International Journal of Toxicology
29(Supplement 4) 274S-287S

DOI: 10.1177/1091581810385957
http://ijt.sagepub.com

Final Amended Safety Assessment of Hydroquinone as Used in Cosmetics

F. Alan Andersen, PhD, Wilma F. Bergfeld, MD, FACP, Donald V. Belsito, MD, Ronald A. Hill, PhD, Curtis D. Klaassen, PhD, Daniel C. Liebler, PhD, James G. Marks, Jr, MD, Ronald C. Shank, PhD, Thomas J. Slaga, PhD, and Paul W. Snyder, DVM, PhD

Abstract

Hydroquinone is an aromatic compound that functions in cosmetics as an antioxidant, fragrance, reducing agent, or polymerization inhibitor. Hydroquinone is also used as a skin bleaching agent. Safety and toxicity information indicate that hydroquinone is dermally absorbed in humans from both aqueous and alcoholic formulations and is excreted mainly as the glucuronide or sulfate conjugates. Hydroquinone is associated with altered immune function in vitro and in vivo in animals and an increased incidence of renal tubule cell tumors and leukemia in F344 rats, but the relevance to humans is uncertain. Quantitatively, however, the use of hydroquinone in cosmetics is unlikely to result in renal neoplasia through this mode of action. Thus, hydroquinone is safe at concentrations of $\leq$1% in hair dyes and is safe for use in nail adhesives. Hydroquinone should not be used in other leave-on cosmetics.

Keywords

cosmetics, hydroquinone, safety

Introduction

The Cosmetic Ingredient Review (CIR) Expert Panel first assessed the safety of hydroquinone (HQ) as a cosmetic ingredient in 1986[1] and concluded that HQ was found to be safe for use at limited concentrations for certain formulations (primarily hair dyes) A subsequent review was conducted following the completion of the National Toxicology Program (NTP) report on HQ. That amended safety assessment,[2] of HQ dealt with the use of HQ in cosmetic leave-on preparations and was published in 1994 with the conclusion from the CIR Expert Panel that HQ "... is safe at concentrations of $\leq$1% for aqueous cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone should not be used in leave-on, nondrug cosmetic products."[2] This amended safety assessment will address the current uses of HQ in cosmetic products and assess information on the safety of HQ in cosmetic products that have become available since the last (1994) published report.

Chemistry

Definition and Structure

Hydroquinone (CAS No 123-31-9) is the aromatic compound that can function as an antioxidant, a fragrance ingredient, a hair colorant, a reducing agent, and a skin bleaching agent.[3] The structure of HQ is presented in Figure 1. Technical names for this ingredient are presented in Table 1.[3]

Physical and Chemical Properties

In its pure form, HQ is a colorless crystalline solid. Commercial preparations of HQ are usually white to off-white crystalline materials. The physical and chemical properties of HQ are presented in Table 2.[4]

Manufacture and Production

There are 5 grades of HQ: photographic, technical, US pharmacopeia (USP), inhibitor, and polyester. Photographic and technical grades are produced in the highest volume, most commonly through either hydroperoxidation of *p*-diisopropylbenzene, hydroxylation of phenol, or oxidation of aniline.[4]

Corresponding Author:
F. Alan Andersen, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA
Email: cirinfo@cir-safety.org

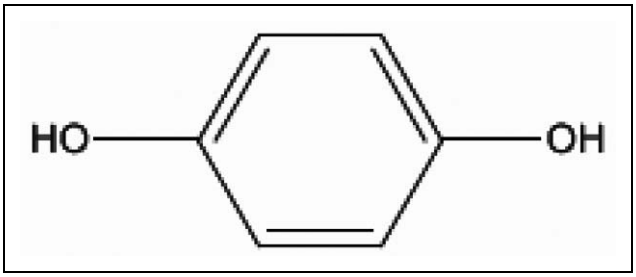

Figure 1. The structure of hydroquinone.

Table 1. Technical Names for Hydroquinone[3]

Ingredient Name	Other Technical Names
Hydroquinone	1,4-Benzenediol 1,4-Dihydroxybenzene p-Dihydroxybenzene 4-Hydroxyphenol p-Hydroxyphenol

Table 2. Physical and Chemical Properties of Hydroquinone[4]

Property	Value
CAS No	123-31-9
Molecular weight	110.11
Color, form	Colorless, crystals
Melting point (°C)	172
Boiling point (°C)	285-287 @760 mm Hg
Vapor pressure @ 25 °C, Pa	2.34×10^{-3}
$LogP_{ow}$	0.50-0.61
Solubility, g per 100 g solvent (30°C)	
Solvent	Value
Ethanol	46.4
Acetone	28.4
Water	8.3

Impurities

Resorcinol (1,3-benzenediol) and catechol can be present in HQ preparations depending on the method of manufacture.[4] In addition, impurities that impart a yellow color to the crystals are often present in technical-grade HQ but can be removed through specialized sublimation processes to produce more refined grades of HQ.

Analytical Methods

Hydroquinone can be analyzed by a number of techniques, including spectroscopic, chromatographic, titrimetric, and electrochemical methods.[4]

Reactions

In solution, HQ is rapidly converted to *p*-benzoquinone via an oxidation reaction upon exposure to air. The rate of this oxidation is increased in alkaline solution.[4] Hydroquinone, which acts as a regulating agent allowing some control of the color-forming coupling reactions, is a "consumable" in the hair dyeing procedure, with its actual concentration decreasing as the color-forming reaction proceeds.[5]

Use

Cosmetic

Hydroquinone is an aromatic compound that is used in cosmetic formulations as an antioxidant, fragrance ingredient, hair colorant, and a reducing agent. Hydroquinone is present in the liquid component of artificial nail systems where it functions to inhibit the polymerization of the methacrylate monomers.[6] According to information supplied to the US Food and Drug Administration (FDA) as part of the Voluntary Cosmetic Registration Program (VCRP), the use of HQ has decreased from 206 uses in 1993 to 151 uses in 2007 to 32 reported uses in 2009 (Table 3).[7,8] A survey of current use concentrations conducted by the Personal Care Products Council reported a use of 0.5% for HQ in other nail care products.[9]

Hydroquinone is added to the methacrylate monomers at a concentration of 0.02% or less than 200 ppm to inhibit their polymerization during transport and storage. In a study to determine the concentration of HQ that remains following the polymerization process, methacrylate polymer was analyzed using the titration method with UV detection (limit of detection [LOD] = 10 ppm) and the concentration of HQ in the finished product was below the LOD.[11]

In 2008, the European Union (EU) banned the use of HQ in hair dyes due to a lack of safety information. They have stated that there is no evidence that HQ as found in hair dye products is safe for human health.[12] Hydroquinone is approved for use in the EU in artificial nail systems, for professional use only, up to a maximum concentration of 0.02% after mixing.[13]

Noncosmetic

Medical. Hydroquinone is used in topical formulations as a skin bleaching and depigmenting agent and is used in the treatment of melasma (chloasma), freckles, senile lentigines, and postinflammatory hyperpigmentation. This use of HQ is considered a drug use that falls under the purview of the FDA. Hydroquinone is available via a prescription and over-the-counter (OTC) products that range in concentration from 0.4% to 5% HQ.[14] These OTC products are used to lighten the skin and are applied all over the face and body with varying use patterns among consumers. It is recommended that exposure to sunlight be avoided when using these products.

The FDA has issued a Federal Register notice on their intent to designate OTC skin bleaching products as no longer generally recognized as safe and effective (GRASE). Any products currently on the market would be considered new drugs that require an approved new drug application (NDA) for continued marketing.[14]

Table 3. Historical and Current Cosmetic Product Uses and Concentrations for Hydroquinone[7-10]

Product Category (FDA 2008)	1993 Uses (FDA 1993)	2007 Uses (FDA 2007)	2009 Uses (FDA 2009)	2009 Concentrations (%; Council 2009)
Hair coloring products				
Dyes and colors	185 (1112)	139 (2481)	13 (2481)	–
Makeup				
Lipstick	2 (937)	1 (1912)	–	–
Nail care products				
Other[a]	–	– (124)	– (124)	0.5
Skin care products				
Cleansing creams, lotions, liquids, and pads	–	1 (1368)	1 (1368)	–
Face and neck creams, lotions, etc	–	– (1195)	2 (1195)	–
Moisturizers	–	1 (2039)	2 (2039)	–
Fresheners	1 (246)	– (285)	2 (285)	–
Other	18 (848)	9 (1244)	12 (1244)	–
Total uses/ranges for hydroquinone	206	151	32	0.5

Abbreviations: FDA, US Food and Drug Administration.
[a] Nail adhesive.

The EU banned the use of HQ in OTC skin lightening products in 2001.[15,16]

Industrial. Hydroquinone is used in the development of black and white film, as an inhibitor of polymerization, as a stabilizer in paints, varnishes, motor fuels and oils, and as an antioxidant for fats and oils.[17]

General Biology

Inhibition of Tyrosinase

The ability of HQ to decrease melanogenesis when applied topically has made this a useful agent for decreasing skin pigmentation. Hydroquinone has been shown to be a weak substrate for the enzyme tyrosinase in kinetic studies but is able to compete with the endogenous substrate, tyrosine in vivo and inhibit the conversion of tyrosine to dopa and dopa to dopaquinone, thus decreasing melanin formation.[18,19]

Absorption, Distribution, Metabolism, and Excretion

Hydroquinone is rapidly absorbed and excreted in urine in rats following oral administration.[2] Absorption from an alcohol vehicle is greater than from an aqueous solution. Hydroquinone in an aqueous solution was absorbed through human skin at a rate of $0.55 \pm 0.13 \mu g/cm^2$ per h

Proposed pathways for the metabolism of HQ in rats and humans are presented in Figure 2. The principal products observed from the metabolic process are the sulfate and glucuronide conjugates. Oxidation to 1,4-benzoquinone results in a reactive metabolite that forms mono- or polyglutathione conjugates.[17] The glutathione conjugates are believed to be the causal agents in rodent nephrotoxicity and renal carcinogenesis.

In vitro. Human skin (500 μm thick) from 6 donors was used to examine the permeation of a 2% [^{14}C]hydroquinone cream alone and following pretreatment with 2% sodium azide.[20] No difference was observed in the permeation of HQ between sodium azide treated (42.7%) and untreated (43.3%) skin. A lag time of 8 hours was noted and believed to be attributed to the time it takes for the HQ cream to penetrate through the skin and into the receiving fluid. Using this model system, 100% of the dose was accounted for.

Metabolism was also examined in this system, with no significant difference in HQ recovery with ($28.1\% \pm 18.4\%$) and without ($28.5\% \pm 12.9\%$) sodium azide treatment. The amount of benzoquinone recovered was significantly decreased, however, from $10.8\% \pm 5.7\%$ without sodium azide pretreatment to $4.1\% \pm 2.0\%$ with sodium azide pretreatment.[20]

The metabolic rate constants for the conversion of HQ to the monoglutathione conjugate (HQ-SG) and subsequently to the mercapturic acid (HQ-Cys) in hepatocyte cultures isolated from F344 rats and humans were measured.[21] The substrate was added to the culture system and the disappearance and subsequent appearance of downstream metabolites were followed using sequential sampling (from 5 to 75 minutes) and high-performance liquid chromatography (HPLC) analysis. In this model system, glucuronidation was the initial favored step in both rat and human hepatocytes, with human hepatocytes having a higher V_{max} and intrinsic clearance (V_{max}/Km) than rat hepatocytes (15.2 vs 3.85 nmol/min per 10^6 cells, respectively). The human hepatocytes also demonstrated a higher capacity for metabolism of the HQ-SG to HQ-Cys than the rat hepatocytes. Acetylation was favored over deacetylation in both species. Overall, the authors concluded that the capacity for the metabolism of HQ and HQ-SG is greater in humans than in rats, suggesting a greater capacity for the detoxification of the glutathione conjugates. These metabolic constants were incorporated into an existing physiologically based pharmacokinetic (PBPK) model, which subsequently predicted that the body burden of these metabolites would be much higher in rats than in humans.[21]

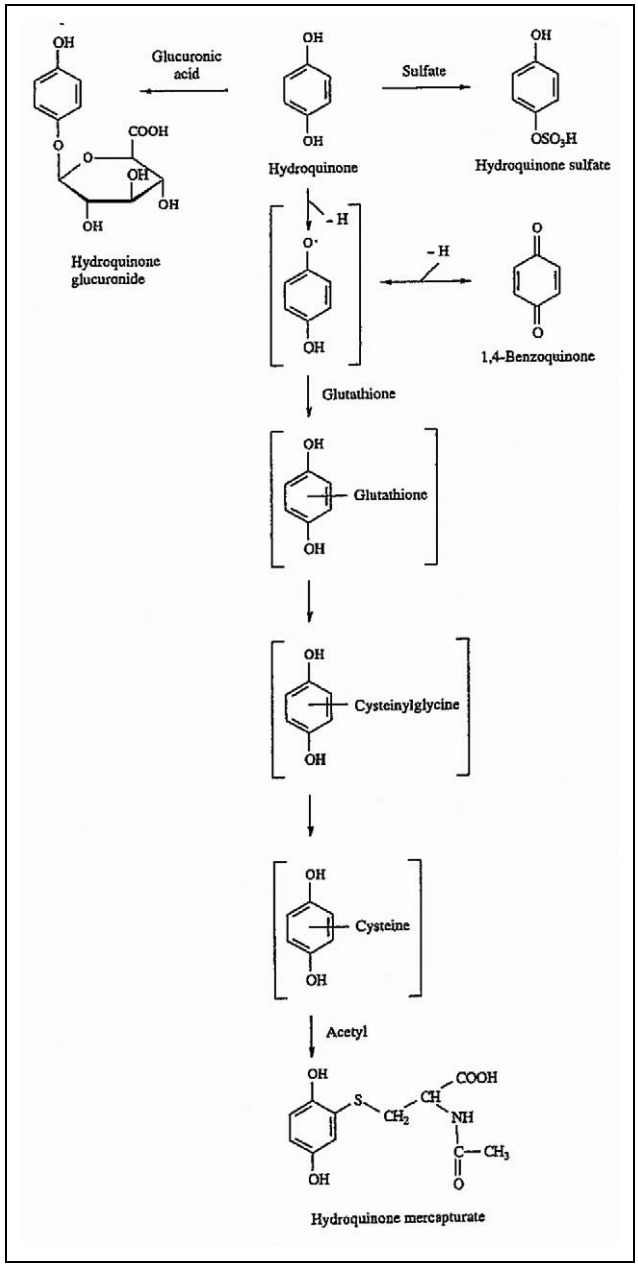

Figure 2. Proposed metabolism of hydroquinone.[17]

In vivo. Male F344 rats (number not specified) were given either a single dose of 1.8 mmol/kg [^{14}C]HQ in corn oil or 14 daily doses of HQ (1.8 mmol/kg in corn oil) followed by a single dose of 1.8 mmol/kg [^{14}C]HQ on day 15 by gavage.[22] Urine was collected for 72 hours and analyzed for total radioactivity. Following a single gavage dose, 21% of the dose was recovered in the 0- to 5-hour urine sample with an additional 35% excreted in the next 19 hours. The major metabolites identified, via HPLC-UVEC, in the 0- to 24-hour urine samples were HQ glucuronide (21%), HQ sulfate (15%), and HQ mercapturate (13%). Following subchronic administration, 46% of the radioactivity was recovered in the 0- to 5-hour urine sample and 31% excreted in the next 19 hours. Subchronic treatment changed the metabolic profile, with an increase in the amount of radioactivity excreted as the glucuronide conjugate (2 fold) and the glutathione conjugate (1.4 fold), while the percentage of the dose that was excreted as the sulfate remained the same. The authors state that these findings lend support to their hypothesis that subchronic administration of HQ increases the rate and extent of HQ metabolism to nephrotoxic glutathione conjugates.[23]

Table 4. Concentrations of Arbutin in Foods Used in the Human Diet Experiments[24]

Food Product	Arbutin Concentration (μg/g)
Tea	0.14 ± 0.02
Coffee	0.31 ± 0.04
Pear (Bosc)	3.84 ± 0.74
Pear (d'Anjou)	15.09 ± 11.69
Wheat cereal	1.04 ± 0.09
Wheat germ	10.65 ± 3.61
Whole wheat bread	2.04 ± 0.35

Hydroquinone occurs as the glucose conjugate, 4-hydroxyphenyl-β-D-glucopyranoside (arbutin), in the leaves of several plants, including cranberries, blueberries, and some varieties of pear. Arbutin is easily hydrolyzed to D-glucose and HQ in hot, dilute acid.[4] To determine the contribution of the diet to total HQ levels in the body, Deisinger et al (1996) measured the concentration of arbutin and HQ in foods and in human plasma and urine samples, respectively, using gas chromatography with an electron capture detector (GC-ECD).[24] They found certain foods to be high in arbutin as shown in Table 4. The researchers measured the concentration of HQ in 2 groups of human volunteers (2/gender per group) who consumed a high- or low-HQ breakfast. Blood samples were collected at 30, 50, and 120 minutes after completion of the meal and urine was collected every 2 hours for 7 to 8 hours after the morning meal. A meal low in HQ was provided for lunch for both the high- and low-HQ breakfast groups. Hydroquinone and phenol concentrations were determined in acid hydrolyzed samples and HQ concentrations were statistically significantly elevated at the 1- and 2-hour time points (exact values not given) in blood plasma, as compared to background concentration. Mean plasma total phenol concentrations did not change with time. A similar pattern was observed for HQ in the urine samples. The concentration of HQ was statistically significantly increased at 2 to 3 hours after the high-HQ meal and decreased slowly after 3 hours. Urinary total phenol excretion rates averaged ~800 μg/h and showed high interparticipant variability but little mean variability over time. Selected, unhydrolyzed blood and urine samples were analyzed and very little free HQ or phenol was found in these samples. It was determined from this that HQ absorbed from these exposures is rapidly conjugated and is not detectable as free HQ or phenol. Plasma and urine levels of HQ and phenol in the low-HQ breakfast group showed a decline (not statistically significant) in their levels over time.

The in vivo bioavailability of HQ was examined in 3 different experiments using a total of 14 healthy males (age

Table 5. Radioactivity Recovery Following Topical Application of 25 μCi of [^{14}C]Hydroquinone to Human Forehead Skin for 24 Hours[20]

	Percentage Administered Dose[a]	
Time (h)	Urine	Skin Surface Wash
24	37.0 ± 9.8	5.2 ± 3.2[b]
48	7.1 ± 2.1	
72	0.9 ± 0.6	
96	0.4 ±0.2	
Total	45.3 ± 11.2	5.2 ± 3.2

[a] Values are mean ± SD (n = 6).
[b] Dosed skin site washed with soap and water after 24-hour dosing period.

Table 6. Radioactivity Recovered Via Tape Stripping, Following Topical Application of 1 μCi of [^{14}C]Hydroquinone to Human Forearm Skin[20]

	Percentage Administered Dose[a]	
Time (h)	Skin Wash Recovery	Skin Tape Strips
0	82.3 ± 8.1	1.2 ± 0.4
1	67.5 ± 25.3	5.4 ±2.5
3	54.8 ± 18.0	8.6 ±4.5
6	53.6 ± 17.3	15.8 ± 4.2
24	15.0 ± 4.5	6.6 ± 2.1

[a] Values are mean ± SD (n = 4).

18-80) following dermal application without occlusion, as the product is normally applied.[20] In group A (n = 6), 25 uCi of ^{14}C-hydroquinone cream (containing 2.5 mg HQ) was applied to a 25 cm^2 area of skin on the forehead. The next day, the area was washed by cotton balls with a 50% soap solution and rinsed with deionized water. The cotton balls were collected and analyzed for radioactivity. Four-day urine samples were collected by the volunteers and submitted to the researchers for analysis. Table 5 provides the results of this study. A total of 45.3% ± 11.2% of the dose was recovered in the urine. 5.2% ± 3.2% was recovered from the cotton ball wash. Thin layer chromatography was used to identify the metabolites in urine and the majority of the recovered dose was excreted as the glucuronide conjugate with lesser amounts of the sulfated conjugate and the parent compound identified.

In group B (n = 4), 5 skin sites (1 cm^2 each) on the ventral forearm were treated with 1 uCi of ^{14}C-hydroquinone cream (containing 0.1 mg HQ). The treated surfaces were washed 5 times and tape stripped 10 times with cellophane tape at time intervals of 0, 1, 3, 6, and 24 hours. The tape strips were analyzed for radioactivity. Table 6 provides the results of this study. The researchers concluded that while HQ can be removed from the skin through washing early after exposure, absorption over time will decrease the amount that can be removed.

In group C (n = 4), 25 uCi of ^{14}C-hydroquinone cream (containing 2.5 mg HQ) was applied to a 25 cm^2 area of skin on the left forearm. Catheters were placed in the vein draining the treated area on the left forearm, the ipsilateral site, and in the same area on the right forearm, the contralateral site. Blood samples (10 mL) were taken from both sites at 0 hour (prior to dosing) and at 0.5, 1, 4, and 8 hours after dosing. The treated skin site was washed by cotton balls with a 50% soap solution and rinsed with deionized water. The cotton balls were not analyzed for radioactivity in this substudy. Urine samples were collected at 24, 48, 72, and 96 hours after dosing, by the volunteers and submitted to the researchers for analysis. The researchers found that initially, the concentration on the ipsilateral side was higher than on the contralateral side; but by 4 hours, the concentration was the same on both sides. The peak plasma concentration of 0.04 ug-Eq/mL occurred at the 4-hour time point. The researchers calculated that 8.0% ± 4.1% of the dose was excreted in the urine for the 8-hour dosing period and that 45.3% of the dose was absorbed. The researchers assumed 100% excretion in making these determinations.

Immunological Effects

Hydroquinone inhibited IL-2-dependent T cell proliferation in primary human T lymphoblasts (HTLs) in vitro.[25] Exposure of HTLs to 50 μmol/L HQ decreased interleukin 2 (IL-2)-dependent proliferation by >90%. Cell viability was not affected by this concentration. Hydroquinone reversibly inhibited DNA synthesis in these cells, but this effect could be reversed up to 6 hours after treatment by removing HQ. No effect on glutathione levels was observed with up to 24 hours of treatment. Hydroquinone did not block binding of 125I-IL-2 to the cells but interfered with the IL-2-dependent progression of the HTLs through S phase of the cell cycle.

Macrophage-mediated immune responses were also inhibited by HQ treatment.[26] Tumor necrosis factor-α (TNF-α) production was inhibited by a 6-hour treatment with 100 μmol/L HQ in mouse RAW264.7 cells. Hydroquinone treatment decreased lipopolysaccharide (LPS)-mediated nitric oxide (NO) production, but HQ alone up to 100 μmol/L had no effect on NO levels. Human U937 cells (a macrophage cell line) and platelets showed a decrease in cell adhesion molecules in response to HQ treatment. The authors believe these effects occur through inhibition of the PI3K/Akt signaling pathway because inhibition of this pathway produced similar effects to those seen with HQ and a synergistic effect was observed with HQ and inhibitors of this pathway.

Animal Toxicology

Oral administration of HQ to rats resulted in dose-dependent mortality, lethargy, tremors, and increased liver and kidney weights.[2] Hydroquinone was found to be cytotoxic to rat hepatoma cells in culture and nephrotoxic in male rats dosed orally by gavage.

Acute Toxicity

Oral. Hydroquinone was found to induce indicators of nephrotoxicity in male and female F344 rats but not in male

Table 7. Functional Observational Battery (FOB) of Sprague-Dawley Rats Treated With 0, 20, 64, or 200 mg/kg per d Hydroquinone 5 d/week for 13 weeks: Statistically Significant Differences[28]

Observation	Change	N	Dose Group	FOB Observation Period
Differences attributed to hydroquinone exposure				
Home-cage activity	Decreased	6/10	200 mg/kg males	6 h
Behavior while removing from cage	Decreased	10/10	200 mg/kg females	1 h
Tremors	Increased	10/10	200 mg/kg females	1 h
Locomotor activity	Decreased	9/10	200 mg/kg males	6 h
Urine stains	Urine discolored papers	10/10	200 mg/kg males	1 and 6 h, days 1, 30, and 60
	under home cages brown	10/10	64 mg/kg males	Days 1, 30, and 60
	when left to stand overnight	10/10	20 mg/kg males	Days 1 and 60
		10/10	20, 64, and 200 mg/kg females	Days 30 and 60
Differences not attributed to hydroquinone exposure				
Urination	Increased	–	200 mg/kg males	1 h
			20 mg/kg males	1 h
Defecation	Increased	–	20 mg/kg males	1 h
	Decreased		200 mg/kg males	1 h
Spontaneous vocalizations	Increased	–	200 mg/kg females	Day 91
Approach response	Increased	–	64 mg/kg females	Day 30
Auditory orientation	Increased	–	200 mg/kg males	Day 60
	Decreased		20 and 200 mg/kg females	Day 91
			64 mg/kg females	Days 30 and 60
Olfactory orientation	Increased	–	64 mg/kg males	Day 7
Visual orientation	Increased	–	64 mg/kg males	Days 30, 60, and 91
Pinna touch response	Decreased	–	200 mg/kg males	Day 14
Tail pinch response	Increased	–	200 mg/kg	Day 30
Grip strength quantitative	Decreased	–	64 mg/kg females	Pre-exposure

or female Sprague-Dawley (SD) rats or B6C3F1 mice.[27] Rats (4-6/group) were gavage dosed with 0, 200, or 400 mg/kg HQ in water. Mice (number not specified) were gavage dosed with 0 or 350 mg/kg HQ in water. Urine and blood samples were collected from all animals. For rats, samples were collected at 0 (prior to dosing), 8, and 24 hours, at 24-hour intervals up to 96 hours after dosing. For mice, urine was collected 16 hours prior to dosing and at 12, 24, and 48 hours after dosing. The samples were examined for markers of kidney toxicity. Female F344 rats were the most sensitive to the indicators examined with statistically significant increases in the urinary activities of the enzymes, alanine aminopeptidase (ALP), alkaline phosphatase (AAP), γ-glutamyl transpeptidase (γ-GT), and *N*-acetyl glucosaminidase (NAG) at the 400 mg/kg dose level 8 hours after dosing. Creatinine (8 hours) and glucose (24 hours) were also statistically significantly increased at the 400 mg/kg dose. Blood urea nitrogen (BUN) was statistically significantly increased at the 400 mg/kg dose at the 48-hour time point. While many of these same parameters were increased in the 200 mg/kg dose group, only the increase in NAG was statistically significant at this dose level.

Male F344 rats had a similar pattern with statistically significant differences at the 400 mg/kg dose, but the change was of a smaller magnitude than that observed with the female F344 rats. At the 8-hour time point, AAP, NAG, and γ-GT activities were statistically significantly increased. Urinary creatinine and BUN were not changed by HQ treatment in male F344 rats. No significant differences were observed at the 200 mg/kg dose level.

Blood urea nitrogen was decreased in female SD rats at the 400 mg/kg dose level. No other changes were observed in the other parameters in male or female SD rats.

Blood urea nitrogen was statistically significantly increased in male and female B6C3F1 mice at the only dose tested (350 mg/kg). The urinary enzyme activities were also increased, but the differences were not statistically significant.

Urinary osmolality was decreased in all groups of rats dosed at the 400 mg/kg level, with statistically significant changes observed in the male and female F344 rats and the female SD rats. Statistically significant changes in diuresis were observed only in female F344 rats at both dose levels. No statistically significant changes in red or white blood cell counts in the urine were observed in any of the treated animals.

Histopathologically, F344 rats of both genders exhibited changes such as cell regeneration, slight mineralization, slight focal necrosis, and the presence of granular casts in the kidneys. The authors described any histopathological changes observed in male SD rats as minimal at either dose level. The kidneys from female SD rats and from mice were not examined.

A range-finding study was conducted in 7 female SD rats (age 10-13 weeks, 1 animal/group) using a single gavage dose of 500, 400, 375, 350, 300, 200, or 100 mg HQ/kg body weight (bw).[28] Table 7 provides the results of this study. Mortality occurred in the 500 and 400 mg/kg bw dose groups. Based on these results, an acute oral study was designed using 7 male

SD rats (age 9 weeks) and 5 female SD rats (age 14-15 weeks). The animals received a single gavage dose at concentrations of 3.75%, 3.45%, 3.15%, and 2.85% in degassed water, which correspond to dose groups of 375, 345, 315, and 285 mg/kg, respectively. Animals were observed 3 times on the day of dosing and once per day on days 1 to 14. Body weight was measured on days 0 (prior to dosing), 7, and 14.

Mortality occurred within 1 hour of dosing in both male (1 of 5) and female (3 of 5) rats at 375 mg/kg and in female rats at 345 (1 of 5) and 285 (1 of 5) mg/kg. The researchers observed mild-to-moderate tremors in all animals after dosing and noted minor convulsions in 1 of 5 males and 3 of 5 females at 375 mg/kg, 1 of 5 males and 1 of 5 females at 345 mg/kg and for 2 of 5 females at 285 mg/kg. With the exception of the male at 345 mg/kg, the animals that convulsed died prior to the 1-hour observation. No signs of tremors or convulsion were observed at the 4-hour observation. Brown discolored urine stains were present in the cages of all surviving animals between day 0 and day 2. From day 3 to the end of the study, all surviving animals appeared to be clinically normal. At necropsy, the researchers noted minor-to-moderate thymus hemorrhage for some of the animals that died on the day of dosing (details not provided) and minor hydronephrosis of the right kidney for a single 345 mg/kg male rat that survived to the end of the study. The hydronephrosis was not considered treatment related, however, as the animal was not in the highest dose group.

Dermal. New Zealand White SPF rabbits (5/gender) were shaved to expose the dorsal skin and a 2000 mg/kg bw limit dose of HQ was applied to the skin, covered and wrapped to secure the treatment in place for 24 hours.[28] The residual HQ was removed with running water after 24 hours. Animals were observed once daily from days 1 to 14 and body weights were measured on days 0 (prior to treatment) 7, and 14.

The researchers observed brown discolored urine stains in the cages of all animals on day 2. There were no adverse dermatological effects at the site of application, no adverse neurobehavioral effects, no changes in weight gain, and no mortalities during the study. No treatment-related findings were observed at necropsy. The dermal LD_{50} was reported as >2000 mg/kg.

Subchronic Toxicity

Dermal. Dermal exposure to a HQ cream did not produce renal toxicity in a subchronic study.[29] F344 rats (20/gender/group) were shaved and treated dermally with 0%, 2%, 3.5%, and 5% HQ in an oil-in-water emulsion cream (1.6 mL/kg bw on a 4 × 6 cm area on the back, with occlusion) 5 days/week for 13 weeks. Body weights and feed and water consumption were monitored and the animals were observed for clinical signs of toxicity and dermal irritation. Urine was collected from all animals to look for markers of cell damage, including NAG, γ-GT, ALP activities, and creatinine concentrations. The authors implanted 5 animals/gender/group with osmotic pumps loaded with 20 mg/mL BrdU (delivery rate 10 μL/h). Animals were then treated dermally with HQ cream as described above and killed 3 days later during the 3rd, 6th, and 13th weeks of treatment to look at renal cell proliferation. No clinical signs of toxicity were observed in any of the treated animals. Erythema was observed in male and female treated animals with increasing concentrations producing increased severity. This finding abated when exposure stopped. In addition, brown discoloration of the skin and scaly skin were observed with higher incidences occurring in the higher treatment groups although no clear dose-related trend was evident. A minimal to minor epidermal hyperplasia was observed at the site of application. No evidence of exogenous ochronosis was observed in this study.

No significant differences in absolute or relative organ weights were observed. Serum levels of protein and alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities were statistically significantly increased as compared to controls in the 5% HQ-treated male rats, but the authors did not feel this represented an adverse effect of HQ treatment because the magnitude seen was not clinically relevant. At 13 weeks, there were no differences in the urine parameters examined in male rats and only the osmolality value was statistically significantly decreased at the highest concentration tested. No histopathological changes and no changes in cell proliferation were observed in males after 13 weeks of treatment. In females, the BrdU labeling index in the 3.5% and 5% treated groups was slightly increased for cells in the outer/inner stripe of the distal tubules. No histopathological changes were observed.

Dermal Irritation and Sensitization

In rats, dermal application produced slight-to-severe irritation.[30] In a guinea pig maximization test, induction with 2% HQ injected intradermally, followed by challenge with 0.5% HQ, showed extreme sensitization. Hydroquinone was classified as an extreme sensitizer in a guinea pig maximization test. Briefly, albino Dunkin-Hartley guinea pigs were given a series of 6 intradermal injections of 2.0% HQ in 0.9% saline in the shoulder region to induce sensitization. After 6 to 8 days, a 48-hour occlusive patch of 1.0% HQ in an acetone−polyethylene glycol 400 vehicle was placed over the injection site. The animals were challenged on a previously untreated area of the flank using a 24-hour occlusive patch of 0.5% HQ, which was the maximum nonirritating concentration. After 14 and/or 48 hours, 100% of the animals had a positive response.

Reproductive/Developmental Toxicity

The literature on Reproductive and Developmental Toxicity of HQ was reviewed in a previous safety assessment and was summarized as follows:

> Oral administration of Hydroquinone did not produce embryotoxic, fetotoxic, or teratogenic effects in rats, nor did it produce significant adverse reproductive effects in a

two-generation study. Using rabbits, various teratogenic/reproductive treatment-related effects were observed at doses of 200-500 mg/kg. All dams dosed with 300-500 mg/kg Hydroquinone died. Some maternal toxicity was observed at a number of dose concentrations.[2]

Growth retardation of offspring was reported at concentrations that also caused maternal toxicity.[1] The overall literature in this area has not shown reproductive or developmental effects even following high-dose HQ exposures.[23]

Genotoxicity

Hydroquinone has been found to be mostly negative for mutagenicity in bacterial assays but positive in many mammalian cell assays in vitro and in vivo including micronuclei formation, sister chromatid exchange (SCE), and chromosomal aberrations.[2]

In Vitro

Blood samples were collected from healthy male volunteers (aged 20-40 years) and used to produce whole blood cultures and isolated lymphocytes for a series of experiments examining the genotoxicity of HQ.[31] Cultures were treated with HQ (0.5-50 μg/mL) for 90 minutes at 37°C. Methylmethanesulfonate (MMS) and hydrogen peroxide (H_2O_2) were used as positive controls. Samples were assayed for DNA damage using the Comet assay. Hydroquinone treatment resulted in dose-dependent increases in tail moment values in isolated leukocytes but had no effect on whole blood samples. Methylmethanesulfonate produced an increase in DNA damage in both types of samples, while H_2O_2 produced damage in only the isolated leukocytes. To investigate whether peroxides may play a role in HQ-induced DNA damage, isolated lymphocytes were treated with HQ in the presence or absence of catalase (250 U/mL). The effects of HQ were almost completely suppressed in the presence of catalase, suggesting that the damage could be due in part to an HQ-generated peroxide or other catalase substrate. Cellular metabolism was not required to generate the causative agent, as treatment of lysed cells also produced the previously observed DNA damage. Finally, the isolated lymphocytes were treated with HQ at 5, 10, and 50 μg/mL and the sample split into 2. One half of the sample was immediately tested in the Comet assay, while the other half was cultured for 66 hours and stimulated by phytohemagglutinin (PHA) to look for micronuclei. The DNA damage previously observed was detected in the freshly analyzed sample, but no significant increase in micronuclei was observed in the cultured cells. These experiments suggest that there are proteins and/or enzymes in whole blood samples that can block the actions of HQ, and it is likely that a self-generated peroxide product is the DNA damaging agent in this system.

The ability of HQ to induce chromosomal aberrations in V79 cells was examined in the presence and absence of S9 mix and antioxidant enzymes.[32] Hydroquinone was found to generate hydroxyl radicals in a time- and pH-dependent manner in the presence of Fe^{3+}/EDTA with more alkaline pH producing higher levels of thiobarbituric acid (TBA) reactive products. The addition of catalase to this system decreased the concentration of TBA-reactive products, suggesting that H_2O_2 is the specific agent generated. The presence of chromosomal aberrations was increased in the presence of 80 μmol/L HQ at pH 7.4 and 8.0, but the increase was not significant as compared to each other, despite the earlier finding that more hydroxyl radicals are produced at pH 8.0. This is believed to be due to a concomitant increase in cytotoxicity as measured by the mitotic index. The addition of S9 mix, superoxide dismutase (SOD), and SOD + catalase all significantly decreased the percentage of cells with chromosomal damage, but catalase alone did not have an effect. The authors suggest that while H_2O_2 generation appears to play a role in the clastogenic activity of HQ, the addition of S9, SOD, or SOD + catalase did not completely abolish the chromosomal aberrations; therefore, other reactive metabolites, such as the semi-quinone radical or the quinone, may play a role.

A total of 27 nonsmoking healthy young caucasian volunteers were enrolled in a study to determine the effect of polymorphisms for the glutathione *S*-transferases (GSTs) on HQ-induced genotoxicity to lymphocytes.[33] Blood samples were collected and genotyped and lymphocytes isolated from the samples were tested for micronuclei induction and SCE in the presence of HQ. For the micronuclei induction experiments, lymphocytes were PHA stimulated and treated with HQ (0, 40, and 80 μmol/L) for 3 hours. Mitomycin C ([MMC] 1.5 μmol/L) was used as a positive control and performed as expected. The number of micronucleated cells was significantly increased at 40 and 80 μmol/L, which were also significantly different from each other. A strong correlation was also observed between the GST phenotype and the frequency of HQ-induced micronuclei, with GSTM1 null lymphocytes exhibiting a significantly higher level with both 40 and 80 μmol/L HQ.

Lymphocyte cultures from these same participants were also PHA stimulated and treated with 0 or 80 μmol/L HQ for 3 hours to test for SCE. The 80 μmol/L HQ treatment induced a significant increase in SCE as compared to controls. No relationship was observed between HQ-induced SCE and the GST polymorphisms examined.

Hydroquinone was tested for induction of DNA damage in HepG2 cells and found to be positive.[34] Cells were exposed to 0, 6.25, 12.5, 25, and 50 μmol/L HQ for 1 hour at 37°C. DNA strand breaks were significantly increased in cells treated with 6.25 up to 25 μmol/L HQ. Cells treated with 50 μmol/L HQ showed a significant increase in DNA strand breaks as compared to controls but were not increased above the 25 μmol/L HQ-treated group. Cell viability was not affected by HQ treatment and no apoptosis was observed (data not shown). The presence of DNA protein cross-links was investigated using a proteinase K posttreatment. Posttreatment with proteinase K did not affect DNA migration in cells exposed to 6.25 to 25 μmol/L HQ; however, cells exposed to 50 μmol/L HQ did

show an increase in DNA migration, suggesting that DNA protein cross-links were present in these cells.

The induction of micronuclei was also examined in HepG2 cells.[34] Cells were treated with 0, 6.25, 12.5, 25, and 50 μmol/L HQ for 24 hours and evaluated for the frequency of micronuclei. Cyclophosphamide (800 μmol/L) was used as a positive control and performed as expected. A dose-dependent increase in micronuclei formation was observed in cells treated with 12.5 to 50 μmol/L HQ. The researchers did further work to look at indicators of oxidative stress. They found that 8-hydroxydeoxyguanosine (8-OHdG) was dose dependently increased in cells treated with 12.5 to 50 μmol/L HQ, reactive oxygen species (ROS) were statistically significantly increased in the 2 highest concentrations, and all treated cells showed statistically significant decreases in glutathione (GSH) content.

In Vivo

Hydroquinone increased the incidence of micronuclei in the bone marrow cells of male Swiss albino mice.[35] Animals were given 1 intraperitoneal (ip) injection of HQ and killed 12, 24, and 36 hours after treatment. The mice (5/dose per time period) were dosed at 0, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 75, and 100 mg/kg body weight. The frequency of micronucleated polychromatic erythrocytes (MPCE) was highest at 24 hours after treatment and was statistically significant as compared to controls at 6.25 mg/kg body weight up to 100 mg/kg body weight for all 3 time points. The frequency of micronucleated normochromatic erythrocytes (MNCE) followed a similar pattern and was highest at 24 hours after treatment and was statistically significantly increased at 6.25 mg/kg body weight up to 100 mg/kg body weight for all 3 time points. The frequency of MNCE reached a plateau at the 75 mg/kg dose. The PCE/NCE ratio decreased in a dose-dependent manner and suggests that HQ treatment resulted in a dose-dependent inhibition of erythropoiesis.

Male Swiss albino mice (4/dose) were given a single dose of HQ (0, 0.5, 1, 2, 4, and 8 mg/kg body weight; ip) per day for 6 days and killed 24 hours after the last treatment.[36] Splenocytes were isolated from these animals, cultured for 72 hours and examined for micronuclei formation. No signs of HQ-induced toxicity were observed over the treatment period. A statistically significant, dose-dependent increase in the number of micronucleated binucleate splenocytes (MNBNS) was observed. The 3 highest doses of HQ also produced increases in the number of BNS.

Carcinogenicity

Hydroquinone administered to rats orally by gavage 5 times/week for up to 103 weeks at doses of 25 or 50 mg/kg resulted in a significant increase of renal adenomas in males at the 50 mg/kg dose and of mononuclear cell leukemia in females with both doses.[37] At doses of 50 or 100 mg/kg on the same schedule, there was a significant increase in hepatocellular adenomas in both male and female mice.[37] Other previously reviewed studies of HQ showed no significant difference in tumors between control and exposed groups, and marginal to no activity as a tumor promoter.[1]

Chronic

Hydroquinone is metabolized to several glutathione conjugates including 2,3,5-tris(glutathione-S-yl)HQ (TGHQ), and this compound was tested for its ability to induce renal cancer in Eker rats.[38] Eker rats are susceptible to renal tumor development because they carry a germline mutation in the tuberous sclerosis 2 (Tsc-2) tumor suppressor gene. Eker rats have 1 mutated copy and 1 wild-type copy and loss of function of the wild-type copy is the rate limiting step for the development of preneoplastic and ultimately neoplastic lesions.

Two groups of male Eker rats (40/group) were given either 0 or 2.5 μmol/kg TGHQ 5 d/week ip in 0.5 mL of saline for 4 months and then 0 or 3.5 μmol/kg TGHQ for an additional 6 months. (These doses represent $\sim$600-800 nmol/rat and are 0.6%-0.8% of the dose of HQ used in the NTP (1989) study.[37] After 4 months, 10 animals/group were killed and examined for cell proliferation in the kidneys.

2,3,5-tris(glutathione-S-yl)HQ treatment for 4 months significantly increased the labeling index of cells in the kidney, as measured by BrdU incorporation, compared with saline controls (133.4 ± 30.9 vs 9.0 ± 1.9 cells/250 μm^2, $P = .016$). Preneoplastic lesions were also observed at this time point, characterized by a peritubular fibrosis surrounding a dilated tubule. Kidney tumors were not observed at this time point.

Following 10 months of treatment, statistically significant increases in basophilic dysplasias, adenomas, and renal cell carcinomas were observed. Most of the renal cell tumors were in the region of TGHQ-induced acute renal injury and using HPLC-based DNA fragment analysis and laser capture microdissection, the researchers found that there was a loss of the wild type *Tsc-2* gene within the preneoplastic lesions.

Renal Tubule Cell Tumor Mode of Action

Hydroquinone treatment has been associated with an increased incidence of renal tubule cell tumors in male F344 rats but not in female F344 rats or B6C3F1 mice. A mode of action (MOA) has been proposed for the HQ-induced renal tubule cell tumors in male F344 rats. This MOA involves HQ or a metabolite interacting with the rat kidney to exacerbate chronic progressive nephropathy (CPN). The combination of CPN, along with HQ-induced cell proliferation in the kidney, promotes neoplasm formation.[39]

Hydroquinone is metabolized in rats to the glucuronide, sulfate, and glutathione conjugates, with increasing levels of the glucuronide and the mercapturic acid measured in the urine following either increasing treatment time or dose.[22,40] Intravenous injection of the glutathione metabolite 2,3,5-(triGSyl)HQ shows that the kidney is a target of this compound, and it produces an increase in cytotoxicity and oxidative DNA damage

as measured by markers of kidney damage in the blood and 8-OHdG in the kidney, respectively.[22] Subchronic treatment (6 weeks) of male F344 rats with 25 and 50 mg/kg HQ (via gavage) produced increased cell proliferation in the kidneys, and dose-dependent and statistically significant increases in the incidence of degenerative and regenerative foci in the tubules.[41] This same treatment regimen using 2.5 mg/kg HQ did not produce these effects in male F344 rats and none of the tested doses (2.5, 25 or 50 mg/kg) produced these effects in female F344 rats or in male SD rats. Glutathione metabolites can be reabsorbed in the proximal tubules of the kidney and metabolized by γ-glutamyl transferase (also γ-GT) where the released compound could generate ROS or covalently bond to cellular targets (ie, proteins or DNA). DNA adducts were not observed in F344 rat kidney following HQ treatment using ^{32}P-postlabeling, suggesting that this is not the target macromolecule.[42] Protein adducts have been identified following gavage and ip treatment with HQ.[43,44]

The localization of a reactive molecule to the kidney along with increased cell proliferation in the kidney seems to also coincide with the occurrence of CPN in these animals.[39,45] Chronic progressive nephropathy is a spontaneous lesion of the kidneys that occurs with age in certain strains of rats, including F344 and SD rats. Male rats are usually more affected than females and the incidence and severity of CPN has been shown to be affected by castration, hormonal status, and caloric intake, with protein overnutrition thought to be a causal factor.[46,47] These lesions are characterized by an increase in basophilic tubules with a thickened basement membrane in the cortex that begin as small discrete areas that expand as the disease progresses. These foci gradually merge into areas of tubule alteration and eventually become areas of frank glomerular pathology with infiltration of inflammatory cells.[48] The impact of CPN on HQ-induced carcinogenicity is unclear, but there does appear to be an association between the severity of CPN and the increased tumor response.[47] The increased proliferation associated with nephrotoxic glutathione conjugates of HQ could leave cells more susceptible to oxidative DNA damage, with subsequent initiation and promotion of neoplasm formation.

The relevance of this process to humans has been questioned on the basis of a proposed lack of a correlative process in humans to the CPN observed in rats. While there does not appear to be a specific human disease that shares all of the features of rodent CPN, humans do experience a glomerular sclerosis of 10% to 30% of nephrons as they age.[46,49,50] In addition, the authors of the NTP analysis cautioned that the presence and severity of CPN in rats did not fully explain the renal tubule cell tumors observed even though a positive correlation between CPN and RTCNs was observed. Their analysis also found that there were many animals with severe CPN that did not develop renal tubule cell neoplasms.[47] Thus, qualitatively, the MOA proposed in animals may have some relevance to humans. Quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

Role of HQ in Benzene Toxicity

Because HQ is a metabolite of benzene, its possible synergistic effect in benzene toxicity has been studied. Possible mechanisms of benzene toxicity have included the consideration of the role of benzene's metabolites in the resulting myelotoxicity. Benzene is a known human carcinogen, inducing leukemia and aplastic anemia. Human bone marrow is a known target, and numerous studies have been conducted to try to determine the mechanism for this effect and the responsible agents. In addition to HQ, benzene metabolites catechol and phenol have also been studied for their effects, both alone and in combination. In the models examined, a synergistic effect does appear to occur depending on the end point under study.[51-55] In examining HQ alone however, there does not appear to be a great deal of similarity in the toxicities observed with the individual compounds.[17,56] Whereas benzene produces leukemia and aplastic anemia in humans, no carcinogenic response has been identified in HQ exposed occupational populations.[17,23] The cancer profiles of the 2 compounds are also different in animals. In NTP studies conducted by the same route of exposure and in the same species of animals, benzene produced neoplasms at multiple sites in all 4 gender-species studies, while HQ induced neoplasia in 1 site in each with the exception of male mice; and all were at different sites.[56] Therefore, it is not clear what role, if any, HQ plays in benzene carcinogenesis. The experimental data suggest that HQ produced at the site of action and in combination with other benzene metabolites probably contributes to the overall toxic profile of benzene, but there are definite differences between species in sensitivity and in the capacity for activating and detoxifying processes that should be considered.

Clinical Assessment of Safety

Exogenous Ochronosis

Use of creams containing at least 1% to 2% HQ has been associated with exogenous ochronosis in people of Asian, Latin American, and African descent.[37,57-59] Ochronosis is the bluish black discoloration of tissues and has been observed in people exposed to several substances in addition to HQ including phenol, trinitrophenol, resorcinol, mercury, picric acid, benzene, and antimalarials. This form of ochronosis has been named exogenous because it does not share any of the systemic complications or the urinary abnormalities observed with the autosomal recessive metabolic disorder alkaptonuric ochronosis even though histologically, the hyperpigmentation in the skin is the same. This condition is associated with prolonged use (>6 months) of HQ containing skin-lightening products and while the mechanism is not understood may involve HQ's effects on tyrosinase, or on homogentistic acid oxidase and resulting deposition of pigment.

Dermal Sensitization

In 80 patients known to be sensitive to aromatic compounds, 0.5% HQ elicited no reactions.[1] Hydroquinone (1%) did not

produce a positive reaction in a cross-reactivity study.[60] Twenty-two volunteers who were classified as sensitized to p-phenylenediamine ([PPD] 9 male; 13 female, 19-72 years old) were patch tested to a serial dilution of benzoquinone (0.1%, 0.2%, 0.5%, and 1.0%) and with other compounds that could be metabolized to benzoquinone including HQ.[30] The control population (n = 8 male and 12 female, 18-77 years old) was tested with the same compounds and concentrations and were selected for no known previous allergy to PPD or other para-benzene derivatives. Among the PPD-sensitized participants, only 1 had reactions to all of the benzoquinone dilutions, while 4 reacted to either 0.2% or 1.0% benzoquinone. Of these participants, 3 presented with erythema, swelling, and papules. In the fourth participant, vesicles were also present

Hair Dye Epidemiology

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. Hydroquinone functions as a coupler in the hair dye reaction and is a "consumable" in the hair dyeing procedure. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information and have been considered by the CIR Expert Panel.

In 1993, an International Agency for Research on Cancer (IARC) working group evaluated 78 epidemiology literature citations and concluded that "personal use of hair colorants cannot be evaluated as to its carcinogenicity" and that "occupation as a hairdresser or barber entails exposures that are probably carcinogenic."[61] The IARC report did not distinguish between personal use of oxidative/permanent versus direct hair dyes, or distinguish among the multiple chemical exposures in addition to hair dyes to which a hairdresser or barber might be exposed.

Rollison et al (2006) reviewed the available epidemiology literature published from1992 through February 2005, which includes over 80 citations on personal hair dye use published since the IARC review.[62] The authors found that hair dye exposure assessment ranged from ever/never use to information on type, color, duration, and frequency of use. The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. The review highlighted well-designed studies with an exposure assessment that included hair dye type, color, and frequency or duration of use, which found associations between personal hair dye use and development of acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin lymphoma. These findings, however, were not consistently observed across studies.

Several studies addressing the possible link between hair dye use and bladder cancer, lymphoma and leukemia, other cancers, reproductive and developmental outcomes, and other end points published since the above review also have been considered.

In February 2008, an IARC Working Group re-evaluated the epidemiology literature including studies considered in the 1993 evaluation as well as all studies subsequently published. For personal use of hair colorants, the Working Group considered the epidemiological evidence inadequate and concluded that personal use of hair colorants is "not classifiable as to its carcinogenicity to humans." The Working Group considered the studies of occupational exposures in hairdressers and barbers as providing limited evidence of carcinogenicity and reaffirmed the previous conclusion made in 1993 regarding occupation as a hairdresser or barber.[63]

A summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

Summary

Hydroquinone is reportedly used in hair dye preparations, skin care products, nail products, and as recently as 2007 in lipstick. Information provided to the FDA through the VCRP indicates that the use of HQ has decreased from 206 uses in 1993 to 151 uses in 2007 to 32 reported uses in 2009. Hydroquinone is a component of artificial nail products because it is added to all types of acrylic monomers to prevent the polymerization of these materials. Upon polymerization of the acrylic monomers, HQ is oxidized and is no longer detectable in the final polymer using analytical techniques for identifying trace amounts in a solid matrix. Any residual HQ is trapped in the polymer and is therefore unavailable and not likely to be absorbed.

While an earlier in vitro study suggested that HQ would be considered a "slow permeant," a more recent in vivo study demonstrated that HQ is in fact rapidly absorbed through the skin from an aqueous preparation. Hydroquinone is metabolized to the sulfate and glucuronide conjugates, with oxidation to 1,4-benzoquinone, resulting in a reactive metabolite that forms mono- or polyglutathione conjugates. The glutathione conjugates are believed to be responsible for the nephrotoxicity observed in rats. In addition to nephrotoxicity, HQ has some immunotoxic effects and has been positive in many mammalian cell assays in vitro and in vivo including micronuclei formation, SCE, and chromosomal aberrations despite being mostly negative in in vitro bacterial mutagenicity assays. The induction of renal cell tubule tumors in male F344 rats has raised concern regarding the nephrocarcinogenicity of HQ and has led to several mechanistic studies which suggest that the male F344 rat is more susceptible to the glutathione conjugates of HQ due to the spontaneous occurrence of CPN which nearly all rats develop as they age. There is no human disease that shares all of the features of rodent CPN, however, there are histopathological similarities between human chronic renal disease and CPN that do not allow the proposed MOA to be ruled out entirely on a qualitative basis. Quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

Hydroquinone has been reported to cause exogenous ochronosis in several ethnic populations following prolonged use (>6 months) of at least a 1% to 2% cream. These effects along

with the NTP cancer study findings have led the FDA to reconsider the GRASE label for HQ in leave-on drug products.

The most recent comprehensive review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

Discussion

The 1994 conclusion of the CIR Expert Panel included the term "aqueous" in discussing the safety of HQ as a cosmetic ingredient. This term was added following discussions of the absorption of HQ from what was termed an alcoholic vehicle (ie, $\sim 70\%$ ethanol), which resulted in estimated HQ absorption of up to 66%. The only other data at the time was an in vitro study that used the stratum corneum of human abdominal skin to examine HQ absorption. The preparation used in that study was described as a 5% aqueous solution of HQ, and the authors calculated a permeability constant of 9.3×10^{-6} which suggested that HQ was a slow permeant with respect to human skin. Since that study, commercial preparations of HQ (commercial cream containing 2.5 mg of HQ) have been shown to be readily absorbed when applied to forehead skin; that is, assuming 100% excretion, 45% of the dose was absorbed. The in vivo studies taken together demonstrate that absorption of HQ will occur if the skin is exposed, and that this absorption occurs from both aqueous and alcohol-based products.

Concerning the use of HQ in hair dye formulations, the question remains regarding the percentage of alcohol in these products. Information provided to the CIR Expert Panel in 1993 suggests that hair dye formulations at the time did contain alcohols but the percentage in the formulation was not provided. Hydroquinone is considered a consumable in the hair dye reaction process and its concentration decreases considerably over time. Therefore, the amount of HQ that may be absorbed during the hair dyeing process is limited by both the decreasing concentration available and by the length of time the hair dye is applied before being rinsed off.

The use of HQ in artificial nail products is considered a safe use because HQ is added to all types of acrylic monomers to prevent the polymerization of these materials. Upon their use, the HQ is oxidized and is no longer present in the preparation and minimal dermal exposure and absorption is expected to occur from this application. Absorption of HQ from other leave-on cosmetic products could be appreciable, and the CIR Expert Panel reiterates that HQ should not be used in these leave-on cosmetics.

The CIR Expert Panel examined the association between oral HQ treatment and the development of renal tubule cell tumors in rats and determined that, while the qualitative relevance of the MOA in humans could not be ruled out, quantitatively, the use of HQ containing hair dyes or nail adhesives is unlikely to result in renal neoplasia through this MOA.

In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other end points, based on lack of strength of the associations and inconsistency of findings.

Amended Conclusion

The CIR Expert Panel concluded that HQ is safe at concentrations of $\leq 1\%$ for cosmetic formulations designed for discontinuous, brief use followed by rinsing from the skin and hair. Hydroquinone is safe for use in nail adhesives in the practices of use and concentration described in this safety assessment. Hydroquinone should not be used in other leave-on cosmetic products.

Authors' Note

The 2010 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, MD, FACP; Donald V. Belsito, MD; Ronald A. Hill, PhD; Curtis D. Klaassen, PhD; Daniel C. Liebler, PhD; James G. Marks Jr, MD, Ronald C. Shank, PhD; Thomas J. Slaga, PhD; and Paul W. Snyder, DVM, PhD. The CIR Director is F. Alan Andersen, PhD.

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th St., Suite 412, Washington, DC 20036, USA.

Declaration of Conflicting Interests

The author(s) declared no potential conflict of interest relevant to this article. F. Alan Andersen and Lillian C. Becker are employed by the Cosmetic Ingredient Review.

Funding

The articles in this supplement were sponsored by th Cosmetic Ingredient Review. The Cosmetic Ingredient Review is financially supported by the Personal Care Products Council.

References

1. Final report on the safety assessment of hydroquinone and pyrocatechol. *J Am Coll Toxicol*. 1986:5(3):123-165.
2. Andersen FA. Addendum to the final report on the safety assessment of hydroquinone. *J Am Coll Toxicol*. 1994;13(3):167-230.
3. Gottschalck TE, Bailey JE. *International Cosmetic Ingredient Dictionary and Handbook*. 12th ed. Washington, DC: CTFA; 2008.
4. Hudnall PM. *Hydroquinone*. 5th ed. New York: Wiley-VCH; 1994:(A13), 499-505.
5. L'Oreal. Rate of disappearance of Hydroquinone during the oxidation of hair dyeing formulation in presence of hair. 1990.
6. Scientific Committee on Cosmetic Products. The Use of Benzoyl Peroxide (BPO), Hydroquinone (HQ), Hydroquinone Methylether (MeHQ) in Artificial Nail Systems. Report No. 0486/01; 2009:1-15.
7. FDA. *Frequency of Use of Cosmetic Ingredients*. Washington DC: FDA; 2007.
8. FDA. *Frequency of Use of Cosmetic Ingredients*. Washington DC: FDA; 2009.
9. Personal Care Products Council. Concentration of Use - Hydroquinone. 2008.

10. FDA. *VCRP Total Number of Products in Each Product Category; March 2008*. Washington, DC: FDA; 2008.
11. Steinberg DC. Submission: Letter from ESSTECH RE: Analysis of HQ/MEHQ content of Finished Nail Matrix, 2000. 2009.
12. EU. Commission directive 2008/88/EC concerning cosmetic products. *Official Journal of the European Union*. 2008; L25612-L25616.
13. EU. Commission directive 2003/83/EC relating to cosmetic products. *Official Journal of the European Union*. 2003; L23823-L23827.
14. FDA. Skin bleaching drug products for over-the-counter human use; Proposed rule. *Federal Register*. 2006;71(167):51146-51155.
15. EU. Commission directive 2000/6/EC relating to cosmetic products. *Official Journal of the European Union*. 2000;L5642-L5646.
16. EU. Council directive 76/768/EEC. *Official Journal of the European Union*. 2008;1-129.
17. IARC. Hydroquinone. *IARC Monogr Eval Carcinog Risks Hum*. 1999;71(pt 2):691-719.
18. Palumbo A, d'Ischia M, Misuraca G, Prota G. Mechanism of inhibition of melanogenesis by hydroquinone. *Biochim Biophys Acta*. 1991;1073(1):85-90.
19. Parvez S, Kang M, Chung HS, et al. Survey and mechanism of skin depigmenting and lightening agents. *Phytother Res*. 2006; 20(11):921-934.
20. Wester RC, Melendres J, Hui X, et al. Human in vivo and in vitro hydroquinone topical bioavailability, metabolism, and disposition. *J Toxicol Environ Health A*. 1998;54(4):301-317.
21. Poet TS, Wu H, English JC, Corley RA. Metabolic rate constants for hydroquinone in F344 rat and human liver isolated hepatocytes: application to a PBPK model. *Toxicol Sci*. 2004;82(1): 9-25.
22. Lau SS, Peters MM, Kleiner HE, Canales PL, Monks TJ. Linking the metabolism of hydroquinone to its nephrotoxicity and nephrocarcinogenicity. *Adv Exp Med Biol*. 1996;387:267-273.
23. DeCaprio AP. The toxicology of hydroquinone—relevance to occupational and environmental exposure. *Crit Rev Toxicol*. 1999;29(3):283-330.
24. Deisinger PJ, Hill TS, English JC. Human exposure to naturally occurring hydroquinone. *J Toxicol Environ Health*. 1996;47(1): 31-46.
25. Li Q, Geiselhart L, Mittler JN, Mudzinski SP, Lawrence DA, Freed BM. Inhibition of human T lymphoblast proliferation by hydroquinone. *Toxicol Appl Pharmacol*. 1996;139(2):317-323.
26. Lee JY, Kim JY, Lee YG, et al. Hydroquinone, a reactive metabolite of benzene, reduces macrophage-mediated immune responses. *Mol Cells*. 2007;23(2):198-206.
27. Boatman RJ, English JC, Perry LG, Bialecki VE. Differences in the nephrotoxicity of hydroquinone among Fischer 344 and Sprague-Dawley rats and B6C3F1 mice. *J Toxicol Environ Health*. 1996;47(2):159-172.
28. Topping DC, Bernard LG, O'Donoghue JL, English JC. Hydroquinone: acute and subchronic toxicity studies with emphasis on neurobehavioral and nephrotoxic effects. *Food Chem Toxicol*. 2007;45(1):70-78.
29. David RM, English JC, Totman LC, Moyer C, O'Donoghue JL. Lack of nephrotoxicity and renal cell proliferation following subchronic dermal application of a hydroquinone cream. *Food Chem Toxicol*. 1998;36(7):609-616.
30. Picardo M, Cannistraci C, Cristaudo A, DeLuca C, Santucci B. Study on cross-reactivity to the para group. *Dermatologica*. 1990;181:104-108.
31. Andreoli C, Rossi S, Leopardi P, Crebelli R. DNA damage by hydroquinone in human white blood cells: analysis by alkaline single-cell gel electrophoresis. *Mutat Res*. 1999;438(1):37-45.
32. do Ceu Silva M, Gaspar J, Duarte Silva I, Leao D, Rueff J. Mechanisms of induction of chromosomal aberrations by hydroquinone in V79 cells. *Mutagenesis*. 2003;18(6):491-496.
33. Silva Mdo C, Gaspar J, Duarte Silva I, Faber A, Rueff J. GSTM1, GSTT1, and GSTP1 genotypes and the genotoxicity of hydroquinone in human lymphocytes. *Environ Mol Mutagen*. 2004;43(4): 258-264.
34. Luo L, Jiang L, Geng C, Cao J, Zhong L. Hydroquinone-induced genotoxicity and oxidative DNA damage in HepG2 cells. *Chem Biol Interact*. 2008;173(1):1-8.
35. Jagetia GC, Aruna R. Hydroquinone increases the frequency of micronuclei in a dose-dependent manner in mouse bone marrow. *Toxicol Lett*. 1997;93(2-3):205-213.
36. Jagetia GC, Menon KS, Jain V. Genotoxic effect of hydroquinone on the cultured mouse spleenocytes. *Toxicol Lett*. 2001;121(1):15-20.
37. Olumide YM, Akinkugbe AO, Altraide D, et al. Complications of chronic use of skin lightening cosmetics. *Int J Dermatol*. 2008; 47(4):344-353.
38. Lau SS, Monks TJ, Everitt JI, Kleymenova E, Walker CL. Carcinogenicity of a nephrotoxic metabolite of the "nongenotoxic" carcinogen hydroquinone. *Chem Res Toxicol*. 2001;14(1):25-33.
39. McGregor D. Hydroquinone: an evaluation of the human risks from its carcinogenic and mutagenic properties. *Crit Rev Toxicol*. 2007;37(10):887-914.
40. English JC, Deisinger PJ. Metabolism and disposition of hydroquinone in Fischer 344 rats after oral or dermal administration. *Food Chem Toxicol*. 2005;43(3):483-493.
41. English JC, Perry LG, Vlaovic M, Moyer C, O'Donoghue JL. Measurement of cell proliferation in the kidneys of Fischer 344 and Sprague-Dawley rats after gavage administration of hydroquinone. *Fundam Appl Toxicol*. 1994;23(3):397-406.
42. English JC, Hill T, O'Donoghue JL, Reddy MV. Measurement of nuclear DNA modification by 32P-postlabeling in the kidneys of male and female Fischer 344 rats after multiple gavage doses of hydroquinone. *Fundam Appl Toxicol*. 1994;23(3):391-396.
43. Boatman RJ, English JC, Perry LG, Fiorica LA. Covalent protein adducts of hydroquinone in tissues from rats: quantitation of sulfhydryl-bound forms following single gavage or intraperitoneal administration or repetitive gavage administration. *Chem Res Toxicol*. 2000;13(9):861-872.
44. Boatman RJ, English JC, Perry LG, Fiorica LA. Covalent protein adducts of hydroquinone in tissues from rats: identification and quantitation of sulfhydryl-bound forms. *Chem Res Toxicol*. 2000;13(9):853-860.
45. Hard GC, Whysner J, English JC, Zang E, Williams GM. Relationship of hydroquinone-associated rat renal tumors with spontaneous chronic progressive nephropathy. *Toxicol Pathol*. 1997; 25(2):132-143.

46. The Kidney. 1998. *Fundamentals of Toxicologic Pathology*. New York, NY: Academic Press.
47. Seely JC, Haseman JK, Nyska A, Wolf DC, Everitt JI, Hailey JR. The effect of chronic progressive nephropathy on the incidence of renal tubule cell neoplasms in control male F344 rats. *Toxicol Pathol*. 2002;30(6):681-686.
48. Hard GC. Mechanisms of chemically induced renal carcinogenesis in the laboratory rodent. *Toxicol Pathol*. 1998;26(1):104-112.
49. Abrass CK. The nature of chronic progressive nephropathy in aging rats. *Adv Ren Replace Ther*. 2000;7(1):4-10.
50. Clark B. Biology of renal aging in humans. *Adv Ren Replace Ther*. 2000;7(1):11-21.
51. Subrahmanyam VV, Doane-Setzer P, Steinmetz KL, Ross D, Smith MT. Phenol-induced stimulation of hydroquinone bioactivation in mouse bone marrow in vivo: possible implications in benzene myelotoxicity. *Toxicology*. 1990;62(1):107-116.
52. Kalf G, Shurina R, Renz J, Schlosser M. The role of hepatic metabolites of benzene in bone marrow peroxidase-mediated myelo- and genotoxicity. *Adv Exp Med Biol*. 1991;283: 443-455.
53. Smith MT, Yager JW, Steinmetz KL, Eastmond DA. Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. *Environ Health Perspect*. 1989;82:23-29.
54. Barale R, Marrazzini A, Betti C, Vangelisti V, Loprieno N, Barrai I. Genotoxicity of two metabolites of benzene: phenol and hydroquinone show strong synergistic effects in vivo. *Mutat Res*. 1990;244(1):15-20.
55. Robertson ML, Eastmond DA, Smith MT. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. *Mutat Res*. 1991;249(1):201-209.
56. NTP Toxicology and Carcinogenesis Studies of Hydroquinone (CAS No. 123-31-9) in F344/N Rats and B6C3F1 Mice (Gavage Studies). *Natl Toxicol Program Tech Rep Ser.* 1989;366:1-248.
57. Tan SK, Sim CS, Goh CL. Hydroquinone-induced exogenous ochronosis in Chinese—two case reports and a review. *Int J Dermatol*. 2008;47(6):639-640.
58. Merola JF, Meehan S, Walters RF, Brown L. Exogenous ochronosis. *Dermatol Online J*. 2008;14(10):6.
59. Charlin R, Barcaui CB, Kac BK, Soares DB, Rabello-Fonseca R, Azulay-Abulafia L. Hydroquinone-induced exogenous ochronosis: a report of four cases and usefulness of dermoscopy. *Int J Dermatol*. 2008;47(1):19-23.
60. Lisi P, Hansel K. Is benzoquinone the prohapten in cross-sensitivity among aminobenzene compounds? *Contact Dermatitis*. 1998;39(6):304-306.
61. IARC working group on the evaluation of carcinogenic risks to humans: occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. Proceedings. Lyon, France, 6-13 October 1992. *IARC Monogr Eval Carcinog Risks Hum.* 1993;57:7-398.
62. Rollison DE, Helzlsouer KJ, Pinney SM. Personal hair dye use and cancer: a systematic literature review and evaluation of exposure assessment in studies published since 1992. *J Toxicol Environ Health B Crit Rev*. 2006;9(5):413-439.
63. Baan R, Straif K, Grosse Y, et al. Carcinogenicity of some aromatic amines, organic dyes, and related exposures. *Lancet Oncol*. 2008;9(4):322-323.