Final Report on the Safety Assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate[1]

Benzyl Alcohol is an aromatic alcohol used in a wide variety of cosmetic formulations as a fragrance component, preservative, solvent, and viscosity-decreasing agent. Benzoic Acid is an aromatic acid used in a wide variety of cosmetics as a pH adjuster and preservative. Sodium Benzoate is the sodium salt of Benzoic Acid used as a preservative, also in a wide range of cosmetic product types. Benzyl Alcohol is metabolized to Benzoic Acid, which reacts with glycine and excreted as hippuric acid in the human body. Acceptable daily intakes were established by the World Health Organization at 5 mg/kg for Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. Benzoic Acid and Sodium Benzoate are generally recognized as safe in foods according to the U.S. Food and Drug Administration. No adverse effects of Benzyl Alcohol were seen in chronic exposure animal studies using rats and mice. Effects of Benzoic Acid and Sodium Benzoate in chronic exposure animal studies were limited to reduced feed intake and reduced growth. Some differences between control and Benzyl Alcohol–treated populations were noted in one reproductive toxicity study using mice, but these were limited to lower maternal body weights and decreased mean litter weights. Another study also noted that fetal weight was decreased compared to controls, but a third study showed no differences between control and Benzyl Alcohol–treated groups. Benzoic Acid was associated with an increased number of resorptions and malformations in hamsters, but there were no reproductive or developmental toxicty findings in studies using mice and rats exposed to Sodium Benzoate, and, likewise, Benzoic Acid was negative in two rat studies. Genotoxicity tests for these ingredients were mostly negative, but there were some assays that were positive. Carcinogenicity studies, however, were negative. Clinical data indicated that these ingredients can produce nonimmunologic contact urticaria and nonimmunologic immediate contact reactions, characterized by the appearance of wheals, erythema, and pruritis. In one study, 5% Benzyl Alcohol elicited a reaction, and in another study, 2% Benzoic Acid did likewise. Benzyl Alcohol, however, was not a sensitizer at 10%, nor was Benzoic Acid a sensitizer at 2%. Recognizing that the nonimmunologic reactions are strictly cutaneous, likely involving a cholinergic mechanism, it was concluded that these ingredients could be used safely at concentrations up to 5%, but that manufacturers should consider the nonimmunologic phenomena when using these ingredients in cosmetic formulations designed for infants and children. Additionally, Benzyl Alcohol was considered safe up to 10% for use in hair dyes. The limited body exposure, the duration of use, and the frequency of use were considered in concluding that the nonimmunologic reactions would not be a concern. Because of the wide variety of product types in which these ingredients may be used, it is likely that inhalation may be a route of exposure. The available safety tests are not considered sufficient to support the safety of these ingredients in formulations where inhalation is a route of exposure. Inhalation toxicity data are needed to complete the safety assessment of these ingredients where inhalation can occur.

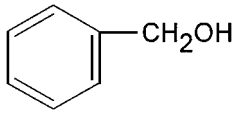
INTRODUCTION

This report is a compilation of data concerning Benzyl Alcohol (CAS No. 100-51-6), Benzoic Acid (CAS No. 65-85-0), and Sodium Benzoate (CAS No. 532-32-1). Reviews of early literature (1920–1977) were prepared for the Food and Drug Administration (FDA) on Benzyl Alcohol and Benzoic Acid (Flavor and Extract Manufacturers' Association 1984) and on Benzoic Acid and Sodium Benzoate (Informatics, Inc. 1972; Federation of American Societies for Experimental Biology [FASEB] 1973). This Cosmetic Ingredient Review (CIR) report includes relevant studies cited in the earlier reviews as well as recent animal and clinical studies.

CHEMISTRY

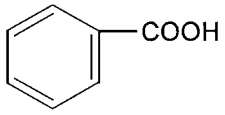
Definition and Structure

Benzyl Alcohol is an aromatic alcohol that conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):



Synonyms for Benzyl Alcohol include Benzenemethanol (Wenninger, Canterbery, and McEwen 2000), Phenyl-methanol, Phenylcarbinol, Phenylmethyl Alcohol (Food and Agricultural Organization of the United Nations/World Health Organization [FAO/WHO] 1994); Hydroxytoluene, α-Hydroxytoluene, (Lewis 1993).

Benzoic Acid is an aromatic acid that conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):



Received 15 May 2001; accepted 12 July 2001.

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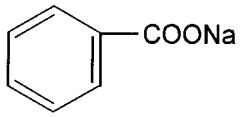
International Journal of Toxicology, 20(Suppl. 3):23–50, 2001

1091-5818/01 $12.00 + .00

Synonyms for Benzoic Acid include Benzeneformic acid, Benzenemethanoic Acid, Benzoate, Carboxybenzene, Dracylic Acid, Phenylformic Acid, Benzenecarboxylic Acid, Phenylcarboxylic Acid (Budavari 1989; Lewis 1993).

The sodium salt of Benzoic Acid, Sodium Benzoate conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):



Synonyms for Sodium Benzoate include sodium salt of benzenecarboxylic acid, sodium salt of phenylcarboxylic acid (FAO/WHO 1994).

Physical and Chemical Properties

Table 1 lists physical properties of Benzyl Alcohol and Benzoic Acid.

Benzyl Alcohol is a combustible liquid. When heated to decomposition it emits acrid smoke and fumes (Lewis 1993).

Benzoic Acid burns rapidly in oxygen and is combustible when exposed to heat or flame. It can react with oxidizing materials. When heated to decomposition it emits acrid smoke and fumes (Lewis 1993).

When Sodium Benzoate is heated to decomposition it emits toxic fumes of Na_2O. It is cautioned that oral doses of 8 to 10 g can cause nausea and vomiting; small doses have little or no effect (Lewis 1993).

Method of Manufacture

Benzyl Alcohol is found naturally in many foods such as apricots, snap beans, cocoa, cranberries, mushrooms, and honey (Flavor and Extract Manufacturers' Association 1984). Benzyl Alcohol is also found in the essential oil of many plants including jasmine, hyacinth, and ylang-ylang (Lewis 1993). Large scale production of Benzyl Alcohol is achieved by the action of sodium or potassium carbonate on benzyl chloride (Budavari 1989).

Benzoic Acid is also found naturally in many foods such as apricots, snap beans, cocoa, cranberries, mushrooms, and honey (Flavor and Extract Manufacturers' Association 1984).

Analytical Methods

The Benzoic Acid content of cosmetic formulations can be determined by high-performance liquid chromatography (Gagliardi et al. 1984).

Impurities

The Cosmetic, Toiletry, and Fragrance Association (CTFA) lists the following specifications for Benzyl Alcohol, 0.2% maximum aldehyde (as benzaldehyde) and 0.005% maximum sulfated ash. Other characteristics such as chlorinated compounds, specific gravity, refractive index, and distilling range must match the standards of the National Formulary (NF) or United States Pharmacopeia (USP) (Nikitakis and McEwen 1990).

CTFA specifications for Benzoic Acid and Sodium Benzoate with regard to congealing range, equivalent weight, water content, and alkalinity follow standards set by the USP and NF (Nikitakis and McEwen 1990).

USE

Cosmetic

Benzyl Alcohol

Benzyl Alcohol is used in cosmetics as a fragrance component, preservative, solvent, and viscosity-decreasing agent (Wenninger, Canterbery, and McEwen 2000). In January 1998, Benzyl Alcohol was reported to be used in 322 cosmetic formulations (FDA 1998). (See Table 2.) Concentration of use data

TABLE 1
Physical properties of Benzyl Alcohol and Benzoic Acid

Property	Benzyl alcohol	Benzoic acid	Reference
Appearance	Liquid		Budavari 1989
	—	White crystalline powder	Lewis 1993
Odor/taste	Faint aromatic odor, sharp burning taste	—	Budavari 1989
Molecular weight	108.14 Da	122.12 Da	Lide 1993
Boiling point (°C)	205.3	249	Lide 1993
Melting point (°C)	−15.3	122.13	Lide 1993
Density	1.0419	1.0749, 1.2659	Lide 1993
Solubility	Water; alcohol; ether; acetone; benzene	Alcohol; ether; acetone; chloroform; benzene	Lide 1993
Flash point (°F)	213 (CC)	250 (CC)	Lewis 1993
Refractive index	−0.002	—	Nikitakis and McEwen 1990

TABLE 2
Frequency of use of Benzyl Alcohol (FDA 1998)

Product category	No. of formulations in category	No. containing ingredient
Baby shampoos	21	5
Baby lotions, oils, powders, creams	53	3
Other baby products	29	2
Bath oils, tablets, and salts	124	2
Other bath preparations	159	3
Eyeliner	514	3
Eye shadow	506	4
Eye makeup remover	84	10
Mascara	167	5
Other eye makeup preparations	120	4
Colognes and toilet waters	656	1
Other fragrance preparations	148	2
Hair conditioners	636	7
Hair sprays (aerosol fixatives)	261	4
Rinses (noncoloring)	40	1
Shampoos (noncoloring)	860	7
Tonics, dressings, and other hair grooming aids	549	14
Hair dyes and colors	1572	130
Hair rinses (coloring)	33	18
Hair color sprays (aerosol)	4	1
Other hair-coloring preparations	59	2
Face powders	250	1
Foundations	287	2
Lipstick	790	1
Makeup bases	132	1
Other makeup preparations	135	2
Deodorants (underarm)	250	1
Feminine hygiene deodorants	4	2
Other personal cleanliness products	291	1
Aftershave lotion	216	4
Preshave lotions (all types)	14	1
Shaving cream	139	3
Cleansing	653	10
Face and neck skin care (excluding shaving)	263	12
Body and hand skin care (excluding shaving)	796	16
Foot powders and sprays	35	2
Moisturizing	769	8
Night skin care	188	3
Paste masks (mud packs)	255	4
Skin fresheners	184	4
Other skin care preparations	692	11
Suntan gels, creams, and liquids	136	2
Other suntan preparations	38	3
1998 total for Benzyl Alcohol		**322**

are no longer reported to the FDA (FDA 1992). Data from FDA (1984) indicated that Benzyl Alcohol was used at concentrations ≤25%. Studies cited in the Clinical Assessment of Safety section of this report tested mascara formulations containing 0.65% Benzyl Alcohol (Hill Top Research 1997a, 1997b).

Benzoic Acid and Sodium Benzoate

Benzoic Acid is used as a pH adjustor and preservative and Sodium Benzoate is used as a preservative (Wenninger, Canterbery, and McEwen 2000). In January 1998, Benzoic Acid and Sodium Benzoate were reported to be used in 223 and 156 cosmetic formulations, respectively (FDA 1998) (See Table 3). Data from 1984 indicated that although Benzoic Acid and Sodium Benzoate were used at up to 5% and 25%, respectively, the majority of use of both ingredients was at ≤1% (FDA 1984).

Studies cited in the Clinical Assessment of Safety section of this report tested eye shadow formulations containing 0.1% Benzoic Acid (Biosearch Inc. 1991; TKL Research 1991) and liquid/powder foundation formulations containing 0.2% Benzoic Acid (Biosearch Inc. 1992a, 1992b, 1992c, 1992d; Education and Research Foundation 1992).

International

Benzyl Alcohol

The European Union (EU) has stipulated that when used as a preservative, Benzyl Alcohol is restricted to a maximum concentration of 1% (EU 1995).

Benzyl Alcohol is listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category (CLS)*. Benzyl Alcohol, which conforms to the specifications of the *Japanese Standards of Cosmetic Ingredients*, has precedent for use without restriction in the following *CLS* categories: soaps, face cleansing products, shampoos, hair rinses, hair coloring preparations, and eye creams, eyeshadows, and mascaras. There has been precedent for use of Benzyl Alcohol at concentrations up to 5% in the following categories: hair care products, creams and milky lotions, shaving creams and lotions, suntan and sun cream, lotion and oil formulations, shaving lotions, cosmetic oils, powders, foundations, perfumes, packs, nail creams, nail enamels, nail makeup removers, cheek color products, eyebrow products, and bath preparations. There has been no precedent regarding its use in eyeliners, lipsticks, lip creams, and dentifrices (Santucci 1999).

According to Notification 990 of the Pharmaceutical and Medical Safety Bureau of the Japan Ministry of Health and Welfare, issued September 29, 2000, Benzyl Alcohol is not prohibited or restricted (beyond the limits in the CLS discussed above) in its use beyond a basic obligation of manufacturers to use all ingredients in a manner which guarantees safety (Japan Ministry of Health and Welfare 2000).

Benzoic Acid and Sodium Benzoate

The EU has stipulated that when used as preservatives, Benzoic Acid, its salts and esters are restricted to a maximum concentration of 0.5% (acid) (EU 1995).

Benzoic Acid and Sodium Benzoate are listed in the *CLS* and must conform to the specifications of the *Japanese Standards of Cosmetic Ingredients*. Precedent to use Benzoic Acid at concentrations up to 0.2% has been established in all *CLS* cosmetic categories except eyeliners. Precedent to use Sodium Benzoate at concentrations up to 1% has been established in all *CLS* categories (Santucci 1999).

According to Notification 990 of the Pharmaceutical and Medical Safety Bureau of the Japan Ministry of Health and Welfare, issued September 29, 2000, use of Benzoic Acid is restricted to 0.2 g per 100 g of any cosmetic formulation (Japan Ministry of Health and Welfare 2000).

Noncosmetic

Benzyl Alcohol

Benzyl Alcohol is approved for use as a food additive (Rothschild 1990). In 1979, the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) established an acceptable daily intake (ADI) level of 0 to 5 mg/kg body weight. This ADI concerned Benzyl Alcohol and benzyl benzoate and "applies to the benzyl/benzoic moiety related to benzoic acid representing total benzoate from all food additive sources" (FAO/WHO 1994).

Benzyl Alcohol can be used as an active ingredient in over-the-counter (OTC) drug preparations (Wenninger, Canterbery, and McEwen 2000). In 1982, the FDA Panel on OTC Dentifrices and Dental Care Products recommended that Benzyl Alcohol not be used in the treatment of dental pain in children younger than 2 years of age (Grad and Grushka 1986).

In human pain studies, Benzyl Alcohol was an effective local anesthetic. Subjects complained of less pain after receiving intramuscular (IM) injections of various medications that contained Benzyl Alcohol (Wightman and Vaughan 1976; Gouyette et al. 1982; Rasmussen, Zachmann, and Nilsson 1989; Frenken, van Lier, and Koene 1994; Jørgensen 1994; Williams and Howe 1994).

Benzoic Acid and Sodium Benzoate

Benzoic Acid and Sodium Benzoate both have the status "generally recognized as safe" (GRAS) (Rothschild 1990). In 1983, the JECFA established an ADI of 0 to 5 mg/kg body weight for Benzoic Acid, its salts, benzyl acetate, alcohol and benzoate (FAO/WHO 1994).

Benzoic Acid (USP) is a component of benzoic and salicylic acid ointments (USP), a topical antifungal agent (Taylor 1988).

Since the late 1970s, Sodium Benzoate has been used in the treatment of hyperammonemia in patients with inborn errors of the urea cycle. The treatment is based on the synthesis and excretion of hippurate as an alternative pathway to eliminate nitrogen versus urea synthesis (Brusilow et al. 1979, 1980).

An extensive review of this therapeutic use of benzoates by Tremblay and Qureshi (1993) noted that laboratory models have yet to corroborate clinical findings. Further, animal studies demonstrated that Sodium Benzoate, at some doses, potentiated

TABLE 3
Frequency of use of Benzoic Acid and Sodium Benzoate (FDA 1998)

Product category	No. of formulations in category	No. containing ingredient
Benzoic Acid		
Other baby products	29	1
Bath oils, tablets, and salts	124	2
Bubble baths	200	26
Other bath preparations	159	32
Eye shadow	506	5
Eye makeup remover	84	3
Mascara	167	1
Other eye makeup preparations	120	1
Other fragrance preparations	148	2
Hair conditioners	636	2
Hair straighteners	63	1
Shampoos (noncoloring)	860	3
Tonics, dressings, and other hair grooming aids	549	5
Other hair preparations	276	2
Blushers (all types)	238	1
Face powders	250	3
Foundations	287	7
Lipstick	790	37
Makeup bases	132	1
Other makeup preparations	135	1
Cuticle softeners	19	1
Other manicuring preparations	61	1
Mouthwashes and breath fresheners	49	12
Other oral hygiene products	6	2
Bath soaps and detergents	385	8
Aftershave lotion	216	4
Shaving cream	139	2
Cleansing	653	12
Face and neck skin care (excluding shaving)	263	2
Body and hand skin care (excluding shaving)	796	8
Moisturizing	769	8
Night skin care	188	1
Paste masks (mud packs)	255	3
Skin fresheners	184	9
Other skin care preparations	692	9
Suntan gels, creams and liquids	136	2
Indoor tanning preparations	62	3
1998 total for Benzoic Acid		**223**
Sodium Benzoate		
Bath oils, tablets and salts	124	1
Other bath preparations	159	1
Eyeliner	514	3
Eye shadow	506	4
Eye makeup remover	84	5
Other eye makeup preparations	120	2
Hair conditioners	636	5
Hair sprays (aerosol fixatives)	261	24

(Continued on next page)

TABLE 3
Frequency of use of Benzoic Acid and Sodium Benzoate (FDA 1998) *(Continued)*

Product category	No. of formulations in category	No. containing ingredient
Hair straighteners	63	1
Rinses (noncoloring)	40	3
Shampoos (noncoloring)	860	20
Tonics, dressings, and other hair grooming aids	549	5
Wave sets	55	1
Hair bleaches	113	2
Face powders	250	2
Other makeup preparations	135	1
Dentifrices	38	6
Mouthwashes and breath fresheners	49	1
Other oral hygiene products	6	2
Bath soaps and detergents	385	1
Deodorants (underarm)	250	1
Other personal cleanliness products	291	4
Aftershave lotion	216	15
Shaving cream	139	7
Other shaving preparation products	60	1
Cleansing	653	5
Face and neck skin care (excluding shaving)	263	4
Body and hand skin care (excluding shaving)	796	8
Moisturizing	769	6
Night skin care	188	3
Paste masks (mud packs)	255	2
Skin fresheners	184	1
Other skin care preparations	692	9
1998 total for Sodium Benzoate		**156**

ammonia toxicity in mice (O'Connor et al. 1982, 1989) and rats (Maswoswe et al. 1986).

Conditions for benzoate therapy typically include reduced nitrogen intake and a priming intravenous (IV) dose of 250 mg/kg administered over 0.5 to 2 hours, followed by an additional 250 to 500 mg/kg/day administered with meals (Tremblay and Qureshi 1993).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

The available human absorption, distribution, metabolism, and excretion data were sufficiently extensive that animal data were not included. Therefore, the following section cites clinical studies only.

Benzyl Alcohol

When metabolized, Benzyl Alcohol is converted to Benzoic Acid by simple oxidation (Flavor and Extract Manufacturers' Association 1984). The relevant data, therefore, relate to Benzoic Acid and Sodium Benzoate.

Benzoic Acid and Sodium Benzoate

Even after administration of high doses of Sodium Benzoate, the hourly excretion of hippuric acid increases to a maximum and then remains constant until all but a small portion is eliminated (Quick 1931). The rate of hippuric acid formation in humans after oral administration of 5 g Benzoic Acid increased with the concomitant administration of glycine.

Bridges et al. (1970) reported on the metabolism of Benzoic Acid in humans and various animal species. The FASEB (1973) and GRAS reports (Informatics 1972) reported that Benzoic Acid and Sodium Benzoate are rapidly absorbed from the gastrointestinal tract of mammals and conjugated with glycine in the liver. The resulting hippuric acid is excreted in the urine rapidly (75% to 100% of the dose is excreted within 6 hours; the remaining dose is excreted within 2 to 3 days). The availability of glycine was the rate-limiting factor in the formation of hippuric acid. When insufficient glycine was available benzoyl glucuronide was formed.

Feldman and Maibach (1970) reported that 42.6% ± 16.5% of a dermally applied [^{14}C]-Benzoic Acid dose (4 μg/cm^2; in acetone) was excreted in the urine within 24 hours. When applied

in petrolatum, 60.5% of the dose was absorbed (Bronaugh and Franz 1986).

By quantifying 24-hour urine excretion, Rougier et al. (1986) demonstrated that dermal application of 1000 nmol [^{14}C]-Benzoic Acid (10^{-3} μCi/nmol) produced the following penetration scale: forehead > abdomen > thigh > chest > arm > back. The 4-day penetration through the forehead (27.65 $\pm$ 3.61 nmol/cm^2) was three times greater than absorption through the back (8.55 $\pm$ 1.32 nmol/cm^2). Benzoic Acid had been applied to two sites of each body area; one site was tape stripped to determine the amount of test material in the stratum corneum. The quantified values from the urine were comparable to predicted values estimated from the tape stripping.

In a study investigating the effects of aging on dermal absorption, Roskos, Maibach, and Guy (1989) applied [^{14}C]-Benzoic Acid (in acetone) to the forearm of two groups of panelists, "young" (22 to 40 years) and "old" (>65 years). A 24-hour protective patch was placed on the skin and the site was washed after patch removal. A second protective patch was then applied and remained in place until day 7. Analysis of 7-day urine excretion indicated that 36.2% $\pm$ 4.6% of the applied dose was absorbed by the young panelists, whereas 19.5% $\pm$ 1.6% was absorbed by the old panelists. The difference was statistically significant ($p < .01$).

Kubota and Ishizaki (1991) demonstrated that biotransformation of Benzoic Acid to hippuric acid follows saturable or Michaelis-Menten kinetics in humans following ingestion of Sodium Benzoate.

No statistical difference ($p > .05$) was found by Lotte et al. (1993) in the percutaneous absorption of Benzoic Acid by Asian, Black, and Caucasian panelists. [^{14}C]-Benzoic Acid (1 μmol/cm^2) was applied to two sites of the upper arm and the sites were washed after 30 minutes of contact. (The two applications occurred on contralateral arms and were made 48 hours apart.) Urine was collected for 24 hours and one site was tape stripped to measure Benzoic Acid in the stratum corneum. Amounts absorbed were 1.43% $\pm$ 0.27% by Asian skin, 1.07% $\pm$ 0.17% by black skin, and 1.2% $\pm$ 0.19% by Caucasian skin.

Gregus et al. (1993, 1996) reported that both lipoic acid (1993) and valproic acid (1996) reduced the clearance of Benzoic Acid in rats that had been "loaded" with glycine. Both acids reduced the availability of hepatic coenzyme A that is needed for the adenosine triphosphate (ATP)-dependent conjugation with glycine.

Cellular Effects

Benzyl Alcohol

Benzyl Alcohol is a membrane "fluidizer" that affects lipid bilayer structure (Ebihara et al. 1979). It has been demonstrated to act on membranes of erythrocytes (Burgen et al. 1970; Bassé et al. 1992) and hepatocytes (Gordon et al. 1980).

Studies reported Benzyl Alcohol to increase activity of membrane-bound Ca^{2+}-dependent enzymes such as adenylate cyclase (Voorheis and Martin 1982; Martin, McConkey, and Stokes 1985; Needham and Houslay 1988) and thiol proteinase (Ahkong et al. 1980). Conversely, Benzyl Alcohol inhibited activities of various glycosyltransferases of the rat liver Golgi membrane (Mitranic, Boggs, and Moscarello 1982). The activities of erythrocyte-bound *p*-nitrophenylphosphatase and acetylcholinesterase were increased at some concentrations of Benzyl Alcohol and inhibited by others (Tanaka 1984). The effect on cell membranes was considered the mechanism by which Benzyl Alcohol inhibited lymphocyte-mediated cytolysis in vitro (Kemp and Berke 1973a, 1973b).

Benzyl Alcohol induced time-, dose-, and temperature-dependent hemolysis of erythrocytes (Ohmiya and Nakai 1978).

Benzoic Acid and Sodium Benzoate

Sodium Benzoate inhibited activity of D-amino acid oxidase (Brada and Bulba 1980; London and Gabel 1988).

In an in vitro study, Sodium Benzoate at doses $\geq$500 μg/ml suppressed the activities of marker enzymes in the mitochondria and cytosol of rat liver hepatocytes. Suppression of DNA synthesis was noted at 100 μg/ml (Oyanagi et al. 1987).

Radical Scavenging Activity

Benzoic Acid and Sodium Benzoate

Benzoic Acid and Sodium Benzoate are recognized as hydroxyl radical scavengers and researchers have reported that benzoates inhibited mechanisms that generated free radicals. In in vitro studies, benzoates reduced the cytotoxicity of drugs/chemicals such as hydroxyurea in L5178Y cells (Przybyszewski and Malec 1982), 6-hydroxydopamine in mouse pancreatic islets (Grankvist, Sehlin, and Taeljedal 1986), doxorubicin in a Dox-sensitive human ovarian cancer cell line (Cervantes et al. 1988), and inhibited argemone oil–induced enzymatic and nonenzymatic hepatic lipid peroxidation in rat cells (Upreti, Das, and Khanna 1991). In other in vitro studies, benzoates inhibited some chemical-induced DNA lesions (Kaneko et al. 1984; Sugioka et al. 1984; Daniel, Mao, and Saffiotti 1983; Mahmood et al. 1993). In in vivo studies using rats, Sodium Benzoate had a protective effect against gentamicin-induced renal failure (Walker and Shah 1988), and demonstrated a dose-dependent reduction in ethanol-induced gastric lesions (Evangelista and Meli 1985). However, Rotstein and Slaga (1988) reported that the scavenging activity of Sodium Benzoate did not significantly inhibit tumor progression when tested in a murine skin multistage carcinogenesis model.

Benzoic Acid and Sodium Benzoate also inhibited immune responses that relied on reactive oxygen intermediates such as natural killer cells (Suthanthiran et al. 1984), some neutrophil activity (Cheung, Archibald, and Robinson 1984; Thomas, Smith, and Pang 1991; Kumar, Anand, and Ganguly 1993), and phagocytes (Weitzman and Stossel 1982; Weitberg et al. 1985). However, Kraut, Segal, and Sagone (1982) reported that granulocyte aggregation in response to cell membrane injury was not affected by oxygen radical scavengers.

Glycine Competition

Benzoic Acid and Sodium Benzoate

The metabolism of the benzoates depletes glycine concentrations and can therefore alter the glycine-dependent metabolism of other compounds. Amsel and Levy (1969) and Levy, Amsel, and Elliott (1969) demonstrated that Benzoic Acid or Sodium Benzoate successfully competed with aspirin for glycine, resulting in increased concentration and persistence of salicylic acid in the body. Almost total inhibition of salicyluric acid formation in humans was achieved using either 2.7 g Benzoic Acid or 3.2 g Sodium Benzoate.

The GRAS report (Informatics Inc. 1972) cited studies in which ingestion of Sodium Benzoate reduced the glycine-dependent formation of creatine, glutamine, urea, and uric acid and increased the effects of procaine, lidocaine, cocaine, tetracaine, and dibucaine. Under conditions of severely restricted fluid and salt intake, benzoates increased and prolonged the concentration of serum penicillin.

Enzyme Inhibition

Benzyl Alcohol

Messiha (1991) reported that short-term intake of 2% Benzyl Alcohol in the drinking water resulted in an inhibition of hepatic alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase isoenzyme activities in female rats. The effects were not noted in male rats.

Compared to control rats, Benzyl Alcohol noncompetitively inhibited activity of hepatic alcohol dehydrogenase (L-ADH) of rats maintained for a short term on 5% ethanol (Messiha, Pasi, and Morniroli 1992).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Benzyl Alcohol

The literature review by the Flavor and Extract Manufacturers' Association (1984) cited the following oral LD_{50} values for Benzyl Alcohol: mouse 1580 mg/kg; rat 1230 to 3200 mg/kg (four studies), and rabbit 1040 mg/kg.

Benzoic Acid and Sodium Benzoate

The RTECS (Registry of Toxic Effects of Chemical Substances) cited that the human low lethal oral dose of Benzoic Acid was 500 mg/kg (RTECS 1995).

The oral LD_{50} of Benzoic Acid in mice was 1996 mg/kg (Flavor and Extract Manufacturers' Association, 1984). In rats, the oral LD_{50} for Benzoic Acid was 2000 to 2500 mg/kg, for Sodium Benzoate it was 2100 to 4070 mg/kg. The LD_{50} of Sodium Benzoate in rabbits and dogs was 2000 mg/kg. The oral LD_{100s} for Benzoic Acid for rabbits, cats, and dogs were 1520 to 2000, 2000, and 2000 mg/kg, respectively (FASEB 1973).

Short-Term Oral Toxicity

Benzyl Alcohol

In a gavage study by the National Toxicology Program (NTP 1989), technical grade Benzyl Alcohol (99% pure) in corn oil at doses of 125, 250, 500, 1000, or 2000 mg/kg was administered to groups of 10 F344/N rats and $B6C3F_1$ mice (5 of each sex). Animals were dosed 5 days a week for 16 days (total of 12 doses). Feed and water were provided *ad libitum*. On days 8 and 9, both rats and mice of the 125-mg/kg group received doses that were 10-fold too high.

All rats that received 2000 mg/kg and two of five males and three of five females that received 1000 mg/kg Benzyl Alcohol died before the end of the study. Rats of the two highest dose groups had blood around the nose and mouth, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tracts. Final body weight of male rats of the 1000 mg/kg group was 18% less than that of vehicle controls. Lethargy was observed in rats of the two highest dose groups; rough coats were noted in males of the 500- and 1000-mg/kg groups and in females of the 250- and 500-mg/kg groups. No compound-related histopathologic changes were noted.

All mice that received 2000 mg/kg and one of five males and two of five females that received 1000 mg/kg Benzyl Alcohol died before the end of the study. Lethargy and rough coats were noted in males that received $\geq$500 mg/kg and in females that received $\geq$1000 mg/kg. Blood in the urinary bladder was noted at necropsy in mice of the two highest dose groups. No compound-related histopathologic changes were noted (NTP 1989).

Reviewing this study, the United States Environmental Protection Agency (EPA) determined that the lowest-observable-adverse-effect level (LOAEL) was $\leq$500 mg/kg for male rats, and $\leq$250 mg/kg for female rats. EPA determined that the no-observable-adverse-effect level (NOAEL) was $\leq$250 mg/kg for male mice and $\leq$500 mg/kg for female mice (EPA 1989).

Benzoic Acid and Sodium Benzoate

Fujitani (1993) fed groups of 10 F344/N rats and $B6C3F_1$ mice (5 of each sex) 1.81%, 2.09%, or 2.40% (rats) or 2.08%, 2.50%, or 3.00% (mice) Sodium Benzoate for 10 days. The doses were selected based on earlier reports that repeated dosing with 2.5% Sodium Benzoate was lethal to rats.

One male rat of the high-dose group had signs of "hypersensitivity" and died on day 8. Rats of the mid- and high-dose groups had significantly reduced mean body weight as compared to nontreated controls. Relative liver and kidney weights, as well as serum concentrations of albumin and total protein, were significantly increased in male rats of the mid- and high-dose group and in female rats of the high-dose group. Serum γ-glutamyltranspeptidase activity was significantly increased in males and significantly decreased in females of the high-dose group. Serum cholesterol was significantly decreased in males of the high-dose group and in all dosed females as compared to controls. Changes in other parameters such as serum phospholipid

and uric acid concentrations were sometimes significant but were non–dose-dependent. Enlarged hepatocytes with glassy cytoplasm were noted at microscopic examination of tissues from males of the high-dose group.

All mice of the high-dose group had signs of "hypersensitivity"; 3 of 10 had convulsions and 2 of the 3 (both females) died before the end of the study. Mean body weights of mice of the treated groups were not significantly different from untreated controls. A dose-dependent increase in absolute and relative liver weight was noted; the increase was significant in mice of the high-dose group. Female mice of the high-dose group also had greater relative kidney weights. Serum cholesterol and phospholipid concentrations in male mice of the high-dose group, serum cholinesterase activities in male mice of the mid- and high-dose groups, and serum γ-glutamyltranspeptidase activities of female mice of the mid-dose group were significantly greater than those of the control group. No significant changes were noted in serum concentrations of triglyceride, uric acid, and urea nitrogen, and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) or in the AST/ALT ratio. Enlargement, vacuolation and necrosis of hepatocytes were noted in male mice of the 3.0% group (Fujitani 1993).

The GRAS report (Informatics, Inc. 1972) and the literature review by the Flavor and Extract Manufacturers' Association (1984) cited several short-term oral studies of Benzoic Acid and Sodium Benzoate toxicity (Table 4). Supporting the above findings of Fujitani, Kreis et al. (1967) reported significantly decreased weight gain in rats dosed with 1.1% Benzoic Acid for 35 days, and toxicity following five days of dosing with 3%. Studies in which approximately 2% Sodium Benzoate was administered for 4 to 8 weeks recorded: no adverse effects (Kramer and Tarjan 1962), lesions (Smyth and Carpenter 1948), or significant weight reduction (noted in male rats only) (Fanelli and Halliday 1963). Severe reduction of growth rate was recorded at slightly larger doses of Sodium Benzoate (White 1941). Sodium Benzoate was toxic at 5% (Kieckebusch and Lang, 1960; Fanelli and Halliday 1963).

Subchronic Oral Toxicity

Benzyl Alcohol

Groups of 20 F344/N rats and B6C3F$_1$ mice (10 of each sex) received 50, 100, 200, 400, or 800 mg/kg Benzyl Alcohol, 5 days a weeks for 13 weeks (NTP 1989). Experimental conditions were the same as in the 16-day study. The death of five rats was attributed to rupture caused by the gavage procedure. Gavage-related deaths were considered to result from the trauma of the gavage procedure combined with the neurotoxic/anesthetic effect of the compound. Aside from these, four male rats and one female of the 800-mg/kg group, as well as one female of the 400-mg/kg group and one male of the 200-mg/kg group died on study. The 800-mg/kg group had signs of neurotoxicity, including staggering, labored breathing, and lethargy after dosing. Blood around the nose and mouth was noted in 5 of 10 males of this group after week 8. Compared to vehicle controls, final mean body weights were 7% and 5% smaller, respectively, in male and female rats of the highest dose group. At histopathologic evaluation, lesions observed in rats of the highest dose group included necrosis of the dentate gyrus of the hippocampus in 7 of 7 males and 9 of 9 females; skeletal muscle necrosis in 5 of 10 males, thymic congestion, hemorrhage, and atrophy in 8 of 10 males, and nephrosis in 6 of 9 males. Renal lesions were similar to those noted in age-related spontaneous renal disease.

Nine of 10 deaths (mice) were attributed to the gavage procedure. Final mean body weights of females of the 400- and 800-mg/kg groups were 5% and 8% lower, respectively, than the vehicle control. Staggering was noted during the first and second weeks of dosing in mice of the high-dose group. No compound-related histopathologic alterations were observed. A Sendai virus infection was suspected (NTP 1989).

Reviewing the 91-day study, the EPA extrapolated the high dose for rats and mice into human doses of 84 and 39 mg/kg/day, respectively (for a 70-kg person). The EPA determined the NOAEL was 143 mg/kg for female rats, "which were the more sensitive sex." Using this level, and applying an uncertainty factor of 100 (10 for interspecies extrapolation multiplied by 10 to protect unusually sensitive individuals), resulted in a human reference dose (RfD) for subchronic oral exposure of 1.43 mg/kg/day, which was rounded to 1 mg/kg/day. The subchronic or partial lifetime RfD was described as "an estimate of an exposure level which would not be expected to cause adverse effects when exposure occurs during a limited time interval, i.e., for an interval which does not constitute a significant portion of the lifespan" (EPA 1989).

Benzoic Acid and Sodium Benzoate

Subchronic oral studies on the benzoates which were cited in the GRAS report (Informatics Inc. 1972) and in the literature review by the Flavor and Extract Manufacturers' Association (1984) are summarized in Table 4.

Reviewing the studies, the GRAS report (Informatics Inc. 1972) concluded that " . . . at a level of approximately 1%, the benzoates are at maximum nontoxic level; higher than this, they result in decreased food intake, depressed growth, and toxic effects on test animals."

Despite feed consumption comparable to controls, significant reduction in weight gain was noted in mice treated for 3 months with 80 mg/kg/day Benzoic Acid (Shtenberg and Ignatév 1970), and in rats treated for 90 days with 8% Sodium Benzoate (Deuel et al. 1954).

Chronic Oral Toxicity

Benzyl Alcohol

Groups of 100 F344/N rats (50 each sex) were dosed with 200 or 400 mg/kg Benzyl Alcohol in corn oil, 5 days per week for 103 weeks. Groups of 100 B6C3F$_1$ mice were dosed with 100 or 200 mg/kg Benzyl Alcohol following the same schedule. During week 80, mice were mistakenly dosed for four days

TABLE 4
Multiple-dose oral toxicity studies on Benzoic Acid and Sodium Benzoate

Protocol	Results/comments	Reference
Benzoic Acid		
40 Sprague-Dawley rats (20 each sex) received feed containing either 0.5% or 2% for 1 year. Some other groups also received sorbic acid	No effect noted at 0.5%; slight reduction of growth rate noted at 2%. No additive toxicity noted of Benzoic Acid plus sorbic acid	Ohno et al. 1978*
Royal Wistar rats dosed with 3% for 1, 2, 3, or 5 days (~1500 mg/kg/day); basal diet followed for 19 to 30 days	Fourteen of 35 rats dosed for 5 days died; necrosis of parenchymal cells noted in brain in all 5-day treated rats and occasionally in 3-day treated rats	Kreis et al. 1967
Royal Wistar rats (number not stated) dosed with 1.1% for 7, 14, or 35 days (~550 mg/kg/day)	Significantly poor weight gain; no signs of neurotoxicity or pathological changes in the brain	Kreis et al. 1967
100 mice (50 each sex) dosed for 3 months with 80 mg/kg/day (oral intubation)	Weight gain in treated animals was 66% (females) and 71% (females) of gain in controls, values significant; however, feed intake comparable	Shtenberg and Ignatév 1970
50 mice (25 each sex) dosed with 40 mg/kg/day; fed as a paste for 17 months, followed by 5 days of oral intubation	Major finding was a reduced response to physiological stress in treated animals compared to controls	Shtenberg and Ignatév 1970
Mice (number not stated) dosed with 40 or 80 mg/kg/day for 3, 8, or 18 months	Negative effect on body weight and viability; treatment-related carcinogenic effects noted (not specified); increased liver weights, enlarged spleens, ovaries, and lungs	Ignatév 1965
20 rats (10 each sex) dosed with 40 mg/kg/day; fed as a paste for 18 months, followed by 13 days of oral intubation	Developed increased tolerance to lethal doses of Sodium Benzoate; daily feed and water intake significantly less for treated males; limited data reported	Shtenberg and Ignatév 1970
Rats (number not stated) dosed with 40 or 80 mg/kg/day for 3, 8, or 18 months	No apparent effect on body weight or viability; no changes noted in parenchymatous organs; developed increased tolerance to lethal doses of Benzoic Acid	Ignatév 1965
50 Wistar rats (20 female, 30 male), 20 male Wistar rats, and 20 male Osborne-Mendel rats, dosed with 1.5% in feed for 18 months	Decreased feed intake and reduced growth	Marquardt 1960
Four generations of Bayer-Elberfeid rats dosed with 0.5 or 1.0% in feed	No adverse effect noted; increased life span noted in treated rats	Kieckebusch and Lang 1960
Sodium Benzoate		
28 rats dosed with 5% in feed	Nineteen of 28 died within two weeks of dosing; remaining 9 died by end of week 3	Kieckebusch and Lang 1960
12 Sherman rats (6 each sex) dosed with 2% or 5% in feed for 28 days	Slight weight depression (significant in males) noted at 2%; 5% toxic to all rats	Fanelli and Halliday 1963
Groups of 10 Sherman rats (5 each sex) dosed with 16 to 1090 mg/kg/day (four doses) for 30 days	No toxic effects; increased body weight, reduced appetite (compared to control), noted. Lesions of adrenal glands, upper intestine, kidneys, liver, and spleen	Smyth and Carpenter 1948

(Continued on next page)

TABLE 4
Multiple-dose oral toxicity studies on Benzoic Acid and Sodium Benzoate *(continued)*

Protocol	Results/comments	Reference
Rats (numbers not stated) dosed with 1947 to 2195 mg/kg/day for 3 to 6 weeks	Severe reduction of growth rate	White 1941
Wistar rats dosed with 1.5% in feed for 6 or 8 weeks (after week 4, carotene was added to diet)	No significant effect noted. Vitamin A content in liver and kidneys comparable to control	Kramer and Tarjan 1962
Groups of 10 Sherman rats (5 each sex) dosed with 1%, 2%, 4%, and 8% in feed for 90 days	No adverse effects at ≤4%. At 8% reduced growth rate (feed consumption comparable to control), significantly increased liver and kidney weight with lesions noted	Deuel et al. 1954
White rats (number not stated) dosed with 1.5%, 2.0%, 2.5%, 3.0% in feed for unknown duration	No effects noted in rats of ≤2.5% groups; distinct growth reduction noted in rats of 3.0% group though feed intake was comparable to control. One third of rats of this group died	Griffith 1929

*Study completed since the GRAS report (see text), but included for completeness.

with 375 (low-dose group) and 750 mg/kg (high-dose group) of α-methylbenzyl alcohol. No adverse effects were apparent (NTP 1989).

Mean body weights were comparable among dosed and vehicle control rats throughout the study. A number of accidental deaths was due to gavage procedures in female rats of both dose groups (17 deaths, low-dose; 13 deaths, high-dose) and in males of the 400 mg/kg group (14 deaths). Survival of female rats of the low- and high-dose groups was significantly lower than that of vehicle controls after weeks 71 and 50, respectively. At the end of the study, 17 female rats survived from each of the dose groups, compared to 35 female vehicle-controls; 27 low-dose males and 24 high-dose males survived, compared to 28 male vehicle controls. Clinical signs characteristic of sialodacryoadenitis (cervical swelling, pink eyes, red exudate around eyes) were observed in dosed and vehicle-control rats. The diagnosis was confirmed by serum analysis. Epithelial hyperplasia of the nonglandular stomach was noted in four high-dose males. A squamous cell papilloma was noted in 1 of 19 low-dose and 1 of 50 high-dose males. (It was not stated why only 19 low-dose male rats were examined.) No other compound-related clinical signs were observed.

Mean body weight was comparable among dosed and vehicle control mice throughout the study. Survival of female vehicle controls was significantly lower than that of the high-dose group after week 74 (female: vehicle control, 26/50; low dose, 32/50; high dose, 36/50). Corpora amylacea (foci of mineralization in the thalamus) was observed at an increased incidence in high-dose mice (male: vehicle control, 15/49; low dose, 21/48; high dose, 22/50; female: 14/50; 15/48; 25/50), but was noted to be a common and spontaneously occurring lesion (NTP 1989).

Reviewing the 2-year study, the EPA extrapolated the rat high dose to a human dose of 52 mg/kg/day (for a 70-kg person). The EPA determined that the LOAEL was 286 mg/kg for male rats. (Data from male rats were selected because mortality in female rats was not definitively associated with Benzyl Alcohol treatment.) Using this level, and applying an uncertainty factor of 1000 (10 for interspecies extrapolation, 10 to protect unusually sensitive individuals, and 10 to extrapolate a LOAEL to a NOAEL) resulted in a human RfD for chronic oral exposure of 0.286 mg/kg/day, which was rounded up to 0.3 mg/kg/day (EPA 1989).

Benzoic Acid and Sodium Benzoate

Table 4 summarizes chronic oral studies of the benzoates that were included in the GRAS report (Informatics Inc. 1972) and in the literature review by the Flavor and Extract Manufacturers' Association (1984). Decreased feed intake and reduced growth were noted in rats fed 1.5% Benzoic Acid for up to 18 months (Marquardt 1960). No adverse effects were noted in most rat studies that used ≤1% Benzoic Acid (Ohno et al. 1978; Ignatév 1965; Kieckebusch and Lang 1960). One 18-month study reported significantly decreased feed and water intake in male rats fed 40 mg/kg/day (Shtenberg and Ignatév 1970). A dose-dependent response to doses of Benzoic Acid well below 1% was noted in mice (Ignatév 1965; Shtenberg and Ignatév 1970).

Acute Inhalation Toxicity

Benzyl Alcohol

Three groups of six Sherman rats were exposed for 4 hours to a 2000-ppm concentration of Benzyl Alcohol vapor in normal atmosphere. Nine rats died within 14 days of exposure. The investigators considered the compound to be a moderate hazard (Carpenter, Smyth, and Pozzani 1949).

Smyth, Carpenter, and Weil (1951) reported that rats could inhale air saturated with Benzyl Alcohol vapor for a maximum of 2 hours. Similar to the results reported by Carpenter, Smyth, and Pozzani (1949), inhalation at a concentration of 1000 ppm for 8 hours caused death of three of six animals within 14 days of exposure.

Acute Parenteral Toxicity

Benzyl Alcohol

In a study to determine the toxicity of various vehicles, Montaguti, Melloni, and Cavalletti (1994) administered undiluted Benzyl Alcohol intravenously (via the tail vein) to groups of 10 mice (5 of each sex). Three different mice strains were used with the following dose ranges. CD_2F_1 mice received 0.05 to 0.2 ml/kg, $B_6D_2F_1$ mice received 0.05 to 0.4 ml/kg, and C57BL/6N mice received 0.025 to 0.1 ml/kg. All mice weighed between 14 and 18 g. The highest dose given did not exceed the LD_{50}. Body weight was determined prior to the start of dosing, and 1 week thereafter. Animals were observed for 14 days and postmortem examinations were performed on day 15. Blood samples were withdrawn from the abdominal aorta and analyzed for hemolysis and precipitation potential.

Convulsions, dyspnea, and reduced mobility were noted at the first 24-hour observation in mice treated with all but the lowest dose of Benzyl Alcohol. Decreased body weight gain or slight decrease in body weight was noted in $B_6D_2F_1$ and C57BL/6N mice treated with all but the lowest dose. Postmortem alteration included hyperemia and edema in most animals that had died during the observation period (number not reported). Occasional hemorrhagic foci were observed in the spleen of C57BL/6N mice from all dose groups that had survived Benzyl Alcohol treatment. The blood from Benzyl Alcohol–treated mice had a potential for hemolysis and precipitation. Undiluted Benzyl Alcohol was ranked the most toxic of the five vehicles tested, which included dimethyl sulfoxide, polyethylene glycol 400, dimethylformamide, and absolute ethanol (Montaguti, Melloni, and Cavalletti 1994).

The literature review by the Flavor and Extract Manufacturers' Association (1984) cites several earlier animal studies in which Benzyl Alcohol was administered as either single or multiple doses via the intraperitoneal, intravenous, and subcutaneous routes.

Neurotoxicity

Benzoic Acid and Sodium Benzoate

In response to concerns about the role of food additives in cases of childhood hyperactivity, Crane and Lachance (1985) performed a neurobiological study of Sodium Benzoate using rats. Groups of eight Wistar dams received feed containing 0.1%, 0.5%, or 1.0% Sodium Benzoate beginning on gestation day (GD) 5 and continuing throughout pregnancy and lactation. The control group was untreated. At birth, the number of pups in each litter was equalized to eight. Locomotor activity of the pups was measured on various days. One pup from each litter was killed on days 9, 15, and 21 and the brain removed and examined. On day 22, pups were weaned onto the same diet as their respective dam. On day 24, one male pup from each litter was caged individually and monitored for spontaneous locomotor activity. Rats were killed on day 45 and brain concentrations of norepinephrine, dopamine, and serotonin were measured. No significant difference was noted in feed intake and body weight gain of dams and pups of the treated groups compared to controls. No consistent differences in motor activity and monoamine concentrations were noted.

Dermal Irritation

Benzyl Alcohol

In a primary irritation study 10% Benzyl Alcohol in squalane was applied (0.3 ml) in a 24-hour occlusive patch to the back of eight male albino rabbits. The sites had been clipped free of hair and were abraded in four rabbits. Sites were evaluated according to the Draize scoring system at the time of patch removal and 72 hours later. No irritation was observed; there was a score of zero on a scale of 0 to 8 (Shiseido Research Center 1972).

In a cumulative irritation study, three male albino guinea pigs received a daily open application of 10% Benzyl Alcohol in squalane (0.3 ml) on the back for 3 successive days. Sites were evaluated for erythema and edema 24 hours after each application and scored on a scale of 0 to 4. Benzyl Alcohol in squalane received a cumulative score of 0.4, falling in the $\leq$2.0 range of "none to weak irritant" (Shiseido Research Center 1972).

A polyvinyl chloride (PVC) cup containing 10% *w/v* Benzyl Alcohol was fastened (using surgical tape) to the dorsal side of three male nude mice for 24 hours of contact. The mice (MF 1 h) were 4 weeks old and weighed 10 to 22 g. Following exposure, mice were immediately killed and specimens of the exposed areas and of an adjacent untreated area were taken for microscopic examination. The skin sections were fixed in formalin, dehydrated, and embedded in paraffin. Sections were stained with hematoxylin and eosin and scored using the Ingram & Grasso system. A typical section from Benzyl Alcohol–treated areas had severe compact hyperkeratosis, acanthosis, spongiosis, intracellular edema, and some areas of ulceration of the epidermis. The collagen bundles in the dermis appeared slightly fragmented and slight cell infiltration of the area was noted. The final score for Benzyl Alcohol was 22, the modal score for at least three animals. Scores greater than 21 were considered "unacceptably severe damage." The investigators acknowledged that male nude mice were not an ideal model for human skin; however, the study was done to establish the relative dermal tolerance of various penetration enhancers (Lashmar, Hadgraft, and Thomas 1989).

Benzoic Acid and Sodium Benzoate

RTECS (1995) cited that the human low toxic dermal dose of Benzoic Acid was 6 mg/kg.

Phototoxicity

In Vitro

Suspensions of human erythrocytes were incubated with Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. Each material was tested at 10^{-5}, 10^{-4}, and 10^{-3} mol/l. Erythrocyte-free samples were also incubated with the test materials and used as controls. Following incubation, suspensions and samples were exposed to varying amounts of ultraviolet A (UVA) light from one of three sources. Hemolysis was measured as a function of absorbance of 550 nm light. None of the three substances produced significant photohemolysis (Eberlein-König et al. 1993).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oral Studies

Benzyl Alcohol

In a study by Inveresk Research International Ltd. (1983), a group of 50 specific pathogen–free (SPF) CD-1 mice received 750 mg/kg/day Benzyl Alcohol (in distilled water) by gavage on GDs 7 to 14. (Earlier toxicity studies had determined the maximum tolerated dose was between 645 and 1300 mg/kg/day, and the 750-mg/kg/day dose was selected for the reproduction study.) The concurrent vehicle control had 50 mice. Mice were individually caged and feed and water were available *ad libitum*. Clinical observations were made daily. Maternal body weights were recorded prior to dosing, on day 18, and on postnatal day 3; the weight on day 7 determined the dose volume administered over the entire treatment period. Mice were allowed to deliver their litters and nurse the pups for 3 days.

There were 18 compound-related deaths during the dosing period, and one on GD 15. Mice that died were discarded without necropsy. No procedure-related deaths (i.e., gavage error) were recorded. Body tremors, hunching, subdued behavior, prostration, ataxia, swelling, and/or cyanosis of the abdomen and piloerection were noted in mice that died during the study as well as those that produced litters.

No significant differences in reproductive and gestation indices, or in mean gestation length were noted between treated and control mice. A significantly lower day 18 mean body weight and a marginally reduced maternal weight on postpartum day 3 were noted in dosed dams. Decreased mean litter mean pup weight was noted on postpartum days 1 ($p < .01$) and 3 ($p < .001$). On postpartum days 1 to 3, a decreased mean litter weight change ($p < .05$) and decreased mean litter mean pup weight ($p < .001$) were noted. No significant differences were noted between treated and control pups in group litter viability. The investigators considered Benzyl Alcohol a suspect reproductive hazard and recommended further investigation (Inveresk Research International Ltd. 1983). Citing that study, the EPA (1989) noted that the extrapolated human dose (for a 70-kg person) was 58 mg/kg/day.

In screening a new developmental toxicity assay, 50 pregnant CD-1 mice were gavaged on GDs 6 to 13 with Benzyl Alcohol at a rate of 750 mg/kg/day. The dose selected was the LD_{10} value determined in preliminary dose-finding studies. Mice were allowed to deliver. Litter size, birth weight, and neonatal growth and survival to postnatal day 3 were measured. Nineteen (38%) of the dams of the Benzyl Alcohol group died prior to delivery; the corresponding vehicle control group (which received water) had no maternal death. (Mice that died were not necropsied.) Maternal weight was significantly less changed in the Benzyl Alcohol group (6.2 ± 3.6 g) as compared to controls (7.9 ± 2.3 g). Viability in the Benzyl Alcohol group was 21 of 22 litters (controls had 29/29 viability) with an average of 10.0 liveborns per litter. Birth weight (1.6 g/pup) and 3-day weight gain (0.5 g/pup) for pups of the Benzyl Alcohol treatment group were significantly less ($p < .05$) than the corresponding values in controls (1.7 and 0.7 g/pup, respectively). The reduced birth weight was classified as "some evidence of developmental toxicity." The researchers noted the 10% false-negative rate for toluene, a "presumptive teratogen" (Hardin et al. 1987).

A group of 50 pregnant SPF CD-1 albino mice was dosed with 550 mg Benzyl Alcohol/kg/day on GDs 6 to 15 by gavage. The Benzyl Alcohol was dissolved in corn oil; a vehicle-control group was maintained. Maternal status (survival, body weight changes), gestation index (length of gestation), reproductive index, postnatal survival, average litter weight, and average pup weight were comparable between treated and control animals (Environmental Health Research & Testing, Inc. 1986).

Benzoic Acid and Sodium Benzoate

Benzoic Acid at doses of 6, 30, 60, and 600 mg/kg was administered by stomach tube to groups of 21 to 24 pregnant golden hamsters on GDs 6 to 10. Two negative-control groups were maintained; one was treated with water, the other with 0.5% carboxymethylcellulose. A positive-control group received either thalidomide or aspirin. Dams were killed on day 16. No adverse effect in maternal survival was noted. A significant number of resorptions was noted in hamsters which received ≥30 mg/kg. The incidence of fetal malformations reached statistical significance at >600 mg/kg (Polish Academy of Sciences 1977).

Benzoic Acid at doses of 5, 25, 50, and 500 mg/kg was administered by stomach tube to groups of 20 pregnant Wistar rats on GDs 6 to 15. Two negative-control groups were maintained; one was treated with water, the other with 0.5% carboxymethylcellulose (used to keep the Benzoic Acid in suspension). A positive-control group received either thalidomide or aspirin. Dams were killed on day 21. Maternal survival was similar for treated and control groups. A significant number of resorptions was noted in rats which received ≥25 mg/kg. The incidence of fetal malformations in Benzyl Alcohol treated rats did not reach statistical significance (Polish Academy of Sciences 1977).

No evidence of teratogenicity was noted in rats administered 510 mg/kg of Sodium Benzoate by gavage on GDs 9 to 11 (Kimmel, Wilson, and Schumacher 1971).

Sodium Benzoate at doses of 1.75, 8, 38, and 175 mg/kg was administered by oral intubation to groups of at least 20 pregnant

TABLE 5
Benzyl Alcohol genotoxicity studies

Assay	Concentration/method	Results/comments	Reference
Bacterial Cells			
Ames: *S. typhimurium* TA 100	100, 250, 500, 1000 μg/plate	Negative	Ball Foxall-Van Aken, and Jensen 1984
Ames: *S. typhimurium* TA 98, TA 100	Not stated	Negative	Rogan et al. 1986
Ames: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537	100; 333; 1000; 3333; 5000; 6666 μg/plate ± S9	Negative	NTP 1989
Mammalian Cells			
L5178Y tk+/tk− forward mutation in mouse lymphoma cell line	[cells incubated with agent for 4 h, then plated to determine thymidine resistance]		McGregor et al. 1988
	(−) S9:		
	156.25; 312.5; 625; 1250; 2500; 5000 μg/ml	Negative ≤2500; lethal at 5000 μg/ml	
	2500; 3000; 3500; 4000; 4500; 5000 μg/ml	POSITIVE at 4500; lethal at 5000 μg/ml	
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
	3200; 3400; 3600; 3800 μg/ml	Negative	
	(+) S9:		
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
L5178Y tk+/tk− forward mutation in mouse lymphoma cell line	(−) S9:		NTP 1989
	156.25; 312.5; 625; 1250; 2500; 5000 μg/ml	POSITIVE at 5000 μg/ml	
	2500; 3000; 3500; 4000; 4500; 5000 μg/ml	POSITIVE at 4500; lethal at 5000 μg/ml	
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
	(+) S9:		
	250; 500; 1500; 2500; 3500 μg/ml	Negative ≤2500; lethal at 3500 μg/ml	
Chromosome aberration in Chinese hamster ovary cells (ABS in CHO)	(−) S9: [cells incubated with agent for 8–10 h]		NTP 1989
	160; 500; 1600; 5000 μg/ml	Negative	
	2000; 3000; 4000; 5000 μg/ml	POSITIVE	
	250; 500; 1600; 3000 μg/ml	Negative	
	500; 1600; 3000; 4000 μg/ml	Negative	
	(+) S9: [cells incubated with agent for 2 h]		
	50; 160; 500; 1600; 5000 μg/ml	Negative	
	500; 1600; 3000; 4000 μg/ml	WEAK POSITIVE	
	1600; 3000; 4000; 5000 μg/ml	WEAK POSITIVE	
	1600; 3000; 4000; 5000 μg/ml	WEAK POSITIVE	

ABS in CHO	(−) S9:		Anderson et al. 1990
	160; 500; 1600; 5000 μg/ml	Negative	
	2000; 3000; 4000; 5000 μg/ml	Negative	
	250; 500; 1600 μg/ml	Negative	
	500; 1600; 3000 μg/ml	Negative	
	(+) S9:		
	50; 160; 500; 1600; 5000 μg/ml	Negative	
	500; 1600; 3000; 4000 μg/ml	POSITIVE at 4000 μg/ml	
	1600; 3000; 4000 μg/ml	POSITIVE at 4000 μg/ml	
	1600; 3000; 4000 μg/ml	POSITIVE at 4000 μg/ml	
Sister Chromatid Exchange (SCE) in CHO	(−) S9: [cells incubated with agent for 2 h, Brd-U added, then incubated for another 24 h]		NTP 1989
	16; 50; 160; 500; 1600 μg/ml	Negative $\leq$500; lethal at 1600 μg/ml	
	500; 750; 1000; 1250; 1500 μg/ml	WEAK POSITIVE; lethal at 1500 μg/ml	
	(+) S9: [cells incubated with agent for 2 h, Brd-U added after cells washed]		
	16; 50; 160; 500; 1600; 5000 μg/ml	Negative $\leq$1600; lethal at 5000 μg/ml	
	500; 1600; 3000; 4000; 5000 μg/ml	WEAK POSITIVE; lethal at 5000 μg/ml	
SCE in CHO	(−) S9:		Anderson et al. 1990
	16; 50; 160; 500 μg/ml	Negative	
	500; 750; 1000; 1250 μg/ml	WEAK POSITIVE at 1250 μg/ml	
	(+) S9:		
	16; 50; 160; 500; 1600 μg/ml	Questionable results	
	500; 1600; 3000; 4000 μg/ml	WEAK POSITIVE at 4000 μg/ml	
In vivo: Fruit Fly			
Sex-linked recessive lethal in *Drosophila melanogaster*	Following dosing either orally with 5000 ppm or injected with 8000 ppm, males were mated with untreated females, progeny observed as indication of mutagenic activity	Negative	Foureman et al. 1994
In vivo: Mammalian			
Replicative DNA synthesis (RDS)	Male rats were killed at various times post dosing (300 or 600 mg/kg by either single oral gavage or SC injection) and hepatocytes obtained from perfused liver samples were incubated with [^{3}H]thymidine. RDS measured as percentage of [^{3}H]thymidine-incorporating cells relative to 2000 hepatocytes	Negative; at various times cell viability for Benzyl Alcohol–treated cells was significantly reduced	Uno et al. 1994

albino CD-1 outbred mice and Wistar albino rats on GDs 6 to 15. Groups of 21 to 22 pregnant hamsters were dosed with 3, 14, 65 or 300 mg Sodium Benzoate/kg on GDs 6 to 10. Groups of 10 Dutch-belted rabbits were artificially inseminated and then dosed by oral intubation with 2.5, 12, 54 or 250 mg Sodium Benzoate/kg on GDs 6 to 18. Dams were individually caged and feed and water were available ad libitum. Positive-control groups for mice, rats, and hamsters received aspirin. A positive-control group of rabbits received 6-aminonicotinamide. Sham groups for each animal type served as negative controls. Caesareans were performed on mice, rats, hamsters, and rabbits on days 17, 20, 14, and 29, respectively. Neither adverse effects on maternal or fetal survival nor a significant increase in fetal abnormalities in either soft or skeletal tissues was noted in any of the animals (Food and Drug Research Labs Inc. 1972).

Parenteral Studies

Benzyl Alcohol

In a study which assayed the teratogenic activity of ethinyloestradiol sulfonate in Wistar rats, a vehicle control group that was treated with Benzyl Alcohol/peanut oil was maintained. On GDs 10, 13, 6 to 10, or 10 to 14, rats (number not stated) received intraperitoneal (IP) injections of either the test material or an unspecified amount of vehicle. Fetuses were removed on day 21 and examined. No teratogenic effect was noted (Chemnitius, Oettel, and Lemke 1979).

Benzoic Acid and Sodium Benzoate

Sprague-Dawley rats were injected intraperitoneally with 100, 315, or 1000 mg/kg Sodium Benzoate on GDs 9 to 11 or 12 to 14. Reduced fetal body weight, increased in utero deaths (by 12%), and gross anomalies were noted at the highest dose (Minor and Becker 1971).

GENOTOXICITY

Benzyl Alcohol

Benzyl Alcohol was negative in the Ames test with and without metabolic activation (Ball, Foxall-Van Aken, Jensen 1984; Rogan et al. 1986; NTP 1989), sex-linked recessive lethal (flies) (Foureman et al. 1994), and replicative DNA synthesis (male rats) (Uno et al. 1994) assays. McGregor et al. (1988) considered results of a mouse lymphoma forward mutation assay in the absence of S9 activation to be "questionable," whereas NTP (1989) reported a positive response at concentrations associated with toxicity. Both studies were negative with S9 activation. Benzyl Alcohol, with S9 activation, was positive in the chromosome aberration test in Chinese hamster ovary (CHO) cells (NTP 1989; Anderson et al. 1990). Equivocal results were noted in the sister chromatid exchange (SCE) assay (NTP 1989; Anderson et al. 1990). Genotoxicity studies concerning Benzyl Alcohol are summarized in Table 5.

Benzoic Acid and Sodium Benzoate

Benzoic Acid was negative in the Ames (Fujita and Sasaki 1986; Zeiger et al. 1988) and SCE assays (Oikawa et al. 1980).

Sodium Benzoate was negative in the host-mediated (Litton Bionetics 1974), Ames (Prival, Simmon, and Mortelmans 1991), dominant lethal (rats) (Litton Bionetics 1974), and cytogenetics (both in vitro and in rats) (Litton Bionetics 1974) assays. When tested in the CHO cell line, Sodium Benzoate was positive in the chromosomal aberrations assay (Ishidate and Odashima 1977) and, at a high dose (2 mM), in the SCE assay (Abe and Sasaki 1977).

Njagi and Gopalan (1980) reported that incubation of adenosine, guanosine, uridine, or calf thymus DNA with Sodium Benzoate for up to 12 hours resulted in small shifts in the UV spectra. No shifts in the absorption peaks of the nucleoside cytidine were noted following incubation with Sodium Benzoate. The investigators noted that "DNA fragments do not have such shifts, thus the DNA must have remained intact during the course of incubation." Sodium Benzoate was considered not to act at the genetic level.

Genotoxicity studies concerning Benzoic Acid and Sodium Benzoate are summarized in Table 6.

CARCINOGENICITY

Oral Studies

Benzyl Alcohol

The 2-year gavage study performed by the NTP (detailed in the Oral Toxicity—Chronic section of this report) also tested for Benzyl Alcohol-induced carcinogenicity in rats and mice. Dose-related negative trends were noted in the incidences of anterior pituitary gland neoplasms in female rats (vehicle control, 29/50; low dose, 17/47; high dose, 9/49) and of Harderian gland adenomas in male mice (8/50; 3/50; 2/50). Epithelial hyperplasia of the nonglandular stomach was noted in 4 of 50 high-dose male rats; it was not found in controls or low-dose male rats. An increased incidence of adenomas of the adrenal cortex noted in high-dose male mice (0/48; 0/44; 3/48) was within historical range and not considered compound-related (NTP 1989). The NTP investigators considered the study negative for Benzyl Alcohol–induced carcinogenicity. However, reviewing the study, the EPA (1989) considered the 3 of 48 incidence of adrenal cortex adenoma to be "equivocal evidence of carcinogenic activity rather than negative."

Benzoic Acid and Sodium Benzoate

For 18 to 24 months, groups of Fischer 344 rats (50 males and 52 females per group) received feed containing 2% or 1% Sodium Benzoate. The doses corresponded to the maximum tolerated dose (MTD) and $^1/_2$MTD as determined in 6-week toxicity studies. A control group of 25 male and 43 female rats received untreated feed. Average daily Sodium Benzoate intake was 280 and 202 mg, respectively, for male and female rats of the 2% group, and 141 and 102 mg, respectively, for male and female

TABLE 6
Benzoic Acid and Sodium Benzoate genotoxicity studies

Assay	Concentration/method	Results/comments	Reference
Bacterial Cells			
Host-Mediated	Mice orally dosed (either single dose or five doses each 24 h apart) with 50, 500, 5000 mg/kg Sodium Benzoate, then inoculated with *Salmonella* TA-1530, G-46, and *Saccharomyces* D3; 3 h later animals were killed and the bacteria were removed (by peritoneal wash) and plated	Negative (slight increases in mutation frequencies noted; non-dose dependent)	Litton Bionetics 1974
Ames: *S. typhimurium* TA 97A, TA 102	33–10000 μg Benzoic Acid/plate ± S9	Negative	Fujita and Sasaki 1986
Ames: *S. typhimurium* TA 97, TA 98, TA 100, TA 1535, TA 1537	Benzoic Acid at 100–6666 μg/plate or 100–10,000 μg/plate ± S9 (either rat or hamster liver)	Negative	Zeiger et al. 1988
Ames: *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538; *E. coli* WP2	0.033–10 mg Sodium Benzoate per plate ± S9	Negative	Prival, Simmon, and Mortelmans 1991
Mammalian Cells			
SCE in CHO	1, 3, 10 mM Benzoic Acid	Negative	Oikawa et al. 1980
SCE in CHO	1, 2, 5, 10 mM Sodium Benzoate	POSITIVE at ≥2 mM (considered a high dose)	Abe and Sasaki 1977
ABS in CHO	Maximum effective dose: 2.00 mg/ml (138.8×10^{-4} M) Sodium Benzoate	POSITIVE: aberrations noted in 38%	Ishidate and Odashima 1977
Cytogenetics (human embryonic lung cells)	2, 20, 200 mg/kg Sodium Benzoate	Negative (checked for aberrations in anaphase chromosomes)	Litton Bionetics 1974
In vivo: Mammalian			
Dominant lethal (rats)	Following dosing by oral intubation (50, 500, 5000 mg/kg Sodium Benzoate either single dose or five doses each 24 h apart), male rats were mated with two females per week for 8 weeks. Corpora lutea, early and late fetal deaths, and total implantations monitored	Negative	Litton Bionetics 1974
Cytogenetic (rats)	Rats dosed by gastric intubation (50, 500, 5000 mg/kg Sodium Benzoate either single dose or five doses each 24 h apart, killed at various times after dosing (were given colcemid to arrest cells in metaphase)	Negative (checked for aberrations in bone marrow metaphase chromosomes)	Litton Bionetics 1974

rats of the 1% group. No clinical signs of toxicity, differences in average body weight or mortality rates were noted in treated rats when compared with controls. Neoplasms that were present in treated rats were similar in type and number to those in controls. No evidence of Sodium Benzoate–related carcinogenicity was observed (Sodemoto and Enomoto 1980).

In a life time drinking water study, 100 Albino Swiss mice (50 of each sex) were supplied with water containing 2% Sodium Benzoate. A control group of 200 mice was supplied with untreated water. Average daily intake of Sodium Benzoate was 124.0 and 119.2 mg for males and females, respectively. Sodium Benzoate treatment did not affect survival. No carcinogenic effect attributable to treatment was noted at necropsy (Toth 1984).

In Vitro Studies

Benzoic Acid and Sodium Benzoate

In an in vitro study hippurate and its parent compound Sodium Benzoate had antitumor effects on cells derived from Skalsky lymphoma, Németh-Kellner lymphoma (LYNK), L-asparaginase–sensitive 6C3HED Gardner lymphoma (GS), and LP-2 plasmacytoma. In a follow-up in vivo study, mice received subcutaneous implantations of GS or LYNK cells followed by twice daily IP injections of hippurate. A "high level of significance" in inhibition of tumor growth was reported. The in vivo study was not done using Sodium Benzoate (Spustová and Oravec 1989).

Dermal Studies

Jacobs et al. (1984) performed a skin-painting study using groups of 120 Eppley Swiss mice (60 each sex). A nonoxidative hair dye containing 2.0% Benzyl Alcohol and 0.016% Benzoic Acid was painted onto the skin at a dose of 0.05 ml/application, three times weekly for 20 months. Sites were shaved of hair 24 hours before each application and a new bottle of dye was used each week. Two groups of control animals were shaved but not treated. Nine months into the study, 10 mice/sex/group were killed. Body weights and survival differed little between treatment and control groups. Varying degrees of chronic dermal inflammation were noted in all groups, including the controls. A significant ($p < .01$) increase in malignant lymphomas was noted in treated females (23/60). However, the researchers noted that one concurrent control group had a very low incidence (7/60 or 12%) for that tumor type. The rate was 22% for the other control group and had averaged 33% for three control groups in previous studies. Thus, the findings were not considered treatment related. The incidence of pulmonary adenomas and hepatic hemangiomas, which are common to this mouse strain, were similar between treated and control groups. No unusual neoplasms were observed.

CLINICAL ASSESSMENT OF SAFETY

Clinical Experience

Benzyl Alcohol

In 1981 and 1982 several neonatal deaths were ascribed to Benzyl Alcohol present as a preservative in isotonic saline (9 mg/ml) that had been used to flush catheters (Brown et al. 1982; Gershanik et al. 1982). The syndrome consisted of metabolic acidosis, central neural depression, respiratory distress progressing to gasping respiration, hypotension, renal failure, and sometimes seizures and intracranial hemorrhages. In alerting pediatricians of the findings, the FDA (1982) reported an estimated daily intake of 99 to 404 mg/kg, which was 20 to 90 times the 4.5-mg/kg dose considered safe for healthy adults (Kimura et al. 1971). Although the infants involved had "serious underlying disease," biochemical evidence of Benzyl Alcohol toxicity was found. Blood and urine specimens contained high concentrations of Benzyl Alcohol, Benzoic Acid, and hippuric acid. The FDA stated that no cases of toxicity were observed in older infants, children, or adults.

In May 1985, FDA published a notice of intent that it was considering prohibiting use of all antimicrobial preservatives in single-dose containers of parenteral drug products for human use, and requiring multiple-dose parenteral drugs that contain any antimicrobial preservative to bear a warning that caution should be used in the administration to newborn infants. This intent was withdrawn in 1989 with the explanation that manufacturers of bacteriostatic water for injection and bacteriostatic sodium chloride injection had voluntarily agreed in 1982 that these two classes of products would contain a warning label against their use in newborns. Further, a 1983 revision of the US Pharmacopeia monograph required that these two classes of products bear the warning "not for use in newborns." The withdrawal by the FDA noted that the increased awareness brought about by these steps, in conjunction with the lack of additional reports of toxicity, prompted the decision that further regulation was not necessary.

Studies that compared infants born before and after the discontinuation of Benzyl Alcohol–containing solutions have generally supported the above measures (Menon et al. 1984; Benda, Hiller, and Reynolds 1986; Hiller et al. 1986; LeBel et al. 1988; Jardine and Rogers 1989; Cronin, Brown, and Ahdab-Barmada 1991).

Reports are available contraindicating the use of neuromuscular blocking agents containing Benzyl Alcohol (Craig and Habib 1977; Hahn, Feasby, and Gilbert 1983). Use of these agents was not advised in neonates (van der Hal et al. 1987) or in the epidural space (King and Hart 1994).

Reynolds and Smith (1995) reported that nebulizers of bacteriostatic saline containing Benzyl Alcohol as a preservative can cause bronchitis in healthy adults.

Benzoic Acid and Sodium Benzoate

FASEB (1973) reported no adverse effects following ingestion of Benzoic Acid at doses of 100 mg/day (82 doses in 86 days), 500 and 1000 mg/day (for 44 days), and 1000 mg/day (88 doses in 92 days). The number of participants was not reported.

In another study, participants initially ingested Benzoic Acid at 1000 mg/day for 5 days and progressed to 1500 then 2000

and finally 2500 mg/day staying at each protocol for 5 days before increasing the dose. Three of the 12 participants took the entire dose of 35 g in 20 days. This total dose produced marked symptoms of discomfort and malaise, which included nausea, headache, weakness, esophageal burning and irritation, hunger, and indigestion (Wiley and Bigelow 1908).

In an early study (Lucas 1909), 12 men drank apple juice containing 0.1% Sodium Benzoate and had the following signs and symptoms: burning taste, fullness in the head, headache, nervousness, nausea, vomiting, itching of the skin, unusual perspiration, constipation, decreased urine flow, increased specific gravity of the urine, and albuminuria. A liter of filtered cider containing 0.2% to 0.3% Sodium Benzoate (2 to 3 g) caused albuminuria within 3 hours of ingestion. However, Lucas himself ingested as much as 6 g/day for 3 successive days without adverse effect.

A single oral dose of 33 g of Sodium Benzoate to a 60-kg individual resulted in "clear signs of poisoning." Signs and symptoms including deep pallor, weak and infrequent pulse, general discomfort, cephalea, and nausea. Similar effects were noted after ingestion of 50 g of Sodium Benzoate over a 5-hour period (Bignami and Boraccia 1924).

Dermal Irritation

Benzyl Alcohol

Benzyl Alcohol (3%) was applied in a polypropylene chamber to the same site on the back of nine healthy female panelists for 4 consecutive days. The duration of exposure was not specified. Sites were visually evaluated on the fifth day. Benzyl Alcohol was an irritant according to the Frosch-Kligman scoring system (Harvell et al. 1994).

Benzoic Acid and Sodium Benzoate

A liquid/powder foundation containing 0.2% Benzoic Acid was applied in a 24-hour occlusive patch to the backs of 12 panelists. A total of three exposures occurred within 1 week. Sites were evaluated at the time of patch removal and 24 hours later (i.e., prior to application of the subsequent patch). No reactions were observed (Biosearch Inc. 1992a).

Forty-eight female panelists participated in an in-use study that investigated the acnegenic and irritation potential of a liquid/powder foundation containing 0.2% Benzoic Acid. Complying with the test protocol, approximately half of the test population had "mild to moderate" acne. Panelists were instructed to apply the product to the entire face and neck area at least twice a day for 45 days. Acne lesions and irritation were evaluated by a dermatologist on days 0, 3, 7, 10, 28, and 45. Objective and subjective evaluations of irritation were made by a nurse or technician on days 15, 21, and 35. Panelists also maintained daily response logs. The dermatologist noted "no significant changes the lesion counts of the non acne subjects and the acne subjects had a decrease. All objective irritation grades were 0's." Transient grade 1 irritation was noted by the technician; panelists' logs recorded occasional instances of dryness, itching, and flakiness (Education and Research Foundation, Inc. 1992).

Dermal Sensitization

DeGroot (1994) compiled the following recommended patch test concentrations from the published literature: Benzyl Alcohol at 1%, 5%, or 10% in petrolatum, Benzoic Acid at 2%, 5%, or 10% in petrolatum (with an advised test concentration of <5%), and Sodium Benzoate at 2% or 5% in petrolatum (with a note that the 5% concentration may be an irritant).

Benzyl Alcohol

A repeat-insult patch test (RIPT) was conducted using a nonexclusive group of 110 panelists. Two mascara formulations each containing 0.65% Benzyl Alcohol were tested. During a 3-week induction period nine occlusive 24-hour patches (containing ~0.15 g of test material) were applied to the same site on either the upper arm or back. Sites were evaluated 24 hours after patch removal (i.e., prior to application of subsequent patch). Following a 12- to 20-day nontreatment period, a challenge patch was applied to both the original site and a previously unexposed site. Challenge sites were evaluated at 24 and 48 hours after patch removal. No reactions were noted during induction or at challenge to either formulation (Hill Top Research 1997a, 1997b).

Patch testing with Benzyl Alcohol (5% in petrolatum) was part of the American standard series (Adams 1982) and the North American Contact Dermatitis Group (NACDG) perfume screening series (Emmons and Marks 1985).

The Research Institute for Fragrance Materials, Inc. (RIFM) report on Benzyl Alcohol cited an unpublished Kligman Maximization study that tested 10% Benzyl Alcohol in petrolatum using 25 male volunteers (skin types: 10 were Caucasian and 15 were Black). Benzyl Alcohol (and three other test materials) was applied under occlusive patches to the forearm of panelists. A total of five 48-hour exposures occurred during induction and each was preceded by a 24-hour occlusive pretreatment of the sites with 5% aqueous sodium lauryl sulfate (SLS). Following a 10-day nontreatment period, panelists were challenged on the scapular back with a 48 hour patch. Challenge sites were pretreated for 1 hour with 10% SLS. Challenge sites were examined at 48 and 72 hours. No reactions were observed (Kligman 1970; Opdyke 1973).

Adams and Maibach (1985) compiled patch test results from 12 dermatologists over a 6-year period. Patches had been applied to the upper back for 48 hours of contact, and sites were evaluated at 48 and 72 hours. Three cutaneous reactions to 5% Benzyl Alcohol in petrolatum were noted among 713 cosmetic dermatitis patients.

Van Joost, Stolz, and Van der Hoek (1985) reported four positive patch tests to 6.5% Benzyl Alcohol among 242 patients with histories of contact allergy of varying origin. An index of simultaneous reactivity in which the number of reactions to other perfume ingredients was divided by the number of positive

reactions to Benzyl Alcohol had a value of 0.50 (one individual responded to eugenol and another to isoeugenol).

Broeckx et al. (1987) reported results of a cosmetic intolerance assay that patch tested 5202 patients with possible allergic contact dermatitis (537 of the patients had a history of "intolerance," allergy, or irritation to cosmetics). Patch test conditions were not specified. A reaction was noted in 48 (0.92% incidence) to Benzyl Alcohol. Reactions were noted in 2 of the 155 patients with cosmetic allergy.

Cross-sensitization to Benzyl Alcohol has been reported in subjects sensitized to Peru balsam (Opdyke 1973).

Benzoic Acid and Sodium Benzoate

A liquid/powder foundation containing 0.2% Benzoic Acid was tested in a modified Draize repeat-insult patch test using 75 panelists. Nine 24-hour occlusive patches were applied to the back during a 3-week induction period. Following a 2-week nontreatment period, panelists were challenged at a previously unexposed site. Sites were evaluated at 24 and 48 hours after patch removal. No reactions were noted during induction or at challenge (Biosearch Inc. 1992b).

The RIFM report on Benzoic Acid cited an unpublished maximization test that tested 2% Benzoic Acid in petrolatum using 25 volunteers (skin types: 5 Black females, 2 Black males, 5 Caucasian females, 14 Caucasian males). During induction, a total of five 48-hour occlusive patches were applied to the same site (either forearm or back). Each was preceded by a 24-hour occlusive pretreatment of the site with 2.5% SLS. Following a 10-day nontreatment period, panelists were challenged at a different site with a 48-hour occlusive patch; the site had been pretreated for 1 hour with 5% to 10% SLS. Challenge sites were examined at the time of patch removal and 24 hours thereafter. No reactions were observed (Kligman 1977; Opdyke 1979).

Benzoic Acid (5% in petrolatum) did not elicit an allergic reaction when applied to the skin of 10 panelists who, in a previous Kligman-maximization assay, had tested positive for benzoyl peroxide sensitivity (Leyden and Kligman 1977).

Broeckx et al. (1987) reported results of a cosmetic intolerance assay that patch tested 5202 patients with possible allergic contact dermatitis (537 of the patients had a history of "intolerance," allergy, or irritation to cosmetics). Patch test conditions were not specified. A reaction to Benzoic Acid was noted in 34 (0.7% incidence). A reaction was noted in 1 of the 155 patients with cosmetic allergy.

Urticarial Reactions

Benzyl Alcohol and especially Benzoic Acid and Sodium Benzoate are among various compounds (such as some food additives) recognized in the published literature to induce nonimmunologic contact reactions in certain populations. Lahti (1980) reported that these agents "produce the reaction without any previous sensitization in most or almost all exposed persons." The hypersensitivity has been indicated by flexural dermatitis, rhinitis, and/or asthma. However, cutaneous changes such as urticaria, angioneurotic edema, and contact urticaria were the more common manifestations (Emmons and Marks 1985; Hannuksela and Haahtela 1987; Fisher 1990; DeGroot 1994). The terms nonimmunologic contact urticaria or nonimmunologic immediate contact reactions were used to describe the occurrence. Using Benzoic Acid, Kligman (1990) demonstrated that immediate reactions to urticariogens were concentration dependent and ranged from wheals induced with the highest test concentration, erythema with a fivefold dilution, and pruritus alone with a 25-fold dilution. (The panelists had been selected because they had developed a raised wheal to 1.0% Benzoic Acid.)

The methodology, test population, and results of various clinical studies demonstrating urticarial reactions are cited in Table 7.

In addition to the study cited in Table 7, Lahti (1980) presented results of various tests using dermatologic patients. Significantly more ($p < .001$) redness and edema were produced by Benzoic Acid (5% in petrolatum) under conditions of the open-test method as compared to the chamber test (both tests were conducted on upper back of 51 atopic and 55 nonatopic patients). Further, reactions were significantly more frequent ($p < .01$) in nonatopic dermatologic patients than in atopic patients when tested using the chamber test; the difference was almost significant in the open test ($p < .05$). However, when summarizing the findings Lahti ultimately concluded that "no significant differences were found in the frequency or strength of the nonimmunologic contact urticaria reactions between atopics and nonatopics." Most reactions appeared within 45 minutes and disappeared within 2 hours, with some persisting for longer than 24 hours (0.1 ml of test material was applied to the volar forearm of 29 atopic and 74 nonatopic physicians and nurses, evaluations were made every 15 minutes for 6 hours). The substance produced more reactions in a water vehicle than when applied in petrolatum; the lowest concentration needed to elicit a wheal and flare reaction was 0.050% Benzoic Acid in water or 0.10% Benzoic Acid in petrolatum (tested on 16 atopic and 16 nonatopic patients). The back, chest, dorsal sides of the forearm and upper arm, and thighs were the most sensitive areas. Neither scratching (11 atopics and 11 nonatopics) nor stripping (7 patients) of the skin prior to Benzoic Acid application increased the severity of the reaction. A diminished skin response was noted after repeated application of Benzoic Acid. In this aspect of the study, 0.1 ml of 5% Benzoic Acid in petrolatum was applied for 40 minutes to the dorsal side of the forearm of 17 nurses and physicians and 1 patient with a venous leg ulcer. The application was repeated on the same site 14 times at 2-hour intervals on the two subsequent days. Sites were evaluated after each exposure. A histamine scratch test was performed on two subjects after the skin had stopped reacting to Benzoic Acid (on the second day). Similar reactivity to histamine was noted between the test site forearm and the control arm. The finding suggested that the decreased reaction to Benzoic Acid resulted from an "emptying of the storage of mediator(s) in the skin rather than . . . fatigue of the dermal vessels and thus a failure to react." Lastly, Lahti

TABLE 7

Clinical patch and oral provocation tests demonstrating urticarial reactions

Test Population; method	No. of reactions (incidence)/comments	Reference
Benzyl Alcohol		
5% Benzyl Alcohol in petrolatum, 15 patients with eczematous dermatitis, 16 with history of cosmetic sensitivity, 19 controls; open testing (45-min exposure) and 48-h patch test	Open testing: contact urticaria noted in 7/15 (47%), 10/16 (63%), and 15/19 (79%) No positive patch tests	Emmons and Marks 1985
Benzoic Acid and Sodium Benzoate		
200 volunteers with no specific skin condition at test site; 125 and 500 mM applied for 20 min to volar forearm using Finn Chamber	To 125 mM: (on scale of 0–8) no. with erythema/no. with edema: score of 0 was 53/175, score of 1 was 44/19, score of 2 was 41/5, score of 3 was 25/1, score of 4 was 26/0, score of 5 was 10/0, score of 6 was 1/0 (none scored higher) To 500 mM: erythema/edema score of 0 was 43/164, score of 1 was 35/30, score of 2 was 41/5, score of 3 was 31/1, score of 4 was 39/0, score of 5 was 9/0, score of 6 was 2/0 (none scored higher)	Basketter and Wilhelm 1996
110 dermatological patients (36 atopics, 23 chronic urticaria, 26 nonatopic dermatitis, 25 non-allergic patients); chamber method with 20-min occlusion to upper back	5% Benzoic Acid in petrolatum, 43 positive reactions (39%)	Lahti 1980
80 housewives (none with known perfume allergy); 20-min occlusive patch to forearm	2% Benzoic Acid in petrolatum, 76 erythematous reactions of varying severity (95%), 15 incidences of edema (19%), 17 incidences of itching, stinging, burning, irritation sensations (21%)	Safford et al. 1990
125 children; patch tested over 7-year period (observed at 20 min for palpable pruritic erythema)	14 positive reactions (11%) to Benzoic Acid	Rademaker and Forsyth 1989
40 patients with urticaria, bronchial asthma, or chronic rhinitis; oral provocation test	Intolerance demonstrated in 2.5% of rhinitis patients and in 11.5% of asthma patients	Wüthrich and Fabro 1981
25 patients with clinical symptoms suggestive of food allergy; oral provocation test to Sodium Benzoate and 4-methylhydroxybenzoate	Positive reaction to benzoates noted in 34.21%	Ibero et al. 1982
132 patients with chronic urticaria and angioneurotic edema (suggested link to food additives); in a double-blind, placebo control study; oral provocation	5 positive reactions (4%) to Sodium Benzoate	Montaño García and Orea 1989
10 subjects with chronic urticaria and angioneurotic edema who had ≥1 positive reaction in histamine equivalent skin test; oral provocation test	1 positive reaction (10%) to Benzoic Acid	Malanin and Kalimo 1989
100 mg Sodium Benzoate—46 patients with history of chronic or acute urticaria/angioedema, chronic urticaria, chronic angioedema, or anaphylactoid reactions; DBPC oral challenge	15 positive reactions (32.6%); 12 reactions were in the 37 patients with chronic urticaria/angioedema	Sanchez-Borges and Suarez-Chacon 1992

noted the lack of correlation between dermal and oral exposure studies and concluded that the skin test "could not be used to predict sensitivity to preservatives taken perorally."

Shriner and Maibach (1996) investigated the variation of the nonimmunologic contact urticaria response in different areas of the body within and between two groups of panelists, "young" (10 women aged 23 to 47), and "old" (5 women aged 72 to 90). Panelists were not selected if they had a current or chronic history of dermatitis and/or current antihistamine use. A closed application method was used to apply 2.5% Benzoic Acid to the forehead, nose, nasolabial area, cheek, perioral area, chin, neck, and volar forearm for 20 minutes of exposure. In both age groups, the neck area was the most reactive followed by the perioral and nasolabial areas; the forearm was the least reactive. The younger group consistently demonstrated greater reactivity to Benzoic Acid at each site. The investigators noted that an earlier study had found the cheek to be the most responsive site.

Studying the mechanism behind these reactions, many investigators suggested a nonspecific histamine release (Forsbeck and Skog 1977; Guin et al. 1984; Larsen 1985), whereas Lahti (1987) argued for other (undetermined) mechanisms.

A 29- to 8000-fold increase in plasma concentrations of prostaglandin PGD_2 and a 72- to 370-fold increase in 9α, 11β-PGF_2 concentrations (the stable metabolite of PGD_2) was noted in four healthy panelists following topical application of 10% Benzoic Acid in petrolatum. Benzoic Acid had been applied to the forearm and covered with plastic wrap for 60 minutes of contact; blood had been drawn from the antecubital vein from the treated sites. The changes were not observed in blood drawn from the contralateral arm. The increased PGD_2 biosynthesis was dose-dependent over a concentration range of 0.01% to 15%. No cutaneous erythema was noted at <1%, patchy erythema was noted at 1%, maximal and confluent erythema was noted with ≥5% Benzoic Acid. Pretreatment with oral acetylsalicylic acid resulted in no erythema. The increased PGD_2 synthesis was not accompanied by histamine release. The investigators concluded that PGD_2 mediated the vasodilation associated with topical application of Benzoic Acid (Downard, Roberts, and Morrow 1995).

Lahti, Pylvänen, and Hannuksela (1995) reported that washing of the upper arm skin with a liquid detergent enhanced the immediate reactivity of the skin to Benzoic Acid. Benzoic Acid (10 μl) was applied without occlusive patches to test sites on the upper left and right arms of 12 healthy panelists on days 0, 3, and 6. The upper right arm was treated with the vehicle, a mixture of 2-propyl alcohol and 1,2-propylene glycol. Panelists were instructed to wash their upper left arm with a diluted dishwashing liquid, twice a day for 6 days. Sites were graded visually, and blood flow (measured by a LDF flowmeter), skin color, transepidermal water loss (TEWL), and electrical capacity were measured. Washing alone increased TEWL and decreased electrical capitance. Benzoic Acid produced immediate skin reactions in all panelists. The reactions were stronger on washed skin, suggesting that "even subclinical changes in the skin caused by repeated washing increase the skin response to benzoic acid."

Phototoxicity/Photosensitization

Benzoic Acid and Sodium Benzoate

Clinical studies have demonstrated that ultraviolet (UV) light can produce a dose-dependent inhibition of Benzoic Acid–induced nonimmunologic immediate contact reactions (Larmi, Lahti, and Hannuksela 1988; Larmi 1989a, 1989b).

Biosearch Inc. (1991) tested a matte eye shadow and base formulation each containing 0.1% Benzoic Acid under the conditions of the Draize-Shelanski repeat-insult patch test using 77 panelists. The test materials were applied in 48-hour occlusive patches to one of three sites on the back. Every third patch was applied to the same site. (This protocol allowed for the observation of delayed reactions.) Sites on the back were irradiated for 1 minute with UV light (365 nm, at a distance of 12 inches) following removal of induction patches 1, 4, 7, and 10. At the same time, the materials were applied in 48-hour open patches to the volar aspect of the right forearm. The protocol was followed for a total of 10 applications within a $3\frac{1}{2}$-week period.

Following a 2-week nontreatment period, closed and open challenge patches were applied to previously unexposed sites. Sites on the back were irradiated after removal of the challenge patch. No reactions were noted during induction or at challenge and no reactions were noted in response to irradiation (Biosearch Inc. 1991).

A liquid/powder foundation containing 0.2% Benzoic Acid was applied at two sites to the back of 10 panelists with Fitzpatrick Skin types I, II, and III. Sites were not covered. One site on each panelist was irradiated with UV light; the exposure was initiated 30 to 60 minutes after test material application. Sites were irradiated with 0.5 of the previously determined minimal erythema dose (MED) of UVA and UVB light (290 to 400 nm from a Model 12S ultraviolet solar simulator), followed by a total of 14 Joules/cm^2 of UVA (290 to 320 nm). A control site that had not been dermally treated was also irradiated. Panelists were instructed to avoid natural or artificial sunlight exposure throughout the study. All sites were evaluated at 24, 48, and 72 hours after irradiation. No reactions were observed (Biosearch Inc. 1992c).

A liquid/powder foundation containing 0.2% Benzoic Acid was tested in a photosensitization study using 30 panelists with varying Fitzpatrick Skin Types (degree of pigmentation was stated not to interfere with UV light response or skin reaction evaluation). During induction, six 24-hour occlusive patches were applied to the back within a 3-week period. At the time of patch removal sites were irradiated with 2.0 MEDs of UVB light and 4 Joules/cm^2 of UVA light. Following an 18-day nontreatment period panelists were challenged at two previously unexposed sites. Challenge sites were scored after 24 hours of exposure and one site was then irradiated with 0.5 MED of UVB and 4 Joules/cm^2 of UVA. Another site, not dermally treated, was also irradiated at challenge and served as the UV light

control. Challenge sites were evaluated at 24, 48, and 72 hours postirradiation. Panelists were instructed to avoid natural or artificial sunlight exposure throughout the study. No reactions were observed (Biosearch Inc. 1992d).

Ocular

Benzyl Alcohol

In each of two double-blind studies, 25 patients suffering from early progressive idiopathic cataracts, subcapsular or cortical in site, received one drop of saline containing 0.07% Benzyl Alcohol every 8 hours (Testa et al. 1987). The eyelid was held open for at least 2 minutes. Treatment continued for 22 months. In one study, a control group received placebo, whereas in the other study, the control group received an anticataract medication. Clinical findings were recorded every 30 days for the first 14 months, then patients were followed for up to 18 and 22 months.

A significant ($p < .01$) increase in visual acuity (VA) was observed in patients treated with Benzyl Alcohol after 30 and 60 days as compared to those receiving either placebo or the medication. Compared to those placebo or medication treated, a significant ($p < .01$) decrease in lens opacity was noted in 19 and 17 patients treated with Benzyl Alcohol, respectively.

In the course of the studies, a significant increase in the number of surgeries for cataracts was noted in patients not receiving Benzyl Alcohol. One patient treated with Benzyl Alcohol required surgery after 22 months compared to 38 total who had received either placebo or medication. Benzyl Alcohol was well tolerated except in two patients (4%) where tolerance was fair in one and poor in the other. The investigators encouraged a large scale prospective trial noting that Benzyl Alcohol is already contained (though not at anticataract concentrations) in ophthalmic solutions (Testa et al. 1987).

Benzoic Acid and Sodium Benzoate

An eye shadow plus base containing 0.1% Benzoic Acid was tested in a 28-day in-use study using 52 women. Half of the panelists were contact lens wearers. Panelists were instructed to use the product (one of four eye shadow colors plus base) at least twice a day. Examinations were made at the beginning and end of the study by an ophthalmologist, weekly by a nurse or technician, and the panelists maintained daily logs. Slight conjunctival hyperemia without chemosis was noted in eight women; the condition was nonpersistent in all cases. Another two women presented with slight hyperemia and were also experiencing allergy symptoms; erythema was noted around the eye of one of these two panelists. One panelist had an incidence of red and mildly swollen caruncles at the fifth observation. Four other panelists reported occasional itching; dryness was reported by another two panelists. One panelist reported redness, puffiness, and irritation of the left upper eyelid that prevented her from wearing her contact lenses for 2 days. The investigators concluded that "any reactions observed or sensations perceived were minor, transient and/or sporadic and there were no apparent differences among the four shades of eye shadow. Based on these findings, all of the four products tested were regarded as safe for their intended use" (TKL Research 1991).

SUMMARY

Benzyl Alcohol

Benzyl Alcohol is an aromatic alcohol that is used in cosmetics as a fragrance component, preservative, solvent, and/or viscosity decreasing agent. As of January 1998 it was used in 322 formulations. Data from 1984 indicated use at ≤25%.

Benzyl Alcohol is used as a food additive, in OTC drug preparations, and in clinical settings. It is a membrane fluidizer and a local anesthetic.

Benzyl Alcohol is metabolized to Benzoic Acid, which is then conjugated with glycine and excreted as hippuric acid. EPA reviews of mouse and rat oral-dosing studies conducted by the NTP determined subchronic and chronic oral reference doses for humans of 1 and 0.3 mg/kg/day, respectively. Earlier, the WHO established an ADI of up to 5 mg/kg.

Investigators considered Benzyl Alcohol to be a moderate respiratory hazard and toxic when administered by the parenteral route. It produced severe irritation when applied to the skin of nude mice.

In oral-dose teratogenicity studies using mice, Benzyl Alcohol was negative in one study (550 mg/kg/day), gave questionable results in another (750 mg/kg/day), and was considered a suspect reproductive hazard in the third (750 mg/kg/day [which EPA extrapolated to a human dose of 58 mg/kg/day]).

Mutagenicity studies reported both positive and negative results. It was negative for carcinogenicity when dermally tested on mice at 2.00% in a nonoxidative hair dye. NTP considered it negative for carcinogenicity following 2 years of oral dosing in rats and mice, but EPA considered the results equivocal.

In clinical settings, Benzyl Alcohol can produce nonimmunologic contact urticaria or nonimmunologic immediate contact reactions. It was not a sensitizer when tested in a maximization test at 10% in petrolatum, and demonstrated a low incidence of sensitization in provocation studies. Therapeutic ocular studies indicated it may be beneficial in the management of cataracts.

Benzoic Acid and Sodium Benzoate

Benzoic Acid is an aromatic acid that is used in cosmetics as a pH adjustor and/or preservative. Sodium Benzoate is its sodium salt and is used in cosmetics as a preservative. As of January 1998 they were used in 223 and 156 cosmetic formulations, respectively. Data from 1984 indicated use primarily at ≤1% (with some use at 5% and 25%, respectively).

Both substances are GRAS ingredients. WHO established an ADI of up to 5 mg/kg. Benzoic Acid can be used in ointments and antifungal agents. Sodium Benzoate has been used clinically in the treatment of hyperammonemia. The benzoates are recognized hydroxy radical scavengers.

Benzoic Acid is rapidly absorbed following dermal application. Its metabolism can deplete glycine supplies.

In animal multiple-dose oral toxicity studies decreased feed consumption, depressed growth, and toxic effects were noted at doses of Benzoic Acid or Sodium Benzoate >1%. A neurobiological study was negative.

In oral-dose teratogenicity studies, Benzoic Acid (600 mg/kg) produced significant results in hamsters, but was negative in two rat studies (up to at least 500 mg/kg/day). Sodium Benzoate was negative for teratogenicity in mice and rats (175 mg/kg/day), hamsters (300 mg/kg/day), and rabbits (250 mg/kg/day).

Benzoic Acid was negative in mutagenicity studies. Sodium Benzoate was positive in assays done on the CHO cell line, but negative in other studies. Benzoic Acid was negative for carcinogenicity when dermally tested on mice at 0.016% in a nonoxidative hair dye. Sodium Benzoate was negative for carcinogenicity when administered orally at up to 2% to rats (in feed for up to 2 years) or mice (in a life-time drinking water study).

In clinical studies, toxic symptoms were noted following doses far exceeding the ADI established by the WHO. The benzoates are recognized to produce nonimmunologic contact urticaria or nonimmunologic immediate contact reactions, but it is not clear whether the reactions, are histamine or prostaglandin mediated. Dermal sensitization, phototoxicity, and photosensitization studies were negative.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel was satisfied that results of toxicity, mutagenicity, carcinogenicity, reproductive/developmental, and sensitization studies cited in this report support the safety of these ingredients in cosmetic formulations.

The focus of the Panel's safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate concerned the ability of these ingredients to induce contact urticaria or other contact reactions. The Panel was interested in knowing the threshold and frequency of occurrence of these so-called nonimmunologic reactions. The Panel used the studies cited in Table 7 to establish a pattern. Dermal studies demonstrated that 5% Benzyl Alcohol elicited a reaction in a sizeable portion of the population. One study noted reactions in almost all panelists following brief exposure to 2% Benzoic Acid. The Panel was of the opinion that these urticarial reactions were strictly cutaneous, possibly involving a cholinergic mechanism and not immunoglubolin E (IgE) mediated. Further, predictive clinical sensitization studies using the maximization protocol indicated that 10% Benzyl Alcohol and 2% Benzoic Acid were not sensitizers. In provocative studies, Benzyl Alcohol had a low incidence of sensitization. Utilizing all of the dermal exposure data, the CIR Expert Panel was of the opinion that these ingredients could be used safely at concentrations up to 5%. However, cosmetic manufacturers should consider the nonimmunologic contact urticaria phenomena when using these ingredients in formulation, especially in products designed for use on infants and children.

The Expert Panel received comments suggesting that the available data support the safety of Benzyl Alcohol in hair dyes at concentrations up to 10%. The Panel recognized that hair dye use involves limited body area exposure, has a controlled exposure time per use, and has limited frequency of use (weeks or months between uses). Because of this pattern of use, the Expert Panel concluded that contact urticaria would not be a concern. Therefore, the Panel was of the opinion that Benzyl Alcohol could be used up to 10% in hair dye formulations.

Frequency of use data indicated that these ingredients are used in formulations where inhalation is a route of exposure. The Expert Panel decided that the toxicity data contained in this report were insufficient to assess the inhalation risk of these ingredients. 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 Procedures, the Expert Panel informed the public of its decision that the data were not sufficient for determination whether the ingredients, under relevant conditions of use, were either safe or unsafe. The Panel released a Notice of Insufficient Data on April 4, 1997, requesting inhalation toxicity data. No comments were received.

CONCLUSION

Based on the available data, the CIR Expert Panel concludes that Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate are safe for use in cosmetic formulations at concentrations up to 5%. The available data are insufficient to support the safety of these ingredients in cosmetic products in which a primary route of exposure is inhalation. Benzyl Alcohol is safe for use in hair dyes at concentrations up to 10%.

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