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Final Report on the Safety Assessment of Hydroquinone and Pyrocatechol

Hydroquinone and Pyrocatechol, two benzenediol isomers, are used as couplers in oxidative hair dyes at concentrations of less than 1.0%. Both compounds are absorbed from the gastrointestinal tract; Pyrocatechol is also readily absorbed through the skin. Both compounds are excreted in the urine, mainly as the ethereal sulfate. In acute oral studies Hydroquinone is practically nontoxic to moderately toxic; the data from subchronic feeding studies of Hydroquinone indicated that it was not toxic at 1% but was at higher concentrations. Pyrocatechol was moderately toxic in acute studies. Subchronic oral studies of Pyrocatechol at 0.25% produced hepatic cell hyperplasia in rats. Hydroquinone was a weak depigmenter but not an irritant when tested at 1.0%. The ingredient was a sensitizer when injected at 2.0%. The acute dermal LD_{50} of Pyrocatechol was 0.8 g/kg. Pyrocatechol did not depigment rabbit skin at 1.0% but did at 3.0%; skin irritation was observed at 5.0%. Guinea pigs were sensitized when Pyrocatechol was injected at concentrations above 0.2 μM. Undiluted product formulation containing 2.0% Hydroquinone produced mild conjunctivitis in 3 of 6 animals; undiluted Pyrocatechol is an extreme ocular irritant.

Hydroquinone was not teratogenic in three separate studies. The results of mutagenesis assays of Hydroquinone varies with the assay system used. In four *Salmonella typhimurium* strains, both with and without activation, the mutagenesis assay was negative. Hydroquinone produced positive results both with and without activation in the HeLa DNA synthesis test but was not considered mutagenic in assays using Chinese hamster cells. Hydroquinone induced SCE and delayed cell-turnover time in human lymphocyte studies. Oral doses of Hydroquinone did not inhibit testicular DNA synthesis in male mice, and was nonmutagenic in the mouse sperm-head abnormality test. In multigeneration studies with rats, topically applied hair dyes containing 0.2% Hydroquinone had no effect on reproduction; the dye was neither embryotoxic or teratogenic. Dermally applied hair dyes containing Hydroquinone were not carcinogenic. Hydroquinone when applied topically was neither a tumor promoter nor a cocarcinogen in mice. The mutagenicity of Pyrocatechol also varies with the test system used. In most studies, Pyrocatechol was nonmutagenic, both with and without metabolic activation, in the Ames' assay. The compound was negative in the *Escherichia coli* DNA polymerase assay, but

was positive in the yeast, *Saccharomyces cerevisiae*. Pyrocatechol was negative in the HeLa DNA synthesis test and with Chinese Hamster V79 cells. The compound increased the numbers of chromatid breaks and exchanges in Chinese hamster ovary cells and induced SCE and delayed cell turnover time in human lymphocyte cultures. The compound given by intraperitoneal injection to mice was negative in the sperm-head abnormality test but was positive in the bone marrow assay.

In three studies in mice, topically applied Pyrocatechol was not a tumor promotor. However, topically applied Pyrocatechol was a cocarcinogen for mouse skin in two other studies. Positive sensitization reactions to Hydroquinone were reported in 8.9% of 536 dermatologic patients. Two of 38 patients treated with an ointment containing 5.4% Hydroquinone became sensitized. A cosmetic formulation containing 2% Hydroquinone produced one or more mild irritation reactions in 69 of 90 subjects in the induction phase of a sensitization test; 22 of the 69 subjects were mildly sensitized when challenged. The use of ointments containing 2, 3, and 5% Hydroquinone produced at least minimal depigmentation in white but not black subjects. It is concluded that Hydroquinone and Pyrocatechol are safe for cosmetic use at concentrations of ≤1.0% in formulations that are designed for discontinuous, brief use followed by rinsing from the skin and hair.

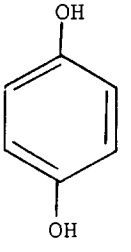
INTRODUCTION

Hydroquinone and Pyrocatechol are used in cosmetic products as couplers in oxidative hair dyes and colors. This presentation and evaluation of the published and unpublished safety data is directed toward the cosmetic product use of these two ingredients.

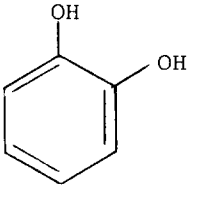
CHEMICAL AND PHYSICAL PROPERTIES

Hydroquinone (CAS No. 123-31-9) and Pyrocatechol (CAS No. 120-80-9) are two benzenediol isomers, 1,4-benzenediol and 1,2-benzenediol. Throughout this report the ingredient name Pyrocatechol will be used instead of the current preferred name of catechol. Pyrocatechol is the recognized name to be used for ingredient labeling on cosmetic products for catechol. The chemical structures of these compounds are as follows[1]:

HYDROQUINONE



PYROCATECHOL



Hydroquinone is composed of colorless crystals that sublime. Solutions turn brown in air due to oxidation; this occurs rapidly in alkali. Hydroquinone is soluble in water, alcohol, ether, acetone, and carbon tetrachloride and is slightly soluble in benzene at room temperature.

Pyrocatechol is a colorless to white crystalline solid with a phenolic odor and taste. The crystals discolor in air and light. They sublime and are volatile with steam. Pyrocatechol is soluble in water, alcohol, ether, acetone, aqueous alkalies, carbon tetrachloride, benzene, and chloroform. Aqueous solutions soon turn brown.[2-5] The melting range of Hydroquinone is 169 to 171°C; upon ignition a maximum residue of 0.07% remains. Pyrocatechol is listed in two grades. The assay for the CP Grade is 99.1% and for the Resublimed Grade at least 99.7%. The freezing points are 103.6°C and 103.9°C, respectively, for the two grades. Each has a maximum residue on ignition of 0.05%.[6] A summary of the physical properties of the two benzenediols is presented in Table 1.

Hydroquinone and Pyrocatechol undergo chemical reactions typical of phenols and may give rise to ethers and monoesters and diesters. Ring substitutions may be made by halogenation, sulfonation, alkylation, nitration, Kolbe and Reimer-Tiemann reactions, Friedal-Crafts acylation, and condensation with aldehydes, esters, or ketones. The benzenediols may take part in reactions leading to multiring systems.[2,7]

Pyrocatechol has the highest redox potential of the three benzenediols. Hydroquinone is a widely used organic reducing agent.[2] Quinones result from the oxidation of Hydroquinone and catechol.[8] Both benzenediols can act as antioxidants.[2]

In aqueous solution and in the presence of oxygen, Hydroquinone undergoes autoxidation at a rate depending on the solution pH; autoxidation increases with increasing pH. The initial products are 1,2-quinone and hydrogen peroxide; the quinone has a positive catalytic effect upon the reaction. Continued oxidation gives condensation products with quinoid structures that account for the dark color of old solutions of Hydroquinone.[2] At pHs of 7–9, Hydroquinone readily autoxidizes; the autoxidation of Pyrocatechol occurs only to a limited extent.[9]

Several studies have explored the effects of the benzenediols on amine N-nitrosation. Hydroquinone and Pyrocatechol at concentrations of 0.06 M were added to an aqueous solution (pH of 4.05 and at a temperature of 37°C) of diethylamine and sodium nitrite at concentrations of 0.5 M and 0.016 M, respectively. Hydroquinone and Pyrocatechol inhibited the formation of N-nitrosodiethylamine by the consumption of nitrite in their oxidation to quinone. Under alkaline conditions (pH 11.0) N-nitrosodiethylamine was formed in the presence of Hydroquinone and Pyrocatechol.[10,11] In an acidic solution (pH 3–4) at 37°C containing concentrations of 0.5 M diethylamine and 0.2 M sodium nitrite, Pyrocatechol at concentrations of 0.005–0.050 M inhibited the nitrosation reaction; smaller concentrations of catechol had no effect on the reaction.[12] In a solution at a pH of 3.0 and at a temperature of 37°C that contained 0.010 M sodium nitrite and 0.001 M dimethylamine, diethylamine, pyrrolidine, and piperidine, a 0.001 M concentration of Pyrocatechol inhibited the formation of the respective nitrosamines.[13,14]

Qualitative and quantitative determinations of Hydroquinone and catechol

TABLE 1. Physical Properties

	Hydroquinone	*Pyrocatechol*	*Reference*
Formula	$C_6H_6O_2$	$C_6H_6O_2$	4
Molecular weight	110.11	110.11	4
Specific gravity at:			
15/4°C		1.371	2
–	1.324–1.328		2
15°C/		1.341	3
20/4°C	1.358		3
–			
15°C/	1.328		4
21°C/		1.1493	4
–		1.344	5
/15°C	1.332		5
Melting point (°C)	166	104	2
	170.5	105	3
Stable			
Unstable			
	173–174	105	4
	170–171	105	5
Boiling point (°C) at:			
–		245	2
–	286.2	246	3
760 mm Hg	285–287	245.5	5
750 mm Hg		245	4
730 mm Hg	285		2
730 mm Hg	285		4
400 mm Hg		221.5	5
200 mm Hg		197.7	5
100 mm Hg		176	5
60 mm Hg		161.7	5
40 mm Hg		150.6	5
20 mm Hg		134	5
10 mm Hg		118.3	5
5 mm Hg		104	5
Vapor pressure (mm Hg) at:			
118.3°C		10	3
132.4°C	1		3

are made by colorimetric methods, gravimetric procedures,[2,15] differential calorimetry,[16] spectrophotometric methods including mixed color photometry,[2,17,18] atomic absorption spectroscopy,[19] ultraviolet spectroscopy,[20] a ring-oven technique,[21] colorimetric analysis,[22] titrimetric procedures including oxidimetry, iodometry, and potentiometry, paper chromatography,[2,17] thin-layer chromatography,[17,23] gel chromatography,[2,20,24] high-pressure liquid chromatography,[25,26] gas chromatography,[2,20,27,28] gas-liquid chromatography[29] and mass spectrometry,[15,20,30] and a field-ionization mass-spectrometric method.[15]

Positive identification of the benzenediols can be made by comparison with

published infrared and ultraviolet absorption spectra, nuclear magnetic resonance, mass and Raman spectra, and x-ray diffraction patterns.[2,4,17]

Hydroquinone has been found in cigarette smoke (up to 80 μg/cigarette)[2,20,24] and in effluents resulting from the production of coal tar chemicals.[17]

Pyrocatechol has been found in cigarette smoke (80–300 μg/cigarette),[2,20,24,27,30] in onions, in crude beet sugar, in crude wood tar, in waters from bituminous shale, in coal,[2] in effluents resulting from the production of coal tar chemicals,[17] and in lignin, wood, and other plant materials.[2,31]

Although a variety of methods had been developed for the production of the benzenediols, only a few methods are commonly used in their commercial manufacture. Hydroquinone is produced by the oxidation of aniline with manganese dioxide and sulfuric acid, followed by reduction with iron dust and water. Also, it may be produced by the alkylation of benzene with propylene to produce a mixture of di-isopropylbenzene isomers, followed by the isolation of the *p*-isomer which is oxidized with oxygen to produce the corresponding dihydroperoxide and treated with acid to produce acetone and Hydroquinone. Pyrocatechol is produced by the alkaline fusion of *o*-chlorophenol, by recovery from the lignin-containing wastes of wood pulping operations, or by the oxidation of benzene with hydrogen peroxide.[2,17]

The Cosmetic, Toiletry and Fragrance Association (CTFA) Standards[6] for Pyrocatechol require it to assay a minimum of 99% and to ash a maximum of 0.05%. The Japan Cosmetic Industry Association standards require a minimum of 98% Pyrocatechol.[32]

USE

Cosmetic

Hydroquinone and Pyrocatechol are used in cosmetics largely as couplers in oxidative hair dyeing where the colored material is produced inside the hair fiber by oxidation of colorless intermediates.[33] Hydroquinone may also be used in some skin care products.[34]

Product types and the number of formulations containing Hydroquinone and Pyrocatechol reported voluntarily to the Food and Drug Administration in 1981 are presented in Table 2. Voluntary filing of this information by cosmetic manufacturers, packagers, and distributors conforms to the prescribed format of preset concentration ranges and product types as described in the Code of Federal Regulations (21 CFR 720.4). Some cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, and, therefore, the value reported by the cosmetic formulator or manufacturer may not necessarily reflect the true concentration of the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold overestimation in the assumed ingredient concentration. In 1981, Hydroquinone was an ingredient of 147 hair dyes

TABLE 2. Product Formulation Data[34]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)			
			Unreported concentration	>1–5	>0.1–1	≤0.1
Hydroquinone						
Hair dyes and colors	811	147	–	–	73	74
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	1	–	1	–	–
Skin lighteners	44	21[a]	–	19	2	–
Other skin care preparations	349	1	–	1	–	–
1981 TOTALS		170	–	21	75	74
Pyrocatechol						
Hair dyes and colors	811	36	–	–	16	20
Hair tints	15	4	–	–	–	4
1981 TOTALS		40	–	–	16	24

[a]FDA has issued a Notice of Proposed Rule-making for the use of Hydroquinone as a skin bleacher in drugs at concentrations between 1.5 and 2.0%.[36]

and color preparations and 23 skin care products, including products intended for medical use as skin lighteners. The concentration of use ranges from ≤0.1% to between 1 and 5%. Pyrocatechol was reported to be an ingredient of 40 cosmetic formulations at concentrations ranging from ≤0.1% to 1%.[34]

Hydroquinone and Pyrocatechol are primarily ingredients in hair dyes and colors, although they are also used in other hair and skin products. Cosmetic formulations containing the benzenediols may be applied to the hair and may come in contact with the skin, eyes, hair, and nails (Table 2).[34]

Hydroquinone has been evaluated in the FDA over-the-counter (OTC) drug review program. FDA has issued a Notice of Proposed Rule making for the safe and effective use of Hydroquinone as a skin bleacher in drug products with concentrations between 1.5 and 2.0%.[36]

Medical Use

Hydroquinone has been used by dermatologists as a depigmenting agent since 1961. Previously, it had been discovered that a sunscreen containing Hydroquinone was being purchased and used as a bleaching agent rather than as a sunscreen.[37] Hydroquinone is used in products designed to lighten small areas of hyperpigmented skin; it is used in the treatment of melasma (chloasma), freckles, senile lentigines, and postinflammatory hyperpigmentation. In addition, products containing Hydroquinone are sometimes used to lighten facial skin. Avoidance of sunlight exposure is necessary for successful skin lightening.[38–40]

Pyrocatechol has been used in antiseptic solutions and ointments for application to wounds and burns and in other medicines[2] but is no longer used for these purposes.[8]

Industrial Use

Hydroquinone is listed in the Code of Federal Regulations as an indirect food additive. There are no concentration limits for its use in adhesives used as components of articles intended for use in packaging, transporting, or holding food or for its use as an optional adjuvant substance/inhibitor in cross-linked polyester resins used as articles or components of articles intended for repeated use in contact with food. Hydroquinone, at no specific concentration limits, may be used as an inhibitor for monomers in the coated or uncoated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packaging, processing, preparing, treating, packing, transporting, or holding aqueous and fatty foods.[35]

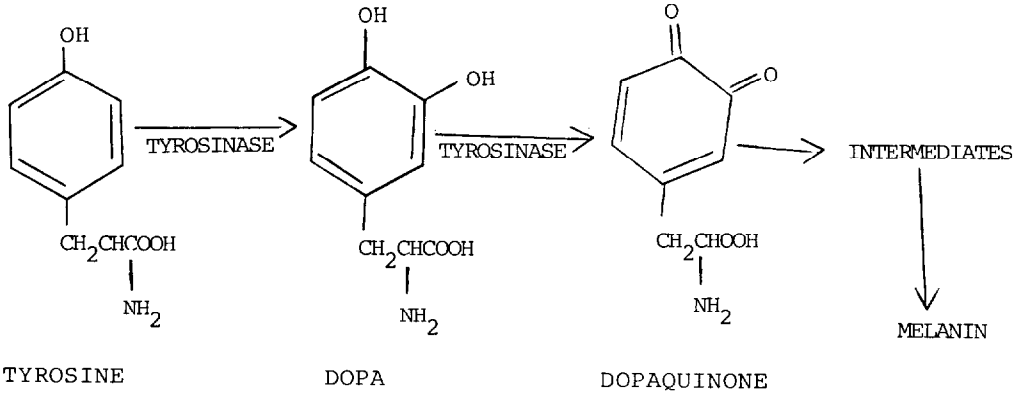
Hydroquinone is used as a laboratory reagent, as a chemical intermediate, particularly in rubber processing chemicals, as a photographic developer, as an antioxidant and polymerization inhibitor, and in pharmaceuticals and dyes.[2,5,17]

Pyrocatechol is used as an analytical agent, as an antioxidant, in polymerization inhibitors, in tanning, in photography, in the synthesis of pharmaceuticals and pesticides, in dyes, and in the rubber and metal plating industrys.[2,5,17]

GENERAL BIOLOGY

Hydroquinone

Hydroquinone affects melanogenesis in vitro and in vivo. A particular reaction of interest is the conversion of tyrosine to melanin as follows[41]:



Denton et al.[42] used tyrosinase from mouse melanomas and determined that Hydroquinone, at 12 times the molar concentration of tyrosine (exact concentrations unspecified), completely inhibited the reaction of conversion of tyrosine to

DOPA. No inhibition was observed when DOPA was the substrate. Iijima and Watanabe[43] reported that 1.7×10^{-7} to 1.7×10^{-4} M Hydroquinone slightly inhibited and 1.7×10^{-3} M Hydroquinone completely inhibited the DOPA reaction in human epidermis that had been surgically removed from the axillary region for the treatment of osmidrosis.

Two tyrosinases were obtained from human melanoma cells. A concentration of 1×10^{-3} M Hydroquinone inhibited the tyrosinases, but that concentration was toxic to melanocytes in tissue culture.[44] Hydroquinone, at a concentration of 0.625 μg/ml, inhibited the growth of B16 mouse melanoma cells. Pigmented cells were particularly sensitive, and treated cultures contained only a few pigmented cells (in contrast to the controls). Growth was completely inhibited at concentrations of 1.25–2.5 μg/ml. A concentration of 1 μg/ml Hydroquinone had no effect on melanin granule movement; 2.5 μg/ml induced cessation of granule movement, vacuolization of the cytoplasm, and clumping of melanin granules. Concentrations of 20–40 μg/ml Hydroquinone abruptly stopped granule movement in the same way as a fixative.[45] Pawelek et al.[46] investigated the effect of Hydroquinone (concentration unspecified) on five cell lines: nonpigmented Vero green monkey kidney cells, an amelanotic mouse melanoma (Cloudman S-91), two melanotic mouse melanomas (Cloudman S-91), and a melanotic hamster melanoma (Greene). Hydroquinone was highly toxic to all the cell lines and was not selectively toxic to melanotic cells. The in vitro actions of Hydroquinone alone or in combination with beta-mercaptoethanolamine on B-16, Cloudman S-91, and Harding-Passey murine melanomas grown in vivo have been investigated. The cells were incubated with the chemicals for 5 to 240 or 480 minutes at 37°C. Hydroquinone, at a concentration of 100 μg/ml, inhibited B-16 and stimulated S-91 tyrosinase activity. In Harding-Passey cells, Hydroquinone at first stimulated and then inhibited tyrosinase activity. Except for S-91 in which the cGMP content was not unaltered, cAMP and cGMP values in the other cell lines were elevated after exposure to Hydroquinone. Hydroquinone did not affect peroxidase but inhibited DNA and RNA synthesis in all three cell lines and inhibited protein synthesis in the S-91 and Harding-Passey cells. Hydroquinone in combination with beta-mercaptoethanolamine did not generally affect the cells in the same way that Hydroquinone alone did.[47–50] Recent studies have suggested that the depigmenting effect of Hydroquinone is the result of a selective action on melanocyte metabolism instead of a specific effect on melanin synthesis.[51]

Protein biosynthesis by organ cultures of pig skin was measured by the uptake of arginine and tyrosine; the skin degenerated after 3 days. A 10-mM concentration of Hydroquinone caused marked degeneration of the skin after 1 day and almost complete inhibition of amino acid incorporation into black and white pig skin.[52]

Hydroquinone produced depigmentation in the skin of the guinea pig, man, mouse, rat, cat, and goldfish.[55] In one study guinea pigs received topical application of creams containing 2% and 5% Hydroquinone in an oil-in-water base 6 days a week for 3 weeks or subcutaneous injection of 2 ml of a 1% Hydroquinone in normal saline solution daily for 8 days. It was observed that Hydroquinone affected the nonfollicular and follicular melanocyte system and that it had no effect on keratinocytes. In areas depigmented by Hydroquinone, the changes

were decreased formation, marked alteration of internal structure, and increased degradation of melanosomes. The membranous organelles in the melanocytes were destroyed. Hydroquinone eventually caused necrosis of melanocytes.[54]

Hydroquinone ointments of 2, 3, and 5% were applied to the senile lentigines of a white man. In depigmented areas, the observations included perivascular infiltrates with less pigment in areas of inflammation, less melanin present in supranuclear caps, greater dispersion of melanin, some alterations in melanin granules, approximately the same number of melanocytes, fewer melanin granules, and melanocytic melanin production halved.[55]

Kligman and Willis[56] tested an ointment containing 5.0% Hydroquinone, 0.1% tretinoin, and 0.1% dexamethasone on black human skin. In depigmented areas irritant reactions consisted of an increase in perivascular monocytic cells; dopa-positive melanocytes were increased and were larger and more active enzymically; epidermal melanin granules were fewer and supranuclear caps were absent.

This formulation did not affect melanin granules already in the dermis but affected the synthesis and transfer of melanin. Sun exposure after treatment resulted in rapid repigmentation, with pigmentation even greater than before.[57] Findlay[58] described several cases in which melanocytes overcame the bleaching effect of Hydroquinone. The prolonged use and thorough inunction of Hydroquinone bleaching creams on black facial skin followed by sun exposure can eventually lead to ochronosis and colloid milium production; the colloid milium were mostly darker than normal skin.

After exposure to Hydroquinone (0.5 to 3 mM) human erythrocytes were depleted of reduced glutathione, and methemoglobin was produced. No Heinz bodies were produced and hemolysis was not observed.[59,60] It has been reported that Hydroquinone forms Heinz bodies in animals[61] and that Hydroquinone can cause methemoglobinemia in animals.[62]

Pyrocatechol

Pyrocatechol has antiseptic properties; its phenol coefficient (the ratio of the dilution of Pyrocatechol to the dilution of phenol required to kill a particular strain of a definite organism) is 0.87 for *Salmonella typhosa* and 0.58 for *Staphylococcus aureus*.[8]

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Hydroquinone

Hydroquinone is absorbed through the gastrointestinal tract and possibly through skin and excreted in the urine as free Hydroquinone and quinone or conjugated with glucuronic, sulfuric, and hexuronic acids.[62]

Hydroquinone was "weakly" absorbed by hairless rat skin in vitro and in vivo and by healthy human skin in vitro.[63]

Radioactive Hydroquinone, at doses of 1.3–14 mg/kg, was injected into the lateral tail vein of rats and the rats were killed 2 h later. Radioactivity was found

in bone marrow, in the white pulp of the spleen, and in the thymus. Some radioactivity was also distributed in subcutaneous tissues, in sebaceous glands, in brown fat, and in the white matter of the brain and spinal cord.[64] In another experiment, the lateral tail vein of rats was injected with 14 mg/kg of radioactive Hydroquinone, and some of the rats were killed at 2 h and the others were killed at 24 h. The liver, thymus, and bone marrow were homogenized and examined for radioactivity. The concentration of acid-soluble radioactivity decreased in the liver and thymus and remained approximately the same in the bone marrow between 2 and 24 h. The concentration of covalently bound radioactivity in the liver, thymus, and bone marrow increased between 2 and 24 h; the greatest increase was in the bone marrow. The researchers suggested that the autoxidation of Hydroquinone would result in the formation of semiquinone oxidation products and superoxide radicals, which may be cytotoxic.[65]

Rabbits were given 100–230 mg/kg Hydroquinone orally and the 24-h urine was collected. The urine contained (as a percentage of dose) 30% Hydroquinone ethereal monosulfate, 43% Hydroquinone monoglucuronide, and trace amounts of free Hydroquinone (0.065%). There was no evidence of the oxidation of Hydroquinone to any trihydroxybenzenes.[66] In rabbits given Hydroquinone orally, the rate of glucuronide conjugation was proportional to the body concentration of Hydroquinone. The rate of organic sulfate formation remained approximately constant until almost the whole dose was excreted.[67] Hydroquinone administered orally to rats at a dose of 0.09 g/kg increased the excretion of organic sulfates but, contrary to other reports, had no effect on glucuronic acid excretion.[68]

Radioactive Hydroquinone was administered iv at a dose of 20 mg/kg to cats. The components in the urine were approximately 10% unchanged Hydroquinone, approximately 87% Hydroquinone sulfate, and approximately 3% glucuronide. The researchers suggested that some Hydroquinone might be metabolized to quinone.[69]

A volunteer received 200 mg (3 mg/kg) Hydroquinone orally. The composite 24-h urine contained no free Hydroquinone, but Hydroquinone was excreted as the ethereal sulfate and as the glucuronate.[26]

Hydroquinone was detoxified by the liver.[70] The results of a study with microsomes from rat liver indicated that *p*-benzosemiquinone and/or *p*-benzoquinone was formed from benzene via Hydroquinone; these compounds bind covalently to macromolecules.[71]

Pyrocatechol

Pyrocatechol was absorbed readily from the gastrointestinal tract and through the intact skin of mice. After absorption, some of the Pyrocatechol was oxidized by polyphenol oxidase with the formation of *o*-benzoquinone. Some Pyrocatechol conjugated with hexuronic, sulfuric, and glucuronic acids and the conjugates were excreted in the urine. A small amount of free Pyrocatechol was also excreted in the urine. Pyrocatechol may also be methylated by catechol-*o*-methyltransferase.[8,62]

Radioactive Pyrocatechol, at doses of 1.2–12 mg/kg, was injected into the lateral tail vein of rats, and the rats were killed 2 h postinjection. Radioactivity

was found in the bone marrow, the spleen, and the thymus. Some radioactivity was also distributed in subcutaneous tissue, in sebaceous glands, in brown fat, and in the white matter of the brain and spinal cord.[64] In another experiment, 14 mg/kg of radioactive Pyrocatechol was injected via the lateral tail vein into rats. Some of the rats were killed at 2 h, and those remaining were killed at 24 h. The liver, thymus, and bone marrow were homogenized and examined for radioactivity. The concentration of acid-soluble radioactivity decreased in the liver and thymus and remained the same in the bone marrow between 2 and 24 h postinjection. The concentration of covalently bound radioactivity in the liver and bone marrow was increased and in the thymus remained approximately the same between 2 and 24 h; the greatest increase was in the bone marrow. The researchers suggested that Pyrocatechol may be metabolized to cytotoxic products.[65]

Mice were exposed for 10 minutes to diluted smoke of cigarettes containing radioactive Pyrocatechol. The deposition and distribution of inhaled Pyrocatechol was determined in certain internal tissues, urine, and feces for up to 2 h after exposure. Immediately after exposure, 56% of the radioactivity (in the total body) was in the blood, 14% was in the kidneys, 13% was in the liver, and 8% was in the lungs. The blood contained the greatest percentage of radioactivity at all times (measured up to 2 h after exposure). The radioactivity decreased over time in all tissues; 2 h after exposure, approximately 11% of the radioactivity remained in the body. In another experiment, urine and feces were collected; 91.2% of the radioactivity (in the total body) was excreted in the urine, and 1.5% of the radioactivity was excreted in the feces within 2 h after exposure. Less than 1% of the radioactivity remained in the lungs, turbinate, liver, or kidneys 2 h after exposure.[72]

Rabbits were given Pyrocatechol orally, and the urine was collected over 24 h. The urine contained (as a percentage of dose) 18% catechol ethereal sulfate, 70% catechol monoglucuronide, and 2% free Pyrocatechol. The urine also contained traces of hydroxyquinol as an ethereal sulfate; apparently, there was further in vivo oxidation of Pyrocatechol.[66] Pyrocatechol was administered orally to rats in a dose of 0.06 g/kg, and urine was collected over the next 3 days. Pyrocatechol had little or no effect on the excretion of glucuronic acid or organic sulfate.[68]

Radioactive Pyrocatechol was infused at a rate of 5×10^{-8} mol/min into the renal artery of dogs, and ureteral urine collections were made during infusion. The sulfate fraction of the urine contained 60–70% of the radioactivity; only trace amounts of glucuronide and free Pyrocatechol were detected.[73]

Hirosawa et al.[74] used a color test to examine the urine of workers exposed to workroom air in which the concentration of Pyrocatechol varied with time and the specific environment. Twenty-four-hour urine samples were examined after 7–9 h of exposure to air polluted with catechol and phenol. Twenty-four-hour urine collections taken from the same workers during a 1-month period in unpolluted air served as controls. Average Pyrocatechol values for 24-h urine samples of 6 workers were 24.2 mg upon catechol exposure and 19.2 mg upon exposure to the unpolluted control environment. The differences were insignificant; however, exposure of an individual to air polluted with Pyrocatechol and phenol caused a temporary increase of the urinary excretion by Pyrocatechol;

the urinary Pyrocatechol was measured several times over 24 h. The calculated biological half-life of inhaled Pyrocatechol in humans was 3–7 h. Pyrocatechol inhibited catechol-O-methyltransferase but did not affect catecholamine metabolism; urinary excretion of catecholamines and their metabolites (epinephrine, norepinephrine, metanephrine, and vanylmandelic acid) was within the normal range (for adult, Japanese males) except for a slight decrease in norepinephrine. The 24-h urine of nonsmokers and of cigarette smokers who were on the same restricted diet, was analyzed for Pyrocatechol. The nonsmokers excreted 4.4 ± 1.2 mg and the cigarette smokers 6.8 ± 3.0 mg of Pyrocatechol. This finding indicates that diet was a major factor in determining urinary catechol concentrations.[75]

Catechol-O-methyltransferase (COMT) catalyzed the transfer of a methyl group from S-adenosylmethionine to a Pyrocatechol substrate with the formation of O-methylated products. COMT activity has been found in many mammalian tissues; it is involved in the extraneuronal inactivation of endogenous catecholamines, in the further metabolism of oxidized catecholamine metabolites, and in the detoxification of Pyrocatechol drugs.[76] Pyrocatechol was a substrate for rat liver COMT, and it competitively inhibited the enzyme's activity with other substrates.[77]

ANIMAL TOXICOLOGY

Oral Studies

Acute Toxicity

The acute oral toxicity of Hydroquinone has been studied in rats,[78–82] mice, guinea pigs,[81,82] rabbits,[62] dogs,[79,81,82] cats,[62,79,81,82] and swine[83] (Table 3). The LD_{50} values for rats ranged from 0.1 to 1.3 g/kg; LD_{50}s were lower in fasted rats. In the Hodge and Sterner[84] classification of single-dose oral toxicity for rats, Hydroquinone would be classified as practically nontoxic to moderately toxic.

The acute oral toxicity of Pyrocatechol has been studied in rats,[7,82] mice, guinea pigs,[82] dogs,[2,62] rabbits, cats, and pigs.[62] The LD_{50} values for rats were 0.3 g/kg in one experiment and 0.26 g/kg in another experiment. In the Hodge and Sterner[84] classification of single-dose oral toxicity for rats, Pyrocatechol would be classified as moderately toxic.

Subchronic and Chronic Toxicity

The subchronic and chronic toxicity of Hydroquinone has been studied in rats,[79,82,85] mice,[42,86] guinea pigs,[42] and dogs[79] (Table 4). Concentrations of up to 1.0% Hydroquinone in the diet of rats for 2 years were not toxic. At 5% in the diet for 9 weeks, signs of toxicity were seen. An oral dose of 500 mg/kg given 101 times within 151 days resulted in the death of half of the test rats within 2 months; 1750 mg/kg given orally 9 times within 12 days resulted in 71% mortality within 24 h of the last exposure. A dose of 262 mg/kg per day Hydroquinone in the drinking water or a concentration of 100 mg/kg in the diet of mice resulted in some fur depigmentation (amount not stated). An oral dose of 88 mg/kg per

day to guinea pigs was not toxic. Dogs were fed up to 40 mg/kg per day Hydroquinone in their feed for 50 weeks, 16 mg/kg per day for 80 weeks, or 100 mg/kg per day for 26 weeks; no toxicity was observed.

The chronic toxicity of Pyrocatechol has been studied in rats[8] and in mice.[87] The results of these studies as well as those for Hydroquinone are summarized in Table 4. Rats fed up to 1.0% Pyrocatechol in their diets for 2 years had the "beginning" of hepatic cell hyperplasia. Mortality rates were similar to the control rats. Mice that received up to 0.4% Hydroquinone in their drinking water for 20 weeks had reduced body weight but no other signs of toxicity.

Dermal Studies

Acute Toxicity

Hydroquinone, as a 2% solution in dimethyl phthalate, was applied to the intact and abraded skin of rabbits in doses of 0.08 to 0.19 mg/kg. It was held in contact with the skin for 24 h with a rubber sleeve. No adverse local or systemic effects were observed.[88,89]

Pyrocatechol, in doses of 0.25, 0.50, 1.0, and 2.0 g/kg, was applied to the abraded and intact skin of 4 rabbits for each dose group for 24 h; the rabbits were observed for an additional 14 days. Deaths occurred in the three highest dose animals (1/4, 2/4, and 4/4). The acute dermal LD_{50} of Pyrocatechol was 0.8 g/kg. Dead rabbits had subdermal hyperemia and edema. Pyrocatechol produced moderate erythema and slight edema in all surviving rabbits; necrosis was associated with compound application at abraded skin sites. Slight epidermal flaking persisted, and incrustations of dead tissue were sloughing at the end of the 14-day observation period. The body weight gains of survivors were less than the weight gains of the control rabbits. No lesions were observed at necropsy of the survivors.[7]

Subchronic and Chronic Toxicity

The subchronic and chronic dermal toxicity of Hydroquinone has been studied in rabbits[88,89] and guinea pigs[54,90-92] (Table 5). Hydroquinone, at a concentration of 2% in dimethyl phthalate, was applied dermally daily for up to 90 days in doses of up to 0.08 mg/kg to rabbits; skin lesions and a slight thyroid hyperplasia were observed but no other toxic effects. Ultraviolet radiation for up to 21 days of this regimen increased the severity of the skin lesions.[88] Hydroquinone, at concentrations in ointments and creams from 1–10%, applied daily to black guinea pigs for 1 month produced weak depigmentation at 1% and moderate at 3%. It was an irritant above 3%.[90] The minimal time interval for Hydroquinone and Pyrocatechol to produce uniform depigmentation was dependent upon both concentration and suspending medium.[91] In one study, ointments containing 5–20% Hydroquinone were applied over 90% of the bodies of guinea pigs daily for 50 days; body weight gains were subnormal and the adrenals were enlarged.

A 13-week dermal toxicity study was conducted in rats with a cosmetic formulation containing 2% Hydroquinone.[93] The product was applied to 15 animals at a dose of 886 mg/kg, once daily, at a dorsal-anterior, shaved skin site.

TABLE 3. Acute Oral Toxicity

Material, concentration and vehicle tested	*No. and species of animal*	*Method*	*LD_{50} (g/kg)*	*Comments*	*Reference*
Hydroquinone	Rats	–	0.78	LD_{100} was 1.125 g/kg	78
Hydroquinone, in	6–40 per trial	Given by stomach tube			
glycerin	Priestly rats	Not fasted	1.30	LD_{50} value was lower in fasted rats	79
glycerin	Priestly rats	Not fasted	1.00	LD_{50} value was lower in fasted rats	79
propylene glycol	Sprague-Dawley rats	Not fasted	1.09	LD_{50} value was lower in fasted rats	79
distilled water	Spraque-Dawley rats	Not fasted	1.18	LD_{50} value was lower in fasted rats	79
glycerin	Sprague-Dawley rats	Not fasted	1.08	LD_{50} value was lower in fasted rats	79
propylene glycol	Spraque-Dawley rats	Fasted (for 18 h prior to hydroquinone administration)	0.32	LD_{50} value was lower in fasted rats	79
propylene glycol	Wistar rats	Not fasted	0.73	LD_{50} value was lower in fasted rats	79
propylene glycol	Wistar rats	Fasted	0.30	LD_{50} value was lower in fasted rats	79
Hydroquinone, in sugar-coated tablets	Dogs Cats	Given with small amount of meat	0.30 between 0.04 and 0.09	–	79
Hydroquinone, in water	Rabbits Cats	–	0.2 0.08	–	62
Hydroquinone	Rats	–	0.37–0.39	–	80
Hydroquinone	Rats Mice Guinea pigs Dogs Cats	–	0.32 0.4 0.55 0.2 0.07	Hyperexcitability, tremors, convulsions, salivation in dogs and cats, and emesis and incoordination of the hind legs in dogs occurred within 30–90 minutes; deaths occurred within a few hours; 0.1 g/kg in dogs and 0.07 g/kg in cats caused mild to severe swelling of the area around the eye, of the nicitating membrane, and of the upper lip; routine blood counts in dogs and cats indicated increased activity of the "cell-forming tissues"	81, 82

Hydroquinone, in saline	Young swine (feeder pigs)	Two groups of 2 given 0.18 and 0.35 g/kg by gavage; killed and necropsied 48 to 72 h later	–	No clinically adverse symptoms or lesions noted in the 0.175 g/kg pigs; both 0.35 g/kg pigs died; shaking, nausea, erythema, severe convulsions, and deaths occurred within 30 minutes of dosing; they were markedly hyperglycemic when comatose; isocitric dehydrogenase and serum glutamic oxaloacetic transaminase were slightly elevated; no gross or microscopic changes or bacterial isolates found	83
Pyrocatechol	Rabbits Dogs Cats Pigs	–	0.2 0.3 0.1 0.2	–	62
Pyrocatechol, crystalline	Rats	Four groups of 5 rats given 0.158–1.26 g/kg by stomach intubation; observed for 14 days	0.3	At doses of 0.63 and 1.26 g/kg, 5/5 died within 1 day and 1 h, respectively; dead rats had hyperemia of the stomach and intestines; survivor rats had no gross lesions and all but 1 had body weight gains similar to controls	7
Pyrocatechol	Rats Mice Guinea pigs	–	0.3 0.3 0.2	–	82
Pyrocatechol	Dogs	Given 0.03 and 0.05 g/kg	–	0.03 g/kg caused anemia and "other complications" followed by death in several weeks; 0.05 g/kg caused death within 48 h	2

TABLE 4. Subchronic and Chronic Oral Toxicity

Material tested	*Dose and vehicle*	*Length of study*	*No. and species of animal*	*Results*	*Reference*
Hydroquinone	0.003–0.3% in diet	10 days prior to insemination and 32–33 days after	Female rats	No maternal mortality	85
Hydroquinone	0–1.0% in diet	103 weeks	10 male and 10 female Spraque-Dawley rats of weaning age at each of 4 doses	Final body weights of treated rats were similar to controls; in first month, growth rate was slower in the 0.5 and 1.0% groups; hematological analyses and histopathology normal	
Hydroquinone, heated with lard to 190°C for 30 min	0–0.5% in diet	103 weeks	16–23 male and 16–23 female rats at each of 4 doses	Final body weights of treated rats were similar to controls; hematological analyses and histopathology normal	79
Hydroquinone	0–1.0% in diet, with 0.1% citric acid	103 weeks	20 male and 20 female rats at each of 4 doses	Final body weights of female rats and 0.5% male rats were similiar to controls; slightly lower final weights observed in 0.1 and 1.0% male rats; in first month, growth rate was slower in the 0.5 and 1.0% groups; hematological analyses and histopathology normal	79
Hydroquinone	5% in diet	9 weeks	14 adult rats (14 controls)	Treated rats had a 46% loss in weight; aplastic anemia, average of 66% decrease in bone marrow cellularity with marked atrophy of the hematopoietic elements, atrophy of liver cord cells, splenic lymphoid tissue, adipose tissue, and striated muscle, and superficial ulceration and hemorrhage of stomach mucosa	79

Hydroquinone	500–1750 mg/kg by stomach tube	9 administrations in 12 days	20–48 rats at each of 6 doses	71% of total mortality rate occurred within 24 h of first dose; for next 11 days, average mortality rate was less than 5%/day	79
Hydroquinone	500 mg/kg by stomach tube	101 administrations in 151 days	16 rats	More than half died in first 2 months (no data given); autopsy findings were negative; survivors grew at same rate as controls	79
Hydroquinone, in sugar-coated tablets	16 mg/kg per day in feed	80 weeks	14-month-old dog [2 controls]	Treated dogs grew normally; negative necropsy findings, urinalyses, hematological analyses, pathology; hemosiderosis was usually more marked in spleens, livers, and marrow of controls	79
Hydroquinone, in sugar-coated tablets	1.6 and 40 mg/kg per day in feed	First dose for 31 weeks, second dose for 49 weeks	2 4-month-old dogs		
	100 mg/kg per day in feed	26 weeks	5 adult dogs	Dogs maintained their weight; negative necropsy findings, urinalyses, hematological analyses, pathology	
Hydroquinone, in capsules	Increasing doses: 22–88 mg/kg per day (total dose of 2.38 g)	76 days	5 adult male guinea pigs (5 controls)	No toxic effects; one treated guinea pig showed "questionable depigmentation"	42
Hydroquinone	Increasing doses: 37–262 mg/kg per day (total dose of 247 mg) in drinking water	76 days	4 adult black C57 male mice (control group)	"Questionable pigmentary change" was observed; 2/4 developed alopecia on back of neck	42
Hydroquinone	Increasing doses: 37–262 mg/kg per day (total dose of 247 mg) in drinking water	76 days	4 7-week-old black C57 male mice (control group)	"General" pigmentation was observed in 2/4; all 4 developed alopecia on back of neck	42

TABLE 4. (Continued)

Material tested	*Dose and vehicle*	*Length of study*	*No. and species of animal*	*Results*	*Reference*
Hydroquinone	0.125–2.0% in diet	2 years	Groups of 12–18 rats	Similar to controls in number of live rats at experiment end	82
Hydroquinone	100 mg/kg of diet	20 weeks	Young mice	Most (number unspecified) of mice had depigmentation of fur within 4 to 20 weeks	86
Pyrocatechol	0.0625–1.0% in diet	2 years	Groups of 12–18 rats	Similar to controls in number of live rats at experiment end; at 0.25% there was "beginning" hepatic cell hyperplasia	82
Pyrocatechol	01–4.0 g/l in drinking water	20 weeks	Mice	No adverse effects at 0.1 g/l; at 4.0 g/l, 55% decrease in body weight; some organ weights increased but blood cell counts, bone marrow cell numbers, and spleen colony-forming units were "almost" unchanged	87

TABLE 5. Subchronic and Chronic Dermal Toxicity

Material tested	*No. and species of animal*	*Length of study*	*Dose of material*	*Method*	*Results*	*Reference*
Hydroquinone, 1–10% in creams	8 black guinea pigs	1 month	–	Applied daily 5 times a week to unepilated skin of ear and wax epilated skin of back	Nonirritant up to 3%, irritant above 5%; weak depigmentation at 1%; moderate at 3% and above	90
Hydroquinone, 2% in dimethyl phthalate	Rabbits	21 days (and 14 days further observation)	1.0–4.0 ml/kg	Applied daily by inunction; Also applied two times a week after initial UV irradiation (General Electric Uviarc portable AC ultraviolet light with an arc length of 6 inches and giving 54 W per arc inch) for 20 minutes at distance of 6 inches; over 21 days, exposure period was 10 minutes	Atypical dermatoses that were aggravated by UV appeared at first as small petechiae and observed occasionally in untreated areas; no edema; no gross local or systemic effects (separate testing without UV indicated no evidence of sensitization)	88, 89
Hydroquinone, 2% in dimethyl phthalate	3 groups of 4 rabbits	90 days	0.5–4.0 ml/kg	Applied daily by inunction	Atypical dermatoses appeared and disappeared over 90 days; initial reaction petechiae, then intense erythema; no gross local or systemic effects at 0.5 and 1.0 ml/kg; at 2.0 and 4.0 ml/kg subnormal body weight gains and lowered food intake; slight emaciation; at microscopic examination, moderate dermatitis and slight thyroid hyperplasia	88, 89
Hydroquinone, 0.5 M in dimethyl sulfoxide and 5% in hydrophilic ointment	Groups of 2–5 black guinea pigs	6 months	0.1 ml	Applied daily to 8 epilated dorsal sites and the unepilated skin of nipples	Weak to moderate depigmentation; moderately to severely irritating	91

TABLE 5. (Continued)

Material tested	*No. and species of animal*	*Length of study*	*Dose of material*	*Method*	*Results*	*Reference*
Creams containing 2 and 5% hydroquinone in an oil-in-water emulsion	Groups of 5 guinea pigs	3 weeks	–	Applied daily 6 days a week to epilated backs	Depigmentation visible within 8–10 days and was maximum between 14 and 20 days; no total depigmentation; inflammatory changes and thickening of the epidermis; desquamation was prominent and often seen within 1 week; 5% caused more marked depigmentation and scaling than 2%	54
5–20% hydroquinone ointment (petrolatum vehicle)	30 guinea pigs (5 controls)	50 days	–	Applied to 15–90% epilated body surface daily	Hydroquinone eliminated in the urine; body weight gains less than controls except when 15% of body surface smeared; number of lymphocytes in blood dropped, adrenals enlarged, focal fibrosis found in the myocardium of some of the guinea pigs	92
Pyrocatechol, 1–10% in creams	11 black guinea pigs	1 month	–	Applied daily 5 times a week to unepilated skin of ear and wax epilated skin of back	No depigmentation or irritation at 1%; definite weak irritant and depigmenter at 3%; definite moderate at 5%; very strong depigmenter and irritating at 7–10%	90
Pyrocatechol, 5–10% in hydrophilic ointment	Groups of 2–5 black guinea pigs	6 month	0.1 ml	Applied daily to 8 epilated dorsal sites and the unepilated skin of ears and nipples	Moderate depigmentation; moderately to severely irritating	91

A minimal to moderate skin irritation as well as a brown discoloration were observed at the application site for most animals throughout the study. Results of hematological and clinical chemistry determinations, urinalysis, and gross and microscopic examination of necropsy specimens were negative for compound effects.

Primary Irritation

In a preliminary screening study to establish the concentration of Hydroquinone to be used in a photoallergic study, an aqueous solution of Hydroquinone was slightly irritating at 10% but not at 5.0, 1.0, or 0.5% when tested on 8 guinea pigs.[94]

A product formulation containing 2% Hydroquinone was tested on 9 rabbits using a single-insult occlusive test patch procedure. The average irritation score was 1.22 (scale 0–4).[95]

Pyrocatechol, at a dose of 0.5 g, was placed onto the intact and abraded skin of the bellies of male albino rabbits for 24 h, and the animals were observed for an additional 14 days. The PII was 5.5 (of a possible maximum of 8.0); Pyrocatechol was a primary irritant. Slight to moderate erythema and slight edema of the intact areas and necrosis of the abraded areas were observed; irritation was less at 72 h than at 24 h. After 14 days, the intact areas were free of irritation except for a slight epidermal flaking and the necrotic areas were sloughing.[7]

Intradermal Studies

Hydroquinone, at concentrations of 0.001–0.1% in water and in a dose of 0.1 ml, was administered intracutaneously in the flank of guinea pigs. Hydroquinone was not an intracutaneous primary irritant.[96]

Groups of 8 C57 mice (black) were given 4 separate intradermal injections on their ventral surface of 50 μg or 500 μg Pyrocatechol in dimethylsulfoxide. Local depigmentation was observed in 5 of 8 and 7 of 8 mice, respectively.[97]

Sensitization and Photoallergenicity

The skin sensitization potential of Hydroquinone for guinea pigs has been investigated[88,89,98] (Table 6). Hydroquinone, in dimethylphthalate at 2%, did not sensitize any of 10 guinea pigs. Hydroquinone was a "weak," "strong," and "moderate" sensitizer in a modified Draize procedure, a maximization procedure, and a single injection adjuvant test, respectively. Hydroquinone, in propylene glycol at 5%, was tested for sensitization potential using 10 guinea pigs by the Magnusson-Kligman maximization procedure. No potential for skin sensitization was found.[99] Guinea pigs sensitized to *p*-methoxyphenol also reacted to Hydroquinone.[100]

A group of 20 Hartley albino guinea pigs, equal male and female, was used to evaluate the photoallergenicity of a 10% aqueous solution of Hydroquinone. The nuchal area was clipped and gently stripped with pressure-sensitive tape, and 0.3 ml of the Hydroquinone solution was applied using a Hilltop® chamber. Induction sites were covered by occlusive patches for 2 h, uncovered, and exposed to approximately 10 J/cm^2 of UV (320–400 nm) light. The lumbar region was simultaneously shielded from UV exposure. Six induction treatments, on alternating days, were given. Ten guinea pigs were sham-treated as negative con-

TABLE 6. Sensitization

Material tested	*Concentration*	*Method*	*No. of guinea pigs*	*Results*	*Reference*
Hydroquinone in dimethyl phthalate	2%	Injected guinea pigs every other day or three times a week for a total of 10 injections; 2 weeks later a challenge injection; read at 24 h	10	None sensitized	88, 89
Hydroquinone	Induction injections of 2.5% intradermal challenge of 1.0%, topical challenge of 20%	Modified Draize procedure: 4 simultaneous induction injections at sites overlaying axillary and inquinal lymph nodes; 2 weeks later, intradermal and open topical challenge on opposite shaved flanks; if no sensitization, procedure repeated	10	None sensitized at first trial; 30% at second; a "weak" sensitizer	98
Hydroquinone	Intradermal inductions of 2.0%, topical induction of 10%, chamber challenge of 5%	Maximization procedure: 6 0.1 ml intradermal inductions, 2 of hydroquinone alone, 2 of hydroquinone in 50% Freund's complete adjuvant (FCA), 2 of 50% FCA alone, into the nuchal region; 7 days later a 48-h occluded patch induction over the injection sites; 2 weeks later challenge with 24-h occluded chamber on shaved flank; 3–4 challenges a week apart	10	7/10% sensitized; a "strong" sensitizer; mean patch test reaction score was 1.8 (calculated from sum of all patch test reactions positive at 4 challenges max. possible score = 3.0)	98

Hydroquinone	Intradermal induction of 2.0%, chamber challenge of 50%	Single injection adjuvant test: one intradermal induction of hydroquinone in FCA in the nuchal region; 12–14 days later 6-h occluded chamber challenge on the shaved flank; scored 18–42 h after removal; 3–4 challenges a week apart	10	4/10% sensitized; a "moderate" sensitizer; mean patch test reaction score was 1.2 (calculated from sum of all patch test reactions positive at 4 challenges max. possible score = 3.0)	98
Hydroquinone	Induction at 5% in propylene glycol, challenge at 5% in petrolatum	Magnusson-Kligman Maximization Procedure	10	None sensitized	99
Hydroquinone	Induction at 10% challenged at 2 and 0.1%	Dermal application on alternate days, 6 treatments, 9-day nontreatment period and challenge at 2 and 0.1% UVA and non-UVA exposed sites	20	20/20 sensitized at 10% and 2% challenge; 18/20 responded to 10% induction and 0.1% challenge; severity of response did not increase on separate areas exposed to UVA	94
Pyrocatechol	–	1 induction injection per week for 3 weeks into nuchal area, inquinal-axillary region, and the footpads, successively; an emulsion in FCA, total dose per guinea pig was 1 mg; 4 weeks later, topical challenge – 5 μl of an acetone solution with a ring on animals skin, max. applied was 1 μmol; scored at 48 h; 3 challenges 2 weeks apart	8–12	None sensitized to less than 0.2 μmol; most animals were sensitized; sensitized animals also reacted to 3-methyl and 3-propyl catechol	101

trols. After a 9-day nontreatment period, two pairs of sites on the clipped lumbar region of each guinea pig were challenged with 0.3 ml of 2.0 and 0.1% Hydroquinone, respectively, and covered with occlusive patches for 2 h. The left side and the induction area were subsequently shielded while the right side was exposed to approximately 10 J/cm^2 of UV (320–400 nm) light. Sites were scored at 24 and 48 h. The 10% Hydroquinone used in the induction phase followed by the 2.0% challenge sensitized 20 of 20 guinea pigs. The severity of response when scored at 24 and 48 h after challenge was similar for both the UVA and non-UVA-treated sites. The responses at the 0.1% challenged sites indicated 18 of 20 animals were sensitized in the non-UV treated sites and 15 of 20 in the UV treated sites. The severity of response when scored at 24 and 48 h after challenge was similar for both the UVA and non-UVA-treated sites. The control groups were challenged in a similar manner. All sites were negative, except for one low-grade response in one non-UVA exposed site challenged at 2.0% Hydroquinone. Preliminary irritation screening responses of 8 guinea pigs indicated that the 10% Hydroquinone was slightly irritating at 10% but not at 5.0, 1.0 or 0.5%.[94]

The skin sensitization potential of Pyrocatechol for guinea pigs has been investigated[101] (Table 6). Guinea pigs could be sensitized to Pyrocatechol and sensitized animals also reacted to 3-methyl and 3-propyl catechol.

Eye Irritation

A rabbit eye irritation test of an undiluted product formulation containing 2.0% Hydroquinone produced mild conjunctivitis on day 1 in 3 of 6 animals. The conjunctivitis had disappeared by the second day. A score of 1 of 110 was reported.[102]

A dose of 0.1 g of Pyrocatechol was applied to one eye of 6 male albino rabbits. The conjunctiva was moderately erythematous and edematous, moderate exudate and corneal opacity were observed, and the rabbits appeared markedly uncomfortable. At 24 h, severe conjunctivitis, swelling, and ocular discharge, iritis, and dense corneal opacities were observed. The eyes had not improved by 72 h. At day 14, the eyes had keratoconus and pannus formation. Eye irritation was numerically scored at 24, 48, and 72 h; the scores were 103, 85, and 78 (of a maximum possible of 110), respectively.[7]

Inhalation Studies

No deaths were observed when groups of 6 female Harlan-Wistar albino rats were exposed to 1500, 2000, and 2800 mg/m^3 Pyrocatechol-water aerosols for 8 hours containing 13.0, 14.5, and 17.0% Pyrocatechol, respectively. Weight gains were normal over a 14-day observation period and no treatment-related lesions were seen at necropsy at the end of 14 days. At the end of the 14-day observation period, all the rats in the 2800 mg/m^3 group and 2 of 6 in the 2000 mg/m^3 group had blackened and missing toes and tail tips. At these two doses, tremors appeared within 6–7 h of exposure; these continued through the first 24 h after exposure.[7]

Special Studies

Animal Reproduction

Hair dyes containing 0.2% Hydroquinone (and 23 other ingredients) have been tested for teratological and reproductive effects in multigeneration studies with rats.[103,104] The procedures used in these studies have previously been described.[105,106] The results were negative.

Hydroquinone was fed to two groups of 10 female rats in their diets at concentrations of 0.003 and 0.3%. All the rats were fertile, all had litters, and none died. Gestation length, mean litter size, viability, and lactation index were similar to the untreated control group.[107]

Ten Walter Reed-Carworth Farms female rats in their first gestation were mated and then were given a total of 0.5 g of Hydroquinone (total dose) in their feed during pregnancy. Hydroquinone was not toxic for the rats. The rats were killed 22 days after mating, and the uteri were examined. One or more resorptions were observed in 100% of the Hydroquinone-treated rats and 26.8% of all implantations terminated in resorptions. This resorption rate was substantially increased over the controls; of 126 untreated pregnant rats, 40.8% had one or more resorptions and 10.6% of all implantations ended in resorptions.[108]

Hydroquinone applied topically was evaluated in a teratology study in rats. Daily dermal doses of 54, 210, or 810 mg/kg were administered to 20 animals per group from day 6–19. The positive control group received acetylsalicylic acid orally. No remarkable differences were found at necropsy between the test group and the negative controls. In the positive control group, the results were those associated with fetotoxic and teratogenic compounds.[109]

Mutagenesis

Hydroquinone has been studied in the Ames mutagenesis assay both with and without metabolic activation; it was negative using *Salmonella typhimurium* strains TA1537, TA1538, TA98, and TA100. It was positive with one medium and negative with another for the TA1535 strain with metabolic activation[110–115] (Table 7). A concentration of 6 ppm Hydroquinone caused no increase in the frequency of penicillin- and streptomycin-resistant *S. aureus*.[116] Hydroquinone was mutagenic in the *Escherichia coli* DNA polymerase assay as it induced repairable DNA damage.[117] Hydroquinone was mutagenic in the yeast, *Saccharomyces cerevisiae*, and it caused mitotic recombination.[118]

Hydroquinone, at concentrations of 50 and 100 mM, did not induce sex-linked recessive lethal mutations in the F_2 and F_3 generations of *Drosophila melanogaster*.[112]

Hydroquinone, at a concentration of 1.0×10^{-4} M without metabolic activation and at a concentration of 3×10^{-5} M with metabolic activation, was positive in the HeLa DNA synthesis test; this assay identifies agents that cause DNA damage.[119] Hydroquinone, at concentrations of $0.5–2.0 \times 10^{-5}$, did not increase the frequency of Sister Chromatid Exchanges (SCE) in cultured Chinese hamster V79 cells.[120] Hydroquinone, at concentrations of 1.6×10^{-6} to 2.0×10^{-4} M, induced SCE in human lymphocytes; it also delayed cell turnover time slightly.[121] Morimoto et al.[122] reported that the induction of SCE was dependent upon the optimum activation of Hydroquinone in the test system.

TABLE 7. Ames *Salmonella* Mutagenesis Assay

Material tested	*Dose and solvent*	*Results without metabolic activation*[a]					*Results with metabolic activation*[a]					*Comments*	*Reference*
		TA1535	*TA1537*	*TA1538*	*TA98*	*TA100*	*TA1535*	*TA1537*	*TA1538*	*TA98*	*TA100*		
Hydroquinone	Up to 10 mol/ plate	(−)	(−)	(−)	(−)	(−)	(±)	(−)	(−)	(−)	(−)	Used two minimal media; was postive on one medium for TA1535+S−9	112
Hydroquinone		...	...	...	...	(−)	...	...	...	...	(−)		110
Hydroquinone	2 μmol/ plate, ethanol	(−)	(−)	...	(−)	(−)	(−)	(−)	...	(−)	(−)	Spot tests	111
Hydroquinone	0.1–1000 μg/plate	...	...	...	...	(−)	...	...	...	...	...		113
Hydroquinone	50–5000 μg/plate	...	...	...	...	...	...	...	(−)	...	...		114
Pyrocatechol	10 μg to 10 mg/plate	...	...	...	(−)	(−)	...	...	...	(−)	(−)		128
Pyrocatechol	3 μmol/ plate, ethanol	(−)	(−)	...	(−)	(−)	(−)	(−)	...	(−)	(−)	Spot tests	111
Pyrocatechol	0.03–30 μmol/ plate ethanol	...	...	...	(−)	...	...	...	...	(−)	...	Spot tests	111
Pyrocatechol	0.1–1000 μg/plate	...	...	...	...	(−)	...	...	...	...	...		129
Pyrocatechol	0.1–1000 μg/plate	...	...	...	...	(−)	...	...	...	...	...		113
Pyrocatechol	4 μg/plate	...	...	...	...	(+)	...	...	...	...	(+)		130
Pyrocatechol	1–10 μg/ plate	...	(−)	...	(−)	(−)	...	(−)	...	(−)	(−)		115

[a](−) = nonmutagenic; (±) = weakly mutagenic; ... = no data.

Oral doses of 100 mg/kg Hydroquinone to male mice did not inhibit testicular DNA synthesis.[123] Hydroquinone was negative in the mouse sperm-head abnormality test after ip administration of 0.5–2.0 mmol/kg.[120]

Hydroquinone has been described as a "mitotic poison" for the intestine, lymphoid tissue, and thymus of the mouse.[124] Abnormal metaphases were observed in intestinal cells of mice after ip or subcutaneous administration of 0.15–0.175 mg/g Hydroquinone.[125] These were seen in the bone marrow and in intestinal cells of the golden hamster after ip administration of 0.15–0.20 mg/g Hydroquinone.[126] Abnormal metaphases were also observed in vitro in chick fibroblasts (1×10^{-7} M to 1×10^{-6} M Hydroquinone), in rat liver, bone marrow, and corneal cells after ip administration of 0.15–0.20 mg/g Hydroquinone and in rat corneal cells after instillation of one drop of a 5% Hydroquinone solution.[127]

Mice were given two ip injections of up to 110 mg/kg (1.0 mmol/kg) Hydroquinone 24 h apart. Polychromatic erythrocytes were observed in the bone marrow after two injections.[112] Hydroquinone was administered subcutaneously to mice in doses of 20–100 mg/kg daily for 6 days; the number of micronucleated cells was increased in bone marrow at all doses above 20 mg/kg Hydroquinone.[71] Hydroquinone was positive in the mouse bone marrow micronucleus test after ip administration of 0.5–2.0 mmol/kg.[120]

Pyrocatechol has been studied in the Ames mutagenesis assay both with and without metabolic activation; most researchers reported that Pyrocatechol was negative in *Salmonella* strains TA1535, TA1537, TA98, and TA100.[111,113,128,129] One researcher reported that Pyrocatechol was positive in strain TA100 with and without metabolic activation[130] (Table 7). Yoshida and Fukuhara[115] reported that Pyrocatechol was a comutagen for benzo[a]pyrene in the Ames assay, provided that a sufficient amount of the S9 mixture was used dsuring the incubation

Pyrocatechol was negative in the *E. coli* DNA polymerase assay.[131] Pyrocatechol was mutagenic for the yeast, *S. cerevisiae*; it enhanced ultraviolet-induced mitotic crossing-over, aberrant colony formation, and mutation, and it increased ultraviolet-induced gene conversion.[132]

Pyrocatechol, at a concentration of 2.0×10^{-4} M, with metabolic activation was positive in the HeLa DNA synthesis test.[119] Pyrocatechol, at concentrations of $0.5–2.0 \times 10^{-5}$ M, did not increase the frequency of SCE in Chinese hamster V79 cells.[120] Increased numbers of chromatid breaks and exchanges were observed when Chinese hamster ovary cells were exposed to 0.05 mg/ml Pyrocatechol without metabolic activation; metabolic activation decreased the response.[133] Pyrocatechol, at concentrations of 1.6×10^{-6} to 2.0×10^{-4} M, induced SCE in human lymphocytes; it also delayed cell turnover time.[121] Pyrocatechol was negative in the mouse sperm-head abnormality test after ip administration of 0.5–2.0 mmol/kg.[120]

Pyrocatechol was positive in a mouse bone marrow micronucleus test after ip administration of 0.5–2.0 mmol/kg.[120] Pyrocatechol was subcutaneously administered to mice in daily doses of 5–42 mg/kg for 6 days; the frequency of micronucleated cells in the bone marrow did not change.[11]

Carcinogenesis, Cocarcinogenesis, and Tumor Promotion

Hydroquinone is currently in the chronic phase of a bioassay in the NTP carcinogenesis bioassay program. It is being administered to rats and mice by gavage.[134]

Hydroquinone has been tested for carcinogenicity using a cholesterol pellet bladder implantation method. The pellet remained in the bladder for at least a year. Ten-milligram cholesterol pellets containing 20% Hydroquinone were implanted into the bladders of mice; 19 mice survived to 25 weeks, and 6 of the mice had bladder carcinomas (a tumor incidence of 32%). Cholesterol pellets alone were also implanted; 77 mice survived to 25 weeks, 4 mice had adenomas or papillomas, and 5 had carcinomas (a tumor incidence of 12%). The researchers stated that Hydroquinone was positive for carcinogenicity.[135] In respect to the carcinogenic effect in mice upon urinary implantation of cholesterol pellets containing Hydroquinone, the International Agency for Research on Cancer (IARC) stated[17]:

> Experiments involving a possible action of the vehicle or a physical effect of the agent, such as in studies by subcutaneous injection or bladder implantation, are included [in this evaluation], however, the results of such tests require careful consideration, particularly if they are the only ones raising a suspicion of carcinogenicity.

The carcinogenic potential of a hair dye containing 0.2% Hydroquinone (and 23 other ingredients) has been investigated.[136] The procedures used in this testing program have been described previously.[105,106] The results were negative.

Hydroquinone has been tested for tumor-initiating activity. Twenty-four male mice of the S strain received a single application on the clipped back of 0.3 ml of a 6.7% solution of Hydroquinone (20 mg) in acetone as an initiator. Three weeks later, the promoter, croton oil, was painted on the same area of the skin in 18 weekly applications of 0.3 ml of a 0.5% solution in acetone. One week later, 22 survivors were killed and necropsied. One mouse had a tumor; no evidence of tumor-initiating activity of Hydroquinone was observed.[137]

Fifty female Swiss mice were topically treated with 150 μg benzo(a)pyrene (BaP) as an initiator and 14 days later with 0.1 ml of a Hydroquinone solution in acetone (5 mg Hydroquinone) as a tumor promoter; the Hydroquinone solution was applied 3 times a week until the mice had been on test for 409 days. BaP and Hydroquinone were applied to the back. Fifty mice were treated with acetone alone, 100 were untreated, and two groups of mice were treated with BaP and phorbol myristate acetate (PMA) and anthralin as positive controls. Hydroquinone had no tumor-promoting activity.[138]

The cocarcinogenic potential of Hydroquinone has been investigated. Hydroquinone had a weak inhibitory action on BaP tumorigenicity. Hydroquinone, in a dose of 5 mg, was applied with and without 5 μg BaP in a single solution in acetone three times a week for 368 days to the clipped backs of groups of 50 female Swiss mice. The experiment included mice treated with PMA and anthralin with BaP as positive controls for cocarcinogenicity and mice treated with BaP alone. The first papilloma was observed at 254 days in mice treated with Hydroquinone and BaP; 7 mice had papillomas, a total of 11 papillomas were observed, and 3 mice had squamous carcinomas. No papillomas or squamous carcinomas were seen in mice treated with Hydroquinone alone. The first papilloma was observed at 251 days in mice treated with BaP alone; 14 mice had papillomas, a total of 16 papillomas were observed, and 10 mice had squamous carcinomas. In this bioassay Hydroquinone inhibited the tumorigenicity of BaP.[138]

Hydroquinone reduced the number of melanoma transplantations. Groups of 40 BALB/c female mice were given Harding-Passey melanoma transplants. Two groups were administered 16 mg/kg and 80 mg/kg Hydroquinone in saline subcutaneously daily for 9 days starting the next day. One group was untreated and one group was treated with saline. After 140 days, all surviving mice were killed and melanomas were weighed. Only the group given 80 mg/kg Hydroquinone survived to 140 days (comparable to survival of normal female mice). The incidences of successful melanoma transplantation in the combined control groups (untreated and saline treated), the 16 mg/kg Hydroquinone group, and the 80 mg/kg Hydroquinone group were 91.7%, 55.6%, and 23.7%, respectively. However, the weights of the successfully transplanted tumors did not differ among groups.[139]

Pyrocatechol has been tested for carcinogenicity using the bladder implantation method. Ten-milligram cholesterol pellets containing 20% Pyrocatechol were implanted into the bladder of mice; 19 mice survived to 25 weeks, 1 mouse had a papilloma, and 3 developed carcinomas (a tumor incidence of 20%). Cholesterol pellets alone were also implanted; 77 mice survived to 25 weeks, 4 mice had adenomas or papillomas, and 5 had carcinomas of the bladder (a tumor incidence of 12%). Pyrocatechol was not carcinogenic in mice with bladders implanted with cholesterol pellets.[135]

A 75-μg dose of 7,12-dimethylbenz(a)antracene (DMBA) in acetone was topically applied to a group of 30 female Swiss mice as an initiator. After 10 days a 1% Pyrocatechol solution in acetone was topically applied as a promoter, and it was applied 5 times a week for 67 weeks. No tumor-promoting activity of Pyrocatechol was observed.[140]

Fifty female Swiss mice were treated topically with 150 μg BaP as an initiator and 14 days later with 0.1 ml of a Pyrocatechol solution in acetone (2 mg Pyrocatechol) as a promoter; the Pyrocatechol solution was applied 3 times a week until the mice had been on test for 448 days. The initiator and the Pyrocatechol solution were applied to the back. Fifty mice were treated with acetone alone, 100 were untreated, and two groups of mice were treated with BaP and with PMA and anthralin as positive controls. Pyrocatechol was inactive as a tumor promoter.[138]

Pyrocatechol has cocarcinogenic activity. A group of 50 female Swiss mice received topical applications of 2 mg Pyrocatechol together with 5 μg BaP in 0.1 ml acetone 3 times a week for 52 weeks. Twenty-six mice survived to 52 weeks. A group of 50 mice also received BaP alone; 42 mice survived to 52 weeks. The experiment also included a group of untreated control mice and a group of acetone treated mice. Eighty-six skin papillomas were found in 35 mice receiving Pyrocatechol and BaP; 31 mice had squamous cell carcinomas. Fourteen papillomas occurred in 13 mice receiving BaP alone; 10 squamous carcinomas of the skin were observed. No skin tumors were observed in the untreated or acetone-treated mice. Pyrocatechol was active as a cocarcinogen and enhanced the carcinogenicity of BaP.[141]

Pyrocatechol, in a dose of 2 mg, was applied with and without BaP in a single solution in acetone three times a week for 368 days to the clipped backs of groups of 50 female Swiss mice. The experiment included mice treated with PMA and anthralin with BaP as positive controls for cocarcinogenicity and mice treated with BaP alone. The first papilloma was observed at 299 days in mice

treated with Pyrocatechol and BaP; 36 mice had papillomas, a total of 90 papillomas were observed, and 31 mice had squamous carcinomas. Without BaP, 1 mouse treated with Pyrocatechol had a papilloma at 292 days and 1 mouse had a squamous carcinoma. The first papilloma was observed at 251 days in mice treated with BaP alone; 14 mice had papillomas, a total of 16 papillomas were observed, and 10 mice had squamous carcinomas. Pyrocatechol was cocarcinogenic.[138].

Mice were given Sarcoma-180 cells by subcutaneous injection. Twenty-four hours later and every other day for a total of 7 treatments, the mice were injected in the same place with 0.2 ml of a 5 mg/ml solution of Pyrocatechol. Pyrocatechol inhibited tumor growth.[142]

CLINICAL ASSESSMENT OF SAFETY

General Effects

A variety of tests and clinical observations have been reported. The results are given in Tables 8 and 9 and summarized in the following text.

Patch tests on the back with 1% Hydroquinone in petrolatum were conducted during 1970–1976 on 53 denture-wearing subjects with burning mouth syndrome. The reactions were scored at 48 and 72 h. Only 1 subject had a positive reaction; erythema and infiltration without papules or vesicles were observed.[146]

In Salvador, Brazil, 536 dermatological patients were patch-tested with occlusive patches containing 5% Hydroquinone in aqueous solution. The reactions were evaluated on removal of the patches at 48 h and at 96 h. Positive reactions were observed in 8.9% of the patients; Hydroquinone was a sensitizer in patients with dermatitis.[147]

Five blacks and 3 whites with freckles applied 10 and 30% Hydroquinone in petrolatum on their backs in a continuous patch test for 1 month. Two blacks developed depigmentation; more depigmentation was produced by the 30% than by the 10% Hydroquinone. Two blacks developed dermatitis, and one of them had secondary pigmentation. In the other patients, no skin changes were observed.[42]

Two and five percent Hydroquinone bleaching creams were used on the hyperpigmented skin of 56 black and white patients; the patients were also instructed to use a sunscreen. Both Hydroquinone concentrations were moderately effective in depigmenting the skin of 44 of the 56 patients. Erythema and tingling at the site of application was observed by 32% of the patients using the 5% Hydroquinone bleaching cream; 8% of those using the 2% Hydroquinone bleaching cream had mild reactions. In 1 patient, a questionable leukoderma developed; in another patient there was a possibility of sensitization.[41].

A primary skin irritation of a cosmetic formulation containing 2% Hydroquinone gave an average irritation index of 0.89 for 19 subjects (scale 0–4).[144] The same cosmetic formulation was used in a repeat insult patch test conducted on 90 subjects. The undiluted formulations (0.1 ml) was applied three times per week, occluded for 24 h after each application with Werbil patches, for 3 consecutive weeks. Following a 4-week nontreatment period, the subjects were

TABLE 8. Human Irritation and Sensitization

Ingredient	*Concentration (%)*	*Vehicle*	*Test method*	*Population type*	*No. of subjects*	*Results*	*Reference*
Hydroquinone	1, 2.5, 3.5, 5, and 7	Lotions, creams, ointments	48-h closed patch	Dark-skinned subjects	840	Classified as sensitizer and mild primary irritant; "adverse effects at 3% were negligible"	143
Hydroquinone	5, 6, and 7	Soft paraffin	Open patch, applied at 0 and 24 h	Indians (random selection)	200	6/200 mild erythema at 5.0% at 72 h	143
Hydroquinone	2	Cosmetic formulation	Primary skin irritation	Random	19	PII = 0.89 of 4.0 max	144
Hydroquinone	2	Cosmetic formulation (same as Ref 144)	Repeat insult patch test	Random	90	69/90 showed one or more mild reactions during induction phase 22/90 had a mild reaction on challenge	145

TABLE 9. Summary of Clinical Patient Data

Ingredient	*Concentration (%)*	*Vehicle*	*Test method*	*Population type*	*No. of subjects*	*Results*	*Reference*
Hydroquinone	1	Petrolatum	24-h occluded patch	Denture wearers with burning mouth syndrome	53	1/53 positive reactions erythema and infiltration without papules	146
Hydroquinone	2, 3, and 5	Ointment	Clinical treatment	White males with freckles and lentigines; normal black males	94 white 43 black	Depigmentation at all concentrations; amount increased with concentration; many patients had transient inflammatory reactions before depigmentation started 0/60 sensitized at 2% 0/39 sensitized at 3% 3/39 sensitized at 5%	55
Hydroquinone	2 and 5	Bleaching cream	Used on hyperpigmented skin with sunscreen	Black and white patients	56	44/56 depigmented at both concentrations 5/56 had erythema at 2% 18/56 had erythema at 5%	41
Hydroquinone	5	Ointment with tretinoin and dexamethasone	Clinical treatment	Selected black patients	100	Irritation first month of treatment; all subjects were depigmented; irradiated depigmented sites became hyperpigmented	56
Hydroquinone	5	Ointment with tretinoin and dexamethasone	Clinical treatment	19 patients with melasma; 25 with acne; 11 with pseudofolliculitis; 11 with senile lentigines	66 total	Positive medical benefits for all patients; no contact sensitization or photosensitization was observed	57
Hydroquinone	5	Aqueous	48-h occluded patch test	Dermatological patients	536	48/536 were positive for sensitization	147
Hydroquinone	10 and 30	Petrolatum	Continuous patch test for one month	5 black patients, 3 white	8	2/5 black patients developed depigmentation at 10%; 2/5 blacks developed irritation	42

challenged under conditions similar to the induction period. Sixty-nine of the 90 subjects had one or more mild irritant reactions during the induction patch phase, and 22 subjects reacted to challenge. Of the challenge reactions noted, none were greater than mild, and only three had the same severity of response at both the 24 and 48 h scorings (one mild, the other two barely perceptible). Within the experimental limits the investigator concluded that "this cosmetic product did not have any potential for inducing allergic sensitization."[145]

The freckles and lentigines of 94 white men and the normal skin of 43 black men were treated with 2, 3, and 5% Hydroquinone ointments. More depigmentation was observed with the 3 and 5% Hydroquinone ointments than with the 2% ointment. No depigmentation was observed in the darker black skins. In many patients, transient inflammatory reactions that lasted for about a week were observed before depigmentation; a few inflammatory reactions were not followed by depigmentation. No patients treated with the 2% (60 patients) or 3% (39 patients) Hydroquinone ointments became sensitized. Two of 38 patients treated with the 5% ointment became sensitized to Hydroquinone. The use of a sunscreen prevented repigmentation.[55]

Kligman and Willis[56] applied a 5% Hydroquinine cream (that also contained 0.1% tretinoin and 0.1% dexamethasone) two times a day to the skin of more than 100 black subjects. Irritation was observed only during the first month of treatment. Depigmentation occurred, and after the Hydroquinone treatment ceased, repigmentation began. Irradiated depigmented sites became hyperpigmented. Darker individuals were more susceptible to the depigmenting effect. The Hydroquinone cream was effective in treating melasma, postinflammatory hyperpigmentation, and ephelides and was not effective in treating senile lentigines. Two blacks with vitiligo were treated with the Hydroquinone cream. The unaffected areas were depigmented; the treatment was successful. A 10% Hydroquinone cream was more irritating and depigmentation was more rapid; a 2% cream was less irritating and less potent for depigmentation.

A 5% Hydroquinone cream (contained 0.1% tretinoin and 0.1% dexamethasone) was used to treat 19 women with melasma; 18 achieved satisfactory lightening. The cream was also used to treat postinflammatory hyperpigmentation in blacks; almost all of 25 acne patients and 8 of 11 men with pseudofolliculitis had satisfactory lightening. With the use of the Hydroquinone cream, mild to moderate irritation was observed. No contact sensitization or photosensitization was observed. A sunscreen was necessary to prevent repigmentation.[57]

In blacks, facial ochronosis and pigmented colloid milium were observed after the chronic oversaturation of the skin with creams containing Hydroquinone and exposure to sunlight.[39,58]

Open and closed patch tests with Hydroquinone were performed on dark-skinned subjects. The open patch test was conducted with 5, 6, and 7% Hydroquinone in soft paraffin on 200 Indians. The application sites were examined at 24 h, the Hydroquinone was reapplied, and the sites were examined again at 72 h. Six positive reactions (mild erythema) were observed at 72 h in patients given 5% treatment. Closed 48-h patch tests were also conducted with 1, 2.5, 3.5, 5, and 7% Hydroquinone in lotions, creams, or ointments on 840 dark-skinned subjects; positive reactions were observed, and their frequency varied with the Hydroquinone concentration and with the formulation. Hydroquinone was a

mild primary irritant and a sensitizer. The investigators stated that 3% Hydroquinone produced negligible adverse effects.[143]

Some investigators have stated that Hydroquinone caused inflammatory reactions followed by depigmentation. However, others[148] found that when an inflammatory reaction followed the use of Hydroquinone creams to lighten black skin, the invariable result was hyperpigmentation.

Occupational depigmentation from Hydroquinone followed its use by photographic developers. Other industrial exposures to Hydroquinone have not caused depigmentation.[149] In a review of Hydroquinone uses and abnormal reactions, it was stated that adverse reactions to the use of this ingredient as a bleaching cream are rare and that Hydroquinone may cross-react with resorcinol and Pyrocatechol.

Ocular lesions have been observed in workers involved in the manufacture of Hydroquinone and exposed to Hydroquinone dust and quinone vapors. Conjunctival and corneal staining and precipitation of pigment were observed, usually in persons employed for at least 5 years. In severe cases, vision was reduced. No other systemic effects were noted. Quinone may be the major factor in causing ocular injury, but the Hydroquinone dust may play a contributing role.[150,151] Corneal changes, particularly alteration of curvature, was observed long after exposure to Hydroquinone had ceased and the ocular stain and pigment had disappeared.[152] No studies document serious ocular injury caused by airborne Hydroquinone in the absence of quinone vapor.[26]

No systemic effects have been observed after occupational exposure to 2 mg Hydroquinone dust/m^3.[153] The TLV for Hydroquinone is 2 mg/m^3 and the Short-Term Exposure Limit (STEL) is 4 mg/m^3.[153,154] NIOSH[26] has recommended a 15-minute ceiling of 2 mg/m^3.

Upon contact with skin, Pyrocatechol may cause an eczematous dermatitis. Absorption through the skin has resulted in symptoms resembling those induced by phenol except that convulsions were more marked.[62]

Thirteen workers were exposed to Pyrocatechol and phenol vapors for 2 years. Most of the workers complained of respiratory problems. Nine of 12 had chronic inflammation of the upper respiratory tract. No other systemic effects were observed.[74]

The TLV for Pyrocatechol set by the ACGIH[153,154] is 5 ppm.

SUMMARY

Hydroquinone and Pyrocatechol are two benzenediol isomers, 1,4-benzenediol and 1,2-benzenediol. Both ingredients are used in cosmetics as couplers in oxidative hair dyes at concentrations of less than 1.0%. Hydroquinone, a known skin-depigmenting agent, is also used in cleansing preparations at concentrations between 1 and 5%.

Both Hydroquinone and Pyrocatechol inhibit bacterial growth. Prior to 1960 Pyrocatechol was used for its antibacterial properties in some drugs.

Both compounds are absorbed from the gastrointestinal tract, and Pyrocatechol is also readily absorbed through the skin. Small amounts of nonmetabolized

Hydroquinone are excreted in the urine of rabbits; however, most of the compound is excreted as Hydroquinone ethereal monosulfate and as the monoglucuronide. In rabbits, Pyrocatechol is excreted mainly as the ethereal sulfate and monoglucuronide. The biological half-life of Pyrocatechol in humans is between 3 and 7 h; excretion is predominantly in the urine.

The results of acute oral studies in animals indicate that Hydroquinone is practically nontoxic to moderately toxic; the data from subchronic feeding studies of Hydroquinone indicated that it was not toxic at 1%, slightly toxic at 2% and toxic at 5%. Pyrocatechol was moderately toxic in acute studies. In subchronic feeding studies, Pyrocatechol at a dietary concentration of 0.25% produced hepatic cell hyperplasia in rats, but survival was not affected at 1.0%. The results of a 20-week study in mice were a loss of weight and blood effects when 4.0 g/L Pyrocatechol was included in the drinking water. There were no adverse effects at 1.0 g/L.

No adverse local systemic effects were produced in rabbits when 2.0% Hydroquinone was applied to intact and abraded skin (3.9–9.4 ml/kg). The results of subchronic and chronic dermal studies of Hydroquinone in animals for time intervals up to 6 months indicated that the ingredient was a weak depigmenter at 1.0%. Other animals studies indicated that the time required for depigmentation was dependent upon both the concentration and the dispersion medium used. When 2.0% Hydroquinone was tested in rabbits using a single-insult patch test, a PII of 1.22 (scale 0–4) was reported. Guinea pigs were sensitized to Hydroquinone when injected at concentrations above 2.0%. The severity of the sensitivity reaction induced at 10% Hydroquinone was not increased when exposed to UVA light. The acute dermal LD_{50} of Pyrocatechol was 0.8 g/kg. Pyrocatechol did not depigment rabbit skin at 1.0% but did at 3.0%; skin irritation was observed at 5.0%. Undiluted Pyrocatechol was a primary irritant in rabbits. Guinea pigs were sensitized when Pyrocatechol was injected at concentrations above 0.2 μmol.

In a rabbit eye irritation test, an undiluted product formulation containing 2.0% Hydroquinone produced mild conjunctivitis in 3 of 6 animals evaluated at 24 h. The conjunctivitis had subsided on the second day. Undiluted Pyrocatechol was a severe ocular irritant in rabbits at doses of 0.1 g.

When Hydroquinone (0.003–0.3%) was included in the diet of two groups of 10 pregnant female rats, no differences were found between the test and control groups relative to gestation length, mean litter size, viability, and lactation index. In a second study 0.5 g of Hydroquinone included in the diets of a group of 10 mated female rats produced no significant difference in resorptions when compared to control groups. Hydroquinone was evaluated in a teratology study in which daily dermal exposure of pregnant rats (20 animals/group) was up to 810 mg/kg; no remarkable difference was found between the control and test groups.

The results of mutagenesis assays of Hydroquinone have varied with the assay system used. In four *S. typhimurium* strains, both with and without activation, the mutagenesis assay was negative. One strain tested was positive, with activation using one medium, but not with a second medium. Hydroquinone did not increase antibiotic resistance in *S. aureus*. Hydroquinone was mutagenic in the *E. coli* DNA polymerase and *S. cerevisiae* mitotic recombination assays. Hydro-

quinone produced positive results both with and without activation in the HeLa DNA synthesis test but was not considered mutagenic in assays using Chinese hamster cells. Hydroquinone induced SCE and delayed cell turnover time in human lymphocyte studies. Oral doses of Hydroquinone did not inhibit testicular DNA synthesis in male mice and was nonmutagenic in the mouse sperm-head abnormality test. Hydroquinone is considered a mitotic poison.

In multigeneration rat studies of topically applied hair dyes containing 0.2% Hydroquinone, no effect on reproduction was observed and embryotoxicity and teratogenesis were not produced. The F_{1A} animals were used for carcinogenic assay of the hair dyes. The results were negative. Hydroquinone, when applied topically, was neither a tumor promoter nor a cocarcinogen in Swiss mice. Harding-Passey melanoma transplants were decreased when Hydroquinone was administered after implantation.

Like Hydroquinone, the mutagenicity of Pyrocatechol varies with the test system used. In most studies, Pyrocatechol was nonmutagenic with and without metabolic activation in the Ames assay. However, positive results have been reported for one test strain. The compound was negative in the *E. coli* DNA polymerase assay but was positive in the yeast, *S. cerevisiae*. Pyrocatechol was negative in the HeLa DNA synthesis test and with Chinese hamster V79 cells. The compound increased the numbers of chromatid breaks and exchanges in Chinese hamster ovary cells and induced SCE and delayed cell turnover time in human lymphocyte cultures. The compound given by ip injection to mice was negative in the sperm-head abnormality test but was positive in the bone marrow assay.

In three separate studies in mice, topically applied Pyrocatechol was not a tumor promotor. Topically applied Pyrocatechol was a cocarcinogen for mouse skin in two separate studies.

Hydroquinone studies in humans at doses of 500 mg and 300 mg to males and females, respectively, for 5 months produced no signs of toxicity.

Positive sensitization reactions to Hydroquinone were reported in 8.9% of 536 dermatologic patients challenged with a 5.0% solution. At higher concentrations (10 and 30%) dermatitis was produced in 2 of 5 black subjects. A cosmetic formulation containing 2% Hydroquinone produced one or more mild irritation reactions in 69 of 90 subjects in the induction phase of a sensitization test. In this latter study 22 subjects had a mild reaction when challenged by the same formulation and scored at 24 h. Only 3 of the 22 subjects had either mild or barely perceptible reactions at 48 h. The use of ointments containing 2, 3, and 5% Hydroquinone in 94 white and 43 black men with normal skin produced at least minimal depigmentation in white but not black subjects. Two of 38 patients treated with an ointment containing 5.4% Hydroquinone became sensitized. Other studies on dark-skinned subjects have confirmed these sensitization results.

Ocular lesions but no other systemic effects have been found in workers involved in the manufacture of Hydroquinone. Occupational exposure to Pyrocatechol has been associated with dermatitis and chronic inflammation of the upper respiratory tract. Recommended limits for occupational exposure of Hydroquinone and Pyrocatechol have been set at 2 and 5 ppm, respectively.

DISCUSSION

During the review of these two ingredients, members of the Expert Panel were aware that the Food and Drug Administration had reviewed studies relating to the safety of Hydroquinone and had concluded that Hydroquinone was safe and effective as an agent to bleach or lighten the skin at concentrations between 1.5 and 2.0% in OTC drugs. The CIR Expert Panel has not evaluated the safety of Hydroquinone to lighten the skin, which is regarded by FDA as a drug use of this ingredient. Hydroquinone is a weak depigmenter at 1.0%. Following prolonged exposure it is a skin irritant and a sensitizer in some individuals. Pyrocatechol has toxicological characteristics similar to those of Hydroquinone. The available eye irritation data indicated that Hydroquinone is a mild ocular irritant at low concentrations. Pyrocatechol is extremely irritating to the eye at high concentrations. The Panel noted that the major use of these two ingredients is in rinse-off-type products for intermittent application of relatively short exposure times, at concentrations of 1.0% and less. The Panel considered these uses of Hydroquinone and Pyrocatechol to be safe, provided the formulations containing Pyrocatechol do not come into contact with the eye.

CONCLUSION

The CIR Expert Panel concludes that Hydroquinone and Pyrocatechol are safe for cosmetic use at concentrations of 1.0% and less in formulations designed for discontinuous, brief use followed by rinsing from the skin and hair.

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