

Butylparaben
[CAS No. 94-26-8]

Review of Toxicological Literature

April 2005

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Abstract

Parabens are esters of 4-hydroxybenzoic acid that have recently been reported to have adverse effect on the male reproductive system in rodents. The toxicological database for the most commonly used parabens is quite extensive and generally indicates a low degree of systemic toxicity. In addition, several parabens have been recently reported to have estrogenic activity in experimental cell systems and animal models. Butylparaben is included among the parabens widely used as antioxidants and preservatives in foods, pharmaceuticals, and cosmetics. It is regulated by the U.S. FDA as a synthetic flavoring and adjuvant. Human exposure to butylparaben may occur via inhalation, eye or skin contact, or ingestion. Inhalation exposure causes irritation to the respiratory tract. Contact with the eyes or skin can cause irritation, redness, pain, and/or itchiness, but patch test results show that the sensitization potential of parabens is low. Ingested butylparaben is rapidly absorbed from the gastrointestinal (GI) tract, metabolized, and excreted in the urine. Large doses, however, may cause irritation to the GI tract. In mice, rats, rabbits, and dogs, butylparaben was reported to be practically nontoxic. Results from one chronic feeding study in mice showed that butylparaben caused a high incidence of amyloidosis, affecting the spleen, liver, kidney, and/or adrenal gland. It was cytotoxic in isolated rat hepatocytes and mitochondria and in other animal cells *in vitro*. Reproductive studies in mice and rats suggested that maternal exposure to butylparaben in the diet results in adverse effects on the reproductive system of F1 male offspring. Butylparaben was not mutagenic in several short-term bioassays (e.g., Ames test, Chinese hamster ovary cells, and comet assay) and was reported to be non-carcinogenic in rats and mice.

Executive Summary

Basis for Nomination

Parabens (esters of 4-hydroxybenzoic acid [4-HBA], also known as alkyl *p*-hydroxybenzoates), in particular propylparaben and butylparaben, have recently been reported to have adverse effects on the male reproductive system in rodents. Humans are exposed to parabens through their widespread use as antioxidants and preservatives in foods, pharmaceuticals, and cosmetics. The toxicological database for the most commonly used parabens is quite extensive and generally indicates a low degree of systemic toxicity. In addition, several parabens have been recently reported to have estrogenic activity in experimental cell systems and animal models. The parabens with longer-chain or otherwise bulkier alkyl groups (e.g., benzyl [phenylmethyl]) are more lipophilic/hydrophobic and more potent in binding estrogen receptors (ERs). Butylparaben was nominated by the National Institute of Environmental Health Sciences (NIEHS) for toxicological characterization, including reproductive toxicity studies, based on presumed widespread human exposure and insufficient data to fully characterize potential human health hazards.

Nontoxicological Data

Butylparaben is a crystalline powder commercially available from Ashland Distribution Company, Clariant LSM (America) Inc., J.T. Baker, Mutchler Inc., Penta Manufacturing Co., R.S.A. Corporation, and Nipa Biocides. It is prepared by the esterification of *p*-hydroxybenzoic acid with 1-butanol. Under the 1986, 1990, and 1994 Inventory Update Rule (IUR), an aggregate production volume ranging between 10,000 and 500,000 lb was reported for butylparaben. Butylparaben is used as a preservative in cosmetics (e.g., baby products, manicuring preparations, and deodorants and other cleanliness products), drug formulations (local anesthetic solutions and estrogen tablets), and some foods.

In surveys in Denmark and Japan, butylparaben was not detected in grey wastewater, surface water, or bottom sediment. However, in a survey of endocrine-disrupting chemicals in indoor air and dust, butylparaben was detected in 8% (maximum concentration of 3.2 ng/m³ [4.1 x 10⁻⁷ ppm]) and 22% (maximum concentration of 3.92 µg/g [0.0202 mmol/g]) of the air and dust samples of 120 and 118 homes, respectively, in Cape Cod, MA.

Butylparaben is regulated by the U.S. Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). In 1998 its pesticide registration status was listed as "cancelled." Butylparaben is also regulated by the U.S. Food and Drug Administration (FDA). It is permitted as a synthetic flavoring substance and adjuvant and is to be used in the minimum quantity required to produce its intended effect [21 CFR 172.515].

Human Data

Potential exposure to butylparaben may occur via inhalation, ingestion, or eye or skin contact. Exposure stems from intake of foods or drugs or use of cosmetics and personal care products. The U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition (FDA/CFSAN) estimated a consumption rate of 37 ng/day for butylparaben. Conservative estimates of butylparaben exposures (e.g., long-term use of pharmaceutical formulations or cosmetics containing butylparaben) range from 0.26 to 14.6 mg/day. In the 1983 National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES), an estimated 24,427 employees were exposed to butylparaben in 704 facilities; of the total, 18,593 were females.

When exposed to butylparaben via inhalation, irritation to the respiratory tract results; symptoms include coughing and shortness of breath. Ingestion of large doses of butylparaben may cause irritation to the gastrointestinal (GI) tract. Contact with the eyes causes irritation, redness, and pain, while contact with the skin causes the same symptoms and itchiness. Allergic skin reactions may also occur.

Chemical Disposition, Metabolism, and Toxicokinetics: When given orally, rapid absorption from the GI tract occurs. The parabens are hydrolyzed to *p*-hydroxybenzoic acid (4-HBA), then conjugated, and rapidly excreted in the urine. A recent study demonstrated that at least a proportion of the parabens present in cosmetic, food, and pharmaceutical products can be absorbed and retained in body tissues based on concentrations of paraben esters measured in human breast tumors. The mean concentration for butylparaben was 2.3 ng/g [0.012 nmol/g] tissue.

Immunotoxicity: Patch tests show that the sensitization potential of parabens is low. In 50 human volunteers, butylparaben ($\leq 5\%$) produced no irritation or sensitization when applied to the skin. Photocontact sensitization and phototoxicity tests on product formulations containing 0.1-0.8% methyl-, propyl-, and butylparaben gave no evidence of photoreactivity. However, exposure to butylparaben and sunlight has caused excessive hyperpigmentation. Butylparaben also reportedly intensified dermatitis.

Toxicological Data

Chemical Disposition, Metabolism, and Toxicokinetics

Rabbits orally given butylparaben (0.4 or 0.8 g/kg [2 or 4 mmol/kg]) excreted 0.2-0.9% of the ester by 24 hours. Within the same period, 25-39% 4-HBA, 15-29% *p*-hydroxyhippuric acid, 5-8% *p*-carboxyphenyl glucuronide, 10-18% *p*-hydroxybenzoyl glucuronide, and 7-12% *p*-carboxyphenyl sulfate were recovered.

In dogs, intravenous (i.v.) injection of butylparaben (50 mg/kg [0.26 mmol/kg]) and oral administration of butylparaben (1000 mg/kg [5.148 mmol/kg]) resulted in a 48 and 40% recovery, respectively, of the administered dose in the urine as the 4-HBA conjugate of glucuronic acid within 30 hours. In a similar study in which dogs were given an i.v. injection of butylparaben (100 mg/kg [0.515 mmol/kg]), pure ester was recovered in the brain, spleen, and pancreas, and high concentrations of metabolites were detected in the liver and kidneys.

Numerous *in vitro* and *in vivo* studies have been conducted reporting the permeability of butylparaben through skin. In these studies, butylparaben usually exhibited low penetration, retention in the epidermis, and/or hydrolysis in the skin.

Acute Exposure

In mice, oral LD₅₀ values >5000 mg/kg bw (25.74 mmol/kg bw) of butylparaben were calculated. The intraperitoneal (i.p.) LD₅₀ was 230 mg/kg bw (1.19 mmol/kg bw) in the animals. In rabbits, the dermal LD₅₀ was >2000 mg/kg bw (67.954 mmol/kg bw).

In mice, rats, and dogs, butylparaben was reported to have a low order of acute toxicity; the main effect was an acute myocardial depression accompanied by hypotension that was transient in nature. Intraperitoneal injection of butylparaben (230 mg/kg [1.18 mmol/kg]) resulted in lacrimation in the eyes of mice. Butylparaben (5%) was a mild irritant when applied to the skin of guinea pigs for 48 hours. In rabbits, a product formulation containing 0.2% butylparaben was nonirritating, while a product with 0.3% butylparaben applied for three days to the back of rabbits produced mild irritation with grade ¼ erythema and/or edema.

Short-term and Subchronic Exposure

In mice provided with pellets containing butylparaben (0.6, 1.25, 2.5, 5, or 10%) for six weeks, deaths occurred within the first two weeks in those given the two highest doses. At levels greater than 0.6%, significant atrophy of lymphoid tissue in the spleen, thymus, and lymph nodes and multifocal degeneration and necrosis in the liver parenchyma were observed.

In rats, oral administration of butylparaben (2 or 8%; equivalent to 2000 or 8000 mg/kg [10.30 or 41.18 mmol/kg] bw/day) in the diet for 12 weeks resulted in 100% mortality before the end of the treatment period in males given the high dose. Females also had many early deaths. The high-dose butylparaben diet also produced a significant decrease in body weight for all animals, while the low dose produced no toxic effects. A diet of 4% butylparaben for nine days acted entirely on the prefundic region of the forestomach epithelium adjacent to the fundic mucosa, while oral intubation of butylparaben (0.25 or 50 mg/kg [0.0013 or 0.26 mmol/kg] bw) daily for 13-15 weeks produced no toxic effects.

Chronic Exposure

In mice orally given butylparaben (0.15, 0.3, or 0.6%) in the diet for 102 weeks, a high incidence of amyloidosis affecting the spleen, liver, kidney, and/or adrenal gland was observed in males and females.

Cytotoxicity

In isolated rat hepatocytes, butylparaben (2 mM [0.4 mg/mL]) caused cell death, almost total loss of adenosine triphosphate (ATP), and reductions in the total adenine nucleotide pool and mitochondrial membrane potential. When added to isolated rat mitochondria, butylparaben (0.05, 0.10, or 0.25 mM [9.8, 19, or 49 µg/mL]) increased the rate of state 4 oxygen consumption and inhibited the rate of state 3 oxidation. In normal and α -linolenic acid (LNA)-loaded cultured rat hepatocytes, butylparaben induced severe cell injury accompanied by a significant decrease in cellular levels of both glutathione (GSH) and protein-SH.

Reproductive and Teratological Effects

Reproductive Studies: When mice were administered butylparaben (0.01-1%) in the diet for ten weeks, the absolute and relative weights of the epididymides were significantly increased compared to controls at the high dose. A dose-dependent decrease in serum testosterone concentration and in round and elongated spermatid counts in stages VII-VIII seminiferous tubules was also observed, and the elongated spermatid counts were significantly lower. Exposure of postweaning male rats to butylparaben (0.01-1%) in the diet for eight weeks caused decreases in the cauda epididymal sperm reserve, daily sperm production in the testis, and in serum testosterone concentration. Butylparaben (400-1200 mg/kg [2.06-6.178 mmol/kg]) injected for three consecutive days caused a significant increase in uterine weight in ovariectomized and immature rats.

Developmental Studies: Subcutaneous (s.c.) administration of butylparaben (100 or 200 mg/kg [0.515 or 1.03 mmol/kg]) in pregnant rats from gestation day 6 to postnatal day 20 decreased the proportion of pups born alive and the proportion of pups that survived up to the weaning period. In neonatal rats, daily s.c. injection of butylparaben (2 mg/kg [0.01 mmol/kg]) for up to 18 days had no effects on the development of the testicular excurrent ducts. Pregnant rats were s.c. administered butylparaben (100 or 200 mg/kg [0.515 or 1.03 mmol/kg]) from gestation day 6 to postnatal day 20. On postnatal day 49, body weights were significantly decreased in F1 females, while the weights of testes, seminal vesicles, epididymides, and prostate glands were significantly decreased in F1 males. Additionally, the number and motility of sperm in the epididymis were significantly lowered. No changes in the weights of female reproductive organs were observed. The results suggested that maternal exposure to butylparaben has adverse effects on F1 male offspring.

In an oral study, rats were administered butylparaben (10, 100, or 1000 mg/kg [0.051, 0.515, or 5.148 mmol/kg]) in 0.5% carboxymethylcellulose daily on gestation days 6-19. At 1000 mg/kg, decreased maternal weight gain was statistically significant during gestation days 18-20. No differences were seen from the control and treated group in any of the developmental parameters measured, including embryo/fetal viability, fetal weight, malformations or variations.

Carcinogenicity

In mice, oral administration of butylparaben (0.15, 0.3, or 0.6%) in the diet for up to 106 weeks produced neoplasms in the hematopoietic system, lung adenomas and adenocarcinomas, and soft tissue myosarcomas and osteosarcomas. Tumor incidences, however, were not significantly different from those of the control group. In rats, butylparaben (0.6 or 1.2%) in the diet for up to 104 weeks did not produce any carcinogenic effect. Butylparaben also showed no enhancing or inhibitory effects on the development of preneoplastic glutathione S-transferase placental form-positive (GST-P⁺) foci in the liver of rats.

Genotoxicity

In Chinese hamster cells, a 1-3% increase in polypoid cell production was observed with butylparaben (0.06 mg/mL [308 μM]). Butylparaben, however, was not mutagenic in the fibroblast cell line and in ovary cells. In addition, negative results were reported for butylparaben (up to 1000 mg/plate [5.148 mmol/plate]) in the Ames test using *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA1535, TA1537, and TA2637. Butylparaben inhibited DNA, RNA, and protein formation in *Bacillus subtilis* and *Escherichia coli*. In the comet assay, butylparaben (2000 mg/kg [10.30 mmol/kg]) did not increase DNA damage in the glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow of mice.

Immunotoxicity

Butylparaben (0.1%) was injected intracutaneously three times weekly and randomly to the back and upper flanks of guinea pigs for a total of ten injections. No reaction was reported 24 hours after the initial injection and 24 or 48 hours after a challenge dose given two weeks later. After intradermal injections of Freund's complete adjuvant and application of butylparaben (5%) to the clipped dorsal skin of guinea pigs every other day for three weeks followed 12 days later by a challenge patch for 48 hours, six of 20 animals reacted to butylparaben. The worst case had spongiosis, squamous crust, and lymphocytic infiltration.

Other Data

Estrogenic Effects: Parabens bind with low affinity to estrogen receptors and regulate estrogen-responsive reporter gene expression in experimental cell systems. The estrogenic activities of the parabens increase as the length and branching of the alkyl ester increase. The ER relative binding activity of parabens is in the following approximate order: 2-ethylhexyl > heptyl > benzyl > butyl > propyl = ethyl > methyl. Additionally, a linear correlation exists between ER binding activity and hydrophobicity.

Effects on Blood: Butylparaben strongly inhibited agonist-induced thromboxane (TXB₂) synthesis irreversibly *in vitro* and inhibited or suppressed platelet function. In mitogen-stimulated peripheral human lymphocytes, butylparaben was the most potent of the parabens tested with C1- to C4-alkyl ester groups in inhibiting release of lysosomal enzymes. In human and rabbit erythrocytes *in vitro*, butylparaben (0.02%) induced hemolysis in 6 and 12% of the cells, respectively.

Miscellaneous Studies: Antimicrobial effects, biochemical effects, and physiological effects of butylparaben have also been reported.

Structure-Activity Relationships

In this section, the reproductive and teratological effects of parabens other than butylparaben and of 4-HBA are presented.

Rats orally given 4-HBA (up to 1000 mg/kg) daily for 45 days in males and from 14 days before mating to day 3 of lactation in females showed no adverse effects on copulation, fertility, pregnancy, parturition, and lactation. In the offspring, no adverse effects on viability, sex ratio, body weights, and morphological

appearance of pups were observed. Subcutaneous injection of 4-HBA to rats at day 9 of gestation and intramuscular (i.m.) injection to mice at day 9 or 12 of gestation produced no teratogenic effects. When immature intact and adult ovariectomized female CD1 mice were s.c. injected daily with 4-HBA (0.5, 5, 50, or 500 $\mu\text{g}/100\text{g}$) for three days, dose-dependent responses on vaginal cornification and uterotrophic activity were reported.

Teratogenic and embryotoxic studies using methylparaben were negative. Methylparaben also gave negative results in the uterotrophic assay. When tested in pregnant rats, ethylparaben at concentrations up to 10% was considered nonteratogenic. In rats, propylparaben (0.01-1.0%) in the diet for four weeks caused a dose-dependent decrease in cauda epididymal sperm reserves and concentrations and in the serum testosterone level. Additionally, daily sperm production and efficiency in the testis were significantly decreased in all treated animals. Propylparaben was reported to be weakly estrogenic (30,000-fold less potent than 17β -estradiol in an *in vitro* yeast-based estrogen assay). In mice, s.c. administration of isobutylparaben (1.2 and 12 mg/mouse) and topical application of benzylparaben (7.5 g/kg bw) for three days produced increases in uterine weight.

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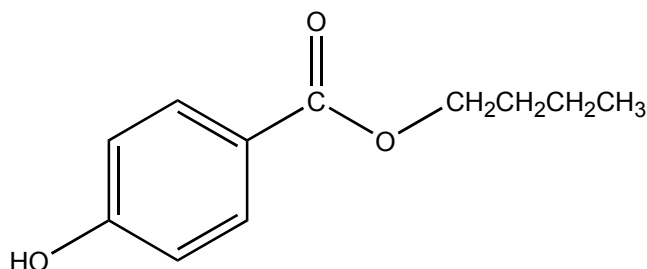
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1.0 Basis for Nomination

Parabens (esters of 4-hydroxybenzoic acid [4-HBA], also known as alkyl *p*-hydroxybenzoates), in particular propylparaben and butylparaben, have recently been reported to have adverse effects on the male reproductive system in rodents. Humans are exposed to parabens through their widespread use as antioxidants and preservatives in foods, pharmaceuticals, and cosmetics. The toxicological database for the most commonly used parabens is quite extensive and generally indicates a low degree of systemic toxicity. In addition, several parabens have been recently reported to have estrogenic activity in experimental cell systems and animal models. The parabens with longer-chain or otherwise bulkier alkyl groups (e.g., benzyl [phenylmethyl]) are more lipophilic/hydrophobic and more potent in binding estrogen receptors (ERs). Butylparaben was nominated by the National Institute of Environmental Health Sciences (NIEHS) for toxicological characterization, including reproductive toxicity studies, based on presumed widespread human exposure and insufficient data to fully characterize potential human health hazards.

2.0 Introduction

Butylparaben
[94-26-8]



2.1 Chemical Identification and Analysis

Butylparaben ($[C_{11}H_{14}O_3]$; mol. wt. = 194.25) is also called:

Aseptofom butyl	<i>n</i> -Butyl parahydroxybenzoate
Benzoic acid, 4-hydroxy-, butyl ester	Butyl parasept
Benzoic acid, <i>p</i> -hydroxy-, butyl ester	Butyl tegosept
Butoben	4-Hydroxybenzoic acid butyl ester
4-(Butoxycarbonyl)phenol	<i>p</i> -Hydroxybenzoic acid butyl ester
Butyl butex	Nipabutyl
Butyl chemosept	Preserval B
Butyl 4-hydroxybenzoate	Solbrol B
Butyl <i>p</i> -hydroxybenzoate	Tegosept B
<i>n</i> -Butyl hydroxybenzoate	Tegosept butyl
<i>n</i> -Butyl <i>p</i> -hydroxybenzoate	

Sources: Budavari (1996); ChemIDplus (undated); HSDB (2003); RTECS (2003)

Chromatography is used for the determination of paraben preservatives in foods, cosmetics, and pharmaceuticals (CIR, 1984). In foods, ion-pair high performance liquid chromatography (HPLC) is employed to simultaneously determine eight kinds of preservatives—dehydroacetic acid, sorbic acid, benzoic acid, ethylparaben, propylparaben, isopropylparaben, isobutylparaben, and butylparaben—and sodium saccharin (Okayama et al., 1998). Liquid chromatography (LC) can be used for the simultaneous determinations of the parabens and benzoic and sorbic acid in meat and nonmeat products, while direct injection gas chromatography (GC) is suitable for simultaneous determination of the preservatives in liquid foods (e.g., vinegar, fish sauce, commercial health drinks, and cold formulas) (Ali, 1985; Lin and Choong, 1999; Lin et al., 2000). In cosmetics, LC with ultraviolet (UV) and fluorescence detection is used (HSDB, 2003). In pharmaceutical dosage forms, simultaneous determination of parabens and their degradation product 4-HBA can be done using high performance thin-layer chromatography (HPTLC) densitometry (Tomankova and Pinkasova, 1990). In human breast fat, parabens can be detected by TLC or HPLC followed by mass spectrometry (MS) (Darbre et al., 2004).

2.2 Physical-Chemical Properties

Property	Information	Reference(s)
Physical State	crystalline powder; small, colorless crystals or white powder; finely divided solid	Budavari (1996); HSDB (2003)
Odor	odorless	HSDB (2003)
Melting Point (°C)	68-69	Budavari (1996)
Water Solubility	very slightly soluble (1:6500)	Budavari (1996)
	0.015 g/100 g @ 25 °C; 0.15 g/100 g @ 80 °C	HSDB (2003)
Soluble in:	acetone, alcohol, ether, chloroform, glycerin (very slightly), and propylene glycol	Budavari (1996)
Octanol-water partition coefficient (log K_{OW})	3.57 (quantitative structure-activity relationship)	Danish EPA (2001); HSDB (2003)

Note: Experimental properties and calculated properties (e.g., boiling point and K_{OC}) are reported in the Registry file.

Parabens are stable in acidic solutions, in hot and cold water, and under conditions of sterilization. They undergo hydrolysis in media above pH 7; in strong basic solutions, they hydrolyze to their corresponding carboxylic acids. As the alkyl chain increases, so does resistance to hydrolysis, as well as antimicrobial activity; water solubility and oil solubility, however, decrease (CIR, 1984). Antimicrobial activity is retained across the pH range of 4 to 8 (Clariant, 2002a). Highly soluble potassium salts of parabens were found to be more microbiocidal against more test organisms than the parabens (Mizuba and Sheikh, 1987).

Although parabens are stable in air, butylparaben may form combustible dust concentrations. When heated to decomposition, carbon monoxide and carbon dioxide may form (Mallinckrodt Baker, Inc., 2003). Parabens interact with cosmetic ingredients, such as gelatin, polysorbates, and cellulose esters; for example, 0.7% Tween 80 was found to deactivate butylparaben (CIR, 1984).

2.3 Commercial Availability

Butylparaben is commercially available from Ashland Distribution Company, Clariant LSM (America) Inc., J.T. Baker, Mutchler Inc., Penta Manufacturing Co., and R.S.A. Corporation (Chemyclopedia, 2004). It is available under the tradename Nipabutyl[®] as a NIPA Product as is the sodium salt from Nipa Biocides (Clariant, 2002a,b). Butylparaben is also present in paraben

mixtures such as Phenonip (Clariant) and LiquaPar Oil (International Specialty Products, 2005) that are often used by formulators of personal care products. Phenonip is a paraben mixture (undefined ratio) containing 60 to 80% phenoxyethanol. LiquaPar Oil contains about 30% each butylparaben and isobutylparaben and 40% isopropylparaben.

3.0 Production Processes

Butylparaben is prepared by the esterification of *p*-hydroxybenzoic acid with 1-butanol in the presence of an acid catalyst such as sulfuric acid (CIR, 1984; HSDB, 2003).

4.0 Production and Import Volumes

Under the 1986, 1990, and 1994 Inventory Update Rule (IUR), an aggregate production volume ranging between 10,000 and 500,000 lb (4.5359×10^6 and 2.26796×10^8 g) were reported for butylparaben; no reports were submitted by companies in 1998 and 2002 (U.S. EPA, 2004). In 1972, 10×10^6 g (~22,000 lb) was produced in the United States, while 2.6×10^6 g (5700 lb) was imported. In 1976, 9.54×10^5 g (2100 lb) was imported (HSDB, 2003).

5.0 Uses

Butylparaben is used as a preservative in some foods, cosmetics, and drug formulations. It has been added to solutions such as commercially prepared low-ionic strength saline (LISS) solutions and beer to retard microbial growth (Judd et al., 1982; Raducan et al., 1994). Parabens in general are most active against molds and yeasts and, to a lesser extent, bacteria. In comparison to other parabens, butylparaben appears to be the best antifungal agent (HSDB, 2003).

5.1 Food Additive

In 2003, butylparaben was cleared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for use as a flavoring agent "at very low levels" [not specified] (CCFAC, 2003; JECFA, 2003). Butylparaben is used as a food additive in Japan (CIR, 1984) and as an additive to beer to retard microbial growth. Recently, a call for data regarding suggested limits for individual heavy metal contents such as lead, with supporting analytical data, was issued for the use of butylparaben as a food additive (JECFA, 2004).

5.2 Cosmetics and Personal Care Products

In 1981, industry's voluntary submissions to the Food and Drug Administration (FDA) indicated the use of butylparaben in 693 personal care/cosmetic products at concentrations up to 5%. This included one baby lotion, bath products, makeup, fragrances, hair coloring, manicure supplies, shaving creams, skin care lotions, suntan oils/lotions, and personal hygiene products (CIR, 1984). An estimated 13% of all cosmetics and personal care products today contain butylparaben while 48% contain propylparaben and/or methylparaben (EWG, 2005). Several products said to have concentrations in the range of >0.1 to 1% often contained $\leq 0.1\%$ butylparaben. While only 1 of 56 baby care products in the 1984 CIR survey contained butylparaben, Internet searches in 2004 and 2005 indicated four baby lotions manufactured by Johnson and Johnson and one by Gerber may contain butylparaben. Two underarm deodorant formulations were also shown to contained butylparaben.

In the European Union a maximum of 0.8% total parabens is allowed in personal care products, so the maximum butylparaben concentration from LiquaPar Oil paraben mixture would be 0.24% in formulated products. However, LiquaPar addition is recommended at 0.3 to 0.6%, so butylparaben concentration range in finished products would be 0.09 to 0.18%. A survey of cosmetics on the Danish market found parabens in 77% of the products at concentrations from 0.01 to 0.87%. The maximum concentration of butylparaben was 0.07% (Rastogi et al., 1995). In Japan the maximum allowable total paraben concentration is 1.0% making the maximum butylparaben concentration 0.3% in products containing paraben mixtures.

5.2.1 Products Used on Body Surfaces

Some body care products, including baby lotions and creams such as Johnson and Johnson Baby Soothing Vapor Cream (used on the chest and neck), cleansing cloths, baby soaps, and sunscreens that are applied to nearly the entire body surface, contain butylparaben (Froogle search [a product search capability that has recently become available on the Google Advanced Search web page], February 2005). The exposure to butylparaben of a child treated dermally with such products is about 1.5 times higher based on body surface as opposed to body weight when compared to the exposure for an adult (U.S. EPA, 2002 [Child Exposure Factors Handbook (interim)]). Application of paraben-containing skin care products to preterm infants could cause exposures greater than expected due to incomplete epidermal development and lowered barrier to skin absorption (Weinberger et al., 2003 [parabens not specifically mentioned]).

Products used by adults that potentially expose large areas of the body surface to butylparaben that were listed in CIR (1984) include:

- Fragrance powders and men's talcum (see section 5.2.3)
- Bath oils, tablets, and salts (8 of 237 products)
- Face, body, and hand skin care preparations (cold creams, lotions, liquids, and pads) (58 of 680 products; one preparation had concentrations in the range >1 to 5%)
- Moisturizing skin care preparations (91 of 747 products)
- Suntan oils/lotions (19 of 192 products; five had concentrations in the range > 0.1 to 1%)
- Arthritis analgesic creams with methylsulfonylmethane (Froogle search, February 2005)

5.2.2 Products that Contact Broken Skin or Mucous Membranes

Unless otherwise indicated, the products listed below were identified in a Froogle search (February, 2005). This group also includes some personal care items considered to be over-the-counter (OTC) topical medications.

- Lipsticks (44 of 3319 products; 20 with concentrations \leq 1%) (CIR, 1984)
- Mascara and other eye make-up (62 of 3549 products) (CIR, 1984)
- Pharyngeal antiseptic. A troche (sublingual dosage form) was reported to contain 0.5 mg butylparaben (HSDB, 2003).
- Acne cleansing products, pore cleansers, exfoliants, and cleansing cloths (lotions, gels, foams, masks, benzoyl peroxide bars, and other soaps)
- Perineal cleansing preparations
- Vaginal preparations (two products)
- Vaginal contraceptives (Song et al., 1991)
- Cradle cap treatment (one product)

- Treatments for eczema and other dermatological conditions (including some baby products)
- Sprays for psoriasis, dermatitis, seborrhea, eczema, yeast infections, jock itch, and hemorrhoids
- Underarm deodorants/antiperspirants (two products) plus one foot-care cream

5.2.3 Products that May Be Inhaled (Froogle search, February 2005 unless otherwise indicated)

- Fragrance powders, dusting and talcum, excluding aftershave talc (14 of 483 products) (CIR, 1984)
- Men's talcum powder (1 of 13 products) (CIR, 1984)
- Spray sunscreens (at least nine products) and artificial tanning products (at least six products)
- Spray hair-relaxer (one product)
- Hair spray for dry hair (one product) and for curling/setting hair (two products)
- Spray hair-conditioner/hair repair (two products)
- Spray lotion, skin toner, resurfacing peel system, body wash, body moisturizer, and lice repellent (one product each category)
- Facial hydrating mist (one product)
- Fragrance/Perfume (one product; for pillows and sheets)

5.3 Pharmaceuticals and OTC Medicines (Comments are based on the results of Froogle searches in February 2005.)

Butylparaben may be used alone or with other parabens, chiefly methylparaben and/or propylparaben, in medications. It is common in many liquid and solid (gel cap) OTC products such as Tylenol, Drixoral, Maalox, and Mylanta. Unfortunately, butylparaben concentrations were seldom identified for OTC or prescription products. No attempt was made to identify butylparaben-containing dietary supplements.

Tylenol (acetaminophen) used in products for treating fever, pain, cough, colds, flu, and sinus symptoms often contains butylparaben. Many of these products are intended for pediatric and infant use. Children's grape- and bubblegum Tylenol contain 0.018% butylparaben (Gao et al., 2004 pat. appl.). The recommended dose for the regular pain reliever varies from 1 tsp (5 mL) for a child weighing 24 to 35 lb to 3 tsp for an 11-year-old weighing 72 to 95 lb. A child taking the maximum dose five times a day would receive a daily butylparaben intake of 13.5 mg. It is possible that a child suffering from a chronic illness (e.g., sickle cell disease or frequent severe headaches) could receive the maximum daily dose of Tylenol for extended periods of time.

Magnesium hydroxide preparations such as Maalox and Mylanta which are often taken for extended periods by persons suffering from stomach ulcers or other symptoms of gastric distress may also contain butylparaben. Seven of 19 Maalox formulations contained butylparaben alone and three contained butylparaben with propylparaben.

Some prescription pharmaceuticals that contain butylparaben or sodium butylparaben include the oral solution of Fosamax[®] (alendronate, Merck); the cholesterol-lowering drug Lescol[®] (fluvastatin, Novartis) (may contain butylparaben in the gelatin capsule); Estinyl[®] Tablets (Schering Corporation) and other estrogen tablets; the antidepressant and tranquilizer Pamelor[®]

(nortriptyline hydrochloride, Novartis); and the antacid Zantac[®] (ranitidine hydrochloride, GlaxoWellcome). The new Fosamax[®] liquid formulation taken once a week contains 0.0075% sodium butylparaben (equivalent to 0.0067% butylparaben). Each dose contains 5.0 mg butylparaben equivalent (Merck and Company, Inc., 2004). A patient taking Pamelor[®] (2 mg/mL) at the daily recommended adult dose (25 mg up to four times a day) would ingest 50 mL/day. If the butylparaben concentration were comparable to that in Fosamax[®] (0.0067%) the daily dose of butylparaben would be 3.4 mg/day. The pediatric dose of Zantac[®] syrup for an 80-lb child is 5 mL; a maximum daily dosage of 300 mg active ingredient would require consumption of 20 mL syrup. Assuming a butylparaben concentration of 0.0067%, the intake at the maximum dosage would be 1.5 mg/day (Glaxo Wellcome, Inc., 2001; Merck and Company, Inc., 2004; Novartis Pharmaceuticals, 2001, 2002; Schering Corporation, 1998).

5.4 Other Household Products

In addition to personal care products, Froogle searches (February 2005) identified one pet care product and several household products that contain butylparaben and are used as sprays, aerosols, or particulates and could contribute to the butylparaben concentrations reported in household dust and air (Rudel et al., 2003). Powders and aerosolized products may be major sources of butylparaben in indoor dust from homes where high concentrations were found (26 of 118 homes had concentrations above 0.2 ppm). Another likely source of butylparaben in household dust is exfoliated skin and hair particles from individuals who use butylparaben-containing personal care products that were able to pass through the sieve used to remove larger particulates when dust samples were collected by vacuum. Exfoliated epithelial cells from bedding may also contribute to parabens in household dust since skin care products are often applied at bedtime.

DHI Water and Environment Force Technology (2004) reported that parabens were found in 18% ($n = 40$) of hand dishwashing products on the European market and 22% ($n = 41$) of all-purpose cleaners, but parabens were not found in 20 cleaners for sanitary facilities. The use of butylparaben in the United States in these types of products was not confirmed by Froogle or Google searches and no surface cleaners were identified that contained butylparaben.

Three U.S. EPA-registered criosine disinfectant formulations containing 0.54% butylparaben were approved for unrestricted use as a pesticide, one as early as 1953, until their cancellations in 1988 and 1989. Between January 1, 1976, and September 29, 1988, the Michel & Pelton Co. "Eureka Products Criosine" formulation had been approved for use on household contents, mortuary instruments, human bedding and clothing, biological specimens (tissues), cadavers, and human stools (PAN Pesticides Database – Pesticide Products, 2004). Since this database had no information on cancellation of criosine use as an oilfield slimicide (Spectrum Laboratories, undated) or cancellation of use in other manufacturers' formulations, other criosine household disinfectant formulations may still be approved (could not be confirmed by Google or Froogle searches).

6.0 Environmental Occurrence and Persistence

In an assessment of the risk for the aquatic environment, several scenarios of parabens from health care products or cosmetics transported with sewage water from the shower/bath to the sewage treatment plant indicated that paraben concentrations in the sewage were at least a factor

of 100 to 1000 below the effect concentrations (acute or chronic effects); however, the risk was considered not conclusive, since data on chronic effect levels are few (Andersen et al., 2001). In a study of the components in grey wastewater, butylparaben was included in a list of chemicals in household products sold in Denmark; however, only ethylparaben and methylparaben were detected at concentrations of 0.6 and 2.6 µg/L, respectively (Eriksson et al., 2003). In surveys of chemicals in the environment and wildlife in Japan, butylparaben was not found in surface water or bottom sediment (Ministry of the Environment, 2003). However, in a survey of endocrine-disrupting chemicals in indoor air and dust, butylparaben was detected in 8% (maximum concentration of 3.2 ng/m³ [4.1 x 10⁻⁷ ppm]) and 22% (maximum concentration of 3.92 µg/g [0.0202 mmol/g]) of the air and dust samples of 120 and 118 homes, respectively, in Cape Cod, MA (Rudel et al., 2003). The possibility exists for environmental release from the use of criosine (0.56% butylparaben) as a biocide in oil recovery packer fluid (Spectrum Laboratories, undated).

7.0 Human Exposure

Estimated daily exposures to butylparaben are given in **Table 1** based on conservative assumptions from exposure to various butylparaben-containing products. The information used to derive these estimates is given in the subsections that follow. These estimates are based on isolated exposure scenarios only (i.e., daily use of a single personal care or pharmaceutical product) and do not necessarily account for aggregated exposures within each of the various categories. The table does not include estimates from food consumption since information on the use and maximum concentrations/limits of butylparaben allowed in the United States for specific food groups was not found.

Table 1. Estimated Butylparaben Exposure for Selected Scenarios

	<u>µg/day</u>	<u>route</u>
Personal care products, adult	14,590	dermal
Pharmaceuticals/OTC, adult	1700	oral
Pharmaceuticals/OTC, child (Tylenol)	13,500	oral
Soil (dust) ingestion, pica	39.2	oral
Soil (dust) ingestion, hand-to-mouth		
child	0.392	oral
adult	0.196	oral
Soil adherence to skin (child)	0.431	dermal
Indoor air		
child	0.032	inhalation
adult male	0.048	inhalation
adult female	0.036	inhalation

7.1 Food Additives

JECFA (2001) calculated a U.S. daily intake of individual food additives by dividing the annual production by 26 x 10⁶ (10% of the population assumed to be "eaters"); 365 days/year; and 0.6, a correction factor for presumed underreporting. The calculated daily intake for butylparaben was 30 ng/day or 0.5 ng/kg bw/day. An FDA/CFSAN exposure estimate for butylparaben from food, cosmetics, and drugs was calculated to be 37 ng/day based on 1987 production divided by 365 days/year (Clydesdale, 1997; cited by Soni et al., 2002). The estimate was far lower than that of

propylparaben (0.78 mg/day) and methylparaben (0.0635 mg/day) which are more commonly used in food and at higher concentrations in personal care products. Without a more recent estimate of relative amounts of the annual U.S. production of butylparaben consumed in particular types of foods, personal care products, and pharmaceuticals, a more realistic estimate of daily intake of butylparaben from these sources is difficult to make.

In surveys in Taiwan, six parabens [including butylparaben] were quantified in vinegar (up to 102 µg/mL total parabens), in soya sauce (243 µg/mL), in pickle condiment liquid (209 µg/mL), and in fish sauce (163 µg/mL) (Lin and Choong, 1999). Maximum values for butylparaben in health drinks, tonic drinks, and cold formulas were 106.8, 120.1, and 184.6 µg/mL (549.8, 618.3, and 950.3 µM), respectively, which exceeded the regulatory limit of 100 µg/mL for preservatives (Lin et al., 2000).

In the 1983 National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES), an estimated 24,427 employees were exposed to butylparaben in 704 facilities; of the total, 18,593 were females (RTECS, 2003).

7.2 Cosmetics and Personal Care Products

An estimate of butylparaben exposure from personal care products could be derived from a survey of product use, such as that reported by Eriksson et al. (2003) for 38 tenants (22 adults and 16 children ages 2-15 years) in a Danish apartment building. The average use of products most likely to contain butylparaben (shampoos, skin care products, soaps, shower gels, and hair styling products) was 19.7 g per person per week. (No lipsticks or other cosmetics were reported to be used.) If only 13% of these products contained butylparaben (EWG, 2005) at the maximum (0.07%) concentration (Rastogi et al., 1995), the daily exposure to butylparaben from personal care products is estimated to be ≤ 260 µg/person/day.

Loretz et al. (2005) supplied 360 U.S. women aged 18-65 with a butylparaben-containing face cream and a body lotion without butylparaben [but containing methyl-, ethyl-, and propylparaben]. They were asked to record the number of daily applications during a two-week period to specific body areas. The remaining product was weighed at the end of the study. Valid diaries for application of body lotion and face cream were returned by 83.3 and 85.6% of study participants, respectively. Face cream consumption at the 50th percentile, the 95th percentile, and the mean was 1.53, 3.99, and 2.05 g/day, respectively. If a butylparaben concentration of 0.07% in the face cream is assumed, the daily applied dose of butylparaben based on the 50th percentile, 95th percentile, and the overall mean would be 1.07, 2.79, and 1.44 mg, respectively. Because butylparaben is also used in some body and baby lotions, application values for the body lotion in the Loretz study are used here to estimate potential exposure if lotions contain butylparaben. The mean body lotion consumption at the 50th percentile, the 95th percentile, and the overall mean was 7.63, 16.83, 8.70 g/day, respectively. Assuming the body lotion contained 0.07% butylparaben, the calculated applied doses of butylparaben would be 5.3, 11.8, and 6.09 mg, respectively. Therefore the total butylparaben dose for both products at the 50th and 95th percentiles would be 6.37 and 14.59 mg, respectively, compared to a mean of 7.53 mg. Based on a mean female body weight of 65 kg (Emission Factors Handbook [EFH], U.S. EPA, 1997) the overall mean daily-applied dose would be 7.53/65 or 0.12 mg/kg.

7.3 Pharmaceuticals and Over-the-Counter Medications

A conservative estimate of exposure to butylparaben via pharmaceuticals can be derived from long-term use of a product such as Maalox for relief of gastric distress. It is reasonable to conclude that a person could consume 6 oz per week (~25 mL/day) of a product containing 0.0067% butylparaben (concentration found in Fosamax). The average daily butylparaben exposure would be 1.71 mg/day. Most children's preparations are intended for short-term use. For each one-teaspoon dose (~5 g) of a preparation containing 0.018% butylparaben, a child would consume 0.9 mg butylparaben.

7.4 Indoor Dust and Air

A survey of dust and air concentrations of butylparaben in 118 homes of women with breast cancer found a wide disparity in concentrations (Rudel et al., 2003). Butylparaben was detected in the air in only 8% of the homes and in the dust vacuumed from flooring, furniture, and windowsills in only 26% of the homes. No mean concentrations or ranges were given and the medians given were below the detection limit. Thus, only a worst-case exposure can be calculated (i.e., 3.92 ppm in dust and 3.2 ng/m³ in air). Default exposure factors were taken from U.S. EPA (1997) EFH and in the U.S. EPA (2002) Child-Specific Exposure Factors Handbook (Interim Report).

A child may ingest 100 mg soil (and dust) per day and an adult 50 mg. Another soil exposure factor for children showed 110 mg/day of soil can adhere to skin. Based on the maximum butylparaben concentration of 3.92 ppm butylparaben in dust reported for one home in the Rudel et al. study, a child might ingest 392 ng butylparaben per day and an adult 196 ng/day. This worst-case calculation assumes time spent in the home to be 100%. The greatest exposure from soil would be from actual ingestion of soil by someone with pica. The exposure factor is 10 g/day so at 3.92 ppm, 39.2 µg butylparaben would be ingested. [Literature values reviewed (U.S. EPA, 1997, 2002) indicated that the indoor dust contribution may be only a quarter of the total daily soil exposure. The "reference child" is a six to eight year old who weighs 15 kg and inhales 10 m³ air/day. The U.S. EPA recommended reference values for inhalation in adults are 15.2 m³/day for males and 11.3 m³/day for females. The maximum butylparaben air concentration would expose a child to 32 ng/day, an adult male to 48 ng/day, and an adult female to 36 ng/day.

8.0 Regulatory Status

Butylparaben is regulated by the U.S. Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). In 1998 its pesticide registration status was listed as "cancelled" (U.S. EPA, 2003). Butylparaben is also regulated by the FDA. It is permitted as a synthetic flavoring substance and adjuvant and is to be used in the minimum quantity required to produce its intended effect [21 CFR 172.515] (FDA, 2003).

In Denmark, the maximum allowable concentration for parabens in food is 300 mg/kg as a preservative. In cosmetics, up to 0.4% of a single paraben and no more than 0.8% of a paraben mixture are permitted (Andersen et al., 2001). There is some concern in the European Union (EU) that parabens might occur in meats. Butylparaben and its sodium salt are not subject to residue limits in foodstuffs of animal origin (CEC, 1999). The EU conditionally permitted the

use of parabens in foods, but they had to be combined with sorbates or sorbates and benzoates. A temporary upper-limit acceptable daily intake (ADI) for methyl-, ethyl-, propyl-, and/or butylparabens was set at 10 mg/kg bw. The European Commission Scientific Committee on Food requested further studies and in 2000 decided to review data for parabens and benzoates. By September 2002, no data for parabens had been submitted. The Committee questioned whether parabens were still used in foods sold in the EU and recommended that the temporary ADI be withdrawn (EC, 2003).

More recently, the European Food Safety Authority (EFSA) concluded that the ADI of up to 10 mg/kg should remain in place for the sum of methyl and ethyl paraben and their sodium salts on the basis of a NOAEL of 1000 mg/kg; an ADI for the propyl ester could not be established because of lack of a clear NOAEL. There is no evidence of demonstrable risk for the development of breast cancer caused by the use of paraben-containing underarm cosmetics (SCCP, 2005a,b).

9.0 Toxicological Data

9.1 General Toxicology

In 1984, the Cosmetic Ingredient Review (CIR) Panel published a report on parabens (methyl-, ethyl-, propyl-, and butylparaben) summarizing published data during the period 1920 to 1982 and unpublished data submitted to them. The panel made the following conclusions regarding the toxicity of parabens:

- acute toxicity studies in animals indicate that they are practically nontoxic by various routes;
- subchronic and chronic oral studies indicate that they are practically nontoxic;
- all animal sensitization tests indicate that they are nonsensitizing;
- mutagenicity studies (Ames test, dominant lethal assay, host-mediated assay, and cytogenic assays) indicate that they are nonmutagenic;
- human studies indicate that they are practically nonirritating and nonsensitizing;
- carcinogenicity studies indicate that methyl- and propylparaben are noncarcinogenic;
- methyl- and ethylparaben were nonteratogenic in rabbits, rats, mice, and/or hamsters; and
- photocontact sensitization and phototoxicity tests on product formulations containing 0.1-0.8% methyl-, propyl-, and/or butylparaben indicated no significant photoreactivity.

The Panel concluded that methyl-, ethyl-, propyl-, and butylparaben were "safe as cosmetic ingredients in the present practices of use" (CIR, 1984).

The following sections contain data from studies using butylparaben. Generally, data for product formulations containing various concentrations of butylparaben with methyl-, ethyl-, and/or propylparaben are not included in this report.

9.1.1 Human Data

When exposed to butylparaben via inhalation, irritation to the respiratory tract results; symptoms include coughing and shortness of breath. Ingestion of large doses of butylparaben may cause irritation to the gastrointestinal (GI) tract. Contact with the eyes causes irritation, redness, and pain, while contact with the skin causes the same symptoms and itchiness. Allergic skin reactions may also occur (Mallinckrodt Baker, Inc., 2003).

Chemical Disposition, Metabolism, and Toxicokinetics

Several *in vitro* and *in vivo* skin penetration studies have been conducted with these chemicals (see **Table 2** for examples). Butylparaben was absorbed "passively" from oral mucosa; the area under the amount in mucosal tissue versus time curve (AUC) and mean transit time (MTT) values in buccal mucosa (4324% dose·min and 16.5 min, respectively) were larger than those in other regions (e.g., 2478 and 10.0, respectively, in dorsum of tongue and 2131 and 10.9, respectively, in labial mucosa) (Kurosaki et al., 1997). When orally given, rapid absorption from the GI tract occurs. The parabens are hydrolyzed to *p*-hydroxybenzoic acid, then conjugated, and rapidly excreted in the urine (CIR, 1984).

Table 2. Absorption Studies Using Human Skin *In Vitro*

Model Membrane	Comments	Reference
Human epidermis in Franz cell diffusion expts. with receptor fluid at 23-45 °C	Transdermal flux from a saturated aqueous solution and epidermal retention increased with increasing temperature. More butylparaben than methylparaben was retained in the epidermis. Amounts increased with higher temperature while the transdermal flux of methylparaben was greater than that of butylparaben. At 37 °C, estimated epidermal diffusivity for methylparaben was $26.54 \pm 4.17 \times 10^{-4}$ cm/s compared to $1.52 \pm 0.22 \times 10^{-4}$ cm/s for butylparaben.	Akomeah et al. (2003 poster, 2004)
Human skin	Fluxes from cosmetic emulsions decreased with increasing chain length.	Dal Pozzo and Pastori (1996)
Human callus	The higher the octanol-water partition coefficient, <i>K_{ow}</i> , the greater the binding to keratinized structures of the stratum corneum. Skin penetration was greater when the compound was highly soluble in the vehicle.	Hagedorn-Leweke and Lippold (1998)
Human erythrocyte ghost membranes	Absorption increased with higher lipophilicity.	Lee and Kim (1994)
Different human skin layers	Four carboxylesterases from skin, subcutaneous fat, and blood had different hydrolyzing capabilities for the parabens at pH 8.0. No hydrolysis at pH 5.0.	Lobemeier et al. (1996)

In a recent study, paraben concentrations measured in human breast tumors were of the esters, demonstrating that at least a proportion of the parabens present in cosmetic, food, and pharmaceutical products can be absorbed and retained in body tissues without hydrolysis to the common metabolite *p*-hydroxybenzoic acid. Mean concentrations for methyl-, propyl-, butyl-, and ethylparabens were 12.7, 2.6, 2.3, and 2.0 ng/g tissue, respectively, while the mean for isobutylparaben was 0.9 ng/g (Darbre et al., 2004).

Using Franz cell experiments in which epidermal sheets were prepared from surgically excised human skin samples from a female non-smoking donor, release rates for methyl and butylparaben were found to increase by a factor of 1.53 and 1.55, respectively with every 7-8 °C rise in receptor temperature. Additionally, increasing the temperature of the receptor enhanced transdermal penetration; a three-fold increase in flux was recorded for butylparaben when the temperature was increased from 37 to 45 °C. Estimated epidermal diffusivity of methylparaben was significantly affected by temperature changes; a 10- to 12-fold difference in epidermal diffusivity was observed between butyl- and methylparaben (Akomeah et al., 2004).

In oral mucosa in humans, perfusion cells were applied to the dorsum and ventral surface of the tongue, labial mucosa, floor of mouth, and buccal mucosa. Using pharmacokinetic analysis of oral-mucosal drug (model drugs used were methyl-, ethyl-, propyl- and butylparaben), absorption rate constant, partition to oral mucosa, and the residence time in oral mucosa increased with lipophilicity of the compound. The disappearance profiles of the alkylparabens were biexponential, suggesting the rapid distribution to the mucosa followed by relatively slow transfer to the circulation (Kurosaki et al., 1997).

Immunotoxicity

Patch tests show that the sensitization potential of parabens is low (Angelini et al., 1997; CIR, 1984; Menne and Hjorth, 1988; Mowad, 2000). Cross-reactivity to each of the paraben esters can occur (CIR, 1984).

In 50 human volunteers, butylparaben (5, 7, 10, 12, and 15%) was applied to the back daily for five days. No irritation was found at a concentration of 5% (CIR, 1984). In a repeated insult patch test, butylparaben (5%) was applied to the skin of 50 volunteers during a period from four to eight hours every other day for a total of ten injections. No irritation was reported. Reapplication of the paraben three weeks later for a period of 24 to 48 hours also resulted in no sensitization (CIR, 1984; Matthews et al., 1956). Photocontact sensitization and phototoxicity tests on product formulations containing 0.1-0.8% methyl-, propyl-, and butylparaben gave no evidence of photoreactivity (CIR, 1984). However, exposure to butylparaben and sunlight has caused excessive hyperpigmentation (Farber, 1998 lett.). In a more recent review, butylparaben was associated with hypersensitivity and was reported to intensify dermatitis compared to the lower alkyl parabens (Soni et al., 2002).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

Note: Pharmacological studies in reviews (e.g., CIR [1984] and JECFA [2001] used below) are from studies conducted in the 1950s and 1960s. The reviews do not specify the strain, sex, age, and pregnancy stage of the tested animals; only the route and dose in the species are given. Many of the studies do not fulfill "present day criteria for conduct of [such] studies" (AFC, 2004). High quality complete studies by the oral or dermal route are not available.

Rabbits orally given butylparaben (0.4 or 0.8 g/kg [2 or 4 mmol/kg]) excreted 0.2-0.9% of the ester by 24 hours. Within the same period, 25-39% 4-HBA, 15-29% *p*-hydroxyhippuric acid, 5-8% *p*-carboxyphenyl glucuronide, 10-18% *p*-hydroxybenzoyl glucuronide, and 7-12% *p*-carboxyphenyl sulfate were recovered (CIR, 1984).

When fasted dogs (groups of three or more) received an intravenous (i.v.) injection of butylparaben (50 mg/kg [0.26 mmol/kg]), 40% of the administered dose was recovered in the urine as the 4-HBA conjugate of glucuronic acid within 30 hours, and metabolites were detected up to six hours postinjection. Oral administration of butylparaben (1000 mg/kg [5.148 mmol/kg]) to the animals resulted in a 48% recovery of the 4-HBA conjugate of glucuronic acid within 30 hours, and metabolites were detected in blood up to 24 hours postingestion (Jones et al., 1956; cited by CIR, 1984 and JECFA, 2001). In a similar study in which dogs were given an i.v. injection of butylparaben (100 mg/kg [0.515 mmol/kg]), the parent compound was recovered in the brain, spleen, and pancreas. High concentrations of metabolites were detected in the liver

and kidneys. [An *in vitro* assay found that esterases in the liver and kidney completely hydrolyzed butylparaben in 30 to 60 minutes] (CIR, 1984; JECFA, 2001).

Numerous *in vitro* studies have been conducted reporting the permeability of butylparaben through skin (see **Table 3**). In these studies, butylparaben usually exhibited low penetration, retention in the epidermis, and/or hydrolysis in the skin.

Table 3. Absorption Studies in Animal Skin *In Vitro*

Model Membrane	Comments	Reference
Rat skin	Ninety-six percent of the penetrated amount of butylparaben was metabolized in the skin to 4-HBA.	Bando et al. (1997)
Rat skin	The relationships among lipophilicity, metabolic rate, and skin permeation were studied. Cutaneous metabolism in the viable layer is important for butylparaben.	Seko et al. (1999)
Guinea pig dorsal skin	Certain agents and vehicles altered permeability.	Kitagawa et al. (1997)
Guinea pig dorsal skin	Addition of Polysorbate 80 or polyethylene glycol 410 reduced penetration of butylparaben in water.	Komatsu and Suzuki (1979; cited by CIR, 1984)
Guinea pig skin	Penetration of butylparaben from liposomes was studied.	Komatsu et al. (1986)
Guinea pig skin	<i>d</i> -Limonene and oleic acid enhanced penetration.	Koyama et al. (1994)
Guinea pig skin	Cyclodextrin complexation decreased percutaneous absorption.	Okamoto et al. (1986)
Guinea pig skin	Skin penetration enhancers increased accumulation in skin.	Okamoto et al. (1991)
Guinea pig skin, two-layer	1-Geranylazacycloheptan-2-one increased skin penetration from an aqueous vehicle.	Yamashita et al. (1993)
Rabbit corneas	The relationship between corneal permeability and lipophilicity was studied.	Lee et al. (1991)
Snake skin	Smaller lipophilic compounds penetrated the skin more readily.	Itoh et al. (1990)
"Idealized skin model membranes" [silastic and demeticone were indexed]	Effects of alcoholic solvents on skin permeation kinetics were studied.	Twist and Zatz (1986)

Using guinea pig skin, the percutaneous absorption of butylparaben (0.015-0.1% aqueous solution) was studied *in vitro*. The amount of compound that passed through the skin depended on the partition coefficient of the system. Solubilizers (e.g., polysorbate 80, propylene glycol, and PEG 400) were found to increase antimicrobial activity but decrease absorption (Komatsu and Suzuki, 1979; cited by CIR, 1984). In addition, *l*-menthol in ethanol and *d*-limonene significantly decreased the absorption of butylparaben, while *N*-dodecyl-2-pyrrolidone and oleic acid had no significant effect (Kitagawa et al., 1997; Koyama et al., 1994). *d*-Limonene significantly enhanced BP penetration through the intact skin; the penetration pattern obtained with the highest dose of *d*-limonene in intact skin (34% at 288 μ mol in the receptor cell) is nearly the same as that obtained in stripped skin (38% in receptor cell). In the absence of these solubilizers, 21% of the butylparaben administered dose was recovered in the receptor cell and 49% was recovered from the skin (Koyama et al., 1994). In another study, penetration through

guinea pig skin from liposome suspension decreased as the lipid content increased (Komatsu et al., 1986).

In rat skin penetration studies, only 4% of butylparaben was not hydrolyzed (Bando et al., 1997). In one such study, the enhancing effects of 1-geranylazacycloheptan-2-one (GACH) were studied *in vitro* and *in vivo* using Wistar rats. Butylparaben recoveries from *in vitro* studies from the donor cell, skin, and the receptor cell after 12-hour diffusion were 13, 14, and 57%, respectively. With GACH, recoveries were 20, 74, and 20%, respectively (with a dose of 6.4 μmol), and 9, 71, and 0.31%, respectively (with 51.0 μmol). [Penetration through tape-stripped skin yielded values of 11, 1, and 76%, respectively.] In *in vivo* absorption experiments, the amounts of butylparaben recovered after four hours were 13% in the donor cell, 10% in the skin, and 47% in urine. With GACH, recoveries of the application amount were 25, 29, and 20%, respectively (with 6.4 μmol), and 16, 52, and 1%, respectively (with 51.0 μmol). The absorbed amount was 51% with butylparaben alone, 24% with the low dose of GACH, 2% with the high dose of GACH, and 72% in tape-stripped skin. *In vivo* skin penetration of butylparaben was considerably greater than that observed *in vitro* (Yamashita et al., 1994). Additionally, urinary excretion profiles indicated that *in vivo* butylparaben absorption was greater than indicated by *in vitro* studies. Although oleic acid enhanced penetration at low doses, it decreased penetration at the high dose. Parabens were rapidly absorbed through the nasal membranes of rats (Aikawa et al., 1998).

9.1.3 Acute Exposure

Acute toxicity values for butylparaben and its salt are presented in **Table 4**.

In mice, rats, and dogs, butylparaben was reported to have a low order of acute toxicity; the main effect was an acute myocardial depression accompanied by hypotension that was transient in nature. In mice, rapid onset of ataxia, paralysis, and deep depression, which was similar to anesthesia, were seen. An increase in motor activity was also observed but very rarely. With nonfatal doses, recovery generally occurred within 30 minutes; with fatal doses, death usually occurred within an hour (Matthews et al., 1956). Intraperitoneal (i.p.) injection of butylparaben (230 mg/kg [1.18 mmol/kg]) resulted in lacrimation in the eyes of mice (Tsuzi, 1956).

Butylparaben (5%) was a mild irritant when applied to the skin of guinea pigs for 48 hours (Danish EPA, 2001; RTECS 2003). In rabbits, a product formulation containing 0.2% butylparaben was nonirritating, although the primary irritation index indicated moderate irritation. In another study, a product with 0.3% butylparaben applied for three days to the back of rabbits produced mild irritation with erythema and/or edema (CIR, 1984).

Table 4. Acute Toxicity Values for Butylparaben and Its Salt

Route	Species (sex and strain)	LD ₅₀ (units bw)	Reference(s)
<i>Butylparaben</i>			
i.p.	Mouse (sex and strain n.p.)	230 mg/kg (1.19 mmol/kg)	RTECS (2003)
oral	Mouse (sex and strain n.p.)	5000 mg/kg (25.74 mmol/kg)	Sokol (1952; cited by JECFA, 2001)
	Mouse (sex and strain n.p.)	13,200 mg/kg (67.954 mmol/kg)	CIR (1984); RTECS (2003); Sado (1973; cited by JECFA, 2001)
dermal*	Rabbit (M and F, albino)	>2000 mg/kg (10.30 mmol/kg)	CIR (1984)
<i>Butylparaben, sodium salt</i>			
i.p.	Mouse (sex and strain n.p.)	~230 mg/kg (1.06 mmol/kg)	Matthews et al. (1956)
s.c.	Mouse (sex and strain n.p.)	2500 mg/kg (11.56 mmol/kg)	CIR (1984)
oral	Mouse (sex and strain n.p.)	~950 mg/kg (4.39 mmol/kg)	Matthews et al. (1956)

*0.2% in eye makeup formulation

Abbreviations: bw = body weight; F = female(s); i.p. = intraperitoneal(ly); LD₅₀ = lethal dose for 50% of test animals; M = male(s); n.p. = not provided; s.c. = subcutaneous(ly)

9.1.4 Short-term and Subchronic Exposure

The no observable effect levels (NOELs) and lowest observable effect levels (LOELs) for butylparaben are listed at the end of Section 9.2 (see **Table 6**) along with results from studies of reproductive and developmental toxicity. The details of the following studies are presented in **Table 5**.

In mice provided with food pellets containing butylparaben (0.6, 1.25, 2.5, 5, or 10%) for six weeks, deaths occurred within the first two weeks in those given the two highest doses. At levels greater than 0.6%, significant atrophy of lymphoid tissue in the spleen, thymus, and lymph nodes were observed. Additionally, multifocal degeneration and necrosis were seen in the liver parenchyma (Inai et al., 1985).

Rats fed a diet of butylparaben (2 or 8%) for up to 12 weeks resulted in 100% mortality before the end of the treatment period in males given the high dose. Females also had many early deaths. The high-dose butylparaben diet also produced a significant decrease in body weight for all animals, while the low dose produced no toxic effects (Matthews et al., 1956). A diet of 4% butylparaben for nine days acted entirely on the prefundic region of the forestomach epithelium adjacent to the fundic mucosa (Rodrigues et al., 1986). Oral intubation of butylparaben (0.25 or 50 mg/kg [0.0013 or 0.26 mmol/kg] bw/day) for 13-15 weeks produced no toxic effects in rats (Ikeda and Yokoi, 1950; cited by JECFA, 2001).

Table 5. Short-term and Subchronic Exposure to Butylparaben

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, ICR/Jcl, 8-wk-old, 10M and 10F per dose group	Butylparaben, purity n.p.	oral; 0.6, 1.25, 2.5, 5, or 10% (900, 1900, 3800, 7500, or 15,000 mg/kg bw/day [0.216, 9.781, 19.56, 38.61, or 77.220 mmol/kg bw/day]) in the diet for 6 wk	At ≥ 7500 mg/kg, all animals died within the first 2 wk. At 1900 or 3800 mg/kg, body weight gain of treated mice was 10% less than that of controls. At $>0.6\%$, marked atrophy of lymphoid tissue in organs (including the spleen, thymus, and lymph nodes) and multifocal degeneration and necrosis in the liver parenchyma were observed. The NOEL was 900 mg/kg bw/day.	Inai et al. (1985)
Rats, Fischer 344, (weanling) age n.p., 5M per dose group	Butylparaben, purity n.p.	oral; 4% in diet for 9-27 days	At 9 days after beginning treatment, thickening of the mucosa with acanthosis, hyperkeratosis, prominent rete pegs, and papillae was observed in the lesser curvature of the forestomach. The lamina propria and submucosa were edematous and infiltrated with eosinophils and lymphocytes.	Rodrigues et al. (1986)
Rats, strain, age, number, and sex n.p.	Butylparaben, purity n.p.	oral intubation; 0.25 or 50 mg/kg bw/day (0.0013 or 0.26 mmol/kg bw/day) in soya bean oil for 13-15 wk	No significant difference in body weights was found between treated and control animals. There were also no sporadic deaths and no significant histological differences from controls. The NOEL was 50 mg/kg bw/day.	Ikeda and Yokoi (1950; cited by JECFA, 2001)
Rats, Wistar, age n.p., 12M and 12F per dose group	Butylparaben, purity n.p.	oral; 2 or 8% in the diet (equivalent to 2000 or 8000 mg/kg bw/day [10.30 or 41.18 mmol/kg bw/day]) for up to 12 wk	At the high dose, 100% mortality was seen for males before the end of the treatment period. Many females also died and showed myocardial depression. Treated animals had decreased body weight and motor activity. In addition, growth rate was slower compared to controls. The NOEL was 2000 mg/kg bw/day.	Matthews et al. (1956)

Abbreviations: bw = body weight; F = female(s); M = male(s); NOEL = no observable effect level; n.p. = not provided; wk = week(s)

9.1.5 Chronic Exposure

In eight-week-old ICR/Jcl mice orally given butylparaben (0.15, 0.3, or 0.6%) in the diet for 102 weeks, a high incidence of amyloidosis affecting the spleen, liver, kidney, and/or adrenal gland was observed. These occurred in 45 and 27% of males and females, respectively, that survived for >78 weeks or died with tumors during the experimental period (Inai et al., 1985).

9.1.6 Synergistic/Antagonistic Effects

In mice, oral administration of any binary combination of sorbic acid, furylfuramide, ethylparaben, propylparaben, butylparaben, sodium dehydroacetate, benzyl alcohol, salicylic acid, and sodium propionate failed to produce synergism in acute toxicity (Sado, 1973).

9.1.7 Cytotoxicity

In isolated rat hepatocytes, butylparaben (2 mM [0.4 mg/mL]) caused 88% cell death, almost total loss of adenosine triphosphate (ATP), and ~60% reductions in the total adenine nucleotide pool and mitochondrial membrane potential. When added to isolated rat mitochondria, butylparaben (0.05, 0.10, or 0.25 mM [9.8, 19, or 49 µg/mL]) increased the rate of state 4 oxygen consumption and inhibited the rate of state 3 oxidation (Nakagawa and Moldeus, 1998; Nakagawa et al., 1999). In normal and α -linolenic acid (LNA)-loaded cultured rat hepatocytes, butylparaben induced severe cell injury accompanied by a significant decrease in cellular levels of both glutathione (GSH) and protein-SH but no lipid peroxidation (Sugihara et al., 1997). Using a menadione-catalyzed hydrogen peroxide production assay (animal cells), cytotoxic events were observed with butylparaben (Yamashoji and Isshiki, 1998, 2002).

9.2 Reproductive and Teratological Effects

The NOELs and LOELs for butylparaben are listed in **Table 6** for reproductive, developmental, and short-term and subchronic toxicity studies. Details of the short-term and subchronic studies are given in **Table 5**. The reproductive and developmental studies are described in the following section and details are presented in **Table 7**. No GLP continuous breeding studies evaluating reproductive effects were located.

Table 6. Selected NOELs and LOELs for Butylparaben

Species, Strain, Age, and Sex	Exposure Route/Duration	NOEL or LOEL (mg/kg bw/day)	Endpoint	Reference
Mice, ICR/Jcl, 8-wk-old, M and F	Oral; diet 6 wk	NOEL = 900	subchronic toxicity: significant atrophy of lymphoid tissue in organs and multifocal degeneration and necrosis in liver parenchyma	Inai et al. (1985)
Mice, Crj:CD-1, 4-wk-old, M	Oral; diet 10 wk	NOEL = 100	reproductive toxicity: significant increase in epididymides weights and decrease in spermatid counts and serum testosterone level	Oishi (2002a)
Rats, Fischer 344, (weanling), M	Oral; diet 9-27 days	LOEL = 1600 ¹	subchronic toxicity: damage to the forestomach epithelium	Rodrigues et al. (1986)
Rats (strain, age, and sex n.p)	Oral; intubation 13-15 wk	NOEL = 50	subchronic toxicity: no significant effects on body weight, no sporadic deaths, and no histological differences	Ikeda and Yokoi (1950; cited by JECFA, 2001)

Table 6. NOELs and LOELs of Butylparaben (Continued)

Species, Strain, Age, and Sex	Exposure Route/Duration	NOEL or LOEL (mg/kg bw/day)	Endpoint	Reference
Rats, Wistar, age n.p., M and F	Oral; diet 12 wk	NOEL = 2000	subchronic toxicity: reduced growth rate, decreased body weight and motor activity, and myocardial depression (in females)	Matthews et al. (1956)
Rats, Alpk:AP, 21- to 22-day-old, F	Oral; gavage 3 days	NOEL = 1200 ²	reproductive toxicity: statistically insignificant increases in uterus wet and dry weights	Routledge et al. (1998)
Rats, Sprague-Dawley, age n.p., F	Oral; gavage GD 6-19	NOEL = 100	reproductive toxicity: decreases in maternal weight gain; statistically significant decreases in weight gain on GD 18-20; decrease in food consumption (GD 6-20)	Daston (2004)
Rats, Sprague-Dawley, age n.p., F	Oral; gavage GD 6-19	NOEL = 1000	developmental toxicity: no changes in embryo/fetal viability, fetal weight, and external, visceral, and skeletal abnormalities	Daston (2004)
Rats, Wistar, 3-wk-old, M	Oral; diet 8 wk	LOEL = 40 ³	reproductive toxicity: significant decrease in epididymides weights and serum testosterone levels	Oishi (2001)
Rats, Alpk:AP, 21- to 22-day-old, F	s.c; 3 days	LOEL = 400 ³	reproductive toxicity: increase in uterus wet weights	Routledge et al. (1998)
Rats, Wistar, 2- to 12-day-old, M	s.c.; 2-18 days	NOEL = 2 ¹	developmental toxicity: testis weight, aquaporin-1 immuno-expression, and effects on rete testis morphology or efferent duct epithelial cell height	Fisher et al. (1999)
Rats, Sprague-Dawley, 9-wk-old, F	s.c.; GD 6 to PND 20	LOEL = 100 ⁴	reproductive toxicity: significant decrease in proportion of pups born alive	Kang et al. (2002b)

¹ based on a single dose level used in the study; ² based on the highest dose reported; ³ based on the lowest dose reported to cause a significant effect; ⁴ based on doses given to pregnant and lactating dams for reproductive toxicity studies

Abbreviations: bw = body weight; F= female(s); GD = gestation day; LOEL = lowest observable effect level; M = male(s); NOEL = no observable effect level; n.p. = not provided; PND = postnatal day; s.c. = subcutaneous; wk = week(s)

Reproductive Studies

When mice were administered butylparaben (0.01-1%) in the diet for ten weeks, the absolute and relative weights of the epididymides were significantly increased compared to controls at the high dose. A dose-dependent decrease in serum testosterone concentration and in round and elongated spermatid counts in stages VII-VIII seminiferous tubules was also observed, and the elongated spermatid counts were significantly lower (Oishi, 2002a).

Exposure of postweaning male rats to butylparaben (0.01-1%) in the diet for eight weeks caused decreases in the cauda epididymal sperm reserve, daily sperm production in the testis, and in serum testosterone concentration (Oishi, 2001). Butylparaben (400-1200 mg/kg [2.06-6.178 mmol/kg]) injected for three consecutive days caused a significant increase in uterine weight in ovariectomized and immature rats (Routledge et al., 1998).

Table 7. Reproductive Toxicity and Teratology of Butylparaben

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, Crj:CD-1, 4-wk-old, 8M/dose group	Butylparaben, ≥99% pure	oral; 0.01, 0.10, or 1.00% (average intake of ~14.4, 146, and 1504 mg/kg [0.0741, 0.752, or 7.743 mmol/kg] per day) in diet for 10 wk; sacrificed at 10 wk	There were no treatment-related effects on the liver, ventral prostates, seminal vesicles, and preputial glands. At 1.00%, absolute and relative weights of the epididymides were significantly increased compared with controls. Round and elongated spermatid counts were dose-dependently decreased in stages VII-VIII seminiferous tubules; the latter was significantly lower in all groups. Serum testosterone level also dose-dependently decreased; significance was seen at 1.00%.	Oishi (2002a)
Rats, Wistar, 2- to 12-days-old (neonatal), number n.p., M	Butylparaben, purity n.p.	s.c.; ~2 mg/kg (0.01 mmol/kg) in corn oil given on days 2-18 inclusive; animals sacrificed (4 h after daily injection) and observed on day 18	No alteration in testis weights compared to controls was seen. Additionally, no gross changes in aquaporin-1 (AQP-1) immun-expression were observed. There was no detectable effect on rete testis morphology or in efferent duct epithelial cell height.	Fisher et al. (1999)
Rats, Sprague-Dawley, 9-wk-old, 22F	Butylparaben, purity n.p.	s.c.; 100 or 200 mg/kg (0.515 or 1.03 mmol/kg) in DMSO given from GD 6 to PND 20, with a 2-day interruption at parturition; animals sacrificed at PND 21; observed up to PND 90	No clinical signs of toxicity or effects on body weight or food consumption were observed. At both doses, the proportion of pups born alive and proportion of pups that survived up to the weaning period were decreased. At 100 mg/kg, vaginal opening occurred several days earlier in treated rats compared to controls. In male F1 offspring: At 100 mg/kg, body weight was significantly decreased on PND 49. Testicular weight was significantly increased at PND 21 but significantly decreased at PND 49. Additionally, prostate gland weight was significantly decreased at PND 49 and PND 90, while the weight of the seminal vesicles was significantly decreased at PND 49. At 200 mg/kg, testicular weight was significantly increased at PND 90. At both doses, the number and motility of sperm in caudal epididymis were significantly decreased. The total cell numbers of round and elongated spermatid in the seminiferous tubules at stage VII were significantly decreased. In female F1 offspring: At both doses, body weights were significantly decreased at PND 49 to 90. There were no effects on the weights of female reproductive organs.	Kang et al. (2002b)

Table 7. Reproductive Toxicity and Teratology of Butylparaben (Continued)

Species, Strain, and Age, Number, and Sex of Animals	Chemical Form and Purity	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Rats, Alpk:AP, 21- to 22-days-old, 5F per dose group	Butylparaben, >99% pure	s.c.; 40, 200, 400, 600, 800, 1000, or 1200 mg/kg (0.21, 1.03, 2.06, 4.12, 5.148, 6.178 mmol/kg) daily for 3 successive days	<i>Immature rats:</i> At 400-800 mg/kg, significantly increased uterus wet weights were reported; at 1200 mg/kg; weights were ~170% that of controls. <i>Ovariectomized rats:</i> At 1200 mg/kg, significantly increased uterus wet and dry weights were up to 150% of controls. At 1000 mg/kg, vaginal cornification was significantly increased. (An increase was seen at 800 mg/kg, but the response was not statistically significant.)	Routledge et al. (1998)
Rats, Alpk:AP, 21- to 22-days-old, 5F per dose group	Butylparaben, >99% pure	oral (gavage); 4, 40, 400, 800, or 1200 mg/kg (0.02, 0.21, 2.06, 4.12, 6.178 mmol/kg) daily for 3 successive days	At 800-1200 mg/kg, small but statistically insignificant increases in uterus wet and dry weights were reported in immature rats.	Routledge et al. (1998)
Rats, Wistar, 3-wk-old, number n.p., M	Butylparaben, purity n.p.	oral; 0.01, 0.10, or 1.00% (average intake of ~10.4, 103, or 1026 mg/kg [0.054, 0.53, or 5.28 mmol/kg] in diet for 8 wk; animals sacrificed at 8 wk	Absolute and relative weights of the epididymides and serum testosterone levels were dose-dependently decreased; statistical significance was seen at ≥0.1%. At all dose levels, the cauda epididymal sperm reserve and daily sperm production in the testis were also significantly lowered compared to controls.	Oishi (2001)
Rats, Sprague-Dawley, age n.p., 25F per dose group	Butylparaben, purity n.p.	oral; 10, 100, or 1000 mg/kg (0.051, 0.515, or 5.148 mmol/kg) daily on GD 6-19; caesarean performed on GD20	At the high dose, decreases in maternal weight gain were observed during some measurement intervals; statistical significance was seen during GD 18-20. Maternal food consumption was significantly decreased over the dosing period (GD 6-20). The maternal NOAEL was 100 mg/kg/day. No differences in developmental parameters (including embryo/fetal viability, fetal weight, malformations, and variations) were seen between treated rats and controls. The NOAEL for developmental toxicity was 1000 mg/kg/day.	Daston (2004)

Abbreviations: DMSO = dimethylsulfoxide; F= female(s); GD = gestation day(s); h = hour(s); M = male(s); n.p. = not provided; NOAEL = no observable adverse effect level; PND = postnatal day; s.c. = subcutaneous(ly); TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; wk = week(s)

Developmental Studies

Subcutaneous (s.c.) administration of butylparaben (100 or 200 mg/kg [0.515 or 1.03 mmol/kg]) in pregnant rats from gestation day 6 to postnatal day 20 decreased the proportion of pups born alive and the proportion of pups that survived up to the weaning period (Kang et al., 2002b). In neonatal rats, daily s.c. injection of butylparaben (2 mg/kg [0.01 mmol/kg]) for up to 18 days had no effect on testis weight, distension of the rete testis and efferent ducts, or epithelial cell height in the efferent ducts (Fisher et al., 1999). Pregnant rats were given s.c. injections of butylparaben (100 or 200 mg/kg [0.515 or 1.03 mmol/kg]) from gestation day 6 to postnatal day 20. On postnatal day 49, body weights were significantly decreased in F1 females, while the weights of testes, seminal vesicles, epididymides, and prostate glands were significantly decreased in F1 males. Additionally, the number and motility of sperm in the epididymis were significantly lowered. No changes in the weights of female reproductive organs were observed. The results suggested that maternal exposure to butylparaben has adverse effects on F1 male offspring (Kang et al., 2002b).

In an oral study, Sprague-Dawley rats were administered butylparaben (10, 100, or 1000 mg/kg [0.051, 0.515, or 5.148 mmol/kg]) in 0.5% carboxymethylcellulose daily on gestation days 6-19. At 1000 mg/kg, decreased maternal weight gain was observed during some of the measurement intervals and was statistically significant during gestation days 18-20; maternal food consumption significantly decreased over the dosing period. No differences were seen from the control and treated group in any of the developmental parameters measured, including embryo/fetal viability, fetal weight, malformations or variations, indicating that butylparaben did not have the potential to cause developmental toxicity in the animals at the highest dose tested (Daston, 2004).

9.3 Carcinogenicity

In eight-week-old female and male ICR/Jcl mice, oral administration of butylparaben (0.15, 0.3, or 0.6%) in the diet for up to 102 weeks produced neoplasms in the hematopoietic system, including thymic lymphoma, non-thymic lymphoid leukemia, and myeloid leukemia. Additionally, a moderately high incidence of lung adenomas and adenocarcinomas and of soft tissue myosarcomas and osteosarcomas were found. Tumor incidences, however, were not significantly different from those of the control group (Inai et al., 1985). AFC (2004) judged this study to be inadequate due to excessive mortality in control and treated groups and high tumor incidences in the control group. Negative results were also reported in another study in mice using the same doses but for a 106 week treatment time (Odashima, 1980). In the rat, butylparaben (0.6 or 1.2%) in the diet for up to 104 weeks did not produce any carcinogenic effects (Odashima, 1980).

9.4 Initiation/Promotion Studies

Two weeks after Fischer rats were administered diethylnitrosamine, the animals were given butylparaben (50,000 mg/kg [257.40 mmol/kg]) in the diet for six weeks, and carcinogenic potential was measured. Butylparaben showed no enhancing or inhibitory effects on the development of preneoplastic glutathione S-transferase placental form-positive (GST-P⁺) foci in the liver of treated rats compared to controls (Ito et al., 1988).

9.5 Anticarcinogenicity

No data were available.

9.6 Genotoxicity

The details of the following studies, except those in Japanese, are provided in **Table 8**.

In Vitro Assays

In Chinese hamster cells, a 1-3% increase in polyploid cell production was observed with butylparaben (0.06 mg/mL [308 µM]). Aberrations included chromatid breaks, chromatid gaps, chromosomal exchanges, and ring formations (Ishidate et al., 1978; cited by CIR, 1984). At a higher dose level, however, butylparaben (60 mg/mL [308 mM]) was not mutagenic in the fibroblast cell line. In addition, negative results were reported for butylparaben (up to 1000 mg/plate [5.148 mmol/plate]) in the Ames test using *Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1535, TA1537, and TA2637 (Haresaku et al., 1985; Ishidate et al., 1984; both cited by JECFA, 2001).

Several Japanese articles have been published regarding the mutagenicity of butylparaben. The data provided in English abstracts are presented here. Butylparaben was nonmutagenic in *S. typhimurium* strains TA97, TA98, TA100, and TA102 (Fujita and Hiraga, 1980; Fujita et al., 1985; Haresaku et al., 1985). Its mutagenicity in *Bacillus subtilis* strains H17A and M45T and *Escherichia coli* strain WP2 were also studied; results were not provided in the available abstracts (Kojima and Hiraga, 1978; Morita et al., 1981). Additionally, butylparaben was found capable of inhibiting DNA, RNA, and protein formation in *E. coli* and *B. subtilis* (Eklund and Nes, 1991; Nes and Eklund, 1983). In Chinese hamster CHO-K1 ovary cells, butylparaben was not mutagenic (Yoshida et al., 1978).

In Vivo Assays

In the comet assay, butylparaben (2000 mg/kg [10.30 mmol/kg]) did not increase DNA damage in the glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow of mice (Sasaki et al., 2002).

9.7 Cogenotoxicity

No data were available.

9.8 Antigenotoxicity

No data were available.

Table 8. Genotoxicity Studies of Butylparaben

Test System or Species, Strain, and Age, Number, and Sex of Animals	Biological Endpoint	S9 Metabolic Activation	Chemical Form and Purity	Dose	Results/Comments	Reference
<i>In Vitro</i> Assays						
<i>S. typhimurium</i> strains TA92, TA94, TA98, TA100, TA1535, TA1537, and TA2637	reverse mutation	+/-	Butylparaben, purity n.p.	1000 mg/plate (5.148 mmol/plate)	negative (-S9)	Ishidate et al. (1984; cited by JECFA, 2001)
<i>S. typhimurium</i> strains TA98 and TA100	reverse mutation	-	Butylparaben, purity n.p.	≤1000 mg/plate (5.148 mmol/plate)	negative (-S9)	Haresaku et al. (1985; cited by JECFA, 2001)
Chinese hamster cells	chromosomal aberrations	n.p.	Butylparaben, purity n.p.	0.06 mg/mL (308 μM) [maximum tolerated dose]	positive A 1-3% increase in polyploid cell production was observed. Aberrations included chromatid breaks, chromatid gaps, chromosomal exchanges, and ring formations.	Ishidate et al. (1978; cited by CIR, 1984)
Chinese hamster fibroblasts	chromosomal aberration	-	Butylparaben, purity n.p.	60 mg/mL (308 mM)	negative (-S9)	Ishidate et al. (1984; cited by JECFA, 2001)
<i>In Vivo</i> Assays						
Mice, ddY, age n.p., 4M per dose group	DNA migration (comet assay)	NA	Butylparaben, >98% pure	2000 mg/kg (10.30 mmol/kg) [oral]	negative DNA damage was measured in the glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow 3 and 24 h after treatment. (Additionally, no death, morbidity, or clinical signs were observed.)	Sasaki et al. (2002)

Abbreviations: h = hour(s); M = male(s); NA = not applicable; n.p. = not provided; + = presence; - = absence

9.9 Immunotoxicity

Butylparaben (0.1%) was injected intracutaneously three times weekly and randomly to the back and upper flanks of guinea pigs for a total of ten injections. No reaction was reported 24 hours after the initial injection. A challenge dose given two weeks later also failed to produce sensitization 24 or 48 hours later (Matthews et al., 1956; Sokol, 1952 [cited by CIR, 1984]). The same results were obtained in a similar experiment using the sodium salt of butylparaben (5%) (Matthews et al., 1956).

Intradermal injections of Freund's complete adjuvant on days 0 and 9 and application of butylparaben (5%) under 48-hour occlusive patches to the clipped dorsal skin of guinea pigs every other day for three weeks (ten applications) were followed 12 days later by a challenge patch for 48 hours. Six of 20 animals reacted to butylparaben. Tissue from two of the six animals showed allergic lesions. The worst case had spongiosis, squamous crust, and lymphocytic infiltration (CIR, 1984).

9.10 Other Data

Estrogenic Effects

Several reviews on potential endocrine disrupting chemicals that include data for parabens have been published in the past several years (Darbre, 2001, 2003; Harvey, 2003; NICEATM, 2002). Results from studies of the estrogenic activity of parabens reported that they bind to ERs from sources such as rodent uterus and MCF7 human breast cancer cells and they regulate estrogen-responsive reporter gene expression as well as progesterone receptor expression (Darbre, 2001, 2003).

The estrogenic activities of the parabens increase as the length and branching of the alkyl ester increase (Darbre et al., 2004). The ER relative binding activity of parabens is in the following approximate order: 2-ethylhexyl > heptyl > benzyl > butyl > propyl = ethyl > methyl. Additionally, a linear correlation exists between ER binding activity and hydrophobicity (Fanget al., 2001). In the yeast estrogenic screen test, methyl-, ethyl-, propyl-, butyl-, and benzylparaben were approximately 3,000,000-, 200,000-, 30,000-, and 10,000- and 4000-fold less potent, respectively, than 17 β -estradiol (Kang et al., 2002a; Miller et al., 2001; Routledge et al., 1998). The estrogenic activity of ten parabens, compared to positive estrogenic controls (i.e., 17 β -estradiol, ethinyl estradiol, and diethylstilbestrol) and exogenous estrogenic agents shown to be carcinogens or reproductive toxicants in rodent studies (i.e., DDE, genistein, and methoxychlor), in various assays are presented in **Table 9**.

Parabens were found to possess weak estrogen receptor activity in a *Saccharomyces cerevisiae*-based Lac-Z reporter assay (Schultz et al., 2002). In a yeast two-hybrid assay using the ER α -TIF2 system, methyl-, ethyl-, propyl-, and butylparaben were positive for estrogenic activity (Nishihara et al., 2000). In another recombinant yeast assay, an additive effect in estrogenic activity was observed when combinations of butylparaben and a strong ER agonist (i.e., 17 β -estradiol) or a weak estrogen (i.e., nonylphenol or bisphenol A) were tested (Kang et al., 2002a).

Table 9. Estrogenic Activities of Some Estrogenic Agents and Parabens

Compound	CASRN	5-day EC ₅₀ (M) ^a	Relative Gene Activation ^a	Mean IC ₅₀ (M) ^b	RBA (%) ^b
Estrogenic Agents					
17β-Estradiol	50-28-2	3.91 x 10 ⁻¹¹	100	8.99 x 10 ⁻¹⁰	100
Diethylstilbestrol	56-53-1	—	—	2.25 x 10 ⁻¹⁰	400
Ethinyl estradiol	57-63-6	—	—	4.73 x 10 ⁻¹⁰	190
Genistein	446-72-0	1.81 x 10 ⁻⁷	2.16 x 10 ⁻²	—	0.45 ^c
<i>o,p'</i> -DDE	3424-82-6	—	—	>5.00 x 10 ⁻⁴	—
<i>p,p'</i> -DDE	72-55-9	—	—	>1.00 x 10 ⁻⁴	—
Methoxychlor	72-43-5	—	—	1.44 x 10 ⁻⁴	0.001
Parabens					
Isoamylparaben	6521-30-8	1.17 x 10 ⁻⁷	3.34 x 10 ⁻²	—	—
Nonylparaben	38713-56-3	1.65 x 10 ⁻⁷	2.37 x 10 ⁻²	—	—
2-Ethylhexylparaben	5153-25-3	1.36 x 10 ⁻⁷	2.88x 10 ⁻²	4.95 x 10 ⁻⁶	0.018
Phenylparaben	17696-62-7	2.28 x 10 ⁻⁷	1.71x 10 ⁻²	—	—
Benzylparaben	94-18-8	1.07 x 10 ⁻⁷	3.65 x 10 ⁻²	3.15 x 10 ⁻⁵	0.003
Heptylparaben	1085-12-7	—	—	1.10 x 10 ⁻⁵	0.008
Butylparaben	94-26-8	2.01 x 10⁻⁶	1.95 x 10⁻³	1.05 x 10⁻⁴	0.0009
Propylparaben	94-13-3	—	—	1.50 x 10 ⁻⁴	0.0006
Ethylparaben	120-47-8	7.52 x 10 ⁻⁵	5.20 x 10 ⁻⁵	1.50 x 10 ⁻⁴	0.0006
Methylparaben	99-76-3	—	—	2.45 x 10 ⁻⁴	0.0004

Sources: ^aSchultz et al. (2002) - using the *S. cerevisiae*-based Lac-Z reporter assay; ^bBlair et al. (2000) - using a standardized ER competitive-binding assay with uteri from ovariectomized rats as the ER source; ^cFang et al. (2001) - using the same standardized ER competitive-binding assay. Abbreviations: EC₅₀ = effective concentration eliciting an activity equal to 50% of 17β-estradiol, IC₅₀ = inhibitory concentration eliciting an activity equal to 50% of 17β-estradiol; RBA = relative binding affinity

When tested in juvenile rainbow trout (*Oncorhynchus mykiss*) for estrogenic activity, butylparaben (100-300 mg/kg [0.515-1.54 mmol/kg]) was positive, inducing yolk protein (vitellogenin). In a receptor-binding assay, butylparaben competed with ³H-estradiol for binding to the rat ER; its affinity was five orders or magnitude less than that of diethylstilbestrol. In an *in vitro* yeast-based estrogen assay, butylparaben was weakly estrogenic; however, it was the most potent out of the other shorter-chain parabens tested. *In vivo*, s.c. and topical administration of butylparaben produced a positive uterotrophic response in rats; oral administration had no effect (Danish EPA, 2001; Darbre et al., 2004). In immature Wistar rats, s.c. administration of butylparaben produced a weak estrogenic response (Hossaini et al., 2000). In pregnant rats s.c. injected with butylparaben (100 or 200 mg/kg [0.515 or 1.03 mmol/kg]) from gestation day 6 to postnatal day 20, expression of ER-α and ER-β mRNAs was dose-dependently decreased in testes at postnatal day 21 and dose-dependently increased at postnatal day 90. At postnatal days 49 and 70, expression patterns were different: on day 49, ER-α was decreased at 100 mg/kg and 200 mg/kg compared to controls, while ER-β was increased at the lower dose and increased at the higher dose; on day 70, ER-α was increased at both doses, while ER-β was decreased at both doses (Kang et al., 2002b).

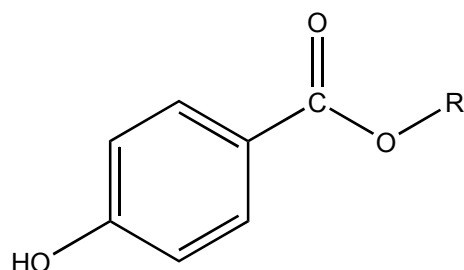
Effects on Blood

Butylparaben strongly inhibited agonist-induced thromboxane (TXB₂) synthesis irreversibly *in vitro* and inhibited or suppressed platelet function (Yamazaki et al., 1998). In mitogen-stimulated peripheral human lymphocytes, butylparaben was the most potent of the parabens tested with C1- to C4-alkyl ester groups in inhibiting release of lysosomal enzymes (45-50% inhibition at 0.06 mM) (Bairati et al., 1994). In human and rabbit erythrocytes *in vitro*, butylparaben (0.02%) induced hemolysis in 6 and 12% of the cells, respectively (CIR, 1984).

Miscellaneous Studies

Antimicrobial effects, biochemical effects (e.g., binding to bovine serum albumin), and physiological effects (e.g., anesthetic effect) of butylparaben have also been reported (CIR, 1984). In experiments using rat pheochromocytoma PC12 cells and cultured rat dorsal root ganglion neurons, the minor irritation and stinging induced by parabens were proposed to be due to their significant effects on the voltage- and ligand-gated channels of neuronal cells (Inoue et al., 1994, 1995, 1996).

10.0 Structure-Activity Relationships



R = CH₃ for methylparaben [99-76-3]
 CH₂CH₃ for ethylparaben [120-47-8]
 (CH₂)₂CH₃ for *n*-propylparaben [94-13-3]
 CH(CH₃)₂ for isopropylparaben [4191-73-5]
 CH₂CH(CH₃)₂ for isobutylparaben [4247-02-3]
 (CH₂)₆CH₃ for heptylparaben [1085-12-7]
 CH₂Ph for benzylparaben [94-18-8]
 H for paraben acid, also called 4-hydroxybenzoic acid [99-96-7]

Several reviews of safety information for parabens are available (e.g., JECFA (1974) [methyl, ethyl, and propylparaben], OECD SIDS (1999) [4-HBA], Soni 2001 [propylparaben] and Soni et al. (2002) [methylparaben]). In this section, the reproductive and teratological effects of parabens other than butylparaben and of 4-HBA are presented.

4-Hydroxybenzoic Acid (4-HBA) [CAS No. 99-96-7]

A Screening Information Data Set (SIDS) Initial Assessment Report, summarizing toxicity data including ecotoxicology, acute toxicity, and genetic toxicity, is available. In the reproductive toxicity study, Sprague-Dawley (Crj:CD) rats orally given 4-HBA (40, 200, or 1000 mg/kg) daily for 45 days in males and from 14 days before mating to day 3 of lactation in females showed no adverse effects on copulation, fertility, pregnancy, parturition, and lactation. In the offspring, no adverse effects on viability, sex ratio, body weights, and morphological appearance of pups were observed. The maternal and F1 offspring no-observable adverse effect level (NOAEL) was 1000 mg/kg/day (OECD SIDS, 1999).

Sprague-Dawley rats given a single oral dose of 4-HBA (333, 667, or 1000 mg/kg) on day 11 of gestation showed no maternal toxic effects (in mortality and body weight). Differences in the number of pregnancy, implantation scars in the uterus, and perinatal loss of offspring, in litter size, total litter weight, and litter biomass between treated and control animals were not reported.

In addition, fetuses had no significant change in pup weight and no overt malformation (OECD SIDS, 1999).

In other studies (details not specified), s.c. injection of 4-HBA to rats at day 9 of gestation and intramuscular (i.m.) injection to mice at day 9 or 12 of gestation produced no teratogenic effects. When immature intact and adult ovariectomized female CD1 mice were s.c. injected daily with 4-HBA (0.5, 5, 50, or 500 µg/100g) for three days, dose-dependent responses on vaginal cornification and uterotrophic activity were reported. At the high dose, the uterotrophic potency of 4-HBA relative to estradiol was 0.0011 and 0.0018 in immature and ovariectomized mice, respectively (OECD SIDS, 1999).

Methylparaben [CAS No. 99-76-3]

Teratogenicity studies using methylparaben were negative. Administration of methylparaben (5.5-550 mg/kg) to pregnant mice or rats for ten days (days 6 to 10 of gestation) had no effect on nidation or on maternal or fetal survival. In rabbits and hamsters, doses up to 300 mg/kg for 13 and 5 days, respectively, produced the same results. The number of abnormalities in soft or skeletal tissues of the treated animals did not differ from that of the controls (CIR, 1984; Soni et al., 2001, 2002). In male Wistar rats, methylparaben (0.1 or 1.0%) administered for eight weeks produced no changes in sperm counts in cauda epididymis and testis and no changes in the levels of testosterone, luteinizing hormone, and follicle stimulating hormone (Oishi, 2004).

Ethylparaben [CAS No. 120-47-8]

Pregnant rats were fed a diet containing ethylparaben (0.1, 1, or 10%) on days 8-15 of pregnancy and killed on day 21. At doses up to 10% ethylparaben, no apparent teratogenesis or toxicity was observed in 363 fetuses. At the 10% level, cerebral hemorrhages and abnormal enlargement in brain ventricles were seen in many fetuses, while hydronephrosis and hypo-osteogenesis were seen in some fetuses. Additionally, some fetuses at the 1% level had no blood in the cardiac ventricle, and some had i.p. hemorrhages. Another group of pregnant rats were given 0.1 or 10% ethylparaben for one week during gestation days 8-15. Neonates, nursed by test dams for a month, had normal growth and no malformations or abnormal behavior. Ethylparaben at concentrations up to 10% was considered nonteratogenic (CIR, 1984).

In male Wistar rats, ethylparaben (0.1 or 1.0%) administered for eight weeks produced no changes in sperm counts in cauda epididymis and testis and no changes in the levels of testosterone, luteinizing hormone, and follicle stimulating hormone (Oishi, 2004).

Propylparaben [CAS No. 94-13-3]

In vitro spermicidal potency of propylparaben assessed in sperm from 19 human subjects was found to be 3 mg/mL, making it an effective spermicide for human spermatozoa. In addition, propylparaben was reported to be weakly estrogenic. It was 30,000-fold less potent than 17β-estradiol in an *in vitro* yeast-based estrogen assay (Soni et al., 2001).

In rats, propylparaben (0.01-1.0%) in the diet for four weeks caused a dose-dependent decrease in cauda epididymal sperm reserves and concentrations, which was significant at doses ≥0.10%, and in the serum testosterone level, which was significant at the high dose. Additionally, daily

sperm production and efficiency in the testis were significantly decreased in all treated animals (Oishi, 2002b).

Isopropylparaben [CAS No. 4191-73-5]

The CIR Panel has published a report on the safety assessment of isopropylparaben; no reproductive toxicity or teratology data were described (CIR, 1995).

Isobutylparaben [CAS No. 4247-02-3]

The CIR Panel has published a report on the safety assessment of isobutylparaben; no reproductive toxicity or teratology data were described (CIR, 1995). In immature female CD1 mice, s.c. administration of isobutylparaben (1.2 or 12.0 mg/mouse) once daily for three days produced an increase in wet uterine weight relative to body weight (Darbre et al., 2002).

Benzylparaben [CAS No. 94-18-8]

The CIR Panel has published a report on the safety assessment of benzylparaben; no reproductive toxicity or teratology data were described (CIR, 1986). In immature female CD1 mice, topical application of benzylparaben (3.3, 10, 33, or 100 mg [equivalent to 0.2-7.5 g/kg bw]) onto unshaven dorsal skin once daily for three days produced a significant increase in absolute uterine weight and uterine weight relative to body weight at a dose of 33 mg (Darbre et al., 2003).

11.0 Online Databases and Secondary References

11.1 Online Databases

National Library of Medicine Databases (TOXNET)

ChemIDplus

EMIC and EMICBACK

HSDB

IRIS

STN International Files

AGRICOLA

IPA

BIOSIS

MEDLINE

BIOTECHNO

NIOSHTIC

CABA

NTIS

CANCERLIT

Registry

EMBASE

RTECS

ESBIOBASE

TOXCENTER

National Archives and Records Administration

Code of Federal Regulations (CFR)

In-House Databases

Current Contents on Diskette®

The Merck Index, 1996, on CD-ROM

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Appendix A: Units and Abbreviations

°C = degrees Celsius

µg/L = microgram(s) per liter

µg/m³ = microgram(s) per cubic meter

µg/mL = microgram(s) per milliliter

µM = micromolar

ADI = acceptable daily intake

bw = body weight

CIR = Cosmetic Ingredient Review

EPA = Environmental Protection Agency

ER = estrogen receptor

F = female(s)

FDA = Food and Drug Administration

g = gram(s)

g/mL = gram(s) per milliliter

GC = gas chromatography

GD = gestation day

GI = gastrointestinal

h = hour(s)

4-HBA = 4-hydroxybenzoic acid

HD = high dose

HPLC = high performance liquid chromatography

HSDB = Hazardous Substances Data Bank

i.m. = intramuscular(ly)

i.p. = intraperitoneal(ly)

i.v. = intravenous(ly)

JECFA = Joint FAO/WHO Expert Committee on Food Additives

kg = kilogram(s)

L = liter(s)

lb = pound(s)

LC = liquid chromatography

LC₅₀ = lethal concentration for 50% of test animals

LD₅₀ = lethal dose for 50% of test animals

LD = low dose

M = male(s)

MD = mid dose

mg/kg = milligram(s) per kilogram

mg/m³ = milligram(s) per cubic meter

mg/mL = milligram(s) per milliliter

min = minute(s)

mL/kg = milliliter(s) per kilogram

mm = millimeter(s)

mM = millimolar

mmol = millimole(s)

mmol/kg = millimoles per kilogram

mo = month(s)

mol = mole(s)

mol. wt. = molecular weight

NA = not applicable

NIOSH = National Institute for Occupational Safety and Health

NOEL = no observable effect level

n.p. = not provided

NTP = National Toxicology Program

OSHA = Occupational Safety and Health Administration

PND = postnatal day

ppb = parts per billion

ppm = parts per million

s.c. = subcutaneous(ly)

TSCA = Toxic Substances Control Act

wk = week(s)

yr = year(s)

Appendix B: Description of Search Strategy and Results

Butylparaben (CASRN 94-26-8; ILS CODE X0050)

Nomination

Recently, attention has been drawn to the endocrine disruptive properties of parabens (esters of 4-hydroxybenzoic acid [4-HBA], also known as alkyl *p*-hydroxybenzoates) and their effect on estrogen-sensitive tissues. Parabens may induce cell proliferation and, potentially, neoplasia in these types of tissues. The parabens with longer-chain or otherwise bulkier alkyl groups (e.g., benzyl [phenylmethyl]) are more lipophilic/hydrophobic and more potent in binding estrogen receptors (ERs). Butylparaben, an example of a lipophilic paraben, was nominated for consideration in this review.

Search Strategy

A preliminary PubMed (free MEDLINE) search was done on January 23, 2004, using the search statement: butylparaben OR "butyl paraben" OR (butyl AND paraben*) (95). About 60 records were definitely on butylparaben, but butyl often was a component of a different compound name in the remainder. When these 95 studies were evaluated for suitability for the report along with 17 more PubMed records collected from miscellaneous searches, about 40 were rated suitable and 12, possibly suitable. Nineteen abstracts in English could be used for papers written in Japanese and Chinese; 30 were on other parabens, and 11 were either rated unsuitable or were "false drops." A number of keywords were extracted from the studies to be used in the fee-based searches.

Records for butylparaben, several other parabens, and some salts were retrieved from the Registry file on STN International on January 28, 2004. Few records were on butylparaben salts in the CAPLUS file, so they were not included in the searches. Simultaneous searches of files MEDLINE, CANCERLIT, NIOSHTIC, AGRICOLA, CABA, BIOTECHNO, EMBASE, ESBIODBASE, BIOSIS, IPA, TOXCENTER, and NTIS on STN International were done on January 28 and January 29, 2004. The keywords and strategy used are given in Attachment A. In the January 28 session, reviews and long-term toxicity studies were sought for 4-HBA, butylparaben, and several other esters (except for butylparaben, only the CASRNs were used). In the January 29 session, the answers on butylparaben were kept in two groups. In Group 1, specific keywords developed from the PubMed search were used with numerous butylparaben synonyms and the CASRN. The rest of the butylparaben retrievals were in Group 2. The statistics from these sessions are marred by problems in execution of the search that led to printing of duplicates from the PubMed search. In some cases, these duplicates were retained in the search package in preference to the MEDLINE records from PubMed because of better indexing or because the STN International record was shorter (effort was taken during post processing to get as many records as possible on one page each). Tallies of numbers of records retrieved by database are given in Attachment A.

An EMIC search on February 5, 2004, found two or three references that had not been noticed in earlier searches. A CIS search on February 13, 2004, resulted in no references.

Additional information was sought in materials available on the Internet using specific government agency and organization web sites as well as the Google search engine. At least ten journal articles and numerous other potentially useful materials were retrieved from January 23 to February 16. The most fruitful searches with the Google search engine specified butylparaben or synonyms and required the pdf format. Another important web site was www.inchem.org, which allows searches of IARC and WHO documents. The URLs of potentially useful materials and the dates retrieved may be found in Attachment B.

No information on butylparaben was found in the U.S. EPA OPPT Inventory Update Rule database. [The CASRNs of several parabens and paraben salts were checked in the November 1997 OPPT Inventory. Only 4-HBA and methylparaben were listed.]

Search Results

Most of the parabens in the database records have been identified in the top right corner or in the header of each page. While ILS has given the butylparaben assignment the code X0050, the individual parabens have been given mnemonic codes in which the digit corresponds to the number of carbons in the alkyl or arylalkyl ester moiety and the zeroes have usually been dropped. An "i" was appended for isopropyl, isobutyl, and 2-ethylhexyl esters, and Bz was added to X57 for the benzyl ester. These codes were also used in tabulations of several of the papers to identify the parabens included in each study as well as could be identified from its full database record. Copies of the database records and first (or a few) pages of other materials on butylparaben have been grouped by report topic and are described in the following summaries. [Note: Except for the reviews, which are discussed first, the topics are generally organized in the following discussion in the order in which they would appear in the ILS toxicology review.] In addition, the package contains materials collected on 4-HBA and several other parabens that did not mention butylparaben. For the most part, these are not described in the summaries here. They may, however, be useful for report preparation, especially for the structure-activity relationships.

Authoritative Reviews (Subject Code 05)

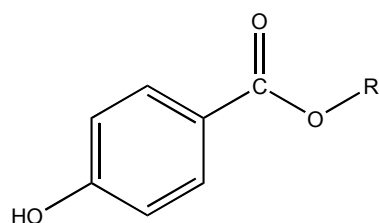
Several safety evaluations of parabens by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) are included in this group.

Other Reviews (11)

Reviews on butylparaben include BIBRA (1989d) (6 pages), Elder (1984) (cosmetic safety assessment of methyl-, ethyl-, propyl-, and butylparabens) (63 pages); and the HSDB (2003) profile. At its panel meeting in November 2002, the Cosmetic Ingredient Review (CIR) announced that the parabens in the 1984 review were likely to be re-reviewed in 2003. No further information about the re-review as located.

Chemical Identification (13a)

This group includes the Registry records for some parabens and butylparaben salts, the ChemIDplus record for butylparaben, and monographs from the 1996 Merck Index (Budavari, 1996; CD-ROM).



R = CH₂CH₂CH₂CH₃ for butylparaben
 CH₃ for methylparaben
 CH₂CH₃ for ethylparaben
 (CH₂)₂CH₃ for *n*-propylparaben
 (CH₂)₆CH₃ for heptylparaben
 CH₂Ph for benzylparaben
 H for paraben acid, also called 4-hydroxybenzoic acid

Chemical-Physical Properties (13b)

The CIR Panel report summarizes physical-chemical properties for butylparaben and other parabens in Table 1 of their review. Additionally, data regarding the solubility and reactivity of parabens are presented in the publication (CIR Panel, 1984). Experimental properties (i.e., melting points) and calculated properties (e.g., boiling point and K_{OC}) are reported in the Registry files for each paraben. Physico-chemical descriptors were also calculated by Cronin et al. (2002). Other data, including the aggregate formation of the parabens in aqueous solutions and photochemical decomposition of the compounds, have been reported (Fukahori et al., 1996a; Giordano et al., 1999; Hensel et al., 1995; Ishizaki et al., 1978; Pongcharoenkiat et al., 2002).

Analytical Methods (13c)

A comprehensive search was not conducted for analytical methods for parabens. The publications compiled here represent search results from the biomedical databases. About half of the publications summarized in the table used high performance liquid chromatography (HPLC) to determine butylparaben and other parabens in pharmaceuticals (e.g., Akhtar et al., 1996), foods (e.g., Ali, 1985), and cosmetics (e.g., Labat et al., 2000). HPLC is also used for monitoring parabens in air (OSHA, 2003 [method for air sampling propylparaben]). Gas chromatography (GC)/mass spectrometry (MS) is applicable to determination of parabens in environmental media (e.g., Grimes et al., 2003), in human plasma (e.g., Kigasawa et al., 1988), and in beverages and aqueous sauces (Ochiai et al., 2002). Newer methods include capillary electrochromatography for pharmaceuticals and cosmetics (e.g., DeRossi and Desiderio, 2002), micellar/microemulsion electrokinetic chromatography (MEKC/MEEKC) for pharmaceuticals (e.g., Driouch et al., 2000; Mahuzier et al., 2001), and capillary zone electrophoresis for cosmetics (e.g., Wang and Chang, 1998).

Commercial Availability (01a,b)

Butylparaben is commercially available from Ashland Distribution Company, Clariant LSM (America) Inc., J.T. Baker, Mutchler Inc., Penta Manufacturing Co., and R.S.A. Corporation (Chemyclopedia, 2004). It is also available as a NIPA Product, both as butylparaben and as the sodium salt (Clariant, 2002a,b).

Production Processes (01d)

Parabens are prepared by an acid-catalyzed esterification of 4-hydroxybenzoic acid with the appropriate alcohol. (Specific information is still being sought.)

Production and Import Volumes (01c)

No data were available for butylparaben.

Uses (01f)

The CIR Panel report summarizes the uses of butylparaben [and the other parabens] in cosmetics (as a preservative), foods (as an additive), and drug formulations (as a preservative) (CIR Panel, 1984). Parabens are the most widely used preservatives in cosmetics and topical medicines, including allergenic extracts used in scratch and intracutaneous testing, in injectable corticosteroid medicaments, and in local anesthetic solutions (Fisher, 1975; Menne and Hjorth, 1988; Rastogi et al., 1995). They are also added to solutions (e.g., commercially prepared low-ionic strength saline (LISS) solutions and beer) to retard microbial growth (Doron et al., 2001; Judd et al., 2001; Parish and Carroll, 1988; Raducan et al., 1994).

Environmental Releases, Occurrence, and Fate (04)

Surveys of chemicals in the environment and wildlife in Japan included butylparaben and several other parabens. Parabens were not found in water or sediment, but ethylparaben was found in fish at one site (Ministry of the Environment, Japan, 2003). Parabens were included in a survey of endocrine-disrupting chemicals in indoor air and dust (Rudel et al., 2003).

Exposure Potential (02)

The greatest exposure potential to parabens comes from foods, pharmaceuticals, cosmetics, and personal care products. The latter provide the greatest potential for exposure by the general public. The Household Products Database (2001) lists 17 cosmetic and personal care products for butylparaben, about 170 for methylparaben, 23 for ethylparaben, about 200 for propylparaben, 4 for isopropylparaben, 2 for isobutylparaben, and none for heptylparaben or 2-ethylhexylparaben. No underarm cosmetics, which have been suggested as an exposure route that explains the occurrence of parabens in human breast tumors, are listed although the shaving cream and gels could be used under the arms. The butylparaben-containing products include eye and face makeup, makeup removers (e.g., cold cream), hair care products, and aftershave and cleanser preparations for men. Baby lotions, creams, and other baby care products from Gerber, Playtex, and Johnson & Johnson were identified as containing parabens in an article recommending a paraben-free line of "organic" cosmetics and baby products (Doctors' Prescription for Healthy Living, October 2003).

The title of a recent article (van Tongeren et al., 2002) implied that links have been made between maternal job exposures and hypospadias in their male babies. However, parabens were lumped together with butylated hydroxyanisole (BHA), phytoestrogens, and synthetic steroids. Of the occupations listed with possible exposure to parabens, the most likely were hairdressers and food processors. However, hairdressers might also be exposed to phytoestrogens in hair products with herbal additives and food processors may be exposed to BHA.

Rudel et al. (2003) sampled indoor air and dust in 120 U.S. homes. Methyl-, ethyl-, and butylparabens were among the endocrine disrupting compounds looked for when the samples were analyzed. No information on the paraben concentrations was given in the database abstract. [Pesticides containing parabens might contribute to the indoor load.]

Regulations (24)

Parabens (methyl- through butyl-, isoamyl, 2-ethylhexyl, heptyl, and benzyl) are regulated by the U.S. Environmental Protection Agency (U.S. EPA) under the Toxic Substances Control Act (TSCA). Only one U.S. Food and Drug Administration (FDA) regulation includes butylparaben:

21 CFR 172.515. Methyl-, propyl-, and butylparabens are permitted as synthetic flavoring substances and adjuvants and are to be used in the minimum quantity required to produce their intended effect.

Toxicological Data

General Toxicology

In 1984, the CIR Panel published a report on parabens (methyl-, ethyl-, propyl-, and butylparaben) summarizing published data during the period 1920 to 1982 and unpublished data submitted to them. The panel made the following conclusions regarding the toxicity of parabens:

- acute toxicity studies in animals indicate that they are practically nontoxic by various routes;
- subchronic and chronic oral studies indicate that they are practically nontoxic;
- all animal sensitization tests indicate that they are nonsensitizing;
- mutagenicity studies (Ames test, dominant lethal assay, host-mediated assay, and cytogenic assays) indicate that they are nonmutagenic;
- human studies indicate that they are practically nonirritating and nonsensitizing;
- carcinogenicity studies indicate that methyl- and propylparaben are noncarcinogenic;
- methyl- and ethylparaben were nonteratogenic in rabbits, rats, mice, and/or hamsters; and
- photocontact sensitization and phototoxicity tests on product formulations containing 0.1-0.8% methyl-, propyl-, and/or butylparaben indicated no significant photoreactivity.

Literature results from the search are described below.

Human Data (18)

The sensitization potential of paraben esters is low (Menne and Hjorth, 1988). Probable cases of systemic contact dermatitis from parabens have been reported but are relatively uncommon (Mochida and Sugai, 1991; Mowad, 2000; Okuda et al., 1992; Roed Petersen and Hjorth, 1976).

ADME (12)

Human *in vivo* studies:

Transdermal absorption of butylparaben in aqueous propylene glycol by human volunteers was studied by Hagedorn-Leweke and Lippold (1995). Buccal and oral mucous membrane absorption of butylparaben by human volunteers was studied by Kurosaki et al. (1997) and Rathbone (1991a,b). Absorption of methyl-, ethyl-, propyl-, and butylparabens was compared in Rathbone (1991b). [Methylparaben has been detected in cord blood and maternal milk (Makino, 2003).]

Animal *in vivo* studies:

Percutaneous absorption of butylparaben trapped in liposomes was studied in guinea pigs (Komatsu et al., 1986a). Yamashita et al. (1994,1995) studied *in vivo* dermal penetration in rats. Urinary excretion profiles indicated that *in vivo* butylparaben absorption was greater than indicated by *in vitro* studies. Although oleic acid enhanced penetration at low doses, it decreased penetration at the high dose. Parabens were rapidly absorbed through the nasal membranes of rats (Aikawa et al., 1998).

Human *in vitro* studies:

Enzyme kinetics were modeled for human catecholamine sulfating sulfotransferase. Quantitative structure-activity relationship (QSAR) models were developed (Sipilae et al., 2003).

Hydrophobic and electrostatic forces may mediate butylparaben and 4-HBA binding to human serum albumin (Otagiri and Perrin, 1977). Pease et al. (2003 abstract) studied methyl-, ethyl-, propyl-, butyl-, heptyl-, and octylparabens in human intestinal epithelial cells (Caco-2 cell line) [no database abstract].

The following studies concern absorption:

Model Membrane	Parabens Studied	Comments	Reference
Human epidermis in Franz cell diffusion expts. with receptor fluid at 23- 45 °C.	X51, X54	Transdermal flux from a saturated aqueous solution and epidermal retention increased with increasing temperature. More butylparaben than methylparaben was retained in the epidermis. Amounts increased with higher temperature while the transdermal flux of methylparaben was greater than that of butylparaben. At 37 °C, estimated epidermal diffusivity for methylparaben was $26.54 \pm 4.17 \times 10^{-4}$ cm/s compared to $1.52 \pm 0.22 \times 10^{-4}$ cm/s for butylparaben.	Akomeah et al. (2003 poster, 2004)
Human skin	X51-X54, X56, X58	Fluxes from cosmetic emulsions decreased with increasing chain length.	Dal Pozzo and Pastori (1996)
Human callus	X54	The higher the octanol-water partition coefficient, K_{ow} , the greater the binding to keratinized structures of the stratum corneum. Skin penetration was greater when the compound was highly soluble in the vehicle.	Hagedorn and Lippold (1998)
Human skin	X51-X54, X58Bz	No abstract in database record.	Hansen and Mollgaard (1990 abstr.)
Human skin	X54	No abstract in database record.	Komatsu and Kurihara (1985) (Japanese)
Human erythrocyte ghost membranes	X51-X54, 4-HBA	Absorption increased with higher lipophilicity.	Lee and Kim (1994)
Different human skin layers	X52-X54	Four carboxylesterases from skin, subcutaneous fat, and blood had different hydrolyzing capabilities for the parabens at pH 8.0. No hydrolysis at pH 5.0.	Lobemeier et al. (1996)

Animal *in vitro* studies:

The tabulated publications below studied absorption. In these studies, butylparaben usually exhibited low penetration, retention in the epidermis, and/or hydrolysis in the skin.

Model Membrane	Parabens Studied	Comments	Reference
Rat skin	X54, 4-HBA	Ninety-six percent of the penetrated amount of butylparaben was metabolized in the skin to 4-HBA.	Bando et al. (1997)
Rat skin	X53, X54	The relationships among lipophilicity, metabolic rate, and skin permeation was studied. Cutaneous metabolism in the viable layer is important for butylparaben.	Seko et al. (1999)
Guinea pig dorsal skin	X51-X54	Certain agents and vehicles altered permeability.	Kitagawa et al. (1997)
Guinea pig skin	X54	Effects of micellar trapping in the presence of nonionic surfactants was studied [no database abstract].	Komatsu and Kurihara (1985) (Japanese)

Model Membrane	Parabens Studied	Comments	Reference
Guinea pig dorsal skin	X54	Addition of Polysorbate 80 or polyethylene glycol 410 reduced penetration of butylparaben in water.	Komatsu and Suzuki (1979)
Guinea pig skin	X54	Penetration of butylparaben from liposomes was studied [no database abstract].	Komatsu et al. (1986b)
Guinea pig skin	X54	<i>d</i> -Limonene and oleic acid enhanced penetration.	Koyama et al. (1994)
Guinea pig skin	X54	Cyclodextrin complexation decreased percutaneous absorption.	Okamoto et al. (1986)
Guinea pig skin	X54	Skin penetration enhancers increased accumulation in skin.	Okamoto et al. (1991)
Guinea pig skin, two-layer	X54	1-Geranylazacycloheptan-2-one increased skin penetration from an aqueous vehicle.	Yamashita et al. (1993)
Rabbit corneas	X51-X56	The relationship between corneal permeability and lipophilicity was studied.	Lee et al. (1991)
Snake skin	X51-X54	Smaller lipophilic compounds penetrated the skin more readily.	Itoh et al. (1990)
"Idealized skin model membranes" [silastic and demeticone were indexed]	X51-X54	Effects of alcoholic solvents on skin permeation kinetics were studied.	Twist and Zatz (1986)

Wefers and Sies (1986) studied the role of glutathione during formation of reactive oxygen species *in vitro* [rats and butylparaben included in MESH terms; no database abstract].

Metabolism by Commensal Organisms

Pseudomonas aeruginosa and *Klebsiella pneumoniae*, bacteria that may be associated with skin and soft tissue infections, converted methyl-, ethyl-, propyl-, and butylparabens to 4-HBA (Nakamori et al., 1975 letter).

Acute Exposure (03)

In mice, the oral and intraperitoneal (i.p.) LD₅₀s were 13,200 and 230 mg/kg, respectively. It was a mild irritant when tested on the skin of guinea pigs. In the rat, butylparaben (504 mg/kg) for 12 weeks produced weight loss or decreased weight gain and death, while subcutaneous (s.c.) injection of butylparaben (1200 mg/kg) for three days intermittently caused uterine weight changes (RTECS, 2003). In mice, s.c. injection of butylparaben (dose n.p. in abstract) did not produce a convulsive effect (Tsuzi, 1956).

The acute toxicity of *p*-hydroxybenzoic acid esters has been investigated in dogs, rats, and mice. All the compounds were reported to have a low order of acute toxicity; the main effect was an acute myocardial depression accompanied by hypotension that was transient in nature (Matthews et al., 1956).

Short-term and Subchronic Exposure (06a)

In rats, butylparaben was observed to affect the perfundic region of the forestomach epithelium adjacent to the fundic mucosa (Rodrigues et al., 1986). In another rat study, the enhancing effect of various hepatocarcinogens, including butylparaben, on the induction of preneoplastic

glutathione S-transferase placental form-positive foci was investigated (Ito et al., 1988). [Results were not specified in the available abstracts.]

Chronic Exposure (06b)

The chronic toxicity of *p*-hydroxybenzoic acid esters has been investigated in dogs, rats, and mice (Matthews et al., 1956). [Results were not specified in the available abstract.]

Synergistic/Antagonistic Effects (22)

In mice, oral administration of any combination of two sorbic acid, furylfuramide, ethylparaben, propylparaben, butylparaben, sodium dehydroacetate, benzyl alcohol, salicylic acid, and sodium propionate failed to produce synergism in acute toxicity (Sado, 1973). Earlier studies assessed the combined effect of the antiseptics sodium benzoate, butylparaben, and sodium dehydroacetate (Kurusu and Kawahara, 1968; Kurisu and Kobayashi, 1968).

Reproductive and Teratological Effects (10)

Subcutaneous injection of butylparaben (100 or 200 mg/kg) in pregnant rats from gestation day 6 to postnatal day 20 decreased the proportion of pups born alive and proportion of pups that survived up to the weaning period, body weights in females, and weights of testes, seminal vesicles, epididymides, and prostate glands in males. Additionally, the number of sperm in epididymis and the sperm motile activity were significantly lowered. The results suggested that maternal exposure to butylparaben has adverse effects on F1 male offspring (Che et al., 2001; Kang and Lee, 2001 abstr.; Kang et al., 2002b). Exposure of postweaning male rats to butylparaben (0.01-1%) in the diet caused decreases in the cauda epididymal sperm reserve, daily sperm production in the testis, and in the secretion of testosterone (Oishi, 2001). Neonatal administration of parabens (2 mg/kg) in rats had no effects on the development of the excurrent ducts of the testis (Fisher et al., 1999). In a sperm-tail hypoosmotic swelling test and supravital stain method, butylparaben killed spermatozoa, suggesting its potential use as a vaginal contraceptive (Song et al., 1991).

When four-week-old mice were administered butylparaben (0.01-1%) in the diet for ten weeks, the absolute and relative weights of the epididymides were significantly increased compared to controls at the high dose. A dose-dependent decrease in serum testosterone concentration and in round and elongated spermatid counts in stages VII-VIII seminiferous tubules were also observed (Oishi, 2002a).

Carcinogenicity (07)

Mice given butylparaben (0.15, 0.3, or 0.6%) in the diet for up to 102 weeks had tumors at various sites including the hematopoietic system, lung, and soft tissue. Tumor incidences, however, were not significantly different from those of the control group (Inai et al., 1985).

Genotoxicity (09)

Butylparaben was nonmutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 (Fujita and Hiraga, 1980; Fujita et al., 1985; Haresaku et al., 1985). Its mutagenicity in *Bacillus subtilis* strains H17A and M45T and *Escherichia coli* strain WP2 were also studied (Kojima and Hiraga, 1978; Morita et al., 1981). Additionally, butylparaben was found capable of inhibiting DNA, RNA, and protein formation in *E. coli* and *B. subtilis* (Eklund and Nes, 1991; Nes and Eklund, 1983).

Chromosome aberration tests were carried out on butylparaben and other parabens (e.g., methylparaben, ethylparaben, and isobutylparaben) using Chinese hamster fibroblast and human cell culture [note: results were not given in the available abstracts] (Ishidate et al., 1978, 1980, 1984; Kawachi et al., 1980; Odashima, 1980). In Chinese hamster CHO-K1 ovary cells, butylparaben was not mutagenic (Yoshida et al., 1978).

DNA damage was evaluated in tissue samples from the glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow of mice orally administered butylparaben (up to 0.5 + LD₅₀ or 2000 mg/kg) using the comet assay (Sasaki et al., 2002).

The mutagenicity of the reaction products from treating butylparaben with potassium nitrate or sodium nitrite while subjected to UV radiation has been studied (Ishizaki et al., 1978).

Other Biological Activities (14)

Endocrine Modulation (20)

Recent reviews on the estrogenicity of parabens include NICEATM (2002) [data from Blair et al. (2000) and Routledge et al. (2000)], Darbre (2001, 2003), Harvey (2003), and Harvey and Everett (2004 editorial). The latter four discuss the hypothesis that estrogenic chemicals, including parabens, in underarm deodorants and antiperspirants may contribute to the prevalence of cancer in the upper outer quadrant of affected breasts. This association of parabens with breast cancer was supported by recent determination of parabens in human breast tumors. Methylparaben (mean concentration 12.8 ± 2.2 ng/g tissue) represented 62% of the total parabens extracted from the tumors. Mean concentrations for propyl-, butyl-, and ethylparabens were 2.6, 2.3, and 2.0 ng/g tissue, respectively, while the mean for isobutylparaben was 0.9 ng/g (Darbre et al., 2004).

Studies in which the estrogenicity and other hormonal effects of butylparaben were evaluated are summarized in Attachment C. The 16 *in vitro* tests included rat uterine cytosol ERs, recombinant yeast ER and reporter gene assays, and assays with MCF7 human breast cancer cells. The four mammalian *in vivo* publications included rat and mouse uterotrophic assays and an experiment with neonatally exposed male rats.

Antimicrobial Activity

Several publications from the PubMed search are included in this group. The mechanisms of paraben antimicrobial activity against several species and of microbial resistance to parabens are topics of most of the papers here. These studies did not seem relevant to the ILS toxicity review. Mizuba and Sheikh (1987) showed that the highly water-soluble potassium salts of methyl-, ethyl-, propyl-, and butylparabens had a wider spectrum of activity than the less soluble parabens. Potassium butylparaben was more active at pH 4-6 than at pH 7-8.

Cytotoxicity (17), Effects on Enzymes (28), and/or Effects on Membranes (23)

Food additives, including parabens, induced cytotoxicity in animal cells (NIH/3T3 and Neuro-2a) within four hours of mixing (Yamashoji and Isshiki, 1998, 2002). Sparnins (1982) studied inhibition of NAD⁺-dependent dehydrogenase activity by several parabens and other aromatic compounds [no database abstract].

The severe cell injury induced in cultured rat hepatocytes by butylparaben was accompanied by increases in cellular glutathione (GSH) and protein-SH, but lipid peroxidation was not observed (Sugihara et al., 1997). Nakagawa and Moldeus (1998) and Nakagawa et al. (1999 [Japanese]) showed that the cytotoxicity of parabens in freshly isolated rat hepatocytes was due to the parent compound, not 4-HBA, and that adenosine triphosphate (ATP) depletion arose from impaired mitochondrial function. The longer the paraben alkyl group, the greater the cytotoxic potency. Other membrane effects are discussed in the next subsection.

Effects on Blood (30)

Butylparaben strongly inhibited agonist-induced thromboxane (TXB₂) synthesis irreversibly *in vitro*. The lipid-soluble preservatives tested, including butylparaben, inhibited or suppressed platelet function (Yamazaki et al., 1998). Zimmer et al. (1981) studied fluidization of human erythrocyte membranes induced by parabens and the "structure-functional aspects on membrane glucose transport" [no database abstract]. Ansell and Cadwallader (1964) reported that butylparaben and many other antibacterial compounds showed hemolytic activity against human and rabbit erythrocytes in normal saline, affecting their permeability and/or membrane integrity. Butylparaben was the most potent of the parabens tested with C1- to C4-alkyl ester groups in inhibiting release of lysosomal enzymes in mitogen-stimulated lymphocyte cultures.

Effects on the Nervous System (19)

Inoue et al. (1994, 1995, 1996) proposed that the minor irritation and stinging induced by parabens may be due to their significant effects on the voltage- and ligand-gated channels of neuronal cells. Cells used in the experiments were rat pheochromocytoma PC12 cells and cultured rat dorsal root ganglion neurons. The paraben concentrations needed were lower than those that induced cytotoxicity as determined by the MTT assay.

Structure-Activity Relationships (25)

Most of the publications on the biological activities of butylparaben include testing of other parabens, most frequently methylparaben, ethylparaben, and/or propylparaben. Often the studies note that the biological activity increases with the size or length of the ester alkyl group and hydrophobicity/lipophilicity. Large numbers of chemicals belonging to other chemical classes are included in many of the experimental endocrine disruptor studies. Several of these experimental studies analyze structure-activity relationships (SAR) as noted in the tables in the Endocrine Disruptor discussion above. The butylparaben articles on SAR that apparently analyzed experimental data from earlier publications are included in this set. Modeling/similarity analyses of the ability of butylparaben, other parabens, and members of several other chemical classes to bind the ER were published by Kaiser and Nicolescu (2001), Klopman and Chakravarti (2003), Saliner et al. (2003), Shi et al. (2001), and Tong et al. (2003).

A few of the SAR articles in this group discussed an endpoint other than those directly related to endocrine disruption. Sakai et al. (1994) analyzed the structural basis for the induction of preneoplastic glutathione-S-transferase (GST) positive foci in the liver. Ethyl- and butylparaben were among the 100 chemicals evaluated. Activity was associated with nonelectrophilic structures. Benigni et al. (1989, 1992) used an electrophilic reactivity parameter in their SAR analysis of 142 compounds, including methyl-, ethyl-, propyl-, and butylparabens, to predict rodent carcinogenicity.

We have also collected cosmetic and food additive safety evaluations for a number of individual parabens including: benzylparaben (Elder, 1986), propylparaben (Soni et al., 2001), methylparaben (Soni et al., 2002), and isobutyl- and isopropylparaben (Willis, 1995). If needed, the HSDB profiles for other parabens will be retrieved. Other reviews on parabens include the following: short BIBRA toxicity profiles on methylparaben (1989a), ethylparaben (1989b), and propylparaben (1989c); Darbre (2001, 2003) on the underarm cosmetics-breast cancer hypothesis; a 44-page review on phenols and parabens (Davidson, 1993); irritation and sensitivities to cosmetic ingredients (De Groot, 1998); food additives toxicological evaluation (JECFA, 1974); and a 10-page review on the safety of pharmaceutical excipients (Pifferi and Restani, 2003). In addition, we have identified carcinogenicity assays, *in vivo* endocrine disruptor assays, and reproductive toxicity studies for several individual parabens.

Attachment A. Search Session Strategies

Tallies of Numbers of Records Retrieved by Database

STN Int. Database	Jan. 28 Reviews & Long- Term	Jan. 29 Specific Keywords	Jan. 29 Other Butylparaben Records	Total
MEDLINE	25	46	24	95
NIOSHTIC	6	1	0	7
AGRICOLA	1	4	2	7
CABA	6	3	7	16
BIOTECHNO	11	9	10	30
EMBASE	201	57	152	410
ESBIOBASE	1	1	1	3
BIOSIS	6	20	29	55
IPA	1	1	13	15
TOXCENTER	69	79	186	334
NTIS	4	1	1	6
TOTAL	331	222	425	1257

History of STN International simultaneous search session for reviews begun at 15:04:31 on 28 January 2004 in files MEDLINE, CANCERLIT, NIOSHTIC, AGRICOLA, CABA, BIOTECHNO, EMBASE, ESBIOBASE, BIOSIS, IPA, TOXCENTER, and NTIS

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L1      689 S 94-26-8
L2      2714 S 99-76-3
L3      3887 S 99-96-7
L4      1579 S 94-13-3
L5      108 S 94-18-8
L6      744 S 120-47-8
L7      48 S 4191-73-5
L8      73 S 4247-02-3
L9      16 S 5153-25-3
L10     28 S 1085-12-7
L11     3470 S L2 OR L4 OR L5 OR L6 OR L7 OR L8 OR L9 OR L10
        SAVE X50SRN/A L11
L12     28 S (L1 OR BUTYLPARABEN) AND (REVIEW? OR REVIEW/DT)
        SET DUPORDER FILE
L13     22 DUP REM L12 (6 DUPLICATES REMOVED)
L14     22 SORT L13 1-22 TI
        SAVE L14 X54REVU/A
L15     7398 S (4 OR P) (W)HYDROXYBENZOIC (W)ACID OR L3
L16     7392 S L15 NOT L12
L17     203 S L16 AND (REVIEW? OR REVIEW/DT)
L18     173 DUP REM L17 (30 DUPLICATES REMOVED)
L19     173 SORT L18 1-173 TI
        SAVE L19 X50ACIDREVU/A
L20     2584 S L11 NOT (L1 OR L15)
L21     71 S L20 AND (REVIEW? OR REVIEW/DT)
L22     60 DUP REM L21 (11 DUPLICATES REMOVED)
L23     60 SORT L22 1-60 TI
        SAVE L23 X50SREVU/A
L24     502 S (L15 OR L11) AND (CHRONIC OR SUBCHRONIC OR WEEK? OR MONTH?
        OR 90 (w)DAY)
L25     351 DUP REM L24 (151 DUPLICATES REMOVED)
L26     134 S (L15 OR L11) AND (CHRONIC OR SUBCHRONIC OR 90 (W)DAY)
L27     106 S L26 NOT (L17 OR L12 OR L21)
L28     76 DUP REM L27 (30 DUPLICATES REMOVED)
L29     76 SORT L28 1-76 TI
        SAVE L29 X50SLONGTERM/A

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History of STN International simultaneous search session for butylparaben-specific information begun at 14:54:34 on 29 January 2004 in files MEDLINE, CANCERLIT, NIOSHTIC, AGRICOLA, CABA, BIOTECHNO, EMBASE, ESBIODBASE, BIOSIS, IPA, TOXCENTER, and NTIS

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L1          689 S 94-26-8
L2          26 S BENZOIC(W)ACID(W) (4 OR P) (W)HYDROXY(W)BUTYL(W)ESTER
L3          221 S (N(W)BUTYL OR BUTYL) (W) (4 OR P) (W)HYDROXYBENZOATE
L4          1 S BUTYL (W)PARAHYDROXYBENZOATE
L5          0 S BUTYL(W)PARAOXYBENZOATE
L6          0 S 4(W)BUTOXYCARBONYL(W)PHENOL
L7          9 S (4 OR P) (W)HYDROXYBENZOIC(W)ACID(W)BUTYL(W)ESTER
L8          0 S ASEPTOFORM(W)BUTYL
L9          1 S BUTOBEN
L10         0 S BUTYL(W)(BUTEX OR CHEMOSEPT OR PARASEPT OR TEGOSEPT)
L11         2 S NIPABUTYL OR PRESERVAL(W)B OR SOLBROL(W)B OR TEGOSEPT(W) (B OR BUTYL)
L12         256 S L2 OR L3 OR L4 OR L7 OR L9 OR L11
L13         317 S BUTYLPARABEN
L14         398 S BUTYL PARABEN
L15         398 S BUTYL(W)PARABEN
L16         861 S L1 OR L13 OR L14
L17         962 S L12 OR L16
L18         101 S L12 NOT L16
           SAVE L17 X54NAMES&RN/Q X54NAMESRN/Q
L19         28 S L17 AND (REVIEW? OR REVIEW/DT)
           SET DUPORDER FILE
L20         22 DUP REM L19 (6 DUPLICATES REMOVED)
L21         6 S L20 AND (2000-2004)/PY
L22         6 SORT L21 1-6 TI
L23         16 S L20 NOT L21
L24         14 S L23 AND ENGLISH/LA
L25        0 S L23 AND ENG/LA
L26         14 SORT L24 1-14 TI
L27         103 S L17 AND (ESTROGEN? OR OESTROGEN? OR ERALPHA OR ERBETA OR ER
           OR HER OR ERE(W) CAT)
L28         94 S L17 AND (RECEPTOR? OR REPORTER?)
L29         5 S L17 AND (ESCREEN OR E(W)SCREEN)
L30         0 S L17 AND (CV1 OR CV(W)1)
L31         33 S L17 AND (MCF7 OR MCF(W)7)
L32         7 S L17 AND (XENOESTROGEN? OR XENOOESTROGEN?)
L33         7 S L17 AND (XENOESTROGEN? OR XENOOESTROGEN?)
L34         26 S L17 AND ((GENE OR GENES) (6A)EXPRESS?)
L35         29 S L17 AND ((GENE OR GENES) (10A)EXPRESS?)
L36         29 S L17 AND (GENE OR GENES) AND EXPRESS?
L37         8 S L17 AND (ANTIESTROGEN? OR ANTIOESTROGEN?)
L38         71 S L17 AND (UTERO? OR UTERINE OR UTERUS OR VAGINA? OR INTERUTERINE)
L39         9 S L17 AND (PREGNAN? OR PUBERTY OR LORDOSIS)
L40         0 S L17 AND HERSHBERGER
L41         196 S L17 AND (ENZYM? OR HUMAN)
L42         36 S L17 AND (PENETRAT?(6A) (PERCUTANEOUS? OR SKIN))
L43         145 S L17 AND (METAB? OR HYDROLY?)
L44         52 S L17 AND PHARMACOKINETIC?
L45         6 S L17 AND TOXICOKINETIC?
L46         6 S L17 AND (PHARMACODYNAMIC? OR TOXICODYNAMIC?)
L47         11 S L45 OR L46
L48         13 S L17 AND (ZR(W)75(W)1 OR MDA(W)MB(W)231)
L49         10 S L17 AND MITOCHONDRIA?
L50         65 S L17 AND (CYTOTOX? OR PROLIFERAT?)
L51         55 S L17 AND (ANDROGEN? OR TESTES OR TESTIS OR SPERM? OR EPIDIDYM)
L52         46 S L17 AND (SEMINAL OR PREPUTIAL OR TESTOSTERONE)
L53         11 S L17 AND (CHRONIC OR SUBCHRONIC)
L54         11 S L53 NOT (REVU OR REVU/DT)
L55         9 S L53 NOT (REVIEW OR REVIEW/DT)
L56         65 S L17 AND (TUMOR? OR TUMOUR? OR CARCINO? OR CANCER?)
L57         422 S L27 OR L28 OR L29 OR L31 OR L32 OR L33 OR L34 OR L37
L58        540 S L17 NOT L57
L59         222 DUP REM L57 (200 DUPLICATES REMOVED)
L60         222 SORT L59 1-222 TI
           SAVE L60 X54GROUP1/A
L61         425 DUP REM L58 (115 DUPLICATES REMOVED)
L62         425 SORT L61 1-425 TI
           SAVE L61 X54GROUP2/A [Unsorted answer set was saved and printed.]

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Attachment B. URLs for Internet Search Results for Butylparaben (ILS Code X0050)

January 23, 2004 [Google search engine; search terms butylparaben and then butyl paraben (limited to pdf format); combined butylparaben with (1) estrogens OR oestrogens and then with (2) estrogenic OR oestrogenic]

KANG et al. (2002)

http://www.ksvs.or.kr/pdf/3_1/2.pdf

Harvey and Everett (2004 editorial)

http://www.vidyya.com/6pdfs/fulltext_ID=106600318&PLACEBO=IE.pdf

370 pp.

<http://iccvam.niehs.nih.gov/methods/endodocs/final/erbnbrd/erbdappx/D1.pdf>

ICCVAM estrogen receptor binding report

<http://iccvam.niehs.nih.gov/methods/endodocs/final/erbnbrd/ERBd034504.pdf>

94 pp.

<http://iccvam.niehs.nih.gov/methods/endodocs/final/panelrpt.pdf>

MILLER et al. (2001)

<http://ehpnet1.niehs.nih.gov/members/2001/109p133-138miller/miller.pdf>

http://www.cdc.gov/exposurereport/pdf/nominated_chemicals.pdf

CIR (2003) Mentions possible re-review of parabens in 2003

<http://www.dep-facts.com/upload/documents/document2.pdf>

Resolution to request Avon to remove parabens from its products

<http://www.bcaction.org/PDF/ParabensResolution.pdf>

Recent Progress in Endocrine Disruptor Research, 45th Int. NIBB Conference, March 3-5, 2001, Okazaki, Japan

http://www.nibb.ac.jp/~cib-bioe/abstract_L.pdf

Parabens often formulated in estrogenic pharmaceuticals

<http://www.usp.org/pdf/drugInformation/mgEstrogensVI.pdf>

Macy et al. (2002)

<http://xnet.kp.org/permanentejournal/fall02/skintest.pdf>

Eriksson et al. (2003)

<http://www.wrc.org.za/publications/watersa/2003/april/1543.pdf>

USP paraben anal. stds. available

<http://www.ricerca.com/documents/USP%20Jan-Feb-2003.pdf>

Andersen et al. (2001) in Royal Danish School of Pharmacy Annual Report 2000-2001

http://www.dfh.dk/publikationer/annual_report/dfh_beretning.pdf

JECFA (2003) Approval for parabens at low concns. in food

<http://www.mvo.nl/voedselveiligheid/download/A10312ae.pdf>

Anonymous article in *Doctors' Prescription for Healthy Living*

<http://www.freedompressonline.com/DPHL/v7i10/710-p16-17.pdf>

Siren Sept. 2001

http://www.fbu.org.uk/regions/sections/nwc/siren/s0901/s0901_13.pdf

Akomeah et al. (2003 poster) [Note: An article was published in 2004.]

Royal Pharmaceutical Society of Great Britain, Biopharmaceutics Poster Session 2, 140th British Pharmaceutical Conference Monday 15 to Wednesday 17 September 2003 Harrogate [BPC Science Programme 2003]

<http://www.rpsgb.org.uk/pdfs/bpc03sciproc86-92.pdf> [pp. 86-92]

Women's Environmental Network (Dec. 2003)

http://www.wen.org.uk/cosmetics/reports/cosmetics_norefs.pdf

EUROCAT (2003)

European Surveillance of Congenital Anomalies, Special Report on Hypospadias in Europe

<http://www.eurocat.ulster.ac.uk/pdf/Hypospadias.pdf>

Darbre et al. (2004)

<http://www.mindfully.org/Pesticide/2004/Parabens-Breast-Tumours1jan04.htm>

Stehlin (1995) (orig. 1991)

<http://vm.cfsan.fda.gov/~dms/cos-safe.html>

Padilla-Zakour (1998)

[*Venture* (summer 1998) NY State Agric. Exp. Station, Cornell Univ.]

http://www.nysaes.cornell.edu/fst/fvc/Venture/venture3_chemical.html

Mallinkrodt (2003)

<http://www.jtbaker.com/msds/englishhtml/b7270.htm> Butylparaben MSDS

Danish Environmental Protection Agency (2001)

Environmental and Health Assessment of Substances in Household Detergents and Cosmetic Detergent Products 8. Preservatives 8.2 Parabens

http://www.mst.dk/udgiv/publications/2001/87-7944-596-9/html/kap08_eng.htm

January 30, 2004

Google engine searches on Cosmetic Ingredient Review, American College of Toxicology, and butylparaben.

Unreferenced summary of the toxic potential of parabens.

<http://livingnature.com/talkwithus/parabens.cfm>

February 2, 2004

Google search for "butyl 4 [OR p] hydroxybenzoate" limited to pdf format. Search at INCHEM web site for hydroxybenzoate.

Nishihara et al. (2000) ER binding study

[http://jhs.pharm.or.jp/46\(4\)/46\(4\)p282.pdf](http://jhs.pharm.or.jp/46(4)/46(4)p282.pdf)

Commission of the European Communities (1998)

http://pharmacos.eudra.org/F2/register/regpdf/1998_07_17-1570.pdf

Commission of the European Communities (1999)

http://pharmacos.eudra.org/F2/register/regpdf/1999_03_04-0508%20Consolidated.pdf

Ministry of the Environment, Japan (2003)

<http://www.env.go.jp/chemi/en/kurohon/http2001e/01-tables/table2-2.pdf>

<http://www.env.go.jp/chemi/en/kurohon/http2001e/01-tables/table2-1.pdf>

Clariant parabens (esters)

[http://www.safewing.de/fun/e2wtools.nsf/lookupDownloads/NIPA_ESTERS.pdf/\\$FILE/NIPA_ESTERS.pdf](http://www.safewing.de/fun/e2wtools.nsf/lookupDownloads/NIPA_ESTERS.pdf/$FILE/NIPA_ESTERS.pdf)

Clariant paraben salts

[http://fun.clariant.com/fun/e2wtools.nsf/lookupDownloads/PARABENS.pdf/\\$FILE/PARABENS.pdf](http://fun.clariant.com/fun/e2wtools.nsf/lookupDownloads/PARABENS.pdf/$FILE/PARABENS.pdf)

[http://www.safewing.de/fun/e2wtools.nsf/lookupDownloads/Sodium_PARABENS.pdf/\\$FILE/Sodium_PARABENS.pdf](http://www.safewing.de/fun/e2wtools.nsf/lookupDownloads/Sodium_PARABENS.pdf/$FILE/Sodium_PARABENS.pdf)

JECFA (2004) Call for data on butylparaben

<http://www.who.int/pcs/jecfa/call63.pdf>

Okayama et al. (1998)

<http://www.jpsfc.com/kaisi-jsfc/PDF/5/5-2-9.pdf>

KANG et al. (2002b) ?

[http://plaza.snu.ac.kr/~kangpub/paraben\(JVMS\).pdf](http://plaza.snu.ac.kr/~kangpub/paraben(JVMS).pdf)

JECFA (1974)

<http://www.inchem.org/documents/jecfa/jecmono/v05je13.htm>

JECFA (2001)

http://www.inchem.org/documents/jecfa/jeceval/jec_219.htm

JECFA (1967)

<http://www.inchem.org/documents/jecfa/jecmono/40abcj03.htm>

OECD SIDS (1999) 4-Hydroxybenzoic acid

http://www.chem.unep.ch/irptc/sids/volume6/part1/v6_hydroxybenzoic.pdf

OECD SIDS/IRPTC Legal file excerpts

http://www.chem.unep.ch/irptc/sids/volume6/part1/99_96_7IRPTC.pdf

Trumpower et al. (1972)

<http://www.jbc.org/cgi/reprint/247/8/2499>

February 4, 2004

Miscellaneous Google searches.

European Commission (April 2003)

http://europa.eu.int/comm/food/fs/sc/scf/out182_en.pdf

February 10, 2004

See methylparaben in pregnant women and neonates

<http://www.med.or.jp/english/pdf/jmaj/v46no03.pdf>

Parabens in the Japanese environment

<http://www.env.go.jp/en/topic/pops/Appendix/03-CIE/Summary2001.PDF>

van Tongeren et al. (2002) Hypospadias and job exposures to endocrine disruptors

<http://reo.nii.ac.jp/journal/HtmlIndicate/Contents/SUP0000003000/JOU0003000023/ISS0000014730/ART0000167507/ART0000167507.pdf>

JECFA (2002) WHO Tech. Report Ser. No. 909

<http://www.who.int/pcs/jecfa/trs909.pdf>

February 13, 2004

Institute of Food Technologists (2001) for FDA

<http://foodsci.rutgers.edu/schaffner/pdf%20files/Busta%20CRFSFS%202003.pdf>

Miller (1998) 4-HBA from toluene by fermentation

http://www.epa.gov/greenchemistry/docs/gc_conference_1997_1998_proceedings2.pdf

February 17, 2004

European Agency for the Evaluation of Medicinal Products (1996)

<http://www.emea.eu.int/pdfs/vet/mrls/005195en.pdf>

Attachment C: Studies of Endocrine Modulation

In Vitro Studies of Endocrine Modulation

Assay	Parabens Studied	SAR Discussed	Reference
Estrogen receptors (ERs) in uteri from ovariectomized Sprague-Dawley rats	X51-X54, X57, X57Bz, X58i	Structure-activity relationships (SAR) for 188 chemicals were discussed.	Blair et al. (2000)
Rat uterine cytosol ER competitive binding assay, the "gold standard for in vitro ER assays." Binding activity was detd. by concentration (concn.) needed to give 50% inhibition of tritiated estradiol (IC ₅₀). Relative binding affinity (RBA) was calculated by dividing the IC ₅₀ of estradiol by that of the competitor and multiplying by 100.	X51-X54, X57, X58i Activity: X58i > X57 > X57Bz > X54 > X53 = X52 > X51	Yes. 230 chemicals studied (130 active). Authors found five distinguishing criteria essential for xenoestrogen activity. Phenols had the lowest mean RBA (0.0015).	Fang et al. (2001) (NCTR)
<ul style="list-style-type: none"> • Rat ER • Yeast ER 	X51-X54	Yes	Routledge et al. (1998)
Non-R1 receptor (ER α & ER β) binding assay	X51-X54, X53i, X54i, 4-HBA	Only parabens evaluated. Isobutylparaben was almost as potent as bisphenol A. Butylparaben was the next most potent.	Satoh et al. (2000) (Japanese)
Recombinant yeast expressing the human estrogen receptor (hER)	X54	Yes	Kang et al. (2002a)
Yeast two-hybrid assay to detect thyroid hormone receptor binding	X51-X54, X57Bz	Yes, but all parabens were negative in the assay.	Kitagawa et al. (2003)
Recombinant yeast estrogen assay	X51-X54, X57Bz	More potent compounds had unhindered phenolic group in para position and mol. wt. 140-250 Da	Miller et al. (2001)
Yeast two-hybrid assay	X51-X54, 4-HBA	517 chemicals. More potent compds. were phenols with hydrophobic moiety at para position without bulky ortho groups.	Nishihara et al. (2000)
<ul style="list-style-type: none"> • Recombinant yeast with hER (YER) or androgen receptor • E-Screen assay with MCF7 cells 	X54	Yes. Only testosterone showed androgenicity. Butylparaben showed a dose-response in the YER assay.	Park et al. (2000)
Recombinant yeast LacZ reporter gene assay	X52, X54, X56Ph, X57Bz, X58, X59	Size and other parameters evaluated.	Schultz et al. (2000)
Recombinant yeast LacZ reporter gene assay	X52, X54, X55i, X56Ph, X57Bz, X59	120 aromatic chemicals evaluated. Size and shape were compared to those of 17 β -estradiol	Schultz et al. (2002)
<ul style="list-style-type: none"> • MCF7 human breast cancer cells competitive binding to ERs • Increased expression of transfected ERE-CAT reporter gene and endogenous pS2 estrogen-regulated genes in MCF7 cells • Increased proliferation of MCF7 cells 	X51-X54	Only parabens compared.	Byford et al. (2002)
<ul style="list-style-type: none"> • MCF7 cell proliferation • ERα and progesterone receptor (PR) expression (gene & protein) • ERα and ERβ binding 	X51-X54, X53i, X54i	Parabens compared to 17 β -estradiol and diethylstilbestrol. No other compd. classes screened.	Okubo et al. (2001)
Evaluated as inhibitors of estrone sulfatase in treatment of hormone-dependent breast cancer by determining hydrophobicity (log P) and pKa.	X51-X59, 4-HBA (X55 probably isoamyl [isopentyl] according to CAS RN in indexing)	Only parabens evaluated. Inhibition activity increased with increasing log P. Hydrophobicity more important than pKa for butylparaben.	Owen et al. (2002)
Evaluated as a substrate for bovine adrenal estrogen sulfotransferase	X51-X54	Sulfation rates relative to estrone were 0.010, 0.017, 0.019, and 0.032, respectively.	Rozhin et al. (1974)

***In Vivo* Studies of Endocrine Modulation**

Assay	Parabens Studied	SAR Discussed	Reference
Alteration of the structure of the excurrent ducts of the testes of neonatally exposed rats from puberty to adulthood	X54 (2 mg/kg/day)	Yes	Fisher et al. (1999)
Mouse and rat uterotrophic assays	X51-X54, 4-HBA	Ethylparaben was inactive in mice even at 1000 mg/kg bw/day. Butylparaben was weakly estrogenic at 600 mg/kg/day.	Hossaini and Larsen (2000)
Uterotrophic assay with ovariectomized mice	X54	Yes	Park et al. (2000)
Yolk protein induction in sexually immature rainbow trout (<i>Oncorhynchus mykiss</i>)	X52-X54, 4-HBA	Only parabens evaluated. Butylparaben comparable to previously detd. estrogenicity of bisphenol A.	Pederson et al. (2000)
Rat uterotrophic assay	X51-X54	Yes. Parabens positive when given s.c. but not when given orally.	Routledge et al. (1998)