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Final Report on the Safety Assessment of Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate

Dibutyl Phthalate (DBP), Dimethyl Phthalate (DMP), and Diethyl Phthalate (DEP) are dialkyl phthalates used primarily in cosmetics at concentrations of less than 10 percent as plasticizers, solvents, and perfume fixatives.

These phthalates are rapidly absorbed, metabolized, and excreted. Acute animal feeding studies indicate that these ingredients are nontoxic. The results of most subchronic and chronic tests indicate that these ingredients are relatively nontoxic to rats. The oral administration of DBP produced testicular atrophy in various test rodents. The available data are not adequate to prove that these ingredients are teratogenic agents to experimental animals. This was not observed after the administration of DMP and DEP. Undiluted DBP, DMP, and DEP produced only minimal irritation to eyes of rabbits.

The mutagenic activity of DBP, DMP, and DEP toward *Salmonella typhimurium* mutants is essentially negative, but some assays reported positive findings. Carcinogenesis was not observed in DBP feeding studies.

Limited clinical data on DBP, DMP, and DEP indicate that these ingredients are not human skin irritants, sensitizers, or phototoxic agents. On the basis of the available data, it is concluded that these compounds are safe for topical application in the present practices of use and concentration in cosmetics.

INTRODUCTION

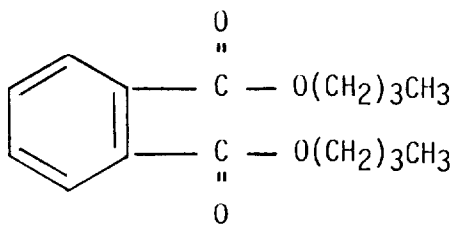
This report reviews the published information and unpublished data supplied by the cosmetic industry on Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate. Di(2-ethylhexyl)phthalate, a compound currently of great concern, is not used in cosmetics.

CHEMICAL AND PHYSICAL PROPERTIES

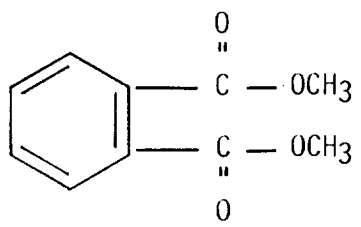
Structure

Dibutyl Phthalate (CAS No. 84-74-2) (DBP), Dimethyl Phthalate (CAS No. 131-11-3) (DMP), and Diethyl Phthalate (CAS No. 84-66-2) (DEP) are dialkyl phthalates. DBP, DMP, and DEP are the aromatic diesters of butyl, methyl, and ethyl alcohol, respectively, and phthalic acid. The chemical formulas of these alkyl phthalates are as follows[1]:

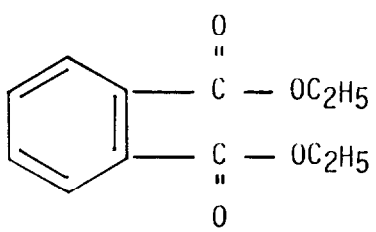
Dibutyl Phthalate



Dimethyl Phthalate



Diethyl Phthalate



Properties

DBP, DMP, and DEP are colorless, oily liquids, soluble in alcohol, ether, and other common organic solvents and almost insoluble in water. DMP is insoluble in petroleum ether and other paraffin hydrocarbons. DBP is odorless. DMP and DEP have no to slight odors, and DEP has a bitter, disagreeable taste.[2-7] DBP is soluble in a solution simulating human sweat (an aqueous solution containing 2.5 g sodium phosphate, 0.2 g triolein, and 2 drops Tween 85/1), and its solubility in this solution increases with an increase in pH.[8] Chemical and properties of DBP, DMP, and DEP are presented in Table 1.

TABLE 1. Chemical and Physical Properties

Property	*DBP*	*DMP*	*DEP*	*Reference*
Molecular weight	278.34	194.19	222.23	
Specific gravity at:				
14/4°C			1.232	7
15.6/15.6°C		1.196		7
20°C	1.0459, 1.0465			7
20/20°C		1.940		7
20/20°C	1.047, 1.049			2
20/20°C		~1.19		3
20/20°C			~1.12	4
20/20°C	1.0484			6
25/25°C		1.189	1.120	6
25/25°C		1.189		7
Boiling point (°C) at:				
760 mm Hg		283.7		7
400 mm Hg		257.8		7
200 mm Hg		232.7		7
100 mm Hg		210.0		7
60 mm Hg		194.0		7
40 mm Hg		182.8		7
20 mm Hg		164.0		7
10 mm Hg		147.6		7
5 mm Hg		131.8		7
1.0 mm Hg		100.3		7
Not specified	340	282	295	5
Not specified	340.0	282	298	6
Not specified	340		295	7
Melting point (°C)				
	−35		−40.5	6
		5.5		7
Vapor pressure (mm Hg) at:				
20°C		<0.1		6
20°C		<0.01		7
150°C	1.1			6
163°C			14	6
182°C			30	6
295°C			734	6
Refractive index at:				
14°C			1.5049	7
20°C	1.4900	1.5168		7
25°C	1.4915	1.5138	1.5002	6

Reactivity

The alkaline hydrolysis products of phthalate esters are mono- and diacids. The second-order alkaline hydrolysis rate constants in water at 30°C are 1.0×10^{-2}, 6.9×10^{-2}, and 2.5×10^{-2} M^{-1} sec^{1} for DBP, DMP, and DEP, respectively. Acid hydrolysis is generally slower than alkaline hydrolysis, and neutral hydrolysis is generally too slow to be detected.[9] DBP is stable in solutions with a near neutral pH.[2]

The products of the thermal decomposition at 250 to 500°C of DBP are 1-butene, butanol, phthalic anhydride, and small amounts of benzoic acid, butyl

benzoate, phthalic acid, and monobutyl phthalate.[10] The major products in the pyrolysis at 730°C of DBP are isobutene, butene, and propylene.[11]

DBP can be degraded by radiolysis. The major product of a 1 ppm aqueous DBP solution at pH 7 after a dose of 3×10^4 rad of gamma radiation is monobutyl phthalate.[12]

Methods of Manufacture and Impurities

Phthalate esters can be prepared by the reaction of phthalic acid with alcohol. DBP, DMP, and DEP are produced industrially by the reaction of phthalic anhydride with butyl alcohol, methyl alcohol, and ethyl alcohol, respectively.[6,7,13] DBP is manufactured by the esterification of phthalic anhydride with an excess of *n*-butyl alcohol. Vacuum stripping removes the unreacted *n*-butyl alcohol. Steam sparging ensures low odor. The phthalate is alkali refined to give a low acid number and is filtered to produce a clear product.[2] The exact manufacturing processes for DMP and DEP are proprietary information. DEP may contain DMP or ethyl methyl phthalate as impurities.[3,4]

DMP and DEP, for use in cosmetics, should contain minimums of 99 percent DMP and DEP, respectively, as determined by gas–liquid chromatography.[2-4,14,15]

Analytical Methods

Qualitative and quantitative determinations of the phthalate esters are made by gravimetric procedures,[15-17] titrimetric analysis,[15] spectrophotometric methods,[18,19] spectrophotofluorometric analysis,[20] the isotope dilution technique,[21] thin-layer chromatography,[22,23] liquid chromatography,[16] liquid chromatography–mass spectrometry,[24] high-performance liquid chromatography,[25,26] gas–liquid chromatography,[3,4,27,28] gas chromatography,[25,29,30] gas chromatography–mass spectrometry,[31,32] high-resolution mass spectrometry, mass fragmentography,[32] gas chromatography with flame ionization,[25] vibration spectroscopy,[33] IR spectroscopy,[14,16,17,34,35] UV spectroscopy,[25,35,36] and NMR spectroscopy.[34,35]

USE

Purpose in Cosmetics

DBP is used in cosmetics as a perfume solvent and fixative, as a suspension agent for solids in aerosols, as a lubricant for aerosol valves, as an antifoamer, as a skin emollient, and as a plasticizer in nail polish, fingernail elongators, and hair spray. DMP is used as a solvent, particularly for artificial musk, and as a plasticizer in fingernail elongators. DEP is used as a solvent for cellulose acetate in nail polish and dopes, as a fixative for perfume, as an alcohol denaturant in toilet preparations, and as a plasticizer in fingernail elongators.[2-5,17,37]

Scope and Extent of Use in Cosmetics

Product types and the number of product formulations containing DBP,

DMP, or DEP and reported voluntarily to the Food and Drug Administration (FDA) in 1981 are presented in Table 2. Voluntary filing of this information by cosmetic manufacturers, packagers, and distributors conforms to the prescribed format of preset concentration ranges and product types as described in the Code of Federal Regulations (21 CFR 720.4)[38] Some cosmetic ingredients are supplied by the manufacturer at less than 100 percent concentration, and, therefore, the value reported by the cosmetic formulator or manufacturer may not necessarily reflect the true concentration of the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration. In 1981, DBP was reported as an ingredient in a total of 590 cosmetic formulations at concentrations ranging from ≤ 0.1 percent to between 10 and 25 percent. DMP was reported as an ingredient in 11 cosmetic formulations at concentrations ranging from ≤ 0.1 percent to between 10 and 25 percent. DEP was reported as an ingredient in 67 cosmetic formulations at concentrations ranging from ≤ 0.1 percent to between 25 and 50 percent.[39]

Surfaces to which Commonly Applied

Cosmetic products containing DBP, DMP, or DEP may be applied to or come in contact with skin, eyes, hair, nails, mucous membranes, and respiratory epithelium (Table 2).[39]

Frequency and Duration of Application

Product formulations containing DBP, DMP, or DEP may be applied as many as several times a day and may remain in contact with the skin for variable periods following application. Daily or occasional use may extend over many years (Table 2).[39]

Potential Interactions with Other Cosmetic Ingredients

No interactions of DBP, DMP, or DEP with other cosmetic ingredients are reported. In typical formulations, the compounds are stable.[2-4]

Noncosmetic Uses

DBP, DMP, and DEP are used as solvents and plasticizers for nitrocellulose, cellulose acetate, and cellulose acetate-butyrate compositions. They are used in the manufacture of varnishes and plastics and in insecticides and insect repellents. DBP is used as a plasticizer in explosives and elastomers, such as polyvinyl, as a textile lubricating agent, as a resin solvent, and in safety glass, printing inks, paper coatings, and adhesives. DMP is used as a camphor substitute in the manufacture of celluloid, as a wetting agent, and as an alcohol denaturant.[6,7]

DBP, DMP, and DEP may be used, at no specific concentration limits, in adhesives used as components of articles intended for packaging, transporting, or holding food (21 CFR 175.105).[38] DBP may be used as a catalyst and crosslinking

TABLE 2. Product Formulation Data[39]

Product Category	*Total No. of Formulations in Category*	*Total No. Containing Ingredient*	*No. of Product Formulations within Each Concentration Range (percent)*					
			>25–50	*>10–25*	*>5–10*	*>1–5*	*>0.1–1*	*≤0.1*
Dibutyl Phthalate								
Other hair preparations (noncoloring)	177	3	–	–	–	–	3	–
Other hair coloring preparations	49	3	–	–	–	–	3	–
Other makeup preparations (not eye)	530	1	–	–	–	–	1	–
Nail basecoats and undercoats	44	36	–	–	8	28	–	–
Nail polish and enamel	767	522	–	3	61	168	127	163
Nail polish and enamel remover	41	3	–	1	–	1	1	–
Other manicuring preparations	50	14	–	1	2	9	–	2
Other personal cleanliness products	227	5	–	–	–	5	–	–
Aftershave lotions	282	3	–	–	–	–	3	–
1981 TOTALS		590	–	5	71	211	138	165
Dimethyl Phthalate								
Hair conditioners	478	2	–	–	–	–	2	–
Tonics, dressings, and other hair grooming aids	290	2	–	–	–	1	1	–
Wave sets	180	2	–	–	–	–	2	–
Other hair preparations (noncoloring)	177	4	–	–	–	–	4	–
Hair rinses (coloring)	76	1	–	–	–	–	1	–
1981 TOTALS		11	–	–	–	1	10	–

Diethyl Phthalate								
Bath oils, tablets, and salts	237	3	–	–	–	1	–	2
Other bath preparations	132	2	–	–	–	–	–	2
Eye shadow	2582	1	–	–	–	–	–	1
Colognes and toilet waters	1120	19	–	–	–	1	10	8
Perfumes	657	23	1	–	–	1	7	14
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	1	–	–	–	–	1	–
Sachets	119	3	–	–	–	1	2	–
Other fragrance preparations	191	2	1	–	–	–	1	–
Hair sprays (aerosol fixatives)	265	5	–	–	–	2	3	–
Wave sets	180	1	–	–	–	–	1	–
Nail polish and enamel remover	41	1	–	–	–	1	–	–
Bath soaps and detergents	148	1	–	–	–	–	1	–
Aftershave lotions	282	3	–	–	–	–	3	–
Face, body, and hand skin care preparations (excluding shaving preparations)	832	1	–	–	–	–	–	1
Other skin care preparations	349	1	–	–	–	–	1	–
1981 TOTALS		67	2	–	–	7	30	28

agent for epoxy resins, and DEP may be used as a plasticizer, at no specific concentration limits, in the resinous and polymeric coatings of the food-contact surface of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food. DBP may be used in coatings of containers having a capacity of ≥1000 gallons and intended for repeated use with alcoholic beverages of less than or equal to 8 percent alcohol by volume (21 CFR 175.300, 175.320).[38] There are no concentration limits for the use of DBP as a component of the uncoated or coated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packaging, processing, preparing, treating, packing, transporting, or holding aqueous and fatty foods (21 CFR 176.170).[38] DBP may be used in the base sheet or coating of cellophane used in packaging food, but total phthalates must not exceed 5 percent by weight of the finished cellophane (21 CFR 177.1200).[38] DBP and DMP may be used, at no specific concentration limits, as solvents for inhibitors, accelerators, and catalysts in crosslinked polyester resins used as articles or components of articles intended for repeated use in contact with food (21 CFR 177.2420).[38] DBP may be used as a plasticizer in rubber articles intended for repeated use in producing, manufacturing, packing, processing, preparing, transporting, or holding food. Total DBP may not exceed 30 percent by weight of the rubber product (21 CFR 177.2600).[38] There is no concentration limit for the use of DMP in semirigid and rigid acrylic and modified acrylic plastics used as articles intended for use in contact with food (21 CFR 177.1010).[38] There is no limit in the amount of DEP that may be used in surface lubricants used in the manufacture of metallic articles that contact food (21 CFR 178.3910).[38] DEP may be used as a plasticizer in the manufacturer of food-packaging materials with no specific limits. This DEP will not be considered a "food additive" if of good commercial grade, suitable for association with food, and used in accordance with good manufacturing practice; the amount of DEP that migrates into food as a result of its use in food-packaging materials should not be intended to accomplish any physical or technical effect in the food itself and should be reduced to the least amount reasonably possible (21 CFR 181.22, 181.27).[38]

GENERAL BIOLOGY

Microbial Metabolism and Toxicity

A variety of bacteria can use DBP or DMP as a carbon source. The corresponding monoesters, phthalic acid, and protocatechuic acid are intermediates in the degradation of these chemicals.[40,41]

The growth of *Pseudomonas aeruginosa* was not inhibited by concentrations of up to 1000 ppm DMP. A 1500 ppm solution slightly inhibited the growth of the organism. After a 24-hour incubation, the concentration of a 98 ppm DMP solution decreased to 88 ppm, suggesting some bacterial utilization of the compound.[41] The concentration of neutralized DEP that inhibited the multiplication of *Pseudomonas putida* was greater than 400 ppm.[42] The minimum inhibitory concentration of a 10 percent solution of DEP in 95 percent ethanol was 1000 ppm for *Corynebacterium* sp. and greater than 1000 ppm for *Staphylococcus aureus* and *Escherichia coli*.[43]

The growth of the blue-green alga, *Microcystis aeruginosa*, was inhibited by 100 to 300 ppm of DMP and suppressed for 3 days by 400 ppm DMP. After 4 days, cellular lysis was observed in the 400 ppm DMP culture. Concentrations of DMP from 500 ppm to 800 ppm completely destroyed the cells within 72 hours.[44,45] Neutralized DEP inhibited the multiplication of *M. aeruginosa* at a concentration of 15 ppm and inhibited the multiplication of the green alga, *Scenedesmus quadricauda*, at a concentration of 10 ppm.[42,46]

A 10 ppm solution of DBP in phosphate buffer at pH 7 decreased the percent survival of the yeast, *Saccharomyces cerevisiae*, throughout a 48-hour incubation; a 20 ppm solution was even more toxic.[47] The minimum inhibitory concentration of a 10 percent (w/v) solution of DEP in 95 percent ethanol was 500 ppm for the fungus, *Candida albicans*.[43]

A concentration of 50 ppm of DBP completely inhibited the growth of cells of the protozoan, *Tetrahymena pyriformis*. Other phthalate esters were inhibitory as well.[48] A concentration of 1000 ppm of DMP markedly inhibited the growth rate of *T. pyriformis*.[44,49] Neutralized DEP inhibited the multiplication of the flagellate protozoan, *Entosiphon sulcatum* at a concentration of 19 ppm.[42]

In Vitro Cell Toxicity

The metabolism and toxicity of DBP, DMP, and DEP in cultures of mouse fibroblast and rat cerebellum and various human cell lines have been investigated.

Dose–response curves were produced, and the ID_{50} for the mouse fibroblast cultures, defined as the dose required to inhibit growth by 50 percent, was determined for the phthalates. The ID_{50}s for DBP, DMP, and DEP were 1×10^{-4}, 7×10^{-3}, and 3×10^{-3} mole/l, respectively. DMP was highly toxic to the cells when they were undergoing significant protein turnover.[13] The effect of DMP on a replicating mouse fibroblastic cell culture was investigated. A radioactively labeled amino acid mixture (^{14}C) was added to the cultures, and the radioactivity was followed over a 96-hour incubation. Cells were relatively insensitive to growth inhibition by DMP, as measured by uptake of radioactivity, for the first 24 hours. However, between 24 and 96 hours, the uptake of radioactivity decreased continuously.[50]

Toxicity to mouse fibroblasts was also investigated using the cell overlay method. Pads containing 0.05 ml of a 50 mg/ml emulsion of the phthalates were placed on the agar surface (2.5 mg phthalates/pad), and the cells were observed for 48 hours. DMP and DEP were toxic to the cells and DBP was not.[51] In another study, mouse fibroblastic cells were incubated for 24 hours with paper discs containing pure DMP and DEP or saline solutions saturated with DMP and DEP at pH 6. Only the pure DMP was toxic to the cells.[52] Other researchers have reported that all three phthalates were toxic in a 24-hour incubation of mouse fibroblastic cells.[53] The response of mouse fibroblastic cells to 1, 5, 10, and 50 percent suspensions of DBP, DMP, and DEP was studied by Oser et al.[54] All the suspensions were toxic except the 1 and 5 percent suspensions of DBP. In cell suspensions with DBP and DEP, the cellular ATP concentrations decreased over a 6-hour incubation.

The effects of DBP, DMP, and DEP on the outgrowth of nerve fibers and fibroblasts in primary cultures of rat cerebellum were investigated. The phthalates were added directly to the nutrient media. DBP and DEP completely inhibited

outgrowth at concentrations greater than or equal to 1.17×10^{-3} and 1.53×10^{-3} M, respectively. DMP did not completely inhibit outgrowth at concentrations less than or equal to 3.05×10^{-3} M.[55]

Human embryonic lung cell cultures were studied after the addition of 40 μg/ml of DBP to the culture medium. DBP inhibited cell growth and caused morphological changes in the cells, the appearance of lipid drops in the cytoplasm, and the accumulation of triacylglycerol in the cytosol.[56]

Thelestam et al.[57] found that DBP and DEP were inactive in a test in which the extent of membrane damage in human lung fibroblasts was determined by measuring the amount of a radioactively labeled cytoplasmic marker released into the media. The ID_{50} of DBP, defined as the concentration that caused 50 percent growth inhibition, for human diploid cell strain WI-85 was 1.35×10^{-4} M.[58]

Guess and Haberman[52] studied the effects of DBP, DMP, and DEP on human amnion and KB human cancer cells in culture. All three compounds killed and lysed the cells. Saline solutions saturated with DMP and DEP at pH 6 did not cause hemolysis of human erythrocytes.

HeLa cells were incubated for 7 days after the addition of DBP, DMP, and DEP to the culture medium. The 7-day IC_{50}s, the geometrical mean values between the totally inhibitory concentrations and the maximal completely noninjurious ones, were 3.1×10^{-2} M for DBP, 7.7×10^{-2} M for DMP, and 6.3×10^{-2} M for DEP.[59]

Effects on Enzymes

Phthalate esters have a variety of different effects on mammalian enzymes, both in vivo and in vitro. DBP and DMP affect drug-metabolizing enzymes in mammalian liver. Single-dose intraperitoneal administration of 3.05 ml/kg of DBP and 3.6 ml/kg of DMP to rats inhibited the activity of hepatic aminopyrine N-demethylase and aniline hydroxylase and had no effect on glucose-6-phosphatase, NADPH-cytochrome c reductase, and tyrosine aminotransferase activity. The activities of these enzymes were not decreased when the phthalates were administered intraperitoneally every day for 7 days.[60,61] Results of another study indicated that DBP weakly enhanced the activity of aminopyrine N-demethylase from rat hepatic 10,000 *g* supernatant.[62] The oral administration of 5 mmole/kg per day of DBP for 6 days to male rats increased the hepatic cytochrome P-450, had no effects on glutathione-S-transferase activity or the monooxygenase activities dependent on cytochrome P-450, increased the epoxide hydratase activity, and increased the conjugation of *o*-aminophenol and 4-methylumbelliferone with glucuronic acid. Rat liver incubated in vitro with 2×10^{-3} M DBP had no effect on epoxide hydratase or glutathione-S-transferase activities, decreased the monooxygenase activities, and decreased the conjugation of *o*-aminophenol and 4-methylumbelliferone with glucuronic acid.[63]

DBP, DMP, and DEP inhibited mitochondrial respiration. Concentrations of 5×10^{-5} to 1×10^{-3} M of the phthalates inhibited the respiration of isolated mitochondria from rat liver primarily by uncoupling oxidative phosphorylation rather than by inhibiting electron transport or energy transfer.[64,65] Other researchers using the same concentrations have suggested that the contrary is probably true; the phthalates inhibited electron transport or energy transfer.[66] In some studies,

DBP and DMP inhibited the activities of succinate dehydrogenase and ATPase, enzymes of the rat liver inner mitochondrial membrane, after intraperitoneal administration, and in in vitro assays at concentrations of 1×10^{-4} to 1.5×10^{-3} M.[62,65,67] DBP stimulated ATPase activity and induced swelling of rat liver mitochondria.[68]

Administration of 0.7 percent DBP or 0.5 percent DMP in the diet of male rats for 21 days increased hepatic weights and reduced serum cholesterol concentrations. Acetate incorporation into triglycerides and the steryl ester plus squalene and mevalonate incorporation into squalene plus sterols in liver minces were inhibited by dietary DBP. These results were not observed with DMP. DMP administration resulted in a decrease in total hepatic cholesterol and lipid. This was not observed with DBP.[69] The intraperitoneal administration of 20 mg/kg per day of DBP to mice for 16 days did not significantly lower serum cholesterol but did lower serum triglycerides. DBP, at a concentration of 2.5×10^{-6} M inhibited mouse liver homogenate acetyl-CoA synthetase, citrate lyase, and acetyl-CoA carboxylase but not fatty acid synthetase. These enzymes are involved in the cholesterol and triglyceride synthesis pathways.[70] A 5×10^{-6} M concentration of DBP and DEP inhibited in vitro human blood lecithin/cholesterol acyltransferase. DMP, at the same concentration, inhibited the enzyme slightly.[71]

DBP elevated the activities of mouse and rat serum lactate dehydrogenase, glutamic-oxalacetic transaminase, and glutamic-pyruvate transaminase.[72-74] DBP increased the activity of alkaline phosphatase in mice[72] but had no effect on this enzyme in rats.[73]

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

DMP was absorbed through human skin, and some of its metabolites were detected in human urine.[75]

A homogenate of rat epidermis metabolized DMP at approximately 1.5 percent of the rate of the metabolism of DMP by a homogenate of rat liver. The homogenates were compared on a mg wet weight basis. DMP was bound to the epidermis in quantities seven to eight times greater than those in which it was bound to an equal dry weight of hepatic tissue.[76]

DEP was absorbed through the skin of rabbits. Labeled DEP (^{14}C) was applied topically, and the application sites were covered with cotton patches. Analysis of urine indicated that approximately 9 percent of the radioactivity was excreted after 24 hours, 14 percent after 48 hours, and 16 to 20 percent within 72 hours. After 3 days of topical exposure, tissue distribution was determined by autoradiography. Radioactivity was detected in the lung, heart, liver, kidney, gonads, spleen, and brain. It was not detected in the skin and subdermal fatty tissue at the site of application.[13]

DBP was administered by gavage to male rats in two doses of 0.2 ml 24 hours apart. Urine was collected for 48 hours after the first dose, and DBP and its metabolites were quantitated. A total of 24.6 percent of the phthalate moiety was recovered in the urine. The recovered phthalate moiety consisted of 89.8 percent monobutyl phthalate (MBP), 2.7 percent phthalic acid (PA), 0.4 percent intact DBP, and four other metabolites in very small amounts. The researchers suggested that DBP was metabolized by hydrolysis of one ester bond and both terminal and subterminal oxidation of the remaining alkyl chain. The resulting primary

and secondary alcohols were, presumably, further oxidized to acids and ketones, respectively. DMP was administered by gavage to male rats in a single dose of 0.1 ml, and the urine was collected for 24 hours. A total of 44.6 percent of the phthalate moiety was recovered in the urine, and it consisted of 77.5 percent monomethyl phthalate, 14.4 percent PA, and 8.1 percent intact DMP. DMP appeared to be metabolized only by hydrolysis of one or both ester groups.[77]

Male mice were administered labeled DBP (^{14}C) orally or intravenously. The radioactivity accumulated in the liver and kidney within 6 hours of oral administration and within 1 hour of intravenous administration. The radioactivity was rapidly excreted in the urine and feces.[78]

DBP interacted with DNA in vitro, but after oral administration of labeled DBP (^{14}C) to mice, no radioactivity was recovered from hepatic DNA. DBP and its metabolites appeared not to be transported into the nuclei.[79]

Labeled DBP (^{14}C) was administered orally in dimethyl sulfoxide in a dose of 60 mg/kg or intravenously in saline in a dose of 10 mg/kg to male rats. Urine and feces were collected, and the amount of radioactivity excreted was determined. The percentage of administered radioactivity excreted varied from 81.4 to 97.7 in the urine and from 1.0 to 8.2 in the feces in the first 24 hours after oral or intravenous administration of DBP. Several rats were killed, and tissue distribution of radioactivity was determined. Brain, heart, liver, lung, spleen, muscle, adipose, stomach, prostate, and thymus tissues, blood, and the intestinal contents were examined 24 hours after oral or intravenous administration of DBP. Very little radioactivity was recovered. The elimination of DBP from tissues and organs was rapid, and no organ had any significant affinity for accumulation. Rats were administered labeled DBP (^{14}C) orally, and bile was collected. From 27.6 to 52.8 percent of radioactivity was excreted in the bile within 24 hours after oral administration of DBP. Since more radioactivity was excreted in the bile than in the feces, there was apparently good absorption of DBP and its metabolites from rat intestine. Urinary metabolites were identified in male rats, male hamsters, and male guinea pigs given a single oral dose of 60 mg/kg DBP. All 24-hour urine samples contained MBP as the major product, intact DBP, PA, MBP glucuronide, and two other MBP oxidation products. The hamster urine contained an additional oxidation product. The livers from rats were examined 1 hour after intravenous dosing of DBP, and the data obtained indicated that DBP was rapidly hydrolyzed to MBP by the microsomal fraction. No PA was detected. The bile contained MBP and intact DBP but not PA. Since PA was detected in the urine, it was suggested that its formation must occur at other sites than the liver. It was concluded that the hydrolysis of DBP to MBP occurred in the liver, that there was enterohepatic circulation of DBP and its metabolites and good absorption from the intestine, and that MBP was the main metabolite of DBP and was primarily excreted in urine.[62]

DBP and DMP, in concentrations of 0.4 mg/ml, were incubated at 37°C with rat liver and kidney homogenates. DBP and DMP almost completely disappeared after 2 hours of incubation with rat liver homogenates. The action of rat kidney homogenates was slower; however, approximately 90 percent of the DBP and 95 percent of the DMP disappeared during a 5-hour incubation. The phthalates were found not to be degraded spontaneously under these experimental conditions.[80]

A 500 mg/kg dose of labeled (^{14}C) DBP in ethanol was administered by gastric

intubation to male rats and the bile was collected every hour for 6 hours. Six hours after oral administration of DBP, 4.5 percent of the radioactivity was recovered in the bile. Five hours after intravenous injection of DBP, 10 percent of the radioactivity was detected in the bile. DBP bile metabolites included MBP, intact DBP, PA, an MBP glucuronide, and traces of other glucuronides. A small amount of DBP appears to be absorbed unaltered from the intestine, and the excretion of DBP through the biliary route has a role in its metabolic fate.[80]

Labeled DBP, DMP, and DEP (^{14}C) were incubated with rat, ferret, and baboon hepatic postmitochondrial supernatant and with intestinal-mucosal cell homogenates. All of the diesters were hydrolyzed by cell homogenates. They were all hydrolyzed by all the preparations, and greater than 90 percent of the total metabolite formed was the corresponding monoester. Baboon liver preparations hydrolyzed the diesters faster than rat liver preparations; ferret liver preparations were the least active. Baboon intestinal-mucosal cell preparations hydrolyzed the diesters faster than rat intestinal-mucosal cell preparations, and ferret intestinal-mucosal cell preparations were the least active.[81] Hepatic preparations from humans also catalyzed the monohydrolysis of DBP, DMP, and DEP. The toxic effects of phthalates administered orally may depend on the properties of the corresponding monoesters and/or alcohols.[82]

DBP, DMP, and DEP, in concentrations of 1 mg/ml, were incubated for 16 hours at 37°C with the contents of rat stomach, small intestines, or cecum or with suspensions of human feces. The phthalates were metabolized rapidly to the corresponding monoesters when incubated with the contents of rat small intestine. Metabolism was slower in the presence of rat cecal contents and only DMP was metabolized to any extent by rat stomach contents. Human feces were almost inactive in metabolizing the phthalates; DBP and DMP were metabolized faster than DEP. The intestinal contents of younger male rats metabolized DBP and DMP at a slower rate than intestinal contents from more mature male rats. Among adults, intestinal contents from male rats metabolized DBP at a faster rate than intestinal contents from female rats. The monoesters were the only products of metabolism; complete hydrolysis to PA did not occur. It may be significant toxicologically that there is a good correlation between rate of phthalate hydrolysis and the acute oral toxicity to rats that is reported in the literature. The more rapidly hydrolyzed phthalate esters are more toxic. In another experiment, rat intestinal contents were incubated at 37°C for 90 minutes or centrifuged or filtered before addition of DMP. Preincubation reduced the ability of the small intestine contents to degrade DMP. The enzymes involved in DMP metabolism appeared to be labile in vitro. Both centrifugation and filtration reduced the rate of DMP hydrolysis. The effect of antibiotics was studied by adding antibiotics to the incubation mixture or to the intestinal contents during the 90-minute preincubation period. The antibiotics used in the experiments were antibacterial enzymes. They had no effect on the rate of metabolism of DMP by small intestine contents, suggesting that the involved enzymes are not bacterial and more probably are mammalian in origin. Mucosal cell enzymes may be involved in DMP metabolism. The low rate of phthalate hydrolysis by rat cecal contents and human feces might be explained by the presence of a low number of active intestinal mucosal cells.[83] DBP was hydrolyzed by crude pancreatic lipase solution.[84]

The in vitro intestinal absorption of DBP and DMP was studied using an everted gut-sac preparation from the rat small intestine. In one experiment with

DBP, S,S,S,-tributylphosphorotrithioate (DEF), administered orally before gut-sac preparation, was used as an esterase inhibitor. Most of the DBP and DMP was hydrolyzed to the corresponding monoester before crossing the intestinal mucosa. Only 4.5 percent of the DBP and 18.8 percent of the DMP crossed the intestine intact. Inhibition of mucosal esterases by DEF reduced the amount of DBP hydrolyzed to MBP. Approximately the same amount of intact DBP was absorbed by the intestine with and without DEF, and DEF did not affect MBP absorption. Intestinal absorption of these compounds may be controlled by the hydrolysis of DBP to MBP.[85]

Labeled DEP (^{14}C) was administered intravenously to pregnant rats on Day 5 or Day 10 of gestation. Diester and/or metabolic products were present in maternal blood, fetal tissue, amniotic fluid, and placentas after Day 8 or Day 11, respectively, and throughout gestation.[86]

Phthalates are ubiquitous in the environment, and human exposure is likely. DBP was found in normal and diseased kidneys,[87,88] adipose tissue at autopsy,[89] in the blood of pregnant women, and in umbilical cords.[90] Possible routes of exposure to phthalates for humans are by oral or dermal contact, inhalation, or as a result of the use of medical devices, such as blood storage bags.[44]

ANIMAL TOXICOLOGY

Oral Studies

Acute Toxicity

The acute oral toxicity of DBP, DMP, and DEP was studied in rats,[84,91-97] mice,[72,91,98-100] rabbits,[91,101] guinea pigs, and chicks[91] (Table 3). The LD_{50} for rats administered DBP orally ranged from approximately 8 g/kg to 23.0 g/kg. The LD_{50} value for rats administered DMP orally was 6.9 ml/kg. In the Hodge and Sterner[102] classification of single-dose oral toxicity for rats, DBP and DMP would be classified as practically nontoxic to relatively harmless and as practically nontoxic, respectively.

The acute oral toxicity of two nail preparations, one containing 9 percent DBP and one containing 6 percent DBP, was studied in rats[104,105] (Table 3). Both preparations were practically nontoxic.

Subchronic and Chronic Toxicity

DBP, DMP, and DEP in corn oil were administered by oral intubation for 4 days to groups of 12 rats in doses of 7.2 mmole/kg per day (approximately 2.0 g/kg per day DBP, 1.4 g/kg per day DMP, and 1.6 g/kg per day DEP). There were no significant changes in food intake or body weight. DMP and DEP administration did not result in significant changes in testes weight, no testicular atrophy was observed, and urinary zinc excretion was unaffected. Administration of DBP decreased weight of testes and produced severe atrophy of the seminiferous tubules. Most of the tubules had complete loss of spermatocytes and spermatids. DBP administration was accompanied by an increase in the urinary excretion of zinc, and there was a decrease in the zinc content of testes on an absolute and relative weight basis.[106-108] The administration of zinc, concurrently with DBP, provided substantial protection against DBP-produced testicular damage.[106]

TABLE 3. Acute Oral Toxicity

Material Tested	*Method*	*Species of Animal*	*LD_{50}*	*Comments*	*Reference*
DBP	–	Rats	23.0 g/kg	–	92,97
DBP	–	Rats	12.5 g/kg	–	93,96
DBP	–	Rats Mice	14.95 g/kg 9 g/kg	–	103
DBP	–	Rats	>20 ml/kg	–	95
DBP	Animals were observed for 7 days following DBP administration	Male mice	9.77 g/kg	–	72,98
DBP	Oral administration of 200 mg DBP to 10 mice	Mice	–	6/10 of the mice died within 7 hours.	99
Undiluted DBP	Oral administration of 4, 8, 16, and 32 g/kg, of DBP to 3, 9, 6, and 6 rats, respectively	Rats	~8 g/kg	0/3, 4/9, 6/6, and 6/6 rats died, respectively. The 4 g/kg dose had no effect on growth, the 8 g/kg dose slightly inhibited growth, and the 16 and 32 g/kg dose groups succumbed too quickly to exhibit significant changes in growth	84
DBP	Animals were observed for 7 days following DBP administration	Male mice	Between 14.8 and 17.0 g/kg	–	98,100
Undiluted DMP	40 rats, 80 guinea pigs, and 120 chicks were fasted prior to DMP administration. 110 mice and 80 rabbits were not fasted. Animals were observed for 6 days following DMP administration. DMP was given to 10 animals/dose	Rats Mice Rabbits Guinea pigs Chicks	6.9 ml/kg 7.2 ml/kg 4.4 ml/kg 2.4 ml/kg 8.5 ml/kg	–	91
DEP	–	Rabbits	1.0 g/kg	–	101
DEP	–	Rats	8.2 ml/kg	–	94
Nail polish, 9 percent DBP	5 male and 5 female animals/dose were fasted 16 hours prior to oral intubation and were observed for 14 days after	Rats	>5 ml/kg	No signs of gross pathology on necropsy of rats receiving 5 ml/kg	104
Nail preparation, 6 percent DBP	Preparation administered by oral intubation to 10 animals	Rats	>5 g/kg	"Nontoxic"	105

DBP produced testicular atrophy in the rat, mouse, guinea pig, and ferret, but not in the hamster after oral administration in a dose of 2.0 g/kg per day for 10 days.[108]

Long-term oral toxicity of DBP, DMP, and DEP was studied in rats,[84,94-96,103,109-113] mice,[103] and rabbits[114] (Table 4). Except at dietary concentrations of 1.25 percent DBP for 1 year, 8.0 percent DMP for 2 years, and 5.0 percent DEP for 16 weeks,the phthalates were relatively nontoxic to rats in subchronic and chronic oral tests.

Dermal Studies

Acute Toxicity

The acute dermal toxicity of DMP to rabbits was determined by placing DMP in contact with the clipped skin and holding it in place with a rubber cuff. The rabbits were exposed for 24 hours and then observed for 2 weeks. The acute dermal LD_{50} of DMP to rabbits was greater than 10 ml/kg.[91]

Subchronic and Chronic Toxicity

DBP and DMP were tested for long-term dermal toxicity by applying 0.5, 1.0, 2.0, and 4.0 ml/kg per day for 90 days to the clipped, intact skin of rabbits. The chemicals were applied to approximately 10 percent of the body surface. The subchronic dermal LD_{50} of DBP to rabbits was greater than 4 ml/kg per day for 90 days. DBP was slightly irritating to skin and very irritating to rabbit penile mucosa. A slight dermatitis was observed, and in the 4 ml/kg dosed rabbits, slight renal damage (not further described) was observed.[95] The subchronic dermal LD_{50} of DMP to rabbits was also greater than 4 ml/kg per day for 90 days. No skin irritation or dermatitis was observed, although DMP was irritating to rabbit penile mucosa. Pulmonary edema and slight renal damage were observed in the rabbits that died during the study. Rabbit survivors had varying degrees of nephritis (not further described) at the two highest doses.[91]

Primary Irritation

DBP and DMP were applied to the clipped, intact, and abraded skin of 3 rabbits. The rabbits were exposed to 0.5 ml of the chemicals for 24 hours with an occluded patch. DBP caused "very slight irritation." DMP was not irritating except in molting areas and the Primary Irritation Index (PII) was 0.7.[91,95]

DMP was treated for primary irritation to rabbits using a pill box device. Pill boxes were affixed to shaved rabbit skin, and 0.1 ml of a 20 percent solution of formalin ("as the primary irritant") was painted onto the skin and allowed to dry. Discs containing 0.2 ml of DMP were placed in the pill box and the box was closed. A 0.25 ml volume of a 0.5 percent sterile Evans blue solution was injected intravenously. After 18 hours, the blue color at the pill box sites was evaluated and correlated with irritancy. Ten to 15 separate observations were made. DMP had an irritation score of 0.8 on a scale of 0 to 3; DMP was less than slightly irritating.[115]

Sensitization

No evidence of sensitization was observed in rabbits receiving daily topical applications of DBP and DMP at doses of up to 4.0 ml/kg per day for 90 days.[95]

Intradermal Irritation

The intradermal irritation of phthalates to rabbits was measured by injecting the phthalates into the skin of the shaven backs. A trypan blue solution was injected into the marginal ear vein, and the extravasated trypan blue at the injection site was used as a measure of the extent of the inflammatory response. In one study, 0.2 ml of 100 mg/ml phthalate emulsions was injected. DBP gave a mild inflammatory response after 10 minutes and a moderate response after 26 minutes. A rapid and marked inflammatory response to DMP and DEP was noted.[51] Other researchers used cottonseed oil as a diluent for DBP, DMP, and DEP. DBP was not irritating, but DMP and DEP produced a significant degree of irritation.[116] In another study, saline solutions saturated with the phthalates were administered. No response was observed to DMP and DEP.[52]

Eye Irritation

The eye irritation potential of DBP, DMP and DEP was studied in rabbits.[116-118] The eye irritation potential of nail preparations containing 9 percent DBP and 6 percent DBP also was investigated[105,119] (Table 5). DBP, DEP, and nail preparations containing DBP were relatively nonirritating to the rabbit eye. With long contact time, undiluted DMP may be injurious to the eyes of rabbits.

Inhalation Studies

Male rats were exposed to 1.5 mg/m^3 of DBP vapor for 6 hours per day and 6 days per week for approximately 1 month. There were no significant effects on body or organ weights when the rats were compared to controls. No significant toxic effects were observed.[73] Rats were exposed to 0.5 mg/m^3 and 50 mg/m^3 of DBP mist for 6 hours per day for 6 months. Rats exposed to either concentration had smaller weight gains and greater brain and lung weights than control rats. The higher concentration had a greater effect than the lower concentration.[120]

Intraperitoneal Studies

Acute Toxicity

Acute intraperitoneal toxicity of DBP, DMP, and DEP was studied in mice[51,52,116,121] and in rats[122] (Table 6). The acute intraperitoneal LD_{50}s for rats for DBP, DMP, and DEP were 3.05 ml/kg, 3.38 ml/kg, and 5.06 ml/kg, respectively.

Subchronic and Chronic Toxicity

DEP was administered intraperitoneally in a dose of 2 ml/kg per day to rabbits for 8 days. "Temporary distress" was observed during and after administration. There was no paralysis or other abnormal effect. The intraperitoneal administration of 1.5 ml/kg per day of DEP to guinea pigs for 8 days did not result in any permanent ill effects during or after the experiment.[114] A DEP emulsion was administered intraperitoneally in a dose of 125 mg/kg per day for 6 weeks to 20 to 30 mice. There was slight retardation in weight gain and some evidence of peritonitis. The organ:body weight ratios for liver, heart, lungs, kidneys, spleen, and testes of treated mice were not different from the control mice ratios. No abnormal hematological patterns were observed.[51]

TABLE 4. Subchronic and Chronic Oral Toxicity

Material Tested	*Dose and Vehicle*	*Length of Study*	*Number and Species of Animals*	*Results*	*Reference*
DBP	1 ml/kg in oil 2 times a week	6 weeks	Rats	No adverse effects were reported	109,113
DBP	20 mg/kg	11 weeks and 3 days	Rats Mice	Leukocytosis was observed in rats. Mouse growth was inhibited	103
DBP	0.12 and 1.2 g/kg per day suspensions in olive oil	3 months	10 male and 10 female rats/dose, 40 control rats given only olive oil	1/10 rat from the high dose group died. No specific cause of death was determined. Both DBP doses produced a statistically significant increase in the animals' mean liver weight. No histological evidence of any pathologic changes were found in the liver, kidneys, and spleen	96
DBP	2.5 mg/kg per day	6 months	Rats	No adverse effects were observed	94,112
DBP	0.125 percent in the feed	1 year	20 male and 20 female rats in dosed and in control groups	6/40 rats from the dosed group died. No specific cause of death was determined. No "remarkable" alterations were observed upon gross and histological examination of liver, kidneys, and spleen of dosed rats	96
DBP	0.01, 0.05, 0.25, and 1.25 percent in the feed	1 year	10 rats/dose, 10 control rats	At 0.25 percent in the diet or lower, there was no effect on growth or survival. At 1.25 percent in the diet, 5/10 rats died during the first week. The remaining rats gained weight as did the controls. No rats exhibited significant changes in the number or distribution of elements in the peripheral blood or specific gross pathological changes	84
DBP	1 ml/kg in oil 2 times a week	1½ years	Rats	No pathological changes observed. No effects on hematological parameters or on organ weights	109,111, 133

DMP	2.0, 4.0, and 8.0 percent in the feed	2 years	10 female rats/dose	2.0 percent in the feed had no effect on growth. 4.0 and 8.0 percent had a slight but significant effect on growth. Chronic nephritis seen in rats on 8.0 percent in diet. Mortality rates were not different from those for control rats	95
DEP	3 ml/kg per day	8 days	Rabbits	The rabbits appeared normal for the 8 days and for 2 weeks afterwards. "Temporary distress" was observed after DEP administration	114
DEP	0.2, 1.0, and 5.0 percent in the feed	2, 6, and 16 weeks	5 male and 5 female rats in dosed and control groups on diet for 2 and 6 weeks. 6 rats of each sex, litter mate-paired, in 5.0 percent diet and control groups for 16 weeks. 15 rats of each sex in dosed and control groups on diet for 16 weeks	No changes in behavioral patterns or clinical signs of toxicity were observed. Both sexes on 5.0 percent feed and females on 1.0 percent feed consumed less food and gained less weight than the controls. There was a pattern of reduction in absolute weight and an increase in relative weight of the brain, spleen, heart, kidneys, adrenal glands, gonads, and pituitary of rats on the 5.0 percent diet. A pattern of increases in absolute and relative weights was observed in livers and various parts of the GI tract in these rats. Both liver and kidneys were enlarged but histologically normal	110

TABLE 5. Rabbit Eye Irritation

Material Tested	*Method*	*Results*	*Reference*
DBP	Undiluted DBP instilled into eyes. Eyes examined at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 24.0, and 48.0 hours postinstillation	No grossly observable irritation at any examination time	116
DMP	0.5 ml undiluted DMP applied to corneal center while eyelids are retracted. Lids released after 1 minute. Eye injury scored on a scale of 0–20 points after 18–24 hours	Injury score was >0.1 and <5.0. 5.0 is the level representative of severe injury; necrosis visible after staining and covering ~75 percent of the surface of the eye	117
DMP	0.1 ml undiluted DMP instilled into the conjunctival sac of the eyes. Injury scored on a scale of 0–110 points after 1 and 24 hours	Score was 3.3 after 1 hour and 2.2 after 24 hours	118
DMP	Undiluted DMP instilled into eyes. Eyes examined at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 24.0, and 48.0 hours postinstillation	No grossly observable irritation at any examination time	116
DEP	0.1 ml undiluted DEP instilled into the conjunctival sac of the eyes. Injury scored on a scale of 0–110 points after 1 and 24 hours	Score was 3.2 after 1 hour and 1.5 after 24 hours	118
DEP	Undiluted DEP instilled into eyes. Eyes examined at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 24.0, and 48.0 hours postinstillation	No grossly observable irritation at any examination time	116
Nail polish, 9 percent DBP	0.1 ml instilled into the conjunctival sac of one eye of 9 rabbits. Lids held together for 1 second. In 3 rabbits, treated eye washed at 30 seconds with 20 ml water. Scored on a scale of 0–110 at 24, 48, and 72 hours and 4 and 7 days postinstillation	Unwashed eyes' average score were 11.3, 9.7, 6.8, 4.8, and 0.5 and washed eyes' average scores were 8.3, 7.7, 4.0, 2.7, and 0.3 at 24, 48, and 72 hours and 4 and 7 days postinstillation, respectively	119
Nail preparation, 6 percent DBP	0.1 ml instilled into conjunctival sac of one eye of 6 rabbits. Lids held together for 1 second. Ocular reactions recorded at 24, 48, and 72 hours	No positives for conjunctival redness or chemosis, keratitis, or iritis. "Nonirritating"	105

A series of doses of DBP, DMP, and DEP was injected intraperitoneally into groups of 10 male mice 5 days a week. The apparent LD_{50} was calculated each week until it remained constant for 3 weeks; this was the chronic LD_{50}. DBP, DMP, and DEP reached chronic LD_{50}s in 25, 18, and 14 weeks, respectively. The chronic LD_{50} values were 0.85 ml/kg per day 5 days a week for DBP, 1.18 ml/kg per day 5 days a week for DMP, and 1.39 ml/kg per day 5 days a week for DEP.[116]

Other Studies

The acute intravenous LD_{50} of DBP to male mice was 0.72 g/kg.[72,98] DEP, in a 3 percent acacia suspension, was administered to an anesthetized rabbit through the jugular vein. The DEP was administered in repeated doses of 50 mg/kg to a total dose of 650 mg/kg (time between doses was not given). The first six doses caused a transient fall in blood pressure. The total dose of 650 mg/kg did not cause death or significant change in the animal. Five doses of the 3 percent acacia vehicle did not produce any blood pressure changes.[51] A 0.25 ml/kg dose of DEP in saline was injected slowly into the femoral vein of a dog. At first, respiration was stimulated and then it was paralyzed. The intravenous administration of 0.5 ml of DEP into a rabbit ear vein caused convulsions "similar to those produced by strychnine" within a few minutes. The symptoms "soon" disappeared and the rabbit appeared normal. A larger dose was fatal to rabbits by causing paralysis of respiration.[114]

The intramuscular administration of DBP in a dose of 4 g/kg to 3 rats and 8 g/kg to 3 rats did not result in any deaths, and there was no effect on the growth of the rats.[84]

The subcutaneous LD_{50} of DEP to guinea pigs was greater than or equal to 3 g/kg.[44,101]

SPECIAL STUDIES

Animal Reproduction and Teratology

DBP in doses of 2 and 4 ml/kg, DMP in doses of 0.5, 1, and 2 ml/kg, and saline in a dose of 4 ml/kg were administered intraperitoneally on Days 3, 6, and 9 of gestation to groups of 5 pregnant female rats. Day 1 of gestation was the day sperm were found in vaginal smears. Five control rats survived, and four of those implanted. Five and four rats survived, and four and three implanted, respectively, in the 2 and 4 ml/kg DBP groups. DBP administration resulted in a 50 percent reduction in the number of pups weaned per litter. Two male pups, one from each of two litters in the 2 ml/kg DBP group, had no eyes. In the 0.5, 1, and 2 ml/kg DMP groups, 5, 2, and 5 rats survived, and 4, 1, and 5 implanted, respectively. The numbers of pups weaned were not significantly different from the controls.[123] In another study in which Day 1 of gestation was the day after sperm were found in vaginal smears, groups of 5 pregnant female rats were administered DBP, DMP, and DEP intraperitoneally, in doses of 1/3, 1/5, and 1/10 of a previously determined acute intraperitoneal LD_{50} (3.05 ml/kg for DBP, 3.4 ml/kg for DMP, and 5.06 ml/kg for DEP), on Days 5, 10, and 15 of gestation (Table 7). Control rats were untreated or were administered distilled water, normal saline, or cottonseed oil. The rats were killed on Day 20, 1 day before expected

TABLE 6. Acute Parenteral Toxicity

Material Tested	*Method*	*No. and Species of Animals*	*LD_{50}*	*Comments*	*Reference*
DBP	Single IP injection of 4 dose levels ranging from 0.5 to 16 g/kg	Mice	4.00 g/kg	–	51
DBP	Single IP injection. Pathological changes observed up to 72 hours. Deaths recorded for 7 days	Female mice	14.9 mmole/kg	Pulmonary congestion, edema, and petechial hemorrhage, toxic reaction in spleen, and renal tubular degeneration observed after 72 hours	121
DBP	Undiluted DBP administered and animals observed 7 days for deaths; 2 ml/kg administered IP, 2 mice sacrificed at Days 1, 2, 4, 7, and 10, and heart, lung, kidneys, spleen, liver, pancreas, and bowel examined histologically for irritation	10 male mice/dose to determine LD_{50}; 10 mice of unspecified sex for histology	3.57 g/kg (3.41 ml/kg)	No evidence of significant intraperitoneal irritation	116
DBP	Animals observed for 7 days after IP injection	Female rats	3.05 ml/kg	–	122
DMP	Single IP injection of 4 doses ranging from 0.5 to 16 g/kg	Mice	1.58 g/kg	–	51
DMP	Single IP injection of saline saturated with DMP. 25 ml/kg DMP	Mice	–	No deaths observed	52
DMP	Single IP injection. Pathological changes observed up to 72 hours. Deaths recorded for 7 days	Female mice	18.8 mmole/kg	Pulmonary congestion and atelectasis, toxic reaction in spleen and lymph nodes, and renal tubular necrosis observed after 72 hours	121
DMP	Undiluted DMP administered and animals observed 7 days for deaths; 2 ml/kg administered IP, 2 mice sacrificed at Days 1, 2, 4, 7, and 10, and heart, lungs, kidneys, spleen, liver, pancreas, and bowel examined histologically for irritation	10 male mice/dose to determine LD_{50}; 10 mice of unspecified sex for histology	3.98 g/kg (3.35 ml/kg)	No evidence of significant intraperitoneal irritation	116
DMP	Animals observed for 7 days after IP injection	Female rats	3.38 ml/kg	–	122

DEP	Single IP injection of 4 doses ranging from 0.5 to 16 g/kg	Mice	2.83 g/kg	–	51
DEP	Single IP injection of saline saturated with DEP. 25 ml/kg DEP	Mice	–	No deaths observed	52
DEP	Single IP injection. Pathological changes observed up to 72 hours. Deaths recorded for 4 days	Female mice	12.4 mmole/kg	Pulmonary congestion, edema and petechial hemorrhage, toxic reaction in spleen, and renal tubular degeneration observed after 72 hours	121
DEP	Undiluted DEP administered and animals observed 7 days for deaths; 2 ml/kg administered IP, 2 mice sacrificed at Days 1, 2, 4, 7, and 10, and heart, lungs, kidneys, spleen, liver, pancreas, and bowel examined histologically for irritation	10 male mice/dose to determine LD_{50}; 10 mice of unspecified sex for histology	3.22 g/kg (2.87 ml/kg)	No evidence of significant intraperitoneal irritation	116
DEP	Animals observed for 7 days after IP injection	Female rats	5.06 ml/kg	–	122

TABLE 7. Embryotoxic and Teratogenic Effects of Phthalates[122]

Treatment Groups	*Volume Injected* (ml/kg)*	*Number of Corpora Lutea*	*Number of Resorptions†*	*Number of Dead Fetuses†*	*Number of Live Fetuses†*	*Number of Gross Abnormalities‡*	*Number of Skeletal Abnormalities***
Untreated controls	None	60	0	0	59 (100)	0	0
Distilled water	10.00	59	4 (6.8)	0	55 (93.2)	0	0
Normal saline	10.00	62	7 (11.5)	0	54 (88.5)	1 (1.9)	4 (14.3)
Cottonseed oil	10.00	59	4 (6.8)	0	55 (93.2)	1 (1.8)	3 (10.7)
	5.00	54	3 (6.4)	0	44 (93.6)	0	0
DBP	1.017	64	23 (36.5)	0	40 (63.5)	0	8 (33.3)
	0.610	56	2 (3.6)	0	53 (96.4)	0	7 (24.1)
	0.305	56	4 (7.3)	0	51 (92.7)	0	6 (20.7)
DMP	1.125	55	17 (32.1)	5 (9.4)	31 (58.5)	4 (11.1)	9 (75.0)
	0.675	55	0	1 (1.9)	52 (98.1)	4 (7.5)	6 (35.3)
	0.338	65	21 (33.3)	0	42 (66.7)	4 (9.5)	4 (25.0)
DEP	1.686	57	2 (3.6)	0	54 (96.4)	0	13 (81.3)
	1.012	59	0	0	57 (100.0)	0	8 (47.1)
	0.506	65	28 (44.4)	0	35 (55.6)	0	5 (26.3)

*5 pregnant female rats injected IP on Days 5, 10, and 15 of gestation and sacrificed on Day 20.

†Numbers in parentheses are percent values based on total number of implantations.

‡Numbers in parentheses are percent values based on total number of viable and nonviable fetuses.

**Numbers in parentheses are percent values based on total number of stained fetuses. Generally 30–50 percent of the fetuses were stained.

parturition. Phthalate administration did not interfere with fertility, as reflected by corpora lutea:implantation site ratio. However, there were significant effects upon embryonic and/or fetal development. The average weights of the fetuses from the treated groups and those administered saline were significantly lower than the average weight of the fetuses from the untreated controls. The investigator normally selected 30 to 50 percent of the fetuses for visualization of skeletal abnormalities. There was a significantly higher number of skeletal abnormalities in the fetuses from the test group as compared to the controls.[122] The failure to include historical control data, as well as a positive control in the test program, makes it difficult to evaluate the significance of the results.

DBP was administered in the feed to pregnant mice throughout gestation, and the mice were killed on Day 18. Day 0 of gestation was the day on which a vaginal plug was found. DBP was administered in five dietary concentrations from 80 to 2100 mg/kg. Implantation was not affected, but resorptions and fetal deaths increased with dosage. Maternal weight gain was depressed at the higher dosages and was due to increased embryonic or fetal death. Two of three live fetuses from the 2100 mg/kg DBP group had neural tube defects. Ossification was depressed, but malformation and resorption rates and fetal weights were not significantly affected by DBP administration up to 350 mg/kg per day.[124] In another study, 120 and 600 mg/day of DBP in olive oil were administered by gavage to groups of 10 female rats for approximately 3 months prior to their being mated. Additional groups of female rats received the same doses for 21 days following fertilization. The uteri and fetuses from all the rats were removed on Day 21 of gestation. Fetuses from treated and control rats did not differ significantly in number of sternum ossification foci, in development of the bones of the base of the skull or in the paws of the front and hind extremities, and in rib fusion. The administration of DBP before gestation did not cause any significant changes in other measured parameters. Administration of DBP to pregnant rats did result in lower placental weights, and fetal weights were significantly lower in the high DBP dose group. There were 4, 2, and 22 resorptions in the control, 120, and 600 mg/day DBP groups, respectively.[96]

The dietary administration of DBP, in doses of 10 and 100 mg/kg per day, to two mouse strains for three generations increased the formation of renal cysts in the F_1 and F_2 generations.[98,125] In another three-generation reproduction study, female rats were dosed daily for 6 weeks with 50 percent DBP solution in oil, at a dose of 1 ml/kg, and then were paired with untreated males. The offspring were bred to produce two additional generations; it is not known whether the second and third generations were dosed with DBP. No impairment of reproductive performance was noted. Development, growth, and fertility were normal for all three generations.[109,111,113]

Mutagenesis

The mutagenic activity of DBP, DMP, and DEP for *Salmonella typhimurium* mutants depended on the assay protocol. In the standard Ames test,[126] DBP and DEP were negative in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without metabolic activation.[98,127,128] DEP was also negative in these strains when using a preincubation protocol.[129] In a liquid suspension assay with a 4-hour incubation, DBP, DMP, and DEP were positive in strain TA100

without metabolic activation and negative with metabolic activation.[130] In a modified Ames test in which histidine and biotin were incorporated into the bottom agar, DBP, DMP, and DEP were negative with and without metabolic activation in strain TA98, and DBP was negative with and without metabolic activation in strain TA100. DMP and DEP were positive in strain TA100 without metabolic activation, and the response was dose related. They were negative in strain TA100 with metabolic activation.[76,131] DBP and DEP were not mutagenic to *E. coli*.[98,132]

DNA repair enzyme-deficient *Bacillus subtilis* and *E. coli* were equally or less sensitive to DBP and DEP than the wild-type bacteria.[98,128] Rosenkranz and Leifer[133] reported that DBP did not affect either the wild-type or the DNA repair enzyme-deficient *E. coli*. There were no measurable zones of growth inhibition for either strain.

DBP was tested for mutagenic activity by reversion analysis of the yeast, *S. cerevisiae*. DBP had no mutagenic effect on the yeast whether the test was conducted with or without metabolic activation.[47]

The effects of DBP on Chinese hamster cell chromosome aberrations and sister chromatid exchanges (SCEs) have been investigated in several studies. In one study DBP was negative for chromosome aberrations and SCEs.[128] In another study, the mitotic index was not appreciably decreased when the cells were exposed to DBP in ethanol. A significant increase over the vehicle for the number of SCEs was found, but no dosage effect was found for chromosome aberrations or SCEs.[134] DBP in a 0.2 percent bovine albumin solution was examined in a third study. The percentages of chromosome aberrations with DBP and with bovine albumin were 6 and 1.8 percent, respectively. These results did not conclusively prove that DBP caused chromosome aberrations; DBP was called a suspicious compound by the researchers.[135] DBP, DMP, and DEP, in doses of 0.25 mg/ml, had no effect on chromatid aberrations in human leukocyte cultures compared to controls.[136]

Carcinogenesis

DBP was noncarcinogenic, but specific details of experiments are lacking.[128,134] Carcinogenesis has not been observed in 18-month or longer DBP feeding studies in rats.[137]

Di(2-ethylhexyl)phthalate (DEHP), a compound currently of great concern, is not used in cosmetics. DEHP was tested in a National Toxicology Program carcinogenesis bioassay and was carcinogenic in both rats and mice.[138]

CLINICAL ASSESSMENT OF SAFETY

Dermal Studies

Patch tests have been performed on human subjects with the phthalates.[139,140] The cosmetic industry has conducted studies on the skin irritation, sensitization, and photosensitization of a variety of products containing DBP[141-148] (Table 8). DBP, DMP, and DEP in concentrations of 2 percent in petrolatum and DBP at 5 percent in petrolatum were nonirritating in 48-hour closed patch tests; the 2 percent concentrations were tested on 1532 subjects with 1

positive reaction, and the 5 percent DBP was tested on 53 subjects with no positive reactions. Products containing DBP in concentrations ranging from 4.5 to 9 percent were tested at a concentration of 100 percent. A nail polish containing 9 percent DBP was slightly irritating in a 23-hour patch test on 13 subjects and not irritating in a 48-hour patch test on 25 subjects. The nail polish was tested in a modification of the maximization test on 25 subjects, and no contact sensitization was observed. A deodorant containing 4.5 percent DBP was tested in an antiperspirant efficacy test on 43 subjects; the deodorant was not irritating. It was slightly irritating in a 21-day cumulative irritancy test on 12 subjects. The deodorant was not an allergen in a modification of the repeated insult patch test on 200 subjects. A nail preparation containing 6 percent DBP was tested on 99 subjects in a prophetic patch test, on 48 subjects in a repeated insult patch test, and on 47 subjects in a controlled use study; the nail preparation was nonirritating and nonsensitizing. The nail preparation was also tested for photosensitization in the prophetic patch test and the repeated insult patch test; it was nonphotosensitizing.

Other Studies

A chemical worker accidentally swallowed approximately 10 g of DBP. The worker's symptoms included nausea, vomiting, dizziness, headache, pain and irritation in the eyes, conjunctivitis, and toxic nephritis. He recovered completely after 2 weeks.[94,149]

Proper treatment of a human corneal burn caused by DMP resulted in healing within 48 hours and no loss of vision.[150]

The health status of 147 workers subjected to prolonged occupational exposure to mixtures of phthalate plasticizers (including DBP) was investigated; many workers had a moderately pronounced toxic polyneuritis.[151]

SUMMARY

DBP, DMP, and DEP are dialkyl phthalates. They are primarily used in cosmetics at concentrations of less than 10 percent as plasticizers, solvents, and perfume fixatives.

Some bacteria can use DBP and DMP as carbon sources. These two phthalates and DEP may inhibit the growth of or be toxic to bacteria, algae, yeast, and protozoa. The phthalates may also inhibit the growth of or be toxic to mouse fibroblast, rat cerebellum, and various human cell lines. The phthalate esters have a variety of different effects on mammalian enzymes, both in vivo and in vitro.

Radioactive DBP, after oral administration to rats, hamsters, and guinea pigs, is rapidly metabolized to monobutyl phthalate and other products, and these metabolites are excreted in the urine and feces. In rats, the biliary route seems to be important in the metabolic fate of DBP. Only small amounts of radioactivity are found in rat tissues and organs after oral administration of labeled DBP (^{14}C). DMP is absorbed through human skin. Labeled DEP (^{14}C) was absorbed through the skin of rabbits, and the radioactivity was distributed throughout the body and excreted in the urine. Within several days of the intravenous administration of DEP to pregnant rats, DEP and its metabolic products were found in maternal blood, fetal tissue, amniotic fluid, and placentas.

TABLE 8. Skin Irritation and Sensitization

Material Tested	*Concentration (percent)*	*Method*	*Number of Subjects*	*Results*	*Reference*
DBP	5 percent in petrolatum	48-hour closed patch test on back. Readings 48 and 72 hours after patch application	7 men and 46 women who wore dentures and suffered from "burning mouth syndrome"	No positive reactions	139
DBP, DMP, and DEP	All phthalates at 2 percent in petrolatum	48-hour closed patch test. Joint study by International Contact Dermatitis Research Group	1532	1 positive reaction	140
Nail polish, 9 percent DBP	100	21 23-hour patches on same site on back. 1 hour rest. Scored (0–3) 1 hour after patch removal	2 men and 11 women	Composite total score was 247 (out of a possible maximum of 819). "Slightly irritating"	147
Nail polish, 9 percent DBP	100	48-hour occluded patch to back or forearm	25	No irritation observed	148
Nail polish, 9 percent DBP	100	Modification of the maximization test.[152] 5 48-hour occluded induction patches on back or forearm with 24-hour rests in between them; 24-hour sodium lauryl sulfate (SLS) pretreatment before first patch. 10-day rest period. 1-hour SLS pretreatment followed by 48-hour challenge patch; scored at patch removal and 24 hours later	25	"No instances of contact sensitization"	148
Deodorant, 4.5 percent DBP	100	Antiperspirant efficacy test, normal use conditions (Federal Register 43:46694-732, October 10, 1978). Applied 0.5 g/day for 2 days	43	"No irritation observed" in the axillary region	146
Deodorant, 4.5 percent DBP	100	Modification of the repeated insult patch test.[153,154] 8 48-hour induction patches of 0.2 g on upper arms. 2-week rest 72-hour challenge at original and new sites. Scored at 48 and 96 hours (0–3)	41	9 reactions of 1 (mild erythema) at induction and 1 equivocal reaction at original site at 96-hour challenge observation. "Not an allergen under conditions of the test"	144

Deodorant, 4.5 percent DBP	100	21-day cumulative irritancy test.[155] 21 24-hour occluded patches of 0.3 g applied to the back over 21 days. Each patch scored at removal (0–4)	1 man and 11 women	Total score calculated on the basis of 10 subjects was 140.8 out of a possible maximum of 840. "Slightly irritating"	143
Deodorant, 4.5 percent DBP	100	Modification of the repeated insult patch test.[153,154] 10 24–48 hour occlusive induction patches of 0.2 g ~ 3 times a week. Patches applied to the back. 10-day rest. 72-hour challenge patch. Scored after patch removal (0–4)	159	4 equivocal reactions and 1 score of 1 (erythema) during induction. One equivocal and 1 score of 1 at challenge. "Does not appear to be an allergen under test conditions"	145
Nail preparation, 6 percent DBP	100	Prophetic patch test.[156] 2 24-hour open and closed patches 10 to 14 days apart. 1 open patch irradiated for 1 minute at a distance of 12 in with a Hanovia Tanette Mark I lamp (UV). Scored at patch removal and daily for 5 days thereafter (1+ to 3+)	99	No positive reactions were observed. "Nonirritating, nonsensitizing, nonphotosensitizing"	141
Nail preparation, 6 percent, DBP	100	Repeated insult patch test.[157] 10 24-hour open and closed induction patches 24 hours apart. 2–3 week rest. 48-hour challenge patches. Open and closed patches at inductions 1,4,7, and 10 and at the challenge were irradiated for 1 minute at a distance of 12 in with a Hanovia Tanette Mark I lamp (UV). Scored at patch removal (1+ to 3+)	48	Five 1+ and one 2+ reactions to open patches at induction. One reaction to an open patch at UV challenge. "Nonirritating, nonsensitizing, nonphotosensitizing"	141
Nail preparation, 6 percent DBP	100	Controlled use study for 4 weeks. Fingernails and eyes were examined each week (1+ to 3+)	47	No positive reactions were observed. "Nonirritating"	142

DBP and DMP are degraded by renal homogenates from rats. Both of these phthalates and DEP are hydrolyzed by rat, ferret, and baboon liver and intestinal-mucosal cell homogenates. The phthalates are also metabolized by human liver homogenates and rat intestinal contents. The enzymes involved in DMP metabolism by rat intestinal contents are labile in vitro and mammalian in origin. DMP is hydrolyzed by the gastric contents of rats. Human feces are relatively inactive in degrading the phthalates.

Phthalates are ubiquitous in the environment, and human exposure is likely. DBP has been found in the kidneys, adipose tissue, blood, and umbilical cords of humans.

The acute oral LD_{50} value of DBP for rats ranged from approximately 8 g/kg to 23.0 g/kg; DBP was practically nontoxic to relatively harmless. DMP was practically nontoxic; it had an acute oral LD_{50} value for rats of 6.9 ml/kg. The LD_{50} for rabbits for DEP administered orally was 1.0 g/kg. No adverse effects were reported after the oral administration of doses of DBP of 2.5 mg/kg per day for 6 months or 1 mg/kg two times a week for 1½ years to rats, or of a DBP concentration of 0.25 percent in the feed of rats for 1 year. At doses of 20 mg/kg of DBP for 80 days, growth was inhibited in mice and leukocytosis was observed in rats. At a concentration of 1.25 percent DBP in the diet, 5 of 10 rats died within a week, but the remaining rats survived the diet for a year and appeared normal. A 2.0 percent dietary concentration of DMP fed for 2 years to rats had no effect on growth, 4.0 and 8.0 percent inhibited growth, and rats fed 8.0 percent had chronic nephritis. Doses of DEP of 3 ml/kg per day for 8 days and a 0.2 percent concentration of DEP in the diet for up to 16 weeks had no adverse health effects in rabbits and rats, respectively. Concentrations of 1.0 and 5.0 percent DEP in the diet for up to 16 weeks reduced the growth of rats.

The oral administration of DBP produced testicular atrophy in the rat, mouse, guinea pig, and ferret but not in the hamster in a dose of 2.0 g/kg per day for 10 days. The simultaneous administration of zinc provided substantial protection against testicular damage in the rat. Testicular atrophy was not observed after the administration of DMP and DEP in oral doses of 7.2 mmole/kg per day (approximately 1.4 g/kg per day DMP and 1.6 g/kg per day DEP) to rats.

The acute dermal LD_{50} for DMP for rabbits was greater than 10 ml/kg. The subacute (90-day) dermal LD_{50}s of DBP and DMP to rabbits were greater than 4 ml/kg per day. At doses of 0.5 to 4.0 ml/kg per day, DBP was slightly irritating to skin, DMP was irritating in molting areas only, and there was no evidence of sensitization by either of the phthalates. Renal damage was observed in rabbits that died during this study, and survivors receiving 2.0 to 4.0 ml/kg per day of DBP and DMP had varying degrees of nephritis. Phthalate emulsions were injected intradermally into rabbits; DBP produced a mild to moderate inflammatory response, and DMP and DEP produced a marked inflammatory response. The results of other such experiments varied with the vehicle used.

Undiluted DBP, DMP, and DEP were instilled into the eyes of rabbits; irritation was minimal. However, with long contact time, DMP may be irritating to the rabbit eye.

The inhalation and intraperitoneal, intravenous, intramuscular, and subcutaneous administration of the phthalates have been studied in a variety of laboratory animals. Results depended on the route, the species, and the dose.

Several studies suggest that the administration of DBP, DMP, and DEP to

pregnant rats may increase the number of resorptions and have significant effects upon embryonic and fetal development. Gross and skeletal abnormalities in offspring have been observed in some cases. The dietary administration to three generations of mice of doses up to 100 mg/kg per day DBP has increased the formation of renal cysts in the second and third generations.

The mutagenic activity of DBP, DMP, and DEP toward *S. typhimurium* mutants depends on the assay protocol; studies have been both negative and positive in the same strains. DBP and DEP were not mutagenic for *E. coli*. DNA repair enzyme-deficient *B. subtilis* and *E. coli* were not more sensitive to DBP and DEP than the wild-type bacteria; one study reported that DBP did not affect either the DNA repair enzyme-deficient *E. coli* or the wild-type. DBP was negative in a *S. cerevisiae* reversion analysis with and without metabolic activation. DBP was both negative and positive for chromosome aberrations and sister chromatid exchanges in Chinese hamster cells; it has been called a "suspicious compound." DBP, DMP, and DEP have no effect on chromosome aberrations in human leukocyte cultures.

DBP was not carcinogenic in chronic (18-month or longer) feeding studies in rats.

There were no positive reactions among 53 human subjects patch tested with 5 percent DBP. One positive reaction was observed when 1532 subjects were patch tested with DBP, DMP, and DEP at a 2 percent concentration. Cosmetic formulations containing up to 9 percent DBP were tested in a variety of patch test procedures; in some procedures some of the formulations were slightly irritating. In other cases, no irritation was observed. Sensitization and photosensitization were not observed.

DISCUSSION

A comparison of the chemical structures of the phthalates suggests that DBP may have the greatest toxicological significance. Data are limited for both DMP and DEP, and, in particular, there are clinical phototoxicity and photosensitivity data only for a preparation containing DBP. However, the Panel believes that the information contained in this report is adequate for a safety assessment of all three phthalates.

DBP but not DMP and DEP caused testicular injury in laboratory animals. The combined teratogenic test data available to the Expert Panel are not adequate to conclude that DBP, DMP, or DEP are proven teratogens. The concentrations used in cosmetic products and the rapid metabolism and elimination of these ingredients, as indicated by experimental studies, minimize the significance of the observations of testicular damage by DBP and the conflicting teratogenic test results. The Panel notes that the information provided in the literature on the carcinogenicity of DBP is limited and does not permit an evaluation of the assays performed and the results obtained. The results of mutagenesis studies, however, are essentially negative.

CONCLUSION

On the basis of the available data, the Panel concludes that Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate are safe for topical application in the present practices of use and concentration in cosmetics.

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