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Final Report on the Safety Assessment of Butyl Benzyl Phthalate

Butyl Benzyl Phthalate (BBP) is an aromatic ester that is used as a plasticizer at concentrations below 1.0%.

After oral and intravenous (iv) administration, BBP was rapidly excreted. The oral LD_{50} was: 2.3 g/kg for F344/N rats, and 4.2 - 6.2 g/kg for B6C3F1 mice. Rats and mice exposed to high concentrations of BBP lost weight, had testicular atrophy, hemorrhages, and hepatomegaly. No toxicity was reported in an oral subchronic toxicity study using dogs.

BBP was not a reproductive toxin to CD-1 and B6C3F1 mice; however, BBP caused dose-dependent microscopic degenerative changes in the testes of male F344 rats. BBP was nonmutagenic in the Ames Test, L5178Y TK mouse lymphoma mutagenesis assay, dominant lethal mutagenicity assay, and BALB/3T3 cell transformation assay. An increase in the incidence of mononuclear cell leukemia in female F344/N rats was not considered to be related to the exposure to high doses of BBP. BBP was not carcinogenic in studies using B6C3F1 and A/St mice.

Slight transient dermal and ocular irritation was produced by undiluted BBP in rabbits. In human dermal studies, BBP was not a significant irritant nor sensitizer.

On the basis of data presented in this report, it is concluded that butyl benzyl phthalate is a safe cosmetic ingredient in the present practices of use and concentration.

INTRODUCTION

Butyl Benzyl Phthalate and other phthalate esters are used widely as plasticizers. As such, they impart to the plastic flexibility and other characteristics necessary for its intended use. The plasticizers are not incorporated in the plastic polymer matrix, and may migrate from the plastic under certain conditions. Because of their widespread use, their high concentration in plastics, and their ability to migrate from the plastic, phthalate esters have become ubiquitous in the environment.

CHEMISTRY

Definition and Structure

Butyl Benzyl Phthalate (BBP) is an aromatic ester which conforms to the formula:[1]

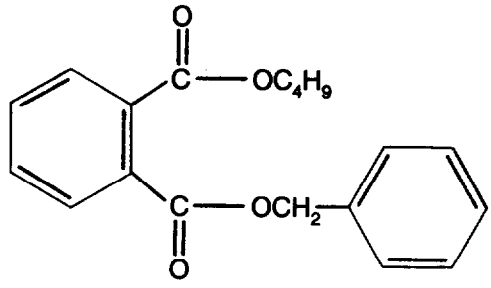
TABLE 1. BBP SPECTRAL DATA: ULTRAVIOLET/VISIBLE LIGHT[11]

λ max (nm)	$\epsilon \times 10^{-3}$
281s	1.17 ± 0.01 (δ)
275	1.30 ± 0.02 (δ)
269	1.20 ± 0.02 (δ)
264	1.16 ± 0.02 (δ)
257s	1.31 ± 0.02 (δ)

No absorbance between 350 and 800 nm (visible region).

Solvent: Methanol.

Instrument: Cary 118.



BBP (CAS No. 85-68-7) is also known as 1,2-benzenedicarboxylic acid, butyl phenylmethyl ester,[1] benzyl *n*-butyl phthalate,[2] and as the benzyl butyl ester of phthalic acid.[2]

BBP is a member of a class of chemicals known as phthalate esters.[3] Generally, the structure of phthalate esters consists of a benzenedicarboxylic acid ring with paired ester groups. Only those structures in the *ortho* configuration are termed phthalate esters; when the esters are in the *meta* and *para* configurations, the chemicals are referred to as isophthalates and terephthalates, respectively.[3,4]

PROPERTIES

BBP is a clear, oily liquid,[5,6] with a slight odor.[5] Its molecular weight is 312.4.[6] The boiling point of BBP has been reported to be 370°C[6,7] and 377°C (at 760 torr),[8] and the melting point has been reported as −35°C,[8] < −35°C[6] and −45°C.[7] The flashpoint for BBP is 390°C.[6] BBP has a vapor pressure of 1.9 mmHg at 200°C.[7] The solubility of BBP in water was 2.82 μg/ml and the octanol/water partition coefficient (K_{ow}) of BBP was 4.91.[9]

The absorption spectrum of BBP in methanol is presented in Table 1.

IMPURITIES

Benzyl chloride and benzal chloride were impurities in BBP used for the manufacture of surface and patterned films for polyvinyl floor tiles.[10] In the floor tile films,

benzyl chloride occurred at 0.03% in the surface film and at 0.004% in the patterned film. Benzal chloride occurred at 0.3% in surface film and at 0.04% in patterned film. Floor tiles containing BBP will release both benzyl and benzal chloride over a period of time. The authors noted that a sampling of various commercial sources of BBP used for vinyl flooring contained benzyl chloride in a concentration range of 0.01–0.05% and a concentration of benzal chloride in the range 0.001–0.4%. They also noted that benzyl chloride may contain up to 1% benzal chloride.

In a study of the chemical properties of phthalic acid esters (PAEs), it was noted that the PAE samples submitted by various manufacturers contained less than 1 % of non-PAE materials.[12]

No information was available regarding the specific impurities in BBP used for cosmetic products.

METHOD OF MANUFACTURE

The phthalate esters, including BBP, are manufactured commercially by the condensation of phthalic anhydride with the appropriate alcohols.[3] Esterification catalysts, including sulfuric acid and *p*-toluene sulfonic acid, are used to promote the reaction.[8] The reaction occurs in the presence of excess alcohol, which is later recovered and recycled. The reaction is carried out at a temperature of 150°C, and the mixture is agitated during the reaction process. The phthalate ester is purified by either vacuum distillation or with activated charcoal, or by a combination of both procedures. The product yield from this manufacturing method is > 90%.[8] In the United States, the majority of benzyl chloride produced is used as an intermediate in the production of BBP.[13]

Specifically, BBP is manufactured by the reaction of the monobutyl ester of phthalic acid with benzyl chloride.[10]

ANALYTICAL METHODS

BBP may be analyzed by gas chromatography with flame ionization detection,[9,12] by gas chromatography-mass spectrometry (GC/MS), and by high performance liquid chromatography (HPLC).[12]

BBP may be determined in drinking water by concentration of the organic material using sequential reverse osmosis, followed by extraction and analysis by gas chromatography/mass spectrometry.[14] BBP also may be determined in water by a method of adsorption/thermal desorption to a fused silica capillary gas chromatography column under whole column cryotapping conditions; recovery of BBP by this method was nearly 100%.[15] This procedure was considered useful for determining trace amounts of contaminants in water.

BBP may be determined in air samples by liquid chromatographic separation using a florisil adsorbent and 10% 2-propanol in hexane as the eluent followed by gas chromatography (GC) analysis.[16]

BBP can be determined in mixtures of semivolatile organic pollutants using gas chromatography/Fourier transform infrared spectroscopy (GC /FTIR) with wall-coated open tubular (WCOT) capillary columns.[17] Combining this technique with GC/MS

allows for better and faster identification of the components of complex mixtures of environmental pollutants.

During the use of gas-liquid chromatography (GLC) with SE-30 or OV-1 low polarity stationary phases and with selective detectors (flame ionization, electron capture, or phosphorus/nitrogen detection) for the determination of drugs in toxicologic analyses, it was found that the selectivity of the detectors provided increased sensitivity to traces of drugs, but that previously they also detected noninterfering nondrug substances. A major class of these previously undetected chemicals was the plasticizers, including BBP.[18]

USE

Cosmetic

United States

BBP is used in cosmetics as a plasticizer in hairsprays.[19] According to data supplied to the Food and Drug Administration (FDA) under the voluntary registration program, BBP is used in a total of two aerosol fixative hairsprays, at a concentration of <1.0% (Table 2).[20] Data supplied to the FDA in 1984 indicated that BBP was used in four aerosol hairspray formulations, with one formulation containing BBP at a concentration of 0.1-1.0%, and the remaining three formulations containing BBP at a concentration of 0.1%.[21]

The FDA cosmetic product formulation computer printout[20,21] is compiled through voluntary filing of data in accordance with Title 21 part 720.4 of the Code of Federal Regulations.[22] Ingredients are listed in preset concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration.

International

BBP is included on the Japanese Ministry of Health and Welfare list of traditional cosmetic ingredients.[23]

Table 2. Product Formulation Data for Butyl Benzyl Phthalate[20]

Product use category	*Total number of formulations in category*	*Total number containing ingredient*	*Number of product formulations within each concentration range <1.0%*
Hair sprays (aerosol fixatives)	261	2	2
1989 Totals		2	2

NONCOSMETIC

The major noncosmetic use of BBP is as a plasticizer in vinyl flooring tiles.[24,25] BBP is a preferred plasticizer for vinyl flooring materials because it resists staining and migration into (and subsequent softening of) flooring adhesives.[8] BBP is also used in synthetic leathers, acrylic caulking, plastic auto body filler, and tacky adhesives for medical devices.[25] It also has uses in other household products.[24] BBP has a minor use as a plasticizer for regenerated cellulose films used for packaging confectionary products.[26] BBP has been used as a plasticizer for cellulose plastics, polyvinyl acetates, polysulfides, and polyurethanes.[8]

There are also instances in which BBP is used for applications other than as a plasticizer.[8] These include uses as a carrier and dispersant for pesticides, colorants, solvents, catalysts, munitions, industrial oils, insect repellants, and perfumes.[8,27]

BBP is approved for use in adhesives, as a plasticizer in polymeric substances, as a component of paper and paperboard in contact with aqueous and fatty foods and with dry foods, in resinous and polymeric coatings in contact with foods, and in resinous and polymeric coatings for polyolefin films, and in crosslinked polyester resins.[28] These uses are subject to restrictions set down in the Code of Federal Regulations.[29–35]

GENERAL BIOLOGY

Because of evidence indicating that di-(2-ethylhexyl) phthalate (DEHP) is a hepatic carcinogen in both rats and mice, and because DEHP caused increased peroxisome production in the liver, a number of phthalate esters, including BBP, were tested for their potential to induce peroxisome proliferation in the livers of rats.[36] The chemicals were fed to groups of 5 male and 5 female Fischer 344 rats in the diet at concentrations of 0.6, 1.2, and 2.5%. The rats were fed the diet for 21 days; feed consumption was measured throughout the study to determine actual intake of the phthalate ester. At the end of the study, the rats were killed and the livers were removed and weighed. A small part of each liver was removed for microscopic study and the remainder was homogenized for the measurement of cyanide-insensitive palmitoyl-CoA oxidation and lauric acid 11- and 12-hydroxylases. Control groups consisted of untreated rats and rats receiving DEHP in the diet. There was a dose-dependent reduction in body weight of the male rats fed the BBP diet when compared with the negative control animals. This result also was observed in the female rats, but to a lesser extent. For the male rats fed 2.5% BBP, there was a moderate increase in peroxisome proliferation equal to that seen in male rats fed a diet containing 0.6% DEHP. The results were similar for female rats, except that the extent of peroxisome proliferation in the females was equivalent to that observed in female rats fed diets containing either 0.6% or 1.2% DEHP (moderate proliferation). BBP in the diet caused a dose-dependent increase in relative liver weights and in palmitoyl CoA oxidation activity in both male and female rats, while serum triglycerides were reduced in a dose-dependent manner in male rats but increased in female rats. The lauric acid 11- and 12-hydroxylase activity was sharply increased in a dose-dependent manner in male rats and only slightly increased in a dose-dependent manner in female rats. In the overall study, BBP had moderate activity when compared with the activity of DEHP.

NERVOUS SYSTEM EFFECTS

A study was undertaken to determine the toxicity of BBP to newborn rat cerebellum in tissue culture.[37] Cerebella from 8-day-old rats were cultured on collagen-coated plastic dishes in a culture medium of equal parts calf serum and Eagle's minimal essential medium (MEM). BBP was dissolved in calf serum at a known concentration and the serum was added to the culture medium in amounts necessary to achieve a particular concentration of the phthalate ester. The toxic effects of BBP were determined by measuring the following parameters: outgrowth of nerve fibers and fibroblasts in combination, outgrowth of nerve fibers, outgrowth of glial cells, and the number of explants that had outgrowth of nerve fibers. At a BBP concentration of 1.8×10^{-4} M, effects on outgrowth were not readily apparent, though a depressed outgrowth of nerve fibers and granules in the fibroblasts was evident in some explants. At a BBP concentration of 7.0×10^{-4} M, nerve fiber and glial cell development were depressed, granulations were observed, and the development of fibroblasts was depressed, with the formation of vacuoles and granules and a deformation of shape. BBP, at a concentration of 17.5×10^{-4} M, caused some explants to have no outgrowth of nerve fibers and glial cells and caused granules and vacuoles in fibroblasts. In some explants that did have outgrowth of nerve fibers, granulation and lytic change of the fibers were noted. BBP inhibited nervous system outgrowth and produced degenerative tissue changes. Of the three phthalate esters tested (BBP, *n*-butyl lauryl phthalate, and butyl phthalyl butyl glycolate), BBP was the most potent inhibitor of nervous system outgrowth.

Because phthalate esters are thought to be environmental pollutants, their effects on the heart rate of goldfish and carp were studied.[38] Three phthalate esters were tested (these were found in the liver of carp): BBP, DEHP, and di-*n*-butyl phthalate. Both di-*n*-butyl phthalate and BBP decreased the heart rate of goldfish when the fish were placed in sonicated suspensions of phthalate ester and water. The decrease in heart rate occurred at much lower concentrations for the di-*n*-butyl phthalate (12 ppm) than for BBP (200 ppm). It was possible that contamination of the BBP with a small amount of di-*n*-butyl phthalate (chromatographic analysis indicated 1.5% di-*n*-butyl phthalate and 0.3% other impurities in the BBP) was the cause of the reduced heart rates apparently caused by the BBP solution. The authors also suggested that the differences in water solubility of the tested compounds may have had an effect on the results of the study. Atropine reversed the effects of the phthalate esters, suggesting that the phthalate esters were acting on the nervous systems of the fish.

CYTOTOXICITY

The cytotoxicity of BBP to HeLa cells was determined using the metabolic inhibition test with microscopy of cells after 24 h of incubation (MIT-24 test system).[39] The test compound was suspended in the culture medium using microdiluters. The test cups containing the HeLa cells in Parker's medium 199, 5% calf serum, antibiotics, glucose, and test substances were sealed with liquid paraffin and mylar film. The initial pH change as determined by the color of phenol red in the culture medium was recorded, and the cultures were incubated at 37°C for 7 days. After 24 h, microscopy was used to determine cell viability; cultures with all round cells were considered completely inhibited and cultures containing fewer fusiform cells when compared with

control cultures were considered partially inhibited. After 7 days, inhibition was determined by the color change of phenol red. Violet cups were considered totally inhibited, red cups partially inhibited, and orange cups uninhibited. At 24 h, total inhibition was observed with 160 mg/ml BBP and partial inhibition was observed with 31 mg/ml BBP. At 7 days, BBP was completely inhibitory at a concentration of 31 mg/ml. The maximum concentration of BBP at which precipitation did not occur was 1.3 mg/ml. The authors noted the study was affected by several characteristics common to plasticizers: insolubility in water, resulting in a concentration in the aqueous phase that does not correspond to the total dose, and probably resulting in the suffocation of the test cells by the test substance; specific gravities between 0.9 and 1.2, which could affect the determination of precipitates and their influence on toxicity; and the breakdown of the plasticizer over time to acid and alcohol components, resulting in increased acidity of the culture medium over time, and making it difficult to determine the 7-day toxicity of the test compounds. The plasticizers in general have low toxicity, but this could be due to their insolubility in water. It was also noted that the compounds tend to become more toxic over time, due to degradation and hydrolysis. Some of the plasticizers were then tested in the presence of 10% calf serum to determine if the toxicity of the compounds or their metabolites would be increased. The 24 h toxicity of BBP remained the same and the 7-day toxicity of BBP was decreased, possibly due to protection of the HeLa cells by the serum or enhancement of their growth by the additional serum.

Porous pads were wet with 0.5 ml of a 50 mg/ml emulsion of BBP.[40] The pads were then placed on the agar of L 929 mouse fibroblast and chick embryo cell cultures. The cultures were observed for signs of cell death over a 48 h period. BBP in the concentration tested was not toxic to either L 929 mouse fibroblasts or to chick embryo cells.

The toxicity of BBP to mouse lymphoma L5178Y cells was determined with and without metabolic activation.[41] The BBP was tested at ten concentrations, ranging from 9.77 to 5000 nl/ml. Tests were performed in duplicate at each concentration, with and without metabolic activation. The mouse lymphoma cells were cultured in media containing the various concentrations of test material. Cells were counted 24 h after initiation of treatment, at which time those cultures containing greater than 4.0×10^5 cells/ml were diluted to 3.0×10^5 cells/ml, and cultures containing fewer than 4.0×10^5 cells/ml were not diluted. The cultures were returned to the incubator for an additional 24 h, and 48 h cell counts were then obtained. Without metabolic activation, BBP was nontoxic at concentrations up to 39.1 nl/ml, moderately toxic at 156 nl/ml,highly toxic from 625 nl/ml to 1250 nl/ml, and lethal at concentrations above 2500 nl/ml. In the absence of metabolic activation, the test material was soluble at concentrations up to 313 nl/ml and oily droplets were present in solution at the greater concentrations, including the greatest concentration (5000 nl/ml), which also contained a white precipitate. Metabolic activation slightly reduced the toxicity of the test compound. The 2500 and 5000 nl/ml concentrations were lethal, the 1250 nl/ml concentration was moderately toxic, and the remaining concentrations induced low to moderate toxicity. The BBP was soluble in the presence of metabolic activation at concentrations up to 1250 nl/ml. At greater concentrations, the test solutions contained globules of test material.

The cytotoxicity of BBP to BALB/3T3 cells was examined prior to a mutagenicity study using the BALB/3T3 cell transformation assay.[42] BBP was diluted in cell culture medium at concentrations ranging from 0.49 nl/ml to 8.0 µl/ml. Three culture sets were

used for each concentration of BBP, and six control cultures were used. BBP concentrations of 1.0 μl/ml and greater were lethal to the test cells. At a BBP concentration of 15.6 nl/ml, the cell survival rate was 71% and at concentrations ranging from 0.49 to 7.81 nl/ml, survival rate was greater than or equal to that of control cell colonies.

METABOLISM, DISTRIBUTION, AND EXCRETION

[^{14}C]BBP was administered orally at doses of 2, 20, 200, or 2000 mg/kg to male Fischer-344 rats.[25] The rats were housed individually in metabolism cages in which urine and feces were collected separately, and were provided with feed and water *ad libitum*. The BBP was administered in a solution of 1:4 ethanol/Emulphor EL-620, except for the greatest dose, which was administered undiluted. Urine and feces were collected daily and analyzed for radioactivity using direct liquid scintillation counting and digestion and oxidation followed by liquid scintillation counting, respectively. At the end of the study, the rats were necropsied and tissues were removed for evaluation of ^{14}C content; total tissue weight was also obtained. In a second part of the study, 20 mg/kg [^{14}C]BBP was administered intravenously (i.v.) via the tail vein to male Fischer-344 rats in a solution of water/ethanol/Emulphor. At 0.5, 1, 2, 4, 8, 12, and 24 h postadministration, rats were exsanguinated, and the brain, lungs, liver, kidneys, spleen, testes, small intestine, renal fat, thigh muscle, and abdominal skin were removed. Urine and intestinal contents also were collected. To determine the half-life in blood, blood samples were taken from rats 5, 10, 15, 20, and 30 minutes after administration of BBP via the saphenous vein. Rats dosed with BBP via the saphenous vein were analyzed for excretion of BBP in the bile. Bile was collected 10, 20, 30, 60, 120, 180, and 240 min after BBP administration and urine was also drained from the bladder at the end of this phase of the experiment.

After oral administration of [^{14}C]BBP, radioactivity was excreted rapidly. Within 24 h, 75–84% of the total dose was excreted, and within 4 days, 92% of the total dose had been excreted; of the total amount excreted, 75% was eliminated in the urine and 20% was eliminated in the feces at all except the highest dose. At the 2000 mg/kg dose, 72% of the total dose was recovered from the feces and 22% was recovered from the urine. Monophthalate (MP), MP-glucuronide, and unidentified metabolites were excreted in the urine. At the 200 mg/kg dose, the amount of free MP and the ratio of free MP to the MP-glucuronide was increased when compared with those values for the lower dosage groups. Because of this, and because elimination of the metabolites was altered at the highest dose, the i.v. studies were performed using 20 mg/kg BBP.

BBP was distributed to the tissues and eliminated rapidly after i.v. administration of 20 mg/kg BBP. MP was also rapidly formed and distributed in the tissues after i.v. administration of BBP. Elimination of both BBP and MP from the various tissues examined followed either monoexponential or biexponential decay curves, depending on the tissue and phthalate examined.

Four hours after the i.v. administration of BBP, 55% of the radioactivity had been excreted in the bile and 34% had been excreted in the urine. No BBP was found in the bile. However, 26% and 13% of the dose was excreted as monobutyl phthalate (MBuP) glucuronide and monobenzyl phthalate (MBeP) glucuronide, respectively; 1.1% and 0.9% as MBuP and MBeP, respectively; and 14% as unknown metabolites. The urine contained 15% MBuP glucuronide, 2% MBeP glucuronide, 1.8% MBuP, and 0.3% MBeP.

The major route of excretion of BBP was into the bile (indicating that BBP undergoes enterohepatic circulation) where the metabolites were then reabsorbed and excreted in the urine. At the greatest dose, the BBP metabolites were largely excreted in the feces; this probably was due to incomplete absorption of BBP or its metabolites during enterohepatic circulation. BBP is preferentially hydrolyzed to the monobutyl phthalate glucuronide rather than the monobenzyl phthalate glucuronide. The authors noted that MBuP caused testicular atrophy in rats, alterations in lipid metabolism, and mitochondrial toxicity, and that BBP may indirectly (through metabolism to MBuP) have the same effects. The increase in the ratio of the monophthalate to the monophthalate glucuronide at higher doses of BBP may indicate a saturation of the conjugation pathway. The half-life of BBP and its metabolites averaged 6 h; this is similar to the half-lives of both DEHP and di-*n*-butyl phthalate. Finally, though the phthalate esters are lipophilic compounds, the rapid metabolism precluded accumulation in the fat.

BBP is partially hydrolysed by intestinal and other esterases to monobutyl phthalate, monobenzyl phthalate, *n*-butanol, and benzyl alcohol, with the monobutyl phthalate being the major hydrolysis product.[43]

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

In range finding studies performed prior to a carcinogenicity bioassay, BBP in corn oil was administered by gavage to F344/N rats and B6C3F1 mice.[11] The estimated oral LD_{50} for BBP in F344/N rats was 2.33 g/kg, and for B6C3F1 mice the estimated LD_{50} was 6.16 g/kg and 4.17 g/kg for males and females, respectively.

In Sprague-Dawley rats, the oral LD_{50} of BBP was 20.4 g/kg,[44] practically nontoxic according to the classification of Hodge and Sterner.[45]

An oral dose of 4 g/kg was fatal to rats (strain not specified).[46] Death occurred 4–8 days after dosing. Weight loss, reduced activity, and leukocytosis were observed. Toxic splenitis, degenerative lesions of the central nervous system with congestive encephalopathy, myelin degeneration, and glial proliferation were observed upon microscopic examination.

Dermal

BBP was applied undiluted to the skin of groups of one or two New Zealand white rabbits in order to determine acute toxicity.[44] Death of 50% of the test animals was not obtained at a dose of 10,000 mg/kg.

Intraperitoneal

The intraperitoneal (i.p.) LD_{50} for BBP in Swiss Webster mice was 3.16 g/kg. This value was estimated by dosing four groups of mice with BBP at concentrations between 0.5 and 16 g/kg, with the doses spaced in geometric progression, increasing by a factor of two.[40]

Doses of 1.8 g/kg BBP were fatal when administered i.p. to rats (strain unspecified).[46] Generally, the injected material (25 plasticizers were tested) remained in the peritoneal cavity where it was surrounded by fibrous tissue.

Short-Term Toxicity

Oral

Prior to the start of a carcinogenicity bioassay, BBP was administered in the diet for 14 days to groups (10 animals of each species, evenly divided by gender) of F344/N rats and B6C3F1 mice.[11] The rats were fed diets that contained BBP at concentrations of 12,500, 25,000, 50,000 or 100,000 ppm; and the mice were fed diets containing BBP at concentrations of 600, 3100, 6300, 12,500, or 25,000 ppm. Untreated controls were included for each species. On day 15, all animals were fed the control diet and on day 16, the animals were killed for necropsy. No deaths occurred in rats during the study. Weight gain was reduced in rats of both sexes receiving BBP at dietary concentrations of 25,000 ppm and greater. The rats of the 100,000 ppm group lost weight and had thymic and testicular atrophy. Testicular atrophy was also noted in rats of the 50,000 ppm group. One male mouse died in each of the 3100 and 6300 ppm diet groups. All mice, except the males of the 1600 ppm group, lost weight when compared with the control mice; however, the weight loss was not dose-dependent. No treatment-related changes were noted at necropsy.

A series of short-term range finding studies was performed prior to beginning subchronic tests.[44] Of the five studies, which ranged in duration from 2 to 6 weeks, three were feeding studies and two were gavage studies. In the feeding studies, BBP was incorporated into the diet of groups of 5–10 male and female Sprague-Dawley rats and concentrations of BBP in the feed were adjusted to maintain dosages between 500 and 4000 mg/kg/day. In the gavage studies, BBP in corn oil was administered to groups of six male Sprague-Dawley or Wistar rats at dosages between 160 and 1600 mg/kg/day. Two of the test groups were maintained for observation at the termination of their respective studies in order to determine if the effects of BBP were reversible. The rats were necropsied at the time of death or at the termination of the study. The liver and testes of the rats in the gavage studies were evaluated histopathologically. Tissues from the central and peripheral nervous systems from Sprague-Dawley rats in the six-week study were also examined. Of the other rats which were necropsied, gross lesions, the liver, kidneys, gonads, adrenal glands, skeletal muscle from three locations, peripheral nerve, two sections of the spinal cord, and brain were examined (this included nearly all of the rats in one of the four-week studies).

Only in one study group were there any treatment-related deaths. In this group (4-week feed study), some rats died between the second and third weeks of the study and mortality occurred as follows: 2/5 at 1500 mg/kg, 8/10 at 2000 mg/kg, 7/10 at 3000 mg/kg, and 9/10 at 4000 mg/kg. Dose-related decreases in growth were noted in all of the study groups at doses above 1000 mg/kg. Feed consumption was greatly decreased for the dosage groups in the 4-week studies receiving more than 2000 mg/kg. Feed consumption of the rats receiving dosages of 1500 and 3000 mg/kg was reduced during the first 2 weeks of the 6-week study, but by the third week feed consumption was normal. The rats receiving doses of 2000–4000 mg/kg in the diet had stiffness of the hind legs when walking; this first appeared two weeks after the start of the study and affected mainly male rats. Bleeding around the nares occurred in the high-dose rats of both 4-week studies. Of the rats which died during the test, dehydration and blue discoloration and/or inflammation of the extremities were observed. Upon gross and microscopic examination, hemorrhages were observed in these rats. This was not observed in the high-dose rats of the 6-week study, though some rats also had stiffness of the hind limbs. The stiffness cleared within 1–2 days of removal of the rats from the

test diet.[44] At the end of the six-week treatment period, half of the rats were sent to a separate facility for neuropathologic examination.[47] The rats were examined for signs of neurologic dysfunction. The rats were then killed, the brain, spinal cord, and peripheral nerves were removed, and sections of each were prepared for examination by light microscopy. No significant changes were noted in the tissues of the peripheral nervous system. Pathologic changes occasionally were noted in the central nervous system tissue samples from some of the rats. Significant structural abnormalities were observed in the medulla oblongata of several rats, including both treated and control animals, and this sometimes was accompanied by other minor pathologic changes; but the types and occurrences of the abnormalities were not consistent with those observed with known neurotoxins. Under the conditions of the study, treatment with BBP did not result in neuropathologic responses consistent with the structural breakdown of nervous system tissues.[47]

In addition to hemorrhages, testicular atrophy also was observed in rats of the two gavage studies.[44] When the testicular tissues were evaluated histopathologically, the changes observed were dose-related. Minimal testicular lesions were observed in 1 of 6 Sprague-Dawley rats administered 480 mg/kg of BBP, whereas no testicular lesions were observed in Wistar rats given 480 mg/kg BBP or in any of the rats receiving equivalent amounts of BBP in the feeding studies. Of the rats receiving doses of BBP greater than 1500 mg/kg, testicular lesions were observed upon histopathologic evaluation. Upon microscopic examination of the male rats which died during one of the 4-week studies, hemorrhages were observed in the brain, spinal cord, testes, prostate, bladder, skeletal muscle, eyes, thymus, colon, pancreas, lymph nodes, and optic and peripheral nerves. After a 4-week recovery period, the 6 rats surviving the high-dose group were necropsied. No hemorrhages were observed upon microscopic examination. Testicular atrophy was observed in a few of the rats.

BBP was fed in the diet at concentrations of 0.625, 1.25, 2.5, or 5.0% to groups of 10 male F344 rats for 14 days.[43] An additional group of 10 rats served as untreated controls. The rats were weighed at the beginning of the study and every 3–4 days thereafter, including the last day of the study (day 15). Feed consumption was monitored for each box of two rats. Prior to necropsy, blood samples for the analysis of complete blood count, clotting time, and endocrine analysis were obtained from all of the rats, and the brain, liver, kidneys, spleen, thyroid gland, thymus, pituitary gland, testes, epididymis, prostate, seminal vesicles, and mesenteric lymph nodes were removed for microscopic examination. Also, both femurs from each rat were removed and preserved for analysis of bone marrow nucleated cells.

No deaths occurred during the study. The rats from the 2.5 and 5.0% dose group appeared weak and had reduced activity, and had significantly reduced weight gains. Feed consumption was slightly reduced in the 2.5% dose group and markedly reduced in the 5.0% dose group. Liver weights were significantly increased in the rats of the 0.625 and 1.25% groups, liver weights of the rats from the 2.5% group were comparable to those of the control group, and liver weights of the rats from the 5.0% group were significantly reduced when compared with control rats. Absolute kidney weights were also significantly increased in rats of the two lower dosage groups, while kidney and thymus weights were significantly reduced for the rats of the 2.5 and 5.0% groups. Significant increases in liver and kidney weights were noted for all dosage groups when the weights of these organs were expressed relative to body weight, while relative thymus weights for the 2.5 and 5.0% groups were significantly decreased. The authors noted that hepatomegaly may be an early indicator of BBP-induced effects in

rats. At microscopic examination, mild multifocal chronic hepatitis was observed in 50% of the rats of the 5.0% dose group and cortical lymphocytolysis of the thymus in 9 of 10 rats of this dosage group. Renal proximal tubular regeneration was observed in some of the rats of all treatment groups. The incidences of hepatitis and proximal tubular regeneration were small and did not appear dose-dependent, and, thus, a connection could not be made between ingestion of BBP and the hepatic and renal lesions. Focal thymic medullary hemorrhages of minimal severity were observed in a few animals of each dosage group, but the occurrence was not dose-dependent. BBP in the diet did not significantly effect blood cell counts. The plasma testosterone concentration was significantly decreased in rats of the 5.0% group. The 2.5% group also had a reduced plasma testosterone concentration, but the difference was not significant from control values. Plasma follicle-stimulating hormone (FSH) concentrations were increased significantly in a dose-dependent manner in rats of the 2.5 and 5.0% groups. Plasma luteinizing hormone (LH) concentrations also were significantly increased in rats of the 2.5% group; the concentration of this hormone in rats of the 5.0% group was also increased, but there were insufficient plasma sample volumes for detection of this hormone for all but three rats of that diet group. Prothrombin times were unaffected by the administration of BBP in the diet. Partial thromboplastin times were not changed significantly, although there were increases in mean values and variability in the rats fed 2.5 and 5.0% diets. The authors commented that these results were inconsistent with speculation that treatment with BBP causes internal bleeding in rats, but they also noted that bleeding may not be evident in a short-term study. BBP at concentrations of 2.5 and 5.0% in the diet significantly decreased bone marrow cellularity, and the reduction occurred in a dose-dependent manner. Decreased feed consumption and toxic effects may have contributed to this effect in the 5.0% diet group rats, but because feed consumption was normal (after an initial decrease) and generalized toxicosis was not observed in the rats of the 2.5% group, the reduction in bone marrow cellularity would appear to be a direct effect of ingestion of BBP. Degenerative changes of the reproductive organs were observed in rats fed diets containing 2.5 and 5.0% BBP; the details of these changes are noted in the Reproductive Toxicity section of this report. The changes observed in the reproductive organs were not responsible for the decreased plasma concentrations of testosterone, though the decreased concentrations may have caused atrophy of the seminiferous tubules; however, there was no direct evidence that such was the case. The increase in plasma FSH and LH concentrations coupled with decreased testosterone concentrations and testicular atrophy were evidence of a negative feedback response and indicated that pituitary-hypothalamus function was not impaired.

Inhalation

Two studies using 6–8-week old Sprague-Dawley rats were performed to assess the short-term inhalation toxicity of BBP.[44] In both studies, the rats were exposed to BBP 6 h/day, 5 days/wk for 4 weeks. Group 1 consisted of groups of 10 rats, evenly divided by gender receiving concentrations of 49, 144, or 526 mg/m^3 BBP (determined by passing inhalation chamber air through a filter or impinger and subsequent GLC analysis) and nominal concentrations of 86, 336, or 1230 mg/m^3 (as determined by dividing the amount of BBP metered through the inhalation chamber by the total volume of air used per exposure period). Group 2 consisted of 40 rats per exposure group, evenly divided by gender, receiving 360, 1000, or 2100 mg/m^3 BBP and nominal concentrations of 1600, 6800, or 15,000 mg/m^3 BBP. Particle size analyses

performed periodically for the rats in group 2 indicated that more than 90% of the aerosol particles were less than 10 μm in diameter. Rats from both groups were necropsied at the end of the studies. Brain, heart, liver, kidneys, liver, gonads, lungs, and spleen weights were recorded for the Group 1 rats. Microscopic examination also was performed on various tissues from the high-dose rats of Group 1 and from the control rats.

The Group 1 rats had reduced weight gains in both genders of the high-dose group when compared with weight gains of the control group rats. Results of hematology, blood chemistries, and urinalyses were not changed following exposure to BBP. No significant changes in relative organ weights were noted for the rats of this group. Hemorrhages were the only microscopic changes considered treatment related.

In Group 2, three male and four female rats of the high-dose group died before completion of the study. These rats had hemorrhages in various tissues. The high-dose rats had chromodacryorrhea, and a few of the rats in each dosage group had alopecia and skin abrasions, probably due to the rubbing against the cages in response to being coated with BBP condensate. In the high-dose group, a few of the rats had bleeding around the nares, and body weight gains were significantly reduced. Atrophy of the spleen and testes was also noted in this group at necropsy.

Intraperitoneal

Groups of 20–30 white mice (strain unspecified) received 500 mg/kg i.p. injections of a BBP emulsion for 6 weeks. Control mice received a 3% acacia solution.[40] Weights were recorded weekly. At the end of the study, the mice were necropsied and organ/body weight ratios were recorded for the liver, kidneys, lungs, heart, spleen, and testes. Various tissues were retained for microscopic examination. Hemoglobin, hematocrit, erythrocyte count, and total and differential leukocyte counts were obtained at the beginning and end of the study.

During the study, body weight gains for the mice receiving BBP were equivalent to those recorded for the control mice. Though the weight gains at the end of the study were approximately the same, the mice which were treated with BBP gained weight at a slower rate than the control mice. The difference in rates of weight gain was likely due to decreased feed consumption probably resulting from peritoneal cavity irritation. No significant differences were noted in organ-to-body weight ratios for treated mice when compared with controls. There were no significant differences in hematology parameters for the treated mice. At necropsy, the BBP-treated mice had acute peritonitis, periportal hepatitis, and extramedullary hematopoiesis of the liver. An abscess of unknown origin was found in one testis.

Subchronic Toxicity

Oral

BBP was fed in the diet for 13 weeks at concentrations of 1600, 3100, 6300, 12,500, or 25,000 ppm to groups of 20 F344/N rats and B6C3F1 mice, evenly divided by gender.[11] Untreated control groups of each species were included in the study. The animals were observed twice daily for signs of abnormal behavior and were weighed once weekly. All animals surviving to the end of the study were killed and necropsied. Tissues were taken from the high-dose and control animals for microscopic examination.

Two male rats, one receiving 6300 ppm and one receiving 12,500 ppm BBP, as well as two female control rats, died during the study. Reduced weight gains and testicular degeneration were noted in male rats of the 25,000 ppm dosage group. No compound-induced effects were noted in the female rats.

Four female mice of the high-dose group died as the result of an accident during the study. The only adverse effect noted was reduced weight gains in mice of all dosage groups.

The subchronic toxicity of BBP was studied in Sprague-Dawley rats.[48] For 3 months, groups of 20 rats, evenly divided by gender, were fed diets with BBP concentrations of 0.25, 0.5, 1.0, 1.5, and 2.0% (w/w). Two negative control groups, and one positive control group receiving 2.15% butyl phthalyl butyl glycolate (BPBG) in the diet were included in the study. In addition, paired feeding studies were performed on rats receiving 2.0% BBP and rats receiving 2.15% BPBG. The paired control rats were fed the amount of control diet equal to the amount of test diet that was consumed on the previous day by their respective paired test rats.

Body weights were recorded on the first day of the study and weekly thereafter. The rats were observed daily for signs of abnormal behavior. Weekly weights and daily observations also were recorded for the rats of the paired feeding studies. Five rats of each gender per test group were randomly selected for hematologic studies and urinalyses. Hemoglobin concentration, erythrocyte count, and total and differential leukocyte counts were obtained at the beginning of the study and every month thereafter. Urinalyses for reducing substances, albumin, and microscopic substances were also performed prior to the study and monthly thereafter, with the urine of the male and female rats pooled prior to analysis. Necropsy was performed on all rats that died during the study as well as on those which survived to the end of the study. Body weights, as well as brain, heart, liver, kidneys, spleen, and either testicular or ovarian weights, were recorded. Microscopic examination of heart, lungs, liver, pancreas, stomach, small intestine, colon, spleen, lymph nodes, kidneys, urinary bladder, pituitary gland, adrenal glands, thyroid gland, parathyroid gland, skeletal muscle, bone marrow, and brain was performed on five rats of each gender from the control groups, including the treated control group, and from the 1.5 and 2.0% BBP groups, and from three rats of each gender from each of the remaining test groups. In addition, the testes and prostate of male rats and ovaries and uterus of female rats were subjected to microscopic evaluation.

No deaths occurred and no treatment-related changes in behavior were noted during the 3-month test period. There was a slight growth depression of male rats fed the 1.5 and 2.0% BBP diets and of the male rats fed the 2.15% BPBG diet. The results of the paired feeding study indicated that the growth depression observed in male rats from the high-dose BBP group of the subchronic study was probably the result of decreased feed consumption due to lack of palatability rather than because of a direct effect of treatment with BBP. No significant differences were noted in hematologic and urinalysis parameters of treated rats as compared with control rats. There were significant differences in liver weights and their ratio to body and brain weights for the female rats of the 1.5 and 2.0% BBP diets as well as for the rats of both genders receiving 2.15% BPBG in the diet. No treatment-related abnormalities were noted upon microscopic examination of various tissues.

Subchronic toxicity of BBP was also studied in groups of 27–45 Wistar rats of each gender.[44] The rats were 4–6 weeks old at the beginning of the study, and they were fed BBP at concentrations of 171, 422, or 1069 mg/kg/day (females) or 151, 381, or 960

mg/kg/days (males) in their diets for 3 months. Hemoglobin concentration, erythrocyte count, total and differential leukocyte counts, hematocrit, reticulocyte count, prothrombin time, glucose, blood urea nitrogen (BUN), albumin, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum alkaline phosphatase (SAP) were measured for 6–27 Wistar rats of each gender. Urinary volume, specific gravity, pH, glucose, hemoglobin, and bile salts also were determined for these rats. Renal function was determined in those rats for which hematologic evaluations and urinalyses were obtained by determining urine volume and refractive index 2 h and 17–23 h after dosing orally with 25 mg/kg water. All of the rats in this study were necropsied, and absolute and relative organ weights were determined for the brain, heart, liver, kidneys, gonads, adrenal glands, full and empty cecum, pituitary gland, stomach, and thyroid gland. All of the rats of the high-dose group and the control group were examined microscopically, as well as the liver, kidneys, testes, and pancreas of three rats of each gender from each of the lower dosage groups.

Although some deaths occurred in various dosage groups, no dose-related trends were observed, and the deaths were not attributed to the ingestion of BBP. Weight gain was reduced in male and female rats at doses above 1000 mg/kg. There were occasional incidences of reduced weight gain in rats receiving lower doses of BBP, but this was not dose related. Feed consumption was reduced in the lower dosage groups, but remained normal in the high-dose group, indicating that reduced weight gain at lower dosages was not due to ingestion of BBP in the diet, while the reduction of weight gain at the high dose was likely due to the ingestion of BBP.

Male rats receiving the high-dose BBP diet had slight anemia and male rats of mid-dose and high-dose groups had decreased urinary pH. There were compound-related changes in relative liver and kidney weights of both male and female rats, and in relative cecum weights of female rats. There was also an increased incidence of red spots on the liver of male rats from the mid-dose and high-dose groups. Small areas of necrosis were observed in the livers of the high-dose male rats. Changes were reported in the pancreas of male rats from the mid-dose and high-dose groups. In the endocrine pancreas, the changes included islet enlargement with cellular vacuolization, peri-islet congestion, and occasionally peri-islet inflammatory cell infiltration with brown pigment, and slight fibrosis. In the exocrine pancreas, pyknotic nuclei, acinar atrophy, and peri-acinar inflammatory cell infiltrate were observed.

The subchronic toxicity of BBP was also studied in adult Beagle dogs.[49] Three groups of 6 dogs each, evenly divided by gender, were fed diets containing 1.0, 2.0, or 5.0% (w/w) BBP. There was an untreated control group of six dogs, three of each gender, as well as a positive control group of six dogs, evenly divided by gender, which received 5.38% BPBG in the diet. The 5.0% BBP diet and the BPBG diet had poor palatability, resulting in decreased feed consumption. After 39 days on these diets, the dogs were in poor physical condition and it was necessary to change the test regimen to prevent deaths due to malnutrition. Consequently, the animals in these groups received BBP or BPBG in capsule form after day 39. Capsules were administered to the dogs immediately after they had consumed their ration of the control diet, and capsule doses were adjusted to compensate for daily feed consumption so that the dogs received the equivalent of the dietary feeding of either 5.0% BBP or 5.38% BPBG. Weights were obtained on all dogs at the start of the study, and were recorded weekly thereafter. The dogs were observed daily for signs of toxicity. Hemoglobin concentration, erythrocyte count, and total and differential leukocyte counts were obtained at the start of the study and monthly thereafter (at 30, 60, and 90 days). Urine also was analyzed at these time

intervals for the presence of reducing substances, albumin, and cellular components. Hepatic and renal function tests were also performed at these intervals using the sulfobromophthalein and phenosulfonphthalein methods, respectively. Necropsy was performed on all dogs at the end of the study. Gross and microscopic examination was made of the heart, aorta, trachea, lungs, liver, gallbladder, pancreas, esophagus, stomach, small intestine, large intestine, spleen, lymph nodes, kidneys, urinary bladder, gonads, pituitary gland, adrenal glands, salivary glands, thyroid gland, parathyroid glands, skeletal muscle, bone, bone marrow, and portions of the central and peripheral nervous systems. In male dogs, the prostate gland and seminal vesicles also were examined, as was the uterus in the female dogs.

No significant differences were noted in weight gains of dogs of the low and mid-dose groups when compared with that of the control group. As noted previously, the high-dose and treated control dogs lost weight, but this was due to lack of palatability of the test diet rather than to a direct effect of treatment. Feed consumption was equivalent for the controls and the low and mid-dose groups for the entire study, and for the high-dose and treated control groups after day 39. No deaths occurred and no treatment-related behavioral abnormalities were noted during the course of the study. No significant differences were found in either the hematologic or urinary parameters, and the renal and hepatic function tests were normal for all of the groups studied. No treatment-related tissue changes were noted at gross and microscopic evaluation.

Inhalation

Groups of 50 Sprague-Dawley rats, evenly divided by gender, were exposed to BBP in air for 6 h/day 5 days/wk for 13 weeks.[44] The rats were exposed to analytical concentrations of 51, 218, or 789 mg/m^3 BBP and nominal concentrations of 60, 460, or 1440 mg/m^3 BBP. A particle size separator prevented nonrespirable particles from entering the inhalation chamber, and particle size analyses were conducted periodically. At 7 and 13 weeks, erythrocyte count, total and differential leukocyte counts, hemoglobin, hematocrit, mean corpuscular hemoglobin and hematocrit, serum alkaline phosphatase, bilirubin, blood urea nitrogen, glucose, total protein, serum glutamic pyruvic transaminase, urinary volume, specific gravity, pH, and ketone were measured for 10 rats of each gender per dosage group. At necropsy, adrenal glands, brain, heart, kidneys, liver, and testes weights were obtained. Microscopic examination was also performed on various tissues of the high-dose and control rats.

There were no changes in body weight gains in the test rats as compared with the control rats. Serum glucose was significantly reduced in high-dose males at 13 weeks. There was a significant increase in relative liver and kidney weights for both male and female high-dose rats. Hemorrhages were noted in some tissues at microscopic examination.

OCULAR IRRITATION

When 0.1 ml of undiluted BBP was placed into the conjunctival sac of the eyes of six New Zealand white rabbits, irritation was slight and subsided within 48 h.[44]

DERMAL IRRITATION

Undiluted BBP, 0.5 ml, was applied to the intact and abraded skin of six New Zealand white rabbits.[44] There was practically no irritation to the rabbit skin exposed to BBP for 24 h.

TERATOGENICITY AND REPRODUCTIVE TOXICITY

The reproductive toxicity of BBP was evaluated in a dominant lethal assay using CD-1 and B6C3F1 mice.[50] BBP was administered by subcutaneous (s.c.) injection to 24 male CD-1 mice and to 36 male B6C3F1 mice on days 1, 5, and 10 of the study. Doses were equivalent to 400–600, 1280–1840, or 3200–4560 mg/kg/day. There were 12 mice of each strain which served as untreated controls, as well as 6 positive control B6C3F1 mice which received injections of triethylene melamine. The mice were mated with three virgin females of the same strain at 4-day intervals, beginning on days 2, 6, 11, 15, 22, 29, 42, and 49. The females were killed 17 days after the start of their mating period and the uterine contents were examined for total implant sites, dead and viable fetuses, and resorptions. There was no significant increase in fetal death rates during any of the mating periods. BBP was not considered a dominant lethal mutagen or a reproductive toxicant to CD-1 or B6C3F1 mice under the conditions of the study.

Field et al.[51] exposed CD rats to 0, 0.5, 1.25, or 2.0% BBP in the diet on gestation day 6 to 15. The no-observed-adverse effect level (NOAEL) for all maternal and embryo effects was established at the average dose of 0.419 g/kg/day. Significant maternal and fetal toxicity was reported at the average dose of 1.64 g/kg/day.

The National Toxicology Program[52] evaluated the maternal and developmental toxicity in CD-1 mice. BBP (0, 0.1, 0.5, 1.25, or 2.0% in feed) was administered on day 6 through 15 of the gestation period. The NOAEL for maternal and developmental toxicity was in the groups receiving 0.1% BBP (0.182 g/kg/day). At the 0.5% dose level, there was evidence of minimal maternal toxicity and significant developmental toxicity. At the two higher concentrations, there were significant maternal and embryo effects, including 90% prenatal mortality.

Groups of 10 male F344 rats were fed BBP at concentrations of 0.625, 1.25, 2.5, or 5.0% (w/w) in the diet.[43] An additional group of 10 rats served as controls. The rats were maintained on their respective diets for 2 weeks, then killed on day 15. The testes, seminal vesicles, epididymis, and prostate gland weights were significantly reduced for the rats receiving 2.5 and 5.0% BBP; this reduction in weight was dose- dependent. The organ-to-body weight ratios were reduced similarly for the testes, epididymis, and seminal vesicles of the two highest dosage groups. There were significant dose-dependent microscopic degenerative changes of the testes, seminal vesicles, and prostate gland of the rats from the 2.5 and 5.0% groups. These changes included atrophy of the seminiferous tubules, seminal vesicles, and prostate gland, aspermatogenesis in the testes, atrophy of the epididymis, sperm granuloma, degeneration or necrosis of the tubular epithelium of the caput epididymis, and the presence of immature spermatogenic cells in the tubular lumen of the epididymis. The effects of PAEs not only on the testes, but also on the accessory gender organs, is a phenomenon that has been noted in studies of di-(2-ethylhexyl) phthalate and di-*n*-butyl phthalate. The authors noted that the metabolism of BBP produces monobutyl phthalate (MBP), and that MBP also has been implicated in causing testicular atrophy, but that it was not

known if this was the mechanism by which BBP caused gonadal toxicity. As was mentioned earlier in this report, plasma concentrations of testosterone were significantly decreased in rats receiving diets containing 2.5 and 5.0% BBP. In addition, plasma FSH and LH concentrations were increased in rats of both of these dosage groups (significantly for the 2.5% group). As reported earlier, the decrease in plasma testosterone paralleled an increase in incidence and severity of atrophy of the seminiferous tubules, but it was not known whether there was a direct correlation between the two events.

MUTAGENICITY

BBP, analyzed as 97.2% pure, was assayed for mutagenicity in the Ames test using *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100.[53] Solvent and positive controls were included in the study, and all tests were performed twice. A positive response was defined as a dose-dependent increase in the number of histidine-independent colonies over the spontaneous incidence of increase. BBP was tested at five concentrations, separated by half log intervals, between 333.0 and 11,550.0 μg/plate, with and without metabolic activation. BBP was not mutagenic under the conditions of the study.

BBP, at concentrations up to 1000 μg/plate, was negative for mutagenic activity in a modified Ames test using *S. typhimurium* strains TA100 and TA98, with and without metabolic activation.[54]

BBP, at a concentration of 30 mg/plate, was not mutagenic to *Bacillus subtilis* (rec A-), *Escherichia coli* (uvrA-, PolA-, recA-, wild), or *S. typhimurium* (TA98 and TA100, with metabolic activation).[55,56]

BBP was nonmutagenic, with and without metabolic activation, in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, or TA100 and in *Saccharomyces cerevesiae* strain D4 at doses up to 10 μl/plate.[57]

BBP was not mutagenic in the mouse L5178Y TK lymphoma forward mutation assay.[57]

BBP was negative for mutagenicity, with and without metabolic activation, in the L5178Y TK+ mouse lymphoma mutagenesis assay.[58]

The mutagenic potential of BBP was also studied in the BALB/3T3 *in vitro* cell transformation assay.[42] A preliminary cytotoxicity test was performed to determine the optimal concentration of test material. The concentration of BBP which resulted in a survival rate of 10–30% was considered optimal since significant transformation frequencies were obtained for a set of model carcinogens over this survival range. The BBP was tested at five concentrations: 10.0, 20.0, 40.0, 80.0, and 160.0 nl/ml. This concentration range included a concentration resulting in 10–50% survival, a concentration resulting in 10–20% survival, and three concentrations from the descending part of the survival curve as determined in the cytotoxicity assay. The spontaneous transformation rate as measured from 35 control cultures (after $\log_{10}$ analysis) was 0.29 focus/flask, within the expected spontaneous transformation range of 0–0.5 focus/flask. Two positive controls, 3-methylcholanthrene (MCA) and *N*-methyl-(*N*-nitro-*N'*-nitroguanidine) (MNNG), were used at concentrations of 2.5 and 1.25 μg/ml, respectively, in 18 cultures each. The transformation rates resulting from treatment with MCA and MNNG were 16.9 and 1.3 foci/flask, significantly higher than the negative controls. The number of transformed foci resulting from treatment with BBP ranged

from 0.31 foci/flask at a BBP concentration of 10.0 nl/ml to approximately 0.04 foci/flask at the highest BBP concentration. None of the transformation values obtained from treatment of BALB/3T3 cells with BBP were significantly different from those of the control cultures, and there was no evidence of a dose-related response to BBP treatment. BBP was considered nonmutagenic under the conditions of the study.

CARCINOGENICITY

BBP was tested for carcinogenic activity in feeding studies using F344/N rats and B6C3F1 mice.[11] Groups of 100 animals (evenly divided by gender) of each species were fed either control diets or diets containing BBP at concentrations of 6000 or 12,000 ppm for 103 weeks, followed by a 1–3 week observation period. Because of poor survival, male rats of both dosage groups were killed between weeks 29 and 30 of the study. The animals were observed twice daily, and weights were recorded once a month. Animals found in a moribund state, as well as those which survived to the end of the study, were killed and necropsied. Animals found dead during the study also were necropsied when the bodies were not autolyzed or cannibalized. At necropsy all major organs, tissues, and lesions were examined. Microsopic examination was made of the skin, abnormal lymph nodes, mandibular lymph nodes, mammary glands, salivary glands, bone marrow, costochondral junction, thymus, larynx, trachea, lungs and bronchi, heart, thyroid glands, parathyroid glands, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mouse), pancreas, spleen, kidneys, adrenal glands, urinary bladder, brain, and pituitary gland. In addition, the seminal vesicles, prostate gland and testes of males and ovaries and uterus of females were examined.

Average body weights of both male and female rats were lower than those of the control rats throughout the study. Feed consumption of the female rats was also lower than that of the controls. Survival of the male rats was significantly reduced in all dosage groups, while the survival rate of the female rats was comparable with that of the controls. Deaths of the male rats appeared to be due to internal bleeding, but this was not confirmed by microscopic examination. Examination for neoplasia or other lesions indicated a significant increase in the incidence of mononuclear cell leukemia for females of the high-dose group; this was the only effect which was considered treatment related. The incidence of mononuclear cell leukemia in the high-dose female rats was also significantly higher than the laboratory's historical controls. The tissue distribution and cytologic characteristics of the leukemias observed in the high-dose rats did not differ from those observed in the rats of the low-dose and control groups.

BBP was considered as probably carcinogenic to female F344/N rats, and the male rat study was considered inadequate for the determination of carcinogenic potential.

Though average weight gains were reduced for treated mice throughout the study, no differences in survival were noted between treated and control groups. The numbers and kinds of lesions found at necropsy were comparable for both treated mice and control mice, and BBP was considered noncarcinogenic to B6C3F1 mice under the conditions of the study.

BBP was administered by i.p. injection to groups of 20 male A/St mice.[57,59] The mice received injections of 160, 400, or 800 mg/kg BBP in tricaprylin three times a week for six weeks (total of 24 injections). The control group received injections of tricaprylin. Eighteen weeks after the last injection, the mice were killed and examined

for the presence of pulmonary adenomas. There were no significant differences in the incidences of pulmonary adenomas in treated and control mice, and no dose–response relationship was observed.

In the IARC monograph on BBP,[57] the Working Group concluded that the available data were inadequate to judge the carcinogenicity of BBP for rats and mice.

Analysis of structure/activity relationships of 222 chemical tested for carcinogenicity and mutagenicity indicated that the phthalic acid substituent of BBP was not likely to be a carcinogen.[60] The structure of BBP was analyzed for potential DNA reactive sites, and the *Salmonella* mutagenicity assay results and level of carcinogenicity in rats and mice were recorded. BBP was included in a group of structurally similar chemicals whose carcinogenic activity was not predicted by either structure or mutagenicity assays. It was suggested that DNA reactivity did not appear to be a mechanism of carcinogenicity in any of these compounds.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation and Sensitization

In a repeat insult patch test, patches of undiluted BBP were applied to the skin of 200 volunteers for 24 hr, three times a week for 5 weeks.[44] After a two-week non-treatment period, the subjects were rechallenged at previously untreated sites on the skin. The skin was examined during the induction and challenge phases for signs of irritation/sensitization. None of the subjects had primary irritation or sensitization reactions.

SUMMARY

BBP, a rather biologically inert organic chemical, is an aromatic ester used as a plasticizer in hairsprays at concentrations less than 1.0%. Cytotoxicity studies indicated that BBP was toxic to HeLa cells, mouse lymphoma L5178Y cells, and BALB/3T3 cells at higher concentrations. BBP was rapidly excreted unchanged after oral and i.v. administration; the major route of excretion was into the bile.

The oral LD_{50} was estimated for various animals: 2.33 g/kg for F344/N rats; 6.16 and 4.17 g/kg for male and female B6C3F1 mice, respectively; and 20.4 g/kg for Sprague-Dawley rats. In feeding and gavage studies with rats and mice, BBP caused weight loss, testicular atrophy, hemorrhages, hepatomegaly, and a few treatment related deaths. No toxicity was reported in an oral subchronic toxicity study using dogs.

The intraperitoneal LD_{50} for Swiss Webster mice was 3.16 g/kg. Intraperitoneal administration of BBP caused hepatic lesions in mice. Weight loss and hemorrhages were observed in rats during an inhalation study. BBP was nontoxic in dermal studies using rabbits.

Slight dermal and ocular irritation was produced by BBP in rabbits. These reactions were transient.

BBP produced negative results in a reproductive toxicity test using CD-1 and B6C3F1 mice; however, BBP caused dose-dependent microscopic degenerative changes in the testes of male F344 rats.

BBP was nonmutagenic in the Ames test, L5178Y TK mouse lymphoma mutagenesis assay, dominant lethal mutagenicity assay, and BALB/3T3 cell transformation

assay. A significant increase in the incidence of mononuclear cell leukemia in female F344/N rats was attributed to a high dose of BBP. BBP was not carcinogenic in studies using B6C3F1 and A/St mice.

In human dermal studies, BBP caused little or no irritation and was not a sensitizer.

DISCUSSION

The Expert Panel noted that BBP absorbs UVB light and that no photosensitization data on this ingredient and its hydrolysis products are available. However, the members agreed that there is no phototoxicological concern for its use in cosmetics because it is used at low concentrations, <1%, and has low skin exposure. The mononuclear cell leukemia reported in the rat feeding study is a common disease in the F344 rat, and was considered unrelated to BBP exposure. Therefore, this result has no biological significance in respect to the use of BBP in cosmetics.

CONCLUSION

On the basis on data presented in this report, the CIR Expert Panel concludes that Butyl Benzyl Phthalate is safe as a cosmetic ingredient in the present practices of use and concentration.

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