*, 1991). In this study, renal tubular hyperplasia also developed in male $B6C3F_1$ mice, but with no CPN association (Shibata *et al.*, 1991). Severity of CPN increased only slightly in female rats (Shibata *et al.*, 1991).

CPN enhances the proliferation of renal tubular cells and a dose-related increase in this proliferation was observed with hydroquinone (Hard *et al.*, 1997). Renal tubular cell proliferation in response to damage may promote tumor development in that area (Whysner *et al.*, 1995). Cell proliferation in response to treatment with hydroquinone or its metabolites has been noted in segments of the renal proximal tubules (English *et al.*, 1994b; Peters *et al.*, 1997).

Hydroquinone administered via gavage for 6 weeks at 50 mg/kg to male F344 rats caused proximal tubular damage, as evidenced by increases in the rate of excretion of enzymes and histopathological assessment (English *et al.*, 1994b). Cell proliferation was measured by incorporation of bromodeoxyuridine (BrdU) in cells of the 3 segments of the proximal tubules (Figure 2), P1 (the portion of the *pars convoluta* adjacent to the glomerulus), P2 (the distal portion of the *pars convoluta* and the proximal portion of the *pars recta*), and P3 (the distal portion of the *pars recta* adjacent to Henle's loop, found in the OSOM). Significantly increased ($P < 0.001$) cell proliferation was observed in segments P1 (87%) and P2 (50%), but the increase in P3 cell proliferation (34%) was not significant (English *et al.*, 1994b). Female F344 rats and male SD rats receiving the same treatment did not exhibit the same effects.

Increased proliferation of renal tubular cells in response to damage caused by the metabolite 2,3,5-(triGSyl)HQ has also been observed (Peters *et al.*, 1997). In F344 rats administered 7.5 μmol/kg 2,3,5-(triGSyl)HQ i.v., cell proliferation, measured by incorporation of BrdU, increased over controls by 3-fold at 24 hr, 19-fold at 48 hr, and 18-fold at 72 hr in only the OSOM, and particularly in the S_3M segment (P3 segment) of the renal proximal tubules

(Peters *et al.*, 1997). The 2,3,5-(triGSyl)HQ was approximately 600 times more potent than hydroquinone, therefore, although it is a minor metabolic intermediate, very little is required to cause nephrotoxic effects (Peters *et al.*, 1997). The location of damage and regeneration in this study is in contrast to that of the above gavage study by English *et al.* (1994b). Peters *et al.* (1997) noted necrosis in only the S_3M region with both hydroquinone and 2,3,5-(triGSyl)HQ, and suggested that differences in the design of the studies (chronic vs. acute) or administration routes (gavage vs. i.v.) may be responsible for the differences.

Dermal application of 5.0% hydroquinone in an oil-in-water emulsion cream to F344 rats 5 days/wk for 13 weeks (applied doses of 73.9 mg/kg/day in males and 109.6 mg/kg/day in females) resulted in no renal lesions nor sustained cell proliferation in the kidneys (measured by BrdU incorporation) (David *et al.*, 1998). At 3 wk the labeling index (LI), a measure of mitotic activity, of each segment of the renal proximal tubule of male rats was higher than that of controls, however, only the P1 segment LI was higher by a statistically significant margin (David *et al.*, 1998). No changes in LI over controls were reported at 6 wk or 13 wk, however, indicating no sustained cell proliferation.

γ-Glutamyl Transpeptidase:

Using enzyme inhibitors, several studies have shown that the nephrotoxicity of the glutathione conjugates, and that of hydroquinone, requires the activity of γGT (Lau *et al.*, 1988; Lau *et al.*, 1995; Peters *et al.*, 1997). This enzyme is found on the surface of epithelial cells. Its responsibilities include the extracellular breakdown of glutathione into its constituent amino acids, providing cells with cysteine and helping to maintain the intracellular glutathione level, and the conversion of glutathione-conjugates into mercapturic acids (Hanigan, 1998; Chikhi *et al.*, 1999). Many human tumors express high levels of γGT, as do many carcinogen-induced tumors in animals (Vanderlaan and Phares, 1981; Hanigan, 1998).

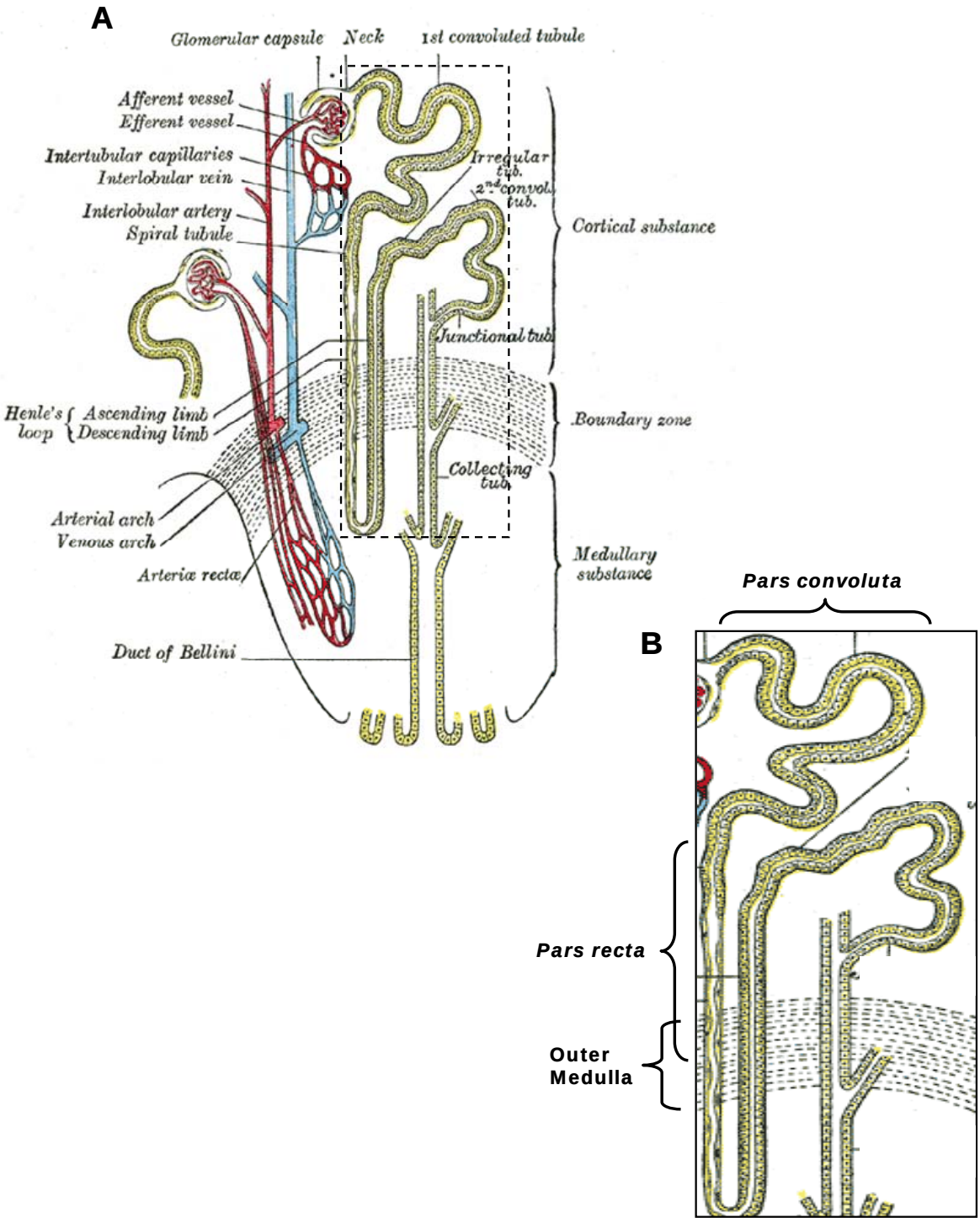


Figure 2. Structure of a renal nephron. A) Nephron with vascular supply. A dotted line indicates the portion shown enlarged in panel B. B) Renal tubule with proximal tubule *pars convoluta* and *pars recta* and kidney outer medulla (OM) labeled. The *pars recta* extends into the OM. Tubules are generally depicted as thicker than Henle's loop (shown same size here). (From the 20th U.S. edition of *Gray's Anatomy of the Human Body*, 1981.)

The presence of γGT in the brush border membrane of renal proximal tubular cells may serve to focus the toxicity of hydroquinone-glutathione conjugates during reabsorption, causing damage at the junction of the medullary rays and the OSOM (Welbourne and Dass, 1982; Peters *et al.*, 1997). The glutamyl groups are cleaved from the hydroquinone-glutathione conjugates [HQ-$(GSyl)_{1-4}$] by γGT, leaving cysteine-glycine conjugates, which are metabolized to the cysteine conjugates by a dipeptidase [HQ-$(Cys)_{1-4}$] (Figure 1). HQ-$(Cys)_{1-4}$ may be further metabolized by *N*-acetyltransferase into detoxified mercapturic acid metabolites (Figure 1) (DeCaprio, 1999).

Under physiological oxygen conditions (5% O_2/5% CO_2/90% N_2) in renal proximal tubule (PT) cells from male F344 and SD rats *in vitro*, 2-(GSyl)HQ was more cytotoxic than 2,3,5-(triGSyl)HQ, as measured by lactate dehydrogenase leakage (viability) and the ATP to ADP ratio (Boatman *et al.*, 2004). This may be explained by the metabolism of 2-(GSyl)HQ to 2-(CysSyl)HQ, which was observed as early as 1 hr after treatment (Boatman *et al.*, 2004). Additionally, 2-(Cysyl-Glycyl)HQ and 2-(*N*-acetylCysSyl)HQ were identified as metabolites of 2-(GSyl)HQ in this system (Boatman *et al.*, 2004). Among the compounds tested, the order of toxicity to isolated renal PT cells was: 2-(CysSyl)HQ > 2-(GSyl)HQ > hydroquinone. In this system, 2,3,5-(triGSyl)HQ was not cytotoxic (Boatman *et al.*, 2004).

Under high oxygen conditions (95% O_2/5% CO_2) in the absence of BSA, *in vitro* renal PT cells from male F344 rats were more susceptible than those of male SD rats to hydroquinone, as evidenced by decreases in the ATP to ADP ratios and in cell viability (Boatman *et al.*, 2004). At physiological oxygen levels, PT cells from both species were equally susceptible. The SD PT cells appeared to have a higher capacity to respond to oxidative stress than those of F344 rats, due to their higher concentration of ATP per cell, which may explain the differences seen in susceptibility to hydroquinone under high oxygen conditions (Boatman *et al.*, 2004).

The activity and regulation of γGT differ among rodent species, and may contribute to differences in susceptibility to 2,3,5-(triGSyl)HQ (Lau *et al.*, 1995). Rats (F344 and SD) had the highest γGT activity levels and were the most sensitive to 2,3,5-(triGSyl)HQ (20 mmol/kg i.v.), while hamsters had the lowest activity and were not sensitive (Lau *et al.*, 1995). The γGT activities of mice ($B6C3F_1$ and BALB/c) and guinea pigs were similar (30-45% that of rats), but only guinea pigs were sensitive to 2,3,5-(triGSyl)HQ (200 μmol/kg i.v.) (Lau *et al.*, 1995). The ratio of *N*-deacetylase to *N*-acetylase activity was highest in the guinea pig (4.57, as compared to 0.16 for the next highest in BALB/c mice), which may explain the sensitivity of this species, since this ratio favors formation of the cysteine conjugate over

the detoxified mercapturate (Lau *et al.*, 1995). The authors note that rat γGT is more susceptible to inhibition by AT-125 (acivicin) than that of human, bovine, canine, or porcine, which may indicate differences in γGT regulation among these species (Lau *et al.*, 1995).

Skin Irritation

Reports of skin irritation or allergy in association with hydroquinone occur infrequently. Individual case reports include allergic contact cheilitis and somatitis from the use of an acrylic dental prosthesis (Torres *et al.*, 1993), allergic contact hypersensitivity associated with exogenous ochronosis due to the use of a 2% hydroquinone cream for melasma (Camarasa and Serra-Baldrich, 1994), and allergic contact dermatitis in areas treated with a 5% hydroquinone, 10% glycolic acid cream following peeling with 70% glycolic acid (Barrientos *et al.*, 2001). Studies involving larger groups generally note mild, transient adverse effects, usually in a fraction of subjects, that include erythema, skin peeling, burning sensation and irritant patch-test reactions (Bentley-Phillips and Bayles, 1975; Kanerva *et al.*, 1999; Taylor *et al.*, 2003; Torok *et al.*, 2005; O'Donoghue, 2006). At least one group has questioned the status of hydroquinone as a "known allergen", and suggested that only additional testing, including re-patch tests, repeated open application tests, and provocative use tests, may definitively determine whether hydroquinone is an allergen or an irritant (Lalloo *et al.*, 1997).

Contact Leukoderma / Vitiligo

Vitiligo, or leukoderma, is a skin disease characterized by the loss of pigment due to the death or dysfunction of melanocytes. Contact leukoderma may occur when skin is exposed to a chemical with a structure similar to that of tyrosine (Fisher, 1994). Hydroquinone-induced leukoderma most commonly occurs from contact with photographic developer (Fisher, 1994).

At least 5 cases of contact leukoderma have been reported in association with the use of hydroquinone-containing skin creams (Fisher, 1982; Romaguera and Grimalt, 1985). The creams contained 2% (Fisher, 1982) or 3% (Romaguera and Grimalt, 1985) hydroquinone. While the creams were being used for depigmentation to lighten areas of the skin, in these cases complete depigmentation, rather than lightening, was achieved in treated areas (Fisher, 1998). The condition may lead to splotchy areas of depigmentation with confetti-like areas of hyperpigmentation (leukoderma-en-confetti) (Fisher, 1998). The condition is not an allergic response and is not detected by patch-tests (Fisher, 1998). In at least one case, the contact leukoderma delayed surgery, as depigmentation occurred in the center of a

lentigo maligna lesion, indicating that hydroquinone can affect abnormal melanocytes in addition to normal melanocytes (Fisher, 1998).

Exogenous Ochronosis

Ochronosis describes the skin discoloration associated with alkaptonuria, an inherited condition in which the enzyme homogentisic acid (HGA) oxidase is missing, allowing HGA to accumulate. The blue-black discoloration associated with the condition is due to polymerized HGA deposition in collagen-containing structures. Systemic effects associated with alkaptonuria include: premature osteoarthritis symptoms, persistent and painless ocular and cutaneous pigmentation, cartilage and prostate gland calcification, darkened urine, and calcification and stenosis of heart valves (Levin and Maibach, 2001).

Exogenous ochronosis is characterized by hyperpigmentation of the skin associated with topical exposure to various compounds, including hydroquinone. No systemic symptoms are associated with exogenous ochronosis. Because hydroquinone absorbs ultraviolet light in the "sunburn waveband" (peak at 293 nm), sunlight likely aggravates and accelerates exogenous ochronosis. The presence of other active derivatives and the use of penetrating vehicles may exacerbate this effect (Hardwick *et al.*, 1989; DeCaprio, 1999). Clinical observations have also indicated that sunlight plays a part in the development of exogenous ochronosis (Hull and Procter, 1990). Histopathological analyses of exogenous ochronosis describe curled, banana-shaped bundles of ochre-colored elastoid fibers in the papillary dermis associated with collagen fibril bundles (Martín *et al.*, 1992; DeCaprio, 1999; Levin and Maibach, 2001; Olumide *et al.*, 2008). The ochronotic fibers appear to form from existing normal collagen fibrils (Phillips *et al.*, 1986).

Three stages of exogenous ochronosis have been described (Dogliotti and Leibowitz, 1979). Stage I is distinguished by erythema and mild hyperpigmentation of the face and neck. Stage II is marked by black "colloid milium-like" or "lumps of caviar-like" papules and mild atrophy. Stage III is characterized by the formation of papulonodular lesions that are usually found in the firm connective tissues and bony areas of the face.

Theories of Mechanism:

Although the mechanism of hydroquinone-induced exogenous ochronosis is not known, several theories have been suggested by multiple reviewers. An *in vitro* assay in black goldfish skin found that 9×10^{-4} M hydroquinone suppressed the activity of tyrosinase, while 4.5×10^{-3} M hydroquinone increased the enzyme's activity (Chen and Chavin, 1975). Based on this study, some reviewers have suggested that high concentrations of

hydroquinone may stimulate the production of melanin (Lawrence *et al.*, 1988; Martín *et al.*, 1992; DeCaprio, 1999; Levin and Maibach, 2001; Olumide *et al.*, 2008).

Another theory, called "bypass effect" or "melanocyte recovery", suggests that, following constant exposure to hydroquinone, melanocytes may become resistant, no longer selectively taking up hydroquinone, and start over-production of melanin. This "recovery" would be accompanied by an increased transfer of hydroquinone into the papillary dermis, where it would be taken up by fibroblasts, leading to an alteration of elastic fibers and production of abnormal fibers (Martín *et al.*, 1992; DeCaprio, 1999; Levin and Maibach, 2001; Olumide *et al.*, 2008).

Some reviewers have pointed to hydroquinone studies involving HGA oxidase, suggesting that hydroquinone may inhibit the enzyme, leading to an accumulation of HGA, as seen in endogenous ochronosis (Lawrence *et al.*, 1988; Martín *et al.*, 1992; DeCaprio, 1999; Levin and Maibach, 2001; Olumide *et al.*, 2008).

Some theories for the mechanism of hydroquinone induction of exogenous ochronosis involve hydroquinone metabolites. Oxidation of hydroquinone to form quinones may activate tyrosinase (Levin and Maibach, 2001), or may lead to the formation of colored hydroxylated indoles that are similar to melanin precursors (Martín *et al.*, 1992; DeCaprio, 1999). Benzoquinone acetic acid, which is formed during hydroquinone oxidation, may damage collagen fibers, allowing deposition of pigmented fibers (DeCaprio, 1999; Olumide *et al.*, 2008).

Melanocytes are apparently required for development of ochronosis, since in at least one case of hydroquinone-induced exogenous ochronosis, areas of vitiligo, containing no basal melanocytes, within the area of treatment did not develop exogenous ochronosis (Hull and Procter, 1990).

Treatment:

Early treatment for exogenous ochronosis generally involved avoiding skin lightening creams and application of sunscreens along with hydrocortisone and/or retinoic acid (Lawrence *et al.*, 1988; Martín *et al.*, 1992; Camarasa and Serra-Baldrich, 1994; Levin and Maibach, 2001). Additional treatments include antibiotics; dermabrasion, alone or in combination with CO_2 laser; and Q-switched ruby laser (Levin and Maibach, 2001; Levitt, 2007; Tan *et al.*, 2008). All of these treatments have at least some efficacy, although some take time and may provide transient or variable results (Levin and Maibach, 2001).

Epidemiology:

The first hydroquinone-containing skin lightening creams were sold in the U.S. in 1955 and in South Africa in 1961 (Hardwick *et al.*, 1989). By 1969 an increasing number of patients with hyperpigmentation on their faces and necks were noted in South Africa, and by 1979 the numbers had reached "epidemic proportions" (Hardwick *et al.*, 1989). In 1975 Findlay *et al.* determined that skin-lightening creams containing hydroquinone caused exogenous ochronosis (Findlay *et al.*, 1975). In a 1982 tentative final monograph, the U.S. FDA limited the concentration of hydroquinone to 2% in OTC skin bleaching creams (21 CFR Part 358, FDA, 1982). In an attempt to control the "epidemic", in 1983 the South African government limited the concentration of hydroquinone in skin-lightening creams to 2% and required the addition of sunscreen with a minimum sun protection factor (SPF) of 5 to the formulations (Hardwick *et al.*, 1989). By 1985 three cases of exogenous ochronosis in the U.S. had been reported in the literature (Hoshaw *et al.*, 1985). According to a literature review, a worldwide total of 789 cases of exogenous ochronosis had been reported through the end of 2006, including 22 cases in the U.S. and 756 cases in South Africa (Levitt, 2007).

The majority of exogenous ochronosis cases have been reported in South African blacks, due to the marked use of skin bleaching products in that population. An epidemiological study (all black outpatients at the hospital) found that, among black outpatients in two South African hospitals, 8 of 53 (15%) males and 60 of 142 (42%) females had exogenous ochronosis (Hardwick *et al.*, 1989). In addition, 60 of 87 (69%) individuals who used skin bleaching products were affected (Hardwick *et al.*, 1989).

In their 1992 review, Martín *et al*. noted that, of the 8 U.S. cases reported by that time, 7 had involved African-American females and 1 had involved a Mexican-American female (Martín *et al.*, 1992). The first Asian cases of exogenous ochronosis were reported in 2008, following treatment with hydroquinone for melasma in 2 Chinese females (Tan *et al.*, 2008).

A number of exogenous ochronosis cases have been reported with use of creams with relatively low concentrations (≤ 2%) of hydroquinone. In a review, Levitt (2007) noted that long-term use of hydroquinone-containing products, rather than high concentration, may be the greatest risk factor for development of exogenous ochronosis.

Factors suggested to have contributed to the relatively high incidence of exogenous ochronosis cases in South Africa include: 1) high concentrations of hydroquinone used in South Africa prior to 1984; 2) the use of additional depigmentation agents, such as t-butyl alcohol and mercuric compounds

prior to 1986; 3) use of resorcinol, another ochronotic agent, separately and in combination with hydroquinone; 4) the addition of hydroalcoholic lotion in South African formulations to increase hydroquinone penetration; 5) sunny climate with greater average solar exposure; and 6) relatively less regular use of sunscreens in South Africa (Levin and Maibach, 2001; Levitt, 2007). Studies have indicated that at least some products contain concentrations of hydroquinone that are higher than those indicated on labels (Boyle and Kennedy, 1986; Martín *et al.*, 1992; Mahé *et al.*, 2003). This labeling discrepancy may be responsible for an enhancement of exogenous ochronosis incidence around the world, even though most countries have limited the concentration allowed in skin bleaching products.

While at least one review draws attention to the relatively low overall incidence of exogenous ochronosis (only 789 reported cases worldwide between 1955 and 2007) (Levitt, 2007), others suggest that the condition is underreported (Martín *et al.*, 1992; Tan *et al.*, 2008). In support of the idea that the condition is underreported, at least 2 cases of exogenous ochronosis were originally misdiagnosed as failed melasma treatment (Tan *et al.*, 2008).

E. Genotoxicity and Mutagenicity

Exposure route significantly affects the genotoxicity and mutagenicity of hydroquinone. Reviews of hydroquinone revealed that, although most *in vitro* studies are positive, *in vivo* studies are generally negative, most likely due to rapid detoxification, except when parenteral administration is used (Whysner *et al.*, 1995; DeCaprio, 1999). Negative mutagenicity results have been obtained by a number of studies: in various strains of *Salmonella typhimurium* or *Escherichia coli;* in *Saccharomyces cerevisiae* strain D4; in *Drosophila;* in an *in vivo* mouse spot test; and in a dominant lethal assay in male rats following oral doses of 30, 100, or 300 mg/kg/day hydroquinone for 10 weeks (Whysner *et al.*, 1995; DeCaprio, 1999). Fewer positive tests have been reported for hydroquinone mutagenicity using *Salmonella* strains (caused base-pair changes in the TA1535 tester strain and was mutagenic in the oxidant sensitive tester strains TA104 and TA2637); *S. cerevisiae* strain D3; and in culture of rodent cells (Whysner *et al.*, 1995; DeCaprio, 1999).

Although a number of studies have shown *in vitro* formation of DNA adducts following exposure to hydroquinone, similar adduction does not occur *in vivo* and is not likely involved in hydroquinone toxicity (Reddy *et al.*, 1990; DeCaprio, 1999). In their review of hydroquinone tumorigenicity, Whysner *et al.* (1995) noted that hydroquinone bound to DNA in *in vitro*, but not *in vivo* assays, although it did bind to rat bone marrow tissues *in vivo*. The review also listed positive results from *in vitro* assays for 1) oxidative DNA damage

in human bone marrow cells; 2) DNA strand breakage and repair in a bacteriophage and with activation in hamster bone marrow cells; and 3) inhibition of DNA replication in HeLa cells (with and without activation), in mouse lymphoma cells (with activation), in rabbit bone marrow mitochondria and in mouse bone marrow cells, and inhibition of rat liver mitochondrial DNA polymerase (Whysner *et al.*, 1995). Negative *in vitro* results were listed for DNA strand breakage and repair, for inhibition of DNA replication in mouse lymphoma cells, and for inhibition of DNA replication in rat liver mitochondria (Whysner *et al.*, 1995). An *in vivo* oxidative DNA damage test was negative in mouse bone marrow tissue (Whysner *et al.*, 1995).

The hydroquinone tumorigenicity review noted several studies indicating that hydroquinone, or its metabolite, benzoquinone, may inhibit microtubule assembly, impairing spindle formation and causing chromosome lagging (*i.e.* anaphase lag) (Whysner *et al.*, 1995). Benzoquinone reacts with or oxidizes the sulfhydryl groups of proteins, including microtubule subunits (Si *et al.*, 1987). Bone marrow cytotoxicity may be due to the metabolism of hydroquinone to benzoquinone by myeloperoxidase, and the subsequent inhibition of microtubule polymerization (Si *et al.*, 1987; Whysner *et al.*, 1995).

Although most studies indicate no direct mutagenicity, many have shown that hydroquinone causes chromosomal aberrations, abnormal mitoses, formation of micronuclei (MN), aneuploidy, and sister chromatid exchange (SCE) (DeCaprio, 1999). The review of hydroquinone tumorigenicity listed positive results from a number of *in vitro* and *in vivo* chromosomal aberration tests, with one negative in a fungus (*Aspergillus nidulans* strain 19) with activation (the same test was positive without activation) (Whysner *et al.*, 1995). The review also listed positive results for numerous *in vitro* and *in vivo* tests for aneuploidy and micronucleus formation, with a single negative test in Chinese hamster DON and LUC cells *in vitro* for micronucleus formation (Whysner *et al.*, 1995). Four studies showed positive *in vitro* SCE tests in human lymphocytes (Whysner *et al.*, 1995). The review also listed a single positive *in vitro* test for inhibition of microtubule polymerization in F344 rat brain microtubulin (Whysner *et al.*, 1995).

Silva Mdo, *et al*. (2004) examined the effects of glutathione-*S*-transferase (GST) polymorphisms, *GSTM1*, *GSTT1*, and *GSTP1*, on the performance of hydroquinone in genotoxicity tests. The *GSTM1* null genotype, which has been implicated as a risk factor for acute childhood myeloid leukemia and lymphoblastic childhood leukemia, produced higher MN frequency, but did not affect SCE or chromosomal aberrations (Silva Mdo *et al.*, 2004). The other two polymorphisms did not affect any of the three genotoxicity tests.

F. Carcinogenicity

In a 2-year gavage study in which hydroquinone was administered in water to F344/N rats (25 or 50 mg/kg) and to B6C3F$_1$ mice (50 or 100 mg/kg), survival rates were lower than those of controls and *some evidence* of carcinogenic activity was observed in all groups except the male mice (Kari, 1989). Male rats developed renal tubular cell adenomas when given 25 mg/kg (4 of 55) and 50 mg/kg (8 of 55); female rats developed mononuclear cell leukemia when given 25 mg/kg (15 of 55), 50 mg/kg (22 of 55) and controls (9 of 55); female mice developed hepatocellular adenomas or carcinomas when given 50 mg/kg (16 of 55), 100 mg/kg (13 of 55) and controls (3 of 55) (Kari, 1989).

At 15 months, 10 animals from each group in the 2-year gavage study above were sacrificed for additional analyses. The mean kidney weights, relative to body weights, of male rats at 50 mg/kg was significantly higher than those of controls (Kari, 1989). Male rats experienced a hydroquinone-related increase in severity of nephropathy. All male rats, including controls, exhibited at least mild nephropathy, while 50% at 25 mg/kg and 60% at 50 mg/kg exhibited moderate nephropathy (Kari, 1989). Nephropathy was less severe in female rats, in which mostly minimal and mild lesions were noted in 50-70% of animals, with only one control animal having moderate lesions (Kari, 1989). The mean relative weights of mouse liver (male and female) and brain (female) at 100 mg/kg, and of female kidney at 50 and 100 mg/kg were significantly higher than those of controls (Kari, 1989). All male mice at 100 mg/kg and at least 80% at 50 mg/kg exhibited liver-associated lesions, while few female mice (at most 5 of 10) exhibited such lesions (Kari, 1989). At 100 mg/kg, significant increases were observed in the hematocrit value, erythrocyte count, serum albumin concentration, total protein concentration and the activities of serum alkaline phosphatase and sorbitol dehydrogenase of male mice and for the serum albumin and total protein concentration for female mice (Kari, 1989). The high dose female mice also had significantly lower alanine aminotransferase and sorbitol dehydrogenase activities (Kari, 1989).

Hard *et al*. reevaluated kidney sections from the 1989 NTP study (TR-366, Kari, 1989) and determined that hydroquinone did not directly cause renal tumor development, but rather exacerbated existing CPN in the high-dose male rats and stimulated proliferation of renal tubular cells in the advanced stages of CPN (Hard *et al.*, 1997). In their evaluation, the authors found that 3 of 55 and 7 of 55 male rats given 25 and 50 mg/kg hydroquinone, respectively, had developed benign renal adenomas. In addition, the kidneys of 2 of 55 and 11 of 55 male rats (receiving 25 and 50 mg/kg, respectively) had 1 or 2 foci of atypical tubule hyperplasia (Hard *et al.*, 1997). The

authors noted that the low-dose male rats exhibited some atypical hyperplasias and adenomas, which were not associated with an increase in CPN, suggesting that stimulation of proliferation of rat kidney proximal tubule cells may be involved in hydroquinone toxicity as well (Hard *et al.*, 1997). Because CPN is a spontaneous, age-related renal disease that affects various strains of rats but has no counterpart in humans, the authors suggest that this mechanism of hydroquinone toxicity has little relevance for humans (Hard *et al.*, 1997; Hard, 1998). In a review that focused on the potential for hydroquinone carcinogenicity and risk to humans, McGregor concluded that hydroquinone is carcinogenic only in the context of end-stage CPN, which is irrelevant in humans (McGregor, 2007).

Assessment of a cohort of lithographers in the Danish Cancer Register from 1974 to 1989 revealed 5 cases (2 exposed to hydroquinone) of malignant melanoma, with 1.5 expected (relative risk 3.4, 95% confidence interval 1.2–7.5) (Nielsen *et al.*, 1996).

In a 1999 monograph, the International Agency for Research and Cancer (IARC) determined that hydroquinone was *not classifiable as to its carcinogenicity to humans (Group 3)* due to *inadequate evidence* in humans and *limited evidence* in experimental animals for carcinogenicity (IARC, 1999).

Leukemia

Most studies investigating a possible function for hydroquinone in the development of leukemia have focused on its role as a metabolite of benzene, a known leukemogen. Phenolic metabolites of benzene (phenol, hydroquinone, and catechol) are formed in the liver and transported to the bone marrow, where they have been implicated in benzene leukemogenesis.

A review of hydroquinone noted that it effects stem cell differentiation (DeCaprio, 1999). In a human promyelocytic leukemic cell line (HL-60), some studies have indicated that hydroquinone suppressed monocytic, but not granulocytic, differentiation in a dose-related manner, while other studies suggested that the pattern of suppression by hydroquinone is dependent on the inducer involved (Kalf and O'Connor, 1993; DeCaprio, 1999). For example, hydroquinone did not prevent the monocytic induction of HL-60 cells induced by IL-1 (Kalf and O'Connor, 1993), and did prevent the granulocytic induction of these cells by retinoic acid (DeCaprio, 1999). One study indicated that pretreatment of bone marrow cells (murine and human) enhanced the clonogenic response to granulocyte-macrophage colony stimulating factor (GM-CSF), but not to IL-3 (Irons and Stillman, 1996), while another study suggested that hydroquinone inhibited GM-CSF

activity in a dose-related fashion (Colinas *et al.*, 1994). Reviews of hydroquinone have noted its ability to induce myeloblast differentiation to the myelocyte stage, but not beyond (DeCaprio, 1999; Westerhof and Kooyers, 2005).

Hydroquinone and its major metabolite in the bone marrow, benzoquinone, have been shown to inhibit apoptosis of proliferating myelocytes by inhibiting CPP32 protease (caspase 3) (Hazel *et al.*, 1996; Westerhof and Kooyers, 2005). In addition, hydroquinone inhibition of apoptosis has been attributed to its enhancement of the activity of c-Myb, a regulator of cell proliferation, differentiation, and apoptosis (Wan *et al.*, 2005). Hydroquinone also inhibits p53 tumor suppression, which is significant for the development of leukemia, by increasing the expression of Bcl2, which blocks Bax activity, and therefore, apoptosis (Westerhof and Kooyers, 2005).

The loss (monosomy) of one copy of chromosomes 5 and 7, and the gain (trisomy) of an extra chromosome 8 are common clonal chromosomal abnormalities associated with acute myelogenous leukemia (AML). As a metabolite of benzene, the effect of hydroquinone on specific chromosomes was tested in peripheral blood lymphocytes. Hydroquinone exposure produced a dose-dependent increase in monosomy of chromosomes 1, 5, 6, 7, 8, 9, 11, and 12, but not of chromosome 21 (Zhang *et al.*, 2005). The effects were highly significant ($P<0.0001$) for chromosomes 5, 7, 9, and 11, and the incidence rate ratios (IRRs) were highest (>3) for chromosomes 5, 6, and 12. Monosomy of chromosomes 5 and 7 was more sensitive to hydroquinone than any other chromosome tested over all concentrations ($P=0.0025$), and at low exposure levels (0-25 μM; $P=0.029$). Hydroquinone exposure also caused trisomy in a dose-dependent manner, especially of chromosomes 7, 8, and 21 (0-100 μM; $P<0.0001$) (Zhang *et al.*, 2005).

AML may also arise from damage to specific DNA regions, since in at least 2 AML patients, the *GRAF* (GTPase regulator associated with the focal adhesion kinase $pp125^{FAK}$) gene at 5q31 of chromosome 5 has been fused with the *MLL* (mixed lineage leukemia) gene at 11q23 of chromosome 11 (Borkhardt *et al.*, 2000; Panagopoulos *et al.*, 2004). AML has been associated both with deletions at 5q31 from treatment with alkylating agents or exposure to benzene, and with translocations of 11q23 from treatment with topoisomerase II inhibitors (Felix, 2001; Larson and Le Beau, 2005; Pedersen-Bjergaard, 2005). Escobar *et al.* (2007) studied hydroquinone-induced DNA damage in TK6 lymphoblastoid cells and found a dose-dependent increase in damage at both locations, with significantly more DNA damage at 5q31 than at 11q23.

McDonald et al. (2001) presented a hypothesis linking hydroquinone and phenol to *de novo* leukemia, especially in those with no known exposure, where the varying background levels of both compounds found in the blood and urine, likely due to their presence in food, cigarette smoke, and OTC medications, contribute to development of the disease (McDonald *et al.*, 2001). The authors suggest that, although sulfation may initially detoxify the compounds, bone marrow contains high concentrations of sulfatase, which could produce unconjugated hydroquinone and phenol in the bone marrow (McDonald *et al.*, 2001). Admitted shortcomings of the hypothesis included the fact that hydroquinone and phenol are not considered carcinogens, the rapid metabolism and elimination of the compounds, the fact that the mechanism of benzene leukemogenicity is unknown, and the large variability in susceptibility to hydroquinone-mediated hematotoxicity from benzene exposure (McDonald *et al.*, 2001).

Several reviews have dismissed any role for hydroquinone in the development of leukemia, outside of its function as a benzene metabolite. The significance of the observation of increased rates of leukemia in female F344 rats by the 1989 NTP gavage study (Kari, 1989) has been questioned, especially in light of the fact that a similar gavage study using higher concentrations of hydroquinone did not observe the same increase and the fact that the observed incidence of leukemia was not statistically higher than that of historical controls (Whysner *et al.*, 1995; DeCaprio, 1999; Levitt, 2007). In addition, leukemia in rats originates in the spleen, while in humans it originates in the bone marrow (Levitt, 2007). No epidemiological evidence of hydroquinone-induced leukemia has been reported in exposed populations (Pifer *et al.*, 1995; DeCaprio, 1999; Westerhof and Kooyers, 2005).

G. Reproductive and Developmental Toxicity

Hydroquinone substantially increased resorption rates during pregnancy in Walter Reed-Carworth Farms rats, from 40.8% of control litters to 100% of litters treated with a total of 0.5 g of hydroquinone in the diet (time frame not reported, Telford *et al.*, 1962). Among all implantations, 10.6% in controls and 26.8% in treated animals ended in resorption (Telford *et al.*, 1962).

A 1999 review examined a number of studies involving the effects of hydroquinone on reproduction and development (DeCaprio, 1999). The review found that, in one study of cultured rat embryos, those treated with hydroquinone exhibited growth retardation at 45 μM and 68 μM and structural defects at 68 μM; and in another study, no effects were noted at 10 μM or 50 μM, but the embryos did not survive 100 μM or 200 μM (DeCaprio, 1999). Oral administration of 200 mg/kg/day hydroquinone to

female rats for 2 weeks inhibited estrus and caused significant toxicity, including clonic seizures, respiratory effects and 30% mortality (DeCaprio, 1999). In studies using s.c. administration in rats, hydroquinone at 10 mg/kg/day for 11 days interrupted estrus in females, and at 100 mg/kg/day for 51 days inhibited spermatogenesis, caused testicular changes, and decreased fertility in males (DeCaprio, 1999). The review also noted that dermal exposure of up to 810 mg/kg/day hydroquinone on gestational days (gd) 6-19 in SD rats did not produce teratogenic effects (DeCaprio, 1999). No treatment-related effects on reproductive parameters were noted when male mice were exposed to up to 300 mg/kg/day oral hydroquinone for 10 wk (5 days/wk) and then mated to untreated females (DeCaprio, 1999).

Decreased maternal body weights and food consumption were noted when hydroquinone was administered orally to New Zealand White rabbits at 75 or 150 mg/kg on gd 6-18; or to SD rats at 30-300 mg/kg on gd 6-15; or when 300 mg/kg/day was administered by gavage to COBS-CD-BR rats on gd 6-15 (Krasavage *et al.*, 1992; DeCaprio, 1999). In the rabbits, the no-observable-effect-level (NOEL) for maternal toxicity was 25 mg/kg, and for developmental toxicity (in the presence of maternal toxicity) the NOEL was 75 mg/kg/day, with no statistically significant effects (Murphy *et al.*, 1992). In COBS-CD-BR rats, the NOEL for maternal and developmental toxicity was 100 mg/kg (Krasavage *et al.*, 1992).

A two-generation study in CD SD rats (one litter per generation) found NOELs for general and reproductive toxicity of 15 and 150 (highest dose) mg/kg/day, respectively (Blacker *et al.*, 1993). The only statistically significant effects observed were in body weights of the F1 parental males at 50 and 150 mg/kg/day at several points from premating through postmating (Blacker *et al.*, 1993).

LD_{50} values for hydroquinone administered by injection into air sacs of chick embryos (55-65 g eggs) were 8.59 μg/egg when administered at 72 h incubation and 15.63 μg/egg when administered at 96 h incubation (Burgaz *et al.*, 1994). Several signs of toxicity were higher in embryos receiving 0.0625-20 μg/egg at both time points, however, none were significantly different than those in controls. While hydroquinone was embryocidal at high doses (5-20 μg/egg), its teratogenic potential was determined to be "unremarkable" (Burgaz *et al.*, 1994). In contrast, an *in vitro* study referenced by the FDA in its most recent proposed rule involving hydroquinone (FDA, 2006) indicated that hydroquinone inhibited the normal growth of 7 out of 15 ovaries from 10-day chick embryos.

In his review of hydroquinone, DeCaprio (1999) described results from a number of reproductive and developmental studies (including virtually all of those mentioned in this section). In general, he found that doses required to produce fetotoxicity were high enough to cause maternal toxicity, which was similar to that seen in non-pregnant animals at the same doses. DeCaprio (1999) concluded that, even at high levels of hydroquinone exposure, reproductive or teratogenic effects were not prominent.

H. Environmental Fate and Aquatic Toxicity

Reviews describing the environmental and health risks of hydroquinone concluded that, because of the physicochemical properties of the compound, its environmental distribution is mainly in the water compartment (Devillers *et al.*, 1990; Gillner *et al.*, 1994). In water, hydroquinone may be degraded under aerobic or anaerobic conditions, and may undergo direct photolysis. Bioaccumulation in aquatic organisms, if any occurs, is expected to be minimal (Devillers *et al.*, 1990; Gillner *et al.*, 1994; HSDB, 2005d). In soil, hydroquinone may be subject to aerobic biodegradation, photolysis (on the surface), or oxidation (HSDB, 2005d).

The 96 h LD_{50} values for hydroquinone in flow-through bioassays with fathead minnows and rainbow trout were 0.044 mg/L and 0.097 mg/L, respectively, and the 48 h LD_{50} value with *Daphnia publicaria* was 0.162 mg/L (DeGraeve *et al.*, 1980). Hydroquinone produced a dark solution in the assay, due the fact that 52% was oxidized to *p*-benzoquinone (DeGraeve *et al.*, 1980).

I. Current Regulation

Other Countries

From 1965 to 2007, 10 reports of adverse events were received by Health Canada that were suspected to be associated with the use of OTC skin lightening products (Canada, 2008). Hydroquinone is on Canada's Cosmetic Ingredient Hotlist, which prohibits its use in cosmetic products for application on the skin or mucous membranes (Canada, 2008). The Canadian Government is planning to propose additional restrictions on the use of hydroquinone in nail systems and hair dyes and to propose more strict regulation of hydroquinone-containing products as prescription drugs rather than OTC (Canada, 2008).

Hydroquinone has been banned in Europe and Japan for use in OTC skin lightening products due to questions about its safety (Briganti *et al.*, 2003; Westerhof and Kooyers, 2005; Draelos, 2007). It was banned in the European Union in 2001 due to concerns about leukoderma-en-confetti

(splotchy areas of complete depigmentation with confetti-like areas of hyperpigmentation) and exogenous ochronosis (Westerhof and Kooyers, 2005).

U.S. FDA Proposed Rule

In August 2006, as part of its review of OTC products, the FDA published a proposed rule in the Federal Register stating that OTC skin bleaching drug products are no longer generally recognized as safe and effective (GRASE) and are misbranded (21 CFR Part 310, FDA, 2006). The proposed rule withdrew the September 1982 tentative final monograph that had recognized OTC skin bleaching drug products containing 1.5–2.0% hydroquinone as GRASE (47 FR 39108, FDA, 1982). In the proposed rule the FDA cited a number of studies, published since the 1982 tentative final monograph, that have prompted the agency to conclude that insufficient data exist to make a final determination on the safety of hydroquinone and a regulatory position on hydroquinone.

The new studies described in the 2006 proposed rule, which indicate potential data gaps, fell into 4 categories: fertility, absorption, carcinogenicity and occurrence of exogenous ochronosis (21 CFR Part 310, FDA, 2006). The FDA noted that existing studies involving the effects of hydroquinone on fertility have produced conflicting results. The rate of absorption of hydroquinone by human skin caused the agency to recommend additional dermal studies using OTC concentrations of hydroquinone. In addition, an NTP report concluded that *some evidence* exists for hydroquinone carcinogenicity. Finally, the FDA noted a number of studies and case reports describing disfiguring effects (exogenous ochronosis) resulting from the use of hydroquinone-containing skin bleaching agents.

Responses to Proposed Rule

In a joint response to the FDA proposed rule, the American Health & Beauty Aids Institute (AHBAI) and the Dermatology Section of the National Medical Association (NMA) requested that the FDA: 1) consider all of the pharmacology/toxicology data on hydroquinone that has been published since the 1982 tentative final monograph, 2) consider undertaking a pharmacoepidemiological study to determine the risks involved with the use of prescription hydroquinone products, and 3) consider only the cases of exogenous ochronosis reported in the U.S., and not those in Africa (Duncan Jones, 2006; Palm and Toombs, 2007). The response listed 22 references with toxicology data published since the 1982 tentative final monograph as "additional data for FDA review" that had not been cited in the 2006 proposed rule (Table 1, Duncan Jones, 2006). In addition, the authors argue

that the majority of exogenous ochronosis cases have been reported in Africa, where hydroquinone is used much more extensively and at higher concentrations (6-8.5%) than in the U.S. (Duncan Jones, 2006; Palm and Toombs, 2007). The AHBAI and NMA suggest that exogenous ochronosis cases reported in the U.S. are rare, considering the volume of hydroquinone product sales, and are categorized as Stage I, generally responding well to treatment, while those reported in Africa represent a "more aggressive disease" and are categorized in the more severe Stage III (Duncan Jones, 2006; Palm and Toombs, 2007).

The American Academy of Dermatology Association (AADA) also wrote to oppose the FDA's 2006 proposed rule on hydroquinone. The AADA's objections to the proposed rule focused on: 1) the FDA's underestimation of the benefits of OTC hydroquinone products to patients with dyschromias, which are associated with significant morbidity; 2) the effects of a ban on OTC hydroquinone products on the underrepresented minority groups, who suffer disproportionately more dyschromias and are disproportionately less likely to receive medical attention; 3) the rarity of exogenous ochronosis in the U.S. population (10 million users in the past 40 years vs. 28 cases reported in the U.S. between 1983 and 2000); and 4) the unproven association between cancers observed in animal studies and risk to humans (Stone, 2006). In light of its assessment that the benefits of hydroquinone products to the U.S. public far outweigh the risks, the AADA recommended "no change in the status of either [OTC] or prescription hydroquinone containing products" (Stone, 2006).

Also in response to the 2006 FDA proposed rule concerning hydroquinone, a dermatologist's review concluded that "the FDA overstates the risks and minimizes the benefits of hydroquinone therapy" (Levitt, 2007). The reviewer suggested that inappropriately extended use, rather than the concentration used, is an important risk factor for exogenous ochronosis and that hydroquinone should be available only by prescription so that its use may be supervised by a physician.

J. Regulatory Position and Recommended Studies

In August 2006, as part of its review of OTC products, the FDA announced in a proposed rule that OTC skin bleaching drug products are no longer generally recognized as safe and effective (GRASE) and are misbranded (21 CFR Part 310, FDA, 2006). This notice withdrew the September 1982 tentative final monograph that had recognized OTC skin bleaching drug products containing 1.5–2.0% hydroquinone as GRASE (47 FR 39108, FDA, 1982). The following were cited by the 21 CFR Part 310 notice as indications

that insufficient data exist to make a final determination on the regulation of hydroquinone:

1. Additional fertility studies are needed due to conflicting studies on the effects of hydroquinone;
2. Because hydroquinone has a high absorption rate in humans, additional studies to determine the safety of dermal use of products containing 2% hydroquinone are required;
3. NTP Report TR 366 indicated *some evidence* of carcinogenicity in male and female rats and in female mice with oral exposure to hydroquinone (Kari, 1989);
4. Topical use of hydroquinone has been shown to cause disfiguring effects (ochronosis) even at low (1–2%) concentrations.

The Carcinogenicity Assessment Committee (CAC) of the FDA's Center for Drug Evaluation and Research (CDER) "indicated that a dermal carcinogenicity study, conducted in an appropriate model with functioning melanocytes, must be performed on hydroquinone to assess both its topical and systemic tumorgenicity" (21 CFR Part 310, FDA, 2006).

The FDA is recommending dermal toxicity and carcinogenicity studies be conducted in pigmented test animals, and reproductive toxicity studies be conducted to address fertility.

Table 1. Publications Suggested for FDA Review (Duncan Jones, 2006).

Reference	Comments
Blacker *et al*. (1993)	No adverse effects on reproduction or fertility in a 2-generation study of SD rats.
Boatman *et al*. (1996)	Pronounced species, sex, and strain dependent differences in acute nephrotoxicity.
Boatman *et al*. (2000)	No correlation between hydroquinone protein binding and clinical signs of nephrotoxicity.
Corley *et al*. (2000)	Development of a physiologically based pharmacokinetic model for hydroquinone.
David *et al*. (1998)	No renal toxicity with dermal administration.
DeCaprio (1999)	Review of hydroquinone toxicology.
Deisinger *et al*. (1996)	Noted the contribution of dietary sources and cigarette smoke to hydroquinone exposure.
Deisinger & English (1999)	No increase in blood levels with simulated hydroquinone inhalation up to 1h at $2mg/m^3$.
Doepker *et al*. (2000)	No significant increase in micronuclei in cultured human lymphocytes.
English *et al*. (1994a)	No covalent DNA adducts in rat kidneys with repeated oral nephrotoxic doses.
English *et al*. (1994b)	Hydroquinone-induced cell proliferation may contribute to renal tumors in male F344 rats.
English & Desinger (2005)	Reported rapid dermal absorption, extensive early metabolism, and urinary excretion. Major metabolites from oral administration were glucuronide and sulfate conjugates.
Hard *et al*. (1997)	Hydroquinone exacerbated rat CPN.
Krasavage *et al*. (1984)	Dominant lethal assay in male rats.
Krasavage *et al*. (1992)	No adverse effects on reproduction or development in rats.
Murphy *et al*. (1992)	NOELs for maternal and developmental toxicity in rabbits of 25 and 75 mg/kg/day.
O'Donoghue *et al*. (1999)	Hydroquinone reduced micronuclei in mouse bone marrow caused by $KBrO_3$
Pifer *et al*. (1995)	No increased incidence of cancer in workers that manufacture and use hydroquinone.
Poet *et al*. (2004)	Metabolic model indicated more hydroquinone activation in rat and deactivation in humans.
Topping *et al*. (2007)	Acute neurobehavioral effects were not exacerbated by subchronic exposure.
Williams *et al*. (2007)	Hydroquinone reduced carcinogen-induced DNA adducts in rat liver.
Whysner *et al*. (1995)	Review of hydroquinone tumorigenicity.

K. References

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