JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 4, Number 5, 1985
Mary Ann Liebert, Inc., Publishers

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Final Report on the Safety Assessment of p-Hydroxyanisole

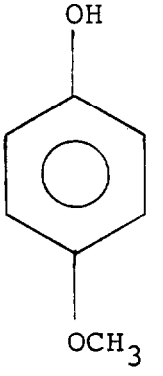
p-Hydroxyanisole is used as an antioxidant in cosmetic products at concentrations of up to 1.0 percent. The acute oral LD_{50} of p-Hydroxyanisole in rats was estimated as 1630 mg/kg. Undiluted p-Hydroxyanisole is a severe skin and ocular irritant in rabbits but produced minimal eye irritation at 0.1 percent and minimal rabbit skin irritation at 5 percent. Skin sensitization to p-Hydroxyanisole occurred when guinea pigs were treated at 0.5 M. p-Hydroxyanisole is a skin-depigmenting agent at concentrations approximating those used in cosmetic products. p-Hydroxyanisole was nonmutagenic in the Ames assay. No local toxic changes or tumors were observed following long-term application of 5 and 10 percent p-Hydroxyanisole. The antioxidant was inactive as a tumor promoter. Solutions of p-Hydroxyanisole produced embryotoxicity but not teratogenicity.

The function of p-Hydroxyanisole in cosmetics is that of an antioxidant; it is not intended for use as a skin lightener or skin-depigmenting agent. Because of the depigmenting action of p-Hydroxyanisole in black guinea pigs at reported concentrations approaching those used in cosmetics, it is concluded that p-Hydroxyanisole is unsafe for use as a cosmetic ingredient.

CHEMISTRY

Definition and Structure

p-Hydroxyanisole (CAS No. 150-76-5) is the substituted phenolic compound with the formula:[1]



p-Hydroxyanisole is also known as Antioxidant 221, 4-hydroxyanisole, p-methoxyphenol, 4-methoxyphenol, and hydroquinone monomethyl ether.[1-4]

Method of Manufacture and Impurities

p-Hydroxyanisole can be made by reaction of hydroquinone with dimethyl ether over a mixture of silica and alumina at 250 to 300°C.[5] The compound is also produced commercially by the methylation of hydroquinone with dimethyl sulfate.[6]

p-Hydroxyanisole used for cosmetic purposes typically has a purity of 99.5 percent. Impurities include an "unidentified compound with a high boiling point" (approximately 0.4 percent) and hydroquinone dimethyl ether (about 0.1 percent). Hydroquinone normally is not detected.[5]

Properties

p-Hydroxyanisole is a white, waxy solid that has an odor of caramel and phenol.[2,4,6] During storage, the compound is quite stable.[6] It is soluble in water, aqueous ethanol, acetone, ether, ethylacetate, and benzene.[2,3] As indicated by its ionization constant (pk) of 10.25, p-Hydroxyanisole has acidic properties characteristic of phenols.[7] Peak absorbance of ultraviolet light occurs at approximately 340 nm.[8] Additional chemical and physical data for p-Hydroxyanisole are presented in Table 1.

p-Hydroxyanisole readily forms hydrogen bonds with itself and with water. Sublimation pressure studies of this antioxidant indicate a high energy of molecular association (6.2 cal/mol), which suggests that the methoxy groups are hydrogen bonded with the hydroxyl groups of adjacent molecules.[14] Because the phenolic moiety of p-Hydroxyanisole also forms hydrogen bonds with water molecules, the acidic properties of this compound are more variable in aqueous systems than such nonaqueous solvents as benzene. The acidic reactivity of p-Hydroxyanisole increases with temperature as a result of the dissociation of these hydrogen bonds.[15] Strong hydrogen bonds are also formed between p-Hydroxyanisole and such proteins as bovine serum albumin and mitochondrial proteins from yeast cells and rat liver. The binding with these proteins is nonspecific.[16,17]

p-Hydroxyanisole is readily oxidized. For example, it is converted by bromine in water to a quinone. This reaction apparently involves the nonionized form of the compound.[18] p-Hydroxyanisole may also undergo a variety of other reactions, including alkylation, halogenation, and other substitutions on the aromatic nucleus. These reactions may occur without loss of the ether group.[9]

Analytical Methods

Analytical methods for the determination of p-Hydroxyanisole include gas and thin-layer chromatography.[5,10,12,19-25] Other reported analytical methods for the determination of p-Hydroxyanisole include gel permeation chromatography,[26,27] a polarographic procedure,[28] and a quantitative oxidimetric procedure.[29]

TABLE 1. Chemical and Physical Data for p-Hydroxyanisole

		References
Appearance	White flakes	9
Formula	$CH_3OC_6H_4OH$	2, 4
Molecular weight	124	4, 6
Boiling point	234°C	2, 3, 6, 10
	243°C	11
	246°C	4
Refractive index	1.5370	10
Melting point	52.5 to 53°C	2, 4, 6, 11, 12
pK (ionization constant)	10.25 at 20°C	7
Heat of vaporization	13.9 Kcal/mole	13
Density	1.413 at 60°C	13
Specific gravity	1.55 at 20/20°C	2, 4
Solidification point	54.0°C	9
Flash point (Cleveland open cup)	132°C (270°F)	9
Fire point	135°C (275°F)	9
Autoignition temperature	421°C (790°F)	9
UV absorbance	340 nm (peak)	8
Solubility at 25°C (g/100 g solvent):		9
Water	4	
10 percent NaOH	> 50	
Acetone	426	
Ethyl alcohol	456	
Ethyl acetate	245	
Benzene	70	
Animal oil (lard)	> 50	
Vegetable oil (cottonseed)	> 50	
Hexane	< 1	

NONCOSMETIC USE

p-Hydroxyanisole has a number of noncosmetic applications, including use as an antioxidant, as a polymerization inhibitor, as a chemical intermediate, and as a stabilizer. It is also used to inhibit the effects of ultraviolet light on the skin.[2,6,9,11]

As an antioxidant, p-Hydroxyanisole is used in concentrations of 0.001 to 0.01 percent to inhibit the development of acidity and discoloration in chlorinated hydrocarbons, aldehydes (such as crotonaldehyde and furfural), and oils of turpentine. It is used also as an antioxidant in synthetic lattices and to inhibit peroxide formation in ethers.[2,9]

As a polymerization inhibitor, p-Hydroxyanisole is employed in combination with hydroquinone or methylhydroquinone to reduce gel time drift in unsaturated polyester resins. The antioxidant is used also to inhibit the polymerization of vinylidene chloride, acrylonitrile, acrylic and methacrylic esters, and various vinyl monomers.[2,9,11]

As a synthetic intermediate, p-Hydroxyanisole is used in the manufacture of dyes, pharmaceuticals, plasticizers, and stabilizers. Butylated hydroxyanisole, which is a food grade antioxidant, is prepared by the alkylation of p-Hydroxyanisole.[2,6,9]

As a stabilizer, p-Hydroxyanisole is used in concentrations of 0.05 to 0.3 percent to inhibit thermal degradation of polyether polyols. The antioxidant can be added at concentrations of 0.5 to 3.0 percent to ethylcellulose in order to maintain the latter compound's viscosity, flexibility, and color. Degradation and formation of aldehydes is retarded by the addition of p-Hydroxyanisole to polyoxyalkylenes. p-Hydroxyanisole is also used as as stabilizer for chlorinated hydrocarbons, textile lubricating oils, and liquid detergent colors.[2,6,9,11]

COSMETIC USE

p-Hydroxyanisole is used in cosmetics as an antioxidant.[5] Data submitted to the Food and Drug Administration (FDA) in 1981 by cosmetic firms participating in the voluntary cosmetic registration program indicated that p-Hydroxyanisole was used that year as an ingredient in 31 cosmetic products (Table 2). Product types in which p-Hydroxyanisole was most frequently used included sachets, makeup bases, and skin care preparations. Cosmetic formulations contained this antioxidant at concentrations of >0.1 to 1.0 percent (8 products) and ≤ 0.1 percent (23 products).[30,31]

Voluntary filing of product formulation data with the FDA by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21 Part 720.4 of the Code of Federal Regulations.[32] Because data are only submitted within the framework of preset concentration ranges, opportunity exists 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 error in the assumed ingredient concentration.

Cosmetic products containing p-Hydroxyanisole are applied to or have the potential to come in contact with skin and eyes. These products may be used from once a week to several times a day. Many of these products may be expected to remain in contact with the skin for as briefly as a few hours to as long as a few days. Each cosmetic product formulated with p-Hydroxyanisole has the potential for repeated application over the course of several years.

BIOLOGY

Skin Depigmentation

Depigmentation has been observed in guinea pigs and mice following application of p-Hydroxyanisole to the skin. The depigmenting action of this antioxidant has been typically observed at concentrations of 3 to 30 percent. However, Pathak[33] in an unpublished study reported skin depigmentation with this com-

TABLE 2. Product Formulation Data for p-Hydroxyanisole[30,31]

Product Category	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (percent)	
			>0.1–1	≤0.1
Eye shadow	2582	1	–	1
Other eye makeup preparations	230	2	–	2
Colognes and toilet waters	1120	2	1	1
Perfumes	657	1	–	1
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	1	–	1
Sachets	119	7	–	7
Makeup foundations	740	1	–	1
Lipstick	3319	2	–	2
Makeup bases	831	5	–	5
Rouges	211	1	–	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	2	1	1
Face, body and hand skin care preparations (excluding shaving preparations)	832	2	2	–
Moisturizing skin care preparations	747	1	1	–
Night skin care preparations	219	1	1	–
Skin lighteners	44	1	1	–
Suntan gels, creams, and liquids	164	1	1	–
1981 TOTALS		31	8	23

pound at a concentration as low as 0.25 percent (Table 3). The onset of depigmentation at the site of application varies according to concentration, duration of exposure, vehicle, and other test conditions. Results of a number of studies are summarized below.

The subchronic and chronic effects of p-Hydroxyanisole on guinea pig skin were examined in an unpublished study by Pathak.[33] For the subchronic exposure, an unspecifed cream vehicle containing either 0, 0.1, 0.25, 0.5, or 1.0 percent p-Hydroxyanisole was applied daily for 6 weeks (42 days) to the epilated skin of the back of 30 black guinea pigs. The treated and vehicle control groups consisted of 6 animals each. Guinea pigs exposed to 0.25, 0.5, and 1.0 percent p-Hydroxyanisole developed hypopigmentation or depigmentation of the skin and hair at the site of treatment. Only 1 guinea pig of the 0.1 percent group developed depigmentation at the treated site. The skin and hair of all animals of the vehicle control group (0.0 percent p-Hydroxyanisole) had no color change. For the chronic exposure, an unspecified vehicle containing 0.0, 0.5, or 1.0 percent p-Hydroxyanisole was applied daily for 6 months to the ear and epilated backs of

TABLE 3. Skin Depigmentation to p-Hydroxyanisole

Animal	*Duration of p-Hydroxyanisole Exposure*	*p-Hydroxyanisole Concentration at Which Skin Depigmentation Was Observed*	*Reference*
Black guinea pig	6 weeks	0.1,* 0.25, 0.5, 1.0% in unspecified cream	33
Black guinea pig	6 months	0.5 and 1.0% in unspecified cream	33
Guinea pig	Not reported	3%	34
Black guinea pig	1 to 6 months	0.1, 0.25, 0.5 and 1.0 M in acetone, dimethylsulfoxide, or hydrophilic ointment	35
Black guinea pig	18 days	5 to 20% in petroleum jelly	36–38
Black guinea pig	1 to 8 weeks	20% in lanolin	39
Black guinea pig	13 days	20% in dimethylsulfoxide	40
Black guinea pig	7 to 46 days	20% in lanolin	41
Black guinea pig	4 weeks	20% in petroleum jelly	42
BLA mice	4 weeks	20% in petroleum jelly	42
Black guinea pig	30 days	20 and 30% in dimethylsulfoxide	43

*Following exposure to a p-Hydroxyanisole concentration of 0.1 percent, 1 of 6 guinea pigs developed skin depigmentation; the remaining 5 animals had no change in skin or hair color at the site of application.

3 groups of black guinea pigs (12 animals per group). The skin of both treatment groups (0.5 and 1.0 percent) appeared hypomelanotic and amelanotic after 4 months. Animals exposed to 1.0 percent p-Hydroxyanisole developed moderate to severe skin and hair depigmentation at the site of application. Guinea pigs exposed to 0.5 percent of the antioxidant developed either skin depigmentation or skin hypopigmentation. The skin of the vehicle control group (0.0 percent p-Hydroxyanisole) appeared normal.

Pathak et al.[34] examined the depigmenting activity of p-Hydroxyanisole on the epilated skin of the backs and ears of guinea pigs. A 3 percent concentration of the compound "produced marked depigmentation" and had a "selective cytotoxic effect" on melanocytes. Further details have not been published.

Total depigmentation of the back, ear, and nipple was observed in black guinea pigs treated topically with 0.1, 0.25, 0.5, and 1.0 M p-Hydroxyanisole. The antioxidant in either acetone, dimethyl sulfoxide, or hydrophilic ointment was applied in a 0.1 ml dose each weekday for 1 to 6 months. The number of days for complete depigmentation varied with vehicle and p-Hydroxyanisole concentration. No depigmentation was noted on areas distant from the treated site. The histological changes found at the treated sites included reduction or absence of melanin, acanthosis, and an increase in mononuclear–histiocytic cells.[35]

Twenty percent p-Hydroxyanisole was applied topically once daily to the back of the ear of black guinea pigs for periods ranging from 1 to 8 weeks. Depigmentation was observed after 5 to 10 days of treatment. After 5 to 6 weeks of treatment, large areas were completely depigmented. None of the 34 treated animals developed depigmentation extending beyond the area of application. Following the cessation of treatment, depigmented regions slowly became repigmented. Areas of depigmentation persisted for as long as 6 months.[39]

Twenty percent p-Hydroxyanisole in dimethylsulfoxide was applied to the epilated skin of the ear and mammilla of 10 black guinea pigs. Applications were made once daily for 13 days. Depigmentation of the treated areas was observed in 4 animals on the fifth day of antioxidant exposure. Varying degrees of depigmentation and whitening of black hairs was observed in all animals 25 days after the initial exposure. Repigmentation of the treated skin began approximately 1 month after termination of treatment. Microscopic changes in the treated skin included hyperkeratosis, acanthosis, a decrease in the number of DOPA-active melanocytes, and a transposition of melanin granules from the epidermis into the dermis. The amount of melanin granules in the epidermis decreased progressively as the duration of treatment increased.[40]

Riley and Seal[41] applied 20 percent p-Hydroxyanisole in lanolin to the backs of the ears of black guinea pigs. Applications were made daily, 5 days a week for periods ranging from 5 days to 46 days. Skin depigmentation and formation of keratinocyte pseudopods (microinvasion) were observed after 7 days of treatment. Similar treatment of guinea pig ears with 1 percent of the antioxidant in lanolin produced no skin depigmentation or pseudopodial extensions of the basal cell cytoplasm.

The skin-depigmenting property of p-Hydroxyanisole was assessed in black guinea pigs and BLA mice. A single application of 10 percent p-Hydroxyanisole in petroleum jelly was made daily to the ear of each of 5 guinea pigs for 4 weeks; 20 percent p-Hydroxyanisole in petroleum jelly was applied in a similar fashion to a second group of 5 guinea pigs. A 10 percent concentration of the antioxidant in petroleum jelly was applied to the neck of 10 mice daily for 8 weeks, whereas 20 percent p-Hydroxyanisole in petroleum jelly was applied to the neck of 10 mice daily for 4 weeks. Skin irritation (acanthosis) and skin depigmentation were observed in both species with 20 percent p-Hydroxyanisole; 10 percent of the antioxidant produced no observable skin effects.[42]

Twenty and thirty percent p-Hydroxyanisole in dimethylsulfoxide was applied 6 times a week for 30 days to the skin of the right ear and right mammilla of black guinea pigs. The skin of the left ear and left mammilla served as untreated control. After 2 to 4 days of antioxidant exposure, a decrease in DOPA-positive cells of the dermoepidermal junction was observed. A reduction of melanin granules in the epidermis and an increase in acid phosphatase activity in early melanosomes were also noted. Skin depigmentation was visible in treated areas after 5 days. A progressive decrease in melanocytes was observed throughout the course of the study.[43]

Riley[39] and Dumishev[40] reported that leukoderma induced by p-Hydroxyanisole in experimental animals resembles vitiligo in man with respect to the following: (1) loss of skin pigmentation long after termination of treatment with the antioxidant, (2) a tendency for hair follicles to depigment last, (3) repigmentation is initially perifollicular, (4) DOPA-positive epidermal melanocytes are reduced in or absent from the depigmented sections, (5) the degree of depigmentation is inversely proportional to the amount of melanin in the epidermis, (6) grafted pigmented skin remains pigmented in the area of leukoderma, whereas skin depigmented by p-Hydroxyanisole grafted in a normally pigmented zone is slowly repigmented from the periphery, and (7) adenosine triphosphatase-positive basal and dendritic cells are unchanged, whereas the suprabasal cells are damaged in the depigmented zone.

Proposed Mechanism for Skin Depigmentation

The mechanism by which p-Hydroxyanisole causes skin depigmentation in guinea pigs appears to be a result of the preferential destruction of melanocytes. In vitro studies suggest that the melanocytotoxicity of p-Hydroxyanisole is related to its structural similarity to the amino acid tyrosine. As a tyrosine analog, p-Hydroxyanisole is oxidized by tyrosinase and gives rise to cytotoxic, free radical oxidation products. It is hypothesized that these cytotoxic oxidation products damage the cellular membranes of the guinea pig melanocyte by initiating lipid peroxidation.[39,44-47]

Pathak[33] proposed that the cytotoxic effect of p-Hydroxyanisole is related to 3 modes of action: (1) the relative inability of melanocytes to degrade the radical oxides formed as a result of oxidation of p-Hydroxyanisole, (2) the ability of the antioxidant to act as a sulfhydryl scavenger, depriving melanocytes of essential growth factors (e.g., cysteine, methionine), and (3) the ability of p-Hydroxyanisole to inhibit melanocyte growth through the inhibition of DNA synthesis.

Effect on Normal Melanocytes and Keratinocytes

Twenty percent p-Hydroxyanisole in lanolin was applied to the skin of black guinea pigs daily for periods up to 6 months. Microscopic changes in the treated skin included formation of basal keratocyte pseudopodia. The pseudopodia extended from the epidermis through the basal lamina into the underlying dermis. This reaction, termed "microinvasion," was reversed upon termination of antioxidant treatment.[41,48-50] Topical application of 20 percent p-Hydroxyanisole in lanolin to the cheekpouch epithelium of hamsters induced similar extensions of basal cell pseudopodia into the dermis.[51] Grasso and Rostron[52] suggested that both the microinvasion reaction and the epithelial proliferation resulting from topical application of p-Hydroxyanisole are manifestations of an irritant effect on the epidermis and are not indicative of carcinogenic potential.

A cream vehicle containing 0.5 or 1.0 percent p-Hydroxyanisole was applied daily for 6 weeks (42 days) to the left ear and epilated back of black guinea pigs. A selective cytotoxic effect on melanocytes was noted. However, no atypical keratinocytes or melanocytes were observed.[33]

An unspecified vehicle containing either 0.0, 0.5, or 1.0 percent p-Hydroxyanisole was applied daily for 6 months to the left ear and epilated back of black guinea pigs. The antioxidant-treated sites had a number of ultrastructural alterations including a marked decrease in the number of melanized melanosomes, a decrease in the number of actively functioning melanocytes, irregular and disorganized melanosomal lamellae, and swelling and disintegration of outer melanosomal membranes. Melanocytes were observed in which the nuclear envelope and other membranous organelles were vacuolated. The mitochondria of these melanocytes were swollen and in many instances had other changes of degeneration. Degenerative changes in keratinocytes included cytoplasmic vacuolation, swelling and disruption of mitochondria, endoplasmic reticulum and nuclear envelope, dendritic degeneration, intranuclear vacuolization, and convolutions of the nuclear membrane.[33]

The cytotoxic action of p-Hydroxyanisole on guinea pig melanocytes was reported by Riley.[46] Normal guinea pig melanocytes exposed in vitro to the anti-

oxidant selectively incorporated the compound into the melanosome. This selective incorporation was a function of the state of pigmentation of the cells and their tyrosinase activity. Heavily pigmented cells had a greater uptake of p-Hydroxyanisole. The cytotoxic effect on the melanocyte was dependent upon both antioxidant concentration and duration of exposure. At 10^{-3} M, p-Hydroxyanisole was extremely toxic to melanocytes, causing cytoplasmic blebbing and rupture of cell membranes within 30 minutes. The antioxidant had a melanocytotoxic effect at concentrations as low as 10^{-8} and 10^{-9} M, although progressively longer exposure periods (24 to 36 hours) were required. No effects were observed in guinea pig melanocytes after 36 hours of exposure to 10^{-10} M p-Hydroxyanisole.

Electron microscopy was used to assess the effects of p-Hydroxyanisole on normal human melanocytes in both organ culture and disperse tissue culture. In disperse tissue culture, no specific toxic effect on human melanocytes was observed following a 45-minute exposure to either 10^{-2} M or 10^{-3} M p-Hydroxyanisole. Plasma membranes, nucleus, and cytoplasmic organelles, including melanosomes, were unaffected. Keratinocytes likewise had no morphological changes following antioxidant exposure. Whole epidermis exposed to 10^{-1} M p-Hydroxyanisole for 1, 5, and 24 hours had extensive damage to melanocytes and keratinocytes; damage was much less severe, however, at an antioxidant concentration of 10^{-2} M. Melanocytes of PUVA-treated skin exposed up to 24 hours to 10^{-2} M or 10^{-3} M p-Hydroxyanisole had no morphological damage at the ultrastructural level. This study failed to demonstrate a specific toxic effect of p-Hydroxyanisole to normal human melanocytes in dispersed tissue and organ culture.[53]

Human melanocytes and keratinocytes in tissue culture were exposed to tyrosinase (15 μg/ml) and p-Hydroxyanisole (5×10^{-4} M to 5×10^{-2} M) for 1 to 24 hours. No damage was noted in either type of cell exposed below 5×10^{-3} M p-Hydroxyanisole for 6 hours. However, higher concentrations and longer exposures extensively damaged the cells. After 6- and 24-hour exposures to 5×10^{-3} M p-Hydroxyanisole and 15 μg/ml tyrosinase, most melanocytes had less dense cytoplasm, poorly defined cytoplasmic membranes, numerous lipid droplets, fewer mitochondria, and swollen and disrupted mitochondria as compared to control cells. The nuclei of treated melanocytes were morphologically unchanged. Nonkeratinized keratinocytes appeared swollen and had loss of cytoplasmic matrix and filaments, with a virtual absence of mitochondria and an accumulation of electron-dense round bodies (probably of a lipid nature). In many keratinocytes, the nucleus had loss of substance as compared to control cells. One hour exposure to 10^{-2} M p-Hydroxyanisole and tyrosinase (15 μg/ml) produced similar results. A 24-hour exposure of melanocytes and keratocytes to 10^{-2} M p-Hydroxyanisole also produced very severe damage, with loss of practically all cytoplasmic organelles and loss of definition or disruption of the plasma membrane. As with lower concentrations of the antioxidant, the nuclei of melanocytes retained more substance than those of nonkeratinized keratinocytes. Exposure of cells washed free of culture medium to both tyrosinase and 10^{-3} M p-Hydroxyanisole for 1 hour resulted once again in extensive damage. The damage could not be attributed to addition of tyrosinase per se to the medium, since controls with tyrosinase alone had no damage to either cell type. These findings suggested that an early-formed product of the reaction between tyrosinase and

p-Hydroxyanisole was inactivated by constituents of the medium. This was confirmed by liquid chromatography and scanning spectrophotometry. A toxic p-Hydroxyanisole quinone immediately reacted with nucleophilic substances in the medium to form products that, on accumulation, were probably responsible for the damage (6 hours plus) to melanocytes and keratinocytes.[54]

In the previously cited reports of Riley[46] and Breathnach et al.,[53] p-Hydroxyanisole was cytotoxic to guinea pig melanocytes at a concentration of 10^{-3} M but had no toxic effect at 10^{-2} M or 10^{-3} M on human melanocytes. The apparent discrepancy of results may be due to:[53,54] (1) species differences between man and the guinea pig (although the basic structure of the melanocyte and general process of melanogenesis are, as far as is known, identical in the two species), (2) an inability of p-Hydroxyanisole to traverse the plasma membrane and enter the human melanocyte, (3) differences in the reaction between human tyrosinase and p-Hydroxyanisole and in the reaction between the guinea pig enzyme and p-Hydroxyanisole, and (4) the need for human tyrosinase to be present in particularly high concentrations (in Riley's view, the presence of active tyrosinase within the cell is essential for p-Hydroxyanisole to exert its toxic effect).

Effect on Malignant Melanocytes

The selective lethal effect of p-Hydroxyanisole on cell cultures of malignant melanocytes was studied by Bleehan.[55] Two cell lines of human malignant melanoma, as well as Harding-Passey and B16 mouse melanoma cells were exposed in vitro for either 30 or 60 minutes to p-Hydroxyanisole concentrations of 10^{-6} M to 10^{-3} M. A dose-dependent cytotoxic effect was observed. Considerable disruption of cytoplasm, cytoplasmic organelles, and nucleus was noted after 30 minutes of exposure to 10^{-3} M p-Hydroxyanisole. Even at 10^{-5} M, the antioxidant had a marked lethal effect on Harding-Passey and B16 melanoma cells, especially on those cells that were pigmented. No effect was observed on human or mouse fibroblast cultures exposed in vitro to 10^{-3} M p-Hydroxyanisole.

The in vitro effects of p-Hydroxyanisole on mammalian melanocytes were assessed in an unpublished study by Pathak.[33] The study consisted of 3 phases. In the first phase, 2 cell lines of mouse melanoma, S-91A (pigmented) and S-91B (nonpigmented), were exposed for 48 hours to p-Hydroxyanisole concentrations ranging from 10^{-5} M to 10^{-1} M. Concentrations of 10^{-4} M to 10^{-1} M were "clearly cytotoxic," whereas concentrations of 10^{-6} M and 10^{-5} M had a "minimal" cytotoxic effect. Inhibition of thymidine, uridine, and leucine incorporation was also observed (the concentration at which this inhibition was noted was not specified). In the second phase of the study, the same 2 cell lines were exposed to p-Hydroxyanisole concentrations of 10^{-5} M, 10^{-4} M, and 10^{-3} M for 1 hour. The antioxidant had minimal or no effect in either cell line on RNA or protein synthesis at the 3 concentrations evaluated. At 10^{-4} M (0.125 percent), synthesis of DNA was inhibited by 73 percent and 50 percent in S-91A and S-91B cells, respectively. At 10^{-3} M (1.25 percent), the inhibitory effect on DNA synthesis was even greater. No significant effects were observed at 10^{-5} M (0.0125 percent) p-Hydroxyanisole. It was concluded that 10^{-5} M "appeared to be nontoxic" to mouse melanoma cells, whereas 10^{-4} M (0.125 percent) was "definitely cytotoxic." The third phase of the study was conducted to determine the effect of p-Hydroxyani-

sole on the tyrosinase activity of the S-91A cell line. The mouse melanoma cells were incubated with L-tyrosine-3,5-^{3}H and 1×10^{-6} M to 1×10^{-3} M p-Hydroxyanisole in culture medium for an unspecified period of time. p-Hydroxyanisole concentrations of 10^{-6} M and 10^{-5} M did not inhibit tyrosinase activity, whereas 10^{-4} and 10^{-3} M "strongly inhibited" tyrosinase activity. At 10^{-6} M, the antioxidant stimulated tyrosinase hydroxylation.

Dewey et al.[56] reported that p-Hydroxyanisole (0.1 to 0.6 μmol/ml) inhibited the incorporation of ^{3}H-thymidine into Harding-Passey melanoma cells in vitro. Addition of tyrosinase to the culture was associated with increased toxicity of the antioxidant to the melanoma cell. The authors suggested that cells producing the enzyme were preferentially killed by the antioxidant.

Intraperitoneal injection of 2.5 mg p-Hydroxyanisole in saline for periods up to 15 days delayed the appearance of tumors in mice inoculated with B16 melanoma cells.[57] Intratumor injection of 12.5 mg of the antioxidant in saline twice a day for 2 weeks caused increased survival time, reduced tumor size, and in many cases resulted in complete loss of tumor in mice inoculated with Harding-Passey melanoma cells.[56]

Effect on Carcinogen-Induced Tumors

In a series of investigations by Wattenberg et al.,[58-60] p-Hydroxyanisole inhibited both beta-propiolactone- and benzo(a)pyrene-induced neoplasia in the nonglandular area of the stomach of female HCR/Ha mice. Although the mechanism of inhibition was not established, tumor inhibition was observed when the antioxidant was given in the diet or by oral intubation prior to carcinogen administration. When p-Hydroxyanisole was fed to HCR/Ha mice subsequent to benzo(a)pyrene exposure, no significant suppressive effect on gastric neoplasia was observed. In experiments with A/HeJ mice, dietary administration of p-Hydroxyanisole prior to benzo(a)pyrene exposure had no effect on the incidence of carcinogen-induced pulmonary adenoma.

Effect on Enzymes

Marked increases in glutathione S-transferase activity and acid-soluble sulfhydryl concentrations were observed in the esophagus and nonglandular stomach of female HCR/Ha mice fed diets containing p-Hydroxyanisole. The antioxidant was administered daily in the amount of 0.03 nmol/g of food for either 3 or 9 days. Enhancement of glutathione S-transferase activity has been associated with a reduced carcinogenic response in the stomach of mice exposed to benzo(a)pyrene.[61,62]

p-Hydroxyanisole was examined for its ability to induce in vivo changes in hepatic mono-oxygenase and detoxification enzyme activities and to act as a mono-oxygenase inhibitor when added in vitro. Female CD-1 mice were fed a diet containing 42 nmol of p-Hydroxyanisole per kg of food for 12 days. No changes in liver weights were noted. Hepatic microsomal activities of aniline hydroxylase, TMPD:CHP peroxidase, glutathione S-transferase, and epoxide hydratase were increased, whereas the hepatic microsomal activity of aminopyrine N-demethylase was decreased. Dietary administration of p-Hydroxyanisole also caused depressed activities of hepatic cytochrome P450 and a reduced ability of

microsomes to catalyze the binding of benzo(a)pyrene metabolites to DNA. The antioxidant did not inhibit benzo(a)pyrene metabolism or DNA binding when added in vitro to hepatic microsome preparations at concentrations up to 300 μM. Test results were consistent with the hypothesis that inhibition of benzo(a)-pyrene-induced neoplasia by p-Hydroxyanisole was related to inducibility of detoxification enzymes.[63]

The effect of p-Hydroxyanisole on the induction of ornithine decarboxylase (ODC) activity in mouse epidermis by the tumor promoter 12-o-tetradecanoyl-phorbol-13 acetate (TPA) was assessed by Kozumbo et al.[64] Graded doses of p-Hydroxyanisole over a 2-log range were topically applied to the shaved skin of female CD-1 mice 30 minutes prior to skin treatment with TPA. The ID_{50}, the dose that causes 50 percent inhibition of enzyme activity, was determined from the generated dose–response curve. The ID_{50} for p-Hydroxyanisole was >50 μmol, indicating that the antioxidant was relatively ineffective in inhibiting promoter-induced ODC activity.

Induction of microsomal enzymes by p-Hydroxyanisole was measured in livers of female weanling rats. The rats received the antioxidant in daily oral doses of 1.5 mM/kg for 6 days. A weak but significant increase in the activities of hexobarbitone oxidase and aminopyrine demethylase was observed.[65]

Effect on Human Erythrocytes

The in vitro effects of p-Hydroxyanisole on human erythrocytes was examined in several studies. The antioxidant (1 to 20 mM) caused lysis of human erythrocytes in the presence of the enzyme, tyrosinase. A correlation was observed between the in vivo depigmenting action of this compound and the ability of hydroxyanisole isomers to act as substrates for tyrosinase and to cause lysis of erythrocytes in the presence of this enzyme.[45]

In other studies, p-Hydroxyanisole protected human erythrocytes from hypotonic hemolysis in vitro. The high coefficient of correlation (0.963) between the 50 percent antihemolytic concentration of p-Hydroxyanisole (6.0×10^{-3} mol/liter) and the octanol-water partition coefficient suggested a hydrophobic interaction between the compound and the erythrocytic membrane. The antihemolytic effect was associated with membrane expansion.[66,67]

Antimicrobial Activity

p-Hydroxyanisole was bactericidal to *Pseudomonas aeruginosa*[68] and inhibited spore production at a concentration of 10^{-4} M in *Candida (Monilia) fructicola* and *Alternaria oleracca*.[69] The compound produced complete inhibition of *Mycobacterium tuberculosis* growth at a concentration of 25 mg/ml of growth medium,[70] whereas 1.0 mg/ml of the antioxidant was inactive against poliomyelitis virus in tissue culture.[71]

Other Biological Effects

p-Hydroxyanisole (1 mg) was mixed with 100 IU of pregnant mare serum gonadotropin, incubated at 37°C, and injected subcutaneously into 3 immature

female rats. Seventy-two hours after injection, the animals were killed. The average ovarian weight of treated animals was 114 mg, whereas the average ovarian weight of control animals receiving pregnant mare serum gonadotropin alone was 127 mg. These results suggested no in vitro inhibition of gonadotropic activity.[72]

Cultured ascites sarcoma BP8 cells exposed in vitro to 0.01 to 1.0 mM p-Hydroxyanisole had a 15 to 93 percent reduction in cell growth. It was suggested that this inhibition of growth resulted from the interference of the antioxidant with the electron transport function of the sarcoma cell.[73]

Results from an in vitro study by Riley[45] indicated that p-Hydroxyanisole interferes with ribonucleic acid and protein synthesis and with mitochondrial respiration. Addition of 5 mM of the antioxidant to rat liver slices inhibited protein synthesis by 80 percent, whereas addition of 5 mM to HeLa cell cultures caused a 56 percent inhibition of ribonucleic acid production. A 25 to 70 percent inhibition of mitochondrial respiration in isolated rat liver was produced by 0.5 mM p-Hydroxyanisole. The author reported that no direct correlation was observed between these in vitro effects and the in vivo depigmenting action of the compound.

The role of p-Hydroxyanisole as an uncoupling agent on oxidative phosphorylation in isolated rat liver mitochondria was studied by Wynn and Fore.[74] Phosphate and oxygen uptake in mitochondria were measured over a 10-minute period following addition of 5×10^{-5} M p-Hydroxyanisole to the liver preparation. The phosphate:oxygen ratio (P:O ratio) in p-Hydroxyanisole-treated liver was 2.0. Measures of the P:O ratio in nontreated control and positive control liver preparations were 2.5 and 1.2, respectively. The P:O ratio of 1.2 for L-thyroxine (positive control) indicated extensive uncoupling of mitochondrial oxidative phosphorylation.

The ability of p-Hydroxyanisole and other phenols to produce chromosome fragmentation in onion roots (*Allium cepa*) was reported by Levan and Tjio.[75] In a more recent investigation, chromosomal aberrations were observed in barley caryopsis (*Hordeum vulgare*) and onion roots (*A. cepa*) treated with 0.25 mM of the antioxidant.[76]

Denaturation of DNA was observed in T_4 bacteriophage treated in vitro with 0.09 M p-Hydroxyanisole.[77]

ABSORPTION, METABOLISM, AND EXCRETION

The rate of skin absorption of p-Hydroxyanisole was examined in vitro by Riley.[39] A 5×10^{-2} M concentration of the antioxidant was applied to the keratin surface of full-thickness guinea pig skin (0.96 cm^2) excised from the back of the ears. The rate of skin penetration as measured by spectrophotometric analysis was 8.05×10^{-4} mol per cm^2 per minute.

p-Hydroxyanisole was administered by stomach tube to female rabbits weighing 2.5 to 3.5 kg at a dose of 0.7 g as a suspension in water. The compound was excreted mainly as conjugates of glucuronic and sulfuric acids; a small amount was partly demethylated to give hydroquinone. The average percentage

of the dose excreted was 13 percent (range: 10 to 15 percent), 69 percent (range: 65 to 73 percent), and 1 percent (range: 0 to 2 percent) for ethereal sulfate, ether glucuronide, and free phenol, respectively. The average percentage of the single dose accounted for was 82 percent.[78]

The metabolism and excretion of butylated hydroxyanisole and its isomers were determined in the rat. The commercial butylated hydroxyanisole used in the study consisted of a mixture of 15 percent or less of 2-tert-butyl-4-hydroxyanisole (isomer A) and 85 percent or more of 3-tert-butyl-4-hydroxyanisole (isomer B). Chromatographic analysis indicated that isomer A contained 3 to 5 percent p-Hydroxyanisole as an impurity. Isomer A was administered by gastric intubation to 6 rats as a 50 percent (W/V) solution in corn oil. Each rat was given successive daily doses of 0.5 g/kg for 5 doses (total average dose/rat was 0.77 g). For the duration of dosing, urine samples were collected and pooled. The urine was subsequently analyzed for free butylated hydroxyanisole, glucuronide, and ethereal sulfate, which accounted for 5, 25, and 30 percent of the dose fed, respectively. Of isomer A, 0.6 percent of the dose was excreted as the impurity p-Hydroxyanisole and 2.6 percent as its glucuronide, as estimated enzymically and chromatographically. The authors concluded that p-Hydroxyanisole was not demethylated by the rat but that it was largely excreted as a glucuronide.[79]

TOXICOLOGY

Acute Oral Toxicity

Fasted rats weighing 129 to 160 g were given p-Hydroxyanisole by stomach tube as a single oral dose ranging from 150 to 350 mg per rat. Size of the 5 test groups varied from 1 to 10 animals. A dose of 200 mg killed 4 of 10 rats; a dose of 300 mg killed 5 of 6. The acute oral LD_{50} was estimated at 1630 mg/kg.[8]

Corn oil containing 10 percent p-Hydroxyanisole was administered to rats as oral doses of 1000 mg/kg or 2000 mg/kg. Two rats were given each dose (4 total). No deaths resulted from the 1000 mg/kg dose, but "kidney injury" was observed at necropsy. The 2 rats given 2000 mg/kg developed convulsions within 10 minutes after dosing; death ensued within 2 hours.[80]

The acute oral toxicity of 50 percent p-Hydroxyanisole in corn oil was assessed in Sprague-Dawley rats by the methods described by Hagan[81] and Weil.[82] Five to ten animals were used in each test group (number of groups and dose range were not specified). The LD_{50} of the corn oil suspension was 740 mg/kg.[83]

Three cosmetic products containing the antioxidant were also evaluated for acute oral toxicity. Each of the products was tested by the procedures described by Hagan[81] and Weil.[82] Test groups consisted of 5 to 10 rats. However, number of groups and dose range were not reported. Reported acute oral LD_{50} values were as follows:

Test Material	*Oral LD_{50} of Test Material (Rat)*	Reference
Moisturizing lotion containing 0.1 percent p-Hydroxyanisole	>21.5 g/kg	84
Moisturizing lotion containing 0.05 p-Hydroxyanisole (product given as a 50 percent suspension in corn oil)	>15.9 g/kg	85
Blusher containing 0.1 percent p-Hydroxyanisole	>15.8 g/kg	86

Intraperitoneal Toxicity

Groups of mice were given various doses of p-Hydroxyanisole by intraperitoneal injection. Mortality was recorded over a 7-day observation period following the single exposure. The LD_{50} was 250 mg/kg.[87]

The acute toxicity of p-Hydroxyanisole following intraperitoneal injection was assessed in mice, rats, and rabbits by Hodge et al.[8] *Mice:* Eight groups of mice (10 to 22 animals/group) weighing 25 to 28 g were given single injections ranging from 4 to 18 mg. A "wobbly gait" immediately following injection was observed. This was followed in a few minutes by paralysis of the hind quarter and spasms. At the "higher doses," the mice developed narcosis 15 minutes after injection. Some mice had "degrees of anesthesia" for as long as 18 hours after the single exposure. No deaths occurred in the group receiving the 4 mg dose; however, all mice in the 18 mg group died. The estimated LD_{50} was 430 mg/kg of body weight. *Rats:* Six groups of albino rats (5 to 15 animals/group) were injected once with 1 of 6 p-Hydroxyanisole doses ranging from 100 to 150 mg per rat. The adult rats ranged in body weight from 150 to 193 g. No deaths were noted at the 100 mg dose, whereas all rats died at the 150 mg dose. The LD_{50} was 730 mg/kg. The acute toxic effects in the rats were similar to those observed in the mice. *Rabbits:* Adult rabbits weighing 2.7 to 4.8 kg of various breeds were given a single intraperitoneal injection ranging from 100 to 3300 mg. Only 1 rabbit was tested at each dose. Doses up to 2800 mg were tolerated without lethal effects; the rabbit treated with 3300 mg died within 24 hours. The LD_{50} was estimated at 720 to 970 mg/kg.

Dental Pulp Irritation

The potential of 100 percent p-Hydroxyanisole to produce dental pulp irritation was assayed in two monkeys (*Macaca fascicularis*). The test material was placed in contact with the pulpal walls of prepared class V cavities in each of 8 quadrants. A piece of gold foil was subsequently placed over the test substance,

and the cavity was then filled with a zinc oxide/eugenol material. Twenty-one days later, the animals were killed, and the teeth were processed for histopathologic evaluation. Pulp irritation was graded on a scale of 0 (no inflammation) to 4 (abscess formation or pulp necrosis). The average pulp score was 0.25 (±0.20), indicating minimal to mild irritation or inflammation. In 1 of the 8 quadrants, there was a single 2.0 response indicative of moderate irritation. Differences in dentin thickness did not lead to differences in pulp response. No abscess formations or "lesions predominating in leukocytes" occurred.[88]

Eye Irritation

The ocular irritating effects of p-Hydroxyanisole were assessed in a 1959 range finding study (unpublished). Both "undiluted" p-Hydroxyanisole and 10 percent p-Hydroxyanisole in propylene glycol were instilled into the eyes of an unspecified number of rabbits. In some rabbits, treated eyes were given a 2 minute water rinse following antioxidant exposure. The propylene glycol solution containing 10 percent p-Hydroxyanisole produced slight conjunctivitis in both the rinsed and unrinsed eye; this irritation dissipated 1 hour after instillation of the test material. No corneal injury was observed. Undiluted p-Hydroxyanisole produced corneal injury, moderate conjunctivitis, and slight iritis in both rinsed and unrinsed eyes. This irritation had "essentially subsided" 1 week after treatment in those rabbits given no water rinse and had "completely subsided" after 1 week in those rabbits given a water rinse.[80] It was not indicated whether or not nontreated or vehicle (propylene glycol) control eyes were used or whether or not treated eyes received single or multiple instillations of the test material.

Undiluted p-Hydroxyanisole and 1.0 percent p-Hydroxyanisole in aqueous solution were evaluated in a second eye irritation study. The procedures used were a modified version of the test methods outlined by Draize.[89] Each test material was instilled into the eyes of 3 to 6 (exact number not specified) New Zealand rabbits. One percent p-Hydroxyanisole in water produced minimal conjunctival irritation 1 hour postinstillation (average score, 2; maximum possible score, 20). The conjunctival irritation dissipated by the 24-hour evaluation. No iridial or corneal lesions were observed. For the undiluted material, average corneal, iridic, and conjunctival irritation scores 1 hour postinstillation were as follows: (1) cornea: score, 20 (max, 80); (2) iris: score, 5 (max, 10); (3) conjunctivae: score, 4 (max, 20). Average ocular irritation scores for the undiluted antioxidant 7 days postinstillation were 80, 10, and 14 for cornea, iris, and conjunctiva, respectively, indicating severe ocular irritation.[83] A test material is considered a severe eye irritant when corneal and iridial lesions have not cleared by the seventh day.[89]

The Draize procedure[89] was used to evaluate the ocular irritation potential of 3 cosmetic products containing p-Hydroxyanisole. The undiluted products were tested on groups of 3 to 6 New Zealand rabbits. A moisturizing lotion and a blusher each containing 0.1 percent p-Hydroxyanisole produced no eye irritation. A moisturizing cream formulated with 0.05 percent of the antioxidant produced minimal conjunctival irritation by the 1-hour evaluation (average score, 4; maximum possible score, 20). This irritation had completely dissipated by the 24-hour evaluation.[84-86]

Skin Irritation

The potential of p-Hydroxyanisole to produce skin irritation in rabbits and guinea pigs was assessed in 3 separate studies.

In a 1959 range finding study (unpublished), p-Hydroxyanisole was tested for skin irritation in 4 separate trials. In the first trial, a single application of undiluted p-Hydroxyanisole was made to the intact skin of an unspecified number of rabbits for either 3 or 7 hours. No irritation was observed on test sites exposed to the antioxidant for 3 hours, whereas treatment sites exposed for 7 hours developed "very slight hyperemia." In the second trial, the undiluted ingredient (0.5 g) was applied for 24 hours under an occlusive patch to the abraded and intact skin of the abdomen of an unreported number of rabbits. "Extensive edema and necrosis" were observed following the single 24-hour exposure. Moderate eschar formation was noted 21 days posttreatment. In the third trial, a single 1 g/kg dose (dose based on solids) of 50 percent p-Hydroxyanisole in dipropylene glycol monomethyl ether was applied to the clipped skin of the torso of 2 rabbits. The single applicatiion was made under an impervious plastic sleeve for 24 hours. "Slight hyperemia" of the skin developed. In the fourth trial, 10 percent p-Hydroxyanisole in dipropylene glycol monomethyl ether was applied to the intact skin of the ear (3 applications), intact skin of the abdomen (3 applications), and abraded skin of the abdomen (3 applications) of 1 rabbit. Applications were made under a cotton pad to each test site daily for 3 consecutive days (total of 9 24-hour applications). No irritation was observed on the intact skin of the ear. However, intact and abraded treatment sites on the abdomen developed "slight hyperemia" and slight to moderate edema.[80]

An abbreviated, unpublished study was conducted in 1950 to evaluate skin irritation and antioxidant absorption following topical application of p-Hydroxyanisole to guinea pig skin. A patch containing 40 percent p-Hydroxyanisole in an acetone/olive oil mixture (92:8) was applied for 24 hours in a single 10 or 20 ml/kg dose to the clipped and depilated skin of 2 guinea pigs (1 animal/dose). Slight to moderate skin irritation was observed.[90]

Five percent p-Hydroxyanisole in sweet almond oil was applied for 24 hours under patch to the clipped skin of 6 New Zealand rabbits. Skin irritation was subsequently assessed according to the evaluation method described by Draize.[89] The irritation index (average score of 6 animals) was 0.3 on a scale of 0 (no irritation) to 8.0 (severe erythema and edema), indicating minimal skin irritation.[83] (It was not specified whether abraded or intact skin was tested).

Skin Sensitization

The skin-sensitizing potential of p-Hydroxyanisole was evaluated by means of the guinea pig maximization test and the Freund's complete adjuvant test. The procedures were those as described by Van de Walle et al.[91] In the guinea pig maximization test, 0.5 M (6.2 percent) p-Hydroxyanisole was given by intradermal injection into the shoulder of 10 guinea pigs on Day 0. On Day 7, a 48-hour induction patch containing 1 M p-Hydroxyanisole was applied to the injection site. Pretreatment with 10 percent sodium lauryl sulfate in petrolatum was performed 24 hours before the patch induction on Day 7 to obtain moderate irrita-

tion. The challenge phase consisted of 2 24-hour patches. One patch was closed and was applied to the shaved skin of the right flank on Day 21. The second challenge patch was open and was applied to the shaved left flank on Day 35. In the Freund's complete adjuvant test, intradermal injections of 0.5 M p-Hydroxyanisole in Freund's adjuvant were made into the shoulder of 8 guinea pigs on Days 0, 2, 4, 7, and 9 (induction phase). Open, 24-hour challenge patches were applied to the shaved right flank on Day 21 and Day 35 (left flank). Challenge concentrations were not specified. p-Hydroxyanisole produced moderate skin sensitization in both tests.[92]

An unpublished study was conducted in 1950 to assess the skin sensitization potential of p-Hydroxyanisole. Seven drops of a "0.1 M solution of the compound in acetone:dioxane:olive oil (1:1:3)" were applied to the clipped backs of each of 5 Hartley strain guinea pigs. The hair "stubble" was removed from the treatment site the following day. Test sites were evaluated for erythema and edema 24 and 48 hours after application. Immediately following the 48-hour evaluation, 10 drops of the test solution were applied to the back. A third induction exposure of 10 drops was applied 48 hours later. After a 3-week nontreatment period, a challenge application of a fresh solution (7 drops) was applied to the clipped right shoulder. The next day, the test area was "depilated." Skin responses were noted 24 and 48 hours following the challenge application. One week later, the challenge procedure was repeated except that the left shoulder was the site of the challenge application. Skin responses to the 2 challenge applications were "not significantly different from the original application," and it was concluded that no sensitization reactions had occurred. Solvent control and positive control (phenylhydrazine) groups were also employed in the study. The positive control group had a "positive response;" however, the response of the solvent control group was not reported. The number of animals in the 2 control groups and the effect of depilation on the treated skin were also not specified.[90]

Concomitant sensitization to p-Hydroxyanisole and various acrylic monomers was observed in monoacrylate sensitized guinea pigs. However, no relation between the antioxidant concentration and the incidence of these reactions could be determined.[91,92] Cross skin sensitization of guinea pigs to hydroquinone (1 M) and p-Hydroxyanisole (3 M) has also been reported.[92]

Photosensitization

The photosensitization potential of 0.1 and 1.0 percent p-Hydroxyanisole in physiological saline and DAE* (20:80) was evaluated in 19 Hartley albino guinea pigs. The phototest consisted of both an induction phase and a challenge phase. During the induction phase, 0.1 ml of 1.0 percent p-Hydroxyanisole was applied 4 consecutive days a week for 3 weeks to the shaved skin of the nuchal area of the back. One hour after application, test sites were irradiated with UV light at 1/2 the minimal erythemic dose. (The minimal erythemic dose, or MED, was determined prior to the study). UVA irradiation was administered during the first

*DAE, 40 percent dimethylacetamide, 30 percent acetone, and 30 percent ethanol.

week of the induction phase, whereas UVB was administered during the second and third week of the induction phase. On the first and third day of the second and third week and before test material application, each guinea pig was given a 0.1 ml intradermal injection of Freund's complete adjuvant in physiological saline (1:1). Adjuvant injections were administered to 4 different areas surrounding the induction site (nuchal area). For the challenge phase, the lumbar area of the shaved back was divided into 6 exposure sites (3 sites on the left of the back, 3 sites on the right). The 3 sites on the left side of the back were treated with 0.1 ml of 1.0 percent p-Hydroxyanisole for 3 consecutive days. One hour after application, these 3 sites were exposed to either no UV light, UVB (1/2 MED), or UVA (1/2 MED). The 3 sites on the right side of the back were treated for 3 consecutive days with 1.0 percent p-Hydroxyanisole and then similarly exposed to UV irradiation as the left side. Each guinea pig was given 3 challenge UV exposures at each of the 2 concentrations (0.1 and 1.0 percent). The time between challenge exposures was not specified. The light source for the second and third week of induction and for challenge consisted of a 150 W Xenon Lamp, which emitted in the UVA (320 to 410 nm), UVB (280 to 320 nm), and visible light (410 nm and greater) range. The same lamp was used for the first week of induction and challenge but was fitted with a WG-345 glass filter to remove UVB waves. Distance between the light source and exposure site was approximately 4.6 cm. Skin reactions were evaluated 24 hours after each UV exposure. A reaction was considered a photocontact sensitization reaction if the skin response was at least 1 grade greater than that observed during the first week of induction. One of 19 guinea pigs reacted to both UVA and UVB irradiation at 0.1 and 1.0 percent. A second animal reacted when challenged at 1.0 percent to UVB irradiation. All skin responses to p-Hydroxyanisole were found after the third consecutive challenge and consisted of minimal erythema. Historical data on 5 percent 6-methylcoumarin (positive control) and undiluted DAE/physiological saline (vehicle control) were positive and negative, respectively, for photosensitization. On the basis of "the limited number of animals responding and the low magnitude of the dermal responses following three consecutive challenge periods," the investigator concluded that the findings with regard to p-Hydroxyanisole were not significant and were not indicative of a photoallergic response. It was also noted that the induction concentration of 1.0 percent p-Hydroxyanisole represented a 10-fold exaggeration of concentration normally used in cosmetics.[93]

Acute Dermal Toxicity

An unpublished study assessed the skin absorption and dermal toxicity of p-Hydroxyanisole. A dipropylene glycol monomethylether solution containing 10 percent p-Hydroxyanisole was applied to the intact skin of the ear (3 applications), intact skin of the abdomen (3 applications), and abraded skin of the abdomen (3 applications) of one rabbit. Applications were made under a cotton pad to each test site daily for 3 consecutive days (total of 9 24-hour applications). The rabbit died within 4 days following the last treatment. It was reported that the cause of death was "possibly from absorption through the abraded skin area."[80]

The skin absorption and acute dermal toxicity of a dipropylene glycol monomethylether solution containing 50 percent p-Hydroxyanisole was assessed in a

second trial by the same investigators. A single 1000 mg/kg application (dose based on solids) was made to the clipped torso of each of 2 rabbits. The test material remained in contact with the skin for 24 hours under an impervious plastic sleeve. No deaths or "untoward reactions" were observed.[80]

An abbreviated, unpublished study was conducted in 1950 to evaluate antioxidant absorption following topical application of p-Hydroxyanisole to guinea pig skin. The study consisted of 2 trials. In the first trial, 20 percent p-Hydroxyanisole in a mixture of acetone and olive oil (92:8) was applied in a single dose to the clipped and depilated skin of 3 guinea pigs. The single dose consisted of a 24-hour patch containing either 5, 10, or 20 ml/kg of body weight of the test solution; 1 animal was tested at each dose. The guinea pig treated with 20 ml/kg (about 4 g of p-Hydroxyanisole/kg of body weight) died during the 14-day observation period following application. In the second trial, a patch containing 40 percent p-Hydroxyanisole in an acetone/olive oil mixture (92:8) was applied for 24 hours as a single 10 or 20 ml/kg dose to the clipped and depilated skin of 2 guinea pigs (1 animal/dose). Both animals survived.[90]

It should be noted that these 3 studies conducted in the 1950s were exploratory or range-finding in nature and were designed primarily to assist in assessing industrial handling hazards and in establishing precautionary measures to be observed for safe manufacturing.

Subchronic Dermal Toxicity

The subchronic dermal toxicity of p-Hydroxyanisole was assessed in guinea pigs, mice, and rabbits.

A water-oil emulsion containing 1.0 percent p-Hydroxyanisole was applied in 0.5 ml doses to the shaved skin of male guinea pigs daily for 30 days. Skin reactions to the emulsion consisted of hyperemia, edema, and slight desquamation. Increased histamine concentrations were also found in the treated skin.[94]

The skin-irritating effects of p-Hydroxyanisole were assessed in both black guinea pigs and BLA mice. A single application of 10 percent p-Hydroxyanisole in petroleum jelly was made daily to the ear of each of 5 guinea pigs for 4 weeks; 20 percent p-Hydroxyanisole in petroleum jelly was applied in a similar fashion to a second group of 5 guinea pigs. A 10 percent concentration of the antioxidant in petroleum jelly was applied to the neck of 10 mice daily for 8 weeks, whereas 20 percent p-Hydroxyanisole in petroleum jelly was applied to the neck of 10 mice daily for 4 weeks. Skin irritation (acanthosis) and skin depigmentation were observed in both species exposed to 20 percent p-Hydroxyanisole. No observable skin effects were produced by 10 percent p-Hydroxyanisole.[42]

A "preliminary" study was conducted to evaluate the dermal toxicity of an alcohol-based suntan lotion containing either 0, 1.0, or 10.0 percent p-Hydroxyanisole. The vehicle control group and the 2 treatment groups each consisted of 6 rabbits (18 total). The suntan lotion was applied in a single 5 ml dose to the clipped and depilated skin of the back and side of each animal. Applications of the product were made daily, 5 days a week for 30 days. Body weights were recorded weekly, and rabbits were killed at the end of the experimental period. Both groups exposed to p-Hydroxyanisole for 30 days had weight losses, but these losses were considered "small." Results of hematological studies and urine

analyses before and during the course of study were normal. Rabbits treated with the lotion containing 0.1 and 10.0 percent p-Hydroxyanisole had a decrease in the average weight of testes and an increase in the average weight of the spleen compared to the vehicle control group. The average weight of heart, lungs, liver, kidneys, brain, and stomach were comparable between control and treatment groups. At necropsy, pitting of the kidney surface (3 rabbits of the 1.0 percent group), an enlarged spleen (1 rabbit of the 1.0 percent group), and an atrophic left testis (1 rabbit of the 10 percent group) were the findings. Principal changes observed in the skin of both control and experimental animals included dryness and crusting of the surface. Microscopic changes ranging from atrophy of the epidermis to ulceration and inflammation were also observed in both control and treatment groups. Rabbits treated with the lotion containing 1.0 and 10.0 percent p-Hydroxyanisole developed "marked irritation" of the skin during the first week of exposure. Control animals treated with the alcohol-based suntan lotion alone had similar but less marked reactions of irritation.[8]

In a follow-up to the previous study, the alcohol-based suntan lotion containing either 0 or 10.0 percent p-Hydroxyanisole was applied to the clipped and depilated skin of the back, sides, and abdomen of each of 12 rabbits (6 rabbits/vehicle control group and 6 rabbits/treatment group). Three hours after the lotion was applied, the treated sites were washed with soap and water, rinsed, and dried. This procedure was repeated daily for 12 days over a 2-week period. The fur was reclipped as required to permit adequate contact of the product with the skin. Vehicle control rabbits (suntan lotion alone) gained weight steadily after an initial small decrease. Rabbits treated with the lotion containing 10 percent p-Hydroxyanisole lost weight during the first week; this weight was largely regained in the succeeding 10 days. None of the weight changes were of "large magnitude." At the conclusion of the 12 days of exposure, skin erythema and escharification were each graded on a scale of 1 (questionable erythema/mild crustiness) to 4 (severe erythema/cracks in the skin). In the vehicle control group, average scores for erythema and escharification were 1.3 and 1.5, respectively, indicating mild skin erythema and mild to moderate skin escharification. In the 10 percent treatment group, average scores for erythema and escharification were 2.1 and 2.3, respectively, indicating moderate skin erythema and moderate skin escharification. Skin from both vehicle control and p-Hydroxyanisole-treated rabbits had "moderate hyperkeratosis" and "slight chronic inflammation." The surface of the skin had red blood cells, necrotic epithelial cells, and keratin. The underlying epithelium was intact in all instances.[8]

Subchronic Oral Toxicity

Subchronic feeding studies were conducted with p-Hydroxyanisole in the rat, rabbit, and dog.

Eighty male and female rats were subdivided into 8 groups (10 rats/group) and then fed diets containing either 0, 0.02, 0.1, 0.5, 2.0, or 5.0 percent p-Hydroxyanisole for 5 to 7 weeks. Two groups of control rats (20 rats total) were fed the diet containing no antioxidant, whereas 2 groups of rats (20 rats total) were maintained on diets treated with 0.5 percent p-Hydroxyanisole. No deaths occurred in any group during the study. A dose-related growth inhibition was ob-

served in males fed 0.1 to 5.0 percent p-Hydroxyanisole and in females fed 0.5 to 5.0 percent of the antioxidant. The inclusion of 2.0 or 5.0 percent p-Hydroxyanisole in the diet produced a marked depression of growth in both sexes. However, it was suggested that the odor and flavor associated with p-Hydroxyanisole at these 2 dietary concentrations may have reduced both the palatability and, thus, the intake of the diet. Qualitative analyses of pooled urine samples collected near the end of the experiment from males revealed slightly elevated concentrations of sugar and p-Hydroxyanisole; protein values were normal. Hematological studies made at various intervals revealed normal red blood cell counts, differential counts, and hemoglobin concentrations. A few high leukocyte counts were observed, but these were likely the result of a mild respiratory infection. Rats of both sexes in the low-dose groups (0.02 to 0.1 percent) had decreased spleen and liver weights; males in these same groups had decreased kidney and testes weights. Organ weights of rats fed 0.5 to 5.0 percent of the antioxidant decreased as the percentage of p-Hydroxyanisole increased. The variations in organ weights (heart, lungs, spleen, liver, kidney, brain, testes) observed at the end of the study period were attributed to general body weight depression. Tissues taken from various organs and the gastrointestinal tract had no changes that could be attributed to dietary administration of p-Hydroxyanisole.[8]

Groups of 6 rabbits were fed diets containing 0, 1.0, 5.0, or 10.0 percent p-Hydroxyanisole for 5 to 9 weeks. Rabbits fed 1.0 and 5.0 percent p-Hydroxyanisole gained weight at the same rate as control animals. However, rabbits fed 10.0 percent had transient weight loss. The "appetite and general condition" of the rabbits fed 1.0 percent p-Hydroxyanisole for 5 weeks was described as "excellent." Urine samples collected at the start and end of the experiment had low concentrations of sugar and protein, but there was "no indication of kidney damage." Blood samples taken midway through the study and at the end of the study generally had normal hemoglobin concentrations and normal white and red cell counts. However, rabbits fed 10.0 percent p-Hydroxyanisole had low red cell counts. Differential leukocyte counts had considerable variation. The average weights of liver and brain were increased in the 10 percent treatment group, whereas average weights were decreased for heart, lungs, spleen, liver, kidney, and testes. These organs and the large and small intestine had no significant pathological changes that could be attributed to the administration of p-Hydroxyanisole.[8]

The subchronic toxicity of p-Hydroxyanisole was assessed in 3 dogs by adding the antioxidant to the diet as follows: (1) dog no. 1 (12.5 kg): 1 g daily for 2 months, (2) dog no. 2 (6.8 kg): 2 g daily for 1 month, then 4 g daily for 6 weeks, (3) dog no. 3 (14.4 kg): 3 g daily for 6 weeks, then 6 g for 1 month, then 12 g for 2 weeks. The body weight of dog no. 1 varied but in general was maintained throughout the study. Dog no. 2 had very little change in body weight. Dog no. 3 fed 3 g daily for 6 weeks lost weight during the first month, regained weight in the next 2 weeks, maintained weight during the period when the dosage was 6 g daily, and then lost weight again when the dosage was increased to 12 g daily. The investigator concluded that up to 6 g of p-Hydroxyanisole daily had no effect on body weight, whereas feeding 12 g a day was associated with body weight loss. The weight loss associated with 12 g a day, however, was not considered excessive. Urine samples examined twice during the study were normal for protein (0.02 to 0.07 percent) and sugar (0.2 to 0.4 percent). Hematological changes in

dog no. 3 included marked decreases in hemoglobin and red blood cell count. The 3 dogs had normal total leukocyte and differential leukocyte counts. Organ weights (heart, lungs, spleen, liver, kidneys, brain, uterus, ovaries) "gave no evidence of toxic effects," and the few pathological changes observed in the lung (diffuse round cell and polymorphonuclear leukocyte infiltration) and spleen (scattered pigment deposit) were not, according to the investigators, attributable to p-Hydroxyanisole.[8]

Other Subchronic Studies

The effects of p-Hydroxyanisole on hamster cheekpouch was assessed by Woods and Smith.[51] A lanolin base containing 20 percent p-Hydroxyanisole was applied to the cheekpouches of golden Syrian hamsters 3 times a week for 45 days. Erythema progressively increased throughout the study. Microscopic examination of treated areas revealed hyperkeratosis, epithelial hyperplasia, formation of small bullae, "disorganization" of basal epithelial cells, "disturbances" at the epithelial-connective tissue junction, "invasion" of basal cell pseudopodia into the lamina densa, and muscle degeneration with scattering of myofilaments.

Chronic Dermal Toxicity

An unspecified vehicle containing either 0.0, 0.5, or 1.0 percent p-Hydroxyanisole was applied daily for 6 months to the left ear and epilated back of 3 groups of black guinea pigs (12 animals per group). The skin of both exposed groups became hypomelanotic and amelanotic after 4 months. Guinea pigs exposed to 1.0 percent p-Hydroxyanisole developed moderate to severe skin and hair depigmentation at the site of application. Animals treated with 0.5 percent of the antioxidant developed either skin depigmentation or skin hypopigmentation. Skin from the vehicle control group (0.0 percent p-Hydroxyanisole) appeared normal when examined grossly and microscopically. At microscopic examination of skin, the treated sites of the two exposure groups had hyperplasia, dyskeratosis, loss of melanin pigment, a decrease in number of DOPA-positive melanocytes, pigment incontinence, decreased pigmentation of melanocytes in the basal layer, focal degeneration of melanocytes, loss of dendritic arborization, presence of melanin-laden macrophages, and loss of pigment in hair follicles. Loss of melanin pigment was more noticeable in skin biopsies of the 0.5 percent exposure group. Maturation disorders of keratinocytes were rare, and the influx of inflammatory cells was minimal suggesting that the p-Hydroxyanisole formulated vehicle was nonirritating and nonsensitizing. There was little or no evidence of atypical cells, vasodilatation, or infiltration of mononuclear cells.[33]

Mutagenicity

p-Hydroxyanisole was nonmutagenic when evaluated in the Ames assay at a concentration of 3 μmol/plate. The qualitative spot test was conducted using 4 histidine-requiring mutants of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537). The antioxidant was tested both with and without metabolic activation using S-9 liver fraction from Aroclor 1254-induced rats.[95]

No mutagenicity was observed in a second assay in which *S. typhimurium* strains TA100 or TA1530 were treated with ≤4 μmol/plate of p-Hydroxyanisole. The S-9 liver fraction of phenobarbitone-induced OF-1 mice provided metabolic activation.[96]

Tumor-Promoting Activity and Carcinogenicity

A benzene solution containing 13.1 percent p-Hydroxyanisole was tested as tumor promoter on the clipped skin of 30 albino mice. The skin was initiated with a single application of 75 μg 7,12-dimethylbenz(a)anthracene (DMBA). A single drop of the benzene solution was applied 1 week later to the DMBA-treated site. Applications of the benzene solution were made twice weekly. After 20 weeks, 1 of the 25 surviving mice developed a benign tumor.[97] Under the experimental conditions, p-Hydroxyanisole was inactive as a tumor promoter.

Acetone solutions of either 5 or 10 percent p-Hydroxyanisole were applied twice a week for life to the dorsal skin of the back of female Swiss albino mice. Each test solution was applied in 0.02 ml doses to a group of 50 animals. The mice developed no local toxic changes or tumors. The incidence of systemic tumors was similar to that of control mice.[98]

The inner surface of the left ear of each of 10 New Zealand rabbits of both sexes was treated twice weekly with 0.02 ml acetone solutions containing either 5 or 10 percent p-Hydroxyanisole. Each test group consisted of 5 rabbits (5 animals per concentration). No local toxic changes or local or distant tumors were observed.[98]

Embryotoxicity and Teratogenicity

A bleach cream containing 5 percent p-Hydroxyanisole and a water-oil emulsion containing 25 percent p-Hydroxyanisole were both assessed for embryotoxicity and teratogenicity. Test animals consisted of groups of 10 to 12 white female rats. Applications of the test materials were made to the skin daily on Days 1 to 20 of pregnancy. Nontreated and cream base control animals were employed. Both test materials produced increased preimplantation mortality of embryos, whereas the bleach cream additionally produced subcutaneous hemorrhages in embryos of treated rats. No significant differences were observed between control and treated groups with respect to skeletal anomalies, postimplantation mortality, craniocaudal dimensions and weight of embryos, or placental weights. To study postnatal development, female rats were also given skin applications of the bleach cream throughout pregnancy; another group of rats served as untreated controls. Newborn rats had subcutaneous hemorrhages, retarded development, and reduced body weight. It was concluded that the bleach cream was embryotoxic but nonteratogenic to rats and that the bleach cream product should not be used by pregnant women.[94]

CLINICAL ASSESSMENT OF SAFETY

Experimental Treatment of Melanoma

A pilot study was conducted to determine the clinical usefulness of p-Hydroxyanisole in treating localized malignant melanomas. Eleven patients with

proven primary or secondary malignant melanomas were given intravenous or intraarterial infusions of p-Hydroxyanisole in physiological saline. Duration of treatment varied from 1 day to 57 days; the total antioxidant dose administered varied from 2 g to 154 g. Evidence of cytotoxic action on melanoma cells and tumor regression was observed in 4 of 5 patients given the antioxidant by the intraarterial route. Intravenous administration of p-Hydroxyanisole had no effect. The investigators noted that the metabolism of the antioxidant and its clearance from the blood were very rapid. No "generalized toxic effects" were observed.[47]

The effect of p-Hydroxyanisole on advanced malignant melonomas was examined in a second clinical study. Twelve of twenty-one cutaneous nodules of malignant melanoma directly injected with 12.5 mg of the antioxidant underwent temporary regression. Intravenous administration of p-Hydroxyanisole was well tolerated in 6 patients up to doses of 1.5 g and total doses of 7.5 g; however, nausea was a frequent complaint. A transient drop in peripheral leukocytes was observed in 1 patient. No other side effects were observed, and 1 patient had a "brief partial response."[57]

Case Reports

A 33-year-old woman developed leukoderma of the face following application of an ointment to a chloasma. The ointment contained an unspecified amount of p-Hydroxyanisole. The duration of application was estimated to have been 2 months. No spontaneous repigmentation of the skin occurred during the 6 months after cessation of ointment usage. PUVA therapy resulted in total repigmentation of the treated skin.[99]

A 56-year-old man developed leukoderma of the hands following occupational exposure to acrylate ester and p-Hydroxyanisole (the latter chemical was referred to by the authors as "methoquinone"). "Plaster experiments" with aqueous solutions containing 10, 5, 2, 1, and 0.5 percent "methoquinone" gave positive results for dermatitis at 48, 72, and 96 hours.[100]

Two of eight workers in a vinylidene chloride plant who had been handling p-Hydroxyanisole for 3 to 3½ years developed depigmentation (occupational leukoderma) of the skin of the forearm and the forehead. In 1 worker, the depigmented areas became erythematous when exposed to sunlight.[101]

The same 2 workers were reexamined by O'Sullivan and Stevenson[102] 8 years later. These 2 authors noted a wider area of vitiligo involvement than initially observed by Chivers.[101] Examination by Wood's light revealed vitiligo of the axillae, groin, feet, shins, and neck. Repigmentation of the previously affected areas was noted in both men. A "blood screen," an "organ-specific antibody screen," and liver function test were normal for both individuals.

A total of 248 men from 3 different factories were screened by Wood's light for vitiligo following occupational exposure to p-Hydroxyanisole. At least 1 of the factories had taken "protective measures" to reduce exposure to the antioxidant. No skin depigmentation was observed.[102]

Skin Depigmentation, Sensitization, and Irritation

The skin depigmentation, sensitization, and irritation potential of both 2.0 percent p-Hydroxyanisole in petrolatum and 2.0 percent p-Hydroxyanisole in

sweet almond oil was examined in 80 male and female test subjects aged 18 to 65. Only individuals with a "moderate degree" of skin pigmentation were tested; fair and dark-skinned individuals were excluded from the study. Each test material was applied to the back under an occlusive patch for 48 hours. Upon removal of the patch, the exposed site was wiped free of excess test material. An identical patch was then applied to the same site and the procedure repeated 3 times weekly for 8 successive weeks. Patches applied on Friday remained in place for 72 hours instead of 48 hours. No patch was applied on the eighth Friday. Seven subjects developed skin reactions to the antioxidant in petrolatum. Two subjects had skin reactions to the antioxidant in sweet almond oil. The majority of these reactions were evaluated as "doubtful;" the few remaining reactions consisted of erythema or erythema plus edema. Most reactions appeared after several weeks of exposure and were transient in nature. Reactions typically lasted for only 2 or 3 successive applications. The investigator considered the 2 test materials as nonirritating and nonsensitizing to the skin. Neither test material caused any observable skin depigmentation.[103,104] The CIR Panel notes that a nontreatment period followed by a challenge is considered a more optimal method for detecting sensitization; such a procedure was not followed in this test. Additionally, it was not reported whether or not Wood's light examination was employed in this study. Screening by Wood's light is often necessary to determine evidence of vitiligo.[102]

A repeated insult patch test was conducted on 102 human panelists to assess the skin sensitization and irritation of 5 percent p-Hydroxyanisole in sweet almond oil. The test material was applied to the skin for 48 hours under an occlusive patch. Patches were reapplied every 48 hours for a total of 10 induction applications. Following a 2-week nontreatment period, a challenge patch was applied. The reported number of patches applied to the 102 subjects totaled 1105. No evidence of skin irritation or sensitization was observed.[83,105]

One hundred human subjects were exposed in a skin irritation study to 5 percent p-Hydroxyanisole in sweet almond oil. The methods employed were those described by Fisher.[106] Occlusive patch test results were reported as "negative." No other details were provided.[83,105]

Three cosmetic products were tested for skin irritation according to the procedures described by Fisher.[106] Occlusive patches containing a moisturizing lotion (formulated with 0.1 percent p-Hydroxyanisole), a blusher (0.1 percent p-Hydroxyanisole), and a moisturizing cream (0.05 percent p-Hydroxyanisole) were applied to groups of 99, 100, and 100 human subjects, respectively. Results were reported as "negative." No other details were available.[84-86,105]

Occupational Exposure to Airborne Concentrations

A "threshold limit value" (TLV) for p-Hydroxyanisole of 5 mg/m^3 is recommended by the American Conference of Governmental Industrial Hygienists (1980). This recommendation is made on the basis of "eye and skin effects and by analogy with hydroquinone." The TLV represents the airborne concentration to which nearly all workers may be repeatedly exposed day after day without adverse effect (assuming an 8-hour workday or 40-hour work week). The TLV serves as a general guide in the control of health hazards in the work environment. As such, it should not be used to differentiate between safe and unsafe airborne concentrations.

SUMMARY

p-Hydroxyanisole is a waxy solid prepared by the reaction of hydroquinone with dimethylether. When used for cosmetic purposes, the compound typically has a purity of 99.5 percent. Impurities consist of hydroquinone dimethylether (about 0.1 percent) and an unidentified compound with a "high boiling point" (about 0.4 percent).

p-Hydroxyanisole has acidic properties characteristic of phenols. It binds by hydrogen bonding to itself, water molecules, and various proteins. The compound is readily oxidized and can undergo a variety of reactions, including alkylation, halogenation, and other substitutions on the aromatic nucleus. Peak absorbance of UV light by p-Hydroxyanisole occurs at about 340 nm.

Noncosmetic uses of p-Hydroxyanisole include applications as an antioxidant, as a polymerization inhibitor, as a chemical intermediate, and as a stabilizer. It is used in cosmetics as an antioxidant.

Data submitted to the FDA by cosmetic firms participating in the voluntary cosmetic registration program indicated that this antioxidant was used in 31 cosmetic products during 1981 at concentrations of >0.1 to 1.0 percent (8 products) and ≤0.1 percent (23 products). Cosmetic formulations containing this compound, such as eye makeup, sachets, makeup bases, and skin care preparations, are normally applied to or have the potential to come in contact with the skin and eyes.

Results of numerous studies indicated that p-Hydroxyanisole is a skin-depigmenting agent. Unpublished data strongly suggested that this cosmetic ingredient was a depigmenter of the skin at concentrations approximating those used in cosmetic products. Skin depigmentation was observed in guinea pigs exposed 6 weeks to 0.25 percent of the antioxidant and in guinea pigs exposed 6 months to 0.5 and 1.0 percent p-Hydroxyanisole. Exposure for 6 weeks to 0.1 percent produced depigmentation at the site of skin application in 1 of 6 guinea pigs. Associated with the skin-depigmenting action of this compound was a selective cytotoxic effect on the melanocyte. The melanocytotoxic effect was dependent upon both antioxidant concentration and duration of exposure. No cytotoxic effects on human melanocytes or morphological changes in human keratinocytes were observed following a 45-minute exposure to either 10^{-2} M or 10^{-3} M p-Hydroxyanisole in disperse tissue culture. However, whole epidermis (human) exposed in vitro to 10^{-1} M for 1, 5, and 24 hours had extensive damage to melanocytes and keratinocytes. Concentrations as low as 10^{-8} and 10^{-9} M were cytotoxic to guinea pig melanocytes in vitro. These latter concentrations are lower than p-Hydroxyanisole concentrations typically used in cosmetics.

p-Hydroxyanisole given orally to rats and mice caused induction and inhibition of various enzymes in the esophagus, nonglandular stomach, and microsomal fraction of the liver. In vitro studies with isolated rat liver suggested that the antioxidant interferes with ribonucleic acid synthesis, protein synthesis, and mitochondrial respiration. The compound inhibited growth or was microcidal in studies with bacteria and fungi. Chromosomal aberrations in plants and denaturation of DNA in bacteriophage were observed following p-Hydroxyanisole exposure.

p-Hydroxyanisole was absorbed by guinea pig skin in vitro. Oral doses of the antioxidant were excreted by rabbits primarily as conjugates of glucuronic and sulfuric acids; small amounts were demethylated and excreted as hydroquinone.

The acute oral LD_{50} of p-Hydroxyanisole in rats was estimated as 1630 mg/kg. The oral LD_{50} in rats of 50 percent p-Hydroxyanisole in corn oil was 740 mg/kg. The acute LD_{50} of the antioxidant when administered by intraperitoneal injection was 250 mg/kg and 430 mg/kg for mice, 730 mg/kg for rats, and 720 to 970 mg/kg for rabbits.

Undiluted p-Hydroxyanisole was a severe skin and ocular irritant in rabbits; a single exposure to the compound produced extensive skin edema and necrosis and corneal injury. Minimal irritation was observed in the eyes of rabbits exposed to a 0.1 percent aqueous solution of the antioxidant and on rabbit skin treated with 5 percent p-Hydroxyanisole in sweet almond oil. Skin sensitization to p-Hydroxyanisole (0.5 M and 1.0 M) was observed in guinea pigs in both the "maximization test" and the "Freund's complete adjuvant test." Cross skin sensitization of guinea pigs to hydroquinone (1 M) and p-Hydroxyanisole (3 M) was also reported. No photosensitization was observed in guinea pigs exposed to both p-Hydroxyanisole (0.1 and 1.0 percent) and UV irradiation.

Application of a water-oil emulsion containing 1.0 percent p-Hydroxyanisole to the skin of guinea pigs for 30 days produced hyperemia, edema, and desquamation. Skin irritation and depigmentation were observed in guinea pigs and mice treated for 4 weeks with 20 percent p-Hydroxyanisole in petroleum jelly and in guinea pigs treated 1 to 6 months with antioxidant concentrations of 0.25 M or 1.0 M in acetone, 0.5 M in dimethylsulfoxide, and 5.0 or 10.0 percent in hydrophilic ointment. Application of 20 percent p-Hydroxyanisole in lanolin base to guinea pig skin for up to 6 months and to hamster cheekpouch 3 times a week for 45 days caused encroachment of basal cell pseudopodia into the dermis. In addition, the hamster cheekpouch had erythema, hyperkeratosis, epithelial hyperplasia, bullae, and muscular degeneration. Rats and rabbits fed diets containing 5 and 10 percent p-Hydroxyanisole and dogs fed up to 12 g daily for 2 weeks had growth inhibition and changes in hematological parameters and organ weights; no other significant toxicological effects were noted.

p-Hydroxyanisole was nonmutagenic in the Ames assay with and without metabolic activation. No local toxic changes or tumors were observed following application of 5 and 10 percent p-Hydroxyanisole in acetone to the skin of mice and rabbits in a lifetime study. The antioxidant (13.1 percent in benzene) was inactive as a tumor promoter when applied for 20 weeks to the DMBA-initiated skin of mice. Application of a bleach cream containing 5 percent p-Hydroxyanisole and a water-oil emulsion containing 25 percent of the antioxidant to the skin of pregnant rats produced embryotoxicity but not teratogenicity.

In clinical studies, p-Hydroxyanisole at a concentration of 2.0 percent in petrolatum and 2.0 percent in sweet almond oil was, at most, minimally irritating to the skin. A 5.0 percent concentration of the antioxidant in sweet almond oil was both nonirritating and nonsensitizing to humans. Several cases were reported in the literature of individuals who developed skin depigmentation following exposure to products containing p-Hydroxyanisole or following occupational exposure to the antioxidant.

DISCUSSION

A few of the available studies on p-Hydroxyanisole were conducted in the 1940s and 1950s and were exploratory in nature. The Panel is aware that many of

these studies do not necessarily reflect current toxicological procedures. In other studies, details of methods, results, and dates of testing were not specified. As a result, the quantity and quality of available information for review was limited. However, it is the Panel's opinion that the sum total of available published and unpublished data support a concern as to the safety of p-Hydroxyanisole as a cosmetic ingredient.

Results of animal studies establish that p-Hydroxyanisole is a skin sensitizer (6.2 percent) and a skin depigmenter (0.25 percent). Results of studies with humans suggest that p-Hydroxyanisole is not a skin sensitizer (2.0 percent). Clinical data on skin depigmentation are inconclusive.

The function of p-Hydroxyanisole in cosmetics is that of an antioxidant. It is not intended for use as a skin lightener or skin-depigmenting agent. Because of the depigmenting action of this compound in black guinea pigs at reported concentrations approaching those used in cosmetics and because of in vitro toxicity to guinea pig melanocytes, p-Hydroxyanisole is an undesirable ingredient in cosmetic products.

CONCLUSION

Based on the available animal data, the CIR Expert Panel concludes that p-Hydroxyanisole is unsafe for use as a cosmetic ingredient.

ACKNOWLEDGMENT

Jonathon T. Busch, Senior Science Analyst and writer, prepared the literature review and technical analysis used by the Expert Panel in developing this report.

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