JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 5, Number 5, 1986
Mary Ann Liebert, Inc., Publishers

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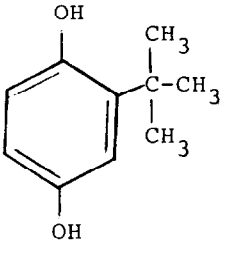
Final Report on the Safety Assessment of t-Butyl Hydroquinone

The safety of this ingredient has not been documented and substantiated. The Cosmetic Ingredient Review Expert Panel cannot conclude that t-Butyl Hydroquinone is safe for use in cosmetic products until such time that the appropriate safety data have been obtained and evaluated. The data that were available are documented in the report as well as the types of data that are required before a safety evaluation may be undertaken.

CHEMISTRY

Definition and Structure

t-Butyl Hydroquinone (CAS No. 1948-33-0) is the aromatic organic compound that conforms to the formula[1]:



This cosmetic ingredient is also known as tert-butylhydroquinone, tertiary-butylhydroquinone, 2-tert-butylhydroquinone, t-butylhydroquinone, monotertiary butylhydroquinone, mono-tertiarybutylhydroquinone, mono-tertbutylhydroquinone, TBHQ, MTBHQ, 1,4-dihydroxy-2-t-butylbenzene, tert-butyl-1,4-benzenediol, 2-tert-butyl-1,4-benzenediol, 2-(1,1-dimethylethyl)-1,4-benzenediol, (1,1-dimethylethyl)-1,4-benzenediol, and Tenox TBHQ.[1-10]

Properties

t-Butyl Hydroquinone (MW 166.22) is a white to light tan, free-flowing, crystalline solid having a very slight but characteristic odor. The melting point ranges from 125 to 130°C depending on the grade and purity of the material; cosmetic-grade t-Butyl Hydroquinone has a melting range of 126.5 to 128.5°C and a boiling point of 295°C. It is soluble in alcohol, acetone, ethyl acetate, ether, and a wide range of fats and oils; it is insoluble in water.[2-4,7,11,12] The solubility characteristics of a purified grade of t-Butyl Hydroquinone in various substances are listed in Table 1.

TABLE 1. Solubility Characteristics of Purified t-Butyl Hydroquinone[11]

Solvent	*Approximate solubility (weight percent)*
Water	Insoluble
Ethyl alcohol	60
Acetone	112
Propylene glycol	30–50
Corn oil	5–10
Lard	3
Glyceryl mono-oleate	10

t-Butyl Hydroquinone is a well-known antioxidant.[2,5-7,11,13-34] It has been suggested that the para-positioned hydroxyl groups, which are quite reactive with oxygen, account for the compound's antioxidant properties.[11]

Studies relating to t-Butyl Hydroquinone and its volatility and decomposition under "frying conditions," gel chromatographic behavior, and autoxidation have been reported.[35-40] The results from one study indicated that fluorescent light accelerates the autoxidation of this compound.[41]

Reactivity

t-Butyl Hydroquinone reacts readily with oxidizing agents to give related quinones.[2]

Analytical Methods

t-Butyl Hydroquinone may be determined by volumetric analysis,[42] fluorimetric and densitometric techniques,[43] colorimetric methods,[7] gel permeation chromatography,[44] thin layer chromatography,[43,45] high performance liquid chromatography,[46-49] and gas and gas–liquid chromatography.[45,50-55] Analytical methods to determine the content of various impurities in t-Butyl Hydroquinone have also been reported.[56-59]

Method of Manufacture

t-Butyl Hydroquinone can be prepared by the acid-catalyzed reaction of hydroquinone with isobutylene or t-butanol.[2,12,60] A method has also been described by Hervert[61] in which hydroquinone is alkylated with isobutene in the presence of a hydrogen fluoride–carbon dioxide complex. After filtration, the solvent and unreacted hydroquinone are removed by distillation, and t-Butyl Hydroquinone is recovered.

Impurities

Both cosmetic and food-grade t-Butyl Hydroquinone (assay: not less than 99.0% of $C_{10}H_{14}O_2$) have the following impurities[2,7,12]:

t-Butyl-*p*-benzoquinone	0.2% maximum
2,5-di-t-Butylhydroquinone	0.2% maximum
Hydroquinone	0.1% maximum
Toluene	0.0025% maximum
Heavy metals (as lead)	10 ppm maximum
Arsenic	3 ppm maximum

USE

Cosmetic Use

t-Butyl Hydroquinone is used in cosmetics as an antioxidant.[2,62] Data submitted to the Food and Drug Administration (FDA) in 1981 by cosmetic firms participating in the voluntary cosmetic registration program indicated that t-Butyl Hydroquinone was used that year as an ingredient in 266 cosmetic formulations at concentrations of >0.1–1% (19 products) and ≤0.1% (247 products) (Table 2). The greatest reported use of the antioxidant was in lipstick (242 products).[63]

Voluntary filing of product formulation data with 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.[64] Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the concentration reported by the cosmetic formulator may not necessarily reflect the true, effective concentration found in the finished product; the effective 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 two- to ten-fold error in the assumed ingredient concentration.

Cosmetic products containing t-Butyl Hydroquinone are applied to or have the potential to come in contact with skin, eyes, and hair. Small amounts of the antioxidant may be ingested from lipstick (Table 2).[63]

TABLE 2. Product Formulation Data for t-Butyl Hydroquinone[63]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)[a]	
			>0.1–1	≤0.1
Eye shadow	2582	1	–	1
Colognes and toilet waters	1120	7	–	7
Perfumes	657	3	–	3
Blushers (all types)	819	3	–	3
Lipstick	3319	242	18	224
Rouges	211	2	–	2
Other makeup preparations (not eye)	530	3	–	3
Depilatories	32	1	1	–
Moisturizing skin care preparations	747	4	–	4
1981 TOTALS		266	19	247

[a]Preset categories and concentration ranges in accordance with federal filing regulations (21 CFR 720.4).

Product formulations containing t-Butyl Hydroquinone can be applied as infrequently as once a week to as often as several times a day. Many of these products have the potential to remain in contact with body surfaces for several days and to be applied hundreds of times over the course of several years (Table 2).[63]

Noncosmetic Use

Because of its antioxidant properties, t-Butyl Hydroquinone is used in fats, oils, and meat products to prevent oxidative rancidity.[5–7,11,33,34] Federal regulations permit the use of t-Butyl Hydroquinone as a food additive provided that the total antioxidant content does not exceed 0.02% of the oil or fat content of the food.[65] Astill et al.[66–68] have reviewed the toxicology and biochemical studies in the petition to establish this food additive regulation.

t-Butyl Hydroquinone has been used to retard polymerization of various polyunsaturated polyesters.[11] A federal regulation has been established that allows use of the compound as a polymerization inhibitor in cross-linked polyester resins that come in repeated contact with food, provided that the quantity of t-Butyl Hydroquinone "does not exceed that reasonably required to accomplish the intended physical or technical effect."[69]

Federal regulations allow use of t-Butyl Hydroquinone as an antioxidant in pesticide formulations. When used as an inert chemical in pesticides for raw agricultural commodities, the antioxidant is exempt from the requirements of a tolerance limit.[70]

Concentrations of 2–5% t-Butyl Hydroquinone have been effective in protecting the color of phenylenediamines or aminophenol compounds that are used as "sweeting accelerators" and antioxidants in gasoline.[11,71] The compound is also a potent antioxidant at concentrations of 0.01–1.0% for oxidizable organic materials, such as chlorinated hydrocarbons.[11] Claims have been made that t-Butyl Hydroquinone is useful for stabilizing polyethylene glycol[72] and polypropylene glycol butyl ether[73] and that it is effective in fly repellents for stabilizing pyridine-dicarboxylic acid esters against ultraviolet exposure.[11,74] One early report suggested that the compound could be used as a body deodorant.[75]

BIOLOGY

Antimicrobial Properties

Results of tests with the antioxidant in both solid and liquid media indicated that a t-Butyl Hydroquinone concentration of 300 ppm was inhibitory to a broad spectrum of gram-negative and gram-positive organisms.[76] A concentration of 25 ppm in trypticase soy broth totally inhibited growth of *Staphylococcus aureus*,[77–79] whereas an antioxidant concentration of 400 ppm in the same growth medium had little or no effect on *Salmonella typhimurium*.[79] Inhibition of growth was observed after exposure of *Clostridium botulinum* to t-Butyl Hydroquinone concentrations of 200–400 μg/ml and exposure of *Saccharomyces cerevisiae* to 200 μg t-Butyl Hydroquinone.[80,81] A synergistic combination of potassium sorbate and t-Butyl Hydroquinone was effective in inhibiting growth of *S. aureus*.[77–79] In two separate studies, t-Butyl Hydroquinone in growth medium at 26 ppm and 0.7 μg/ml inhibited *Tetrahymena pyriformis* growth by 50%.[82,83] Several patents have been issued claiming antifungal and antibacterial properties for this compound.[84–86]

Effect on Cellular Function

Surak[87] added t-Butyl Hydroquinone to cultures of *T. pyriformis*, a ciliated protozoan, at concentrations ranging from 0 to 60 ppm. As the concentration in growth medium increased, cellular contents of tetrahymenol and phosphorus-containing lipids decreased, as did the molar ratio of tetrahymenol to phosphorus-containing lipids. In addition, increasing concentrations of the antioxidant altered the ratio of polar lipids synthesized.

In a second study by Surak,[82] the antioxidant was added to *T. pyriformis* in growth medium at concentrations of 0 to 40 ppm. As the concentration of t-Butyl Hydroquinone was increased to 40 ppm, the synthesis of lipids, protein, RNA, and DNA decreased. Increasing concentrations of t-Butyl Hydroquinone also diminished the oxidation of 1-^{14}C-acetate to $^{14}CO_2$. In addition, the incorporation of ^{14}C-acetate into glycogen increased with concentrations up to 20 ppm, whereas glycogen synthesis decreased at 40 ppm t-Butyl Hydroquinone.

Effect on Mixed-Function Oxidases

Results from both short-term and long-term feeding studies indicate that t-Butyl Hydroquinone does not significantly induce microsomal mixed-function oxidases in rat or dog liver. When the antioxidant was fed to rats at a dietary concentration of 0.2% for 21 days, activities of *p*-nitroanisole demethylase and aniline hydroxylase were slightly increased. These slight elevations probably represented "acclimation." No change in glucose-6-phosphatase activity was observed. In long-term experiments with rats (6, 11, and 20 months) and dogs (2 years), diets containing up to 0.5% t-Butyl Hydroquinone produced no appreciable deviations in enzymatic activities from control values. Further, there was no proliferation of hepatic smooth-surfaced endoplasmic reticulum and no hepatic enlargement.[66]

Influence on the Toxic Effects of Other Substances

Astill and Mulligan[88,89] reported that t-Butyl Hydroquinone inhibited N-nitrosodimethylamine-induced hepatotoxicity in rats. Sodium nitrite (125 mg/kg) and dimethylamine (1000 mg/kg) were given by gastric intubation, followed immediately by t-Butyl Hydroquinone in doses of 25, 75, or 225 mg/kg. Indices of N-nitrosamine formation 48 h after dosing were the activities of serum glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and ornithine-carbamoyl transferase (OCT) and the extent of hepatic necrosis. The nitrosamine-forming mixture alone produced extensive hepatic necrosis and 24-, 19- and 4-fold increases in serum GOT, GPT, and OCT activities over control animals given corn oil. At 225 mg/kg, t-Butyl Hydroquinone provided a "60 percent protection" against hepatic necrosis and significantly suppressed the increases of enzymatic activity caused by the nitrosamine-forming mixture. Antioxidant concentrations of 25 and 75 mg/kg were neither protective nor lethal. The investigators suggested that when used as a food antioxidant, t-Butyl Hydroquinone would unlikely promote N-nitrosamine formation in the stomach and, under low or diminished nitrite intake, could completely inhibit any N-nitrosamine formation.

Pagnotto and Epstein[90] reported that in contrast to certain other antioxidants, t-Butyl Hydroquinone does not confer protection against free-radical, ozone-induced toxicity. Four daily intraperitoneal injections of 10 mg t-Butyl Hydroquinone suspended in saline or tricaprylin were given to Swiss ICR/HA mice. One hour following the last injection of antioxidant, the animals were exposed to 8.8–10.4 ppm ozone for 4 h in a closed chamber. No significant differences in mortality were noted between t-Butyl Hydroquinone-treated animals and controls.

Inhibition of Neoplasia

Wattenberg et al.[91] reported the t-Butyl Hydroquinone added to the diet of mice suppressed benzo(a)pyrene (BP)-induced neoplasia of the nonglandular stomach. Twenty female ICR/HA mice were fed a diet containing the antioxidant at a concentration of 0.03 mmol/g of chow. On the eighth day of this diet, the

animals were given the first of eight doses (two times a week for four weeks) of 1 mg BP in 0.2 ml of corn oil by oral intubation. The experimental diet was fed during the entire period of carcinogen administration and was discontinued 3 days after the last dose of BP, at which time the mice were 98 days old. The animals were then fed a normal diet of chow. The mice were killed for necropsy when 211 days old. In the control group given BP but no dietary antioxidant, 15 of 16 mice developed an average of 4.2 ± 0.6 tumors of the stomach. In the test group (20 mice) given both BP and t-Butyl Hydroquinone, 17 of 20 mice developed an average of 2.4 ± 0.6 tumors of the stomach.

Effect on Prostaglandin Biosynthesis

Boehme and Branen[92] found that low concentrations of t-Butyl Hydroquinone significantly interfered with prostaglandin biosynthesis in vitro. A concentration of 5.49 μM of t-Butyl Hydroquinone caused a 50% inhibition of prostaglandin E_1 synthesis by the microsomal fraction of bovine seminal vesicles, whereas a concentration of 6.07 μM resulted in a 50% inhibition of prostaglandin E_2 synthesis.

Effect on Hepatic Peroxidation

t-Butyl Hydroquinone protected whole rat liver homogenate from lipid peroxidation. Antioxidant concentrations of 1 and 5 μM inhibited peroxidation 20 and 100%, respectively.[93]

Metabolism, Excretion, and Storage

Fate in Rats and Dogs

Investigations by Astill et al.[66] established urinary excretion as the principal route of elimination in rats and dogs for orally administered t-Butyl Hydroquinone (Table 3). In 3–4 days, rats given single oral doses of 100–400 mg/kg eliminated in the urine the O^4-sulfate (57–80%) and the O^4-glucuronide (4%), with 4–12% of the dose remaining unchanged. Both the proportions eliminated in the urine and the rate of elimination decreased as doses were increased. In dogs, single doses of 100 mg/kg given in meat capsules were also principally eliminated in the urine as O-sulfate conjugates, O-glucuronide, and unchanged t-Butyl Hydroquinone. When compared to the rat, less of the dose was accounted for as sulfate and more as glucuronide. Results of additional studies indicated that both sulfate conjugation and O-glucuronide conjugation occurred with the 4-hydroxyl group.

The effect of prolonged feeding of t-Butyl Hydroquinone on the overall metabolic pattern also was monitored by Astill et al.[66] After 2 years of feeding the antioxidant to dogs at concentrations up to 0.5% of the diet, patterns of metabolites excreted in the urine remained unaltered from that described for the single oral dose. In contrast, the proportion of glucuronide was elevated somewhat in rats fed up to 0.5% t-Butyl Hydroquinone in the diet for 20 months. Chromatograms of urine from both rats and dogs had the same metabolites throughout the study.

TABLE 3. Disposition of Single Oral Doses of TBHQ by Rats, Dogs, and Male Humans[a(66)]

Species	Dose (mg/kg)	No. of animals	Percent of dose eliminated in urine[b]				In feces	As CO_2	During (days)	Total accounted for
			Unchanged	O-SO_2O^-	O-glucuronide	Total				
Rat	100	2 × 6	(12)	80	4	---	---	---	3	96
	200	2 × 6	0.6–8.0	60,61	2, 8.5	---	---	---	3	70
		1 × 8	---	---	---	---	---	---	---	---
	400	2 × 6	4	57	4	---	---	---	4	65
Rat	15–920	3	0.8–1.3[c]	57–74[c]	0.9–3.3[c]	82–88	2.4–3.7	<1	4	86–92[d]
(TBHQ-^{14}C)	380,400	2	---	---	---	74–80	6.0,6.0	<1	4	80,86[d]
Dog	100	2 × 3	3	69,85	31,24	---	---	---	3	100,112
Humans (HF)	2	2	<0.1	73,88	15,22	---	---	---	2–3	95,103
(LF)	1–2	8	<0.1	18–51	0–6	---	---	---	2–3	18–51

[a]Values are percent of given dose as averages or ranges. Rats received oral intubation of a 10% corn oil solution of TBHQ; dogs received TBHQ as a meat capsule; humans received TBHQ incorporated in high-fat (HF) or low-fat (LF) content food.

[b]O-SO_2O^-, increased O-sulfate output; O-glucuronide, increased O-glucuronide output.

[c]In 24 h after dose.

[d]Tissues, organs, and carcass account for <0.2 % of the dose.

Experiments with ^{14}C-t-Butyl Hydroquinone confirmed test results obtained with the nonradioactive compound (Table 3). As with the nonradioactive material, single oral doses of the radioactive compound were eliminated in the urine of rats in the first 1–2 days of dosing. The primary metabolite was the O-sulfate conjugate, which accounted for over 90% of the 24-h urinary radioactivity. The proportion of the radioactive dose eliminated through the urine decreased with increasing intake, as was the case with the nonradioactive material. The amount of ^{14}C-labeled CO_2 in expired air was negligible (<0.1%), indicating that t-Butyl Hydroquinone was not handled by intermediary metabolism.[66]

In feeding studies with dogs, Astill et al.[94] identified t-Butyl Hydroquinone as the major metabolite of the food antioxidant butylated hydroxyanisole (BHA). Chromatograms of the urine of dogs receiving BHA or t-Butyl Hydroquinone were almost identical when sprayed with Gibbs' reagent for phenols. It was later suggested by Astill et al.[66] that the presence of t-Butyl Hydroquinone as a metabolic product of BHA "implies the existence of a prior indirect evaluation of its behavior in animal feeding studies."

Fate in Humans

Astill et al.[66] found that t-Butyl Hydroquinone was metabolized similarly in humans as it is in rats and dogs (Table 3). Single doses (0.5–4.0 mg/kg) of the compound in food were given to men. Cumulative excretion of combined and free t-Butyl Hydroquinone indicated that urinary recoveries of the antioxidant depended on the vehicle. In a food medium containing 30% corn oil (high-fat vehicle), almost all of the dose was recovered in the urine within 40 h. In a food medium containing 10% corn oil (low-fat vehicle), urinary excretion accounted for less than half the intake concentrations of the compound in serum, as measured spectrophotofluorometrically. It was concluded that t-Butyl Hydroquinone in a high-fat vehicle was almost entirely absorbed, whereas absorption was much lower in a low-fat vehicle. Single antioxidant doses of 2 mg/kg in a high-fat vehicle were eliminated almost completely in the urine in 2–3 days, with <0.1% unchanged, 73–88% as the 4-O-sulfate, and 15–22% as the 4-O-glucuronide. The authors suggested that in dogs, rats, and humans, t-Butyl Hydroquinone was metabolized by the same biochemical pathways that are commonly found for dihydric phenols in mammals.

Antioxidant Storage in Rat and Dog Tissue

Radioactivity in the liver, kidney, brain, and fat of rats after either single oral doses of radioactive t-Butyl Hydroquinone or after "low-level" dietary feeding of the radioactive compound for 17 days was "inconsequential." Normal background radioactivity in rats, expressed as μg of t-Butyl Hydroquinone/g wet tissues, was: liver, 0.06–0.34; kidney, 0.09–0.38; brain, 0.06–0.56; and fat, 0.06–0.37. Radioactivity of the experimental animals was within background or was less than twice background. The investigators concluded that a short-term feeding of the antioxidant produced no body burden.[66]

Results of long-term feeding studies with rats and dogs indicated no significant accumulation of t-Butyl Hydroquinone (TBHQ) in tissues or organs (Table 4). When radioactive t-Butyl Hydroquinone was fed to rats for 20 months at concentrations of up to 0.5% of the diet, radioactive residues in liver, kidney,

TABLE 4. TBHQ in Tissues and Organs of Rats and Dogs Fed the Antioxidant for 20 Months and 2 Years, Respectively[a(66)]

	Intake			*ng TBHQ in organ*			*Mg TBHQ*
Species	*%*	*Total (g)*	*Sex*[b]	*Liver*	*Kidney*	*Brain*	*in fat*[c]
Dog	0.05	110	M(4)	15	ND[d]	31	4
			F(4)	100	4	ND	ND
	0.16	350	M(4)				5
			F(4)				5
	0.5	1100	M(4)	80	ND	ND	ND
			F(4)	2	ND	7	ND
Rat	0.16	21	M(5)	ND	ND	2	ND
		16	F(5)	9	ND	0	ND
	0.5	63	M(4)	ND	4	ND	ND
		47	F(4)	3	7	ND	ND

[a]Based on average net fluorescence of extractives at sacrifice 24 h after last intake.
[b]Numbers in parentheses are animals whose tissues were analyzed at each level. Organs and fat samples were pooled for each assay.
[c]Estimated total body fat of rats, 30 g; of dogs, 1 kg.
[d]ND, None detected.

brain, and fat were either less than background or negligibly increased. When the radioactive material was fed to dogs at the same concentration for 2 years, small quantities (2–100 ng) were found in the liver. The investigators suggested that these quantities were consistent with the fact that the dogs were killed a few hours after the last antioxidant dose. They also suggested that 4–5 ppm found in fat were a very minute fraction of the total intake and probably originated from circulating t-Butyl Hydroquinone in the vasculature.[66]

Toxicology

Acute Oral Toxicity

The acute oral LD_{50} in rats of t-Butyl Hydroquinone as a 5.0–10.0% suspension in corn oil was between 700 and 1000 mg/kg.[66] The oral LD_{50} of the antioxidant in rats was also reported as 480 mg/kg[83] and 800 mg/kg.[95] According to the classification system of Hodge and Sterner,[96] a test material with a single-dose, oral LD_{50} in rats of 50–500 mg/kg is "moderately toxic," whereas a substance with an oral LD_{50} in rats of 500–5000 mg/kg is considered "slightly toxic."

The acute oral LD_{50} in mice was 1000 mg/kg.[95] In studies with dogs, no estimate of the oral LD_{50} was obtained, since doses of 400 mg/kg or higher caused vomiting with loss of the dose.[66]

Intraperitoneal Administration

The intraperitoneal LD_{50} in rats of t-Butyl Hydroquinone as a 5.0 or 10.0% suspension in corn oil was between 300 and 400 mg/kg.[66]

t-Butyl Hydroquinone was tested for its potential to produce lung damage in mice. Groups of 10 male mice (CRL:CD-1) were given single intraperitoneal injections of 62.5, 125, 250, or 500 mg/kg t-Butyl Hydroquinone (10% in corn oil). Survivors were killed 5 days after treatment, and the lungs were removed, weighed, and processed for microscopic examination. Doses of 250 and 500 mg/kg killed 9 of 10 and 10 of 10 mice, respectively, within 0.5 h of injection. One mouse given 125 mg/kg died within a few hours of treatment, and three mice were found dead on Day 4 of the study. All animals given 62.5 mg/kg survived; thus, the LD_{50} over a 5-day period was 144 mg/kg. Lung weights of the antioxidant-treated mice were comparable to those of controls. No treatment-related lung lesions were observed.[97]

Skin Irritation/Skin Sensitization

Astill et al.[66] reported that t-Butyl Hydroquinone was neither a skin irritant nor a skin sensitizer. Details of the study to establish the irritating ability of this compound were not available.

Subchronic Dermal Toxicity

A hair dye formulation containing 0.3% t-Butyl Hydroquinone was evaluated for systemic toxicity in 12 (6F, 6M) New Zealand albino rabbits. The formulation was mixed with an equal volume of 6% hydrogen peroxide before its application to the clipped skin twice weekly for 13 weeks. The applied dose was 1 ml/kg of the 1:1 oxidation mixture. The application sites on 3 animals of each sex were abraded on the first treatment day of each week. The rabbits were restrained in holding stocks for 1 h after application. Animals were weighed weekly. Hematological and clinical chemistry determinations and examination of the urine were performed at 0, 3, 7, and 13 weeks. These studies included determination of complete blood count, methemoglobin, fasting blood sugar, blood urea nitrogen, alkaline phosphatase, and serum glutamic oxaloacetic transaminase. Urine was examined for color, pH, albumin, glucose, occult blood, and sediment. Survivors were killed after 13 weeks and examined for gross abnormalities. Organ:body weight ratios were determined for liver, kidneys, adrenals, heart, thyroid, spleen, and brain. Twenty-five selected tissues from each animal were examined microscopically. No evidence of formulation-induced toxicity was seen. Body weight gain, relative organ weights, and results of urinalysis of test animals were comparable to those of controls. No gross abnormalities or microscopic lesions were observed that were attributed to formulation administration. Alkaline phosphatase was significantly lower in both males and females as compared to controls, whereas hemoglobin percentage and red blood cell count in females were significantly higher than in controls. These differences were not considered of toxicological significance because of either the direction or continuity of the difference or the fact that they fell within the range of historical control values. The treated skin was slightly thickened, but this was not unexpected due to the frequency of product application. It was concluded that the hair dye formulation containing 0.3% t-Butyl Hydroquinone caused no

systemic toxicity when applied to the skin of rabbits twice a week for 13 weeks.[98]

Subchronic and Chronic Oral Toxicity

In a preliminary 22-day feeding study in rats, t-Butyl Hydroquinone at a concentration of 1.0% of the diet produced a slight impairment of weight gain but no mortality or lesions.[66]

t-Butyl Hydroquinone was fed to 275 male and 275 female Sprague-Dawley rats for 20 months at 0.016, 0.05, 0.16, and 0.5% of the diet. Animals were killed for necropsy at 6, 12, and 20 months. No differences between treated animals and controls were observed in behavior, growth rate, feed intake, diet efficiency, or mortality, and no differences were found at necropsy with respect to absolute or relative weights of lungs, liver, kidneys, heart, adrenals, testes, or spleen. In male rats fed 0.5% and killed at 20 months, a slight decrease from controls in absolute brain weights was noted; however, this decrease was not associated with lesions or behavioral change. Hematocrit, hemoglobin, leukocyte count, and differential counts were determined after 5 months, and all values were normal. Clinical chemistry studies done at the same time for serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SAP), blood urea nitrogen (BUN), and total protein were also normal. Urinalyses, consisting of specific gravity, pH, albumin, sugar, and microscopic examinations, were conducted at termination on female and male survivors in the two high-intake groups and controls; the results were normal. At the time of death or sacrifice, gross and microscopic examinations were made of trachea, lungs, heart, tongue, esophagus, stomach, small intestine, large intestine, liver, kidney, bladder, adrenals, pancreas, thyroid, gonads, uterus, spleen, bone marrow, cerebrum, cerebellum, and eyes. No compound-related changes were found in any of the organs examined. The most frequent tumors seen were cystic fibroadenomas or mammary gland fibroadenomas; however, neoplasms occurred randomly in all groups, including controls, with no relationship to dose.[66]

A 6-month feeding study was conducted to test the effect of heated t-Butyl Hydroquinone on groups of 30 rats (15 males and 15 females per group). The test ingredient was added to cottonseed oil at concentrations of 0.02, 0.10, or 0.5%. Cottonseed oil with added t-Butyl Hydroquinone was unheated or was heated for 1 h to 375°F and held at this temperature for 3 h. These oils were then incorporated into the diet at a concentration of 5.0%. The same parameters as described directly above in the 20-month rat feeding study were evaluated. The growth, behavior, feed intake, urinalyses, hemograms, clinical chemistries, and mortality were "almost all normal," as were the absolute and relative organ weights at necropsy. A slight increase in relative mean liver weight was observed in the 0.5% group as compared to controls. In addition, SGOT activity was slightly decreased at both 3 and 6 months in the male animals given 0.5% t-Butyl Hydroquinone in cottonseed oil. However, this depressed value was not associated with any pathological changes or changes in alkaline phosphatase. Decreased SGOT activities were not seen in the females or in rats treated with the lower concentrations of oil-heated t-Butyl Hydroquinone. The authors noted that a slight statistical decrease in SGOT was not evidence of cellular damage.[66]

Forty Beagle dogs were selected for a 2-year feeding study. Twenty-four of the animals were divided into three groups of 8 (4 males and 4 females/group) and fed diets containing either 0.05, 0.158, or 0.5% t-Butyl Hydroquinone. A group consisting of 8 male and 8 female dogs served as control. Blood and urine were examined twice before initiation of the study and at five intervals throughout the study. Hematological and serum chemistry measurements consisted of hemoglobin, hematocrit, white cell and differential counts, platelet and reticulocyte counts, blood urea nitrogen (BUN), glucose, serum alkaline phosphatase (SAP), lactic dehydrogenase (LDH), protein, total and direct bilirubin, albumin, albumin:globulin (A:G) ratio, and serum glutamic pyruvic transaminase (SGPT). Slight depression of hemoglobin and hematocrit values were observed in the 0.5% group but were within the normal range for these animals. This group also had an increased reticulocyte count during the 99th and 104th week, as did two females in the control group. The final reticulocyte count, however, suggested no pattern relating to t-Butyl Hydroquinone intake. Urinalyses were all normal and included measurements for glucose, albumin, specific gravity, pH, occult blood, ketone bodies, urobilinogen, and sediment. At termination of the study, organ weights of the high intake group were comparable to controls for liver, kidneys, spleen, heart, brain, lung, gonads, adrenal, thyroid, and pituitary. The liver, spleen, gallbladder, stomach, intestines, pancreas, kidneys, urinary bladder, adrenals, gonads/adnexa, pituitary, thymus, thyroid, salivary glands, lymph nodes, heart, lung, marrow, aorta, skin, muscle, spinal cord, and brain from all control and experimental dogs were histologically normal. In addition, kidneys and liver of the 0.5% group animals were examined by electron microscopy and found normal.[66]

Mutagenicity

In studies by Ishizaki et al.,[99] t-Butyl Hydroquinone had "notable mutagenicity" in both the "rec-assay" with wild and recombinationless strains of *Bacillus subtilis* and in the "sensitivity test" with wild and rad mutant strains of yeast.

Teratogenicity

Krasavage[100] found that t-Butyl Hydroquinone produced no teratogenic effects when administered in the diets of pregnant rats. Three groups of pregnant Sprague-Dawley rats (20 animals/group) received diets containing either 0.125 0.25, or 0.50% t-Butyl Hydroquinone. Total antioxidant doses of 970, 1878, and 3,599 mg/kg, respectively, were administered from the sixth to the sixteenth day of gestation. Mean body weight gain and feed consumption of treated dams were comparable to controls. Average number of corpora lutea, implantation sites, viable fetuses, resorptions and body weight of the fetuses per litter, fetal mortality, and sex ratio were unaffected at all doses. A total of 849 fetuses were examined for external anomalies, and all were normal. Approximately half of these fetuses were examined for internal soft tissue anomalies, and 3 were found abnormal. Of these 3 fetuses, 2 were from one litter each of the high dose (0.5%) group and 1 from the control group. The 2 abnormal fetuses of the 0.5% treated group had hydrocephalus, and 1 also had a transposition of the major blood vessels. This latter fetus (a male) had a body weight of 2.2 g as compared to the mean weight of his group (3.9 g) and that of the control group (4.0 g). The 1 ab-

normal fetus (female) from the control group was also unusually small (2.4 g) and had hydrocephalus. None of the abnormalities in the antioxidant-exposed fetuses were attributed to t-Butyl Hydroquinone treatment. Six of the total number of fetuses examined for skeletal variations had 10th, 11th, or 12th thoracic centra split. These fetuses numbered 1, 2, 2, and 1 each from the control, 0.5, 0.25, and 0.125% groups, respectively. All groups including controls had extra 14th ribs and/or a significant number of fetuses with rudimentary ribs; however, the incidence of these variants was nearly two times greater in the control group than in the antioxidant-treated groups. Krasavage[100] concluded that t-Butyl Hydroquinone at doses of 62.5, 125, and 250 times the approved food use level* for humans caused no gross external or internal tissue abnormalities in rat fetuses.

A hair dye formulation containing 0.3% t-Butyl Hydroquinone was applied topically at a dose of 2 ml/kg on days 1, 4, 7, 10, 13, 16, and 19 of gestation to a group of 20 pregnant Charles River CD rats. Body weight gain and food consumption of treated females were similar to controls through Day 20 of gestation, when the animals were killed. No significant differences were noted between treated and control animals in terms of number of corpora lutea, implantation sites and live fetuses, the sex ratio, or number of females with resorption sites or mean resorptions per pregnancy. In addition, no significant differences were observed in the fetuses of treated and control groups with respect to soft tissue anomalies. Normally occurring skeletal variations were present in both control and exposed fetuses, with the most frequent variation being "accessory ribs." It was concluded that the administration of the hair dye containing 0.3% t-Butyl Hydroquinone every third day of the gestation period produced no embryotoxic or teratogenic effects.[98]

Reproduction Studies

A three-generation, six-mating (two/generation) reproduction study was performed on rats receiving 0.5% t-Butyl Hydroquinone in their diet. The F_0 group (15 males and 15 females) was derived from 30 male and 30 female Sprague-Dawley rats. A total of 2090 rats was produced by litters throughout the study. Gonadal function, estrus, mating, conception rates, gestation times, parturition, and lactation of all experimental animals were similar to the controls. A "slight, questionable increase" in the F_1 pup mortality and a "slight, questionable decrease" in F_1 pup growth weight were observed; however, in a second experiment designed to examine these results in more detail, no effect was found. The F_{3b} pups were taken by caesarean section and examined grossly for soft tissue and skeletal abnormalities. No differences between the experimental groups and their controls were noted. The F_{3a} groups were fed for 11 months, at which time they were killed and samples of the liver of some animals examined by electron microscopy; no abnormalities were noted. Pregnant females from the F_{1a} generation were administered ^{14}C-t-Butyl Hydroquinone 1 day before parturition. Elimination of radioactivity was similar to other experimental rats as described previously in this report (see section on Metabolism, Excretion, and Storage).

*Federal regulations permit a total antioxidant content in food of not more than 0.02% (200 ppm) of the oil or fat content.[65]

Traces of ^{14}C, which amounted to approximately 0.2 and 0.02% of the dose, were found in fetuses at 7.6 and 16.7 h after treatment, respectively. According to the investigators, elimination was rapid, indicating that the pregnant females and their fetuses handle t-Butyl Hydroquinone similarly to nonpregnant rats. Radioactivity in amniotic fluid and uteri was similar to those in the fetus and decreased at about the same rate.[66]

Dose–Response Considerations

The results of feeding and metabolic studies referenced in this report to Astill et al.[66] indicate that the concentration at which "untoward effects" are produced by t-Butyl Hydroquinone in dogs and rats exceeds dietary concentrations of 0.5% (over 300 mg/kg per day). These authors estimated that the maximum daily dietary intake of t-Butyl Hydroquinone by humans, assuming a maximum permitted antioxidant intake of 200 ppm, is approximately 20 mg or 0.3 mg/kg per day. The FDA reported that the use of phenolic antioxidants in the American diet leads to a daily intake of these materials of about 4 ppm (< mg/kg per day).[101] Using FDA's calculations and considering the food types likely to contain t-Butyl Hydroquinone, Astill et al.[66] estimated the probable daily dietary intake of this particular compound as 0.02–0.07 mg/kg. In their view, this intake provides safety margins for use of the antioxidant in foods of 1000:1 to 10,000:1.

Clinical Assessment of Safety

Case Reports

A woman aged 71 had dermatitis of the groin, popliteal fossae, elbow flexures, axillae, and submammary areas over 15 years. The woman also had "patches" on the forehead and palms and an acute dermatitis of the vermilion of the lips. In a standard patch test series, this patient was allergic to t-Butyl Hydroquinone.[102]

Skin Irritation and Sensitization Studies

Lipstick products containing 0.054–0.15% by weight of t-Butyl Hydroquinone were tested for skin irritating and sensitizing effects on a total of 271 subjects in four separate RIPTs (Table 5). In three of the four tests, a modified Draize-Shelanski-Jordan patch procedure was used. In this method, the test material was applied under occlusive patches for 24 h every other day for a total of 10 induction patches. After a 13-day nontreatment period, a 48-h challenge patch was applied to the back of each subject. A second 48-h challenge patch was applied 7 days later. The challenge sites were evaluated 48 and 72 h after application. In the modified Draize-Shelanski procedure, the test material was applied under occlusive patches to the same skin site for a total of eight 24-h inductions. After an 11-day nontreatment period, a 48-h challenge patch was applied to the induction site. A second 48-h challenge patch was applied following removal of the initial challenge patch. Challenge sites were evaluated immediately and 24 h after patch removal. In the total of 271 subjects tested, 1 subject developed "intense eyrthema" to the lipstick product containing 0.14% by weight t-Butyl Hydroquinone. This skin response was observed after removal of the initial chal-

TABLE 5. Human Skin Irritation and Sensitization to Lipstick Products Containing t-Butyl Hydroquinone

t-Butyl Hydroquinone (weight percent)	*No. of subjects tested*	*Method*	*Results*	*Reference*
0.054	110 (92 females, 18 males)	Modified Draize-Shelanski repeat insult patch test	No skin irritation or sensitization	103
0.11	55 (49 females, 6 males)	Modified Draize-Shelanski-Jordan repeat insult patch test	No skin irritation or sensitization	106
0.14	54 (all females)	Modified Draize-Shelanski-Jordan repeat insult patch test	One subject developed intense erythema to initial challenge patch, but this reaction was not evident following removal of second challenge patch. This single reaction was classified as "nonspecific" irritation by the investigator	104
0.15	52 (41 females, 11 males)	Modified Draize-Shelanski-Jordan repeat insult patch test	No skin irritation or sensitization	105
TOTAL 271				

lenge patch but was not observed after removal of the second challenge patch. This single reaction was classified by the investigator as a "nonspecific irritant type" skin reaction. It was not ascertained whether this skin reaction was due to t-Butyl Hydroquinone or another ingredient in the formulation. No skin reactions were observed in any other subject during either induction or challenge phases. The four lipstick products containing t-Butyl Hydroquinone were considered nonirritating and nonsensitizing under conditions of the test.[103–106]

SUMMARY

t-Butyl Hydroquinone is a crystalline solid that is prepared by the acid catalyzed reaction of hydroquinone with isobutylene or t-butanol. Reported impurities include t-butyl-*p*-benzoquinone, 2,5-di-t-butylhydroquinone, hydroquinone, toluene, lead, and arsenic. t-Butyl Hydroquinone is easily oxidized to quinones.

Noncosmetic applications of t-Butyl Hydroquinone include use as a food additive, antioxidant, and stabilizer for chemicals. It is used also to retard polymerization of polyesters. In cosmetics, t-Butyl Hydroquinone is used as an antioxidant.

Data submitted to the FDA by cosmetic firms indicated that t-Butyl Hydroquinone was used in 1981 in at least 266 cosmetic products at concentrations of >0.1–1.0% (19 products) and ≤0.1% (247 products). The greatest use of the antioxidant was in lipsticks (242 products). Cosmetic products containing this ingredient are normally applied to or have the potential to come in contact with skin, eyes, and hair. Small amounts of the antioxidant could be ingested from lipstick.

t-Butyl Hydroquinone inhibits the growth of a broad spectrum of gram-negative and gram-positive bacteria and growth of certain yeast and protozoa. In studies with the ciliated protozoan *Tetrahymena pyriformis*, the antioxidant inhibited the biosynthesis of lipids, proteins, RNA, and DNA. Induction of hepatic mixed-function oxidases and liver enlargement were not observed following subchronic and chronic feeding of the antioxidant to rats and dogs. Inhibition of prostaglandin synthesis was noted in bovine seminal vesicles exposed in vitro to low concentrations of the antioxidant (5.49 and 6.07 μM).

In studies with rats, dogs, and humans, oral doses of t-Butyl Hydroquinone were excreted predominantly in the urine, primarily as sulfate conjugates and glucuronides, with smaller amounts excreted as unchanged t-Butyl Hydroquinone. It was suggested that the metabolic pathway for t-Butyl Hydroquinone in rats, dogs, and humans was similar to that of dihydroxybenzenes. Results of subchronic and chronic feeding studies with rats and dogs indicated no significant accumulation of the antioxidant in tissues.

t-Butyl Hydroquinone was slightly to moderately toxic to rats by the oral and intraperitoneal routes of administration. The acute oral LD_{50} in rats was reported in separate studies as 480 mg/kg and 800 mg/kg, respectively; whereas, the acute oral LD_{50} in rats of 5.0 or 10.0% t-Butyl Hydroquinone in corn oil was between 700 and 1000 mg/kg. The intraperitoneal LD_{50} in rats of a 5.0 or 10.0% suspension of the antioxidant in corn oil was between 300 and 400 mg/kg.

Subchronic and chronic studies were conducted with rabbits, rats, and dogs. A hair dye formulation containing 0.3% t-Butyl Hydroquinone produced no systemic toxicity when applied to the skin of rabbits twice a week for 13 weeks. Reduced weight gain was noted in rats fed a diet containing 1.0% of the antioxidant for 22 days. Rats fed a diet containing 0.016, 0.05, 0.16, or 0.5% t-Butyl Hydroquinone for 20 months were comparable to control animals in terms of behavior, growth rate, feed intake, mortality, hematological parameters, clinical chemistry, urinalyses, and tissue changes. A slight decrease in the brain weight of male rats fed 0.5% was noted; however, this decrease was not associated with any pathological or behavioral change. No other organ weight changes were observed. In a 6-month feeding study with rats, the antioxidant was added to heated and unheated cottonseed oil at concentrations of 0.02, 0.10, or 0.5%. The oil and antioxidant were then added to the diet at a 5.0% concentration. An increase in liver weight and a decrease in serum glutamic oxaloacetic transaminase activity were observed in the 0.5% group; no other toxicological effects were reported. Dogs fed diets containing 0.05, 0.158, or 0.5% t-Butyl Hydroquinone for 2 years were comparable to control animals with respect to hematological values, clinical chemistry, urinalyses, and organ weights. Selected tissues and organs were histologically normal in the treated dogs.

t-Butyl Hydroquinone was mutagenic in both the "rec-assay" with wild and recombinationless strains of *Bacillus subtilis* and in the "sensitivity test" with wild and rad mutant strains of yeast. No teratogenic effects were observed when the antioxidant was administered at doses of 970, 1878, or 3599 mg/kg in the diet of pregnant rats. Application of a hair dye containing 0.3% t-Butyl Hydroquinone to the skin of pregnant rats caused no embryotoxic or teratogenic effects. Results of a three-generation reproduction study indicated no differences between control animals and rats fed 0.5% t-Butyl Hydroquinone with respect to gonadal function, estrus, mating, conception rates, gestation times, parturition, lactation, soft tissue, skeletal and liver abnormalities, and accumulation of the antioxidant in amniotic fluid and uteri.

In clinical tests with 271 subjects, lipstick products containing 0.054, 0.11, 0.14, and 0.15% by weight t-Butyl Hydroquinone were nonirritating and nonsensitizing to the skin.

DISCUSSION

There are clinical data to support that t-Butyl Hydroquinone is nonsensitizing and nonirritating to the skin at concentrations used in cosmetics. Although there have been no studies on t-Butyl Hydroquinone as a depigmenter, structural similarity of this compound to hydroquinone raises significant concern about this possibility. Furthermore, there are no data regarding phototoxicity or photosensitization.

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the CIR Procedures, the Expert Panel informed the public of its decision that the data on t-Butyl Hydroquinone are insufficient to determine whether this ingredient, under each relevant condition of use, is either safe or not safe. The Panel released a Notice of Insufficient Data Announcement on October 10, 1984 outlining the data needed to assess the safety of t-Butyl Hydroquinone. The types of data required included:

1. Ninety-day depigmentation study in black guinea pigs or other appropriate animal. At least three concentrations of t-Butyl Hydroquinone shall be evaluated. Appropriate positive controls shall also be employed.
2. In vitro human and animal melanocyte studies at three t-Butyl Hydroquinone concentrations. Appropriate positive controls shall be employed.
3. Photosensitization data—animal data are appropriate.

No response to the Notice of Insufficient Data Announcement was received within an appropriate time period.

On June 21, 1985, the Expert Panel reviewed a request to delay the issuance of the Final Report until the data previously requested could be supplied. The Panel decided to issue the Final Report in accordance with Section 45 of the CIR Procedures. When new data are available, the Expert Panel will reconsider the Final Report in accordance with Section 46 of the CIR Procedures, Amendment of a Final Report.

CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of t-Butyl Hydroquinone as used in cosmetics.

ACKNOWLEDGMENT

Jonathon T. Busch, Senior Scientific Analyst, prepared the literature review and technical analysis of this report. Word processing for the report was performed by Purita S. Ibanez.

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