Amended Safety Assessment of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date (i.e., by **December 19, 2026**) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

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ABBREVIATIONS

ΣAA	sum of anti-androgenic metabolites
AChE	acetylcholinesterase
ADHD	attention-deficit/hyperactivity disorder
AKT	protein kinase B
ALB	albumin
ΣAll	sum of all phthalate metabolites
ALT	alanine aminotransferase
AOP	adverse outcome pathway
AST	aspartate aminotransferase
BAL	bronchoalveolar lavage
BaP	benzo[a]pyrene
CAT	catalase
Cdyn	dynamic compliance
CGRP	calcitonin gene-related peptide
CHO	Chinese hamster ovary
CI	confidence interval
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CRP	C-reactive protein
Cx43	connexin 43
ΣDBP	sum of Dibutyl Phthalate metabolites
DEF	*S,S,S*-tributylphosphorotrithioate
Dictionary	*International Cosmetic Ingredient Dictionary*
DMSO	dimethyl sulfoxide
DNFB	2,4-nitrofluorobenzene
EC	European Commission
ECHA	European Chemicals Agency
EITC	eosin 5-isothiocyanate
ELISA	enzyme-linked immunosorbent assay
EPA	US Environmental Protection Agency
ER	estrogen receptor
FDA	US Food and Drug Administration
Fe^{2+}	ferrous iron
FITC	fluorescein isothiocyanate
FOR	fecundity odds ratio
FT3	free thyroxine
FT4	free thyroxine free thyroxine
GC-MS	gas chromatography – mass spectrometry
GDM	gestational diabetes mellitus
GLUT	glucose transporter
GPER	G-protein-coupled estrogen receptor
GPMT	guinea pig maximization test
GPX4	glutathione peroxidase-4
GSH	glutathione
GSSG	oxidized glutathione
HbA1c	glycosylated hemoglobin
hER	human estrogen receptor
$HER2^{+}$	human epidermal growth factor receptor 2 positive
hEST	human embryonic stem cell test
HPLC	high-performance liquid chromatography
HRIPT	human repeated-insult patch test
IARC	International Agency for Research on Cancer
IC_{50}	50% inhibition of growth and viability
ID_{50}	50% inhibition of differentiation
IgE	immunoglobulin E
IGF	insulin-like growth factor
IL	interleukin
IQ	intelligence quotient
JAK 1	Janus kinase 1
LABC	levator ani plus bulbocavernosus muscles

LC-MS/MS	liquid chromatography-tandem mass spectrometry
l.o.	leave-on
LOAEL	lowest-observed-adverse-effect level
LOEC	lowest-observed-effect concentration
MDA	malondialdehyde
MDI	methylenediphenyl diisocyanate
MNG	multinucleated gonocytes
MoCRA	Modernization of Cosmetics Regulation Act of 2022
MOE	margin of exposure
MOS	margin of safety
MRL	minimal risk level
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	nuclear factor kappa B
NICU	neonatal intensive care unit
NO	nitric oxide
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NR	not reported
Nrf2	nuclear factor erythroid 2-related factor
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
8-OHdG	hydroxydeoxyguanosine
OR	odds ratio
OVA	ovalbumin
OXA	oxazolone
Panel	Expert Panel for Cosmetic Ingredient Safety
PBTK	physiologically-based toxicokinetics
PEITC	phenethyl isothiocyanate
PET	polyethylene terephthalate
PI3K	phosphatidyl inositol 3 kinase
POD	point of departure
PPARα	peroxisome proliferator-activated receptor alpha
PRDX6	peroxiredoxin-6
q-PCR	quantitative polymerase chain reaction
Re	expiratory resistance
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
Res	resveratrol
RfD	reference dose
Ri	inspiratory resistance
RIFM	Research Institute for Fragrance Materials
RITC	rhodamine B isothiocyanate
RLD	Registration and Listing Data
r.o.	rinse-off
ROS	reactive oxygen species
RT-PCR	reverse transcription-polymerase chain reaction
SCCNFP	EC Scientific Committee on Cosmetic and Non-Food Products
SCCP	EC Scientific Committee on Consumer Products
SCCS	EC Scientific Committee on Consumer Safety
SCE	sister chromatid exchange
SCO	Sertoli cell-only
SD	standard deviation
SED	systemic exposure dose
SF-1	steroidogenic factor-1
SHBG	sex hormone binding globulin
SOD	superoxide dismutase
STAT6	signal transducer and activator of transcription 6
STZ	streptozotocin
TDI	toluene 2,4-diisocyanate
TG	test guideline
TMA	trimellitic anhydride

TNF-α	necrosis factor-α
TPN	total parenteral nutrition
TRPA1	transient receptor potential ankyrin 1
TSLP	thymic stromal lymphopoietin
TT3	total triiodothyronine
TT4	total thyroxine
TUNEL	terminal deoxynucleotide transferase-mediated deoxy-UTP nick labeling
US	United States

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate, which are reported to function as fragrance ingredients, plasticizers, and solvents in cosmetic formulations. Diethyl Phthalate is also reported to function as a denaturant. The Panel reviewed the available data to determine the safety of these ingredients. Accordingly, the Panel issued an amended report with a revised conclusion stating that Diethyl Phthalate is safe in cosmetics in the present practices of use and concentration described in this safety assessment; the Panel also concluded that the available data are insufficient to make a determination of safety for Dibutyl Phthalate and Dimethyl Phthalate under the intended conditions of use in cosmetic formulations.

INTRODUCTION

This assessment reviews the safety of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate as used in cosmetic formulations. According to the *International Cosmetic Ingredient Dictionary* (*Dictionary*), these ingredients are reported to function as fragrance ingredients, plasticizers, and solvents in cosmetic formulations (Table 1).[1] Diethyl Phthalate is also reported to function as a denaturant.

Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate are structurally very similar and have similarly reported functions. The Expert Panel for Cosmetic Ingredient Safety (Panel) first reviewed the safety of these ingredients in a report that was published in 1985, with the conclusion that these ingredients are "safe for topical application in the present practices of use and concentration in cosmetics."[2] This conclusion was reaffirmed in re-reviews that were published in 2005 and 2017.[3,4] This report was re-opened in 2024 when Dibutyl Phthalate was placed on the 2024 Priorities List following nomination by the FDA for cause. Excerpts from the summaries of the 1985 report, the unpublished draft amended report that preceded the 2005 published re-review, and the 2005 and 2017 re-review summaries are disseminated throughout the rest of the document, as appropriate, and are identified by *italicized* text. Additionally, where appropriate, data regarding the monoester metabolites have been included in this safety assessment. This report was placed on the 2024 Cosmetic Ingredient Review (CIR) Priorities List following nomination by the US Food and Drug Administration (FDA) for cause.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last performed in September 2026. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some chemical and toxicological data on the phthalate ingredients included in this safety assessment were obtained from robust summaries of data submitted to the European Chemicals Agency (ECHA) by companies as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) chemical registration process.[5-7] These data summaries are available on the database for ECHA, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition and Structure

The definitions and structures of the ingredients included in this review are provided in Table 1. These 3 ingredients are each a short-chain alkyl diester of *o*-phthalic acid, resulting from esterification with butyl, ethyl, or methyl alcohol.

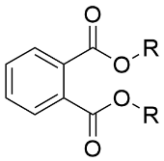


Figure 1. Phthalates, wherein both R substituents are either butyl, ethyl, or methyl.

Chemical Properties

Chemical properties of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate are described in Table 2. Dibutyl Phthalate is a colorless, oily liquid with a molecular weight of 278 g/mol.[2] The log P_{ow} at 30ºC is 4.46.[5] Diethyl Phthalate is a colorless or pale yellow, oily liquid with a molecular weight of 222 g/mol.[2,6] The log P_{ow} at 41ºC is 2.2.[6] Dimethyl Phthalate is a colorless, oily liquid with a molecular weight of 194 g/mol.[2] The log P_{ow} at 25ºC is 1.54.[7]

Method of Manufacture

Phthalate esters can be prepared by the reaction of phthalic acid with alcohol.[2] Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate are produced industrially by the reaction of phthalic anhydride with butyl alcohol, ethyl alcohol, and methyl alcohol, respectively. Dibutyl Phthalate 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. The exact manufacturing processes for Diethyl Phthalate and Dimethyl Phthalate are proprietary information.

Impurities

Diethyl Phthalate may contain Dimethyl Phthalate or ethyl methyl phthalate as impurities.[2] *Additional impurities may be isophthalic acid, terephthalic acid, and maleic anhydride.*[8] *The purity of phthalate esters has been reported to be > 99%.*

Natural Occurrence

Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate have been identified in the organic solvent extracts, root exudates, and essential oils of many different plant species.[9] These phthalates have also been isolated and purified from various algae, bacteria, and fungi.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate in cosmetics. Registration and Listing Data (RLD) obtained from the FDA report frequency of use, and responses to a survey conducted by the Personal Care Products Council (Council) indicate maximum reported concentrations of use; it is these values that define the present practices of use and concentration that are assessed by the Panel. Since 2024, as a result of the Modernization of Cosmetics Regulation Act of 2022 (MoCRA), manufacturers and processors are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-yr period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products, are not included in this exemption.[10] Another change resulting from MoCRA is the addition of tattoo preparations (permanent tattoo inks, temporary tattoo inks, and other tattoo products) to the product categories for which companies need to list their products with FDA. However, evaluating the safety of ingredients as used in tattoo preparations is not within the purview of the Panel; accordingly, such use is not included as part of the present practices of use that are assessed by the Panel.

According to RLD obtained from the FDA in 2025, Diethyl Phthalate is used in 284 formulations, with most of the uses reported in fragrance preparations (211; Table 3).[11,12] Additionally, the RLD reported Dibutyl Phthalate is used in 2 "other" preparations (Category 17) and Dimethyl Phthalate is used in 2 skin care preparations. However, according to communications from the FDA, the 2 uses reported for Dibutyl Phthalate as "other" preparations are actually liquid bandage products, which are not cosmetics, and thereby outside the purview of the Panel. Thus, the Panel's safety assessment conclusion does not apply to these products, and accordingly, there are no cosmetic uses reported for Dibutyl Phthalate, as reflected in Table 3. Between 2004 and 2010, the FDA monitored the use of phthalates in cosmetic products and found that Dimethyl Phthalate and Dibutyl Phthalate were at that time already "used rarely." [14]

The results of the concentration of use survey conducted by the Council in 2025 indicate Diethyl Phthalate has a maximum concentration of use range of 0.1 - 0.15%, with 0.15% reported in leave-on face and neck products.[13] No concentrations of use were reported for Dibutyl Phthalate or Dimethyl Phthalate; however, responses to the survey indicated that Dibutyl Phthalate and Diethyl Phthalate may be present in cosmetics as impurities.

In a study from 2017, Dibutyl Phthalate, Diethyl Phthalate and Dimethyl Phthalate had mean concentrations of 3.2 x 10^{-6} % (0.032 ppm), 0.167% (1670 ppm), and 0.003225% (32.25 ppm), respectively, in 42 branded perfumes from 11 countries, including the US.[15] This research group described the mean concentrations of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate in the perfume manufactured in the US to be 9.5 x 10^{-8} % (0.00095 ppm), 0.000343% (3.43 ppm), and 0.00434% (43.40 ppm), respectively.[16]

The RLD and the results of the concentration of use survey indicate that Diethyl Phthalate is used in products that may come into contact with the eyes (e.g., 1 use was reported in eyelash and eyebrow preparations; concentration not reported).[11-13] Additionally, some of the phthalates may be used in cosmetic sprays, and could possibly be inhaled; for example, Diethyl Phthalate is reported to be used in colognes, perfumes, and other fragrance preparations (concentrations not reported). In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., would not enter the lungs) to any appreciable amount. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable. However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

Products containing Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined by the FDA, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such for some, but not all, product

categories in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available, in some instances. Additionally, the concentration of use surveys are conducted based on product categories as stated in the RLD. None of the reported product categories for these ingredients as listed in the RLD include a designation using airbrush application, so it is possible that this ingredient is used with airbrush delivery systems, but not reported as such. Additionally, no concentration of use data were provided indicating airbrush application. Nevertheless, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

In a review published in 2001, the Research Institute for Fragrance Materials (RIFM) concluded that Diethyl Phthalate is not considered to present any significant toxic liability for its uses as a solvent and vehicle in cosmetic products.[17] No toxicological endpoints of concern were identified. The Expert Panel for Fragrance Safety has not published a monograph on the phthalate ingredients.

Under European regulations for cosmetic ingredients, Dibutyl Phthalate is listed in Annex II, List of Substances Prohibited in Cosmetic Products.[18] In 2004, the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) determined that Dibutyl Phthalate should not be intentionally added to cosmetic products.[19] Dibutyl Phthalate has been classified as a Category 2 toxic substance to reproduction that "may cause harm to the unborn child."

In the US, Diethyl Phthalate is permitted to be used as a denaturant (27CFR§21.71, 21.72, 21.106, and 21.151). Diethyl Phthalate and Dimethyl Phthalate are not restricted from use in any way under the rules governing cosmetic products in the European Union.[18] In 2002, the SCCNFP opined that the safety profile for Diethyl Phthalate supported its use in cosmetic products;[20] this opinion was reaffirmed by the SCCNFP in 2003[21] and the Scientific Committee on Consumer Products (SCCP) in 2007.[22]

Non-Cosmetic

Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate are used as solvents and plasticizers for nitrocellulose, cellulose acetate, and cellulose acetate-butyrate compositions.[2] *They are used in the manufacture of varnishes and plastics and in insecticides and insect repellents. Dibutyl Phthalate 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. Dimethyl Phthalate is used as a camphor substitute in the manufacture of celluloid, as a wetting agent, and as an alcohol denaturant.*

Phthalate esters are ubiquitous in the modern environment, found in clothing textiles, packaged foods, and even in household dust.[8] *Dibutyl Phthalate and other phthalates are primarily used as plasticizers in flexible polyvinyl chloride and in cellulose plastics. Additional applications include uses as solvents, lubricants, fixatives, and detergents. Dibutyl Phthalate is used in the manufacture of medical products including denture base materials, and ocular prosthetic. Other products containing Dibutyl Phthalate include plastic wraps and containers for food, paints, plastic toys and inks. Diethyl Phthalate is found in many plastic consumer products and plastic-coated materials, packaging for food and drug products. Dimethyl Phthalate is used as an insect repellant and, with permethrin, as a fabric treatment on tents to protect campers from mosquitoes.*

Globally, Dibutyl Phthalate has been detected in commercial polyethylene terephthalate (PET) bottled water at concentrations as high as 82.8 µg/l.[23] Analysis of feminine hygiene products (pads, panty liners, and tampons) in the US found Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate to have mean concentrations of 0.90, 1.35, and 0.55 ng/g, respectively.[24] Another study (published in 2020) reported that feminine bactericidal creams and possibly cosmetic deodorant sprays and feminine powders had measurable concentrations of Dibutyl Phthalate (mean 0.52 ng/g), Diethyl Phthalate (mean 0.51 ng/g), and Dimethyl Phthalate (mean 0.60 ng/g).[24]

In 2012, the FDA issued guidance for industry on limiting the use of Dibutyl Phthalate as an excipient in regulated drug and biologic products, recommending that use of this ingredient be avoided if an alternative is available.[25] Dibutyl Phthalate (> 1000 ppm or 0.1%) has been prohibited in the manufacturing, sale, distribution, or importation of children's toys and childcare articles (16CFR§1307). Diethyl Phthalate is permitted to be used as a plasticizer in food packaging material (21CFR§181.27). The European Food Safety Authority established a tolerable daily intake for Dibutyl Phthalate at 0.01 mg/kg bw/d based on reproductive effects.[26]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

The dermal absorption rates of undiluted phthalate esters were determined in an in vitro study using rat and human skin.[8] *The diffusion of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate across the epidermal samples was*

measured for various exposure periods. In rat skin (9-11 samples), the mean steady-state absorption rates for Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate were 93.35 ± 0.94, 413.67 ± 92.79, and 410.55 ± 41.77 µg/cm²/h, respectively. In human skin (11-15 samples), the mean steady-state absorption rates for Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate were 2.40 ± 0.63, 12.75 ± 1.12, and 30.51 ± 6.43 µg/cm²/h, respectively.

In a study of the percutaneous absorption of Dibutyl Phthalate using static diffusion cells with full-thickness rat skin (haired and hairless; 1.76 cm²), an infinite dose of [^{14}C]Dibutyl Phthalate (50 mg/cm²) was applied to the stratum corneum side of the skin samples for 24 h.[8] Aliquots (200 µl) of the receptor fluid on the dermis side of the skin sample were collected periodically over the 24-h period. The percutaneous absorption fluxes of Dibutyl Phthalate across the skin sections of haired and hairless male rats were 26 ± 1 and 39 ±1µg/cm²/h, respectively. All of the radioactivity contained in the receptor fluid had the same HPLC retention time as monobutyl phthalate. Addition of an esterase inhibitor caused a reduction of 99% of the absorption flux of [^{14}C], and monobutyl phthalate was barely detectable in the receptor fluid.

In another absorption study, shaved rat skin and human breast skin were exposed to Diethyl Phthalate.[8] Absorption through the rat skin was rapid and extensive. There was an initial lag for the first 8 h during which the absorption was 1.7%. From 8 - 72 h, absorption was almost linear, reaching 35.9% on occluded skin and 38.4% on unoccluded skin. The mean rate of absorption in rat skin between 12 - 72 h was 0.094 mg/cm²/h (occluded) and 0.103 mg/cm²/h (unoccluded). The percutaneous absorption of Diethyl Phthalate through human skin was slow and incomplete. The initial lag time was 10 h. At 8 h, 0.1% had absorbed in occluded skin and 0.2% in unoccluded skin. At 72 h, 3.9 and 4.8% of applied Diethyl Phthalate had absorbed in occluded and unoccluded skin, respectively. The mean rate of absorption of Diethyl Phthalate through human breast skin between 12 - 72 h was 0.011 mg/cm²/h (occluded) and 0.014 mg/cm²/h (unoccluded). In this in vitro model, the rate of absorption of Diethyl Phthalate through rat skin was significantly ($p < 0.05$) faster than through human skin.

The in vitro permeation of Diethyl Phthalate was studied using abdominal guinea pig skin mounted into a Franz-type diffusion cell.[8] The donor side of the skin was pre-treated for 2 h with 10 mM sodium dodecyl sulfate, 10 mM benzalkonium chloride, or 0.5 % polyoxyethylene (10) oleyl ether. After the surfactant solution was rinsed, a solution of 0.05 % Diethyl Phthalate was added to the donor side for 16 - 24 h. A control condition consisted of Diethyl Phthalate treatment without surfactant pre-treatment. Additional skin samples were treated with 0.05% Diethyl Phthalate in a mixture with each of the respective surfactants described above, without surfactant pre-treatment. Skins pre-treated with sodium dodecyl sulfate, benzalkonium chloride, or polyoxyethylene (10) oleyl ether had respective permeation rates of 190, 174, and 110% of the permeation rate of the control condition. Co-treatment of Diethyl Phthalate with sodium dodecyl sulfate, benzalkonium chloride, or polyoxyethylene (10) oleyl ether resulted in Diethyl Phthalate permeation rates of 82.0, 105.7, or 99.5% of the control. The cumulative amount of Diethyl Phthalate permeation through guinea pig skin without surfactant at 24 h was 21.8 µg/cm².

In an in vitro absorption study, [^{14}C]Dibutyl Phthalate (radiochemical purity > 97%; specific activity 21.1 mCi/mmol) in an oil-in-water emulsion was applied to flow-through diffusion cells (0.64 cm²) using hairless guinea pig dermatomed skin (200 - 320 µm thick) at approximately 0.5 µCi test material/cm².[27] Absorption was measured at 24 and 72 h from the receptor fluid consisting of HEPES-buffered Hanks' balanced salt solution and 4% bovine serum albumin. At the end of the 24 or 72 h experiments, the skin was washed and rinsed to measure unabsorbed test material. Tape stripping was utilized to determine how much test material remained in the skin after 24 h. The absorption of Dibutyl Phthalate in the receptor fluid over 24 h was 27.1 ± 1.9%, with 29.2 ± 2.4% remaining in the skin (total skin penetration over 24 h = 56.3 ± 2.7%). Over 72 h, the amount of Dibutyl Phthalate in the receptor fluid was 59.9 ± 3.2%, with 13.7 ± 3.1% remining in the skin (total skin penetration over 72 h = 75.6 ± 4.7%).

A monolayer of human keratinocytes cultured on a Transwell® insert (24-mm diameter, 0.4-µm polycarbonate membrane; effective diffusion area 4.52 cm²) was used to evaluate the percutaneous penetration of Diethyl Phthalate and Dibutyl Phthalate.[28] A complete culture medium containing 200 or 500 ng/ml of test compound was applied to the apical side (1.5 ml) for 540 min, and 2.5 ml Hanks' balanced salt solution served as the receptor phase. Samples of receptor fluid, apical medium, and cell layers were collected and analyzed by gas chromatography – mass spectrometry (GC–MS). The 9-h absorption factor (AF) values, which included both the fraction recovered in the receptor fluid and that retained in the cell layer, for Diethyl Phthalate were 62 and 39%, and for Dibutyl Phthalate were 53 and 34%, in the low- and high-dose groups, respectively. The corresponding 1-h AF-values were 6.9 and 4.3% for Diethyl Phthalate, and 5.8 and 3.7% for Dibutyl Phthalate. The permeability coefficient values were inversely correlated with the log K_{ow}, while cumulative amounts of phthalates in the cells increased with log K_{ow}, indicating phthalates with higher hydrophobicity were more likely to remain on the skin. This apparent parabolic relationship was broadly consistent with the competing influences of lipophilicity and molecular size described by the Potts–Guy equation.

In an in vitro dermal penetration study, Diethyl Phthalate and Dibutyl Phthalate (> 99% pure; each at 5.4 mM in 40% ethanol/pH 7.4 buffer) were applied for 12 h to full-thickness dorsal skin from 8-wk-old female nude mice or 1-wk-old pigs mounted in Franz diffusion cells (diffusion area = 0.785 cm²; n = 4).[29] Diethyl Phthalate and Dibutyl Phthalate accumulated in both skin types and were detected in receptor fluid. The respective skin accumulation values for Diethyl Phthalate and Dibutyl Phthalate were 1.37 ± 0.26 and 10.8 ± 0.51 nmol/mg in nude mouse skin and 0.13 ± 0.01 and 0.41 ± 0.07 nmol/mg in

pig skin. The respective flux values were 96.5 ± 7.97 and 94.7 ± 6.49 nmol/cm^2/h across nude mouse skin and 26.9 ± 6.82 and 11.9 ± 3.20 nmol/cm^2/h across pig skin. Follicular-cast analysis of nude mouse skin showed deposition of Dibutyl Phthalate, but not Diethyl Phthalate, in hair follicles.

A percutaneous absorption study of Dimethyl Phthalate and 2 other phthalates (dipropyl phthalate and dipentyl phthalate) tested these materials using different carriers, such as ointments, creams, and lotions.[30] The study used rat skin in Franz diffusion cells using phosphate-buffered saline as the receptor fluid. There was 0.2 g of phthalate per 10 g of carrier. The lag time of the ointment, base cream, and lotion containing Dimethyl Phthalate were 0.891, 0.888, and 0.885 h, respectively. The permeation rate of the ointment, base cream, and lotion containing Dimethyl Phthalate were 1.391, 1.216, and 1.120 µg/cm^2/h, respectively. Of the 3 phthalates tested, Dimethyl Phthalate had the fastest permeation rate, regardless of carrier type.

Animal

The dermal absorption of Dibutyl Phthalate was studied in 3 female hairless guinea pigs.[27] [^{14}C]Dibutyl Phthalate (radiochemical purity > 97%; specific activity 21.1 mCi/mmol) in an oil-in-water emulsion was applied in a single dose for 24 h at approximately 0.5 µCi test material/cm^2 (dosage of 1 mg/cm^2; application site 3.0 cm^2). The test site was covered to prevent test material from being rubbed off. Urine and feces were collected during the application period. At the end of 24 h, the guinea pigs were killed and the test site was washed and rinsed. The washes and rinses were analyzed to determine how much test material was not absorbed. Skin from the treatment site, ovaries, livers, and kidneys were analyzed for systemic absorption. Approximately 62.0 ± 2.0% of the applied dose was absorbed systemically. Most of the Dibutyl Phthalate was found in the urine (60.4 ± 1.8%) and the amount systemically absorbed in the ovaries, kidneys, and liver were less than 2%. Approximately 2% of Dibutyl Phthalate remained on the skin.

Absorption, Distribution, Metabolism, and Excretion

In Vitro

Dibutyl Phthalate and Dimethyl Phthalate (0.4 mg/ml each) almost completely disappeared after 2 h of incubation with rat liver homogenates, while action with rat kidney homogenates was slower.[2] Approximately 90% of Dibutyl Phthalate and 95% of Dimethyl Phthalate disappeared during a 5-h incubation. In another study, Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate (1 mg/ml each) were incubated for 16 h with the contents of rat stomach, small intestines, or cecum. The test material was metabolized rapidly to monobutyl phthalate when incubated with the contents of rat small intestine, while metabolism was slower in the presence of rat cecal contents. Monobutyl phthalate was the only product of metabolism; complete hydrolysis to phthalic acid did not occur.

In an in vitro study of the intestinal absorption of Dibutyl Phthalate and Dimethyl Phthalate using an everted gut-sac preparation from the rat small intestine, most of Dibutyl Phthalate and Dimethyl Phthalate hydrolyzed to the corresponding monoester before crossing the intestinal mucosa.[2] Only 4.5% of Dibutyl Phthalate and 18.8% of Diethyl Phthalate crossed the intestine intact. Inhibition of mucosal esterases by an esterase inhibitor reduced the amount of Dibutyl Phthalate hydrolyzed to monobutyl phthalate. Approximately the same amount of intact Dibutyl Phthalate was absorbed by the intestine with and without the esterase inhibitor. Intestinal absorption of this compound may be controlled by the hydrolysis of Dibutyl Phthalate to monobutyl phthalate.

Animal

Dermal

The dermal absorption and distribution/excretion fate of radiolabeled phthalates was examined in using F344 rats.[8] [^{14}C]Dibutyl Phthalate, [^{14}C]Diethyl Phthalate, or [^{14}C]Dimethyl Phthalate (157 µmol/kg each) was applied to a 1.3 cm diameter patch of shaved skin and covered with a plastic cap. Rats were then placed in a metabolic cage that allowed separate collection of urine and feces which were collected every 24 h for 7 d. [^{14}C] was excreted in the urine and feces at a nearly constant rate of 10 - 12% of the [^{14}C]Dibutyl Phthalate dose per 24 h throughout the 7 d of collection. Approximately 24 times as much [^{14}C] was excreted in the urine as in feces. For [^{14}C]Diethyl Phthalate, 24% was excreted in the first 24 h, then slowed to 11% in the second 24 h, and slowed to 5% every 24 h for the remaining period of measurement. The rate of [^{14}C]Dimethyl Phthalate excretion was a relatively constant 6 - 7.5% per 24 h over the entire 7 d. Rats were killed on the seventh day, and various tissues and organs were analyzed for the presence of [^{14}C]. Most of the absorbed phthalate had been excreted in the urine and feces. Very little was retained in the tissues.

In another experiment in this report, 10 µl/cm^2 neat [^{14}C]Dibutyl Phthalate was applied to the shaved backs of male Sprague-Dawley rats and covered with a perforated plastic cap.[8] Groups of 3 to 8 rats were killed at several time points between 0.5 - 72 h after application of the test material. Blood, urine and feces were collected for analysis. Dibutyl Phthalate penetrated the skin rapidly. Approximately 30 min after application, 20% of the applied dose had entered the skin. Within the first 8 h, the absorption flux was 43 µg/cm^2/h. Between 8 - 48 h, the rate of absorption flux increased to 156 µg/cm^2/h. At the 24- and 72-h time points, the radiolabled test material that absorbed into the skin had radially diffused into the surrounding stratum corneum and/or epidermis. Dibutyl Phthalate was hydrolyzed by skin esterase before reaching systemic circulation. At all time points, unchanged Dibutyl Phthalate accounted for 2% of the plasma radioactivity, whereas monobutyl phthalate and monobutyl phthalate-glucuronide combined accounted for 61 - 85% of the plasma radioactivity.

The mean ratio of plasma monobutyl phthalate to monobutyl phthalate-glucuronide was 1.3. In the urine, monobutyl phthalate and monobutyl phthalate-glucuronide together accounted for 50 to 66% of the urinary radioactivity, with no significant correlation to time. The mean ratio of monobutyl phthalate to monobutyl phthalate-glucuronide in urine was 0.73. Over the maximum 72-h exposure, the total urinary excretions of monobutyl phthalate and monobutyl phthalate-glucuronide were 14.1 ± 1 and 20 ± 1% of the applied dose, respectively. Similar experiments were conducted using Sprague-Dawley female rats and hairless rats for comparison. Female haired rats showed similar Dibutyl Phthalate absorption rates as the males. Male hairless rats had a higher percutaneous absorption of Dibutyl Phthalate (75% of the applied dose). The urinary excretion rate of [^{14}C]Dibutyl Phthalate in hairless rats was 2.5-fold higher than that of the haired rats.

[^{14}C]Diethyl Phthalate was applied topically to rabbits.[2] Analysis of urine indicated that approximately 9% of the radioactivity was excreted after 24 h, 14% after 48 h, and 16 to 20% within 72 h. After 3 d 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.

Radiolabeled Diethyl Phthalate was applied on the shaved backs (25 mm diameter; occluded) of 3 female albino rabbits at approximately 299 - 569 mmol per rabbit.[8] Blood samples were collected at 1, 24, 48, 72, and 96 h after application, and urine and feces were collected at 24, 48, 72, and 96 h. At 96 h after application, the rabbits were killed and tissues from the vital organs were collected. The results of the study suggested that Diethyl Phthalate is rapidly absorbed through the skin, widely distributed to tissues, and actively excreted. Approximately 50% of the applied Diethyl Phthalate was excreted in 4 d. It was estimated that it would take approximately 1 mo to eliminate the entire Diethyl Phthalate dose applied. There was evidence of higher deposition of Diethyl Phthalate in the adrenal tissue and kidneys than in other tissues.

Oral

Dibutyl Phthalate was administered by gavage to male rats in 2 doses of 0.2 ml 24 h apart.[2] Urine was collected for 48 h after the first dose, and Dibutyl Phthalate and its metabolites were quantitated. A total of 24.6% of the phthalate moiety was recovered in the urine. The recovered phthalate moiety consisted of 89.8% monobutyl phthalate, 2.7% phthalic acid, 0.4% intact Dibutyl Phthalate, and four other metabolites in very small amounts. The researchers suggested that Dibutyl Phthalate 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.

In a study in which male mice were administered [^{14}C]Dibutyl Phthalate orally, the radioactivity accumulated in the liver and kidney within 6 h.[2] The radioactivity was rapidly excreted in the urine and feces. In another oral study in mice, no radioactivity was recovered from hepatic DNA after administration of [^{14}C]Dibutyl Phthalate. Dibutyl Phthalate and its metabolites appeared not to be transported into the nuclei.

Urinary metabolites were identified in male rats, male hamsters, and male guinea pigs given a single oral dose of 60 mg/kg Dibutyl Phthalate.[2] All 24-h urine samples contained monobutyl phthalate as the major product, intact Dibutyl Phthalate, phthalic acid, monobutyl phthalate glucuronide, and two other monobutyl phthalate oxidation products. The hamster urine contained an additional oxidation product. The livers from rats were examined 1 h after intravenous dosing of Dibutyl Phthalate and the data obtained indicated that Dibutyl Phthalate was rapidly hydrolyzed to monobutyl phthalate by the microsomal fraction. No phthalic acid was detected. The bile contained monobutyl phthalate and intact Dibutyl Phthalate but not phthalic acid. Since phthalic acid 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 Dibutyl Phthalate to monobutyl phthalate occurred in the liver, that there was enterohepatic circulation of Dibutyl Phthalate and its metabolites and good absorption from the intestine, and that monobutyl phthalate was the main metabolite of Dibutyl Phthalate and was primarily excreted in urine.

In another study, at least 90 % of orally ingested Dibutyl Phthalate was absorbed from the gastrointestinal tract.[8] Once absorbed, Dibutyl Phthalate distributed to the liver, adipose, kidney, muscle, and some other tissues, but it ddi not remain in these tissues, thus accumulation in the body is unlikely. Dialkyl phthalates are metabolized by hydrolytic enzymes found in many tissues to the produce the corresponding monoester and alcohol. A second hydrolysis can further metabolize the monoester into an alcohol and phthalic acid, but in primates and man the monoester is more likely to be conjugated to glucuronide for excretion. The major route of excretion of phthalates in rats, primates, and humans is in the urine. Very small amounts of Dibutyl Phthalate metabolites are found in the feces, but they have been detected in the bile at levels up to 44% of the Dibutyl Phthalate dose given to rats (60 mg/kg orally).

Single doses of 0.27 g/kg or 2.31 g/kg of 7-[^{14}C]Dibutyl Phthalate were given by oral intubation to Wistar rats.[8] Urine and feces were collected at 4, 8, 24, and 48 h after dosing. At the same time points, selected animals were killed, and their tissues and organs were weighed and analyzed for metabolites of 7-[^{14}C]Dibutyl Phthalate. By 48 h after dosing, 80 to 90% of the test material administered had been excreted in urine or feces as phthalic acid, monobutyl phthalate, mono(3-hydroxybutyl) phthalate, or mono(4-hydroxybutyl) phthalate. Less than 0.001% of the dose was found in the tissues at 48 h. The radioactivity was cleared more rapidly from the body in the lower dose group (rates not reported). Furthermore, rats fed a diet of 1% Dibutyl Phthalate for 12 wk did not accumulate Dibutyl Phthalate or its monoester metabolites in the tissues or organs.

Dibutyl Phthalate was reported to absorb in the intestines of rats mostly as monobutyl phthalate following oral exposure.[8] However, inhibition of intestinal epithelial esterases by S,S,S-tributylphosphorotrithioate (DEF) (8 mg/kg) did not significantly reduce circulating [^{14}C] after oral administration of [^{14}C]Dibutyl Phthalate at 6, 10, 14, 25, or 30 g/kg in rats. Blood concentrations of circulating [^{14}C]monobutyl phthalate were not measured. Approximately 4 h after a single dose of 837 mg/kg [^{14}C]Dibutyl Phthalate, rats that had been treated with 8 mg/kg DEF had a significantly lower ($p < 0.01$) concentration of the diester in the intestinal lumen, but there was no effect of DEF on the monoester in the lumen.

In both rats and hamsters, the major urinary metabolite of Dibutyl Phthalate or monobutyl phthalate was monobutyl phthalate-glucuronide following oral exposure.[8] However, the levels of unconjugated monobutyl phthalate in rat urine were 3- to 4-fold higher than in hamster urine. The activities of β-glucuronidase in the livers of rats and hamsters was comparable, however the testicular β-glucuronidase activity was significantly higher in rats than in hamsters. Because β-glucuronidase deconjugates monobutyl phthalate-glucuronide to free monobutyl phthalate, this difference in enzyme activity between species may contribute to the quantitative differences in urinary metabolites and to the male developmental toxicity of Dibutyl Phthalate and monobutyl phthalate (see Reproductive and Developmental Toxicity section).

Pregnant Sprague-Dawley rats were given a single oral dose of radiolabeled Dibutyl Phthalate (up to 3 g/kg) on gestation day 14.[8] Tissues from the dams and embryos were analyzed for Dibutyl Phthalate, monobutyl phthalate, and monobutyl phthalate-glucuronide for several time points up to 48 h after dosing. The percent of administered dose in the placenta and embryonic tissue peaked at 0.3 and 0.15%, respectively, at 2 h after dosing. By 48 h after dose administration, no detectable phthalates were found in these tissues nor in the uterus, ovaries, or amniotic fluid. Although Dibutyl Phthalate was shown to cross the placenta into fetal tissues, neither Dibutyl Phthalate nor its metabolites accumulated in the fetus, placenta or in maternal tissues.

Dimethyl Phthalate was administered by gavage to male rats in a single dose of 0.1 ml, and the urine was collected for 24 h.[2] A total of 44.6% of the phthalate moiety was recovered in the urine, and it consisted of 77.5% monomethyl phthalate, 14.4% phthalic acid, and 8.1% intact Dimethyl Phthalate. Dimethyl Phthalate appeared to be metabolized only by hydrolysis of one or both ester groups.

CD-1 female mice were used in a study to determine the distribution of Dibutyl Phthalate (99.6% pure) after single or repeated doses via gavage.[31] Groups of 5 mice/treatment received 0, 1, 10, or 1000 mg/kg Dibutyl Phthalate in tocopherol-stripped corn oil either once or once daily for 10 d. The mice were killed at 2, 6, 12, or 24 h after the single dose or the final dose, and tissues, including liver and ovaries, and serum were collected for analysis of monobutyl phthalate. Regardless of duration of exposure (single or repeated), monobutyl phthalate was detected in the sera and tissues of Dibutyl Phthalate-treated mice. In single dose mice, monobutyl phthalate levels peaked at ≤ 6 h and fell close to background levels by 24 h post-treatment. Following the last repeated dose, monobutyl phthalate levels peaked at ≤ 2 h and fell to background levels by 12 h.

Oral and Intravenous

[^{14}C]Dibutyl Phthalate was administered orally in dimethyl sulfoxide (DMSO) in a dose of 60 mg/kg or intravenously in saline in a dose of 10 mg/kg to male rats.[2] Urine and feces were collected, and the amount of radioactivity excreted was determined. The amount 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 h after oral or intravenous administration of Dibutyl Phthalate. 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 h after oral or intravenous administration of Dibutyl Phthalate. Very little radioactivity was recovered. The elimination of Dibutyl Phthalate from tissues and organs was rapid, and no organ had any significant affinity for accumulation. Rats were administered labeled [^{14}C]Dibutyl Phthalate orally, and bile was collected. Approximately 27.6 to 52.8% of radioactivity was excreted in the bile within 24 h after oral administration of Dibutyl Phthalate. Since more radioactivity was excreted in the bile than in the feces, there was apparently good absorption of Dibutyl Phthalate and its metabolites from rat intestine.

A 500 mg/kg dose of [^{14}C]Dibutyl Phthalate in ethanol was administered by gastric intubation to male rats and the bile was collected every hour for 6 h.[2] At 6 h after oral administration of Dibutyl Phthalate, 4.5% of the radioactivity was recovered in the bile. At 5 h after intravenous injection of Dibutyl Phthalate, 10% of the radioactivity was detected in the bile. Dibutyl Phthalate bile metabolites included monobutyl phthalate, intact Dibutyl Phthalate, phthalic acid, a monobutyl phthalate glucuronide, and traces of other glucuronides. A small amount of Dibutyl Phthalate appears to be absorbed unaltered from the intestine, and the excretion of Dibutyl Phthalate through the biliary route has a role in its metabolic fate.

Parenteral

In a study in which male mice were administered [^{14}C]Dibutyl Phthalate intravenously, the radiolabeled chemical distributed to the liver and kidney within 1 h.[2] In another study, Sprague-Dawley rats were given a single intravenous injection of 1 or 10 mg/kg [^{14}C]Dibutyl Phthalate.[8] After dosing, the animals were placed in individual metabolic cages for collection of urine and feces for 72 h. Arterial blood was collected at 10 s and 72 h after dose administration. The main route of excretion of the radiolabel was in urine (85% of the administered dose), followed by feces (9 - 10% of the administered dose). More than 95% of the radiolabel in urine was excreted within the first 24 h. Approximately 72 h after the dose administration, 0.6 - 2% of the administered dose remained in the exsanguinated rat carcass, and 1.9 - 2.3%

remained in the blood. Unchanged Dibutyl Phthalate was undetectable in the urine. Monobutyl phthalate in urine accounted for 22 ± 3.7% of the administered dose. Monobutyl phthalate-glucuronide in urine accounted for 34.6 ± 3.6% of the administered dose.

Pregnant rats received [^{14}C]Diethyl Phthalate that was administered intravenously on day 5 or day 10 of gestation.[2] *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.*

Human

Dermal

Dimethyl Phthalate was reported to absorb through human skin, and its metabolites were detected in human urine.[2] *(No further details available.)*

The absorption, metabolism, and extraction of Dibutyl Phthalate after dermal application was studied in 26 Caucasian male subjects.[32] The study was single-blinded and the subjects were given daily whole-body topical applications of a basic cream (control; 2 mg/cm^2) for 5 d and then a cream containing 2% (w/w) of Dibutyl Phthalate for another 5 d. Showering, bathing, or swimming were not allowed until 4 h after each application. For each subject, 24-h urine pools were collected in both weeks, except the first 24-h sample of the treatment week, where every single urine sampled was collected individually to prepare a 24-h excretion profiled for the test material. The urine samples were analyzed for urinary total and unconjugated monobutyl phthalate metabolites by liquid chromatography-tandem mass spectroscopy. All 26 subjects had increased excretion of monobutyl phthalate after topical application. Total monobutyl phthalate excreted in the urine during the treated week was 11.8 ± 0.6 mg/24 h. On average, 1.82% of the applied Dibutyl Phthalate was recovered in urine as monobutyl phthalate. The urinary monobutyl phthalate excretion rate was relatively low in the first 4 h following application of the test cream but increased thereafter to a relatively constant rate over the next 20 h. The fraction of unconjugated monobutyl phthalate was 8.0%.

A human dermal physiologically-based toxicokinetics (PBTK) model for Diethyl Phthalate was developed using experimental and published human data.[33] Five adult volunteers received deuterated Diethyl Phthalate in a simulated personal care formulation at 0.127 μg/cm² on each forearm for up to 240 min. Skin-wipe measurements yielded an estimated dermal bioaccessibility of 56.2 ± 13.5%, with an absorption half-life of approximately 30 min; the corresponding model estimate for direct air-to-skin exposure was 100%. Four volunteers subsequently participated in a urinary excretion study following a 240 min application of 0.254 μg/cm², with urine collected for 48 h. Urinary monoethyl phthalate concentrations increased rapidly, reaching a mean maximum concentration of 27.9 ± 6.7 ng/ml at 5.8 ± 1.6 h, and were no longer detectable after 24 h. The model was calibrated using published serum and urinary monoethyl phthalate data and validated against the urinary data from this study. The calibrated model estimated that 24.9% of Diethyl Phthalate entering the exposed skin became systemically available. Model predictions were generally within 2-fold of observed monoethyl phthalate concentrations or amounts in serum and urine.

Dermal and Inhalation

In a controlled chamber study, 6 adult males wearing only shorts were exposed to airborne Dibutyl Phthalate and Diethyl Phthalate for two 6-h sessions.[34,35] During one session, subjects wore breathing hoods that supplied clean air to quantify dermal exposure only ("hood-on"); during the other session, both dermal and inhalation exposure occurred ("hood-off"). Mean chamber air concentrations during the 6-h exposures were approximately 230 - 290 μg/m³ for Diethyl Phthalate and 114 - 140 μg/m³ for Dibutyl Phthalate. Urine was collected before, during, and for 48 h after exposure and analyzed for key Diethyl Phthalate (e.g., monoethyl phthalate) and Dibutyl Phthalate metabolites (e.g., monobutyl phthalate and mono-3-hydroxy-n-butyl phthalate). The results demonstrated high levels of Diethyl Phthalate and Dibutyl Phthalate metabolite excretions while in the chamber and within the first 24 h after leaving the chamber, increasing from background concentrations (~ 1 - 10 μg/g creatinine) to peak values on the order of tens to hundreds of μg/g creatinine and, in some cases, > 1000 μg/g creatinine, under both exposure conditions. When uptake was normalized to airborne concentrations, dermal uptake during the 6-h chamber exposure was comparable to inhalation intake for both compounds. For Diethyl Phthalate, the median dermal uptake was approximately 4.0 μg of absorbed Diethyl Phthalate per 1 μg/m³ of air concentration during a 6-h exposure, compared with an inhalation intake of approximately 3.8 μg per 1 μg/m³. For Dibutyl Phthalate, the median dermal uptake from air was approximately 3.1 μg of absorbed Dibutyl Phthalate per 1 μg/m³ of air concentration during a 6-h exposure, compared with an inhalation intake of approximately 3.9 μg per 1 μg/m³. These findings indicate that dermal uptake directly from air can represent a meaningful exposure pathway for both Diethyl Phthalate and Dibutyl Phthalate under controlled exposure conditions.

Modeling of the urinary metabolite data was subsequently performed by linking inhalation and transdermal exposure modules to a simplified one-compartment pharmacokinetic model that predicted both the timing and magnitude of metabolite excretion. Each exposure route was simulated separately ("inhalation-only" and "dermal-only"), as well as jointly ("combined exposure"), using parameters derived independently from prior experiments. When the model was extrapolated to typical U.S. indoor air concentrations of Dibutyl Phthalate (approximately 0.2 μg/m³), the estimated daily dermal uptake (0.14 μg/kg bw/d) exceeded the inhalation intake (0.042 μg/kg bw/d) by roughly threefold. Based on these steady-state

simulations, the combined dermal and inhalation uptake of airborne Dibutyl Phthalate was estimated to account for ~ 25% of total exposure to Dibutyl Phthalate in the general U.S. population.[34]

Oral

Groups of 8 volunteers received a single oral dose of 255 or 510 µg isotope-labeled Dibutyl Phthalate (type of isotope not reported).[8] *Approximately 24 h after the doses were given, the monobutyl phthalate content in the subjects' urine was determined. Subjects in the low and high dose groups respectively excreted 64 and 73% of the dose as monobutyl phthalate in the first 24 h. The amount of conjugated monobutyl phthalate was not measured.*

In an oral study, an individual male ingested 60 µg/kg deuterium-labeled Dibutyl Phthalate (tetra substituted ring - ^{2}H-3,4,5,6; 5.38 mg total) in decaffeinated coffee served in an edible cup to ensure full dose was consumed (no further description).[36] Urine was collected at 20 time points up to 48 h post-dosing. Serum and saliva were collected no earlier than 15 min after eating or drinking at 7 time points up to 24 h post-dosing. Quantification of metabolites was performed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The majority of the dose (92.2%) was excreted in the first 24 h, with only < 1% of the dose excreted on day 2. The major metabolite was monobutyl phthalate (84%). Various side-chain oxidized metabolites accounted for approximately 8%. The Dibutyl Phthalate metabolites reached peak concentrations between 2 to 4 h post-exposure, followed by a monotonic decline. The elimination halftime of monobutyl phthalate was 2.6 h; oxidized metabolites had elimination halftimes estimated to be from 2.9 to 6.9 h. Monobutyl phthalate was detected in the saliva, and monobutyl phthalate plus side chain oxidized metabolites were detected in the serum.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

The dermal LD_{50} for Diethyl Phthalate in adult albino rats was > 10 ml/kg. The acute dermal LD_{50} for Dimethyl Phthalate in rabbits was > 10 ml/kg.[2]

Oral

In mouse studies, the acute oral LD_{50} values of Dibutyl Phthalate ranged from 9.77 to 17.0 g/kg, while in multiple rat studies, the LD_{50} values ranged from approximately 8 g/kg to 23.0 g/kg.[2] *The approximate lethal dose of commercial- grade Dibutyl Phthalate administered orally to male albino rats was found to be > 7500 mg/kg; no clinical signs of toxicity or pathological findings were observed 11 d after dosing.*[8] *The LD_{50} in rats of a nail polish formulation containing 9% Dibutyl Phthalate was > 5 ml/kg, while in another study of a nail preparations containing 6% Dibutyl Phthalate, the LD_{50} was > 5 g/kg.* [2]

The oral LD_{50} for Diethyl Phthalate was > 5 - 9 ml/kg in rats[2,8] *and 1.0 g/kg in rabbits.*[2] *Undiluted Dimethyl Phthalate had acute oral LD_{50} values for mice, rats, guinea pigs, and rabbits as 7.2, 6.9, 2.4, and 4.4 ml/kg, respectively. Another study reported the acute oral LD_{50} for Dimethyl Phthalate in mice to be 6281 mg/kg.*[8]

Parenteral

The acute intraperitoneal LD_{50} Dibutyl Phthalate in a study with female mice was 14.9 mmol/kg.[2] *In other studies with mice, the LD_{50} ranged from 3.57 g/kg (in males) to 4.00 g/kg (sex not specified). In female rats, the intraperitoneal LD_{50} was reported to be 3.05 ml/kg. The acute intravenous LD_{50} of Dibutyl Phthalate in male mice was 0.72 g/kg. The intramuscular administration of Dibutyl Phthalate at a dose of 4 or 8 g/kg to groups of 3 rats did not result in any deaths, and there was no effect on the growth of the rats.*

In acute intraperitoneal studies with Diethyl Phthalate, the LD_{50} in mice ranged from 2.83 to 3.22 g/kg, and the LD_{50} in a female rat study was 5.06 ml/kg.[2] *The intraperitoneal LD_{50} in mice following administration of Dimethyl Phthalate ranged from 1.58 g/kg to 3.98 g/kg; an intraperitoneal study in female rats reported the LD_{50} to be 3.38 ml/kg. The subcutaneous LD_{50} of Diethyl Phthalate in guinea pigs was $\geq$ 3 g/kg.*

Acute toxicity studies are summarized in Table 4. In oral rat studies, the LD_{50} for Dibutyl Phthalate was 6279 mg/kg bw for both sexes.[5] The LD_{50} for Diethyl Phthalate was > 5 ml/kg bw for both male and female rats.[6] For Dimethyl Phthalate (85% pure), the combined LD_{50} in male and female rats was 5.1 g/kg bw.[7] In inhalation studies with rats, the LC_{50} value for Dibutyl Phthalate in both sexes was estimated to be $\geq$ 15.68 mg/l after a 4 h exposure, and the LC_{50} for Diethyl Phthalate in both sexes was > 4.64 mg/l after a 6 h exposure.[5,6] *Danio rerio* (zebrafish) were exposed to Dibutyl Phthalate (up to 10 mg/l) for 96 h.[5] The LC_0 was 1.3 mg/l and the LC_{100} was 5 mg/ml.

Short-Term Toxicity Studies

Dermal

The National Toxicology Program (NTP) conducted a 4-wk study on the dermal effects of Diethyl Phthalate on mice and rats.[8] *The mice received up to 100 µl of the test material neat and the rats received up to 300 µl. There was no evidence of dermal toxicity and no adverse clinical signs in mice or rats at any dose tested. Feed consumption and weight gains were unaffected by Diethyl Phthalate treatment. The absolute and relative liver weights in female mice in the 25 and 50 µl groups and relative liver weights and relative kidney weights of male and female rats in the 100 µl groups were greater than those of*

control animals. No other adverse effects of Diethyl Phthalate were observed. The no-observed-adverse-effect level (NOAEL) was determined to be 12.5 µl in mice and 75 µl in rats.[6]

Oral

In a short-term study of 6 rats that received repeated oral doses of 7500 mg/kg Dibutyl Phthalate (commercial grade), 2 rats each died after the second and fourth dosing.[8] *The 2 surviving rats (10 doses total) showed clinical signs of toxicity and weight loss but had completely recovered 10 d after the last treatment. In a 14-d feed study in male and female mice exposed to up to 1% Dibutyl Phthalate, body weights were reduced ($p < 0.05$) in males in the 1% exposure group and liver weights were increased ($p < 0.05$) in males and females in the 1% group. There were no effects on feed or water consumption, mortality rate, or incidence of clinical signs due to Dibutyl Phthalate exposure in this study.*

Relative weights of liver, kidney, and spleen were increased in male rats that were fed a powdered diet containing 0.5 or 5% Dibutyl Phthalate or 0.5 or 5% monobutyl phthalate for 34 - 36 d.[8] *Succinate dehydrogenase and pyruvate dehydrogenase activities in liver mitochondria were reduced in rats exposed to 5% Dibutyl Phthalate or 0.5 or 5% monobutyl phthalate, but glutamate dehydrogenase was not affected. Serum chemistry analysis revealed increases in ALP, GOT, GPT, and CPK in rats exposed to 5% Dibutyl Phthalate. Serum globulin was reduced, and A/G was increased at both concentrations of Dibutyl Phthalate and monobutyl phthalate. Both concentrations of monobutyl phthalate decreased TG and uric acid levels. Dibutyl Phthalate and monobutyl phthalate at 5% produced increased liver necrosis (single cells and zonal) and hypospermatogenesis. Microscopic examination of hepatocytes revealed that 5% Dibutyl Phthalate and 5% monobutyl phthalate caused increased peroxisomes, lysosomes, and mitochondria. Increased mitochondrial swelling and decreased liver glycogen was observed in the rats treated with 5% monobutyl phthalate.*

In a 6-wk study in rats that received 1 ml/kg Dibutyl Phthalate in oil 2 times/wk, no adverse effects were reported.[2] *Growth was inhibited in mice and leukocytosis was observed in rats that received 20 mg/kg Dibutyl Phthalate for over 11 wk (frequency of dosing not reported). Rabbits that received 3 ml/kg/d Diethyl Phthalate for 8 d displayed no adverse effects for up to 2 wk after dosing.*

Short-term oral toxicity studies are summarized in Table 5. In a 28-d study, no clinical signs of toxicity were observed in rats that received up to 7000 ppm Dibutyl Phthalate in feed, with or without the addition of 3000 ppm diethylhexyl phthalate.[37] Relative liver weights were increased in males and females of the 7000 ppm Dibutyl Phthalate group and in males of the 1000 ppm Dibutyl Phthalate group. In the groups that received both phthalates, an increase of absolute and relative liver weights were observed in all animals in all combined treatment groups. Mice that received 40 mg/kg bw/d Dibutyl Phthalate for 60 d via gavage had no significant differences in body weight gain or lung function parameters when compared to saline controls.[38] However, oxidative stress biomarkers in lung tissue were significantly altered. Serum total IgE levels showed a statistically significant increase compared to controls, whereas cytokine levels and inflammatory cell counts in bronchoalveolar lavage (BAL) fluid were not significantly different from controls. In a 9-wk oral gavage study, male rats treated with Dibutyl Phthalate at 750 mg/kg bw/d had marked histopathological changes in the lungs, including increased inflammatory cell infiltrations, epithelial cell shedding, and mucus secretion.[39] Hematological analysis showed significant elevations in white blood cells, neutrophils, lymphocytes, eosinophils, and platelets. Compared to controls, the Dibutyl Phthalate group exhibited a significant increase in inflammatory cells in rat BAL fluid, accompanied by elevated Th2 cytokines. Oxidative stress was evident, and increased apoptosis was observed in lung tissue.

No mortalities were observed in a 4-wk study in male rats that received Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate, the respective monoesters, or phthalic acid in corn oil via gavage (500 mg/kg bw/d for diesters, 250 mg/kg bw/d for monoester and acid).[40] Liver weights were significantly increased in groups treated with Dibutyl Phthalate and monobutyl phthalate compared to control group. Red blood cells and hematocrit were significantly lower and mean corpuscular hemoglobin concentration and platelet count were significantly higher in the Dibutyl Phthalate group (the monoester group also had significantly higher platelet counts), and hemoglobin level was reduced only in the Dimethyl Phthalate group.

The NOAEL for male and female rats that received up to 3170 mg/kg/d (5%) Diethyl Phthalate in feed for up to 16 wk was 150 mg/kg/d (0.2%).[6] Male and female rats that received 5% Diethyl Phthalate for 16 wk had statistically significantly lower absolute weights of the brain, heart, spleen & kidneys than controls; reductions in the absolute weights of the female gonads and male heart, spleen, and kidneys were also observed in the 5% groups after 2 and 6 wk of treatment. Increased kidney weights were seen in males & lower pituitary weights in females in the 1% group after 2 wk of treatment. Increased gonad weights were also seen in females of the 1% group after 6 wk.

Inhalation

No significant effects were observed in male rats exposed to 1.5 mg/m^3 of Dibutyl Phthalate vapor for 6 h/d and 6 d/wk for approximately 1 mo.[2] *In another 1 mo study, no significant treatment-related effects were observed in rats exposed to up to 500 mg/m^3 aerosolized Dibutyl Phthalate for 6 h/d and 5 d/wk.*[8] *Particle size of the aerosolized Dibutyl Phthalate ranged from 1.5 to 2.0 µm. The no-observed-effect concentration (NOEC) for inhalation exposure to aerosolized Dibutyl Phthalate was the highest concentration tested in this study, 500 mg/m^3.*

Other Routes

No paralysis or other adverse effects were observed in rabbits that received 2 ml/kg/d Diethyl Phthalate intraperitoneally for 8 d.[2] *The intraperitoneal administration of 1.5 ml/kg/d Diethyl Phthalate to guinea pigs for 8 d did not result in any permanent ill effects during or after the experiment. An emulsion of Diethyl Phthalate administered intraperitoneally at 125 mg/kg/d for 6 wk to mice produced a slight decrease in weight gain and some evidence of peritonitis. No further adverse effects were observed.*

Subchronic Toxicity Studies

Dermal

In a 90-d study, 0.5, 1, 2, or 4 ml/kg/d Dibutyl Phthalate or Dimethyl Phthalate were applied to clipped, intact rabbit skin over approximately 10% of the body surface.[2] *For these ingredients, the subchronic dermal LD_{50} was > 4 ml/kg/d. Dibutyl Phthalate was slightly irritating to skin and very irritating to rabbit penile mucosa. Slight dermatitis was observed, and in the 4 ml/kg dosed rabbits, slight renal damage (not further described) was observed. No dermal irritation or dermatitis was observed following application of Dimethyl Phthalate, although irritation was observed in the penile mucosa. Pulmonary edema and slight renal damage were observed in the rabbits that died during the study. Survivors had varying degrees of nephritis (not further described) at the two highest doses.*

Oral

In a 3-mo study, rats received 0.12 or 1.2 g/kg/d Dibutyl Phthalate in olive oil.[2] *Both doses produced a statistically significant increase in the rats mean liver weight; however, no histological evidence of any pathologic changes were found in the liver, kidneys, or spleen. No changes in behavioral patterns or clinical signs of toxicity were observed in rats that received 0.2, 1, or 5% Diethyl Phthalate in feed for up to 16 wk. Both sexes that received 5% and females that received 1% Diethyl Phthalate consumed less feed and gained less weight than controls. A reduction in absolute weight and an increase in relative weight of the brain, spleen, heart, kidneys, adrenal glands, gonads, and pituitary was observed in the 5% group. A pattern of increases in absolute and relative weights was observed in livers and various parts of the gastrointestinal tract in these rats. Both liver and kidneys were enlarged but histologically normal.*

In another 3-mo dietary study, rats received 400, 2000, or 10,000 ppm (30, 152, or 752 mg/kg/d) Dibutyl Phthalate.[8] *No treatment-related effects were seen in the 400 and 2000 ppm groups. In the 10,000 ppm group the following observations were noted: decrease in triglyceride and triiodothyronine in both sexes; increase in cyano-intensive palmitoyl-CoA-oxidation in both sexes; decrease in number of red blood cells, hemoglobin, and hematocrit in the males; increase in glucose and albumin in the males; increased absolute and relative liver weights in both sexes; and decreased or missing lipid deposition in the hepatocytes of both sexes.*

The NTP conducted a 13-wk dietary study of Dibutyl Phthalate in mice and rats.[8] *The mice received 0, 1250, 2500, 5000, 10,000, or 20,000 ppm of the test material and rats received 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm. All mice and rats survived to study completion. No clinical signs of toxicity attributed to Dibutyl Phthalate were observed in mice; however, male and female rats in the 40,000 ppm dose group were emaciated at the end of the 13-wk dosing period. No other clinical observations were reported in rats. In mice, reduced final body weights and body weight gains were observed starting at 5000 ppm in males and 20,000 ppm in females. Increased relative kidney weights were observed starting at 2500 ppm in females, and increased relative liver weights were observed started at 5000 ppm in both sexes. Female mice also had reduced hematocrit at 20,000 ppm. In male mice, increased serum testosterone and increased zinc in the testes were observed starting at 1250 and 5000 ppm, respectively. An increased incidence of cytoplastic alteration in the liver was observed starting at 10,000 ppm in male mice and 20, 000 ppm in female mice. In rats, reduced final body weights and body weight gains were observed starting at 10,000 ppm in males and 20,000 ppm in females. Reduced feed consumption was observed at 40,000 ppm in both sexes. Both sexes also had increased liver and kidney weights (starting at 5000 ppm in males and 10,000 ppm in females), reduced cholesterol (starting at 20,000 ppm in both sexes), reduced triglycerides (starting at 2500 ppm in males and 10,000 ppm in females), increased palmitoyl-CoA oxidase (starting at 5000 ppm in both sexes), and increased incidence of cytoplasmic alteration in the liver (starting at 10,000 ppm in both sexes). Additionally, male rats had reduced hematocrit (starting at 20,000 ppm), reduced hemoglobin (starting at 5000 ppm), increased platelets (starting at 5000 ppm), increased incidence of germinal epithelium atrophy (starting at 10, 000 ppm), and reduced serum testosterone, reduced testes weights, and reduced zinc in testes (starting at 20,000 ppm of all effects).*

In a 120-d study, rats received 5% (v/v) ethanol, 50 ppm (w/v) Diethyl Phthalate, or 5% ethanol + 50 ppm Diethyl Phthalate in drinking water.[8] *No significant changes were seen in the body weights, liver weights, daily water consumption, and serum glucose levels of any treatment groups. Generally, Diethyl Phthalate caused changes in enzymes and biochemical endpoints in the liver and serum that were not affected by the presence of ethanol, thus there was no interaction between ethanol and Diethyl Phthalate at these doses, but Diethyl Phthalate alone appears to cause toxic injury to the liver under the study conditions.*

Inhalation

Rats exposed to 0.5 mg/m^3 and 50 mg/m^3 of Dibutyl Phthalate mist for 6 h/d for 6 mo had reduced weight gains and increased brain and lung weights than control rats.[2] *The higher concentration had a greater effect than the lower concentration.*

Chronic Toxicity Studies

Oral

No adverse effects were observed in rats that received 2.5 mg/kg/d Dibutyl Phthalate for 6 mo.[2] *In a year-long study, groups of 20 male and 20 female rats received 0 or 0.125% Dibutyl Phthalate in feed. Six rats died before study end, but no specific cause of death was determined. No remarkable alterations were observed upon gross and histological examination of the liver, kidneys or spleen. In another year-long study, groups of 10 rats received 0, 0.01, 0.05, 0.25, or 1.25% Dibutyl Phthalate in feed. No effect of growth or survival was observed in rats that received up to 0.25% test material in their feed. At 1.25%, half of the rats died the first week of the study. The remaining rats gained weight comparable to the control group. No rats exhibited significant changes in the number or distribution of "elements" in the peripheral blood or specific gross pathological changes. No adverse effects on hematological parameters, organ weights, or other pathological changes were observed in an 18-mo study in rats that received 1ml/kg Dibutyl Phthalate in oil (type not specified) 2 times/wk.*

In a 2- yr study, groups of 10 female rats received 2, 4, or 8% Dimethyl Phthalate in feed.[2] *Mortality rates were similar to control rats, and no effects were observed in the 2% dose group. A slight but significant effect was observed on growth in the 4 and 8% dose groups. Chronic nephritis was observed in rats in the 8% group.*

Other Routes

A series of doses of Dibutyl Phthalate, Diethyl Phthalate, or Dimethyl Phthalate were injected intraperitoneally into groups of male mice 5 d/wk.[2] *The apparent LD_{50} was calculated each week until it remained constant for 3 wk; this was the chronic LD_{50}. Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate reached chronic LD_{50}s in 25, 14, and 18 wk, respectively. The chronic LD_{50} values for Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate were 0.85, 1.39, and 1.18 ml/kg/d, respectively.*

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

The numerous developmental and reproductive toxicity studies from the original 1985 report and the data from the 2005 re-review have been summarized in Table 6. Numerous studies available since these documents were published are summarized in Table 7, and extremely brief overviews of the studies summarized in Table 7 are presented below.

A plethora of studies are issued regularly, and many of them are purely cumulative to the studies already summarized in this document. Therefore, some additional studies that are purely cumulative in nature and generally support the developmental and reproductive toxicity profile of Dibutyl Phthalate already summarized in this document are not presently described in this section, but are referred to herein. Studies in rabbits, mice, rats, and zebrafish reported persistent male reproductive-tract effects, impaired sperm motility and morphology, altered Leydig- and Sertoli-cell function and spermatogenesis, and disruption of embryonic motor and sensory neuron development.[41-45] Collectively, these findings were consistent with the existing evidence and did not indicate a qualitatively different toxicity profile. A review of phthalate-induced testicular dysgenesis in rats also discussed parallels with features of human testicular dysgenesis syndrome, as well as relevant species differences and limitations in extrapolating these findings to humans.[46]

Embryotoxic and Teratogenic Effects

The NOEC for malformations was 5.8 ppm for Dibutyl Phthalate (tested at up to 15 ppm) and 17 ppm for Diethyl Phthalate (tested at up to 200 ppm) in in vitro 96-h frog embryo teratogenesis assays using *Xenopus laevis* embryos.[47] The mean 96-h LC_{50}s for Dibutyl Phthalate and Diethyl Phthalate in this study were 12.88 and 64.5 ppm, respectively. Malformations were observed starting at 5 ppm in Dibutyl Phthalate-treated embryos and starting at 50 ppm in Diethyl Phthalate-treated embryos. Additional studies in *Xenopus laevis* with Dibutyl Phthalate also noted defects, with mean percent normal development decreased as concentration and exposure time increased.[48,49]

No significant effects on survival or hatching of zebrafish embryos were observed following exposure to up to 250 µg/l Dibutyl Phthalate; however, body length decrease, yolk sac abnormalities, and immune responses were observed.[50] An acute developmental toxicity of Dibutyl Phthalate (up to 0.2 mg/l), Diethyl Phthalate (up to 10 mg/l), and Dimethyl Phthalate (up to 8 mg/l) in zebrafish saw a malformation rate of 30% with 0.05 mg/l Dibutyl Phthalate (deaths were observed at higher concentrations).[51] The number of dead fish larvae also increased with increased concentrations of Diethyl Phthalate. Dimethyl Phthalate was not correlated with larvae mortality. All phthalates tested induced different developmental abnormalities in the fish larvae, including abnormal movement, decreased heart rate, spinal curvature, and pericardial edema.

In a dermal study in mice, Diethyl Phthalate (tested at up to 5600 mg/kg/d) applied to pregnant mice on gestation days 0 - 17 had a NOAEL < 500 mg/kg/d in dams and 1600 mg/kg/d in offspring.[6] The dams exhibited dose-related abnormal behavior and reduced thymus and spleen weights were observed at all doses; while significantly reduced fetal body weight and an increased incidence of cervical and lumbar rib variations were observed in 5600 mg/kg/d offspring. In a developmental study in which dams receiving 5, 50, or 500 mg/kg/d Dibutyl Phthalate in canola oil on gestation day 14 to postnatal day 21, female offspring in the 500 mg/kg/d group had delayed vaginal opening.[52] Additionally, male and female offspring of the 500 mg/kg/d group had increased fasting glucose levels, but no effects were observed in insulin secretory response. In a 3-generation reproductive and developmental study in mice, Dibutyl Phthalate in olive oil up to 2000 mg/kg bw induced skeletal malformations in F_0 males, with significant mortality in postnatal life and skewing the sex ratio.[53] An

increased frequency of DNA damage in the germ cells was observed in F_1 males; exposure of F_0 males to Dibutyl Phthalate did not affect F_1 male fertility and pregnancy frequency. In multigeneration studies with Dibutyl Phthalate in rats at up to 10,000 ppm in dietary feed, no adverse effects to body weights and body weight gains were observed in parental dams.[54,55] In one study, male offspring in the 10,000 ppm dose group had high incidence of small or absent reproductive organs and undescended testes, gross lesions correlated with microscopic lesions in the male reproductive organs.[55] In another feed study, anogenital distance was significantly decreased in male F_1 and F_2 offspring in the 10,000 ppm dose groups.[54] F_1 males of the 10,000 ppm dose group also had a statistically significant mean delay in preputial separation, testicular descent, and seminiferous tubular atrophy. In a 2-generation rat feed study with Diethyl Phthalate at up to 15,000 ppm, the NOAEL for parental animals was 15,000 ppm, and the NOAEL for development and growth of pups was 3000 ppm.[6,56] Body weight gains before weaning were inhibited in F_1 and F_2 pups and vaginal openings were slightly delayed in F_1 females at 15,000 ppm.

Effects in Female Reproductive Organs

Mouse ovarian antral follicles treated with 1000 µg/ml Dibutyl Phthalate in DMSO for 24 h were significantly smaller than controls, and follicles treated with concentrations greater than 100 µg/ml were significantly reduced after 72 h. [57] No toxicity was observed to the follicles after treatment with monobutyl phthalate at the same concentrations for the same periods of time. In in vitro and in vivo studies on the effects on mouse oocyte maturation, Dibutyl Phthalate (200-800 µM in vitro; 100 or 500 mg/kg/d in vivo) significantly reduced mouse oocyte germinal vesicle breakdown and polar body extrusion rates.[58] Additionally, oocyte cytoskeleton was damaged and cortical granule-free domains were disrupted; Dibutyl Phthalate also induced early apoptosis of oocyte and granulosa cells. Dibutyl Phthalate in tocopherol-stripped corn oil did not cause significant deviations in the expression of transcripts encoding insulin-like growth factor 1 (IGF) binding proteins in female mice that received 10 or 100 µg/kg/d or 1000 mg/kg/d for 20 d when compared to the vehicle control.[59] Total number of ovarian follicles counted per ovary was reduced in the 100 µg/kg/d group when compared to controls; however no significant differences were observed in the 10 µg/kg/d or 1000 mg/kg/d dose groups. Significantly low primordial follicle counts observed in the 100 µg/kg/d group and in the 1000 mg/kg/d group compared to controls, also fewer primary follicles were observed in 100 µg/kg/d mice. In another female mouse study with Dibutyl Phthalate in tocopherol-stripped corn oil (up to 1000 µg/kg/d for 30 d), dose-dependent effects on folliculogenesis and gene expression were observed.[60] At 1000 µg/kg, more atretic follicles were observed in the ovaries. In a dose-dependent manner, Dibutyl Phthalate significantly reduced the expression of genes responsible for homologous recombination, mismatch repair, and nucleotide excision repair. Pregnant rats that received 500 mg/kg Dibutyl Phthalate in corn oil every second day starting at gestation day 14.5 through postnatal day 6 exhibited no adverse effects and delivered exclusively live pups.[61] Offspring also exhibited no adverse effects, and female pups had no effects to anogenital distance or adverse effects in vaginal opening. In an oral uterotrophic assay, female rats that received 100 mg/kg Dibutyl Phthalate in corn oil from postnatal day 21 for 3 d had significantly decrease uterine wet weight and minor variations in ovary wet weight; no abnormal clinical signs or symptoms were observed in any of the treated or control animals.[62] Female rats from a pubertal onset assay that were treated with 10 or 100 mg/kg Dibutyl Phthalate from postnatal day 21 for 20 d had significantly reduced uterus and ovary weights in both treated groups. However, vaginal opening was not observed in any of the animals in the controls and treated groups until postnatal day 42, except in one animal each in vehicle control and the 100 mg/kg dose group. Female offspring of dams that received up to 10,000 ppm on gestation day 15 to postnatal day 21 had marginal increases in the number of cases of extended diestrus at 10,000 ppm, and at adult stage necropsy, relative pituitary weights were decreased in the 10,000 ppm dose group at postnatal week 11, and in the 200 ppm dose group at postnatal week 20.[63] In an oral multigeneration study in which the F_0 pregnant mice received a mixture of phthalates that included 15% Dibutyl Phthalate and 35% Diethyl Phthalate in tocopherol-stripped corn oil at up to 500 mg/kg/d, female offspring of the F_2 generation had increased uterine weight, anogenital distance and body weight.[64] Cystic ovaries and breeding and pregnancy complications were also observed. Similar effects were observed in the females of the F_3 generation.

Pregnant CD1 mice received 0, 10, or 50 mg/kg bw/d Dibutyl Phthalate orally beginning on gestation day 14.5, for 3 d or until sacrifice.[65] For analysis of meiotic progression, mice received 10 mg/kg bw/d on gestation days 14.5 - 16.5, and fetal ovaries were collected on gestation day 17.5. Exposure in utero delayed meiotic prophase I progression, reduced colocalization of the recombination proteins DMC1 and RAD51, and decreased homologous recombination. Additional analyses showed impaired DNA damage-response signaling on gestation day 15.5; DNA double-strand breaks were repaired by gestation day 17.5. Similar recombination defects were observed in fetal ovary cultures exposed to 10 µM Dibutyl Phthalate. Mechanistic experiments suggested that reduced lysine crotonylation of the DNA-repair protein MSH6 impaired its interaction with Ku70, favoring non-homologous end joining, an alternative DNA-repair pathway, over homologous recombination. In follow-up studies of offspring from dams exposed to 10 or 50 mg/kg bw/d, F1 females showed no significant changes in litter size over seven breeding rounds, but pup-licking frequency decreased and pup-retrieval latency increased. At both maternal doses, F2 offspring had increased birth weights without a significant change in sex ratio. F2 females exhibited premature follicular activation, earlier vaginal opening, and anxiety-like behaviors.

In another study, pregnant CD1 mice received 0, 0.4, 2, 10, or 50 mg/kg bw/d Dibutyl Phthalate by oral administration on gestation days 16.5 - 18.5.[66] Ovaries collected from offspring on postnatal day 1 had a significantly reduced proportion of primordial follicles and an increased proportion of germ-cell cysts at 2 mg/kg bw/d and greater; effects were not observed at

0.4 mg/kg bw/d. In fetal ovary cultures, 10 and 50 µM Dibutyl Phthalate similarly disrupted germ-cell-cyst breakdown and primordial-follicle assembly. Mechanistic experiments indicated that Dibutyl Phthalate-induced oxidative stress activated autophagy and reduced NOTCH2 signaling; autophagy inhibition, NOTCH2 intracellular-domain overexpression, or melatonin treatment attenuated the effects. In a repeated-breeding assessment, F1 females derived from dams exposed to 0, 10, or 50 mg/kg bw/d had increased time to mating, decreased fertility and gestational indices across successive litters, and fewer pups. In the 50 mg/kg bw/d group, the gestational index decreased to 33% for the second litter and 0% for the fifth litter, and some females became sterile.

Effects in Male Reproductive Organs

In an in vitro mechanistic study using TM3 (Leydig) and TM4 (Sertoli) cells, exposure to Dibutyl Phthalate at 0, 5, 10, 50, and 100 mg/l for 24 h produced dose-dependent evidence of ferroptosis.[67] Dibutyl Phthalate treatment decreased cellular GSH and mitochondrial membrane potential, and increased malondialdehyde (MDA), reactive oxygen species (ROS), oxidized glutathione (GSSG), and ferrous iron (Fe^{2+}) levels. Dibutyl Phthalate selectively upregulated peroxiredoxin-6 (PRDX6), a negative regulator of ferroptosis, while glutathione peroxidase-4 (GPX4) remained unchanged. Further, Dibutyl Phthalate upregulated SP1 expression, which can directly bind to the PRDX6 promoter and transcriptionally activate its expression. Rat testicular explants exposed in vitro to Dibutyl Phthalate at 10^{-6}, 10^{-5}, and 10^{-4} M for 24 h showed significant upregulation of Notch1, Dll4, and Hey1 at both mRNA and protein levels, while Hes1 expression remained unchanged.[68] Dibutyl Phthalate altered immunoexpression of activated NOTCH1, DLL4, HEY1 and HES5 both in seminiferous epithelium and interstitial tissue, with differential effects across cell types. In rat Leydig cells exposed to 50 mg/l Dibutyl Phthalate, Dibutyl Phthalate + prostaglandin E2, or flutamide for 24 h, expression of testosterone was significantly decreased in all groups.[69] A significant decrease of Cx43 was observed in the Dibutyl Phthalate group. Human sperm exposed to up to 134.7 µg/ml Dibutyl Phthalate for 30 min to up to 96 h had a concentration- and duration-dependent decrease in motility.[70] In another study with human sperm, Dibutyl Phthalate at 6 µM, monobutyl phthalate at 3 µM, and a mixture of both had adverse effects on sperm motility, penetration ability, and capacitation following exposure for 1 to 4 h.[71]

Multiple oral studies have investigated the effects of phthalates on male offspring of female rodents treated during gestation.[61,63,72-83] The NOAEL was 50 mg/kg bw/d for developmental toxicity in a study in pregnant rats that received up to 500 mg/kg bw/d on gestation day1 through postnatal day 21.[72] At doses of 250 mg/kg and higher, reduced birth weight, body weight gain, number of live pups per litter, anogenital distance in males, epididymis weight, and sperm count and motility were observed. Studies in which pregnant rats received up to 600 mg/kg bw/d Dibutyl Phthalate between gestation days 12 - 21 found cryptorchidism, infertility, hypospadias, decreases in anogenital distance, and/or testes abnormalities.[61,73-80] Closer examination of the focal dysgenesis of the testes found adverse effects in the Leydig and Sertoli cells. One study of rats that received Dibutyl Phthalate (500 mg/kg/d) from gestation day 12 through gestation days 16 - 20 saw a significantly increased incidence of multinucleated gonocytes (MNG) in fetal rat testes following in utero exposure to the test material.[78] Sertoli cells exhibited retracted apical processes, disorganized vimentin cytoskeleton, and abnormal contacts with gonocytes. These morphological changes were no longer evident after birth and cessation of exposure. Pregnant dams that received Dibutyl Phthalate (up to 600 mg/kg/d) or Diethyl Phthalate (up to 900 mg/kg/d) on gestation days 8 - 18 experienced no adverse effects, but fetal testicular testosterone production was significantly reduced at doses of 300 mg/kg/d or higher in Dibutyl Phthalate male fetuses.[81] This effect was not observed with Diethyl Phthalate. In a study where pregnant dams received up to 10,000 ppm Dibutyl Phthalate on gestation day 15 to postnatal day 21, male offspring from the 10,000 ppm dose group had decreased neonatal anogenital distance and retention of nipples at postnatal day 14; however, but the adult stage, testicular lesions appeared to be very faint in most cases, but degeneration and atrophy of mammary gland alveoli were observed in males starting at 20 ppm.[63] Exposure to Dibutyl Phthalate (100 mg/kg/d) from gestation day 12 until postnatal day 21 affected the prostate development in male rats, including disseminated inflammatory infiltrate in the stroma and increased androgen receptor expression, proliferation index, and metalloproteinase 9 activity.[82] A phthalate mixture containing Dibutyl Phthalate significantly increased fetal mortality at 40% of top dose (containing 120 mg/kg/d Dibutyl Phthalate) and above in dams that received the test material on gestations days 8 - 18, and testosterone production was reduced in a dose-additive manner starting at 20% of top dose (containing 60 mg/kg/d Dibutyl Phthalate) and above.[81] Phthalate-induced underdevelopment of the testes was observed at 100% of the top dose (300 mg/kg/d).

Additional oral studies have investigated the effects of phthalates on male reproductive organs in male rodents treated directly.[6,40,55,67,84,85] Male rats that received up to 600 mg/kg/d Dibutyl Phthalate in corn oil for 15 d had significantly decreased testicular weight at each dose tested, and sperm count and motility were significantly decreased in a dose-dependent manner.[84] Serum follicle-stimulating hormone, testosterone levels, and testicular lactate dehydrogenase activities were significantly decreased at all doses tested. In a study of male rats exposed to 250 mg/kg Dibutyl Phthalate, with or without BaP for up to 12 wk, no adverse effects were observed in the relative weights of the testes and epididymides, but vacuolization of Sertoli cells was observed in Dibutyl Phthalate-exposed rats after 12 wk.[85] Male rats orally exposed to Dibutyl Phthalate (500 mg/kg/d) for 3 wk, had decreased germ cell layer thickness, and reduced sperm density in testicular tissue.[67] These morphological changes were associated with increased testicular levels of MDA, Fe^{2+}, and GSSG, together with decreased reduced GSH and serum testosterone. No gross lesion were observed in mice that received up to 1393 mg/kg bw/d Dibutyl Phthalate in a 2-yr study, but significantly increased incidences of germinal epithelium degeneration in the testes ad exfoliated germ cells in the epididymal duct were observed.[55] In rats that received 2000 mg/kg/d Diethyl Phthalate

in dietary feed for 7 d, lower levels of testosterone were measured in the testes and serum of the treated animals, but no testicular damage was observed by microscopic examination.[6] Testes weights were significantly reduced in male rats that received Dibutyl Phthalate (500 mg/kg bw/d) for 4 wk, and Dibutyl Phthalate, monobutyl phthalate (250 mg/kg bw/d), and monoethyl phthalate (250 mg/kg bw/d) significantly lowered sperm counts and sperm motility of epididymal sperm.[40] These effects were not observed with Diethyl Phthalate or Dimethyl Phthalate (500 mg/kg bw/d for each diester), monomethyl phthalate (250 mg/kg bw/d), or phthalic acid (250 mg/kg bw/d).

Mice that received Dibutyl Phthalate (2.5 mg/kg/d), with or without diethylhexyl phthalate, for 40 d via implanted osmotic pumps had abnormal sperm morphology, particularly in the mixture group.[86] Significant differences in sperm concentration were observed between control and exposed groups. No clinical signs of toxicity were observed in rats that were injected subcutaneously with Dibutyl Phthalate (up to 20 mg/animal) from postnatal day 5 -14; and no alterations in testicular descent were observed in any treated rats. However, significantly reduced testes, seminal vesicles, levator ani plus bulbocavernosus muscles (LABC), and Cowper's glands weights were observed in the 20 mg dose group when compared to the controls.[87]

In additional studies, 100 mg/kg Dibutyl Phthalate injected into chicken eggs significantly reduced hatching and increased late hatching when compared to controls.[88] Gross malfunctions and severe motor dysfunctions were observed in treated animals. Zebrafish that were exposed to up to 1133 µg/l Dibutyl Phthalate, with or without diisobutyl phthalate had a greater imbalance of the testosterone to estradiol ratio and severe structural damage from the mixture than to just Dibutyl Phthalate.[89] These effects were consistent with the testis transcriptome analysis for which 4570 genes were differentially expressed in the mixture exposure, while 2795 genes were differentially expressed in Dibutyl Phthalate.

One study investigated the effects of Dibutyl Phthalate and its major metabolite, monobutyl phthalate, on testosterone production and Leydig cell aggregation in fetal testis explants from the rat and human.[90] Explants obtained from gestation day 19.5 rats and human fetuses at 15 - 19 wk gestation were cultured for 24 - 48 h with or without the test substances. In rat explants, 10^{-3} M slightly decreased human chorionic gonadotropin-stimulated testosterone production and induced minor Leydig cell aggregation but did not affect basal or 22R-hydroxycholesterol-stimulated testosterone production. These endpoints were unaffected in human fetal testis explants cultured with 10^{-3} M monobutyl phthalate for up to 48 h. The explant results were compared with in vivo findings in Dibutyl Phthalate-exposed rats. In utero exposure following maternal administration of 500 mg/kg bw/d Dibutyl Phthalate for 48 h before gestation day 21.5 substantially decreased intratesticular testosterone concentrations and cytochrome P450 side-chain cleavage enzyme (P450scc) expression and induced Leydig cell aggregation. Aggregation was more extensive following the longer exposure (gestation days 13.5 - 20.5). Effects on testosterone production were also evaluated in vivo in neonatal male marmosets. A single 500 mg/kg dose of monobutyl phthalate significantly decreased blood testosterone concentrations after 5 h, while administration of 500 mg/kg/d for 14 d increased Leydig-cell volume per testis. The authors concluded that Dibutyl Phthalate/monobutyl phthalate suppressed steroidogenesis by fetal-type Leydig cells in rodents and primates, but that these effects could not be reliably evaluated in vitro.

In a prenatal study, pregnant BALB/c mice received 0, 50, 250, or 500 mg/kg bw/d Dibutyl Phthalate by gavage from gestation day 12.5 until birth.[91] Testicular structure and blood-testis-barrier integrity were evaluated in male offspring on postnatal day 22. In utero exposure to 50 mg/kg bw/d was associated with accelerated formation of tight junctions between Sertoli cells and the blood-testis barrier, accompanied by accumulation of the tight-junction protein occludin. At 250 and 500 mg/kg bw/d, testicular histological abnormalities, tight-junction disruption, and vacuoles were observed. In vitro, 0.1 mM monobutyl phthalate increased transepithelial electrical resistance in primary mouse Sertoli cells and TM4 cells. Mechanistic experiments in TM4 cells indicated that monobutyl phthalate reduced matrix metalloproteinase-2 (MMP-2) expression and occludin degradation by inhibiting nuclear factor-κB/cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) signaling pathway.

In a review, phthalate-mediated testicular dysgenesis syndrome, especially in rodents, was assessed.[92] The reviewed outcomes included reduced fetal testicular testosterone and INSL3 expression, fetal Leydig-cell aggregation, shortened anogenital distance, cryptorchidism, hypospadias, and testicular atrophy. The researchers found that phthalates with straight side chains that had carbon chain lengths of 5 or 6 (C5 - C6) were the most potent for causing this adverse effect, while side chains of C4 (Dibutyl Phthalate) or C7 were moderate in effect. Phthalates with side chains of C3 had weak adverse effects and very small (C1 and C2, including Dimethyl Phthalate and Diethyl Phthalate) or very large side chains (C8 or greater) were ineffective. The authors noted that human exposure levels are substantially lower than the doses used in rodent studies and that species-dependent sensitivity requires further investigation.

GENOTOXICITY STUDIES

Some genotoxicity assays are no longer considered appropriate as standard methods for evaluating genotoxic potential under current OECD guidance. (See https://www.cir-safety.org/sites/default/files/Genotoxicity_ResourceDocument_062026.pdf for the identification of such study types.) Although these assays may be reported in the published literature, unpublished reports, or data submissions, the results from these assays (as identified in the guidance) are of limited interpretability and therefore, the studies are not summarized herein.[2,8]

In gene mutation studies, Dibutyl Phthalate was mainly not mutagenic in multiple Ames tests, with and without metabolic activation in several Salmonella typhimurium strains.[2] *In additional studies, Dibutyl Phthalate (up to 2000 µg/plate with and up to 500µg/plate without metabolic activation) was mutagenic in S. typhimurium strain TA100 without activation at all doses tested, but mutagenicity was not observed in strains TA98, TA1535, TA1537, TA1538, or TA2637, with or without metabolic activation or in strain TA100 with activation.*[8] *Mutagenicity was observed with Diethyl Phthalate and Dimethyl Phthalate (up to 2000 µg/plate with and up to 500µg/plate without metabolic activation)in strains TA100 and TA1535 without metabolic activation only. Diethyl Phthalate and Dimethyl Phthalates were mutagenic in Ames tests conducted with S. typhimurium strain TA100.*[2] *In a different Ames test, Dimethyl Phthalate (500 - 4000 µg/plate) was mutagenic in S. typhimurium strain TA100 in a dose-dependent manner without metabolic activation.*[8] *Monobutyl phthalate (up to 4000 µg/plate) was not mutagenic to S. typhimurium strain TA100 without metabolic activation. No mutagenicity was reported in Ames tests performed by the NTP on Diethyl Phthalate (up to 10,000 µg/plate) and Dimethyl Phthalate (up to 6666 µg/plate), with and without metabolic activation. Dibutyl Phthalate and Diethyl Phthalate were not mutagenic in studies with Escherichia coli.*[2] *Dibutyl Phthalate (up to 0.06 µl/ml) and Dimethyl Phthalate (up to 0.6 µl/ml) were found to be mutagenic in a L5178Y mouse lymphoma assay with metabolic activation, but not without.*

Dibutyl Phthalate was negative for chromosome aberrations in Chinese hamster cells in one study (concentrations tested not reported).[2] *Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate (0.25 mg/ml) had no effect on chromatid aberrations in human leukocyte cultures when compared to controls.*[8] *Neither Diethyl Phthalate (up to 10,000 µg/plate) nor Dimethyl Phthalate (up to 6666 µg/plate) induced chromosomal aberrations in Chinese hamster ovary (CHO) cells.*[8] *The genotoxicity of Dibutyl Phthalate (354 µmol/ml) was evaluated human mucosal cells from the upper respiratory tract. An increase in single strand breaks in DNA was observed, with cells of the nasal mucosa having more sensitivity than oropharyngeal epithelia.*

Genotoxicity studies are summarized in Table 8. In gene mutation assays, Dibutyl Phthalate (up to 2000 µg/plate) and Diethyl Phthalate (up to 5000 µg/plate) were not genotoxic in separate Ames tests, with and without metabolic activation.[5,6] In a third Ames test, Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate were mildly genotoxic in *S. typhimurium* strains TA100 and TA1535 without metabolic activation in a dose-dependent manner when tested at up to 2000 µg/plate.[93] Diethyl Phthalate (up to 771 µg/ml) was also not genotoxic in a gene mutation assay in mouse L5178 TK +/- lymphoma cells.[6] Dibutyl Phthalate (up to 80 µM) caused chromosomal damage in a micronucleus test using cultured bovine lymphocytes.[94] In additional chromosomal damage studies, Dibutyl Phthalate (up to 30 µM) caused a non-linear induction of micronuclei percentage and a higher occurrence of chromosomal aberrations in CHO cells at all tested concentrations when compared to the vehicle control.[95] A micronucleus assay of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate tested at 0.05 or 0.2 ppm using human lymphoblast cells found no significant differences in percent frequency between the low and high concentrations of Diethyl Phthalate and Dimethyl Phthalate, but the percent frequency of micronuclei for Dibutyl Phthalate was significantly lower at 0.2 ppm than at 0.05 ppm ($p < 0.001$).[15] Diethyl Phthalate (up to 1780 µg/ml) was not genotoxic to human lymphocytes.[6] In studies of DNA strand breaks, genotoxicity was observed to Dibutyl Phthalate when tested in human mucosal cells, and human peripheral lymphocytes at up to 354 µmol/ml and in cultured bovine lymphocytes at up to 80 µM.[94,96] Genotoxicity was also observed to Dibutyl Phthalate and monobutyl phthalate in human peripheral blood mononuclear cells at up to 10 µg/ml; neither phthalate were capable of creating adducts with DNA.[97]

In a 3-generation alkaline comet assay, groups of pregnant Wistar rats received olive oil or 500 mg/kg/d Dibutyl Phthalate by gavage on gestation days 6 - 20 and throughout lactation.[98] Dibutyl Phthalate significantly increased olive tail moment, tail DNA percentage, and tail length in the examined neural tissues of all 3 generations; DNA damage was generally greatest in F_3 animals. Treatment also reduced DNA and RNA content in several neural regions, decreased superoxide dismutase and catalase activities, and increased malondialdehyde levels.

CARCINOGENICITY STUDIES

Dermal

In a study performed by the NTP, mice were dermally exposed to 0, 9, 18, or 37 µg Diethyl Phthalate in 100 µl acetone 5 d/wk for 103 wk.[8] The 2-yr survivability of treated mice was similar to that of controls. There were no adverse clinical signs and no signs of dermatotoxicity. An increase in liver neoplasms was observed in male and female treated mice. The incidence of adenoma or carcinoma (combined) was increased in 37 µg group males and in the 9 and 15 µg group females, but not in the 37 µg group females. The increased incidence of liver neoplasms was considered equivocal because the incidence of hepatic neoplasms was within the historical range and because there was no clear dose-response relationship in females.

A similar study was performed in rats with 0, 123, or 368 µg Diethyl Phthalate (neat).[8] Survival of treated rats was similar to the controls for the first 15 mo; however, survival to 2 yr was reduced in male rats in all groups, including the controls. The incidence of anterior pituitary adenoma was higher in all groups of male and female rats than the historical incidence. This may have been partially responsible for the reduced survivability of the males. No adverse clinical effects or evidence of carcinogenic activity from treatment with Diethyl Phthalate were observed in male or female rats.

Oral

Carcinogenesis was not observed in rats that were fed Dibutyl Phthalate for 18-mo or longer.[2] *No further details were provided.*

The carcinogenicity potential of Dibutyl Phthalate was evaluated in a 2-yr study in mice and rats performed by the NTP.[55] Groups of 50 male and 50 female B6C3F1/N mice received 0, 1000, 3000, or 10,000 ppm Dibutyl Phthalate in feed. The estimated average chronic chemical consumption was 105 - 112, 329 - 347, and 1306 - 1393 mg/kg bw/d Dibutyl Phthalate for the 1000, 3000, and 10,000 ppm dose groups, respectively. Feed consumption and body weights were measured and clinical observations were made through the study period. Complete necropsies and microscopic examinations were performed on all mice at the end of the study. No exposure-related effects on survival were observed. Mean body weights were lower only in the 10,000 ppm groups when compared to the control groups. Feed consumption was comparable to the controls. There was no exposure-related increase in neoplasms. Effects to the male reproductive system are summarized above in the Developmental and Reproductive Toxicity Studies section and in Table 7. Nonneoplastic lesions observed generally occurred only in the 10,000 ppm group; these included hepatocyte cytoplasmic alteration in male and female mice, multinucleated hepatocytes in male mice, and renal tubule hyperplasia in female mice. The authors of the study concluded that there is no evidence of carcinogenic activity of Dibutyl Phthalate in male or female mice at up to 10,000 ppm.

In the rat study, groups of 45 - 47 time-mated female Sprague Dawley rats were exposed to 0, 300, 1000, 3000, or 10,000 ppm Dibutyl Phthalate in feed starting on gestation day 6 and continuing through lactation.[55] Body weights and feed consumption were measured and clinical observations were made, and complete necropsies and microscopic examinations were performed on all F_1 rats at the end of the study. Post-weaning, groups of 50 male and 50 female F_1 offspring consumed diets with the same exposure concentrations as the dams for 2 yr. The estimated average chronic chemical consumption was 16 - 17, 54 - 57, 152 - 169, and 510 - 600 mg/kg bw/d Dibutyl Phthalate for the 300, 1000, 3000, and 10,000 ppm dose groups, respectively. No exposure-related effect in mortality between exposed and control groups was observed through the study period. Reproductive effects are summarized above in the Developmental and Reproductive Toxicity Studies section and in Table 7.

In the F_1 postweaning animals, body weights were approximately within 20% of the control animals. At 2 yr in male rats, the incidence of pancreatic acinus adenomas was slightly higher in the 10,000 ppm group compared to the control group. The marginal increase may be related to the test material as pancreatic acinus adenomas and carcinomas have been associated with peroxisome proliferator-activated receptor alpha (PPARα) activation, which has been observed with exposure to other phthalates. Again, nonneoplastic lesion observed generally occurred only in the 10,000 ppm group; these included hepatocyte cytoplasmic alteration in male and female rats and pars distalis hypertrophy of the pituitary in male rats. The authors of the study concluded that there was equivocal evidence of carcinogenic activity of Dibutyl Phthalate in male rats based on the marginal increased in the incidence of pancreatic acinus adenomas at up to 10,000 ppm; however, there was no evidence of carcinogenic activity of Dibutyl Phthalate at up to the same concentration in female rats.

Tumor Promotion

Dermal

In a 1-yr initiation/promotion study in male mice, Diethyl Phthalate and Dimethyl Phthalate were tested neat as tumor initiators, with and without promotion from 12-*O*-tetradecanoylphorbol-13-acetate.[8,99] Diethyl Phthalate and Dimethyl Phthalate were also tested neat for the potential as promoters, with and without initiation from 7,12-dimethylbenzanthracene. Neither Diethyl Phthalate nor Dimethyl Phthalate initiated or promoted skin carcinogenesis.

In Vitro Cell Transformation

Normal human breast epithelial cells (MCF-10A) were co-cultured with fibroblasts derived from normal mammary tissue adjacent to estrogen receptor-positive primary breast cancers and exposed to Dibutyl Phthalate (100 nM).[100] Untreated MCF-10A and MCF-10A co-cultured with fibroblasts alone served as controls. Cell viability was assessed using a MTT assay, cell cycle distribution by flow cytometry, and the protein expression of cell cycle regulators and P13K/AKT/mTOR signaling components by Western blot analysis. The co-cultured cells treated with Dibutyl Phthalate exhibited a significant increase in cell viability when compared to the untreated control ($p < 0.001$) and to the co-cultured control ($p < 0.05$). PDK1 expression was significantly higher in the MCF-10A cells treated with Dibutyl Phthalate compared with the control cells ($p < 0.05$) and the co-cultured cells ($p < 0.05$). Significant higher expressions of P13K, p-AKT, and p-mTOR were observed in the MCF-10A cells treated with Dibutyl Phthalate ($p < 0.05$). Dibutyl Phthalate significantly increased cell percentages in the S and G2/M phases compared to the untreated controls ($p < 0.001$) and the co-cultured cells ($p < 0.05$). Elevated expression of cyclin/CDK complexes (cyclin D/DCK4, cyclin E/CDK2, cyclin A/CDK2, cyclin /CDK1, and cyclin B/CDK1) was also observed. The authors concluded that Dibutyl Phthalate may have a role in the development of estrogen receptor positive breast cancer.

In a related study by the same research group, MCF-10A normal breast cells were treated with Dibutyl Phthalate (10 or 100 nM) or 17β-estradiol (10 nM) in co-culture with mammary fibroblasts.[101] Compared to controls, both Dibutyl Phthalate and estradiol significantly increased cell viability, decreased apoptosis (TUNEL assay), and increased cell numbers in the S and G2/M phases. Western blot analysis showed dose-dependent up-regulation of cyclins/CDKs, PDK1, P13K, p-AKT, p-mTOR, and BCL-2, with concomitant down-regulation of Bax protein, cytochrome C, caspase 8, and caspase 3.

In human breast epithelial R2d cells, exposure to Dibutyl Phthalate (1 μM) for 24 h induced expression of mesenchymal markers vimentin and CD90 and decreased expression of epithelial markers cytokeratin 7 and E-cadherin.[102] After 72 h, the cells began to resemble mesenchymal-like morphological traits, such as spindle-like shape and scattered colony growth.

Analysis of MCF-7 and MDA-MB-231 breast cancer stem cells after exposure to Dibutyl Phthalate (10^{-9} M) revealed enhanced tumorsphere formation ability and higher levels of cancer stem cell markers (CD133, CD44, ALDH1A1, OCT-4, Nanog).[103] The promotion of this stemness is proposed to be mediated by the Sonic hedgehog (Shh) signaling pathway and ΔNp63α upregulation.

The tumor promotion potential of Dibutyl Phthalate (up to 10 μM) in DMSO was studied in normal bladder epithelial cells (SV-HUC-1) and human bladder cancer cells (T24 and UM-UC-3).[104] The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Transwell® assays were used to investigate cell proliferation and migration (cell motility through a porous membrane), respectively, and second-generation sequencing was used to determine any differences in gene expression before and after treatment with Dibutyl Phthalate. Differential gene expression was verified by quantitative polymerase chain reaction (q-PCR) and analyzed using bioinformatics. Dibutyl Phthalate exposure reduced migration of SV-UC-1 cells but enhanced migration of T24 and UC-3 cells in a concentration-dependent manner. This indicated that Dibutyl Phthalate enhanced migration in bladder cancer cells. FOSB, JUND, ATP6V1C2, and RHOQ genes were identified as differentially expressed following Dibutyl Phthalate treatment; q-PCR confirmed FOSB upregulation, and FOSB overexpression promoted both proliferation and invasion in bladder cancer cells.

ANTI-CARCINOGENICITY STUDIES

Dibutyl Phthalate (50 μg/ml) suppressed the growth of HL-60 cells.[8] *Human bone marrow granulocyte/macrophage progenitor and human myeloid leukemia assays were used to show that Dibutyl Phthalate has no effect on normal bone marrow cell growth, but growth of leukemic cells was dose- and time-dependently reduced in vitro. In another study, Dibutyl Phthalate (50 μM) inhibited the incorporation of 3H-thymidine into the DNA of Ehrlich ascites tumor cells and inhibited cell proliferation. Diethyl Phthalate and Dimethyl Phthalate did not show these inhibitory effects. The ability of Dibutyl Phthalate to suppress leukemic cell growth may work through an apoptotic mechanism.*

OTHER RELEVANT STUDIES

Effects on Gene Expression

The altered genetic expressions profiles were studied in fetal male rats from dams exposed to 500 mg/kg/d Dibutyl Phthalate via daily gavage on gestation days 12 to 21.[8] *Corn oil was used as a negative control, and the antiandrogen flutamide (50 mg/kg/d) was administered as a positive control. Sample size was 18 dams per exposure condition. Testes of fetal males were collected and examined by microarray analysis on gestation days 16, 19, and 21 (n = 6 litters per group per examination day). Selected genes were quantified by reverse transcription-polymerase chain reaction (RT-PCR). Dibutyl Phthalate reduced the expression of the steroidogenic enzyme cytochrome P450c17 and steroidogenic acute regulatory protein. Testicular testosterone and androstenedione were decreased on gestation days 19 and 20, while progesterone was increased on gestation day 19 in rats of the Dibutyl Phthalate group. Dibutyl Phthalate exposure also caused an increase in the expression of the cell survival proteins testosterone-repressed prostate messenger-2 and bcl-2, which may be involved in Dibutyl Phthalate-induced Leydig cell hyperplasia. A reduction in stem cell factor receptor in the Dibutyl Phthalate group may contribute to gonocyte degeneration.*

In an ecotoxicological study, zebrafish were exposed to up to 2 mg/l Dibutyl Phthalate for up to 28 d.[105] Feeding of the fish stopped for 24 h before the experiment and the fish were randomly selected on days 7, 14, 21, and 28 to determine various indicators, including antioxidant enzymes and gene expression. Oxidative stress, lipid peroxidation, and DNA damage occurred in zebrafish liver according to changes in antioxidant enzymes, MDA, and 8-hydroxydeoxyguanosine (8-OHdG) content. Acetylcholinesterase (AchE) activity was always active and negatively correlated with the Dibutyl Phthalate concentration. The expression of Cu/Zn-sod and gpx genes were similar to that of antioxidant enzymes from 7 to 21 d, while in the end, the inconsistent result appeared due to the time lag effect in protein modification, gene transcription and translation. The mRNA abundance of Caspase-3 and p53 were upregulated, showing a dose-response relationship. The integrated biomarker reaction indicated that the effects of exposure time on zebrafish liver was day 14 > day 28 > day 7 > day 21.

In another study used to assess gene expression, DNA damage, and oxidative stress biomarkers in the brain, zebrafish were exposed to Dibutyl Phthalate (up to 2 mg/l) for 7, 14, 21, or 28 d.[106] Dibutyl Phthalate significantly stimulated SOD and CAT activities, increasing MDA and 8-OHdG contents. On day 28, AChE inhibition rates for 0.08, 0.4, and 2 mg/l were 13.4, 11.9, and 14.7%. The trend of Cu/Zn-sod gene variation was consistent with SOD activity, showing "inhibition-activation-inhibition". Integrated biomarker response values showed a dose-response relationship on day 28.

CD-1 female mice were used in a study of potential alterations by Dibutyl Phthalate (99.6% pure) on metabolizing enzymes in the ovary and liver after single or repeated doses of the test material via gavage.[31] Groups of 5 mice/treatment received 0, 1, 10, or 1000 mg/kg Dibutyl Phthalate in tocopherol-stripped corn oil either once or once daily for 10 d. The mice were killed a 2, 6, 12, or 24 h after the single dose or the final dose, and tissues, including liver and ovaries, and serum

were collected to extract RNA to generate cDNA for enzyme analysis. Hepatic and ovarian expression of *Lpl*, *ldh1a1*, *Adh1*, *Ugt1a6a*, and *Cyp1b1* were altered in the treated mice in a time- and dose-specific manner.

Epigenetic Effects

The effects of Dibutyl Phthalate and its metabolite, monobutyl phthalate, on epigenetic parameters were studied in human peripheral blood mononuclear cells.[107] The cells were incubated with each test material at 0.001, 0.01, or 0.1 µg/ml for 24 h. Methylation in the promoter regions of tumor suppressor genes (P16, TP53) and proto-oncogenes (BCL2, CCND1) were assessed and the expression profile of these genes was analyzed. When compared to controls, a statistically significant reduction of global DNA methylation level was observed in the cells exposed to monobutyl phthalate at 0.001 and 0.01 µg/ml, but no statistically significant changes were observed for Dibutyl Phthalate. For TP53 gene promoter, statistically significant hypermethylation from monobutyl phthalate was observed at all concentrations tested, but for Dibutyl Phthalate, an increase was only observed at 0.001 µg/ml. With P16 gene promoter, Dibutyl Phthalate and its metabolite caused hypermethylation at the 2 highest concentrations. No significant changes were observed in methylation within the promoter of the CCND1 gene with monobutyl phthalate, but Dibutyl Phthalate caused statistically significant hypomethylation within this gene. Both forms of the phthalates caused a decrease in methylation within BCL2 gene promoter at 0.01 and 0.1 µg/ml. A statistically significant decrease in the expression of TP53 and P16 genes was found with monobutyl phthalate at all concentrations and with Dibutyl Phthalate at 0.01 (for TP53 only) and 0.1µg/ml (for both genes). The greatest increase in CCND1 gene expression was observed with Dibutyl Phthalate at 0.001 and 0.01 µg/ml, while no changes were observed with monobutyl phthalate. An increase in BCL2 gene expression was observed with 0.1 µg/ml Dibutyl Phthalate, and Dibutyl Phthalate and its metabolite at 0.01 µg/ml caused a statistically significant increase in this examined parameter.

Endocrine Effects

In Vitro

The estrogenic effects of several phthalate esters were studied in a recombinant yeast assay and in estrogen-responsive human breast cancer cells; Dibutyl Phthalate and Diethyl Phthalate were shown to have extremely weak estrogenic activity.[8] *In another study, MCF-7 cells were transfected with human estrogen receptor construct associated with a luciferase reporter gene. The luciferase response of cells exposed to 10 µM Dibutyl Phthalate was 36% of the response to 10 nM 17-β-estradiol, suggesting a weak estrogenic activity.*

In recombinant yeast assay to determine the estrogenic potential of Diethyl Phthalate, a strain of Saccharomyces cerivisiae contained a DNA sequence for human estrogen receptor (hER) associated with the reporter gene lac-Z (encoding the enzyme β-galactosidase).[8] *Binding to and activation of the hER in this yeast assay results in an increase in the synthesis and secretion of β-galactosidase β-estradiol produced an estrogenic response with an* EC_{50} *of* 3.2×10^{-11} *M. Spectrophotometry of the medium treated with Diethyl Phthalate at concentrations up to* 10^{-3} *M did not differ from that of negative control and untreated cultures.*

In a protein binding assay, several phthalates were evaluated for their potential for binding to sex hormone binding globulin (SHBG) in competition with radiolabeled H^3-E_2. *Dibutyl Phthalate and monobutyl phthalate (each at 200 µM) failed to displace appreciable amounts of* H^3-E_2, *and thus, did not bind to SHBG. In a study evaluating the ability of phthalate esters to inhibit the binding of known (radiolabeled) ligands to endocrine receptors from healthy human uterus and prostate tissues, Dibutyl Phthalate (100 µM), monobutyl phthalate (10 µM), Diethyl Phthalate (100 µM), and monoethyl phthalate (10 µM) did not significantly inhibit the binding of known ligands to the estrogen, progesterone, testosterone, or retinoic acid (uterus and prostate) receptors.*

Dibutyl Phthalate exposure caused a slight increase in the number of progesterone receptors in rat uterine tissue, but the action was one-millionth the efficacy of β-estradiol.[8] *Dibutyl Phthalate at* 1.0×10^{-3} *M had weak binding to estrogen receptors in rat uterine tissue, however it showed no estrogenic activity). In another study, Dibutyl Phthalate did not interact with estrogen receptors as determined in three binding assays involving rat, human, and rainbow trout estrogen binding proteins.*

Diethyl Phthalate (10^{-7} *to* 10^{-3} *M) was evaluated in an estrogen receptor binding assay using rat uterine cytosol.*[8] *The cytosol was acquired from the uterine tissue of 10-wk-old Wistar rats. β-Estradiol was used as a positive control in a saturation binding assay, and caffeine was used as a negative control. Diethyl Phthalate or the positive or negative control material were allowed to compete with radiolabeled* H^3-E_2 *to bind with the estrogen receptors in the rat uterine cytosol supernatant preparations. β-estradiol reduced the binding of* H^3-E_2 *to the estrogen receptors with an* EC_{50} *of* 1.3×10^{-3} *M. Caffeine and Diethyl Phthalate at concentration up to* 10^{-3} *M did not reduce* H^3-E_2 *binding in this assay. A study found that Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate each had no binding affinity for the estrogen receptor and failed to prevent [*^{3}*H]-estradiol from binding to estrogen receptors obtained from the uteri of NCTR:SDN outbred rats.*

Monobutyl phthalate, monoethyl phthalate, and monomethyl phthalate did not affect estradiol production in primary cultures of rat ovarian granulosa cells at concentrations up to 400 µM. Monobutyl phthalate at 100 µM did not affect aromatase RNA and aromatase protein levels in cultured granulosa cells.[8]

The effects of Diethyl Phthalate and monoethyl phthalate on the sex steroid hormone system were studied using human adrenocortical carcinoma (H295R) cells.[108] The cells were exposed to 0, 0.004, 0.04, or 0.4 mM of Diethyl Phthalate or

monoethyl phthalate for 48 h. The media (Dulbecco's modified Eagle's) was collected for sex steroid hormone measurement using ELISA. Gene expression was analyzed from the total RNA extracted from the H295R cells. Diethyl Phthalate significantly reduced testosterone concentrations and significantly increased the17β-estradiol/testosterone ratio starting at 0.004 mM. These reductions and increases were observed with the metabolite, but statistical significance was observed at 0.04 mM with testosterone and 0.4 mM with the 17β-estradiol/testosterone ratio. A statistically significant down-regulation of *StAR* and *3βHSD2* gene and up-regulation of *CYP19A* gene were also observed with both Diethyl Phthalate and monoethyl phthalate.

Animal

Monobutyl phthalate did not promote growth of the uterus in 20- to 22-d-old female rats (6 rats/dose group) that received oral doses of the test material at 0, 10, 100, 500, or 1000 mg/kg/d for 3 d.[8] *A positive control compound, estradiol benzoate (0.5 µg/rat/d) did cause an increase in uterine weight.*

Male zebrafish were exposed to Diethyl Phthalate (up to 10 mg/l) dissolved in DMSO for 14 d in accordance with OECD TG 204.[108] The fish were killed at the end of the exposure period, and liver and testis were dissected out of each fish. Blood samples were collected from the caudal vein. ELISA was used to measure sex steroid hormones and testosterone. Gene expression was analyzed from the total RNA extracted from fish organ samples. No mortalities were observed at any concentration level. Diethyl Phthalate significantly decreased testosterone concentrations and 17β-estradiol at 10 mg/l, decreases were non-significant at the lower concentrations. The test material also significantly up-regulated *cyp19a* gene expression at concentrations of 2 mg/l and higher and down-regulated *StAR* (statistical significance at 10 mg/lg) and *3βHSD* genes.

In a maternal toxicity study, pregnant Sprague-Dawley rats were administered streptozotocin (STZ; 40 mg/kg, intraperitoneally, gestation day 0) and/or Dibutyl Phthalate (750 mg/kg/d, by gavage, gestation days 1 - 3) to establish a gestational diabetes mellitus (GDM) model.[109] Dibutyl Phthalate alone caused unstable blood glucose, whereas combined exposure to STZ and Dibutyl Phthalate significantly increased fasting glucose and oral glucose tolerance test values to the GDM range and decreased serum insulin levels on gestation day 18 ($p < 0.05$). Histological and immunohistochemical examination of the pancreas demonstrated significant suppression of FoxM1 ($p < 0.001$) and increased pSTAT1 expression ($p < 0.05$). Primary islet β cells were exposed to 1, 10, or 100 µM Dibutyl Phthalate. Cell viability, assessed at 24 and 48 h, decreased in a concentration-dependent manner. Insulin levels also decreased with increasing Dibutyl Phthalate concentration. Exposure was associated with reduced FoxM1 expression and increased STAT1 phosphorylation. Cotreatment with 50 µM fludarabine, a STAT1 phosphorylation antagonist, partially attenuated the loss of cell viability at 24 h; whereas IFNγ, a phosphorylation agonist, further suppressed FoxM1. These findings indicate that maternal Dibutyl Phthalate exposure can aggravate GDM through pSTAT1-mediated suppression of FoxM1, leading to impaired β-cell function, reduced insulin secretion, and hyperglycemia.

Adverse Outcome Pathway

In a comprehensive evaluation conducted by a US Environmental Protection Agency (EPA) research group, mechanistic and toxicological data were systematically organized using the Adverse Outcome Pathway (AOP) framework to assess the mechanisms and species relevance of Dibutyl Phthalate-induced male reproductive toxicity.[110] Employing a structured weight-of-evidence approach, the authors performed extensive literature searches across multiple databases (PubMed, Web of Science, Toxline, and TSCA submissions) with no date or language restrictions, supplemented by manual screening of regulatory citations. Eligible studies meeting predefined Population–Exposure–Comparator–Outcome criteria were compiled into a curated database capturing life stage, exposure conditions, and mechanistic endpoints. Evidence from rodent studies, ex vivo human fetal testis tissue cultures, and human fetal xenograft models was evaluated according to the mode-of-action/AOP framework, integrating molecular, cellular, tissue, and organism-level responses. Three relevant biological elements were identified: 1) fetal rats are more sensitive than other rodents and human fetal xenografts to Dibutyl Phthalate-induced anti-androgenic effects, 2) Dibutyl Phthalate-induced androgen-independent adverse outcomes are conserved among different mammalian models and human fetal testis xenografts, and 3) Dibutyl Phthalate-induced anti-androgenic effects are conserved in different mammalian species when exposure occurs during postnatal life stages.

Cytotoxicity

A 10 ppm solution of Dibutyl Phthalate in phosphate buffer at pH 7 decreased the percent survival of the yeast, Saccharomyces cerevisiae, throughout a 48-h incubation; a 20 ppm solution was even more toxic.[2] *A concentration of 50 ppm of Dibutyl Phthalate completely inhibited the growth of cells of the protozoan, Tetrahymena pyriformis. Other phthalate esters were inhibitory as well (no further details available).*

The minimum inhibitory concentration of a 10% Diethyl Phthalate solution (in 95% ethanol) was 1000 ppm for Corynebacterium sp. and > 1000 ppm for Staphylococcus aureus and Escherichia coli.[2] *The minimum inhibitory concentration of a 10% (w/v) solution of Diethyl Phthalate (in 95% ethanol) was 500 ppm for the fungus, Candida albicans. The concentration of neutralized Diethyl Phthalate that inhibited the multiplication of Pseudomonas putida was greater than 400 ppm. Neutralized Diethyl Phthalate inhibited the multiplication of the flagellate protozoan, Entosiphon sulcatum at a concentration of 19 ppm. Neutralized Diethyl Phthalate 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.*

In a validation study for a phototoxicity assay, cultured skin cells were exposed to 10, 30, 50, 70, or 100% Diethyl Phthalate and exposed to UVA and UVB radiation for 30 minutes.[8] *Controls were not irradiated. At concentrations of 30% and higher, virtually all cells in the culture system were killed, with or without radiation. At 10% Diethyl Phthalate, 70% viability of the cells were observed.*

A concentration of 1000 ppm of Dimethyl Phthalate markedly inhibited the growth rate of Tetrahymena pyriformis. The growth of Pseudomonas aeruginosa was slightly inhibited by 1500 ppm Dimethyl Phthalate. The growth of the blue-green alga, Microcystis aeruginosa, was inhibited by 100 to 300 ppm of Dimethyl Phthalate and suppressed for 3 d by 400 ppm. After 4 d, cellular lysis was observed in the 400 ppm Dimethyl Phthalate culture, and concentrations of 500 ppm to 800 ppm completely destroyed the cells within 72 h.

The metabolism and toxicity of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate in cultures of mouse fibroblast have been investigated.[2] *The ID_{50} (dose required to inhibit growth by 50%) for Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate determined from dose-response curves were 1 x 10^{-4}, 3 x 10^{-3}, and 7 x 10^{-3} M, respectively. Toxicity to mouse fibroblasts was also investigated using a cell overlay method where pads containing 0.05 ml of a 50 mg/ml emulsion of Dibutyl Phthalate, Diethyl Phthalate, and/or Dimethyl Phthalate (not clear whether the 3 phthalates were tested together or separately) were placed on the agar surface (2.5 mg per pad), and the cells were observed for 48 h. Diethyl Phthalate and Dimethyl Phthalate were toxic to cells; however, Dibutyl Phthalate was not toxic to cells. In another study, a toxic response of mouse fibroblastic cells to 1, 5, 10, and 50% suspensions of Diethyl Phthalate and Dimethyl Phthalate was observed; Dibutyl Phthalate elicited a toxic response in the 10 and 50% suspensions only. A decrease in cellular ATP concentrations was observed over a 6-h incubation with Dibutyl Phthalate and Diethyl Phthalate.*

The effects of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate on the outgrowth of nerve fibers and fibroblasts in primary cultures of rat cerebellum were investigated.[2] *Dibutyl Phthalate and Diethyl Phthalate completely inhibited outgrowth at concentrations greater than or equal to 1.17 x 10^{-3}M and 1.53 x 10^{-3} M, respectively. Dimethyl Phthalate did not completely inhibit outgrowth at concentrations less than or equal to 3.05 x 10^{-3} M. Dibutyl Phthalate inhibited cell growth in addition to causing morphological changes, the appearance of lipid drops in the cytoplasm, and the accumulation of triacylglycerol in the cytosol of human embryonic lung cell cultures after the addition of 40 µg/ml of the test material in culture medium the cells. Dibutyl Phthalate and Diethyl Phthalate 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. In another study, the ID_{50} of Dibutyl Phthalate for human diploid cell strain WI-85 was 1.35 x 10^{-4} M. Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate killed and lysed human amnion and KB human cancer cells in culture. The 7-d IC_{50} (geometrical mean values between the totally inhibitory concentrations and the maximal completely non-injurious ones) were 3.1 x 10^{-2}, 6.3 x 10^{-2}, and 7.7 x 10^{-2} M for Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate, respectively, in HeLa cells.*

Limb bud cells from embryonic rats (gestation day 12.5) were exposed in vitro to a range of concentrations of Dibutyl Phthalate or monobutyl phthalate for 96 h.[8] *Cytotoxicity was measured by uptake of neutral red, and inhibition of cell differentiation was measured by alcian blue staining. The IC_{50} values for cytotoxicity and cell differentiation were 25.54 µg/ml and 21.21 µg/ml, respectively, for Dibutyl Phthalate. Monobutyl phthalate had respective IC50 values for cytotoxicity and cell differentiation at 307.24 µg/ml and 142.61 µg/ml. Co-treatment with antioxidants, such as catalase or vitamin E, protected the cells from Dibutyl Phthalate cytotoxicity and reduced the Dibutyl Phthalate-inhibition of cell differentiation. However, antioxidants did not have a protective effect in monobutyl phthalate-exposed cells.*

The cytotoxicity potential of Dibutyl Phthalate was tested on bovine cultured lymphocytes using the MTT assay.[94] A concentration-dependent decrease in cell viability was observed when tested on concentrations ranging from 10 µM to 100 µM.

In another study using the MTT assay, human sperm were exposed to 13.47, 67.35, or 134.7 µg/ml Dibutyl Phthalate for up to 96 h.[70] A concentration- and duration-dependent decrease in cell viability to Dibutyl Phthalate was observed. Cells exposed to low, medium and high concentrations at 24, 48, 72, and 96 h showed cell viability varies from 80 to 90%, 75 to 89%, 52 to 72%, and 42 to 65%, respectively. Cytotoxicity was apparent only at highest dose level after 98 h exposure of Dibutyl Phthalate, where cell viability was observed to be 42%.

The human keratinocyte cell line, HaCaT cells, were utilized to assess the potential of cytotoxicity of Dibutyl Phthalate using the MTT assay, immunocytochemical, flow cytometric analysis, and western blotting.[111] Cells were exposed to up to 5 mg/ml Dibutyl Phthalate for 24 h in the cell viability assay and to 1 mg/ml Dibutyl Phthalate for 24 h in the other assays. HaCaT cell viability was 85, 81, 75, 62, and 37% after exposure to 0.1, 0.5, 1, 2, and 5 mg/ml Dibutyl Phthalate, respectively. The lowest-observed-effect concentration (LOEC) was 0.1 mg/ml. Dibutyl Phthalate at 1 mg/ml led to significantly decreased colony formation in HaCaT cells. Flow cytometric analysis showed 10% of the cells underwent apoptosis, with a 4.9-fold apoptotic fraction when compared to the vehicle control (ethanol).

The effects of Dibutyl Phthalate (0.0006 - 1.25 µg/ml), Diethyl Phthalate (0.0002 - 0.5 µg/ml), and Dimethyl Phthalate (0.0061 - 12.5 µg/ml) were studied in an MTT cytotoxicity assay in human prostate cancer cell lines DU145 and PC3.[112] α-Lipoic acid was used as an antioxidant compound. A low dose proliferative effect was observed. In combination with the

antioxidant, at IC_{50} and lower doses an increase of cytotoxic effect was observed for Dibutyl Phthalate (IC_{50} 27.3 ppb; 25.2% increase) in DU145 cells, while a decrease was observed with Diethyl Phthalate (IC_{50} 1905 ppb; 8.12% decrease) and Dimethyl Phthalate (IC_{50} 2390ppb; 9.27% decrease). With the PC3 cells, a decrease was observed for Dibutyl Phthalate (IC_{50} 77.2 ppb; 16.0%), Diethyl Phthalate (IC_{50} 477 ppb; 17.0%), and Dimethyl Phthalate (IC_{50} 1301 ppb; 26.6%)

Enzyme Effects

Dibutyl Phthalate (2×10^{-3} M) incubated with rat liver in vitro 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.[2] *In another study, Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate at concentrations of 5×10^{-5} to 1×10^{-3} M inhibited the respiration of isolated mitochondria from rat liver primarily by uncoupling oxidative phosphorylation rather than by inhibiting electron transport or energy transfer. Other researchers using the same concentrations have suggested that the contrary is probably true; Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate inhibited electron transport or energy transfer. In some studies, Dibutyl Phthalate and Dimethyl Phthalate 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. Dibutyl Phthalate stimulated ATPase activity and induced swelling of rat liver mitochondria.*

Administration of 0.7% Dibutyl Phthalate or 0.5% Dimethyl Phthalate in the diet of male rats for 21 d increased hepatic weights and reduced serum cholesterol concentrations.[2] *Acetate incorporation into triglycerides and the steryl ester plus squalene and mevalonate incorporation into squalene plus sterols in liver minces were inhibited by dietary Dibutyl Phthalate; these results were not observed with Dimethyl Phthalate. Dimethyl Phthalate administration resulted in a decrease in total hepatic cholesterol and lipid that was not observed with Dibutyl Phthalate. The intraperitoneal administration of 20 mg/kg/d of Dibutyl Phthalate to mice for 16 d did not significantly lower serum cholesterol but did lower serum triglycerides. Dibutyl Phthalate, 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. A 5×10^{-6} M concentration of Dibutyl Phthalate and Diethyl Phthalate inhibited in vitro human blood lecithin/cholesterol acyltransferase; whereas the enzyme was only slightly inhibited by the same concentration of Dimethyl Phthalate.*

Single-dose intraperitoneal administration of 3.05 ml/kg of Dibutyl Phthalate or 3.6 mg/kg of Dimethyl Phthalates 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.[2] *The activities of these enzymes were not decreased when the test materials were administered intraperitoneally every day for 7 d. Results of another study indicated that Dibutyl Phthalate weakly enhanced the activity of aminopyrine N-demethylase from rat hepatic 10,000 g supernatant. The oral administration of 5 mmol/kg/d of Dibutyl Phthalate for 6 d 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.*

Dibutyl Phthalate and monobutyl phthalate were reported to inhibit mitochondrial respiration by uncoupling energy-dependent processes and inhibiting succinate dehydrogenase activity, causing a diminished energy supply, altered ion transport, and mitochondrial swelling.[8] *Additionally, Dibutyl Phthalate was reported to inhibit the metabolism of arachidonic acid by cyclooxygenase and lipoxygenase in rat peritoneal leukocytes. Aliquots of the rat leukocytes were pre-incubated with 0, 1, or 10, or 100 µg/ml Dibutyl Phthalate and then incubated with 1.7 nM radiolabeled arachidonic acid for 5 min before reactions were stopped with methanol and formic acid. Cells pre-incubated with Dibutyl Phthalate showed reduced concentrations of prostaglandins, LTB4, 5-HETE, 11-HETE, and 15-HETE.*

To evaluate the effects of Dibutyl Phthalate on mitochondrial enzymes in the liver, male Wistar rats were fed a diet containing 5% Dibutyl Phthalate for 35 to 45 d.[8] *The treated rats had reduced body weights; increased liver, kidney, and spleen organ weights; and reduced testicular weights ($p < 0.01$) when compared to control rats. Respiration in liver mitochondria and succinate dehydrogenase and pyruvate dehydrogenase activities were reduced in Dibutyl Phthalate-treated rats. In in vitro preparations of liver cells, less than 100 µM Dibutyl Phthalate inhibited respiration, and 150 µM Dibutyl Phthalate inhibited dehydrogenase activity.*

The inhalation of Dibutyl Phthalate (0.5, 2.5, or 7.0 ppm) for 5 d dose-dependently decreased lung microsomal cytochrome P-450, and was accompanied by a reduction in n-hexane and benzo[a]pyrene (BaP) metabolism.[8] *Liver cytochrome P-450 was not affected, but n-hexane and BaP metabolism in liver was increased. Additionally, Dibutyl Phthalate was a weak inducer of liver cytochrome P-450 in male Sprague-Dawley rats that were dosed with 0, 0.01, 0.1, or 1.0 mmol/kg via gavage for 5 d. Cytochrome P-450 and NADPH-cytochrome-c-reductase activities in the liver were increased at the lowest Dibutyl Phthalate dose, and the microsomal enzyme activity did not return to normal after a 4-wk recovery period. Liver microsomal metabolism of n-hexane was increased by Dibutyl Phthalate, but the increased n-hexane metabolism did not persist. Metabolism of BaP was not affected by Dibutyl Phthalate.*

Oral intake of Diethyl Phthalate (5.4 mmol/kg/d for 3 d) caused a slight (~1.6 fold) increase and Dibutyl Phthalate (3 mmol/kg/d) caused a 5-fold increase in the activity of hepatic laurate 12-hydroxylase.[8] *Likewise, palmitoyl-CoA oxidase*

activity was only slightly increased by Diethyl Phthalate, but increased 6-fold in animals dosed with Dibutyl Phthalte (no further details available).

In an in vitro study using recombinant human microsomes, Dibutyl Phthalate and its monoester metabolite monobutyl phthalate were evaluated for their effects on CYP2C9 and CYP2C19 enzyme activities.[113] The assays were performed using 5 uM diclofenac (for CYP2C9) and voriconazole (for CYP2C19) as probe substrates, with metabolite formation quantified by ultra-high-performance liquid chromatography (UHPLC) or UHPLC–MS/MS. Dibutyl Phthalate produced concentration-dependent inhibition of both CYP2C9 and CYP2C19 activities across the tested concentration range (0.1 - 100 μM). At 100 μM, CYP2C9 activity was inhibited by 67.3% (IC_{50} = 29.63 μmol/l; Ki = 7.06 μmol/l), while CYP2C19 activity was completely inhibited (100%) with an IC_{50} of 2.63 μmol/l and Ki of 7.01 μmol/l. Kinetic analyses demonstrated that Dibutyl Phthalate was a competitive inhibitor of the respective probe substrates and a noncompetitive inhibitor with respect to the cofactor NADPH. In contrast, monobutyl phthalate had little effect on either enzyme, producing 1.34% inhibition of CYP2C9*1* and 23.58% inhibition of CYP2C19*1* at 100 μM.

Hepatotoxicity

Mice that received up to 50 mg/kg/d Dibutyl Phthalate in Tween 80 via gavage for 28 d had disrupted hepatic architecture, inflammatory cell infiltration, and hepatocellular disorganization.[114] Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were elevated, with the AST/ALT ratio significantly increased. Sirius red staining demonstrated collagen deposition in Dibutyl Phthalate-treated livers, and protein expression of fibrosis markers (α-SMA, TGF-β, Col-I) was significantly upregulated. In a HepG2 and LX-2 co-culture, treatment with inhibitors targeting p38MAPK (SB203580), NF-κB (PDTC), and NLRP3 (MCC950) attenuated LX-2 activation, suppressed p38MAPK/NF-κB signaling, and reduced Dibutyl phthalate-induced pyroptosis.

The effects of Dibutyl Phthalate on hepatic structure and function were studied using adult albino male mice.[115] Groups of 10 mice received the corn oil, Dibutyl Phthalate (250 mg/kg), diethylhexyl phthalate (300 mg/kg), or Dibutyl Phthalate + diethylhexyl phthalate (250 + 300 mg/kg) in corn oil (0.2 ml) via gavage for 28 d. An untreated group served as a negative control. All animals were killed at the end of the treatment period and blood was collected for serum lipid profiles and liver function tests. Body weights and liver weights were measured. Liver samples were also collected for histopathological examination, and microphotographs of hematoxylin and eosin-stained sections were evaluated with micrometry.

In the Dibutyl Phthalate group, the mean body weights of the mice were not significantly affected, but a significant ($p < 0.05$) increase was observed in the diethylhexyl phthalate and Dibutyl Phthalate + diethylhexyl phthalate groups. All phthalate treatment groups had significant increases in liver enzymes, liver proteins, and altered serum cholesterol, triglycerides, LDL, and VLDL levels. In the mice treated with the phthalates, marked alterations leading to hepatic hypertrophy were observed. Additionally, a significant ($p < 0.01$; $p < 0.05$) increase in the mean number of micronucleated cells and binucleated cells per unit area was observed following treatment with Dibutyl Phthalate. Treatment with the phthalates caused disorganized structure of trabeculae arising from the hepatic vein, deshaped mononucleated hepatocytes, binucleated hepatocytes with swollen nuclei, dilated sinusoidal spaces, regeneration zones cellular hypertrophy, and intracellular vacuolation in the liver, when compared to the control group. Dibutyl Phthalate and Dibutyl Phthalate + diethylhexyl phthalate caused a highly significant ($p < 0.001$) increase in the relative nucleo-cytoplasmic index of mononuclear hepatocytes when compared to the control group. The researchers concluded that Dibutyl Phthalate and diethylhexyl phthalate alone or in combination, had toxic potential to hepatic tissue.[115]

A 28-d oral gavage study in male Kunming mice (8 per group) was conducted with Dibutyl Phthalate at 50 mg/kg/d.[116] Dibutyl Phthalate exposure resulted in significant increases in the AST/ALT ratio and decreases in albumin (ALB) levels, indicating hepatocellular injury. Histological evaluation showed hepatocyte edema, unclear hepatic cord structure, sinusoidal narrowing, and central vein dilation. Dibutyl Phthalate treatment also increased hepatic ROS, MDA, and phosphorylated ERK1/2. Co-administration of Vitamin E (antioxidant) or PD98059 (ERK inhibitor) attenuated hepatic biochemical and histological alterations, indicating that Dibutyl Phthalate-induced hepatotoxicity is mediated, at least in part, through oxidative stress and ERK1/2 activation.

Renal Toxicity

A 28-d oral gavage study in Kunming mice (8 per group) also assessed renal function following Dibutyl Phthalate exposure at 50 mg/kg/d.[116] Significant increases in serum creatinine and urea nitrogen were observed compared with controls. Histopathological examination demonstrated glomerular and tubular injury, characterized by cellular swelling and structural disorganization. Mechanistic evaluations revealed elevated renal ROS and MDA levels, and activation of ERK1/2 signaling. Co-treatment with vitamin E or PD98059 mitigated these biochemical and morphological alterations.

Another study investigated the effects of Dibutyl Phthalate (50 mg/kg/d) on renal function of male Kunming mice (7 per group) and the potential protective effects of curcumin (2.5 mg/kg/d).[117] Dibutyl Phthalate was administered orally, while curcumin was administered intraperitoneally for 28 d. Mice were divided into a control group, Dibutyl Phthalate group, curcumin group, and Dibutyl Phthalate + curcumin group. Kidney function, oxidative stress biomarkers, and apoptosis factors as well as Bcl-2 and Bax were investigated. A marked increase of renal dysfunction, oxidative stress and apoptosis level after Dibutyl Phthalate exposure was observed when compared to the control. Administration of curcumin to Dibutyl

Phthalate-treated mice appeared to reduce these adverse biochemical changes when compared with the group that received Dibutyl Phthalate alone.

Renal tubular epithelial cells (HK-2) were exposed to Dibutyl Phthalate (100 μM) to investigate mechanisms of renal fibrosis.[118] Dibutyl Phthalate exposure significantly upregulated connexin 43 (Cx43) expression at mRNA and protein levels ($p < 0.01$), as measured by RT-PCR and Western blot. Knockdown of Cx43 with siRNA attenuated Dibutyl Phthalate-induced epithelial–mesenchymal transition, indicated by restored E-cadherin ($p < 0.01$) and decreased N-cadherin ($p < 0.05$) and Snail1 expression ($p < 0.01$). Inhibitor studies further demonstrated that Dibutyl Phthalate increased Cx43 expression through activation of the Ang II/AMPKα2 signaling pathway.

Splenic Toxicity

The effect of Dibutyl Phthalate (> 99% pure) on the spleen and potential protective effects of antioxidants were studied in male BALB/c mice.[119] Groups of 6 mice received saline (control; oral), 50 mg/kg/d Dibutyl Phthalate (oral), 50 mg/kg/d Vitamin E (oral), 50 mg/kg/d Dibutyl Phthalate + 50 mg/kg/d Vitamin E (intragastric), 2.5 mg/kg/d curcumin (intraperitoneal injection), or 50 mg/kg/d Dibutyl Phthalate + 2.5 mg/kg/d curcumin (intragastric) daily for 3 wk. After the treatment period ended, the mice were killed, and the spleens were collected for tissues sectioning for histological examination and preparation of a homogenate. The homogenate was used to determine the levels of ROS, glutathione, IL-1β, necrosis factor-α (TNF-α), cytochrome C, and caspase-3 and -9. Histopathological changes of the spleen were observed in the 50 mg/kg/d Dibutyl Phthalate group, including inflammation and apoptosis associated with oxidative stress. The antioxidants were observed to alleviate spleen tissue damage; reduce oxidative stress, IL-1β, and TNF-α; and reduce the levels of apoptosis-associated factors in the mice exposed to Dibutyl Phthalate.

In a 90-day oral study, Sprague–Dawley rats were divided into four groups (n = 5/group): control, BaP (1 mg/kg bw/d), Dibutyl Phthalate (50 mg/kg bw/d), and combined BaP + Dibutyl Phthalate.[120] Test substances were dissolved in corn oil and administered once daily by gavage. Dibutyl Phthalate alone induced moderate splenic alterations, including blurred boundaries between the white pulp and marginal zone and an enlarged marginal zone. Serum cytokine levels of IL-1β and IL-18 (~ 1.3-fold increase) were elevated compared to controls. In spleen tissue, Western blot analyses indicated increased expression of the pyroptosis-related proteins IL-1β, ASC, and GSDMD (~ 1.3–1.5-fold increase). Dibutyl Phthalate exposure also led to reduced Arg1 expression (~ 0.8-fold decrease; M2 macrophage marker) and increased iNOS expression (~ 1.4-fold increase; M1 macrophage marker), indicating a shift toward pro-inflammatory macrophage polarization. Co-exposure to BaP and Dibutyl Phthalate produced more severe splenic injury and a stronger inflammatory response than either chemical alone. Compared to Dibutyl Phthalate or BaP exposure, the combined group showed synergistic increases in iNOS (1.79-fold), NLRP3 (2.11-fold), and cathepsin B (1.9-fold) protein levels, accompanied by a further reduction in Arg1 (0.8-fold decrease). In vitro studies using primary splenic macrophages demonstrated that Dibutyl Phthalate alone reduced cell viability and activated caspase-1, consistent with the induction of pyroptosis observed in vivo.

Insulin Effects

In Vitro

A culture system of rat insulinoma cells was used to assess the effect of Dibutyl Phthalate on insulin synthesis and secretion.[121] The cells were treated with 15, 30, 60, or 120 μM Dibutyl Phthalate or just the vehicle, DMSO, alone for 24 h. The contents of insulin in the intracellular and extracellular fluid of the cells were measured, and the apoptosis rate and mitochondrial membrane potential the cells were measured by flow cytometry with annexin V- fluorescein isothiocyanate (FITC) conjugate and PI and JC-1, respectively. Western blot was used to measure oxidative stress. Insulin synthesis and secretion in the insulinoma cells were significantly decreased ($p < 0.01$) in the high dose group. Dibutyl Phthalate also caused a significant increase ($p < 0.01$) in apoptosis rate at 30 and 60 μM, and a significant decrease ($p < 0.01$) in the mitochondrial membrane potential in the insulinoma cells starting at 60 μM. The expression of Bax/Bcl-2, caspase-3, caspase-9, and Cyt-C were significantly increased, starting at 30 μmol/l for capase 9 and at 60 μM for the remaining proteins. Starting at 30 μM, oxidative stress levels (MDA, GSH/GSSG) were increased, and the antioxidant index (SOD) levels were decreased.

Animal

The role of Dibutyl Phthalate (> 99% pure) on the development of type 2 diabetes was studied in male Balb/c mice.[122] Groups of 8 mice received 0, 0.5, 5, or 50 mg/kg/d Dibutyl Phthalate orally for 7 wk in combination with a high fat diet and injections of a low dose of streptozotocin. One group received 50 mg/kg/d Dibutyl Phthalate without the high fat diet and injections. Exposure to 50 mg/kg/d Dibutyl Phthalate alone induced a marked decrease in insulin secretion and glucose intolerance, but had no influence on insulin resistance. Combined with a high fat diet and treatment with streptozotocin, Dibutyl Phthalate exposure markedly aggravated glucose intolerance, insulin tolerance, and insulin resistance. Additionally, this combination induced lesions in the pancreas and kidney. Further investigation of the role of Dibutyl Phthalate on the insulin signing pathway found that Dibutyl Phthalate could disrupt phosphatidyl inositol 3 kinase (P13K) expression and AKT (protein kinase B) phosphorylation, and decrease the level of glucose transporter 2 (GLUT2) in the pancreas.

The effects of Dibutyl Phthalate (98% pure) on glucose homeostasis and insulin secretion was studied in groups of 10 male Sprague-Dawley rats.[123] The rats received corn oil (solvent control), Dibutyl Phthalate (500 mg/kg), diethylhexyl phthalate (750 mg/kg), or both Dibutyl Phthalate and diethylhexyl phthalate (500 + 750 mg/kg) via gavage 5 d/wk for 8 wk.

Body weights were determined weekly and fasting blood glucose was measured in tail vein blood at 0, 2, 4, 6, and 8 wk. At the end of the study, an oral glucose tolerance test and insulin resistance test were performed. The rats were then killed. Blood samples were collected from the heart, and the pancreas, perigenital fat, and one side of the gastrocnemius muscle were collected and weighed. During the study, there were no clinical signs of toxicity or mortality. Starting at 2 wk, rats that received Dibutyl Phthalate + diethylhexyl phthalate had significantly lower ($p < 0.05$) body weights than control rats, and non-significantly lower ($p > 0.05$) body weights than the individual phthalate groups. The organ coefficient did not differ significantly between the treatment groups for pancreatic tissue, perigenital fat, and gastrocnemius tissue. Fasting blood glucose was elevated in the Dibutyl Phthalate + diethylhexyl phthalate group at 2 and 4 wk, and in all treatment groups at 6 and 8 wk. Dibutyl Phthalate alone and with diethylhexyl phthalate caused an increase in insulin resistance index, a decrease in insulin sensitivity, and impairment of the function of pancreatic islet β-cells. Dibutyl Phthalate, with and without diethylhexyl phthalate, triggered an increase in plasma MDA and reduction in SOD activity; a reduction in the phosphorylation of PI3K and phospho-protein kinase B (p-Akt473) proteins; an increase in the relative expression of Bax, Caspase-8, cleaved-Caspase-9, and cleaved-Caspase-3; and a reduction in the relative expression of Bcl-2-related Bax in pancreatic tissue and of gastrocnemius GLUT4 in the gastrocnemius muscle.

Vascular Effects

In Silico

Toxicogenomic data from human vascular endothelial EA.hy926 cells exposed to Dibutyl Phthalate (0, 10^{-9}, 10^{-8}, or 10^{-7} M in DMSO) for up to 4-wk were integrated with in silico analyses to investigate potential effects on vascular function.[124] Gene expression was evaluated by RT-PCR, and nine affected genes, including VCAM1, ICAM1, MMP2, ITGA2, ITGA5, ITGAV, ITGB1, ITGB3, and ITGB5 were used for in silico network, pathway, and disease-enrichment analyses using Cytoscape, DAVID, and the Comparative Toxicogenomics Database. The analyses implicated extracellular matrix–receptor interactions, focal adhesions, and cell adhesion molecules, and identified potential associations with cardiovascular, cerebrovascular, and immune system diseases.

In Vitro

Human endothelial EA.hy926 cells were exposed to Dibutyl Phthalate at 10^{-6}, 10^{-5}, and 10^{-4} M to evaluate its effect on angiogenesis.[125] Dibutyl Phthalate at 10^{-4} M significantly enhanced tube formation without affecting cell viability and upregulated angiogenesis-related genes (VEGFA, KDR, and CDH5). The pro-angiogenic effect was abolished by ERK1/2 inhibitor (U0126), PI3K-Akt inhibitor (wortmannin), and nitric oxide (NO) synthase inhibitor (L-NAME). Short-term exposure (15 - 120 min) to 10^{-4} M Dibutyl Phthalate induced phosphorylation of ERK1/2, Akt, and eNOS and increased NO production after 24 - 48 h. Co-treatment with nuclear estrogen receptor (ER) antagonist ICI 182,780 or G-protein-coupled estrogen receptor (GPER) antagonist G-15 eliminated these effects. This study demonstrated that Dibutyl Phthalate promoted angiogenesis in human endothelial cells through ER- and GPER-dependent activation of ERK1/2, PI3K-Akt, and nitric oxide signaling pathways.

Animal

Groups of 6 Wistar rats received Dibutyl Phthalate (25 mg/kg) or Dibutyl Phthalate plus rutin (50 mg/kg) orally for 28 d.[126] A control group received 0.1% DMSO. Cardiac lipid peroxidation, antioxidants, and inflammatory markers activities were measured. Dibutyl Phthalate reduced catalase (CAT) activity, GSH, and Nrf2. Additionally, Dibutyl Phthalate increased tissue C-reactive protein (CRP) and tissue nuclear factor kappa B (NF-κB). However, rutin efficiently reduced CRP and NF-κB, increasing GSH and Nrf2 levels in Dibutyl Phthalate-exposed rats.

In a mechanistic study evaluating the effects of Dibutyl Phthalate on hypertension, male C57BL/6 strain mice (n = 9/group) were orally administered Dibutyl Phthalate by gavage at doses of 0.1, 1, or 10 mg/kg/d for 6 wk, and their blood pressure was monitored using the tail-cuff plethysmography method.[127] Across all Dibutyl Phthalate dose groups, no significant changes in blood pressure were observed compared to vehicle controls. Dibutyl Phthalate exposure caused a significant increase in serum estradiol levels in the 1 and 10 mg/kg/d groups ($p < 0.01$). Co-treatment with ICI182780, an estrogen receptor antagonist, inhibited the Dibutyl Phthalate-induced elevation in NO levels.

Neurobehavioral Effects

The neurobehavioral effect of Dibutyl Phthalate was investigated in adult male NMRI mice.[128] The mice received Dibutyl Phthalate at 6.25, 12.5, 25, 50, 100, or 200 mg/kg in sunflower oil orally once daily for 14 d. After exposure the mice underwent the behavioral assessments using open field, Y-maze, elevated plus maze, passive avoidance test, rotarod, and grip strength tests. At the end of the tests, the mice were killed and brains from the 0, 25, and 100 mg/kg/d dose groups underwent histological analysis by hematoxylin-eosin staining. Exposure to Dibutyl Phthalate reduced total distance movement (starting at 12.5 mg/kg), impaired memory function (starting at 25 mg/kg), and induced anxiety (starting at 12.5 mg/kg) in mice when compared to negative control. Histological analysis showed a significant nuclei size reduction and condensation in dentate gyrus cells in both groups of Dibutyl Phthalate-treated mice when compared to the negative control.

In another neurobehavioral study, the effects of Dibutyl Phthalate were studied in Wistar rat offspring following in utero and lactational exposure.[129] Pregnant Wistar rats received 0, 0.037, 0.111, 0.333, or 1% Dibutyl Phthalate in dietary feed on gestation day 6 to postnatal day 28. Pregnant rats were examined daily for signs of toxicity. On postnatal day 0, litters were

culled to a standardize size and number of each sex. Pups were weighed on postnatal day 0 and weekly thereafter until postnatal day 28. Functional and behavioral development measurements were conducted on 2 pups of each sex from each litter chosen at random. Procedures included: surface righting (postnatal days 4 and 7), air righting (postnatal day 16), negative geotaxis (postnatal days 4 and 7), cliff avoidance (postnatal day 7), forepaw grip time (postnatal day 10), open field (postnatal day 28), and Morris water maze (postnatal day 35). A prolonged gestation period, decreased body weight in both male and female pups, depressed surface righting (postnatal day 7) in male pups, shortened forepaw grip time, and enhanced spatial learning and reference memory in male pups were observed in the 1% Dibutyl Phthalate group. Shortened forepaw grip time was also observed in the 0.037% dose group, along with inhibited spatial learning and reference in male pups. Male pups appeared to be more susceptible than females with treatment effects; however, Dibutyl Phthalate (all dose groups) did not significantly alter surface righting (postnatal day 4), air righting, negative geotaxis, cliff avoidance, or open field behavior in either sex.

In a study of maternal and offspring neurotoxic effects, groups of 20 pregnant C57BL/6 mice received Dibutyl Phthalate (0, 50 and 100 mg/kg/d) in corn oil mixed with feed from gestational day 13 to postnatal day 15.[130] Maternal behavior was measured using pup retrieval and nest shape test at postpartum day 4. The neurodevelopment of the offspring was measured using negative geotaxis, cliff avoidance at postnatal day 7, and swimming test and olfactory orientation at postnatal day 14. RNA and protein expressions in the brain cortex of the control group and the 50 mg/kg/d were analyzed. Nissl-stained sections at the coronal level of interaural were used for counting of dark cortical neurons in mother and pup mice.

Dams that were treated with 50 and 100 mg/kg/d Dibutyl Phthalate exhibited poor maternal behavior, nesting, and retrieval behavior when compared to the control group. The treated dams also had decreases in protein expression of Nr4a3, Egr1, Arc, BDNF and phosphorylation of AKT and CREB in the brain cortex. The pups of the mice from the Dibutyl Phthalate-treated groups had significantly decreased scores in negative geotaxis, swimming, and olfactory orientation tests. The cortex of the pups from the Dibutyl Phthalate-treated groups had an increase in expression of dopamine receptor D2 gene. Nissl staining showed that dark neurons were increased in the cortex of the Dibutyl Phthalate-treated dams and their offspring.[130]

Gastrointestinal Effects

Animal

In an investigation into the potential effects of a short-term exposure of Dibutyl Phthalate on intestinal histopathology, groups of 16 male and female ICR mice received the test material (0, 50, or 500 mg/kg bw) in corn oil by gavage once a day for 10 d.[131] At the end of the treatment period, the mice were killed and the duodenum and jejunum were collected for histological and immunohistochemical analyses. The intestinal villus and crypt of the duodenum and jejunum had variation among the mice in the treatment groups. Villus height and villus height/crypt depth ratio significantly increased ($p < 0.05$) in the duodenum and decreased ($p < 0.05$) in the jejunum. With increasing Dibutyl Phthalate concentration, goblet cell numbers significantly ($p < 0.05$) decreased in both the duodenum and the jejunum compared to the number in the control group mice. Damage to the structure of the small intestine was accompanied by a marked increase in HSP27 expression and a decrease in the expression of HSP70 and HSP90 in both dose groups.

Immunotoxicity

Animal

Zebrafish embryos (30 per treatment; 3 strains used: transgenic, albino, and AB line) were exposed to 0.02. 0.2, or 2 μM Dibutyl Phthalate.[132] Immune cell formation and phagocytosis ability were measured after 6 - 96 h post-fertilization. Respiratory burst activity was measured to assess the immune-toxic effects of the test material. Analysis of T/B relevant gene expression was also performed. No statistically significant differences in hatching and survival rates among the treatment groups; however, some developmental abnormalities, such as yolk sac resorption and swim bladder inflation were inhibited at 2 μM. Exposure to Dibutyl Phthalate inhibited neutrophil and macrophage formation in a concentration-dependent manner. The ability of macrophage phagocytosis decreased after Dibutyl Phthalate exposure. Respiratory burst was induced, and transcription levels of T/B cell-related genes *rag1/2* were up-regulated.

Adult male C57bl/6N mice received 0, 10, or 100 mg/kg/d Dibutyl Phthalate by gavage once daily for 5 wk and were evaluated 1 wk after the final dose.[133] At 100 mg/kg/d, total circulating leukocytes, classical monocytes, and $CD4^+$ T-helper (Th) cells were significantly decreased, whereas non-classical monocytes were increased. Immunofluorescence analysis of the spleen showed decreased $CD4^+$ staining at both doses; at 100 mg/kg/d, $CD3^+$ staining decreased, whereas $CD11b^+Ly6G^+$ staining, a marker of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), and $CD43^+$ staining increased. Plasma levels of IL-21 and MCP-1 decreased, while M-CSF increased. Changes in phosphorylated STAT3, ARG1, NOX2, nitrotyrosine, GCN2, and phosphorylated eIF2α were interpreted as supporting myeloid-derived suppressor cell expansion and pathways potentially involved in T-cell suppression. The authors considered the changes persisting 1 wk after treatment to be indicative of immunosuppression.

In a murine model of contact hypersensitivity induced with the hapten FITC, Dibutyl Phthalate was identified as an adjuvant that promotes TSLP expression in skin keratinocytes.[134] FITC was resuspended in vehicle (1:1 mixture of Dibutyl Phthalate and acetone) to a concentration of 0.5%. Mice were sensitized with 100 μl of 0.5% FITC or vehicle applied to the shaven or unshaven back or abdomen on days 0 and 2, or days 0 and 1. On day 6, baseline ear thickness was measured with

calipers, followed by challenge with 20 µl of 0.5% FITC on one ear and vehicle on the contralateral ear. On day 7 (24 h post challenge), ear swelling was assessed to calculate the change in thickness. Epicutaneous exposure to Dibutyl Phthalate, either alone or in combination with FITC, significantly upregulated TSLP mRNA and protein in keratinocytes. Functional studies in TSLP receptor–deficient (TSLPR−/−) mice and in mice treated with neutralizing anti-TSLP antibodies confirmed that TSLP is required for the development of Th2-type contact hypersensitivity responses. In a similar mouse study of FITC induced contact hypersensitivity, Dibutyl Phthalate exhibited clear adjuvant activity.[135] Epicutaneous co-exposure to FITC and Dibutyl Phthalate significantly increased the number of FITC-positive $CD11c^+/CD8\alpha^+$ dendritic cells in draining lymph nodes ($p < 0.01$), and a smaller but detectable increase was observed in FITC-positive $CD11c^+/B220^+$ plasmacytoid dendritic cells. Dibutyl Phthalate exposure also markedly enhanced IL-4 production, with lymph node cell cultures yielding ~100 - 150 pg/ml compared to undetectable levels (< 3 pg/ml) in FITC/acetone controls. By contrast, Diethyl Phthalate, tested in a 1:1 (v/v) mixture with acetone (concentration not reported), showed relatively weak activity on FITC-positive dendritic cell migration but still enhanced IL-4 production (~100 pg/ml) in draining lymph nodes.

In a related study with an FITC-induced contact hypersensitivity mouse model, female BALB/c mice were sensitized epicutaneously with 0.5% FITC (in acetone) on shaved forelimbs on days 0 and 7 in the presence of Dibutyl Phthalate (2%), and monobutyl phthalate (2%).[136] On day 14, mice were challenged on the ear with 0.5% FITC in acetone–Dibutyl Phthalate (1:1), and ear swelling was measured at 24, 48, and 72 h. In parallel, transient receptor potential ankyrin 1 (TRPA1) activation was evaluated in vitro using CHO cells expressing human TRPA1. Dibutyl Phthalate significantly enhanced ear swelling responses to FITC compared to acetone controls ($p< 0.001$), whereas monobutyl phthalate did not. In vitro, Dibutyl Phthalate activated TRPA1 with an EC_{50} of 7.3 µM, monobutyl phthalate required > 40-fold higher concentrations (EC_{50} ~313 µM). These results indicate that Dibutyl Phthalate itself, rather than its metabolite, enhances FITC-induced skin sensitization, at least in part via TRPA1 activation.

In another murine model of FITC-induced contact hypersensitivity, long-term dermal exposure to Dibutyl Phthalate enhanced Th2-type immune responses and aggravated atopic dermatitis.[137] Male BALB/c mice (n = 8/group) were dermally exposed to saline or Dibutyl Phthalate (0.4, 4, or 40 mg/kg/d) once daily for 40 consecutive days by topical application (0.1 mL) to the shaved backs. On days 41 and 42, mice were sensitized with 120 µL of 0.5% FITC (in acetone/Dibutyl Phthalate 1:1 v/v) or saline alone. On day 47, baseline ear thickness was measured, and animals were challenged with saline or 0.5% FITC on the right ear and vehicle on the left ear, followed by measurement of ear swelling 24 h later (day 48). High-dose Dibutyl Phthalate (40 mg/kg/d) significantly increased ear swelling and epidermal thickness ($p < 0.01$), with histopathologic evidence of inflammatory cell infiltration and mast cell degranulation. Co-exposure to Dibutyl Phthalate and FITC elevated serum total IgE and skin levels of Th2 cytokines (IL-4, IL-5, IL-13) and Th17 cytokine (IL-17A), while IFN-γ and TNF-α remained unchanged. Eosinophil accumulation, indicated by increased eosinophil cationic protein staining, was also observed. Dermal exposure to Dibutyl Phthalate markedly upregulated TSLP expression in skin, suggesting a TSLP-mediated mechanism in the Dibutyl Phthalate-induced enhancement of Th2-type hypersensitivity.

In a similar FITC-induced contact hypersensitivity model, topical exposure to Dibutyl Phthalate during the sensitization phase promoted Th2-type immune responses via TSLP signaling.[138] Female BALB/c mice were topically sensitized on days 0 and 1 with 0.5% FITC in acetone/Dibutyl Phthalate (1:1 v/v, 400 µl on shaved abdomen or 20 µl on ear), and challenged on the right ear with 0.5% FITC/Dibutyl Phthalate more than 5 days later. Ear thickness was measured before and 24 h after the elicitation. FITC/Dibutyl Phthalate sensitization significantly increased ear swelling ($p < 0.001$), and was associated with elevated IL-4 and TNF-α expression in the ears, and increased plasma total IgE levels. Dibutyl Phthalate alone, but not FITC or acetone, induced TSLP expression in the skin during the sensitization phase. Removal of Dibutyl Phthalate from either the sensitization or elicitation phase abolished ear swelling, which was restored by recombinant mouse TSLP injection during sensitization. Conversely, TSLP knockdown using intradermal siRNA significantly suppressed ear swelling and IL-4 production without affecting IL-12p40 levels. Dibutyl Phthalate also enhanced the migration of $FITC^+/CD11c^+$ dendritic cells to draining lymph nodes, although TSLP did not independently induce this migration.

In a murine model of contact hypersensitivity, the adjuvant effect of Dibutyl Phthalate on skin sensitization to multiple haptens was evaluated.[139] Female BALB/c mice (n = 7 - 10/group) were epicutaneously sensitized on shaved forelimbs with a suboptimal dose of each hapten dissolved in acetone or in an acetone/Dibutyl Phthalate mixture (1:1, v/v). For the isothiocyanate-containing haptens, such as 0.5 % (w/v) eosin 5-isothiocyanate (EITC) and 0.5 % (w/v) rhodamine B isothiocyanate (RITC), mice were treated twice (days 0 and 7); for 2.5 % (v/v) phenethyl isothiocyanate (PEITC), mice were treated four times (days 0, 1, 7, and 8), each time with 160 µl of the hapten solution applied to the shaved forelimbs. On day 14, mice were challenged by applying 20 µl of the same hapten solution (0.5 % for EITC and RITC, 2.5 % for PEITC) in acetone/Dibutyl Phthalate (1:1, v/v) to the right ear, while the left ear received vehicle only. For Th1-type haptens, mice were sensitized with 0.04 % (w/v) 2,4-dinitrofluorobenzene (DNFB) on days 0 and 1 or with 0.1 % (w/v) oxazolone (OXA)on day 0. For Th2-type haptens, sensitization was performed on day 0 using 2.5 % (w/v) trimellitic anhydride (TMA), 0.01 % (w/v) methylenediphenyl diisocyanate (MDI), or 0.05 % (w/v) toluene 2,4-diisocyanate (TDI). Mice were challenged with the corresponding hapten solutions on day 5, and ear thickness was measured before (0 h) and at 24 and 48 h post-challenge to evaluate sensitization. Dibutyl Phthalate did not enhance sensitization to Th1-type haptens (DNFB, OXA) or Th2-type haptens (TMA, MDI, TDI). In contrast, Dibutyl Phthalate significantly enhanced sensitization to isothiocyanate-containing haptens, including EITC, RITC, and PEITC. Mice co-exposed to Dibutyl Phthalate during sensitization exhibited

markedly greater ear-swelling responses upon hapten challenge compared with those sensitized without Dibutyl Phthalate ($p < 0.01$).

In an allergic asthmatic mouse model, male Balb/c mice were sensitized and challenged with ovalbumin (OVA), exposed to Dibutyl Phthalate (40 mg/kg bw/d) by gavage on days 1 - 53, and subsequently exposed to OVA aerosol (30 min/d) on days 54 – 60.[38] Compared to OVA alone, co-exposure to OVA and Dibutyl Phthalate produced significantly greater lung inflammatory cell infiltration, higher serum IgE, and increased Th2 (IL-4, IL-5, IL-13) and Th17 (IL-17A) cytokine concentrations in bronchoalveolar (BAL) fluid. These effects were accompanied by alterations in lung histology and airway hyperresponsiveness ($p < 0.01$), consistent with allergic asthma. Oxidative stress was also enhanced, as evidenced by decreased GSH and elevated MDA and 8-OHdG levels ($p < 0.01$), along with significantly increased lung calcitonin gene-related peptide (CGRP) expression. Co-treatment with melatonin attenuated oxidative stress and CGRP expression and alleviated asthma-like symptoms. Collectively, these results indicate that Dibutyl Phthalate exacerbates allergic airway responses through oxidative stress–mediated up-regulation of CGRP.

Human

In a double-blinded, randomized crossover human exposure study, 16 allergen-sensitized adults (sensitized to grass, n = 5; birch, n = 5; house dust mite, n = 6) were exposed for 3 h in an environmental chamber to filtered air or Dibutyl Phthalate (mean concentration ~150 μg/m³), immediately followed by an inhalation challenge with their specific sensitizing allergen.[140] Dibutyl Phthalate exposure significantly enhanced the early allergen response, resulting in a 21.4% greater decline in FEV1 (forced expiratory volume in 1 second, a standard measure of lung function) area under the curve ($p = 0.03$), and increased airway responsiveness by 48.1% in participants without baseline hyperresponsiveness ($p = 0.01$). Dibutyl Phthalate exposure modestly increased the total number of macrophages in BAL (4.6%; $p=0.07$), but significantly increased the proportion of M2-polarized macrophages (46.9%; $p = 0.04$). Effects on immune mediator levels were generally limited; among 65 analytes assessed, only a few were altered, with more pronounced changes in bronchial wash compared to BAL. Collectively, these findings indicate that Dibutyl Phthalate exposure may exacerbate allergen-induced impairments in lung function and exerts immunomodulatory effects in the airways of sensitized individuals. In a follow-up analysis, Dibutyl Phthalate also altered systemic immune endpoints, increasing circulating $CD4^+$ T helper cells and reducing regulatory T cells at both 3 and 20 h post-exposure, with reductions in non-classical monocytes.[141] Notably, the cells and mediators affected by Dibutyl Phthalate were largely distinct from those altered by allergen inhalation alone, suggesting that Dibutyl Phthalate exerted systemic immunomodulatory effects independent of simple enhancement of allergen responses.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Animal

Slight to moderate dermal irritation was observed in rabbit studies with Dibutyl Phthalate (concentrations not reported).[2,8] *Diethyl Phthalate also caused slight to moderate skin irritation in several rabbit studies, including a study with undiluted material and in a shampoo formulation at 1%.*[8] *Dimethyl Phthalate was not irritating in rabbits or guinea pigs (concentrations not reported).*[2,8]

No sensitization was observed in rabbits that received up to 4 ml/kg/d Dibutyl Phthalate or Dimethyl Phthalate (concentration not reported) for 90 d.[2] *Diethyl Phthalate (50%) and Dimethyl Phthalate (concentration not reported) was not sensitizing to guinea pigs.*[8]

Human

Dibutyl Phthalate at 5% in petrolatum was not irritating in 48-h closed patch tests with 53 subjects.[2] *One positive reaction was reported in 1532 subjects that underwent patching with 2% Dibutyl Phthalate, Diethyl Phthalate, or Dimethyl Phthalate in petrolatum in a 48-h closed patch test (no further details provided). A nail polish containing 9% Dibutyl Phthalate was slightly irritating in a 23-h patch test on 13 subjects and not irritating in a 48-h patch test on 25 subjects when tested neat. Another nail preparation containing 6% Dibutyl Phthalate was not irritating in a 4 wk controlled use study with 47 subjects. A deodorant containing 4.5% Dibutyl Phthalate was not irritating in an antiperspirant efficacy test on 43 subjects. The same formulation was slightly irritating in a 21-d cumulative irritancy test on 12 subjects when tested neat. Diethyl Phthalate was not irritating in a primary irritation study with 10 subjects (concentration not reported); little to no irritation was observed to undiluted Diethyl Phthalate in another primary irritation study with 45 subjects.*[8]

In a modified maximization test in 25 subjects, no contact sensitization was observed to a nail polish containing 9% Dibutyl Phthalate.[2] *A deodorant containing 4.5% Dibutyl Phthalate was considered non-sensitizing in a modified human repeated-insult patch test (HRIPT) in 159 subjects. No sensitization was observed in HRIPTs with nail enamels that contained 2.74 or 5.32% Dibutyl Phthalate (105 and 25 subjects, respectively).*[8] *No sensitization was observed in several HRIPTs with up to 100 subjects and undiluted Diethyl Phthalate. Sensitization was also not reported in an HRIPT with Dimethyl Phthalate (concentration not reported) in 16 subjects.*

Dermal irritation and sensitization studies are summarized in Table 9. Undiluted Dibutyl Phthalate and Diethyl Phthalate were not irritating to rabbit skin.[5,6] When induced topically at 75% and challenged at 50%, Dibutyl Phthalate was not sensitizing in a guinea pig maximization test (GPMT).[5] Diethyl Phthalate at up to 100% was not sensitizing in guinea

pigs.[6] Diethyl Phthalate was also not sensitizing in a local lymph node assay (LLNA) when tested at up to 100%. A mouse ear swelling test was used to determine the adjuvant effect of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate (1:1 v/v with acetone; no further details).[142] The mice were sensitized with FITC. A strong enhancement of ear-swelling response was observed in mice treated with Dibutyl Phthalate, while the response was not as strong with Diethyl Phthalate and Dimethyl Phthalate. Additional studies evaluating the enhancement of FITC-induced contact hypersensitivity by Dibutyl Phthalate and the underlying mechanisms are described in the Immunotoxicity section.

Photosensitization/Phototoxicity

Human

Dibutyl Phthalate (6% in a nail preparation) was non-irritating, non-sensitizing, and non-photosensitizing in a prophetic patch test with 99 subjects and in a modified HRIPT with irradiation at challenge in 48 subjects.[2] *While some primary irritation was observed, 25% Diethyl Phthalate in a fragrance material was not sensitizing or photoallergenic in photoallergy studies with 23-35 subjects.*[8]

OCULAR IRRITATION STUDIES

In Vitro

Dibutyl Phthalate was used in a test to determine the utility of using human corneal endothelial cell line B4G12 to evaluate an in vitro model for eye irritancy testing.[143] Cell proliferation and toxicity were assessed after exposure with up to 200 µmol/l Dibutyl Phthalate (≥ 98% pure). Additionally, gene expression and secretion of inflammatory cytokines were evaluated. Decreased cell proliferation was observed (mean IC_{50} = 57.8 ± 14.8 µmol/l with 5% fetal calf serum supplement; 30.6 ± 12.7 µmol/l without 5% fetal calf serum), as well as cell toxicity (lactate dehydrogenase leakage observed at ≥ 50 µmol/l without fetal calf serum; at 200 µmol/l with fetal calf serum). A significant ($p < 0.05$) increased gene expression and cytokine cell secretion were observed for interleukin-1β and interleukin-8. Increased interleukin-6 secretion was also observed.

Animal

Overall, undiluted Dibutyl Phthalate, undiluted Diethyl Phthalate, and nail formulations containing up to 9% Dibutyl Phthalate were relatively non-irritating to rabbit eyes.[2] *An undescribed material containing 12.5% Diethyl Phthalate caused severe conjunctival irritation, including chemosis and discharge in 3 rabbit eyes.*[8] *Undiluted Dimethyl Phthalate may cause severe injury to the eyes of rabbits after prolonged contact.*[2]

In an ocular study in 3 Vienna White rabbits (2 males and 1 female), 0.1 ml of undiluted Dibutyl Phthalate was instilled into the conjunctival sac of the right eye while the untreated left eye served as control.[5] This study was performed in accordance with OECD TG 405. The eyes were not rinsed and were observed for 72 h. Minimal irritation was observed (only conjunctivae redness) and were reversible within 72 h. Dibutyl Phthalate was considered not an ocular irritant in this study.

CLINICAL STUDIES

Retrospective and Multicenter Studies

In 3 different studies, patch testing was conducted in subjects with allergies to glues and/or plastics (n = 173, 357, and 839, respectively).[8] *No allergic reactions were observed in subjects that were tested with 5% (w/w) Dibutyl Phthalate.*

The German Contact Dermatitis Research Group performed a retrospective study on 251 metalworkers that had suspected metalworking fluid dermatitis in 2002 and 2003.[144] Of the 251 patients, 199 were tested with a metalworking fluid series that included Dibutyl Phthalate in 5% pet. Negative patch results to Dibutyl Phthalate were observed in 197 patients; 2 patients had questionable reactions.

A retrospective review of patch testing resulting using the Mayo Clinic Contact Dermatitis Group extended standard series was performed from 2010 to 2023.[145] During this period, 3823 patients were tested with 5% Dibutyl Phthalate. Only 1 patient had a weak positive allergic reaction, and 12 patients had irritant reactions.

Case Reports

A chemical worker accidentally swallowed approximately 10 g of Dibutyl Phthalate.[2] *The worker's symptoms included nausea, vomiting, dizziness, headache, pain and irritation in the eyes, conjunctivitis, and toxic nephritis. He recovered completely after 2 wk. A patient that exhibited acute swelling and erythema of the groin and genitals after using a cream to treat intertrigo had a positive patch test to Dibutyl Phthalate.*[8] *Another patient exhibited anaphylactic shock from a drug capsule containing Dibutyl Phthalate; undiluted Dibutyl Phthalate induced a 3 cm diameter wheal that expanded over the entire arm in a prick test. Proper treatment of a human corneal burn caused by Dimethyl Phthalate resulted in healing within 48 h and no loss of vision.*[2]

In a case report, a 65-yr-old man presented with an acute erythematous rash in his groin and anogenital region following use of a cream product to treat itching in the peri-anal region.[146] There was no personal, family, or occupational history of note. Patch testing using the European stand and medicament series, the cream product, and constituents of the cream

product was performed. Positive reactions were observed on day 4 to 5% Dibutyl Phthalate pet. (++), the cream product (++), 0.1% benzalkonium chloride aq. (+), balsam of Peru (++), and fragrance mix (++).

Occupational Exposure

The health status of 147 workers subjected to prolonged occupational exposure to mixtures of phthalate plasticizers (including Dibutyl Phthalate) was investigated; many workers had a moderately pronounced toxic polyneuritis.[2]

In a study performed in Europe, assessed the exposure of electronic waste processing workers from 10 companies to phthalates, including Dibutyl Phthalate.[147] This assessment including analyzing urine samples from 106 workers at the beginning and at the end of the work week, comparing the urine samples to those of 63 non-occupationally exposed controls, and analyzing settled floor dust collected on the waste processing plants premises. The median concentration of Dibutyl Phthalate metabolite in the workers was 14.1 µg/g creatinine, compared to 7.54 µg/g creatinine in the controls. However, no significant differences were observed between pre- and post-shift concentrations in the workers. The concentration of Dibutyl Phthalate in dust was correlated with the corresponding urinary metabolite in the workers. Workers that used respiratory protective equipment had significantly lower urinary concentration Dibutyl Phthalate.

Occupational exposure to Dibutyl Phthalate was assessed in 37 manicurists.[148] Pre- and post-shift urine samples were obtained on a single workday, and the urine was analyzed for monobutyl phthalate, mono-3-carboxypropyl phthalate, and mono-isobutyl phthalate, metabolites of Dibutyl Phthalate. Work environments varied, with open windows and doors in less than half of the salons and exhaust hoods used in 22% of salons. Approximately 41% of workers used paper masks and 22% wore gloves during work. More than 75% of workers ate in the salon. All 3 metabolites were detected in workers at baseline, with the highest concentration detected in monobutyl phthalate (58.5 ng/ml overall, n = 37), followed by mono-iso-butyl phthalate (10.7 ng/ml overall). Glove users had higher monobutyl phthalate concentration at baseline (91.6 ng/ml, n = 7) than those who did not use gloves (42.6 ng/ml, n = 27). A significant increase ($p = 0.05$) in specific gravity-adjusted monobutyl phthalate concentrations was observed across the work shift (after work concentration overall = 87.2 ng/ml). A small, but significant increase ($p = 0.05$) was observed in mono-3-carboxypropyl phthalate, but no significant changes were noted in mono-iso-butyl phthalate. In linear regression modeling, individual cross-shift change in urinary monobutyl phthalate concentration was not related to whether doors and/or windows were open. Manicurists in salons without local exhaust had a 54% increase in urinary monobutyl phthalate concentration across shift compared with a 7% decrease across shift for those with exhaust ventilation. Glove use was associated with a significant reduction ($p = 0.04$) in urinary cross-shift monobutyl phthalate concentration. The cross-shift mono-iso-butyl phthalate concentration increased 6% among manicurists who did not wear gloves and decreased 15% in manicurists wearing gloves; however, these findings did not reach statistical significance. No associations between cross-shift mono-3-carboxypropyl phthalate concentration and exhaust or glove use were detected.

Other Clinical Reports

A high incidence of premature breast development (thelarche) was reported in young girls (younger than 8-yr-old) of Puerto Rico.[8] *Serum samples from 41 thelarche patients and 35 control subjects in Puerto Rico were analyzed by GC-MS for detection of phthalate esters and other possible contaminants. No pesticides or pesticide metabolites were detected in the samples tested. Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate were detected in the serum of thelarche patients but not the control subjects. There was no statistically significant evidence that the premature breast development was a consequence of phthalate exposure.*

In a study of healthy college students, semen samples from 5 separate recovery groups (number of students not reported) were analyzed by GC-MS for concentrations of Dibutyl Phthalate and Dimethyl Phthalate, for which the respective average concentrations were 10 ± 1.5 and 59.0 ± 6.5% at the level of 10 ppm. Phthalate exposures were presumably from ambient levels in the environment. The concentrations of Dibutyl Phthalate were compared to the sperm densities of the semen samples. The plot of Dibutyl Phthalate concentration vs. sperm density gave two clusters of points. The two clusters were surmised to represent the subjects' differing abilities to metabolize Dibutyl Phthalate. For those with a lesser ability to metabolize Dibutyl Phthalate, the average concentration was 129 ppb, the correlation coefficient between sperm density and Dibutyl Phthalate concentration was -0.4, and the slope of the regression line was -0.7. The subjects with apparent greater ability to metabolize Dibutyl Phthalate had an average semen concentration of Dibutyl Phthalate of 51 ppb. The correlation coefficient between sperm density and Dibutyl Phthalate concentration for the latter cluster was -0.4, and the slope of the regression line was -0.6. The investigators concluded that in both clusters there was a negative correlation between sperm density and semen Dibutyl Phthalate concentration.

ENVIRONMENTAL EXPOSURE

The semen samples of 58 men were analyzed for phthalates by gas-liquid chromatography.[8] *These samples were found to contain concentrations of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate at 0.89, 0.40, and 0.22 mg/kg, respectively. The phthalates apparently entered the body through general environmental exposure.*

In a study done by the Centers for Disease Control and Prevention, phthalate monoesters were measured in urine samples from 289 adult humans aged 20 - 60-yr-old.[8] *The analyses were performed after the samples were treated with β-glucuronidase to release the phthalate monoesters from the glucuronide conjugates. High concentrations of monobutyl*

phthalate (mean = 41.5 ng/ml, max = 4670 ng/ml) and monoethyl phthalate (mean = 345 ng/ml, max = 16200 ng/ml) were reported. Women of child-bearing age, 20 to 40-yr-old, were found to have higher urinary monobutyl phthalate concentrations (46.9 µg/g creatinine) than any other age/sex group (31.4 µg/g creatinine). These data suggest that the subjects had been exposed to diester phthalates, but the source of the phthalate exposure was uncertain.

In a pilot study, urine samples were collected from 19 young children (17 Hispanic, 2 Caucasian; 14 boys, 5 girls; ages 11.8 to 16.5 mo). [8] *The samples were analyzed for the presence of phthalate monoesters after deconjugation by β-glucuronidase. Monobutyl phthalate and monoethyl phthalate were detected in the children's urine samples at respective mean (± standard deviation; SD) concentrations of 117.4 ± 287.6 ng/ml and 184.1 ± 246.9 ng/ml, respectively. The levels of monobutyl phthalate fall into the 75th to 100th percentile of the levels found in adult urine in the previously described study. The primary care givers of the children were questioned about the parents' cosmetic and perfume use, the presence of a farm or pesticide worker in the household, use of plastic toys, the child's health, and diet habits. None of these parameters correlated significantly with urinary phthalate monoester levels.*

The reproducibility of detecting urinary phthalate metabolites in first morning urine samples was studied in 46 women (ages 35-49) that provided the urine samples on 2 consecutive weekdays.[8] *The samples were analyzed for detection of 7 phthalate monoesters. Of the 7 phthalate monoesters sought, 4 were detected in samples from all subjects. The mean concentrations of monobutyl phthalate was 78 ng/ml (range 0.7 - 251 mg/ml); monoethyl phthalate was 260 ng/ml (range 58 - 1043 ng/ml); monobenzyl phthalate was 39 ng/ml (range 6 - 135 ng/ml); and monoethylhexyl phthalate was 16.5 ng/ml (range 1 - 143 ng/ml). Phthalate levels did not differ between the two sampling days, as determined by Pearson coefficient correlation tests.*

The intrauterine exposure to Dibutyl Phthalate from environmental exposure was measured in the cord blood of 50 postpartum women in China using ultra-HPLC-tandem mass spectrometry and solid-phase extraction for the detection of the metabolites.[149] The mean concentration of Dibutyl Phthalate metabolites was 24.7 µg/, with a detection rate of 95.7%.

RISK ASSESSMENT

Margin of exposure (MOE) is a quantitative ratio calculated for cosmetic ingredients by dividing the point of departure (POD) obtained for an ingredient in an animal experiment by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US Environmental Protection Agency (EPA) and European Commission (EC) Scientific Committee on Consumer Safety (SCCS) guidelines. An MOE value greater than 100 has traditionally been considered an indication of safety. The basis for this MOE value of 100 comes from two multiplication factors: a 10-fold factor for extrapolating data from test animals to human beings (interspecies extrapolation), and an additional 10-fold for differences among the human population (intraspecies extrapolation). Notably, the MOE value is sometimes referred to as the margin of safety (MOS) despite the parameters being definitionally different.

The EC SCCP performed an MOE calculation for Dibutyl Phthalate at the trace levels found in tested perfume products in 2007.[22] The result of the calculation was 10,000. The MOE was based on a LOAEL of 2 mg/kg bw/d (from a rat developmental toxicity study) and an SED of 0.0002 mg/kg/d (calculated from 17.79 g/d (exposure to all cosmetic products) x 0.0014% (maximum trace level detected in perfume) x 1000 (unit conversion) x 5% (estimated worst-case maximum absorption rate)/60 (default human weight)). When the calculation was performed at a concentration of 100 ppm (0.01%) Dibutyl Phthalate, the MOE was calculated to be 1350.

CIR staff has prepared an MOE calculation for Diethyl Phthalate using the results from the 2025 concentration of use survey performed by the Council (Table 10). The maximum reported use concentration was 0.15% for leave-on face and neck skin care products and 0.1% for rinse-off cleansing products.[13] Using a conservative NOAEL of 150 mg/kg bw/d from a 16-wk oral study in rats[6,8] and a dermal absorption rate of 5.5% determined from an in vitro human skin study,[150] the calculated MOE was 28,301. When an NOAEL of 200 mg/kg bw/d for developmental and reproductive toxicity was applied, the resulting MOE was 37,735.

The following parameters were used in the calculation:

- $\Sigma_{\text{Sum of each of the separate exposures}}$ = 5.25 mg/d (daily exposure from skin cleansing products) + 0.481 mg/d (daily exposure from face and neck products) = 5.731 mg/d
- Body weight (adult) = 60 kg
- Skin absorption = 5.5 % (absorption of Diethyl Phthalate through human skin reached 3.9 ± 1.2% (mean ± SD, n = 4) of the applied dose over 72 h when the skin was occluded and 4.8 ± 0.7% (mean ± SD, n = 3) when the skin was unoccluded)[8]
- NOAEL - 150 mg/kg bw/d (repeated-dose toxicity): Diethyl Phthalate was administered in the diet of rat (dose at 0, 0.2, 1.0, and 5% in feed, groups of 15 rats of each sex) for 16 wk[6]
  - 200 mg/kg bw/d (maternal & developmental toxicity): Diethyl Phthalate was administered in the diet of rats (dose at 0, 0.25, 2.5 and 5% in feed) during gestation days 6 -15 (following OECD TG 414 - Prenatal Developmental Toxicity Study)[6]

- 222 mg/kg bw/d (reproductive toxicity): dose at 0 (control) 600, 3000, and 15,000 ppm; approximately 15 wk for male and 17 wk for female parents of the F_0 and F_1 generations (following OECD TG 416 – Two Generation Reproduction Toxicity Study)[6,56]

$$SED_{dermal} = \frac{5.731 \text{ mg/d} \times 5.5\ \%}{60 \text{ kg}} = 0.0053 \text{ mg/kg bw/d}$$

$$MOE_{dermal} = \frac{NOAEL_{repeated\ dose}}{SED_{dermal}} = \frac{150 \text{ mg/kg bw/d}}{0.0053 \text{ mg/kg bw/d}} = 28{,}301$$

To address uncertainty in dermal absorption, a conservative Tier 1 calculation assuming 100% absorption was performed using the same aggregate external exposure estimate (5.731 mg/d), body weight (60 kg), and NOAEL (150 mg/kg bw/d), yielding an SED of 0.0955 mg/kg bw/d and an MOE of approximately 1570. All calculated MOE values exceed the safety threshold of 100, indicating an adequate margin of safety.

$$SED_{100\%} = \frac{5.731 \text{ mg/d} \times 100\ \%}{60 \text{ kg}} = 0.0955 \text{ mg/kg bw/d}$$

$$MOE_{100\%} = \frac{NOAEL_{repeated\ dose}}{SED_{dermal}} = \frac{150 \text{ mg/kg bw/d}}{0.0955 \text{ mg/kg bw/d}} = 1570$$

EPIDEMIOLOGICAL STUDIES

Phthalate Exposure and Metabolic Health

In a study of 1015 elderly men and women from Uppsla, Sweden, blood levels of Dimethyl Phthalate, Diethyl Phthalate, diisobutyl phthalate, and diethylhexyl phthalate were measured.[4] *A correlation was made with measures of insulin resistance and poor insulin secretion in nondiabetic participants. Another study performed by the Centers for Disease Control and Prevention measured urinary concentrations of phthalate metabolites in women with diabetes. The odds ratio for diabetes in women with higher levels of n-butyl phthalate, isobutyl phthalate, benzyl phthalate, 3-carboxypropyl phthalate, and the sum of diethylhexyl phthalate metabolites was greater than the odds ratio for women with the lowest concentrations of these phthalates.*

In a prospective cohort study of 618 women in Mexico City, urinary concentrations of Dibutyl Phthalate metabolites (sum of Dibutyl Phthalate metabolites; ΣDBP) measured during the second and third trimesters of pregnancy were associated with adverse long-term maternal metabolic outcomes assessed 4 - 5 and 6 - 8 yr after delivery.[156] Specifically, ΣDBP exposure was characterized by urinary monobutyl phthalate and monohydroxybutyl phthalate concentrations, with geometric mean monobutyl levels of approximately 79 - 85 ng/ml and monohydroxybutyl phthalate levels of approximately 7 - 8 ng/ml across the second and third trimesters. Higher ΣDBP concentrations were positively associated with elevated plasma glucose increased glucose and insulin levels, insulin resistance, and glycosylated hemoglobin (HbA1c) in mothers several years postpartum. These findings suggest that prenatal exposure to Dibutyl Phthalate may contribute to persistent metabolic dysfunction in mothers, including impaired glucose regulation and adverse lipid alterations, years after pregnancy.

A nested case-control study of 1006 type 2 diabetes mellitus cases and a matching number of controls was performed in China to determine the association of the disease with 6 different phthalates detected in baseline serum.[157] The conditional logistic regression, Bayesian kernel machine regression, and Quantile-based g-computation models were utilized for the evaluation of the associations of risk of type 2 diabetes from individual phthalates or a mixture. No statistically significant association was observed between single or mixed phthalates and the incidence of type 2 diabetes risk in the whole population. However, in subpopulation studies of gender, age, body mass index, smoking status, drinking status, education level, and physical activity, serum levels of Dibutyl Phthalate (odds ratio 2.06, 95% CI 1.11-3.96), Σdibutyl phthalate (sum molar concentrations of diisobutyl phthalate and dibutyl phthalate; odds ratio 1.96, 95% confidence interval 1.06-3.76), and Σlow-molecular- weight phthalate (sum of Dimethyl Phthalate, Diethyl Phthalate, Dibutyl Pthalate, diisobutyl phthalate, and butyl benzyl phthalate; odds ratio 2.27, 95% CI 1.17-4.57) were significantly associated with type 2 diabetes in current drinkers. Significant potential interactions were also observed among some of the phthalates, including Dibutyl Phthalate with drinking status on type 2 diabetes risk (P for interaction = 0.005). A significant association was not observed between

serum phthalate levels and type 2 diabetes in the whole population, but current alcohol drinkers exposed to higher levels of Dibutyl Phthalate, Σdibutyl phthalate, and Σlow-molecular-weight phthalates had a higher risk of type 2 diabetes.

Phthalate Exposure and Neurodevelopment

Several epidemiological studies have explored the relationship between phthalate exposure and neurodevelopmental outcomes in children. While numerous studies suggesting a potential link between gestational/childhood phthalate exposure and neurodevelopment, a review of the broader epidemiological literature concluded that the associations between specific phthalates and behavioral domains are inconsistent, likely due to differences in the behavioral and cognitive domains assessed at different ages and the varied timing of exposure measurements across studies.[158]

A prospective cohort study of 417 Korean mother-infant pairs found that higher maternal urinary concentrations of diethylhexyl phthalate and Dibutyl Phthalate metabolites during late pregnancy were associated with lower Mental and Psychomotor Developmental Index scores in infants at 6 mo of age.[159] Maternal exposure was characterized using third-trimester spot urine samples. Geometric mean concentrations of diethylhexyl metabolites were approximately 8.9 µg/l for mono(2-ethyl-5-hydroxyhexyl) phthalate and 7.4 µg/l for mono(2-ethyl-5-oxohexyl) phthalate, while the geometric mean concentration of monobutyl phthalate was approximately 12.4 µg/l. These effects were especially prominent in male infants.

In a Cincinnati-based cohort study of 350 mother-infant pairs, higher maternal urinary concentrations of Dibutyl Phthalate metabolites measured during late pregnancy were associated with improved behavioral organization in 5-wk-old infants.[160] This effect was reflected by decreased arousal, increased self-regulation, and reduced handling needs on the neonatal intensive care unit (NICU) network neurobehavioral scale. Maternal exposure was assessed using spot urine samples collected at 26 wk of gestation. Urinary concentrations of Dibutyl Phthalate metabolites were summarized as the molar sum of monobutyl phthalate and mono-isobutyl phthalate. The geometric mean concentration of monobutyl phthalate was approximately 20.3 ng/ml, and the geometric mean concentration of the summed Dibutyl Phthalate metabolites was 113 nmol/l.

A prospective study assessed the exposure of 30 preterm neonates who received total parenteral nutrition (TPN) during their stay in the neonatal intensive care unit to phthalates and the risk of neurodevelopmental delays at the age of 2 mo.[161] Urine samples for analyzing phthalate metabolites were obtained at admission and then daily until the last day of receiving TPN. The phthalate content in the daily TPN was also analyzed. The neurodevelopment of the neonates was assessed using a developmental questionnaire at 2 mo of age. Approximately 26% of the samples (n = 195) of TPN contained Dibutyl Phthalate above the method detection limit of 0.169 µg/l. In the neonates, all urine samples collected at admission and before discharge contained monobutyl phthalate above the method detection limit of 1.758 µg/l. The mean urinary level of monobutyl phthalate was 66.7 (standard deviation 101.8) µg/l/µmol/l at admission and 44.5 µg/l/µmol/l (standard deviation 40.7) before discharge. The mean molar sum of the metabolites of Dibutyl Phthalate was calculated to be 0.615 µmol/l (standard deviation 1.254) at admission and 0.398 µmol/l (standard deviation 0.181) before discharge. The daily dose of Dibutyl Phthalate and several other phthalates in the preterm neonates measured from their TPN was much lower than the recommended tolerable daily limit. However, these levels were not correlated with the phthalate parent compounds in the TPN solutions, suggesting other sources of exposure in the neonatal intensive care unit, such as devices, tubings, or staff/family members. The contribution of Dibutyl Phthalate in TPN to the preterm neonates' daily dose ranged between 0.002 and 1.427 µg/kg bw, much lower than the oral reference dose of 100 µg/kg bw. While negative association of neurodevelopment was observed with some of the phthalates tested, this association was not observed with Dibutyl Phthalate.

In another study assessing the effects of prenatal exposure of phthalates on neurodevelopment in infants age 4.5 and 7.5 mo, 5 first-morning urine samples were collected from 481 pregnant women in Illinois.[162] Biomarkers included in the assessment were ΣDBP, monoethyl phthalate, sum of all phthalate metabolites (ΣAll), and sum of anti-androgenic metabolites (ΣAA). Overall, anti-androgenic phthalates were associated with higher (i.e., better) scores; however, a one-unit increase in ΣDBP was associated with a 12% increase in problem solving scores in 4.5-mo-old females but a 85% decrease for 7.5-mo-old females. A one-unit increase in monoethyl phthalate was associated with poorer scores on several overcomes, which were sex- and timepoint-specific: a 52% decrease in personal-social score in 7.5-mo-old males, a 39% decrease in fine motor scores in 7.5-mo-old males, and a 6% decrease in fine motor scores in 4.5-mo-old females. A one-unit increase in ΣAll was associated with a 4% increase in personal-social scores in 4.5-mo-old males, but a 17% decrease in 7.5-mo-old males.

In a cross-sectional study of 667 Korean school-aged children, urinary concentrations of diethylhexyl phthalate metabolites were significantly and inversely associated with Full Scale and Verbal Intelligence Quotient (IQ) scores, whereas Dibutyl Phthalate metabolites showed no such relationship.[163] Both diethylhexyl phthalate and Dibutyl Phthalate metabolites were negatively associated with children's vocabulary subscores, but after adjusting for maternal IQ, only the association with diethylhexyl phthalate metabolites remained significant.

In the Mount Sinai Children's Environmental Health birth cohort in New York City, 188 children aged 4 - 7 yr were evaluated for behavioral and executive functioning.[164] Higher maternal urinary concentrations of Diethyl Phthalate and Dibutyl Phthalate metabolites during late pregnancy were associated with increased externalizing behaviors (aggression, conduct, and attention problems) and poorer emotional control and executive function scores. These behavioral domains are consistent with patterns observed in attention-deficit/hyperactivity disorder–like profiles. A follow-up study in the same

cohort observed more autistic-like behaviors in children aged 7 - 9 yr with higher prenatal exposure to Diethyl Phthalate and Dibutyl Phthalate.[165]

In a cohort of 277 New York City mother–child pairs, higher prenatal urinary concentrations of Dibutyl Phthalate and butyl benzyl phthalate metabolites were associated with more internalizing behaviors (e.g., withdrawn and anxious/depressed symptoms) in 3-yr-old children.[166] A similar study on a birth cohort of 1074 Australian children also investigated the potential of prenatal phthalate exposure on maternal oxidative stress and assessed whether oxidative stress mediates the relationship between prenatal phthalate exposure and early internalizing problems.[167] The researchers found that higher phthalate exposure, including Dimethyl Phthalate and Diethyl Phthalate, was associated with increased maternal oxidative stress, and the phthalate mixture was associated with child internalizing problems at age 2 yr but not at age 4 yr. Causal mediation analysis suggested that greater exposure to phthalates increased child internalizing problems at ages 2 and 4 yr by elevating maternal oxidative stress.

Cognitive performance at approximately 13 yr of age was analyzed using data from mother-child pairs in a birth cohort established in the Netherlands from 2002 - 2006 n = 565 for the prenatal-exposure and n = 539 for the childhood-exposure).[168] A prenatal phthalate and pesticide mixture level at 75th percentile, compared with the median, was associated with a decrease in verbal comprehension and a decrease in matrix reasoning scores[164] Diethylhexyl phthalate and Dibutyl Phthalate were the primary contributing chemicals to the mixture effect for matrix reasoning. Higher childhood levels of these chemical mixtures were associated with higher verbal scores, in contrast to the inverse associations observed for prenatal exposure, but these findings could be due to potential exposure misclassification, selection bias, and residual confounding. The authors concluded that prenatal exposure to a mixture of plasticizers and pesticides may have a long-lasting adverse effect on offspring cognition.

A systematic review and meta-analysis of human epidemiological studies evaluated the potential association between prenatal and early-life phthalate exposure and neurodevelopmental outcomes.[169] Six phthalates, including Dibutyl Phthalate and Diethyl Phthalate, were reviewed. In total, 53 epidemiological studies were identified and evaluated for risk of bias and study sensitivity using predefined domain-based criteria covering exposure assessment, outcome measurement, confounding control, and statistical analysis. Evidence was synthesized separately by phthalate compound and neurodevelopmental outcome, and for studies assessing cognitive and motor development in children aged ≤ 4 yr, a random-effects meta-analysis was conducted. The primary outcome domains included cognition (14 studies), motor development (9 studies), behavioral outcomes including attention-deficit/hyperactivity disorder (20 studies), infant behavior (3 studies), and social behavior, including autism spectrum disorder (7 studies). Overall, the review concluded that there is no consistent pattern linking prenatal or early-life phthalate exposure with adverse neurodevelopmental outcomes. The authors noted that null or inconsistent findings may reflect methodological limitations, including exposure misclassification, differences in susceptibility windows, potential sex-specific responses, and confounding by phthalate mixtures.

A case-control study compared urinary concentrations of 10 phthalate metabolites between adolescents (ages 12 - 17) with attention-deficit/hyperactivity disorder (ADHD; n = 39) and healthy peers (n = 41).[170] Differences in the urinary concentrations of several phthalate metabolites were observed between adolescents with ADHD and controls; however, Dibutyl Phthalate concentrations did not differ significantly between the groups ($p = 0.158$). Detection frequencies varied across analytes, and the researchers warned that findings for analytes with low detection frequency should be interpreted with caution. In a multivariable logistic regression, Dimethyl Phthalate was associated with ADHD status (OR = 6.13; 95% CI: 1.10 – 34.34). In factorial analysis of covariance models adjusting for product-use behaviors, the group differences remained significant only for Dimethyl Phthalate. However, these findings should be interpreted with caution because Dimethyl Phthalate had a low detection frequency of 33.3%.

Phthalate Exposure and Reproductive Health

The relationship between phthalate levels and semen parameters was analyzed in semen samples collected from 52 men in Shanghai, China.[171] Samples were analyzed for Dibutyl Phthalate, Diethyl Phthalate, and diethylhexyl phthalate. The 3 phthalates were detected in most of the samples, with levels ranging from 0.09 - 0.57 mg/l (mean 0.16 mg/l) for Dibutyl Phthalate and 0.13 - 1.32 mg/l (mean 0.47 mg/l) for Diethyl Phthalate. A significant positive association between liquefied time of semen and phthalate concentrations of semen was observed. The correlation coefficient was 0.475 for Dibutyl Phthalate and 0.456 for Diethyl Phthalate. There was no significant difference between phthalate concentrations of semen and sperm density or livability, though the correlation coefficients were negative.

In a study comparing semen of fertile (n = 50) versus infertile (n = 130) men in Lucknow, India, Dibutyl Phthalate was detected in greater than 80% of the samples from infertile subjects.[70] In men that were oligoasthenospermic (low sperm count and low sperm motility), the maximum concentration of Dibutyl Phthalate in semen observed was 13.47 µg/ml. The maximum concentration in semen observed in asthenospermic (low sperm motility alone) men was 4.11 µg/ml. Fertile men had a maximum concentration of Dibutyl Phthalate in semen at 0.80 µg/ml. A significant negative association between sperm motility and Dibutyl Phthalate levels was observed in oligoasthenospermic and asthenospermic men ($r = -0.25, -0.20$; $p < 0.01$, $p < 0.01$).

A study evaluated the relationship of preconception phthalate exposure (measured as metabolites) with fecundability and pregnancy loss in women.[172] Data were collected from the Effects of Aspirin in Gestation and Reproduction trial of 1228

women attempting pregnancy for up to 6 menstrual cycles and throughout pregnancy if one occurred. Urine samples collected at enrollment were tested for 20 phthalate metabolites, including monobutyl phthalate, monoethyl phthalate, and mono-methyl phthalate. Monobutyl phthalate had a fecundity odds ratio (FOR) of 0.82 (95% CI 0.70-0.96), while the FOR mono-ethyl phthalate and mono-methyl phthalate were 0.99 (95% CI 0.86-1.13) and 0.92 (95% CI 0.84-1.01), respectively. No consistent associations were observed with pregnancy loss.

Phthalate Exposure and Pubertal Development

A large prospective cohort study of 1151 girls examined the association between childhood phthalate exposure and pubertal development one year later.[173] Girls with the highest urinary concentrations of low-molecular-weight phthalates, including Diethyl phthalate and Dibutyl Phthalate, had a slightly higher prevalence of stage 2+ breast/pubic hair development compared with those with the lowest exposure (prevalence ratio = 1.06). In contrast, a cross-sectional study of 725 Danish girls reported delayed onset of pubic hair development among those with higher urinary concentrations of phthalate metabolites, particularly from Dibutyl Phthalate and butyl benzyl phthalate.

Phthalate Exposure and Breast Cancer

A case–control study in northern Mexico (233 breast cancer cases and 221 controls) analyzed urinary concentrations of nine phthalate metabolites.[174] Monoethyl phthalate, a metabolite of Diethyl Phthalate, was significantly higher in cases than controls and was positively associated with breast cancer risk (odds ratio (OR) = 2.20; 95% CI 1.33 - 3.63), particularly among premenopausal women (OR = 4.13; 95% CI 1.60 - 10.70). In contrast, monobenzyl phthalate and mono(3-carboxy-propyl) phthalate showed inverse associations, suggesting higher exposure was linked to reduced breast cancer risk.

A nested case–control study within the Women's Health Initiative (419 post-menopausal breast cancer cases and 838 controls) measured 13 urinary phthalate metabolites, including monoethyl phthalate, collected over 1 - 3 yr.[175] Multivariable analyses showed no significant associations between phthalate biomarker concentrations and breast cancer, regardless of hormone therapy use, body mass index, or tumor subtype. Overall, phthalate exposure was not linked to increased postmenopausal breast cancer risk.

In a cohort of 102 women, serum levels of several phthalates, including Dibutyl Phthalate, Diethyl Phthalate, butyl benzyl phthalate, and diethylhexyl phthalate, were detected in all participants.[176] Women with breast cancer—particularly those from Mexico City and those with human epidermal growth factor receptor 2 positive ($HER2^+$) or triple-negative subtypes—showed significantly higher Dibutyl Phthalate and butyl benzyl phthalate concentrations than controls or ER^+ patients, indicating a positive association between phthalate exposure and breast cancer incidence.

Phthalate Exposure and Genotoxic Effect

Semen and urine samples of 168 men recruited from the Massachusetts General Hospital Andrology Laboratory analyzed to investigate whether environmental phthalate exposure was associated with DNA damage in human sperm.[177] Eight phthalate metabolites, including monobutyl phthalate, monoethyl phthalate, and monomethyl phthalate were measured in urine by using HPLC and tandem mass spectrometry (HPLC-MS). Sperm DNA integrity was assessed using the neutral single-cell microgel electrophoresis assay (comet assay). VisComet image analysis software was used to quantify comet parameters, including comet length, percent DNA in tail (tail%), and tail distributed moment (an integrated measure reflecting both comet length and DNA intensity in the tail). Results showed that for each interquartile range increase in specific gravity–adjusted urinary monoethyl phthalate, comet extent increased significantly by 3.6 μm (95% CI: 0.74 - 6.47), and increased by 1.2 μm (95% CI: -0.05 - 2.38). No significant associations were observed for monobutyl or monomethyl phthalates with any comet assay parameters.

Phthalate Exposure and Endocrine Effect

The association of maternal urinary phthalates with markers of the thyroid system was studied in 1996 women.[178] Urinary concentration of phthalate metabolites, including monobutyl phthalate, and serum concentration of thyroid simulating hormone, free and total thyroxine (FT4 and TT4), and free and total triiodothyronine (FT3 and TT3) were measured in pregnant women in early pregnancy (median gestational week 10). Monobutyl phthalate was associated with lower T4/T3 (free and total) ratios and higher FT4/TT4 and FT3/TT3 ratios.

A prospective Danish-Finnish cohort study conducted from 1997 to 2001 analyzed individual breast milk samples of mothers to male infants collected 1 - 3 mo postnatally for 6 phthalate monoesters, including monobutyl phthalate, monoethyl phthalate, and monomethyl phthalate.[179] Of the 130 mothers studied, 62 were cryptorchid males and 68 were healthy males. Serum samples of 74% of all males were also analyzed for gonadotropins, sex-hormone binding globulin, testosterone, and inhibin B. The phthalate monoester content in breast milk ranged from 0.6 - 10,900 μg/l for monobutyl phthalate, 0.07 - 41.4 μg/l for monoethyl phthalate, and < 0.01 - 5.53 μg/l for monomethyl phthalate. No association was found between phthalate monoester levels and cryptorchidism. Positive correlation with sex-hormone binding globulin was observed with monoethyl phthalate and monobutyl phthalate ($r = 0.323$, $p = 0.002$ and $r = 0.272$, $p = 0.01$, respectively); and monomethyl phthalate, monoethyl phthalate, and monobutyl phthalate was positively correlated with luteinizing hormone: free testosterone ratio ($r = 0.21 – 0.323$, $p = 0.002 – 0.044$). Monobutyl phthalate was negatively correlated with free testosterone ($r = –0.22$, $p = 0.033$).

Personal Care Product Use and Urinary Phthalate Metabolites

In a cross-sectional analysis of 100 girls from Salinas, California aged 14 - 18 yr enrolled in the Health and Environmental Research on Makeup of Salinas Adolescents study, girls who reported daily makeup use had higher urinary monoethyl phthalate concentrations than girls who rarely or never used makeup (102.2 vs. 52.4 ng/ml, p = 0.04), and recent use of foundation, blush, or mascara on the day of or day before urine collection was associated with higher monoethyl phthalate and monobutyl phthalate concentrations.[151] In another study with adult men, use of cologne or aftershave within the previous 48 h was associated with higher median urinary monoethyl phthalate concentrations, and each additional type of personal care product used was associated with a 33% (95% confidence interval (CI): 14 - 53%) increase in monoethyl phthalate.[152] Studies in 108 children from Mexico aged 8 – 13 yr, cologne or perfume use within the previous 48 h was positively associated with urinary monoethyl phthalate concentrations in boys, while use of makeup was positively associated with urinary monobutyl phthalate concentrations in girls.[153] A more recent study of 30 articles examining personal care product use and phthalate exposure in infants, children, and adults highlighted prominent positive associations between monoethyl phthalate and the use of perfumes or other fragrance-containing products, including shampoos, body lotions, and hair products.[155]

SUMMARY

According to the *Dictionary*, Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate are reported to function as fragrance ingredients, plasticizers, and solvents in cosmetic formulations. Diethyl Phthalate also functions as a denaturant. The Panel first reviewed the safety of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate in a report that was published in 1985, with the conclusion that these ingredients are "safe for topical application in the present practices of use and concentration in cosmetics." This conclusion was reaffirmed in re-reviews that were published in 2005 and 2017. This report was placed on the 2024 CIR Priorities List following nomination by the US FDA for cause.

According to RLD that CIR received in 2025, Diethyl Phthalate is used in 284 formulations, with most of the uses reported in fragrance preparations. Additionally, the RLD reported Dibutyl Phthalate is used in 2 "other" preparations (Category 17) and Dimethyl Phthalate is used in 2 skin care preparations. However, according to communications from the FDA, the 2 uses reported for Dibutyl Phthalate as "other" preparations are actually liquid bandage products, which are not cosmetics, and thereby outside the purview of the Panel. Thus, the Panel's safety assessment conclusion does not apply to these products, and accordingly, there are no cosmetic uses reported for Dibutyl Phthalate.

The results of the concentration of use survey conducted by the Council in 2025 indicate Diethyl Phthalate has a maximum concentration of use range of 0.1 - 0.15%, with 0.15% reported in leave-on face and neck products. No concentrations of use were reported for Dibutyl Phthalate or Dimethyl Phthalate; however, responses to the survey indicated that Dibutyl Phthalate and Diethyl Phthalate may be present in cosmetics as impurities.

Under European regulations for cosmetic ingredients, Dibutyl Phthalate is listed in Annex II, List of Substances Prohibited in Cosmetic Products. In 2004, the SCCNFP determined that Dibutyl Phthalate should not be intentionally added to cosmetic products. Dibutyl Phthalate has been classified as a Category 2 toxic substance to reproduction that "may cause harm to the unborn child."

In the US, Diethyl Phthalate is permitted to be used as a denaturant. Diethyl Phthalate and Dimethyl Phthalate are not restricted from use in any way under the rules governing cosmetic products in the European Union. In 2002, the SCCNFP opined that the safety profile for Diethyl Phthalate supported its use in cosmetic products; this opinion was reaffirmed by the SCCNFP in 2003 and the SCCP in 2007. Globally, Dibutyl Phthalate has been detected in commercial PET bottled water, and in the US, Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate have been detected in feminine hygiene products (pads, panty liners, and tampons). In 2012, the FDA issued guidance for industry on limiting the use of Dibutyl Phthalate as an excipient in regulated drug and biologic products, recommending that use of this ingredient be avoided if an alternative is available. Dibutyl Phthalate (> 1000 ppm or 0.1%) has been prohibited in the manufacturing, sale, distribution, or importation of children's toys and childcare articles. Diethyl Phthalate is permitted to be used as a plasticizer in food packaging material.

In an in vitro study, the percutaneous absorption of [^{14}C]Dibutyl Phthalate in the receptor fluid over 24 h was 27.1 ± 1.9%, with 29.2 ± 2.4% remaining in the skin of hairless guinea pigs (total skin penetration over 24 h = 56.3 ± 2.7%). Over 72 h, the amount of Dibutyl Phthalate in the receptor fluid was 59.9 ± 3.2%, with 13.7 ± 3.1% remining in the skin (total skin penetration over 72 h = 75.6 ± 4.7%). The 9-h percutaneous penetration of 200 or 500 ng/ml Diethyl Phthalate and Dibutyl Phthalate in human keratinocytes for Diethyl Phthalate was 62 and 39%, and for Dibutyl Phthalate were 53 and 34%, for the low- and high-dose groups, respectively. The respective skin accumulation values for Diethyl Phthalate and Dibutyl Phthalate in a study where both were tested at 5.4 mM in 40% ethanol were 1.37 ± 0.26 and 10.8 ± 0.51 nmol/mg in nude mouse skin and 0.13 ± 0.01 and 0.41 ± 0.07 nmol/mg in pig skin. The respective flux values were 96.5 ± 7.97 and 94.7 ± 6.49 nmol/cm^2/h across nude mouse skin and 26.9 ± 6.82 and 11.9 ± 3.20 nmol/cm^2/h across pig skin. A percutaneous absorption study of Dimethyl Phthalate and 2 other phthalates using different carriers determined the permeation rate of the ointment, base cream, and lotion containing Dimethyl Phthalate to be 1.391, 1.216, and 1.120 μg/cm^2/h, respectively. Of the 3 phthalates tested, Dimethyl Phthalate had the fastest permeation rate, regardless of carrier type. The dermal absorption of

[^{14}C]Dibutyl Phthalate in hairless guinea pigs was 62.0 ± 2.0% of the applied dose. Most of the Dibutyl Phthalate was found in the urine (60.4 ± 1.8%) and the amount systemically absorbed in the ovaries, kidneys, and liver were less than 2%. Approximately 2% of Dibutyl Phthalate remained on the skin.

Female mice were used in a study to determine the distribution of 0, 1, 10, or 1000 mg/kg Dibutyl Phthalate after single or repeated doses via gavage either once or once daily for 10 d. Regardless of duration of exposure (single or repeated), monobutyl phthalate was detected in the sera and tissues of Dibutyl Phthalate-treated mice. In single dose mice, monobutyl phthalate levels peaked at < 6 h and fell close to background levels by 24 h post-treatment. Following the last repeated dose, monobutyl phthalate levels peaked at < 2 h and fell to background levels by 12 h.

In human dermal studies, increased excretion of monobutyl phthalate was observed after topical application of a cream containing 2% Dibutyl Phthalate given as daily whole-body topical applications for 5 d. Total monobutyl phthalate excreted in the urine during the treated week was 11.8 ± 0.6 mg/24 h. On average, 1.82% of the applied Dibutyl Phthalate was recovered in urine as monobutyl phthalate. High levels of Diethyl Phthalate and Dibutyl Phthalate metabolite excretions were observed in a controlled chamber study with males exposed to the airborne phthalates for two 6-h sessions (one "hood-on" and the other "hood-off". Modeling of the urinary metabolite data estimated that the daily dermal uptake (0.14 µg/kg bw/d) exceeded the inhalation intake (0.042 µg/kg bw/d) by roughly threefold. In an oral study, an individual male ingested 60 µg/kg of labeled Dibutyl Phthalate in decaffeinated coffee served in an edible cup. The majority of the dose (92.2%) was excreted in the first 24 h, with only < 1% of the dose excreted on day 2. The major metabolite was monobutyl phthalate (84%). Various side chain oxidized metabolites accounted for approximately 8%.

In oral rat studies, the LD_{50} for Dibutyl Phthalate was 6279 mg/kg bw for both sexes. The LD_{50} for Diethyl Phthalate was > 5 ml/kg bw for both male and female rats. For Dimethyl Phthalate, the combined LD_{50} in male and female rats was 5.1 g/kg bw. In inhalation studies with rats, the LC_{50} value for Dibutyl Phthalate in both sexes was estimated to be > 15.68 mg/l after a 4-h exposure, and the LC_{50} for Diethyl Phthalate in both sexes was > 4.64 mg/l after a 6-h exposure.

In a 28-d study, no clinical signs of toxicity were observed in rats that received up to 7000 ppm Dibutyl Phthalate in feed, with or without the addition of 3000 ppm diethylhexyl phthalate. Relative liver weights were increased in males and females of the 7000-ppm Dibutyl Phthalate group and in males of the 1000 ppm Dibutyl Phthalate group. In the groups that received both phthalates, an increase of absolute and relative liver weights were observed in all animals in all combined treatment groups. Mice that received 40 mg/kg bw/d Dibutyl Phthalate for 60 d via gavage had no significant differences in body weight gain or lung function parameters when compared to saline controls. However, oxidative stress biomarkers in lung tissue were significantly altered. Serum total IgE levels showed a statistically significant increase compared to controls, whereas cytokine levels and inflammatory cell counts in BAL fluid were not significantly different from controls. In a 9-wk oral gavage study, male rats treated with Dibutyl Phthalate at 750 mg/kg bw/d had marked histopathological changes in the lungs, including increased inflammatory cell infiltrations, epithelial cell shedding, and mucus secretion. Hematological analysis showed significant elevations in white blood cells, neutrophils, lymphocytes, eosinophils, and platelets. Compared to controls, the Dibutyl Phthalate group exhibited a significant increase in inflammatory cells in rat BAL fluid, accompanied by elevated Th2 cytokines. Oxidative stress was evident, and increased apoptosis was observed in lung tissue.

No mortalities were observed in a 4-wk study in male rats that received Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate, the respective monoesters, or phthalic acid in corn oil via gavage (500 mg/kg bw/d for diesters, 250 mg/kg bw/d for monoester and acid). Livers weights were significantly increased in groups treated with Dibutyl Phthalate and monobutyl phthalate compared to control group. Red blood cells and hematocrit were significantly lower and mean corpuscular hemoglobin concentration and platelet count were significantly higher in the Dibutyl Phthalate group (the monoester also had significantly higher platelet counts), and hemoglobin level was reduced only in the Dimethyl Phthalate group.

The NOAEL for male and female rats that received up to 3170 mg/kg/d (5%) Diethyl Phthalate in feed for up to 16 wk was 150 mg/kg/d (0.2%). Male and female rats that received 5% Diethyl Phthalate for 16 wk had statistically significantly lower absolute weights of the brain, heart, spleen & kidneys than controls; reductions in the absolute weights of the female gonads and male heart, spleen, and kidneys were also observed in the 5% groups after 2 and 6 wk of treatment. Increased kidney weights were seen in males & lower pituitary weights in females in the 1% group after 2 wk of treatment. Increased gonad weights were also seen in females of the 1% group after 6 wk.

Zebrafish were exposed to Dibutyl Phthalate (up to 10 mg/l) for 96 h. The LC_0 was 1.3 mg/l and the LC_{100} was 5 mg/ml.

The NOEC for malformations was 5.8 ppm for Dibutyl Phthalate (tested at up to 15 ppm) and 17 ppm for Diethyl Phthalate (tested at up to 200 ppm) in in vitro 96-h frog embryo teratogenesis assays using *Xenopus laevis* embryos. The mean 96-h LC_{50}s for Dibutyl Phthalate and Diethyl Phthalate in this study were 12.88 and 64.5 ppm, respectively. Malformations were observed starting at 5 ppm in Dibutyl Phthalate-treated embryos and starting at 50 ppm in Diethyl Phthalate-treated embryos. Additional studies in *Xenopus laevis* with Dibutyl Phthalate also noted defects, with mean percent normal development decreased as concentration and exposure time increased.

No significant effects on survival or hatching of zebrafish embryos were observed following exposure to up to 250 µg/l Dibutyl Phthalate; however, body length decrease, yolk sac abnormalities, and immune responses were observed. An acute

developmental toxicity of Dibutyl Phthalate (up to 0.2 mg/l), Diethyl Phthalate (up to 10 mg/l), and Dimethyl Phthalate (up to 8 mg/l) in zebrafish saw a malformation rate of 30% with 0.05 mg/l Dibutyl Phthalate (deaths were observed at higher concentrations). The number of dead fish larvae also increased with increased concentrations of Diethyl Phthalate. Dimethyl Phthalate was not correlated with larvae mortality. All phthalates tested induced different developmental abnormalities in the fish larvae, including abnormal movement, decreased heart rate, spinal curvature, and pericardial edema.

In a dermal study in mice, Diethyl Phthalate (tested at up to 5600 mg/kg/d) applied to pregnant mice on gestation days 0 - 17 had a NOAEL < 500 mg/kg/d in dams and 1600 mg/kg/d in offspring. The dams exhibited dose-related abnormal behavior and reduced thymus and spleen weights were observed at all doses; while significantly reduced fetal body weight and an increased incidence of cervical and lumbar rib variations were observed in 5600 mg/kg/d offspring. In a developmental study in which dams receiving 5, 50, or 500 mg/kg/d Dibutyl Phthalate in canola oil on gestation day 14 to postnatal day 21, female offspring in the 500 mg/kg/d group had delayed vaginal opening. Additionally, male and female offspring of the 500 mg/kg/d group had increased fasting glucose levels, but no effects were observed in insulin secretory response. In a 3-generation reproductive and developmental study in mice, Dibutyl Phthalate in olive oil up to 2000 mg/kg bw induced skeletal malformations in F_0 males, with significant mortality in postnatal life and skewing the sex ratio. An increased frequency of DNA damage in the germ cells was observed in F_1 males; exposure of F_0 males to Dibutyl Phthalate did not affect F_1 male fertility and pregnancy frequency. In multigeneration studies with Dibutyl Phthalate in rats at up to 10,000 ppm in dietary feed, no adverse effects to body weights and body weight gains were observed in parental dams. In one study, male offspring in the 10,000-ppm dose group had high incidence of small or absent reproductive organs and undescended testes, gross lesions correlated with microscopic lesions in the male reproductive organs. In another feed study, anogenital distance was significantly decreased in male F_1 and F_2 offspring in the 10,000 ppm dose groups. F_1 males of the 10,000-ppm dose group also had a statistically significant mean delay in preputial separation, testicular descent, and seminiferous tubular atrophy. In a 2-generation rat feed study with Diethyl Phthalate at up to 15,000 ppm, the NOAEL for parental animals was 15,000 ppm, and the NOAEL for development and growth of pups was 3000 ppm. Body weight gains before weaning were inhibited in F_1 and F_2 pups and vaginal openings were slightly delayed in F_1 females at 15,000 ppm.

Mouse ovarian antral follicles treated with 1000 µg/ml Dibutyl Phthalate in DMSO for 24 h were significantly smaller than controls, and follicles treated with concentrations greater than 100 µg/ml were significantly reduced after 72 h. No toxicity was observed to the follicles after treatment with monobutyl phthalate at the same concentrations for the same periods of time. In in vitro and in vivo studies on the effects on mouse oocyte maturation, Dibutyl Phthalate (200 - 800 µM in vitro; 100 or 500 mg/kg/d in vivo) significantly reduced mouse oocyte germinal vesicle breakdown and polar body extrusion rates. Additionally, oocyte cytoskeleton was damaged and cortical granule-free domains were disrupted; Dibutyl Phthalate also induced early apoptosis of oocyte and granulosa cells. Dibutyl Phthalate in tocopherol-stripped corn oil did not cause significant deviations in the expression of transcripts encoding IGF binding proteins in female mice that received up to 1000 mg/kg/d for 20 d when compared to the vehicle control. Total number of ovarian follicles counted per ovary was reduced in the 100 µg/kg/d group when compared to controls; however, no significant differences were observed in the 10 µg/kg/d or 1000 mg/kg/d dose groups. Significantly low primordial follicle counts observed in the 100 µg/kg/d group and in the 1000 mg/kg/d group compared to controls, also fewer primary follicles were observed in 100 µg/kg/d mice. In another female mouse study with Dibutyl Phthalate in tocopherol-stripped corn oil (up to 1000 µg/kg/d for 30 d), dose-dependent effects on folliculogenesis and gene expression were observed. At 1000 µg/kg, more atretic follicles were observed in the ovaries. In a dose-dependent manner, Dibutyl Phthalate significantly reduced the expression of genes responsible for homologous recombination, mismatch repair, and nucleotide excision repair. Pregnant rats that received 500 mg/kg Dibutyl Phthalate in corn oil every second day starting at gestation day 14.5 through postnatal day 6 exhibited no adverse effects and delivered exclusively live pups. Offspring also exhibited no adverse effects, and female pups had no effects to anogenital distance or adverse effects in vaginal opening. In an oral uterotrophic assay, female rats that received 100 mg/kg Dibutyl Phthalate in corn oil from postnatal day 21 for 3 d had significantly decrease uterine wet weight and minor variations in ovary wet weight; no abnormal clinical signs or symptoms were observed in any of the treated or control animals. Female rats from a pubertal onset assay that were treated with 10 or 100 mg/kg Dibutyl Phthalate from postnatal day 21 for 20 d had significantly reduced uterus and ovary weights in both treated groups. However, vaginal opening was not observed in any of the animals in the controls and treated groups until postnatal day 42, except in one animal each in vehicle control and the 100 mg/kg dose group. Female offspring of dams that received up to 10,000 ppm on gestation day 15 to postnatal day 21 had marginal increases in the number of cases of extended diestrus at 10,000 ppm, and at adult stage necropsy, relative pituitary weights were decreased in the 10,000-ppm dose group at postnatal week 11, and in the 200-ppm dose group at postnatal week 20. In an oral multigeneration study in which the F_0 pregnant mice received a mixture of phthalates that included 15% Dibutyl Phthalate and 35% Diethyl Phthalate in tocopherol-stripped corn oil at up to 500 mg/kg/d, female offspring of the F_2 generation had increased uterine weight, anogenital distance and body weight. Cystic ovaries and breeding and pregnancy complications were also observed. Similar effects were observed in the females of the F_3 generation.

In an in vitro mechanistic study using TM3 and TM4 cells, exposure to Dibutyl Phthalate at 0, 5, 10, 50, and 100 mg/l for 24 h produced dose-dependent evidence of ferroptosis. Dibutyl Phthalate treatment decreased cellular GSH and mitochondrial membrane potential, and increased MDA, ROS, GSSG, and Fe^{2+} levels. Dibutyl Phthalate selectively upregulated PRDX6, a negative regulator of ferroptosis, while GPX4 remained unchanged. Further, Dibutyl Phthalate upregulated SP1 expression, which can directly bound to the PRDX6 promoter and transcriptionally activated its expression.

Rat testicular explants exposed in vitro to Dibutyl Phthalate at 10^{-6}, 10^{-5}, and 10^{-4} M for 24 h showed significant upregulation of Notch1, Dll4, and Hey1 at both mRNA and protein levels, while Hes1 expression remained unchanged. Dibutyl Phthalate altered immunoexpression of activated NOTCH1, DLL4, HEY1 and HES5 both in seminiferous epithelium and interstitial tissue, with differential effects across cell types. In rat Leydig cells exposed to 50 mg/l Dibutyl Phthalate, Dibutyl Phthalate + prostaglandin E2, or flutamide for 24 h, expression of testosterone was significantly decreased in all groups. A significant decrease of Cx43 was observed in the Dibutyl Phthalate group. Human sperm exposed to up to 134.7 µg/ml Dibutyl Phthalate for 30 min to up to 96 h had a concentration- and duration-dependent decrease in motility. In another study with human sperm, Dibutyl Phthalate at 6 µM, monobutyl phthalate at 3 µM, and a mixture of both had adverse effects on sperm motility, penetration ability, and capacitation following exposure for 1 to 4 h.

Multiple oral studies have investigated the effects of Dibutyl Phthalate in male rodents. The NOAEL was 50 mg/kg bw/d for developmental toxicity of male offspring in a study in pregnant rats that received up to 500 mg/kg bw/d on gestation day1 through postnatal day 21. At doses of 250 mg/kg and higher, reduced birth weight, body weight gain, number of live pups per litter, anogenital distance in males, epididymis weight, and sperm count and motility were observed. Studies in which pregnant rats received up to 600 mg/kg bw/d Dibutyl Phthalate between gestation days 12 - 21 found cryptorchidism, infertility, hypospadias, decreases in anogenital distance, and/or testes abnormalities. Closer examination of the focal dysgenesis of the testes found adverse effects in the Leydig and Sertoli cells. One study of rats that received Dibutyl Phthalate (500 mg/kg/d) from gestation day 12 through gestation days 16 - 20 saw a significantly increased incidence of MNG in fetal rat testes following in utero exposure to the test material. Sertoli cells exhibited retracted apical processes, disorganized vimentin cytoskeleton, and abnormal contacts with gonocytes. These morphological changes were no longer evident after birth and cessation of exposure. Pregnant dams that received Dibutyl Phthalate (up to 600 mg/kg/d) or Diethyl Phthalate (up to 900 mg/kg/d) on gestation days 8 - 18 experienced no adverse effects, but fetal testicular testosterone production was significantly reduced at doses of 300 mg/kg/d or higher in Dibutyl Phthalate male fetuses. This effect was not observed with Diethyl Phthalate. In a study where pregnant dams received up to 10,000 ppm Dibutyl Phthalate on gestation day 15 to postnatal day 21, male offspring from the 10,000-ppm dose group had decreased neonatal anogenital distance and retention of nipples at postnatal day 14; however, but the adult stage, testicular lesions appeared to be very faint in most cases, but degeneration and atrophy of mammary gland alveoli were observed in males starting at 20 ppm. Exposure to Dibutyl Phthalate (100 mg/kg/d) from gestation day 12 until postnatal day 21 affected the prostate development in male rats, including disseminated inflammatory infiltrate in the stroma and increased androgen receptor expression, proliferation index, and metalloproteinase 9 activity. A phthalate mixture containing Dibutyl Phthalate significantly increased fetal mortality at 40% of top dose (containing 120 mg/kg/d Dibutyl Phthalate) and above in dams that received the test material on gestations days 8 - 18, and testosterone production was reduced in a dose-additive manner starting at 20% of top dose (containing 60 mg/kg/d Dibutyl Phthalate) and above. Phthalate-induced underdevelopment of the testes was observed at 100% of the top dose (300 mg/kg/d).

In studies with male rats that received up to 500 mg/kg/d Dibutyl Phthalate in corn oil, residuary phthalate in the testes was increased in a dose- dependent manner and the microscopic structure of seminiferous tubule, spermatogonial cell, or spermatic cell in treated groups was dramatically changed. Testes weights were significantly reduced in male rats that received Dibutyl Phthalate (500 mg/kg bw/d) for 4 wk, and Dibutyl Phthalate, monobutyl phthalate (250 mg/kg bw/d), and monoethyl phthalate (250 mg/kg bw/d) significantly lowered sperm counts and sperm motility of epididymal sperm. These effects were not observed with Diethyl Phthalate or Dimethyl Phthalate (500 mg/kg bw/d for each diester), monomethyl phthalate (250 mg/kg bw/d), or phthalic acid (250 mg/kg bw/d). Adult male rats that received up to 600 mg/kg/d Dibutyl Phthalate in corn oil for 15 d had significantly decreased testicular weight at each dose tested, and sperm count and motility were significantly decreased in a dose-dependent manner. Serum follicle-stimulating hormone, testosterone levels, and testicular lactate dehydrogenase activities were significantly decreased at all doses tested. In a study of male rats exposed to 250 mg/kg Dibutyl Phthalate, with or without BaP for up to 12 wk, no adverse effects were observed in the relative weights of the testes and epididymides, but vacuolization of Sertoli cells was observed in Dibutyl Phthalate-exposed rats after 12 wk. Male rats orally exposed to Dibutyl Phthalate (500 mg/kg/d) for 3 wk, had decreased germ cell layer thickness, and reduced sperm density in testicular tissue. These morphological changes were associated with increased testicular levels of MDA, Fe^{2+}, and GSSG, together with decreased reduced GSH and serum testosterone. No gross lesion were observed in mice that received up to 1393 mg/kg bw/d Dibutyl Phthalate in a 2-yr study, but significantly increased incidences of germinal epithelium degeneration in the testes and exfoliated germ cells in the epididymal duct were observed. In male rats that received 2000 mg/kg/d Diethyl Phthalate in dietary feed for 7 d, lower levels of testosterone were measured in the testes and serum of the treated animals, but no testicular damage was observed by microscopic examination.

Mice that received Dibutyl Phthalate (2.5 mg/kg/d), with or without diethylhexyl phthalate, for 40 d via implanted osmotic pumps had abnormal sperm morphology, particularly in the mixture group. Significant differences in sperm concentration were observed between control and exposed groups. No clinical signs of toxicity were observed in rats that were injected subcutaneously with Dibutyl Phthalate (up to 20 mg/animal) from postnatal day 5 -14; and no alterations in testicular descent were observed in any treated rats. However, significantly reduced testes, seminal vesicles, LABC, and Cowper's glands weights were observed in the 20 mg dose group when compared to the controls.

In additional studies, 100 mg/kg Dibutyl Phthalate injected into chicken eggs significantly reduced hatching and increased late hatching when compared to controls. Gross malfunctions and severe motor dysfunctions were observed in treated animals. Zebrafish that were exposed to up to 1133 µg/l Dibutyl Phthalate, with or without diisobutyl phthalate had a greater imbalance of the testosterone to estradiol ratio and severe structural damage from the mixture than to just Dibutyl Phthalate. These effects were consistent with the testis transcriptome analysis for which 4570 genes were differentially expressed in the mixture exposure, while 2795 genes were differentially expressed in Dibutyl Phthalate. In a review assessing the structure-activity relationship of phthalate-mediated testicular dysgenesis syndrome, phthalates with straight side chains that had C5 - C6 were the most potent for causing this adverse effect, while side chains of C4 (Dibutyl Phthalate) or C7 were moderate in effect. Phthalates with side chains of C3 had weak adverse effects and very small (C1 and C2, including Dimethyl Phthalate and Diethyl Phthalate) or very large side chains (C8 or greater) were ineffective.

Dibutyl Phthalate (up to 2000 µg/plate) and Diethyl Phthalate (up to 5000 µg/plate) were not genotoxic in separate Ames tests, with and without metabolic activation. In a third Ames test, Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate were mildly genotoxic in Salmonella typhimurium strains TA100 and TA1535 without metabolic activation in a dose-dependent manner when tested at up to 2000 µg/plate. Dibutyl Phthalate (up to 80 µM) was genotoxic in micronucleus tests using cultured bovine lymphocytes and CHO cells. In chromosome aberrations tests, Dibutyl Phthalate (up to 30 µM) caused a higher occurrence of chromosomal aberrations in CHO cells at all tested concentrations when compared to the vehicle control; however, Diethyl Phthalate (up to 1780 µg/ml) was not genotoxic to human lymphocytes. Diethyl Phthalate (up to 771 µg/ml) was also not genotoxic in a gene mutation assay in mouse L5178 TK +/- lymphoma cells. Genotoxicity was observed to Dibutyl Phthalate when tested in human mucosal cells and human peripheral lymphocytes at up to 354 µmol/ml and in cultured bovine lymphocytes at up to 80 µM. Genotoxicity was also observed to Dibutyl Phthalate and monobutyl phthalate in human peripheral blood mononuclear cells at up to 10 µg/ml; neither phthalate were capable of creating adducts with DNA.

In a multigeneration alkaline comet assay in rats, Dibutyl Phthalate (500 mg/kg/d in olive oil) significantly increased olive tail moment, tail DNA percentage, and tail length in the examined neural tissues of 3 generations. DNA damage was generally greatest in F3 animals. Treatment also reduced DNA and RNA content in several neural regions, decreased superoxide dismutase and catalase activities, and increased malondialdehyde levels.

In a study performed by the NTP, the 2-yr survivability of mice dermally exposed to 0, 9, 18, or 37 µg Diethyl Phthalate in 100 µl acetone was similar to that of controls. There were no adverse clinical signs and no signs of dermatotoxicity. An increase in liver neoplasms was observed in male and female treated mice. The incidence of hepatocellular adenoma or carcinoma (combined) was increased in 37 µg group males and in the 9 and 15 µg group females, but not in the 37 µg group females. The increased incidence of liver neoplasms was considered equivocal because the incidence of hepatic neoplasms was within the historical range and because there was no clear dose-response relationship in females. A similar study performed in rats with 0, 123, or 368 µg Diethyl Phthalate (neat) saw survivability of treated rats similar to the controls for the first 15 mo; however, survival for 2 yr was reduced in male rats in all groups, including the controls. The incidence of anterior pituitary adenoma was higher in all groups of male and female rats than the historical incidence. This may have been partially responsible for the reduced survivability of the males. No adverse clinical effects or evidence of carcinogenic activity from treatment with Diethyl Phthalate were observed in male or female rats. In an oral study, carcinogenesis was not observed in rats that were fed Dibutyl Phthalate for 18-mo or longer (no further details provided). Certain phthalates and their monoester metabolites can activate PPARα. Humans and rodents differ in the liver cell proliferative response to PPARα activation. This difference suggests that rodent liver tumors arising through this mechanism have limited relevance to human cancer risk.

In a 2-yr dietary study in mice and rats performed by the NTP, no evidence of carcinogenic activity of Dibutyl Phthalate in male or female mice at up to 10,000 ppm. However, there was equivocal evidence of carcinogenic activity of Dibutyl Phthalate in male rats based on the marginal increased in the incidence of pancreatic acinus adenomas at up to 10,000 ppm; there was no evidence of carcinogenic activity of Dibutyl Phthalate at up to the same concentration in female rats.

In a 1-yr initiation/promotion study in male mice, Diethyl Phthalate and Dimethyl Phthalate were tested neat as tumor initiators, with and without promotion from 12-*O*-tetradecanoylphorbol-13-acetate. Diethyl Phthalate and Dimethyl Phthalate were also tested neat for the potential as promoters, with and without initiation from 7,12-dimethylbenzanthracene. Neither Diethyl Phthalate nor Dimethyl Phthalate initiated or promoted skin carcinogenesis.

Dibutyl Phthalate treatment of MCF-10A normal breast cells co-cultured with mammary fibroblasts led to increased cell viability, decreased apoptosis, and enhanced cell-cycle progression (S and G2/M phases), accompanied by upregulation of cyclins/CDKs and PI3K/AKT/mTOR/BCL-2 signaling and downregulation of pro-apoptotic proteins.

The potential for Dibutyl Phthalate (up to 10 µM) to promote tumors was studied in normal bladder epithelial cells and human bladder cancer cells. A decrease in migration of the bladder epithelial cells was observed as the Dibutyl Phthalate concentration increased, while migration of the bladder cancer cells increased with increased Dibutyl Phthalate. FOSB, JUND, ATP6V1C2, and RHOQ were differentially expressed in the second-generation sequencing before and after Dibutyl Phthalate treatment. FOSB expression was confirmed, and overexpression of FOSB increased both proliferation and invasion in the bladder cancer cells. In human breast epithelial cells and breast cancer stem cells, exposure to Dibutyl

Phthalate (up to 1 μM) induced expression of mesenchymal markers vimentin and CD90 and decreased expression of epithelial markers cytokeratin 7 and E-cadherin and enhanced tumorsphere formation ability and higher levels of cancer stem cell markers (CD133, CD44, ALDH1A1, OCT-4, Nanog).

Oxidative stress, lipid peroxidation, and DNA damage occurred in zebrafish liver according to changes in antioxidant enzymes, MDA, and 8-OHdG content following exposure to up to 2 mg/l Dibutyl Phthalate for up to 28 d. AchE activity was always active and negatively correlated with the Dibutyl Phthalate concentration. The expression of Cu/Zn-sod and gpx genes were similar to that of antioxidant enzymes from 7 to 21 d, while in the end, the inconsistent result appeared due to the time lag effect in protein modification, gene transcription and translation. In another zebrafish study using to assess gene expression, DNA damage, and oxidative stress biomarkers in the brain following exposure to Dibutyl Phthalate (up to 2 mg/l) for up to 28 d, Dibutyl Phthalate significantly stimulated SOD and CAT activities, increasing MDA and 8-OHdG contents. On day 28, AChE inhibition rates for 0.08, 0.4, and 2 mg/l were 13.4, 11.9, and 14.7%. The trend of Cu/Zn-sod gene variation was consistent with SOD activity, showing "inhibition-activation-inhibition." Hepatic and ovarian expression of Lpl, ldh1a1, Adh1, Ugt1a6a, and Cyp1b1 were altered in a time- and dose-specific manner in mice treated with up to 1000 mg/kg Dibutyl Phthalate either once or once daily for 10 d. Dibutyl Phthalate exposure induced oxidative stress, lipid peroxidation, and DNA damage in zebrafish liver, as evidenced by increased MDA and 8-OHdG levels and altered antioxidant enzyme activities (SOD, CAT, GPx) and Cu/Zn-sod gene expression. In zebrafish, AChE activity was inhibited in a concentration-dependent manner, while in mice, hepatic and ovarian expression of metabolic genes (Lpl, Ldh1a1, Adh1, Ugt1a6a, and Cyp1b1) was altered in a time- and dose-dependent manner following Dibutyl Phthalate exposure.

Monobutyl phthalate significantly reduced global DNA methylation and altered promoter-specific methylation and gene expression (TP53 and P16) in human blood mononuclear cells, while Dibutyl Phthalate showed more limited but distinct methylation changes across genes of TP53, P16, CCND1 and BCL2. Both phthalates modulated epigenetic patterns and gene expression in a concentration-dependent and gene-specific manner.

Diethyl Phthalate significantly reduced testosterone concentrations and significantly increased the17β-estradiol/ testosterone ratio in human adrenocortical carcinoma cells starting at 0.004 mM, with similar effects observed for its metabolite monoethyl phthalate at higher concentrations. Both Diethyl Phthalate and monoethyl phthalate downregulated steroidogenic genes StAR and 3βHSD2, while upregulating CYP19A,

Diethyl Phthalate (up to 10 mg/l) reduced testosterone and 17β-estradiol levels in male zebrafish and altered steroidogenic gene expression. In pregnant rats, combined exposure to Dibutyl Phthalate (750mg/kg/d, oral, gestation days 1-3) and STZ induced gestational diabetes–like effects with hyperglycemia, reduced insulin, and pancreatic FoxM1 suppression. In primary islet β cells, Dibutyl Phthalate decreased viability and increased apoptosis via pSTAT1 activation, reversible by STAT1 inhibition. Using the AOP framework, three key elements were identified by the US EPA for Dibutyl Phthalate–induced male reproductive toxicity: 1) fetal rats are most sensitive to anti-androgenic effects, 2) androgen-independent adverse outcomes are conserved across mammalian models and human fetal testis xenografts, and 3) anti-androgenic effects are also conserved across species when exposure occurs postnatally.

Dibutyl Phthalate caused concentration-dependent cytotoxicity across multiple cell types. In bovine lymphocytes and human sperm, MTT assays showed reduced viability with increasing concentration (10 - 100 μM in lymphocytes; 13.47 - 134.7 μg/ml in sperm) and exposure duration (up to 96 h). In human keratinocytes, viability declined from 85% to 37% across 0.1- 5 mg/ml (LOEC = 0.1 mg/ml), with decreased colony formation and apoptosis at ≥ 1 mg/ml. In prostate cancer cells, Dibutyl Phthalate exhibited higher cytotoxicity (IC_{50} = 27.32 ppb in DU145; 77.21 ppb in PC3) compared with Diethyl and Dimethyl Phthalates.

In recombinant human microsomes, Dibutyl Phthalate inhibited CYP2C9 and CYP2C19 activities in a concentration-dependent manner (0.1 - 100 μM), acting as a competitive inhibitor toward probe substrates and a noncompetitive inhibitor with respect to the cofactor NADPH. In contrast, its metabolite monobutyl phthalate showed minimal inhibition.

Mice that received up to 50 mg/kg/d Dibutyl Phthalate in Tween 80 via gavage for 28 d had disrupted hepatic architecture, inflammatory cell infiltration, and hepatocellular disorganization. Serum ALT and AST activities were elevated, with the AST/ALT ratio significantly increased. Sirius red staining demonstrated collagen deposition in Dibutyl Phthalate-treated livers, and protein expression of fibrosis markers was significantly upregulated. Mice orally exposed to Dibutyl Phthalate (250 mg/kg/d), Diethylhexyl Phthalate (300 mg/kg/d), or their combination for 28 d showed elevated liver enzymes and proteins, altered lipid profiles, and histological signs of hepatic hypertrophy and structural disorganization. Dibutyl Phthalate (50 mg/kg/d) specifically increased the AST/ALT ratio, reduced albumin, and induced hepatocyte edema, sinusoidal narrowing, and central vein dilation. Increased hepatic ROS, MDA, and phosphorylated ERK1/2 levels, and the protective effects of Vitamin E and PD98059, indicate that DBP-induced hepatotoxicity involves oxidative stress and ERK1/2 activation.

In mice, 28-d oral exposure to Dibutyl Phthalate (50 mg/kg/d) caused glomerular and tubular injury, increased renal ROS and MDA, and activated ERK1/2 signaling, effects mitigated by Vitamin E or PD98059. Co-treatment with curcumin (2.5 mg/kg/d) similarly alleviated DBP-induced renal dysfunction, oxidative stress, and apoptosis. In HK-2 cells, Dibutyl

Phthalate (100 μM) upregulated Cx43 via Ang II/AMPKα2 signaling, and Cx43 knockdown attenuated epithelial–mesenchymal transition.

In mice, 3-wk exposure to Dibutyl Phthalate (50 mg/kg/d) caused splenic inflammation and apoptosis linked to oxidative stress, which were alleviated by antioxidants. In rats, Dibutyl Phthalate (≤ 50 mg/kg/d) induced moderate splenic alterations and increased IL-1β, ASC, and GSDMD expression, with co-exposure to BaP producing greater injury and inflammation than either chemical alone.

Dibutyl Phthalate (≥ 30 μM) induced oxidative stress, mitochondrial dysfunction, and apoptosis in rat insulinoma cells, evidenced by increased MDA, altered GSH/GSSG ratio, elevated pro-apoptotic markers and reduced SOD activity. At 120 μM, insulin synthesis and secretion were markedly decreased.

In male mice, Dibutyl Phthalate (50 mg/kg/d in the diet for 7 wk) alone reduced insulin secretion and induced glucose intolerance without affecting insulin resistance. When combined with a high-fat diet and low-dose STZ, Dibutyl Phthalate markedly aggravated glucose intolerance, insulin resistance, and pancreatic and renal lesions, accompanied by disrupted PI3K expression, reduced AKT phosphorylation, and decreased pancreatic GLUT2 levels.

In rats, an 8-wk exposure to Dibutyl Phthalate (500 mg/kg/d, gavage) alone or with Diethylhexyl Phthalate (750 mg/kg/d) elevated fasting glucose, increased insulin resistance, and impaired pancreatic β-cell function. Both treatments increased oxidative stress, suppressed PI3K and p-Akt phosphorylation, upregulated pro-apoptotic markers (Bax, Caspase-8, -9, -3), and reduced Bcl-2 and GLUT4 expression.

In silico analysis of exposure of human endothelial cells to Dibutyl Phthalate (up to 10^{-7} M in DMSO) determined that 9 vascular genes were affected, which could alter cell adhesion and migration. Dibutyl Phthalate (100 μM) promoted angiogenesis in human endothelial cells by enhancing tube formation and upregulating angiogenic genes, accompanied by ERK1/2, Akt, and eNOS phosphorylation and increased NO production—effects blocked by ER and GPER antagonists. In rats, Dibutyl Phthalate (25 mg/kg, oral, 28 d) reduced CAT, GSH, and Nrf2 while increasing CRP and NF-κB, changes reversed by rutin. In mice Dibutyl Phthalate (≤ 10 mg/kg/d, oral, 6 wk), elevated serum estradiol and NO levels, effects inhibited by the estrogen receptor antagonist ICI182780.

Daily oral exposure to Dibutyl Phthalate (6.25-200 mg/kg/d) in sunflower oil for 14 d reduced total distance movement (starting at 12.5 mg/kg), impaired memory function (starting at 25 mg/kg), and induced anxiety (starting at 12.5 mg/kg) in male mice when compared to negative control. Histological analysis showed a significant nuclei size reduction and condensation in dentate gyrus cells in mice treated with 25 or 100 mg/kg/d Dibutyl Phthalate when compared to the negative control. In a neurobehavioral study of rat offspring that received Dibutyl Phthalate (0.037 to 1% in dam dietary feed) in utero from gestation day 6 to postnatal day 28, a prolonged gestation period, decreased body weight in both male and female pups, depressed surface righting in male pups, shortened forepaw grip time, and enhanced spatial learning and reference memory in male pups were observed in the 1% exposure group. Shortened forepaw grip time was also observed in the 0.037% dose group, along with inhibited spatial learning and reference in male pups. Male pups appeared to be more susceptible than females with treatment effects. In a study of maternal and offspring neurotoxic effects, dams treated with 50 or 100 mg/kg/d Dibutyl Phthalate exhibited poor maternal behavior, nesting, and retrieval behavior, while pups of treated dams had significantly decreased scores in negative geotaxis, swimming, and olfactory orientation tests. Effects were observed in the brain cortexes of both dams and offspring, including increased dark neurons. In a 10-d gavage exposure of Dibutyl Phthalate (0, 50, or 500 mg/kg bw in corn oil), intestinal villus and crypt of the duodenum and jejunum had variation among the mice in the treatment groups. Damage to the structure of the small intestine was accompanied by a marked increase in HSP27 expression and a decrease in the expression of HSP70 and HSP90 in both dose groups.

Dibutyl Phthalate (≤ 0.2 μM) inhibited neutrophil and macrophage formation and reduced macrophage phagocytic activity in zebrafish. Exposure to Dibutyl Phthalate (10 or 100 mg/kg/d in corn oil) in adult male mice decreased the total number of leukocytes and classical monocyte and T helper populations, while non-classical monocyte and polymorphonuclear myeloid-derived suppressor cell populations increased. Also in mice, Dibutyl Phthalate (≤ 40 mg/kg/d) acted as in murine FITC-induced contact hypersensitivity by promoting TSLP expression in keratinocytes, whereas Diethyl Phthalate showed weaker effects but enhanced IL-4 production. In an asthmatic mouse model, Dibutyl Phthalate increased airway inflammation, serum IgE, and Th2/Th17 cytokines. In allergen-sensitized humans, 3-h Dibutyl Phthalate inhalation (~150 $\mu g/m^3$) enhanced early allergen responses, airway reactivity, M2 macrophage polarization, and circulating $CD4^+$ T cells while reducing regulatory T cells and non-classical monocytes. Undiluted Dibutyl Phthalate and Diethyl Phthalate were not irritating to rabbit skin. When induced topically at 75% and challenged at 50%, Dibutyl Phthalate was not sensitizing in a GPMT. Diethyl Phthalate at up to 100% was not sensitizing in guinea pigs. Diethyl Phthalate was also not sensitizing in a LLNA when tested at up to 100%. A mouse ear swelling test was used to determine the adjuvant effect of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate (1:1 v/v with acetone; no further details). The mice were sensitized with FITC. A strong enhancement of ear-swelling response was observed in mice treated with Dibutyl Phthalate, while the response was not as strong with Diethyl Phthalate and Dimethyl Phthalate.

Dibutyl Phthalate (up to 200 µmol/l) was used in a test to determine the utility of using human corneal endothelial cell line B4G12 to evaluate an in vitro model for eye irritancy testing. Decreased cell proliferation was observed, as well as cell toxicity. In rabbit eyes, Dibutyl Phthalate was not considered an ocular irritant when tested undiluted.

In a retrospective study on 251 metalworkers that had suspected metalworking fluid dermatitis, 199 were tested with a metalworking fluid series that included Dibutyl Phthalate in 5% pet. Negative results were observed in 197 patients and 2 had questionable reactions. A 65-yr-old man presenting with an acute erythematous rash following use of a cream product to treat itching in the peri-anal region had positive patch reactions to 5% Dibutyl Phthalate pet. (++), the cream product (++), 0.1% benzalkonium chloride aq. (+), balsam of Peru (++), and fragrance mix (++).

The median urinary concentration of Dibutyl Phthalate metabolite in electronic waste processing workers was 14.1 µg/g creatinine, compared to 7.54 µg/g creatinine in the controls. However, no significant differences were observed between pre- and post-shift concentrations in the workers. The concentration of Dibutyl Phthalate in dust was correlated with the corresponding urinary metabolite in the workers. Workers that used respiratory protective equipment had significantly lower urinary concentration Dibutyl Phthalate. Metabolites of Dibutyl Phthalate were detected in manicurists at baseline, with the highest concentration detected in monobutyl phthalate (58.5 ng/ml overall, n = 37), followed by mono-iso-butyl phthalate (10.7 ng/ml overall). A significant increase in specific gravity-adjusted monobutyl phthalate concentrations was observed across the work shift. Manicurists in salons without local exhaust had a 54% increase in urinary monobutyl phthalate concentration across shift compared with a 7% decrease across shift for those with exhaust ventilation. Glove use was associated with a significant reduction ($p = 0.04$) in urinary cross-shift monobutyl phthalate concentration.

An MOE calculation performed by the EC SCCP for Dibutyl Phthalate at trace levels found in perfume products resulted in a value of 10,000. The MOE was based on a LOAEL of 2 mg/kg bw/d and a SED of 0.0002 mg/kg/d. When the calculation was performed at a concentration of 100 ppm (0.01%) Dibutyl Phthalate, the MOE was calculated to be 1350. CIR staff prepared an MOE for Diethyl Phthalate using concentrations from 2025 concentration of use survey. The resulting value was 28,301 when using a conservative NOAEL of 150 mg/kg/bw/d, and the value was 37,735 when using a NOAEL of 200 mg/kg bw/d. To address uncertainty in dermal absorption, a conservative Tier 1 calculation assuming 100% absorption was performed using the same aggregate exposure estimate (5.731 mg/d), body weight (60 kg), and NOAEL (150 mg/kg bw/d), yielding an SED of 0.0955 mg/kg bw/d and an MOE of approximately 1570.

Epidemiology studies have been performed on the associations of phthalate exposure to maternal metabolic outcomes, type 2 diabetes, neurodevelopmental in children, reproductive health, pubertal development in girls, breast cancer, genotoxic effects, and endocrine effects. Mixed results have been found. Personal care product use, including perfumes and certain cosmetics, has been associated with higher urinary monoethyl phthalate and/or monobutyl phthalate concentrations in children, adolescents, and adults.

DISCUSSION

In accordance with its Procedures, the Panel re-evaluates the conclusions of previously-issued reports approximately every 15 years. In 1985, the Panel first published a final report on Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate and concluded that these ingredients are safe for topical application in the present practices of use and concentration in cosmetics. Upon re-review in 2002, the Panel reaffirmed the original conclusion, as published in 2005. In December 2012, the Panel deliberated on studies separately concerning endocrine disruption and diabetes and Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate, and Butyl Benzyl Phthalate; however, the Panel chose not to re-open the safety assessment of these ingredients and published their discussion as a re-review summary in 2017. The report was re-opened in 2024 when Dibutyl Phthalate was placed on the 2024 Priorities List following nomination by the FDA for cause. The Panel concluded that the available data are sufficient to determine the safety of Diethyl Phthalate; however, the Panel noted a lack of relevant safety data, specifically maximum concentration of use data, for Dibutyl Phthalate and Dimethyl Phthalate.

The Panel considered the voluminous data concerning phthalates and endocrine and developmental effects. Significant adverse effects in male reproductive toxicity have been observed in phthalates with a carbon chain length of 4 or greater. Based on the MOE, the Panel found that Diethyl Phthalate, with high NOAELs for reproductive endocrine effects, greatly exceeded the safety threshold of 100. Even if absorption was 100%, the MOE would still exceed the safety threshold. While it appears that Dibutyl Phthalate, with a carbon chain length of 4, is linked to many adverse endocrine and developmental effects, the Panel cannot assess the risk from cosmetic exposure without a concentration of use. While Dimethyl Phthalate has a smaller carbon chain length and likely does not pose a significant risk (like Diethyl Phthalate, it has extremely high NOAELs), an MOE calculation cannot be performed without knowing concentration of use.

The Panel noted the increased incidences of liver adenoma and carcinomas in 2-yr dermal carcinogenicity study of Diethyl Phthalate in mice; however, the incidence of the tumors described were within the historical range. The Panel also noted the non-genotoxic tumorigenic effects in the 2-yr oral study on Dibutyl Phthalate, specifically increased incidences of pancreatic acinus adenomas. These types of tumors are associated with PPARα activation in mice and rats only and are thereby not relevant to human risk. Based on the overall weight of evidence, the Panel concluded that the available findings did not raise a carcinogenicity concern for the phthalates evaluated in this assessment.

Additionally, the Panel noted the numerous epidemiological studies investigating intrauterine and early-life exposures to phthalates on neurocognitive development. A meta-analysis of these studies concluded that there was no consistent pattern linking prenatal or early-life thyroid exposure with adverse neurodevelopmental outcomes. The Panel also reviewed epidemiological studies of personal care product use contributing to aggregate phthalate exposure, particularly Diethyl Phthalate-related exposure as reflected by urinary monoethyl phthalate. However, the studies generally relied on self-reported product use, and the presence and concentrations of individual phthalates in the products used were generally not analytically confirmed. In addition, urinary metabolites reflect recent aggregate exposure from both cosmetic and non-cosmetic sources. Consequently, the findings cannot be used to quantify exposure from a specific cosmetic formulation or to directly inform the margin of exposure calculations in this assessment. Because these studies evaluate associations between product use patterns and biomarkers of exposure rather than adverse health outcomes, they provide relevant exposure-context information but do not directly inform the toxicological safety determination for Diethyl Phthalate or Dibutyl Phthalate as used in cosmetics.

At least 1 use for the ingredients reviewed in this assessment is reported in the RLD under Category 17, "other preparations." According to communications from the FDA, the 2 uses reported herein within Category 17 are liquid bandage products, and thereby outside the purview of the Panel. Thus, the Panel's conclusion does not apply to these preparations.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (e.g., Diethyl Phthalate is reported to be used in fragrance preparations and a deodorant spray; concentrations not reported). The reported deodorant spray use was conservatively assumed to involve a propellant-based aerosol, and the Panel recognized that respirable droplets or particles could be emitted. Limited available inhalation toxicity data included acute studies in Dibutyl Phthalate and Diethyl Phthalate and short-term and subchronic studies in Dibutyl Phthalate. The lack of toxicity observed in these studies, along with a favorable safety profile for Diethyl Phthalate, mitigated concerns of adverse effects from incidental inhalation. Moreover, the Panel noted that bulk ingredient or in-container formulation particle size alone is insufficient for spray-use inhalation risk assessment. Assessment requires data on emitted aerosol size distribution and airborne concentration in the user's breathing zone during use, or, when available, respiratory deposition fraction or dose, particularly in the deep lung. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern. Although frequency and concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded that if this ingredient is used in airbrush formulations, the data are insufficient to support safe use when applied with such delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Diethyl Phthalate is safe in cosmetics in the present practices of use and concentration described in this safety assessment. The Panel also concluded the available data are insufficient to make a determination of safety for Dibutyl Phthalate and Dimethyl Phthalate under the intended conditions of use in cosmetic formulations.

TABLES

Table 1. Definitions, reported functions, and structures of the ingredients in this safety assessment.[1]

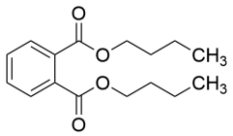
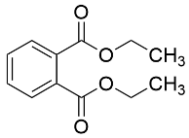
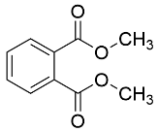
Ingredient & CAS No.	Definition	Function(s)
Dibutyl Phthalate 84-74-2	Dibutyl Phthalate is the aromatic diester of butyl alcohol and phthalic acid. It conforms to the following structure: 	Fragrance ingredient; plasticizer; solvent
Diethyl Phthalate 84-66-2	Diethyl Phthalate is the aromatic diester of ethyl alcohol and phthalic acid. It conforms to the following structure: 	Denaturant; fragrance ingredient; plasticizer; solvent
Dimethyl Phthalate 131-11-3	Dimethyl Phthalate is the diester of methyl alcohol and phthalic acid. It conforms to the following structure: 	Fragrance ingredient; plasticizer; solvent

Table 2. Chemical properties

Property	Value	Reference
	Dibutyl Phthalate	
Physical Form	colorless oily liquid	[2]
Odor	odorless	[2]
Molecular Weight (g/mol)	278	[2]
Density (g/ml @ 20ºC)	1.05	[2,5]
Viscosity (mm^2/s @ 20ºC)	18.8	[5]
Vapor pressure (mm Hg @ 150ºC)	1.1	[2]
(mm Hg @ 20ºC)	7.5×10^{-5}	[5]
Melting Point (ºC)	-35	[2]
Boiling Point (ºC)	340	[2,5]
Water Solubility (mg/l @ 25ºC & pH 6)	11.4	[5]
Other Solubility	alcohol, ether, and other common organic solvents	[2]
log P_{ow} (@ 30 ºC)	4.46	[5]
	Diethyl Phthalate	
Physical Form	colorless or pale-yellow oily liquid	[2,6]
Odor	odorless to slight odor	[2,20]
Molecular Weight (g/mol)	222	[2]
Density (g/ml @ 20ºC)	1.12	[6]
Viscosity (mm^2/s @ 20ºC)	11.5	[6]
Vapor pressure (mm Hg@ 16ºC)	14	[2]
(mm Hg@ 25ºC)	0.0021	[6]
Melting Point (ºC)	-40.5	[2,20]
	-60	[6]
Boiling Point (ºC)	295-298	[2,6]
Water Solubility (mg/l @ 20ºC & pH 7.2)	932	[6]
Other Solubility	alcohol, ether, and other common organic solvents	[2]
log P_{ow}	2.47	[20]
(@ 41ºC)	2.2	[6]
	Dimethyl Phthalate	
Physical Form	colorless oily liquid	[2]
Odor	odorless to slight odor	[2]
Molecular Weight (g/mol)	194	[2]
Specific Gravity (@ 20ºC)	1.19	[7]
Vapor pressure (mm Hg @ 20ºC)	< 0.1	[2]
	7.5×10^{-4}	[7]
Melting Point (ºC)	5.5	[2]
	0.36	[7]
Boiling Point (ºC)	282	[2]
	283	[7]
Water Solubility (mg/l @ 25ºC)	4000	[7]
Other Solubility	alcohol, ether, and other common organic solvents	[2]
log P_{ow} (@ 25ºC)	1.54	[7]

Table 3. Frequency[11,12] and concentration[13] of use according to likely duration and exposure and by product category

	Dibutyl Phthalate		Diethyl Phthalate		Dimethyl Phthalate	
	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use
	RLD (2025)	% (2025)	RLD (2025)	% (2025)	RLD (2025)	% (2025)
Totals*	**NR†**	**NR**	**284**	**0.1-0.15**	**2**	**NR**
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*NR*	*NR*	*350*	*0.15*	*1*	*NR*
Rinse-Off	*NR*	*NR*	*3*	*0.1*	*1*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Permanent Tattoo Ink	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Unknown	*2*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Baby Products	NR	NR	NR	NR	NR	NR
Children's Makeup	NR	NR	NR	NR	NR	NR
Eye Area	NR	NR	1	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	339; 1[a]; 5[b]	NR	2[a]	NR
Incidental Inhalation-Airbrush	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	5[b]	0.15[c]	NR	NR
Dermal Contact	NR	NR	345	0.1-0.15	2	NR
Deodorant (underarm)	NR	NR	1 (spray)	NR	NR	NR
Hair - Non-Coloring	NR	NR	6	NR	NR	NR
Hair-Coloring	NR	NR	2	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Other Preparations (Unknown Exposure Type)	NR†	NR	NR	NR	NR	NR
as reported by product category						
Eye Makeup Preparations (not children's)						
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)			1	NR		
Fragrance Preparations						
Cologne and Toilet Water			70	NR		
Perfumes			211	NR		
Other Fragrance Preparation			58	NR		
Hair Preparations (non-coloring)						
Hair Conditioners			1 (l.o.)	NR		
Tonics, Dressings, Other Hair Grooming Aids			2	NR		
Other Hair Preparations			3 (l.o.)	NR		
Hair Coloring Preparations						
Hair Rinses (coloring)			2 (r.o.)	NR		
Personal Cleanliness						
Deodorants (underarm)			1 (spray)	NR		
Shaving Preparations						
Aftershave Lotions			3	NR		
Skin Care Preparations						
Cleansing			NR	0.1		
Face and Neck (excluding shaving preps)			NR	0.15 (l.o.)	1 (r.o.)	NR
Moisturizing					1	NR
Other Skin Care Preparations			1 (r.o.)	NR		

NR – not reported
l.o. – leave-on; r.o. – rinse-off
*The sum of the counts given for duration of use and by exposure type, and the sum of the frequency reported by product category, may not equal the sum of total uses because each ingredient may be used in cosmetic formulations that are reported under more than one product category.
**Likely duration and exposure are derived from survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)
†Two uses that were reported under "Other Preparations" were identified as liquid bandage products, which are non-cosmetic products, and thereby outside the purview of the Panel and not identified in this table.
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 4. Acute toxicity studies

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
			ORAL			
Dibutyl Phthalate	olive oil	male and female Sprague-Dawley rats, number not reported	50%; no further details available	Acute oral study performed in accordance with OECD TG 401; rats observed for 7 d before being killed and necropsied; no further details available	6279 mg/kg bw for both sexes; no further details available	5
Diethyl Phthalate	neat	groups of 5 male and 5 female Wistar rats	0.5, 1, 2, or 5 ml/kg	Acute oral study performed in accordance with OECD TG 401; rats observed for 14 d before being killed and necropsied	> 5 ml/kg bw; 1 male rat in the 5 ml/kg was killed on day 5 after dosing due to mild depression and body weight loss	6
Dimethyl Phthalate; 85% pure	not reported	groups of 5 male and 5 female Sprague-Dawley rats	2, 3.5, 5, or 8 ml/kg bw	Acute oral study performed in accordance with OECD TG 401; no further details available	Combined LD_{50} = 5.1 g//kg bw, male LD_{50} = 5.7 g/kg bw; female LD_{50} = 4.4 g/kg bw; signs of toxicity included unsteady gait, sluggishness, lacrimation, kyphosis, red crust around nose and eyes, wetness on periurogenital fur, drooping eyelids, piloerection, unkept appearance, and moribund appearance; death of 4 animals (groups not described for deaths or survivors) occurred at 10 - 17 min; most deaths occurred at 1 -3 d ; survivors recovered at 3 - 7 d; necropsy revealed mottled and red to pink lungs, whit to red glandular stomachs with a thickened appearance, red focal areas in a few stomachs, a few gas-filled stomachs, red intestines, tan foci in one liver, tan calculus in one kidney, and small testes in one male (groups not described for necropsy results)	7

Table 4. Acute toxicity studies

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
			INHALATION			
Dibutyl Phthalate	air	groups of 5 male and 5 female Sprague-Dawley rats	0, 12.45, 15.68, or 16.27 mg/l	Rats exposed to test material as an aerosol for 4 h; type of inhalation exposure not specified. Respirable fraction (diameter < 4.7 µm) was 64.4, 56.9, and 59.9% at 12.45, 15.68, and 16.27 mg/l, respectively; the rats were observed for 14 d	In the 15.68 mg/l group, 2 males and 3 females died; no mortalities were observed in the other 2 dose groups. A reduction in respiratory rate was observed in the 15.68 dose group, and poor coat condition was seen in all surviving animals during the observation period due to excessive grooming behavior. Lung/body weight ratios in rats that died prematurely at 15.68 mg/l were elevated, while these ratios were lower than those of controls in males at 12.45 and 16.27 mg/l. Macroscopy of the lungs revealed red/dark foci in several animals scattered among the treatment groups. One male and one female rat in the 15.68 mg/l group had white foci in all lung lobes. Dark red areas were seen in the lungs of 2 females at 12.45 mg/l and in 1 male and 1 female rat at 16. 27 mg/l. Due to the unusual death pattern, no LC_{50} value could be determined, but the LC_{50} value was estimated to be ≥ 15.68 mg/l in this study. No further details available.	[5]
Diethyl Phthalate	air	3 male and 3 female rats; strain not reported	4.64 mg/lg (511 ppm)	Rats exposed to test material via whole body inhalation chamber for 6 h and then observed for 14 d; no further details available	> 4.64 mg/l; no further details available	[6]
			OTHER			
Dibutyl Phthalate	water	*Danio rerio*; 10 fish per tank	0.6, 1.3, 2.5, 5.0, or 10 mg/l	Toxicity study in fish (OECD TG 236); zebrafish were exposed to test material at up to 96 h	After 96 h, the LC_0 is 1.3 mg/l and the LC_{100} is 5 mg/l	[5]

Table 5. Short-term toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
				ORAL			
Dibutyl Phthalate	dietary feed	groups of 5 male and 5 female Wistar rats	28 d	0, 150, 1000, or 7000 ppm Dibutyl Phthalate, with or without 3000 ppm diethylhexyl phthalate	Rats received test material in dietary feed for 28 d. Rats were checked daily for clinical signs of toxicity and mortalities. Feed consumption was determined on days 6, 13, 20, and 27 and body weight was measured on days 0, 3, 6, 13, 20, and 27. Rats were killed at study end and liver and testes weights were measured. Liver tissue homogenates tested for cyanide non-sensitive palmitoyl-CoA oxidation.	No clinical signs of toxicity observed in any dose groups. Body weight gains and feed consumption were not affected in any dose groups, with the exception of a non-statistically significant reduction in the body weight in the 7000 ppm Dibutyl Phthalate + diethylhexyl phthalate males. An increase of absolute liver weight in males and females were observed in the 7000 ppm Dibutyl Phthalate group. Relative liver weights were increased in males and females of the 7000 ppm dose group and in males of the 1000 ppm dose group. In the combined groups, an increase of absolute and relative liver weights were observed in all animals in all combined treatment groups. No effects were observed on absolute or relative testes weights in the any of the Dibutyl Phthalate alone groups, and very weak increases of absolute and relative testes weights were observed in the combined groups (no further details). A statistically significant ($p < 0.01$) increase in cyanide-insensitive palmitoyl-CoA oxidation levels were observed only in the 7000 ppm males; statistically significant increases were observed in all combined groups.	[37]

Table 5. Short-term toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Dibutyl Phthalate, >99% pure	not specified; saline used for control	groups of 6 male Balb/c mice; 6 groups total; 108 mice across 3 rounds of experiments	60 d	40 mg/kg bw/d	From days 1–53, saline or Dibutyl Phthalate was administered to mice via gavage; melatonin (5 mg/kg, oral) was given 5 h post-Dibutyl Phthalate in the melatonin groups. On days 21, 35, 42, and 49, groups received either saline or OVA (83.3 mg with aluminum hydroxide i.p.), followed by aerosol challenge (saline or 1% OVA, 30 min/d) on days 54–60. Following the treatments, endpoints measured include serum total IgE and OVA-specific IgE, BAL fluid cytokines (IL-4, IL-5, IL-13, IL-17A), lung histopathology, airway hyperresponsiveness (AHR; Ri, Re, Cldyn), oxidative stress markers (GSH, MDA, 8-OHdG), and CGRP.	Dibutyl Phthalate exposure group showed no significant differences in body weight gain or lung function parameters (Ri, Re, Cldyn) compared to saline controls. However, oxidative stress biomarkers in lung tissue were significantly altered, including 8-OH-dG, GSH and MDA ($p < 0.05$). Serum total IgE levels showed a statistically significant increase compared to controls ($p < 0.05$), whereas cytokine levels (IL-4, IL-5, IL-13, IL-17A) and inflammatory cell counts in BAL fluid were not significantly different from controls. Compared to OVA alone, mice treated with OVA and Dibutyl Phthalate exhibited significantly greater inflammatory cell infiltration into the lungs, elevated serum IgE levels ($p < 0.01$), and increased concentrations of Th2 (IL-4, IL-5, IL-13) and Th17 (IL-17A) cytokines in bronchoalveolar lavage fluid ($p < 0.01$), accompanied by pronounced alterations in lung histology and airway hyperresponsiveness ($p < 0.01$), which are characteristic features of allergic asthma. Oxidative stress markers were altered, with reduced GSH and increased MDA and 8-OHdG levels in the OVA+Dibutyl Phthalate group compared to OVA alone ($p < 0.01$). In addition, CGRP concentrations in lung tissue were significantly elevated in the OVA+Dibutyl Phthalate group compared to OVA alone ($p < 0.01$). Blocking oxidative stress by co-treatment with melatonin attenuated oxidative stress and CGRP expression, and alleviated the asthma-like symptoms. Taken together, these findings indicate that Dibutyl Phthalate exacerbates asthma-like pathologies by increasing the expression of CGRP mediated by oxidative stress.	[38]

Table 5. Short-term toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Dibutyl Phthalate, >98% pure	corn oil	male Wistar rats, 6–8 wk old; 4 groups (12/group)	9 wk	750 mg/kg bw/d	Rats were randomly divided into four groups: control, Dibutyl Phthalate, Dibutyl Phthalate + resveratrol (Res), and Res. The Dibutyl Phthalate and Dibutyl Phthalate + Res groups received a daily intragastric administration of Dibutyl Phthalate. The Dibutyl Phthalate + Res and Res groups were given Res at 100 mg/kg bw/d. On days 42, 49, and 56, rats in the Dibutyl Phthalate and Dibutyl Phthalate + Res groups were intraperitoneally injected with 1 ml of OVA sensitizer (1 mg OVA and 100 mg aluminum hydroxide in 1 mL saline). From days 57 to 63, rats were nebulized with 1% OVA for 30 min daily, while the control group and the Res group were atomized with 0.9% (w/v) saline. After 24 h of the last treatment, the rats were weighed and anesthetized.	Compared with controls, Dibutyl Phthalate-exposed rats showed lung swelling, congestion, increased lung wet weight and lung/body weight index ($p < 0.05$), along with significant histopathological lesions including inflammatory cell infiltration, epithelial shedding, and mucus secretion. Dibutyl Phthalate-exposed group showed significant increase in inflammatory cell counts in BAL fluid ($p < 0.05$), accompanied by elevated Th2 cytokines (IL-4 and IL-13), as determined by ELISA. OVA-IgE levels and LDH activities were markedly increased in the Dibutyl Phthalate group compared with the control group ($p < 0.05$). In the Dibutyl Phthalate-exposed group, oxidative stress was evident, as indicated by increased MDA and decreased GSH and SOD. TUNEL staining revealed increased apoptosis in lung tissue. Western blot analysis showed elevated the protein levels of thymic stromal lymphopoietin TSLP, JAK1, and phosphorylated STAT6, while the nuclear translocation of Nrf2 and its downstream antioxidant proteins NAD(P)H quinone oxidoreductase 1(NQO1) and heme oxygenase-1 were suppressed. In general, Res co-treatment markedly attenuated the above Dibutyl Phthalate induced effects. In general, Res co-treatment markedly attenuated the above Dibutyl Phthalate induced effects.	[39]

Table 5. Short-term toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate, monobutyl phthalate, monomethyl phthalate, monomethyl phthalate, and phthalic acid	corn oil	groups of 6 male Sprague-Dawley rats	4 wk	250 mg/kg bw/d for monoesters and phthalic acid; 500 mg/kg bw/d for diesters	Rats received test materials via gavage for 4 wk; control group received corn oil only; animals observed for clinical signs of toxicity and mortality daily; body weights were measured every 3 d; feed consumption measured twice per wk; collected urine underwent analysis; organs were weighted at necropsy; blood was also collected at necropsy for analysis; and sperm was collected for sperm count analysis and motility analysis (results presented in the DART section below)	No mortalities observed. Body weights of rats in the Dibutyl Phthalate, monobutyl phthalate, and phthalic acid groups were significantly decreased ($p < 0.05$) after 2-wk administration periods, but no significant differences were observed in feed consumption between treatment groups and controls. Livers weights were significantly increased in groups treated with Dibutyl Phthalate and monobutyl phthalate compared to control group. Red blood cells and hematocrit were significantly lower and mean corpuscular hemoglobin concentration and platelet count were significantly higher in the Dibutyl Phthalate group (the monoester also had significantly higher platelet counts), and hemoglobin level was reduced only in the Dimethyl Phthalate group. Glutamate oxaloacetate transaminase levels in Dibutyl Phthalate and monobutyl phthalate groups and alkaline phosphatase levels in the Dibutyl Phthalate and Dimethyl Phthalate groups were significantly higher than the controls	[40]

Table 5. Short-term toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Diethyl Phthalate	dietary feed	Groups of 5 male and 5 female Sprague-Dawley rats at 2 and 6 wk standard study; groups of 15 males and females in 16 wk standard study; groups of 6 males and 6 females in pair feeding study	2, 6, and 16 wk	0, 0.2, 1, and 5% in standard study; 0 and 5% in pair feeding study (approximately equivalent to 0, 150, 750, and 3170 mg/kg/d)	Rats were fed continuously for 2, 6, and 16 wk for standard study and 16 wk for pair feeding study. Standard study: body weight, feed and water consumption were measured at intervals. At the end of each designated treatment period, the rats were killed and blood samples were collected for hematological and serum examination (16 wk groups only). Necropsy was performed, organs weighed and underwent histological examination. Pair feeding study: groups of 6 rats of each sex, individually housed and fed untreated diet (controls) or diet containing test material. Each control animal was from the same litter as one of the treated rats of the same sex. The treated rats were fed ad libitum and the weight consumed by each one was recorded daily. Each control rat was given a weight equal to that consumed by its paired litter mate in the previous 24 h period. Body weights were recorded at intervals during the treatment period.	NOAEL = 150 mg/kg diet In the standard study throughout treatment, male and females of the 5% group and females of the 1% group gained significantly less weight than the controls, transient lower body weight gains were observed in the 1% males; overall feed consumption was significantly lower than that of controls in 5% male and female rats, and 1% females. In 6 wk groups, the erythrocyte count in 5% males was significantly higher than in controls; this change was associated with a non-significant increase in hemoglobin levels when compared with controls. Male and female rats that received 5% Diethyl Phthalate for 16 wk had statistically significantly lower absolute weights of the brain, heart, spleen & kidneys than controls; reductions in the absolute weights of the female gonads and male heart, spleen, and kidneys were also observed in the 5% groups after 2 and 6 wk of treatment. The weights of stomach of rats in the 5% group increased at times during the study but attained statistical significance with respect to controls after 2 wk (males) and 2 & 16 wk (females). The weight of the full caecum was also increased in females 5% group that received the test material for 16 wk. Increased kidney weights were seen in males & lower pituitary weights in females in the 1% group after 2 wk of treatment. Increased gonad weights were also seen in females of the 1% group after 6 wk. In the 5% group, the relative weights of the brain, kidney (16 wk only), liver, stomach, small intestine, and full caecum were increased in rats of both sexes & of testes of males. In the rats given 0.2 or 1%, there were similar increases in the relative weights of the liver, stomach & small intestine. In pair feeding study, rats that received 5% Diethyl Phthalate lost more weight than their respective controls over the 1st day of treatment; weight gain in the 5% group was also lower than controls thereafter. Statistical significance was attained in week 16. Total feed consumed in the 5% groups were greater than that consumed by their respective controls. No further details were available.	[6]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
GENERAL DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES						
IN VITRO						
monobutyl phthalate	*not reported*	*day 10 rat embryos, no further details*	*0, 0.5, 1, 1.5, 2, 2.5, or 5 mM*	*Whole rat embryos were incubated without antibiotics in cell culture flasks containing growth medium with inactivated rat serum. Embryos were incubated with the test material in culture for 46 h. Embryos with beating hearts were considered viable and examined microscopically for growth and morphological development*	*A decrease was observed in head length at 1 mM and in all growth parameters at 1.5 mM. Morphological abnormalities at 1 mM and higher occurred in the craniofacial region, the prosencephalon and/or optic system, the auditory system, mesencephalon, and in shortening of the trunk. At 5 mM, 20% of the embryos were non-viable and those surviving had severe dysmorphogenesis.*	[8]
DERMAL						
Diethyl Phthalate	*0.5% carboxymethylcellulose*	*groups of 12 pregnant New Zealand White rabbits*	*0, 5, 15, or 50%*	*Rabbits received test material (2 ml/kg) daily on a 10 cm^2 shave area of the dorso-lumbar skin on gestation days 6 through 18; test sites were not occluded and were washed with tepid water 7 h after application; rabbits were killed on gestation day 29*	*The dams showed no clinical signs of toxicity, and performance on behavioral tests in dosed animals showed no difference from vehicle controls. Maternal body weight gain and feed consumption were not affected by the treatments. There were no differences between the groups in the numbers of fetuses, the sex ratio, weights of fetuses, or incidence of dead fetuses. One dam in the 50% dose group had a higher number of early resorptions, but this did not provide a statistical significance between treatment conditions. Fetal examinations revealed 1 fetus with acranie and malformations of the ribs and lumbar and coccygeal vertebrae, and 1 fetus showed hernia umbilicalis with prolapse of abdominal organs and incurved ribs. Both malformed fetuses were in the 50 % treatment group. However, the researchers concluded that these were not treatment-related effects, based on the historical occurrence of these malformations.*	[8]
Dimethyl Phthalate	*paraffin oil*	*groups of 15-25 pregnant outbred Mol:Wis rats*	*0, 0.5, 1, or 2 ml/kg bw*	*Rats received test material on shaved skin for 2 h/d on gestation days 6 to 15 or 1 to 20; test sites were occluded; dams were killed on gestation day 21*	*No treatment-related signs of toxicity in the dams; no treatment-related effects observed in fetal survivability or development*	[8]
ORAL						
Dibutyl Phthalate	*in feed*	*pregnant mice, no further details available*	*80 - 2100 mg/kg/d*	*Mice received test material in feed throughout gestation and were killed on day 18 of gestation*	*Implantation was not affected, but resorptions and fetal deaths increased with dose. Maternal weight gain was depressed at higher doses and was due to increased embryonic or fetal death. 2 of 3 live fetuses from the 2100 mg/kg Dibutyl Phthalate group had neural tube defects. Ossification was depressed, but malformation and resorption rates and fetal weights were not significantly affected by Dibutyl Phthalate up to 350 mg/kg/o*	[2]
Dibutyl Phthalate	*olive oil*	*groups of 10 female rats*	*120 and 600 mg/d*	*Rats received test material via gavage approximately 3 mo prior to being mated; additional groups of female rats received the same doses for 21 d following fertilization; rats were killed on day 21 of gestation*	*There were 4, 2, and 22 resorptions in the 0, 120, and 600 mg/d dose groups, respectively. 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. Dosing for Dibutyl Phthalate before gestation did not cause any significant changes in other measured parameters. Lower placental weights and fetal weights were significantly lower in the high dose group.*	[2]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	*olive oil*	*groups of 11 or 12 pregnant Wistar rats*	*0, 0.5, 0.63, 0.75, or 1.0 g/kg/d*	*Rats received test material via gavage on days 7 through 15 of gestation; rats were killed on day 20 of gestation*	*Maternal weight gain was reduced ($p < 0.05$) on days 15 - 20 in the 0.63 g/kg group and on day 7 to 20 in the 0.75 and 1.0 g/kg groups. Maternal feed consumption was reduced ($p < 0.05$) on days 7 to 15 in the 0.75 g/kg group and on days 7 to 20 in the 1.0 g/kg group. The number of litters and number of implantations per litter were not affected by treatment. Number resorptions, dead fetuses, and post-implantation loss per litter were increased ($p < 0.05$) in the 0.63, 0.75, and 1.0 g/kg groups. The number of litters totally resorbed was increased ($p < 0.05$) in the 0.75 and 1.0 g/kg groups. The number of live fetuses per litter was reduced ($p < 0.05$) in the 0.63, 0.75, and 1.0 g/kg groups. No live pups were born in the 1.0 g/kg group. Body weights of live fetuses were reduced ($p < 0.05$) in the 0.63 and 0.75 g/kg groups. Significant increases ($p < 0.05$) of number of fetuses with external malformations were observed in the 0.75 g/kg dose group*	[8]
Dibutyl Phthalate	*not reported*	*groups of 10 or 11 pregnant rats*	*0, 0.75, 1.0, or 1.5 g/kg/d*	*Study related to the one described above; the dosing schedule was divided to expose pregnant rats to test material by daily gavage either on gestation days 7 to 9, 10 to 12, or 13 to 15; dams were killed on gestation day 20*	*There were no live fetuses in the 1.5 g/kg group. The 0.75 and 1.0 g/kg groups produced similar external, skeletal, and internal malformations as those seen in the study above; however there was a higher incidence of skeletal malformations in the vertebral column and ribs. Of the 3 dosing schedules, the fetuses dosed on days 7 to 9 and on days 13 to 15 showed the higher incidence of fetotoxicity and incidence of malformations. The fetuses exposed on gestation days 10 to 12 showed less severe malformations than those exposed before or after this period.*	[8]
Dibutyl Phthalate	*not reported*	*groups of 11 or 13 pregnant rats*	*0, 0.75, 1.0, or 1.25 g/kg*	*Study related to the two described above; the pregnant rats were dosed by daily gavage on gestation days 7 to 9, 10 to 12, or 13 to 15; dams were killed on gestation day 20*	*There was a significant increase in post-implantation loss at all doses in animals dosed in all 3 dosing schedules. Skeletal malformations were seen in the ribs and vertebrae at all doses in animals dosed on day 7 to 9. No increased incidence of malformation due to Dibutyl Phthalate were observed in animals dosed on days 10 to 12. Increased incidence of cleft palate and fused sternebrae were seen at all doses tested in animals exposed on days 13 to 15. As seen in the study above, fetuses were more vulnerable to Dibutyl Phthalate-induced malformations on days 7 to 9 and on days 13 to 15 than on days 10 to 12.*	[8]
Dibutyl Phthalate	*in feed*	*groups of 10 pregnant rats, strain not reported*	*2%*	*Rats received test material in feed ad libitum on gestation days 0 through 20; most dams were killed on gestation day 20, some were killed on gestation days 7, 9, or 11; no further details available*	*Body weight gain and feed consumption were reduced in dams exposed to test material. Number of implantations and number of corpora lutea were not affected by test material, but post-implantation loss and number of resorptions per litter were increased, and number of live fetuses was reduced in the exposure group. There were no live male fetuses in the treated group. Due to high fetal death, malformations were not observed. Ovarian weight, uterine weight, and serum progesterone were reduced in the treated dams on gestation day 11.*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	*in feed*	*groups of 10 or 15 pregnant rats, strain not reported*	*0, 0.5, 1, or 2% (corresponding to 0, 331, 555, or 661 mg/kg bw/d)*	*Rats received test material in feed ad libitum on gestation days 11 through 21; dams were killed on gestation day 21*	*Body weight gain and feed consumption were reduced in dams in the 1 and 2% dose groups. There were no adverse effects observed on fetal survival, number of resorptions, or sex ratio of live fetuses. Fetal body weights in both sexes were reduced in the 2% dose group. Anogenital distance was reduced in male fetuses in the 1% and 2% dose groups. Cleft palate and fused sternebrae were observed in the 2% dose group. Undescended testes were observed in the 1% and 2% dose groups. An NOAEL was determined to be 331 mg/kg bw/d.*	[8,180]
Dibutyl Phthalate	*not reported*	*groups of 10 - 12 pregnant rats, strain not reported*	*0 or 1500 mg/kg*	*Dams received a single dose of 0 or 1500 mg/kg Dibutyl Phthalate once via gavage between gestation day 6 through 16; dams were killed on gestation day 20*	*A significant increase in post-implantation loss in litters of all treatment days except in animals dosed on days 7 and 11. Cleft palate and fusion of sternebrae were seen in fetuses exposed on day 15. There were malformations of the cervical and thoracic vertebrae and of the ribs in animals exposed on day 8 and 9. Dilatation of renal pelvis was observed in animals exposed on day 9.*	[8]
Dibutyl Phthalate	*not reported*	*groups of 10 - 13 pregnant rats, strain not reported*	*0, 1000, or 1500 mg/kg*	*Dams received daily doses of test material via gavage on gestation days 12 to 14, 15 to 17, or 18 to 20; dams were killed on gestation day 21*	*Body weight gain and feed consumption were reduced in dams exposed to 1000 or 1500 mg/kg in all dosing schedules. Litters exposed to 1500 mg/kg on days 12 to 14 or on days 15 to 17 had increases in the number of litters totally resorbed, in the number of resorptions per litter, in post-implantation death, and in the number of males with undescended testes. Fetal body weights were reduced in both male and female fetuses that had been exposed to 1500 mg/kg at all three dosing schedules. The incidence of undescended testes was increased in fetuses exposed on gestation days 12 to 14 and 15 to 17 but not 18 to 20. The anogenital distance was reduced in males exposed in all treatment schedules.*	[8]
Dibutyl Phthalate or monobutyl phthalate	*olive oil for Dibutyl Phthalate; ammonium chloride for monobutyl phthalate*	*groups of 11 - 15 pregnant Sprague-Dawley rats*	*0, 1.8, 3.6, 5.4, or 7.2 mmol/kg*	*Dams received a single dose of the test materials via gavage on gestation day 10; dams were killed on day 12. Further study to determine the rate of non-viability of fetuses was performed with monobutyl phthalate treated the dams with 0, 5.4 or 7.2 mmol/kg on day 10 and killed the rats on day 12, 13, 14, or 18 (6 to 12 rats/exposure)*	*11 to 15 litters were examined/ dose group. Embryos exposed to Dibutyl Phthalate showed dose-dependent growth retardation and dysmorphogenesis. At the 1.8 mmol/kg dose, few adverse effects were seen. At the 3.6 mmol/kg and higher doses all measures of growth and development were reduced from vehicle and non-treated controls, and the incidence of malformed embryos was increased 4-fold. The observations in the higher groups for Dibutyl Phthalate included defects of the prosencephalon, the optic system, and the mandibular process. The most severely affected embryos also exhibited underdevelopment of the auditory system, maxillary process, and caudal extremity. The total number of viable embryos per litter was not affected by the Dibutyl Phthalate treatment. The embryos exposed to monobutyl phthalate exhibited the same types of growth retardation and malformations, but at higher doses (5.4 and 7.2 mmol/kg). These effects were not observed at the 1.8 or 3.6 mmol/kg monobutyl phthalate doses. In the viability study with monobutyl phthalate, an increase in the rate of non-viable fetuses was observed on days 13 and 14. On day 18, surviving fetuses from the exposed groups had eye anomalies, absent digits, misshapen lower jaws, enlargement of the pericardium and/or cardiac tube, and brain anomalies.*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	*in feed*	*number of pregnant F344/N rats not reported; groups of 10 male and 10 female pups per dose*	*pregnant rats = 0 or 10,000 ppm* *13-wk pups = 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm*	*Dams were exposed to 0 or 10,000 ppm Dibutyl Phthalate in feed from the beginning of gestation through lactation; when pups were able to eat solid food, they were exposed to the same feed conditions as their mothers until they were 8 wk old; at postnatal week 13, pups exposed to 10,000 Dibutyl Phthalate were divided into dosing groups to receive up to 40,000 ppm Dibutyl Phthalate in feed for 13 wk; at the end of the 13 wk, animals were killed for examination*	*The number of live pups born was not different between the control and 10,000 ppm perinatal groups. However, by the first day after parturition the survivability of the exposed pups was significantly lower than that of control pups and remained so through weaning. Pup body weights were also lower in the 10,000 ppm perinatal group from the time of birth through weaning. All rats survived the 13-wk dosing portion of the study. The mean body weights of control animals who had not been exposed to Dibutyl Phthalate perinatally were higher than all animals that had been dosed perinatally. Female rats that had been exposed perinatally but not during the 13-wk dosing period gained weight more rapidly than other groups that had been exposed perinatally, and by the end of the study they had body weights similar to those controls that were not dosed perinatally. The mean body weights of all groups of males exposed perinatally and of females that received 20,000 or 40,000 ppm as adults remained less than those unexposed controls at the end of the study. Males in the 40,000 ppm group lost weight during the study. All male and female animals in the 40,000 ppm group were emaciated at the end of the study and had lower food consumption rates than controls. Males in this group had abnormal posture and ruffled fur and appeared hypoactive in weeks 2 through 4 of dosing. Males that had been exposed perinatally but not during the 13-wk dosing period had lower testicular weights than unexposed control males. No other organ weights differed between these two groups. There were signs of toxicity to the liver and kidneys in both sexes and in the reproductive tissues of the males. There were no dose-related effects on the estrous cycles of the females.*	[8]
Dibutyl Phthalate	*sesame oil*	*groups of 6 pregnant or groups of 6-8 pseudo-pregnant female Holtzman rats*	*pregnant rats = 0, 250, 500, 1000, or 2000 mg/kg/d* *pseudo-pregnant rats = 0, 500, 1000, or 2000 mg/kg/d*	*Study on the maternal effects of test material on fetotoxicity; some females were paired with males for regular breeding., while other females were subjected to artificial stimulation of the cervix by a glass rod during proestrus or estrus in order to induce a pseudopregnancy. Deciduomata were induced surgically to simulate implantation of a blastocyst in pseudo-pregnant rats. All rats were dosed by oral gavage on day 1 through day 8 of gestation or pseudo-gestation. On day 9 rats were killed, and blood was collected. Uteri and ovaries were dissected and cleaned of adjacent tissues and weighed.*	*Dibutyl Phthalate at doses up to 2000 mg/kg/d did not significantly affect whole body weights, uterine weights, ovarian weights, or serum progesterone levels in the pregnant or pseudo-pregnant rats. In pregnant rats, there was no effect of Dibutyl Phthalate on the number of implantations. The authors concluded that any fetotoxic effects of short-term Dibutyl Phthalate exposure are due to the test material's interaction with the fetus and not a result of maternal effects in early pregnancy, and that the viability of preimplantation embryos is not affected by Dibutyl Phthalate*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	*not reported*	*groups of 10-13 pregnant or pseudo-pregnant female rats*	*0, 250, 500, 740, 1000, 1250, or 1500 mg/kg*	*Rats received test material via oral gavage on gestation day 0 through day 8; pregnant rats were killed on day 20 and pseudo-pregnant rats were killed on day 9*	*Body weight gain and feed consumption were reduced in dams exposed to doses of 500 mg/kg and higher. Increases in preimplantation loss were seen in doses of 1250 and 1500 mg/kg. The numbers of resorptions and dead fetuses per litter were increased in 1000 mg/kg and higher doses. Preimplantation loss was increased, and number of live fetuses per litter was reduced at doses of 750 mg/kg and higher. The fetal sex ratio was not affected by Dibutyl Phthalate, but both male and female fetal weights were reduced at all doses tested. Body weight gain and feed consumption were reduced in pseudo-pregnant rats exposed to doses of 750 mg/kg and higher. In the pseudo-pregnant rats ovarian weight, number of corpora lutea, and serum estradiol were not affected by Dibutyl Phthalate, but uterine weight was reduced at doses of 750 mg/kg and higher. Serum progesterone was reduced in pseudo-pregnant rats only at the 1500 mg/kg dose.*	[8]
Dibutyl Phthalate	*in feed*	*2 strains of mice, no further details available*	*10 and 100 mg/kg/d*	*3 generation reproduction study; no further details available*	*An increase in the formation of renal cysts was observed in the F1 and F2 generations; no further details available*	[2]
Dibutyl Phthalate	*oil, kind not specified*	*female rats, no further details available*	*50% solution; daily dose of 1 ml/kg*	*3 generation reproduction study; female rats dosed daily for 6 wk prior to mating with untreated males; the offspring were bred to produce 2 additional generations; no information available on whether the second and third generations were dosed with Dibutyl Phthalate*	*No impairment of reproductive performance was noted. Development, growth, and fertility were normal for all 3 generations. No further details available*	[2]
Dibutyl Phthalate	*in feed*	*groups of 20 male and 20 female COBS Crl:CD-1, (IC)BR mice*	*0, 0.03, 0.3, or 1%*	*Continuous breeding protocol; mice exposed to test material for a 7-d remating period and then assigned to mating pairs within the dose groups; cohabitation period was 98 d; newborn pups were collected within 12 h of birth and discarded after physical data (body weight, proportion of males, number of litters per pair, number of live pups) were recorded. At the end of the cohabitation period, mating pairs were separated for 21 d while Dibutyl Phthalate in the diet continued. During this 21-d period, any remaining litters were delivered and kept for at least 21 d. At the end of the continuous breeding phase, mice in the high dose group were re-paired for crossover mating, to create 3 groups of pairs: control male x control female; control male x 1% female; or 1% male x control female. The offspring of the crossover pairs were analyzed, and the parents were necropsied.*	*The fertility of the breeding pairs, number of litters per pair, number of live pups per litter, and proportion of pups born alive were significantly ($p < 0.01$) reduced in the 1% dose group, compared to control. The lower doses did not affect these parameters. Live pup weight was not different from control in any treatment groups. Pups that were born to control female x 1% male pairs did not differ from pups born to two control parents. However, significantly reduced fertility ($p < 0.01$), number of live pups per litter ($p < 0.01$), proportion of live pups per litter ($p < 0.05$), and live pup weight ($p < 0.05$) were observed when 1% females were mated with control males. In male mice of the 1% dose group, total body weight was reduced ($p < 0.01$), but organ weights were not different from control. Liver weights were increased and uterus weights were reduced in 1% treated female mice, compared to controls ($p < 0.05$). Additionally, there were no differences in sperm motility, sperm concentration, or percentage of abnormal sperm in male mice exposed to 1% dose group, compared to controls*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	*in feed*	*groups of 20 male and 20 female VAF Crl:CD BR outbred Sprague-Dawley albino rats*	*0, 0.1, 0.5, or 1%*	*Similar study to the continuous breeding protocol described above. Crossover pairs were as follows: control male x control female; 1 % male x control female; and control male x 1 % female (n = 20 pairs/crossover group).*	*The number of litters per pair was not different from control in any of the treatment groups. The number of live pups per litter and pup weights was significantly reduced ($p < 0.05$) in all dose treatments, compared to control. Live pup weights were significantly reduced ($p < 0.05$) in the 0.5 and 1.0 % groups, compared to control. The pregnancy rate, fertility, and live birth rate of the three crossover groups were not significantly different. Live pup weights were significantly reduced ($p < 0.05$) in the control male x 1% female group, compared to those in the control male x control female group.* *Body weight, liver weight, and kidney weight were reduced ($p < 0.05$) in 1% female F_0 rats, compared to controls. In 1% male F_0 rats, body weight was not different, but kidney and liver weights were reduced ($p < 0.05$), compared to controls. Sperm endpoints (count, morphology, and motility) were not affected by treatment in F_0 males at the doses tested.* *The mating index (number of females with copulatory plugs per number of cohabited pairs), pregnancy index (number of fertile pairs per number of cohabited pairs), and fertility index (number of fertile pairs per number of females with plugs) were all significantly reduced ($p < 0.05$) in the 1 % F_1 mating pairs. Live pup weights were reduced ($p < 0.05$) in all concentrations tested compared to controls. Necropsy of F_1 rats revealed reduced ($p < 0.05$) body weights for both males and females in the 1 % treatment. In the 1% males, the prostate, seminal vesicles, and right testes had reduced ($p < 0.05$) weights, compared to controls, and the liver and kidneys weights were increased ($p < 0.05$). While epididymal sperm motility, number, and morphology were not affected by treatments, the total spermatid heads per testis and per g testis were reduced ($p < 0.05$) in the 1% dose group, compared to control.*	[8]
Diethyl Phthalate	*in feed*	*groups of male and female Swiss CD-1 mice*	*0, 0.25, 1.25, or 2.5%*	*Continuous breeding protocol; F_1 mice from the control and high dose group were mated within their treatment groups (not cross-mated between groups); no further details available*	*Feed consumption was not affected by test material. There were no effects by Diethyl Phthalate on fertility or reproductive outcome of the F_0 generation. There were no treatment-related differences between the treated and control groups of the F_2 generation. Male and Female F_1 mice of the high dose group had a reduced adult body weight and increased liver weight. Male F_1 mice of the high dose group showed an increased prostate size and a reduced sperm count. However, motility and morphology of the sperm were not affected by Diethyl Phthalate and neither were testicular, epididymal, and seminal vesicle weights.*	[8]
Diethyl Phthalate	*in feed*	*groups of 26 - 32 pregnant Sprague-Dawley rats*	*0, 0.25, 2.5, or 5% (0, 0.2, 1.91, or 3.21 g/kg/d)*	*Dams received diet containing test material on gestation days 6 to 15; dams were killed on gestation day 20*	*5% dose group had reduced body weight gain during the dosing period; however, feed and water consumption were reduced in this group during dosing, so the effect on body weight gain may have been due to palatability of the test material in the feed. There was no effect of the test material on any parameter of fetal viability or development, except for an increased incidence of supernumerary ribs in the 5% group.*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dimethyl Phthalate	*corn oil*	*groups of 36 - 40 pregnant CD-1 mice*	*0 or 3500 mg/kg*	*Mice received test material daily via gavage on gestation days 7 through 15; offspring were examined externally and weighed 12 to 24 h after birth and again on postnatal day 3*	*There were no signs of maternal toxicity or changes in weight gain due to test material. There were no treatment-related effects on fetal survivability, litter size, litter weight, or physical development of the fetus.*	[8]
Dimethyl Phthalate	*in feed*	*groups of 25-30 pregnant Sprague-Dawley rats*	*0, 0.25, 1, or 5% (0, 0.2, 0.84, or 3.57 g/kg/d)*	*Rats received test material in feed on gestation days 6 to 15; dams were killed on gestation day 20*	*The high dose group had increased maternal liver weights and reduced body weight gain. However, feed and water consumption were reduced in the 5% group during dosing, so the effect on body weight gain may have been due to palatability of the test material in the feed. There was no effect of Dimethyl Phthalate on any parameter of fetal viability or development.*	[8]
monobutyl phthalate	*ammonium chloride*	*groups of 11-15 pregnant Wistar rats*	*0, 250, 500, or 650 mg/kg*	*Rats received test material via gavage on gestation days 7 to 15. Maternal body weighs and feed consumption recorded daily. Dams were killed on gestation day 20*	*Maternal body weight gain and feed consumption rates were reduced in the 500 and 650 mg/kg doses ($P < 0.01$). Fetal effects observed at 500 and 650 mg/kg included increased post-implantation loss (resorptions and dead fetuses), reduced number of live fetuses, and lower body weights ($p < 0.01$). An increased incidence of external malformations (cleft palate), skeletal malformations of the cervical and thoracic vertebral arches and sternebrae, and internal malformations (dilation of the renal pelvis) were observed in the 500 and 650 mg/kg fetuses ($p < 0.05$). No developmental effects were observed at 250 mg/kg.*	[8]
monobutyl phthalate	*ammonium chloride*	*groups of pregnant Wistar rats, number per group not specified*	*0, 500, 625, or 750 mg/kg*	*Rats received test material via gavage on gestation days 7 to 9, 10 to 12, or 13 to 15. Maternal body weights and feed consumption recorded daily. All dams were killed on gestation day 20*	*Maternal body weight gain and feed consumption were reduced during the dosing periods. In the 7750 mg/kg group, dams dosed on gestation days 10 - 12 and 13 - 15 had continued reductions in feed consumption and weight gain through gestation day 20. The percentage of post-implantation loss was increased int eh 625 and 750 mg/kg dose groups at all dosing schedules ($p < 0.01$) and was as high as 95% at 750 mg/kg in rats exposed on gestation days 13 - 15. Body weights of live fetuses were most reduced by monobutyl phthalate at all doses in those exposed on gestation days 7 - 9 ($p < 0.05$). Fetuses of the 625 and 750 mg/kg groups exposed during gestation days 13 - 15 had cleft palate ($p < 0.01$). Skeletal malformations of the cervical vertebral arches were observed at all doses exposed during gestation days 7 - 9, and fusion of the sternebrae was observed at the two higher doses expos d on gestation days 13 - 15 ($p < 0.05$).*	[8]
monobutyl phthalate	*not reported*	*groups of 16 pregnant Wistar rats*	*0, 250, 500, or 750 mg/kg*	*Rats received test material on gestation days 15 through 17 and were killed on gestation day 21. Pregnancy outcome and fetal development were recorded; no further details available*	*Maternal feed consumption was reduced in the 750 mg/kg dose group, and maternal body weight gain was reduced in the 500 and 750 mg/kg dose groups. LOELs are listed with the following fetal parameters: increased number of resorptions and dead fetuses per litter (500 mg/kg), percent post-implantation loss per litter (500 mg/kg), reduced body weights for male and female fetuses (750 mg/kg each), increased number of male fetuses with undescended testes (250 mg/kg), reduced anogenital distance of male fetuses (250 mg/kg), and reduce ratio of anogenital distance/body weight (250 mg/kg).*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
monobutyl phthalate	*not reported*	*groups of 16 pregnant or pseudopregnant Wistar rats*	*0, 250, 500, 750, or 1000 mg/kg*	*Rats received test material on gestation days 0 through 8. Pregnant rats were killed on gestation day 20 and pseudopregnant rats were killed on day 9. Pregnancy outcome of the pregnant rats and uterine weights of the pseudopregnant rats were recorded; no further details available*	*The incidence of preimplantation loss was increased at 1000 mg/kg. Uterine decidualization in the pseudopregnant rats was decreased in the 1000 mg/kg dose group.*	[8]
			PARENTERAL			
Dibutyl Phthalate and Dimethyl Phthalate	*saline*	*groups of 5 pregnant female rats, strain not specified*	*2 or 4 ml/kg for Dibutyl; 0.5, 1, or 2 ml/kg for Dimethyl*	*Rats received test materials intraperitoneally on days 3, 6, and 9 of gestation; controls received just 4 ml/kg saline; no further details available*	*1 rat died in the 4 ml/kg Dibutyl Phthalate dose group, implantations occurred in 4 and 3 rats, respectively, in the 2 and 4 ml/kg Dibutyl groups. Dibutyl Phthalate resulted in a 50% reduction in the number of pups weaned/litter. 2 male pups, 1 from each of 2 litters in the 2 ml/kg Dibutyl group had no eyes. In the 0.5, 1, and 2 ml/kg Dimethyl Phthalate groups, 2 rats died in the 1 ml/kg dose group while all survived in the 0.5 and 2 ml/kg dose groups, and only 1 implantation occurred in the 1 ml/kg dose group. The numbers of pups weaned were not significantly different from the controls*	[2]
Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate	*not reported*	*Groups of 5 pregnant female rats, strain not specified*	*3.05 ml/kg for Dibutyl; 5.06 ml/kg for Diethyl; 3.4 ml/kg for Dimethyl*	*Rats received test materials intraperitoneally on days 5, 10, and 15 of gestation; control rats were untreated or received distilled water, normal saline, or cottonseed oil; maternal rats were killed on day 20; no further details available*	*Phthalate administration did not interfere with fertility, as reflected by corpora lutea:implantation site ratios. Significant effects on embryonic and/or fetal development were observed; 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. A significantly higher number of skeletal abnormalities in the fetuses from the test group as compared to the controls was observed. The failure to include historical control data, as well as a positive control in the test program, made it difficult to evaluate the significance of the results.*	[2]
			UTEROTROPHIC AND VAGINAL EFFECTS			
			ORAL			
Dibutyl Phthalate	*not reported*	*groups of 10 immature ovariectomized Sprague-Dawley rats*	*0, 20, 200, or 2000 mg/kg/d*	*Rats received test material via gavage for 4 d; on the day after the fourth dose, all animals were killed and the uteri were weighed; no further details available*	*The uterine weights of the treated animals did not differ from those of control animals; no further details available*	[8]
Dibutyl Phthalate	*not reported*	*mature ovariectomized female Sprague-Dawley rats; number not reported*	*0, 20, 200, or 2000 mg/kg/d*	*Rats received test material via gavage for 4 d; each day of the study, physiological saline was used for vaginal lavage of the rats to determine cornification of vaginal cells., the percentage of cornified cells was used as an indicator of estrogenic activity*	*The percentages of cornification of vaginal cells from the treated rats were similar to those of control rats.*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	*not reported*	*ovariectomized rats; no further details available*	*1000 mg/kg/d*	*Rats were exposed to estradiol benzoate (10 µg) or test material for 2 d; on the third day, the rats were given 0.5 mg progesterone; 6-8 h after the progesterone injection, the females were paired with a sexually active male for observation of lordosis behavior. After the lordosis behavioral observations, the females were killed and their uteri were cleaned of mesenteric fat and weighed*	*Dibutyl Phthalate treatments did not affect uterine weights or lordosis behavior, compared to control (oil) treatment. The estradiol group showed significant increases in these parameters.*	[8]
Diethyl Phthalate	*peanut oil*	*groups of 10 immature female Wistar rats*	*0, 50, 150, 500 mg/kg/d*	*Rats received a daily dose of test material of 0.4 mg/kg β-estradiol (positive control) for 3 d; 24 h after last dose, rats were killed and uteri were removed and weighed*	*The uteri of rats in the β-estradiol positive control group were significantly heavier than those of the vehicle control group, as expected. The weights of the uteri of rats exposed to Diethyl Phthalate were similar to those in the control group.*	[8]
			PARENTERAL			
Dibutyl Phthalate	*not reported*	*ovariectomized rats; no further details available*	*200 or 400 mg/kg/d*	*Rats were exposed to estradiol benzoate (10 µg) or test material subcutaneously for 2 d; on the third day, the rats were given 0.5 mg progesterone; 6-8 h after the progesterone injection, the females were paired with a sexually active male for observation of lordosis behavior. After the lordosis behavioral observations, the females were killed and their uteri were cleaned of mesenteric fat and weighed*	*Dibutyl Phthalate treatments did not affect uterine weights or lordosis behavior, compared to control (oil) treatment. The estradiol group showed significant increases in these parameters.*	[8]
			TESTICULAR EFFECTS			
			IN VITRO			
monoethyl phthalate	*DMSO*	*Wistar rat Leydig cells*	*0 or 1000 µM*	*Cell cultures were exposed test material or vehicle for 3 h. Some cells were exposed to luteinizing hormone to test for Leydig cell function integrity. Cells were fixed and processed for histopathological evaluation*	*Monoethyl phthalate did not affect testosterone output or induce any structural changes in Leydig cell morphology or ultrastructure.*	[8]
			ORAL			
Dibutyl Phthalate or sodium salt of monobutyl phthalate	*corn oil for Dibutyl Phthalate; aqueous solution of sodium salt of monobutyl phthalate*	*Groups of 6 male Sprague-Dawley rats, groups of 10 male TO mice, groups of 5 male Dunkin-Hartley guinea pigs, and groups of 8 male DSN Syrian hamsters*	*0, 2000, or 3000 mg/kg for Dibutyl Phthalate; at least up to 1600 mg/kg/d for monobutyl phthalate solution*	*Animals received test material for 7 to 9 d; histological examinations of the testes were performed; in additional experiments, male rats were exposed to 3000 mg/kg/d Dibutyl Phthalate for 9 d or to 800 mg/kg/d monobutyl phthalate for 5 d*	*Dibutyl Phthalate produced severe atrophy in the seminiferous tubules of rats and guinea pigs, with milder effects in mice. Hamster testes were not affected by Dibutyl Phthalate. Results for monobutyl phthalate not reported. In additional experiments, each treatment produced a significant reduction ($p < 0.001$) in testicular weight, compared to control treatments. Histologically, the treated rats showed > 90 % tubular atrophy in the testes. However, neither 3000 mg/kg Dibutyl Phthalate nor 1600 mg/kg/d monobutyl phthalate produced any changes in testicular weight in hamsters. No further details available*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	*not reported*	*male mice, rats, hamsters, guinea pigs, and ferrets; number and strains not reported*	*2 g/kg/d*	*Animals received test material orally for 10 d; no further details available*	*Testicular atrophy was observed in mice, rats, guinea pigs, and ferrets, but not in hamsters*	[2]
Dibutyl Phthalate	*not reported*	*28 male Wistar rats*	*8.6 mmol/kg/d*	*Rats received single dose of test material; no further details available*	*A decrease in testicular fructose and glucose and caused sloughing of germ cells in the first days of dosing. Testicular iron and zinc levels decreased, and inositol and cholesterol levels increased. After repeated doses, sloughing led to atrophy and dissociation of germ cells from Sertoli cells, and testicular levels of triglycerides, cholesterols, phosphatidyl choline, and phosphatidyl ethanolamine were reduced. Additionally, increases were observed in γ-glutamyl transferase, lactate dehydrogenase, lactate, alkaline phosphatase, and flavin adenine dinucleotide in the testes. There were also decreases in sorbitol, sorbitol dehydrogenase, succinate dehydrogenase, and aldose reductase in the testes. Transferrin was increased in Sertoli cells, Sertoli-germ connection, epididymis and liver and decreased in the seminal vesicle. Ferritin was reduced in the seminiferous lumen.*	[8]
Dibutyl Phthalate	*not reported*	*28 male Wistar rats*	*8.6 mmol/kg/d*	*Rats received oral doses of test material for 7 d; no further details available*	*A decrease in testicular fructose and glucose and caused sloughing of germ cells in the first days of dosing. Testicular iron and zinc levels decreased, and inositol and cholesterol levels increased. After repeated doses, sloughing led to atrophy and dissociation of germ cells from Sertoli cells, and testicular levels of triglycerides, cholesterols, phosphatidyl choline, and phosphatidyl ethanolamine were reduced.*	[8]
Dibutyl Phthalate	*not reported*	*groups of 6 male Wistar rats*	*0, 250, 500, or 1000 mg/kg/d*	*Rats received test material via gavage for 15 d; animals were weighed daily; on day 16 rats were killed and testes were promptly weighed and prepared for histopathological examination and enzyme activity assessments*	*No unscheduled deaths observed, but body weights and testicular weights were reduced in the 500 and 1000 mg/kg doses. Testicular sorbitol dehydrogenase activity was reduced in 500 and 1000 mg/kg dose groups. The activity of testicular acid phosphatase was reduced at all doses. Glucose-6-phosphate dehydrogenase, β-glucuronidase, and γ-glutamyl transpeptidase in the testes showed increased activities at all doses tested. Histopathological examination of the testes revealed dose-dependent damage to the seminiferous tubules. Spermatocytes in the 1000 mg/kg dose group showed pycnotic nuclei.*	[8]
Dibutyl Phthalate	*corn oil*	*pregnant rats; no further details available*	*0 or 500 mg/kg/d*	*Rats received test material via gavage on gestation days 12 to 21; no further details available*	*On gestation days 16-21, Dibutyl Phthalate caused focal hyperplasia of Leydig cells in male fetuses. At gestation day 21, Dibutyl Phthalate exposed fetuses showed testicular atrophy and seminiferous cords were enlarged. Dibutyl Phthalate-exposed male fetuses had reduced testosterone levels, fewer epididymal ducts, and reduced "AR" staining. The Leydig cell hyperplasia was proposed to be a compensatory response to increase steroidogenesis triggered by reduced testosterone. However, the overall androgen concentration was not corrected to the levels necessary to prevent malformations of the reproductive tract.*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Diethyl Phthalate	*corn oil*	*3 male Wistar rats*	*0 or 2 g/kg/d*	*Rats received test material via gavage for 2 d; all rats killed 24 h after the second dose; bodies were perfused with a fixative solution, and the testes were removed, sliced, and stained for histological examination*	*Diethyl Phthalate produced no change in seminiferous tubular structure or Leydig cell morphology. However, the Leydig cells of treated rats showed ultrastructural changes, specifically swelling of the mitochondria and vesiculation and focal dilatation of the smooth endoplasmic reticulum.*	[8]
Diethyl Phthalate	*corn oil*	*5 pregnant Sprague-Dawley rats*	*0 or 750 mg/kg/d*	*Dams received test material on gestation day 14 through postnatal day 3; developing male pup were evaluated for affects; no further details available*	*Maternal weight gain, litter size, pup weight, and sex ratio of pup were not affected by Diethyl Phthalate. No effects from Diethyl Phthalate were observed with weights of the testes and other reproductive tissues, anogenital distance, and incidence of males with areolas.*	[8]
Dimethyl Phthalate	*corn oil*	*5 pregnant Sprague-Dawley rats*	*0 or 750 mg/kg/d*	*Dams received test material on gestation day 14 through postnatal day 3; developing male pup were evaluated for affects; no further details available*	*Maternal weight gain, litter size, pup weight, and sex ratio of pup were not affected by Dimethyl Phthalate. No effects from Dimethyl Phthalate were observed with weights of the testes and other reproductive tissues, anogenital distance, and incidence of males with areolas.*	[8]
Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate	*corn oil*	*groups of 12 male rats, strain not reported*	*2 g/kg/d for Dibutyl, 1.6 g/kg/d for Diethyl, or 1.4 g/kg/d for Dimethyl*	*Test material was administered via gavage for 4 d; no further details available*	*No significant changes in feed intake or body weight observed in any dose group. Diethyl Phthalate and Dimethyl Phthalate did not result in significant changes in testes weight, no testicular atrophy was observed, and urinary zinc excretion was unaffected. Decreased testes weight and severe atrophy of the seminiferous tubules were observed following administration with Dibutyl Phthalate. Most of the tubules had complete loss of spermatocytes and spermatids. An increase in the urinary excretion of zinc was also observed, and there was a decrease in the zinc content of testes on an absolute and relative weight basis. The administration of zinc, concurrently with Dibutyl Phthalate, provided substantial protection against Dibutyl Phthalate-produced testicular damage.*	[2]
monobutyl phthalate	*sesame oil*	*groups of 7 pregnant Wistar-King A rats*	*0 or 3 ml dose of 0.3 g/d*	*Dams received test material on gestation days 15 to 18. On gestation day 20, 3 treated rats and 3 control 3 were killed and the male fetuses were examined for testicular effects. The remaining dams were allowed to deliver and the male offspring were killed on postnatal day 30 or 40 for analysis of the testes position and frequency of cryptorchidism*	*Males that were examined from gestation day 20 had relatively high positioned testes in the abdominal cavity, with some near the kidneys. In the controls of the males killed on postnatal day 30 or 40, the testes were allocated in the scrotum and there was a 0% incidence of cryptorchidism. In the treated males of the postnatal day 30 o4 40 group, 22 out of 26 had cryptorchidism (14 unilateral and 8 bilateral undescended testes). The majority of the undescended testes were in the abdominal cavity, while the remaining were at the external inguinal ring.*	[8]
SPERM EFFECTS						
IN VITRO						
Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate	*no reported*	*semen samples from healthy human volunteers, number not reported*	*not reported*	*Semen was collected, maintained in culture, and exposed to various concentrations of phthalates for 0, 2, or 18 h. At exposure end, sperm motility was measured by a computerized sperm analysis system. Results were presented as EC_{25}, or the concentration of each phthalate to reduce sperm motility parameter by 25% compared to untreated controls*	*The respective EC_{25} values for 0, 2, and 18 h of Dibutyl Phthalate exposure on sperm motility were 1, 30, and 8 mM. The EC_{25} values for 0, 2, and 18 h of Diethyl Phthalate exposure on sperm motility were >3.3, 2.5, 0.7mM, respectively. The respective EC_{25} values for 0, 2, and 18 h of Dimethyl Phthalate exposure on sperm motility were >0.94, >0.94, and 0.4 mM. Likewise, acute exposure to each of the phthalates produced dose-dependent reductions in sperm curvilinear velocity, straight line velocity, and linearity of sperm motion, and Dibutyl Phthalate was the most potent of the three phthalate diesters in inhibiting these parameters.*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
ANOGENIITAL EFFECTS						
ORAL						
Dibutyl Phthalate	*not reported*	*groups of 10 pregnant CD rats*	*0, 250, 500, or 750 mg/kg/d*	*Dams were exposed to test material throughout pregnancy and lactation until postnatal day 20; no further details available*	*The number of live pups per litter was decreased at the 750 mg/kg/d dose, probably due to maternal effects. Anogenital distance was reduced in male (but not female) pups exposed to 500 and 750 mg/kg/d. Other effects observed in male Dibutyl Phthalate-exposed offspring include absent or underdeveloped epididymis, hypospadias, ectopic or absent testes, germ cell loss, testicular atrophy, small testes and seminal vesicles, and absent prostate and seminal vesicles. These are all androgen-mediated effects. Estrogen-mediated development in female offspring such as vaginal opening and estrous cycle were not affected by Dibutyl Phthalate exposure.*	[8]
Dibutyl Phthalate	*corn oil*	*groups of 10 pregnant CD rats*	*0, 250, or 500 mg/kg/d*	*Dams were exposed to test material or flutamide (100 mg/kg/d in 5 rats) on gestation days 12 – 21; male pups were killed and examined either on postnatal day 60 or after postnatal day 100 to 105; female pups were killed and examined on postnatal day 20 to 30; pup examinations were focused on reproductive tissues; an immunohistological evaluation for androgen receptor expression was performed*	*Dibutyl Phthalate exposure induced the following morphological effects in a dose-dependent fashion that was similar to flutamide: reduced anogenital distance in males, increased incidence of thoracic nipple development, hypospadias, absent prostate, absent or partially developed epididymis, absent or underdeveloped seminal vesicles, and degradation of seminiferous epithelium. However, there were some differences between Dibutyl Phthalate and flutamide effects. For example, there was some variation in the sensitivities of androgen-dependent tissues between Dibutyl Phthalate and flutamide. Inguinoscrotal descent, an androgen-dependent event was impaired by flutamide and not Dibutyl Phthalate, whereas transabdominal descent (an androgen-independent event) was affected by Dibutyl Phthalate and not by flutamide. Other androgen-independent events were affected by Dibutyl Phthalate, as well (no further details).*	[8]
Dibutyl Phthalate	*not reported*	*groups of 19 - 20 pregnant CD rats*	*0, 0.5, 5, 50, or 100 mg/kg/d*	*Dams were exposed to test material on gestation days 12 to 21; beginning on postnatal day 1, offspring pups were examined for signs of developmental toxicity, including pup survival, clinical signs of toxicity, pup weight, and anogenital distance. On postnatal day 14, the numbers and locations of nipples and areolas on male pups were recorded. The days of acquisition of preputial separation or vaginal opening were recorded for male or female pups, respectively. Male pups were killed for necropsy on postnatal day 110 ± 10 d, and females were killed for necropsy on postnatal day 80 ± 5 d.*	*There were no effects of Dibutyl Phthalate treatment on rate of pup survival at birth. On postnatal day 14, there was an increase in female pup weights at doses 5 mg/kg/d and higher, but this increase was transient and not observed at later time points. The following observations were made of male pups of the 500 mg/kg/d group: reduced anogenital distance; hypospadias; absent or partially developed epididymis, vas deferens, seminal vesicles, and ventral prostate; decreased weights of testes, epididymis, dorsolateral and ventral prostates, seminal vesicles, and levator anibulbocavernosus muscle; seminiferous tubule degradation; focal interstitial cell hyperplasia; and interstitial cell adenoma. Male pups of the 100 and 500 mg/kg/d groups showed a dose-dependent increase in the number of retained areolas or nipples, and androgen-dependent event. The nipple retention at 100 mg/kg/d represented the LOAEL for Dibutyl Phthalate in this study. The NOAEL in this study is 50 mg/kg/d*	[8]

Table 6. Developmental and reproductive toxicity studies from previous reviews

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	*corn oil*	*8 pregnant Sprague-Dawley rats*	*0 or 500 mg/kg/d*	*Dams were exposed to test material from gestation day 14 to postnatal day 3; at 5 mo of age, male offspring (50 control, 48 Dibutyl Phthalate-exposed) were killed and examined externally and internally for indications of reproductive development; in another experiment of the same report, five pregnant LE rats were given daily oral doses of 500mg/kg on gestation days 16 to 19. Six control rats received corn oil on the same schedule. At 5 mo of age, male offspring were examined.*	*In treated males, there was a significant increase in number of retained nipples ($p < 0.0003$), a decrease in anogenital distance ($p < 0.0001$), and decreases in the weights of ventral prostate, epididymis, cauda epididymis, testes, glans penis, and levator ani-bulbocavernosus ($p < 0.0001$ to 0.03). There was also a non-significant increase in the incidence of hypospadias in treated males.*	[8]
Dibutyl Phthalate	*corn oil*	*5 pregnant LE rats*	*0 or 500 mg/kg/d*	*Dams were exposed to test material from gestation day 16 to 19; at 5 mo of age, male offspring were examined.*	*In the treated males, anogenial distance was reduced ($p < 0.0008$) and number of retained nipples was increased ($p < 0.032$). There were reductions in the weights of seminal vesicle, ventral prostate, and levator ani-bulbocanerosus ($p < 0.03$) of treated males. Hypospadias was not seen in treated rat offspring*	[8]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
EMBRYOTOXIC AND TERATOGENIC EFFECTS						
IN VITRO						
Dibutyl Phthalate and Diethyl Phthalate	DMSO for Dibutyl Phthalate; none for Diethyl Phthalate	*Xenopus laevis* embryos, 20 embryos/dose	0, 1, 3, 5, 7.5, 10, 12.5, or 15 ppm for Dibutyl Phthalate 0, 25, 50, 100, or 200 ppm for Diethyl Phthalate	Effects of Dibutyl Phthalate or Diethyl Phthalate on embryos were analyzed using the 96-h frog embryo teratogenesis assay. Treatments were performed in triplicate.	The mean 96-h LC_{50} obtained from embryos exposed to Dibutyl Phthalate was 12.88 ppm. The teratogenic index for Dibutyl Phthalate was 1.56. The NOEC for malformations was lowest at 5.8 ppm, indicating malformations as the most sensitive endpoint. The mean minimum concentration to inhibit growth was 8.75 (based on 2 of 3 experiments). Embryos from 5 ppm Dibutyl Phthalate exposures had notable edema, loose gut coiling, craniofacial abnormalities, and few reduced eye abnormalities. Embryos exposed to 7.5 or 12.5 ppm concentrations had similar effects, with hemorrhaging as an additional malformation. Severe abnormalities at 15 ppm were observed. A tumor-like growth was observed in one embryo. The mean 96-h LC_{50} obtained from embryos exposed to Diethyl Phthalate was 64.5 ppm. The teratogenic index for Diethyl Phthalate was 1.25. The NOEC for malformation was lowest at 17 ppm, indicating malformations as the most sensitive endpoint for Diethyl Phthalate. The mean minimum concentration to inhibit growth was 41.7 ppm. Embryos exposed to Diethyl Phthalate for 96 h had edema and loose gut coiling at concentrations at 50 ppm and higher, with notable muscular tail kinking, kidney abnormalities, hemorrhaging, craniofacial abnormalities, and a reduced eye abnormality observed at 100 ppm.	[47]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate, > 99% pure	ethanol or DMSO, details not clear	*Xenopus laevis* embryos, 25 - 50 embryos/dose	0, 1, 25, or 200 µM	Embryos were treated 1.5 h after fertilization with control or test material, and were analyzed for abnormalities and mortalities for 2 h intervals for the first 12 h and every 24 h after for up to 96 h. Treatments were performed in duplicate or triplicate	Normal development was observed in the control groups. Many embryos treated with the 3 concentrations of Dibutyl Phthalate had curved body axes in the lateral direction; head, eye, and mouth irregularities; hypertrophied gut and blisters/edema; an overall delayed development, with all embryos in the 200 µm group exhibiting these defects. The mean percent normal development over the span of 96 h decreased as Dibutyl Phthalate concentration and exposure time increased, with the greatest numbers of defects occurring at stage 25 ($p < 0.005$). Many embryos exposed to lower concentrations seemed to self-correct by late tadpole stage 46, as indicted by increased numbers of normal embryos. Dibutyl Phthalate did not result in increased mortality, although the number of normal embryos was still lower than controls at all time points.	[48]
Dibutyl Phthalate, > 99% pure	DMSO	*Xenopus laevis* embryos, 40 embryos/dose	0, 5, 10, 12.5, 15, 17.5, 20, or 30 mg/l	Embryos were treated starting at blastula stage with control or test material. At 24 h intervals, mortalities were determined and after 96 h, surviving embryos (tadpole stage) were counted and fixed for examination. RNA of embryos was also analyzed.	At 20 mg/l, embryonic survival was significantly decreased from 72 h onward, and at 30 mg/l, most of the embryos (67.5%) died before 48 h and all embryos died by 72 h. The 96 h LC_{50} value of Dibutyl Phthalate was calculated to be 18.3 mg/l. Tadpoles that received Dibutyl Phthalate had significantly decreased body length, tail length, and total length at 10, 5, and 10 mg/l, respectively. Treated tadpoles had significantly increased occurrences of abnormal gut coiling, head malformation, and ventral blister at 10 mg/l. The EC_{50} value of Dibutyl Phthalate for malformations by 96 h was calculated to be 7.5 mg/l, although Dibutyl Phthalate did not change the body length parameters of tadpoles *BiP* mRNA levels were significantly increased at 1 mg/l in Dibutyl Phthalate-treated tadpoles, but *atf4* mRNA levels were not significantly changed. Spliced *xbp1* mRNA was significantly increased at 1 mg/l Dibutyl Phthalate. *CHOP* mRNA was not significantly changed up to 20 mg/l Dibutyl Phthalate. *bcl2*, *bax*, *bad*, and *bak* mRNA levels were not significantly changed but the ratio of *bax*/*bcl2* mRNA was increased at 5 mg/l.	[49]
Dibutyl Phthalate, 99% pure	not reported	*Danio rerio* embryos, wild type (AB strain); number not reported	0, 50, or 250 µg/l	Dibutyl Phthalate was dissolved in acetone. Fish embryos were exposed to different concentrations of Dibutyl Phthalate for up to 96 h. Embryos were observed for deaths or developments daily. Teratogenic effects were recorded. Body length along with yolk sac width and height were measured. Lipidomic analysis, immune response, gene expression analysis, and an estrogenic activity test were performed.	No significant effects on survival or hatching of embryos following exposure to Dibutyl Phthalate were observed. Dibutyl Phthalate induced body length decrease, yolk sac abnormalities, and immune responses (up-regulation of immune proteins and genes). At 50 µg/l, Dibutyl Phthalate significantly reduced fatty acid, triglycerides, diacylglycerol, and cholesterol levels. Co-exposure to Dibutyl Phthalate and estrogenic receptor antagonist did not significantly relieve the toxic symptoms compared with exposure to Dibutyl Phthalate alone.	[50]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate	DMSO	*Danio rerio* embryos, 30 per treatment	0.0005 - 0.2 mg/l for Dibutyl; 1.5 - 10 mg/l for Diethyl; 1.5 - 8 mg/l for Dimethyl	The acute developmental toxicity of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate in addition to 3 other phthalate esters on zebrafish embryos; control was DMSO. Fertilized eggs were exposed with 5 ml of the test solution; embryos observed at 12, 24, 48, 72, 96, 120 h post-fertilization. Alcian blue and alizarin red double staining were performed to detect skeletal development of the fish larvae	All phthalates tested induced different developmental abnormalities in the fish larvae, including abnormal movement, decreased heart rate, spinal curvature, and pericardial edema. Bone development was also affected. Malformation rate caused by Dibutyl Phthalate at 0.05 mg/l was 30%, and exposure to higher than that exposure concentration led to death. Dibutyl Phthalate caused the greatest mortality in the fish of the phthalates tested, even at low concentrations; mortality rates varied with the exposure concentrations of the phthalates. The number of dead fish larvae also increased with increased concentrations of Diethyl Phthalate. Dimethyl Phthalate was not correlated with larvae mortality. The inhibitory effect of Dibutyl Phthalate on the movement of zebrafish embryo 24 h post fertilization showed a concentration -dependent effect; Diethyl Phthalate had a weak inhibitory effect at the same interval. Gene expression levels of skeleton-related genes showed the upregulation of runx2b and shha genes after Dibutyl Phthalate exposure	[51]
DERMAL						
Diethyl Phthalate	not reported	groups of 17-20 female Jcl:ICR mice	0, 500, 1600, or 5600 mg/kg/d	Test material was applied to the skin of pregnant mice on gestation days 0-17; no further details available	NOAEL < 500 mg/kg/d in dams; dose-related abnormal behavior (possibly caused by pain) was observed; reduced thymus and spleen weights were observed at all doses and increased adrenal gland weights at 5600 mg/kg/d NOAEL = 1600 mg/kg/d in offspring; when compared to controls, fetal body weight was significantly reduced in the 5600 mg/kg/d group; an increase in the incidence of cervical and lumbar rib variations was also observed; no external, visceral, or skeletal anomalies were observed in the fetuses that were attributed to treatment with Diethyl Phthalate	[6]
ORAL						
Dibutyl Phthalate, 99% pure	canola oil	groups of 7-8 pregnant Wistar rats	0, 5, 50, or 500 mg/kg/d	Developmental toxicity study; dams received Dibutyl Phthalate by gavage from gestation day 13 to postnatal day 21. Body weight gains, organ weights of the dams and litter parameters were measured. Male and female offspring were examined for body weight development, external markers of prenatal androgenization and puberty onset, plasma concentrations of glucose and insulin, insulin tolerance, glucose-stimulated insulin secretion, and the expression of peroxisome proliferator-activated receptor gamma and pancreatic and duodenal homeobox 1 protein.	Dams exposed to Dibutyl Phthalate had no observed effects to body weight gains or organ weights. No significant differences to litter parameters were observed between the control and treated groups. Body weight gains in both male and female offspring were increased significantly ($p < 0.05$) in the 5 and 50 mg/kg dose groups from postnatal day 16 until postnatal day 21; weight variations were not observed at adulthood. Female offspring exposed to 500 mg/kg/d Dibutyl Phthalate had delayed vaginal opening. Male and female rats exposed to 500 mg/kg/d Dibutyl Phthalate had increased fasting glucose levels when compared to controls. Triglyceride levels in female offspring were also increased at this dose level. No effects were observed in the insulin secretory response of male and female offspring exposed to Dibutyl Phthalate	[52]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate; purity not reported	olive oil	Pzh:Sfis outbred mice F_0 and F_1: 18-20 males/dose group mated with 2 females each	0, 500, or 2000 mg/kg bw	A 3-generation reproductive and developmental toxicity study. Male mice were exposed to test material via gavage 3 d/wk for 8 wk; 6 – 7 males from each dose group were killed at 4, 8 and 12 wk after the start of exposure to examine sperm count and quality. At the end of exposure, remaining males mated with 2 unexposed females each; ¾ of females from each dose group were killed 1 d before expected parturition while other females were allowed to deliver and rear litters. Males from the F_1 generation were mated with females from the same group but different litter in order to examine prenatal development of the F_2 generation; remaining F_1 males were killed at the same age to check sperm count and quality. A comet assay was utilized to determine induction of DNA breaks.	Exposure of F_0 males to Dibutyl Phthalate induced skeletal malformations in surviving fetuses, causing significant mortality in postnatal life and a skewing of the sex ratio (F_1 females had higher survival rates); increased frequency of DNA damage in the germ cells was observed in F_1 males; exposure of F_0 males to Dibutyl Phthalate did not affect F_1 male fertility and pregnancy frequency; mean numbers of total live and dead fetuses were not significantly different compared to the control	[53]
Dibutyl Phthalate; > 99% pure	dietary feed	Sprague Dawley rats; 45 - 47 time mated female rats in F_0 generation/ dose group; 50 male and 50 female rats in F_1 generation/dose group	0, 300, 1000, 3000, or 10,000 ppm, equivalent to 16 - 17, 54 - 57, 152 - 169, and 510 - 600 mg/kg bw/d, respectively	2-yr carcinogenicity study (see the Carcinogenicity Studies section for full details); body weights and feed consumption measured (both F_0 and F_1), clinical observations made through study period, and complete necropsies and microscopic examinations performed on all F_1 rats at study end	Maternal mean body weights and body weight gains of the exposed dams during gestation were comparable to the control group; during lactation, minimal differences in mean body weights noted between the high dose group and the control group; feed consumption during gestation significantly ($p < 0.05$; $p < 0.01$) higher only in the 10,000 ppm compared to control group on gestation days 12 - 18, feed consumption during lactation was significantly ($p < 0.05$; $p < 0.01$) increased compared to the control group on days 1 – 4 in all groups except the high dose group In the 10,000 ppm dose group male offspring, high incidence of small or absent organs of the male reproductive tract and undescended testes observed; some gross lesions correlated with microscopic lesions in the testes (germinal epithelium atrophy), epididymis (hypospermia), and prostate and seminal vesicles (decreased secretory fluid) also observed in this dose group. Additional microscopic lesions in the reproductive tract in rats included seminiferous tubule dysgenesis, testicular interstitial cell hyperplasia, testicular edema, and fibrosis and granuloma of the rete testis.	[55]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	dietary feed	groups of 17 male and 17 female Sprague-Dawley rats	1, 4, 10, 30, 100, 1000, or 10,000 ppm	2-generation reproduction toxicity study; 1 ppm dose group served as control. The F_0 generation received test material in feed as adults starting at study day 1 until day before necropsy (weeks 29-32), and were bred to produce F_{1a}, F_{1b}, and F_{1c} offspring. F_{ab} adults were reared and bred to produce F_{2a}, F_{2b}, and F_{2c} offspring. An "Outbreeding Cohabitation" was conducted by mating 10,000 ppm F_1 males with naive females and 10,000 ppm F_1 females with naive males. Body weights, feed consumption, clinical observations, vaginal cytology, reproductive performance, neonatal anogenital distance, pup survival, sexual development, computer-assisted sperm analyses, gross pathology, organ weights, and selected histopathology were performed over the course of the study.	No adverse effects in body weight gains, feed consumption, clinical observations, incidental gross findings, and mortality were observed in any dose levels of Dibutyl Phthalate during any generation of the Outbreeding Cohabitations. Deaths observed during the F_0 and F_1 cohabitations were not treatment-related. No consistent treatment-related effects were observed in litter data from the F_0 and F_1 cohabitations. In the F_0 cohabitation, the mean anogenital distance in the 10,000 ppm F_{1a} male pups was decreased in a statistically significant manner by 15% on postnatal day 1, while the anogenital distance of the 10,000 ppm F_{1b} male pups was not decreased significantly. During F_1 cohabitations, the mean anogenital distance of 10,000 ppm F_{2a}, F_{2b}, and F_{2c} male pups decreased in a statistically significant manner by 13 - 15%. The mean anogenital distance in the Outbreeding Cohabitation of male pups born to 10,000 ppm F_1 dams decreased by a statistically significant 14%. A statistically significant mean delay in preputial separation and testicular descent was observed in 10,000 ppm F_{1b} males when compared to controls. Seminiferous tubular atrophy was noted in 10,000 ppm F_1 males. No other adverse effects were noted in gross pathology, organ weight changes, or microscopic pathology in adult F_0 or F_1 rats. No changes were noted in the sperm endpoints in the F_0 or F_1 males.	[54]
Diethyl Phthalate, 99.8% pure	dietary feed	groups of 24 male and 24 female Crj:CD (SD) IGS rats	0, 600, 3000, or 15,000 ppm	Two-generation reproduction toxicity study performed in accordance with OECD TG 416; males received treated feed for 15 wk and females received treated feed for 17 wk in the F_0 and F_1 generations; F_1 animals were mated at 10 wk of age	NOAEL for general toxicity and reproductive performance in the parental animals is 15,000 ppm (equivalent mean intake = 1016 - 1297 mg/kg/d). No adverse effects observed in clinical findings, body weights, feed consumption, reproductive parameters, or gross or histopathological findings in any treated group of the F_0 and F_1 parental animals. F_0 males in the 15,000 ppm group had increased liver weights and enhanced activities of metabolic enzymes. This group of males also had an increase in the content of CYP3A2, and a decrease in serum testosterone levels was observed in F_0 males of the 3000 and 15,000 ppm groups; however, these effects were not considered adverse since it did not affect the ability to produce offspring. NOAEL for development and growth of pups is 3000 ppm (equivalent mean intake = 222 - 267 mg/kg/d) due to decreased body weight gain at 15,000 ppm (equivalent mean intake = 1150 - 1375 mg/kg/d). Body weight gains before weaning were inhibited in F_1 and F_2 pups and vaginal openings were slightly delayed in F_1 females at 15,000 ppm.	[6,56]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
EFFECTS IN FEMALE REPRODUCTIVE ORGANS						
IN VITRO						
Dibutyl Phthalate and monobutyl phthalate	DMSO	ovarian antral follicles from CD-1 mice, 6 - 12 follicles per treatment group	0.001 - 1000 µg/ml	Mouse ovarian antral follicles were treated with vehicle, Dibutyl Phthalate, or monobutyl phthalate for 24 - 72 h. Follicle diameter, ATP production, q-PCR, and TUNEL were used to measure follicle growth, viability, cell cycle and apoptosis gene expression and cell death-associated DNA fragmentation, respectively.	After 24 h, follicles treated with Dibutyl Phthalate at 1000 µg/ml were significantly smaller than controls, growth inhibition was evident in follicles treated with 500 and 1000 µg/ml after 48 h, and follicles treated with all concentrations of Dibutyl Phthalate > 100 µg/ml after 72 h were significantly reduced compared to the controls ($p \leq 0.05$). Dibutyl Phthalate exposure at ≥10 µg/ml resulted in a significant decrease in the percentage of follicles growing. Cytotoxicity was observed to Dibutyl Phthalate at ≥500 µg/ml. Expression of *Ccnd2* was decreased in follicles treated with Dibutyl Phthalate at 1000 µg/ml at both 48 and 72 h. The expression of the cell cycle arrest gene *Cdkn1a* was significantly increased in follicles treated with the highest concentration of Dibutyl Phthalate at 48 h. *Cdkn2a* expression was increased at 48 h in follicles treated with Dibutyl Phthalate at 0.001 and 10 - 1000 µg/ml, but returned to levels similar to the controls by 72 h. *Bax* and *Bid* expression were increased at 48 h in follicles treated with 1000 µg/ml Dibutyl Phthalate in the absence of *Bcl2* expression changes. *Bax* expression returned to control levels by 72 h, but *Bid* mRNA levels remained increased and included an increase in follicles treated with 100 µg/ml Dibutyl Phthalate. A statistically significant decrease in the expression of *Bcl2* mRNA in follicles treated with 1 µg/ml Dibutyl Phthalate for 72 h. In assessing antral follicle death, a statistically significant difference in DNA fragmentation levels relative to the was observed in follicles treated with 10 and 1000 µg/ml Dibutyl Phthalate. No toxicity to monobutyl phthalate was observed.	[57]
Dibutyl Phthalate	DMSO	oocytes from ICR female mice	0, 200, 500, or 800 µM	Study performed to examine the effects of Dibutyl Phthalate on oocyte maturation. Oocytes were treated with test material obtained from antral follicles of mice after being washed thoroughly with M2 medium. To retrieve prometaphase I oocytes, germinal vesicle oocytes were cultured for 8 h with test material prior to retrieving prometaphase I oocytes, while metaphase II oocytes were obtained after 12 h culture. Additional metaphase II oocytes with first polar body were collected for parthenogenetic activation. Several different staining techniques were performed to assess meiosis spindle morphology, actin cap formation, and apoptosis	In mouse oocytes treated with 400 or 800 µM Dibutyl Phthalate, germinal vesicle breakdown rates and polar body extrusion rates were statistically significantly decreased when compared with the controls. Oocyte pronucleus formation rates in the 500 and 800 µM were also significantly lower than the controls. No significant statistical differences were observed between the 200 µM and the control for these parameters. Metaphase I (MI) spindles in oocytes from the control group displayed a shuttle shape and were slightly translocated to the periphery of the oocyte, while after Dibutyl Phthalate treatment, the typical shuttle shape was destroyed, and spindles showed no poles, multiple poles, or disrupted poles. All Dibutyl Phthalate-treated groups normal spindle morphology rates were significantly lower compared with the control group Additionally, γ-tubulin exhibited abnormal localization in Dibutyl Phthalate-treated groups, and actin cap formation was significantly inhibited. Cortical granules were uniformly located at the plasma membrane of oocytes treated with Dibutyl Phthalate, but were absent in areas close to chromosomes in the controls. Staining indicated that Dibutyl Phthalate treatment caused early apoptosis, but not late apoptosis in the mouse oocyte.	[58]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
			ORAL			
Dibutyl Phthalate	corn oil	6 groups of 50 female ICR mice	0, 100, or 500 mg/kg/d	Study performed to examine the effects of Dibutyl Phthalate on oocyte maturation. Mice received daily oral gavage treatment with test material for 30 or 60 d. At study end, the mice were killed, the ovaries were removed for oocyte collection. The oocytes underwent several different staining techniques to assess meiosis maturation and spindle morphology, actin cap formation, parthenogenetic development, cortical granule-free domains, and apoptosis.	After 30 or 60 d, mice treated with Dibutyl Phthalate had significantly decreased body weights, were less active, and decreased response to stimuli. Loss in hair luster was also observed. Ovarian weight coefficients had a significant decrease in both Dibutyl Phthalate treatment groups after 60 d when compared with controls. TUNEL staining of the treated groups showed clear positive signals in granulosa cell nuclei at both 30-d 60-d in both concentrations tested, while only poor signal was detected in control group. Caspase-3 staining was scarce in granulosa cells of ovarian sections from the control group, but clear staining was observed in the treated groups. After 30 d, germinal vesicle breakdown rates were significantly reduced in both treated groups when compared with the control group; further reduction of rates was detected in the 60 d treatment. Polar body extrusion rates after 30 d in the 500 mg/kg/d group were significantly lower than the controls, but not in the 100 mg/kg/d group. After 60 d, the rates were significantly lower for both treatment groups. Both Dibutyl Phthalate treatments for 30 d significantly reduced oocyte pronucleus formation rates compared with the control group, while 2-cell formation rates did not show any statistical differences from the control. However, pronucleus formation rates after 60-d of treatment with both concentrations were significantly reduced when compared with the control group, and 2-cell formation rates were also significantly reduced when compared with the control. The 30 d treatment groups significantly disrupted spindle morphology and inhibited actin cap formation; the 60 d treatment groups had disruptions and inhibitions at similar rates in both concentrations. Immunofluorescence staining found that all treated oocytes could not form cortical granule-free domains. Early apoptosis, but not late apoptosis, was induced after 30- and 60-d Dibutyl Phthalate treatments.	[58]
Dibutyl Phthalate	tocopherol-stripped corn oil	groups of 16 female CD-1 mice	10 or 100 µg/kg/d or 1000 mg/kg/d	Study to investigate Dibutyl Phthalate exposure on IGF signaling in the ovary and effects on ovarian folliculogenesis; rats received test material daily for 20 consecutive days; animals were subjected to vaginal smears, weighed, and then killed as they reached the proestrus stage; ovaries were dissected from each animal and rid of fat and oviductal tissue; one ovary from each pair was snap-frozen for RNA extraction, while the other was fixed for subsequent histological processing; levels of mRNAs encoding IGF1 and 2 (*Igf1* and *Igf2*), IGF1 receptor (*Igf1r*), and IGF binding proteins 1-6 (*Ifgbp1-6*) were measured in whole ovary homogenates; ovarian follicle counts and immunostaining for phosphorylated IGF1R protein (pIGF1R) were used to evaluate folliculogenesis and IGF1R activation, respectively	Dibutyl Phthalate at 100 µg/kg/d significantly decreased ovarian *Igf1* ($p = 0.028$) and *Igf1r* ($p = 0.048$) mRNA expression without affecting *lgf2* ($p = 0.997$); however, the mRNA levels for all 3 genes did not differ from controls at 10 µg/kg/d or 1000 mg/kg/d. Dibutyl Phthalate did not causes significant deviations in the expression of transcripts encoding IGF binding proteins when compared to the vehicle control; total number of ovarian follicles counted per ovary was reduced in the 100 µg/kg/d group ($p = 0.02$) when compared to controls; however no significant differences were observed in the 10 µg/kg/d or 1000 mg/kg/d dose groups. Significantly low primordial follicle counts observed in the 100 µg/kg/d group ($p=0.006$) and in the 1000 mg/kg/d group ($p=0.02$) compared to controls, also fewer primary follicles were observed in 100 µg/kg/d mice ($p=0.04$). Immunostaining for pIGF1R showed a significantly reduced *lgf1r* mRNA and primordial and primary follicle numbers in 100 µg/kg/d mice, but not in the other doses	[59]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate, 99.6% pure	tocopherol-stripped corn oil	groups of 7 or 8 female CD-1 mice	0, 10, 100, or 1000 µg/kg/d	Females were treated with vehicle or test material for 30 d via oral pipette. At the end of dosing, the mice were killed and ovaries were removed and analyzed for classification and enumeration of ovarian follicles and corpora lutea, RNA extraction, and cDNA synthesis. Gene expression was determined using PCR, and immunostaining was performed for phosphorylated H2AX. Protein was extracted and analyzed with SDS-PAGE and western blotting.	No overt clinical toxicity was observed in the mice. A non-statistically significant increase in body weight gain was observed in the 100 and 1000 µg/kg groups during dosing. No differences in the percentage of time spent in proestrus, estrus, diestrus phases in the treatment groups before or during dosing were noted. Dose-dependent effects on folliculogenesis and gene expression were observed. At 1000 µg/kg, more atretic follicles were observed in the ovaries. Follicle numbers in the 10 and 100 µg/mg doses were comparable to controls. In a dose-dependent manner, Dibutyl Phthalate significantly reduced the expression of genes responsible for homologous recombination (*Atm*, *Braca1*, *Mre11a*, and Rad50), mismatch repair (*Msh3*, *Msh6*), and nucleotide excision repair (*Xpc*, *Pcna*); however, staining for γH2AX was similar between doses. No differential DNA methylation in the *Brca1* promoter was observed, but significantly reduced transcript levels for *Dnmt1* was observed in the ovary.	[60]
Dibutyl Phthalate	corn oil	groups of 8 or 9 pregnant Sprague-Dawley rats	0 or 500 mg/kg bw every second day starting at gestation day 14.5	Dams received test material on gestation days 14.5 to postnatal day 6 via oral gavage. Corn oil was also subcutaneously injected on gestation days 14.5 and 16.5. Pups were culled on gestation days 10, 24, and 90, and surviving pups were weaned on gestation day 21. Anogenital distance was measured on all pups on postnatal days 2 and 10. Vaginal opening was checked daily from postnatal day 29 onwards in female offspring.	Dams and offspring exhibited no adverse effects. Dams in the control and treatment group delivered exclusively live pups, although there was some subsequent perinatal mortality. Female pups showed no effects to anogenital distance. No adverse effects were observed in the vaginal opening when compared to controls.	[61]
Dibutyl Phthalate	corn oil	immature female rats, at least 6 per group, strain not specified	0, 10, or 100 mg/kg	In this uterotrophic assay, rats received Dibutyl Phthalate orally from postnatal day 21 for 3 d. Body weights recorded before treatment, during treatment, and prior to necropsy. Clinical signs and symptoms observed daily. On the 4th day, rats were killed and the uterus was dissected out. Uteri and ovaries were weighed with luminal contents.	No abnormal clinical signs or symptoms were observed in any treated or control animal during study period. Body weight increased significantly in the 10 mg/kg dose group, but was not significantly elevated in the 100 mg/kg dose group. In the 100 mg/kg Dibutyl Phthalate group, the uterine wet weight was significantly decreased, and there were minor variations in the ovary wet weight.	[62]
Dibutyl Phthalate	corn oil	immature female rats, at least 6 per group, strain not specified	0, 10, or 100 mg/kg	In this pubertal onset assay, rats received Dibutyl Phthalate orally from postnatal day 21 for 20 d. Rats examine daily and body weight was recorded before treatment, during treatment, and prior to necropsy. The rats were examined daily for vaginal opening from beginning of postnatal day 21. On the day of vaginal opening, body weight and age was recorded. Vaginal lavage was collected on the day of vaginal opening and estrous cycle was evaluated and stages determined. Rats were killed on postnatal day 42. The uterus, vagina, and ovaries were weighed.	All rats from the treated group and the control group gained body weight during the study period; however, a statistically significant decline in body weight gain was observed at postnatal days 27, 33, and 42, in both treated groups. The weight of uterus and ovary declined significantly and changes in vaginal weight were nonsignificant in both treated groups. However, vaginal opening was not observed in any of the animals in the controls and treated groups until postnatal day 42, except in one animal each in vehicle control and the 100 mg/kg dose group.	[62]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate; > 98% pure	feed	groups of 6-8 pregnant CD(SD)IGS	0, 20, 200, 2000, or 10,000 ppm	Developmental study to assess toxicity of Dibutyl Phthalate exposure from late gestation through lactation; dams received test material on gestation day 15 to postnatal day 21. Body weight and feed intake of all dams were recorded at gestation days 15 and 20 and postnatal days 2, 10 and 21. Methods and results of the male offspring are described in the section below (Effects in Male Reproductive Organs – Oral). On postnatal day 2, the numbers, weights, and anogenital distances of the neonates were recorded, and on postnatal day 3, each litter was culled randomly to 4 females (when possible). Until postnatal day 21, body weights of pups were recorded. Prepubertal necropsy was conducted in groups of 8 females at postnatal day 21 to evaluate weights and histopathology of endocrine-linked organs. All remining female pups were monitored daily for vaginal opening from postnatal day 27. Estrous cyclicity of females was examined by daily microscopic observation of vaginal smears during postnatal weeks 8 - 11 and 17 - 20 for at least 21 d from the first day to the end of each period. Classification was into proestrus, estrus and diestrus, when nucleated epithelial cells, cornified epithelial cells or leukocytes, respectively, were contained. 'Extended diestrus' or 'extended estrus' was concluded when diestrus or estrus continued for at least 4 days. At postnatal weeks 11 and 20, groups of 8 - 10 female offspring were subjected to organ weight measurement and histopathological examination of endocrine-linked organs. Killing was delayed for up to 4 d after the first day of postnatal weeks 11 and 20 until the animal entered the diestrus stage of the estrous cycle.	In the dams, body weight gains were slightly decreased in the 20 and 10,000 ppm during gestation days 15 - 20, but no effects were observed during the lactation period from postnatal day 2 to 21. No effects were observed in feed consumption during gestation or lactation. Female offspring had a slight, non-significant delay in the onset of puberty at 10,000 ppm. Mammary gland changes at low incidence were observed at postnatal day 21 starting at 20 ppm. Also at this time point, population changes of pituitary hormone-immunoreactive cells were observed at 10,000 ppm with a similar pattern of increase in the percentages of luteinizing hormone-positive and decrease in follicle-stimulating hormone and prolactin producing cells, with effects also being evident on follicle-stimulating hormone from 200 ppm and luteinizing hormone from 2000 ppm. During postnatal weeks 8 - 11, marginal increase of the number of cases with extended diestrus was found at 10,000 ppm. At adult stage necropsy, relative pituitary weights were decreased after 10,000 ppm at postnatal week 11, and from 200 ppm at postnatal week 20. The proportion of follicle-stimulating hormone-positive cells in the pituitaries at postnatal week 11 was increased at 10,000 ppm.	[63]
mixture of phthalates including 15% Dibutyl Phthalate and 35% Diethyl Phthalate	tocopherol-stripped corn oil	groups of 12 pregnant CD-1 mice in F_0 generation	0, 20 µg/kg/d, 200 µg/kg/d, 200 mg/kg/d, or 500 mg/kg/d	Dams orally dosed with control or phthalate mixture daily from gestation day 10 to birth. Adult F_1 females born to these dams were used to generate the F_2 generation and adult F_2 females born to F_1 females were used to generate the F_3 generation. Sera, ovaries, uteri, and livers were collected and organ weights, body weights, and anogenital distances were measured from F_2 and F_3 females on postnatal days 1, 4, 8, 21, 60 and at 13 mo of age. Fertility tests were also performed in the F_2 and F_3 generations.	In the F_2 generations, prenatal exposure to the phthalate mixture containing Dibutyl Phthalate and Diethyl Phthalate increased uterine weight, anogenital distance, and body weight. Cystic ovaries and breeding and pregnancy complications were observed. In the F_3 generation, uterine weight was increased, anogenital distance was decreased, and fertility complications were observed.	[64]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	corn oil	Pregnant CD1 mice; number/group not reported for initial fetal-ovary analyses; 5 - 6 F1 females/group for breeding	0, 10, or 50 mg/kg bw/d	Dams received test material beginning on gestation day 14.5 for 3 d or until scheduled killing. Meiotic progression, DNA-damage response, homologous recombination, protein crotonylation, and DNA-repair pathway selection were evaluated in fetal ovaries. F1 females were mated monthly with fertile males for 7 mo; F2 folliculogenesis, vaginal opening, and behavior were evaluated.	At 10 mg/kg bw/d, Dibutyl Phthalate delayed meiotic prophase I progression and impaired homologous recombination; zygotene oocytes increased (55.56% vs. 39.81% in controls, $p < 0.05$), whereas pachytene oocytes decreased (43.79% vs. 58.89%, $p < 0.05$), and DMC1/RAD51 colocalization and RAD51/MLH1 foci were reduced. Despite impaired DNA damage-response signaling, programmed double-strand breaks were repaired. F1 litter size and F2 sex ratio not significantly altered, but F1 maternal-care behaviors were affected, including reduced pup licking on postnatal days 6 and 8 and delayed pup retrieval on postnatal day 3. F2 offspring had increased birth weights, and F2 females exhibited accelerated follicular development, earlier vaginal opening (median vaginal-opening ages were postnatal days 24 and 23 at 10 and 50 mg/kg bw/d, respectively, versus day 28 in controls. Anxiety-like behaviors were also observed in F2 offspring.	[65]
Dibutyl Phthalate	Dibutyl Phthalate	Groups of 4 pregnant CD1 mice; F1 breeding assessment used 6 females/group derived from 3 F0 dams/group	0, 0.4, 2, 10, or 50 mg/kg bw/d	Dams received test material orally on gestation days 16.5 - 18.5. Offspring ovaries were collected on postnatal day 1 for follicle evaluation. F1 females from the 0-, 10-, and 50-mg/kg/d groups were repeatedly mated for at least 5 litters. Supporting fetal-ovary cultures used 2, 10, or 50 µM Dibutyl Phthalate for 4 d, with mechanistic rescue experiments.	The proportion of primordial follicles was significantly reduced and the proportion of germ-cell cysts was significantly increased at 2, 10, and 50 mg/kg bw/d, indicating impaired germ-cell cyst breakdown and primordial follicle assembly. No significant effect on primordial folliculogenesis was observed at 0.4 mg/kg bw/d. F1 females exposed only in utero had a significantly increased time to mating ($p < 0.05$), declining fertility indices over successive litters, and significantly fewer pups. At 50 mg/kg bw/d, the gestational index was 33% for the second litter and 0% for the fifth litter; some females were reported to become sterile. Dibutyl Phthalate at 10 and 50 µM, but not 2 µM, significantly reduced the proportion of primordial follicles and increased the proportion of germ-cell cysts in fetal ovary cultures. At 10 µM, Dibutyl Phthalate increased oxidative-stress and autophagy markers and reduced NICD2 and downstream NOTCH2-signaling proteins. Autophagy inhibition with 0.1 mM 3-methyladenine, NICD2 overexpression, or treatment with 1 µM melatonin partially restored primordial folliculogenesis.	[66]
EFFECTS IN MALE REPRODUCTIVE ORGANS						
IN VITRO						
Dibutyl Phthalate	not reported	TM3 and TM4 mouse cells	0, 5, 10, 50 or 100 mg/l	An in vitro mechanistic study exposing cells to test material for 24-h	Dibutyl Phthalate exposure produced dose-dependent evidence of ferroptosis. Dibutyl Phthalate treatment decreased cellular GSH and mitochondrial membrane potential, and increased MDA, ROS, GSSG, and Fe^{2+} levels. Morphological evaluation by transmission electron microscopy revealed mitochondrial shrinkage and cristae loss. Real-time PCR and Western blot analyses showed that Dibutyl Phthalate selectively upregulated PRDX6, a negative regulator of ferroptosis, while GPX4 remained unchanged. Further, Dibutyl Phthalate upregulated SP1 expression, which can directly bind to the PRDX6 promoter and transcriptionally activate its expression.	[67]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	not reported	rat testicular explants	10^{-6}, 10^{-5}, or 10^{-4} M	Explants were exposed to test material for 24 h to evaluate the effects on juxtacrine communication through Notch signaling pathway	Real-time PCR and Western blotting showed significant upregulation of Notch1, Dll4, and Hey1 at both mRNA and protein levels following Dibutyl Phthalate exposure ($p < 0.05$; $p < 0.01$; $p < 0.001$), while Hes1 expression remained unchanged. Dibutyl Phthalate altered immunoexpression of activated NOTCH1, DLL4, HEY1 and HES5 both in seminiferous epithelium and interstitial tissue, with differential effects across cell types.	[68]
Dibutyl Phthalate	DMSO	Leydig cells isolated from male Sprague-Dawley rats	50 mg/l	Effects of Dibutyl Phthalate on Leydig cells and its mechanism related to gap junction investigated; cells were cultured for 24 h, Dibutyl Phthalate, Dibutyl Phthalate + 10 µM prostaglandin E2, or 40 µM flutamide; radioimmunoassay, semi-quantitative RT-PCR, immunofluorescence, and Western blot were utilized to determine the expression of testosterone and Connexin 43 (Cx43) in the Leydig cells	Expression of testosterone was significantly decreased ($p < 0.05$) in the Dibutyl Phthalate, Dibutyl Phthalate + prostaglandin E2, and flutamide groups. A significant decrease ($p < 0.05$) of Cx43 was observed in the Dibutyl Phthalate and flutamide group. Cx43 was up-regulated with the administration of prostaglandin E2, but there was no significant change in testosterone. Testosterone was down-regulated with a significant decrease of Cx43 in the flutamide group.	[69]
Dibutyl Phthalate, 99.9% pure	not reported	human sperm from 12 healthy volunteers	13.47, 67.35, or 134.7 µg/ml	Healthy mature spermatozoa were seeded in 96-well plate in complete Minimum Essential Medium (MEM) culture medium supplemented with Dibutyl Phthalate. The cells were incubated and analyzed for motility, and percent cell viability was assessed between 30 min and 96 h.	A concentration- and duration-dependent decrease in motility was noted. The decline varied from 20%, 33%, 66%, with respect to control, after 18 h exposure to Dibutyl Phthalate at the low, medium, and high concentrations, respectively.	[70]
Dibutyl Phthalate, > 99% pure and monobutyl phthalate	human tubal fluid medium containing 1% DMSO	human sperm	2 nM to 6 µM for Dibutyl; 1 nM to 3µM for monobutyl	Effects on human sperm function tested in vitro with Dibutyl Phthalate and monobutyl phthalate; sperm treated with Dibutyl Phthalate, monobutyl phthalate, or a mixture of both (2 nM Dibutyl Phthalate + 1 nM monobutyl phthalate to 6 µM Dibutyl Phthalate + 3 µM monobutyl phthalate) for 1 to 4 h; controls were the medium without the phthalates	Results showed that only Dibutyl Phthalate at 6 µM, 3 µM monobutyl phthalate, and the mixture had adverse effects on sperm motility, penetration ability, and capacitation. Additionally, human sperm tyrosine phosphorylation was suppressed at these doses.	[71]
Dibutyl Phthalate, 99% pure; monobutyl phthalate	DMSO (0.027%)	Fetal testis explants from Wistar rat fetuses (gestation day 19.5) and human fetuses (15 - 20 wk gestation)	10^{-6} - 10^{-3} M; cultured for up to 48 h	Rat and human explants were cultured under basal conditions or with human chorionic gonadotropin or 22R-hydroxycholesterol. Testosterone production was measured. Leydig-cell aggregation was quantified in rat explants; human explants were examined histologically	Dibutyl Phthalate at concentrations up to 10^{-3} did not affect basal or stimulated testosterone production in rat fetal testis explants. Monobutyl phthalate at 10^{-3} M significantly decreased human chorionic gonadotropin-stimulated testosterone production ($p < 0.05$) but did not affect basal or 22R-hydroxycholesterol-stimulated testosterone production. This concentration also produced a significant shift from small toward medium/large Leydig-cell clusters ($p < 0.05$), indicating minor Leydig-cell aggregation. In human fetal testis explants, Dibutyl Phthalate or monobutyl phthalate at 10^{-3} M did not significantly affect basal or stimulated testosterone production. Leydig-cell aggregation could not be quantified because Leydig cells were diffusely distributed in human tissue. Central necrosis occurred in most human explants cultured for 48 h.	[90]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
			ORAL			
Dibutyl Phthalate; 99.5% pure	corn oil and Tween-80	groups of 20 female Sprague-Dawley rats	0, 50, 250, or 500 mg/kg bw/d	Pregnant rats received test material via gavage on gestation day 1 through postnatal day 21. Pregnant rats were weighed and observed daily for clinical signs of toxicity. The developmental condition of F_1 rats and reproductive system of mature F_1 male rats were assessed (groups of 20 males per dose group up to postnatal day 70). Dams and female pups were killed on postnatal day 21.	NOAEL = 50 mg/kg bw/d for developmental toxicity. No adverse effects were observed in the dams. At doses of 250 mg/kg and higher, reduced birth weight, body weight gain, number of live pups per litter, anogenital distance (males), epididymis weight, and sperm count and motility were observed. Significantly reduced prostate weight, absent or underdeveloped epididymis, undescended testes, and testicular atrophy were also observed in mature F_1 males at 250 mg/kg and higher.	[72]
Dibutyl Phthalate	corn oil	groups of 5 - 10 pregnant Wistar rats	0 or 500 mg/kg bw/d	Dams rats received Dibutyl Phthalate via gavage on gestation days 13 - 21. Male offspring of both the control and test material groups were tested for fertility in adulthood by mating with a female of proven fertility. Cell-specific immunohistochemistry and confocal microscopy were used to track development of Sertoli, Leydig, germ, and peritubular myoid cells from male fetal life to adulthood. Adult testes of the male offspring of the control and test material groups were examined histologically. Plasma and testicular testosterone were analyzed.	No treatment-related effects were observed in the maternal animals. A high rate (> 60%) of cryptorchidism (mainly unilateral), infertility, hypospadias, and testes abnormalities observed. In scrotal and cryptorchid testes of Dibutyl Phthalate-exposed males, areas of focal dysgenesis were found that contained Sertoli and Leydig cells, and gonocytes and partially formed testicular cords; these dysgenetic areas were associated with Leydig cell hyperplasia at all ages. Suppression (~90%) of testicular testosterone levels on gestation day 19 in Dibutyl Phthalate-exposed males, coincident with delayed peritubular myoid cell differentiation, may have contributed to the dysgenesis. Double immunohistochemistry revealed immature Sertoli cells in dysgenetic areas. The Dibutyl Phthalate-exposed males also exhibited Sertoli cell-only (SCO) tubules, sporadically in scrotal and predominantly in cryptorchid, testes, or foci of SCO within normal tubules in scrotal testes. In all SCO areas the Sertoli cells were immature. Intratubular Leydig cells were evident in the exposed animals and, where these occurred, Sertoli cells were immature and spermatogenesis was absent. Abnormal Sertoli cell ± gonocyte interaction was evident at gestation day 19 in the test material-exposed rats coincident with appearance of multinucleated gonocytes, although these disappeared by postnatal day 10 during widespread loss of germ cells.	[73]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	corn oil	groups of pregnant Crl:CD(SD)Br rats	0 or 500 mg/kg/d	Dams received test material via gavage on gestation days 12 -21. The dams were examined at dosing and several h post-dosing for clinical signs of toxicity. Body weights were recorded daily. Male fetuses or pups were necropsied on gestation days 16, 17, 18, 19, 20, and 21 and on postnatal days 3, 7, 16, 21, 45, and 70. Fetal and pup weights were recorded at necropsy. Anogenital distance and number of areolae were recorded on postnatal days 1 and 13. The reproductive tracts of all male fetuses were removed using a dissecting microscope, and the testes and epididymides were examined. In male pups killed on postnatal days 3, 7, 16, and 21, the testes, epididymides, and vasa deferentia were removed and analyzed. Kidneys and livers were also analyzed in the pups from postnatal days 16 and 21. Pups from postnatal days 45 and 70 had the testes, epididymides, vasa deferentia, prostates, seminal vesicles, levator ani and bulbocavernosus muscles, liver, and kidneys dissected for analysis.	No maternal toxicity was observed, and no decreases in litter size, pup survival, or body weights of offspring were observed. Examination of the phthalate-exposed males revealed gross or histologic lesions at all the time points chosen. A statistically significant decrease (14%) in anogenital distance was observed on postnatal day 1 and a significant increase in the number of retained areolae in the exposed pups was observed on postnatal day 13. In the fetal testes, large aggregates of Leydig cells, multinucleated gonocytes, and increased numbers of gonocytes were first detected on gestation day 17 and increased in incidence to 100% by gestation day 20 and 21. These lesions resolved during the early postnatal period, while decreased numbers of spermatocytes were noted on postnatal day 16 and 21. On postnatal day 45, there was mild degeneration of the seminiferous epithelium, which progressed to severe seminiferous epithelial degeneration on postnatal day 70 This degeneration was concurrent with ipsilateral malformed epididymides, which caused obstruction of testicular fluid flow and secondary pressure atrophy in the seminiferous tubules. In the fetus, the epididymal lesion was observed as decreased coiling of the epididymal duct. The decreased coiling progressed into the early postnatal period and adulthood, at which time malformed epididymides were apparent.	[74]
Dibutyl Phthalate, 99% pure	corn oil	groups of female Wistar rats; numbers per group not clearly reported	0, 4, 20, 100, or 500 mg/kg	Pregnant rats received Dibutyl Phthalate via gavage on gestation days 13.5 to either 20.5 or 21.5. Male fetuses were analyzed for testis dysgenesis. Fetal testis weight and testicular testosterone levels were evaluated. A portion of the male offspring were allowed to live until 90 d of age for mating, following which the males were killed and testes were also examined	A significant decrease in testis weight was observed in offspring in the 500 mg/kg dose group at gestation day 21.5 and in adulthood. Animals exposed to the other dose levels did not have any significant change in testis weight at either gestation day 21.5 or adulthood. Testicular testosterone levels in fetal animals were significantly decreased in the 100 and 500 mg/kg dose groups when compared to controls. No significant effects of Dibutyl Phthalate treatment were observed on fetal body weight or on litter size. Fetal males of the 100 and 500 mg/kg dose groups had a significant increase in the occurrence of multinucleated gonocytes and changes in Leydig cell distribution compared to controls. A significant decrease in total Leydig cell cluster number per testis section was also observed in the 100 and 500 mg/kg dose groups. In the adult male offspring exposed to 500 mg/kg Dibutyl Phthalate, a statistically significant 75% infertility rate was observed. The infertility rates for the 100 and 20 mg/kg groups were 33 and 14%, respectively, and were not statistically significant. The 500 mg/kg doe group also had a significantly elevated incidence of cryptorchidism compared with controls. Sertoli cell-only tubules were present in all cryptorchid testes in the 100 and 500 mg/kg dose groups, and 7 out of 11 cryptorchid testes in the 500 mg/kg group had one or more focal dysgenic areas. These effects were not observed in controls.	[75]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	corn oil	groups of 10 female Wistar rats	0 or 100 mg/kg bw/d	Pregnant rats received Dibutyl Phthalate via gavage on gestation days 12 to postnatal day 21. Five dams of the treatment and control groups were killed on gestation day 20 and testes from male fetuses from these dams were collected for histopathology. The male offspring from the remaining 5 dams of each group were weighed on postnatal day 1, and anogenital distance was measured on postnatal day 4. Remaining rats were killed at postnatal day 90. Blood was collected for hormone assays. The weights of the right testis, epididymis, vas deferens, ventral prostate, and seminal vesicle were recorded. The epididymides and testes were utilized for testicular and serum testosterone, assays, histopathological analysis, immunohistochemistry, proliferation index determination, androgen report analysis, and sperm analysis.	In the male offspring of the treated dams, Leydig cell clusters, the presence of multinucleated germinative cells, and an increased interstitial component were also observed. Anogenital distance was decreased, but this was statistically insignificant. Testosterone levels and reproductive organ weights were similar between the treated and control male offspring. Treatment with Dibutyl Phthalate did not markedly affect relative proportions of epithelial, stromal, or luminal compartments in the epididymis; sperm counts in the testis and epididymis; sperm transit time; or sperm morphology and motility in the adult male offspring The androgen receptor and aquaporin 9 immunoreactivities and proliferation index were similar between the treatment group and control.	[76]
Dibutyl Phthalate, 99% pure	corn oil and feed	groups of 3 pregnant Sprague-Dawley rats	500 mg/kg in corn oil and 6000 ppm in feed	Dams received test material via gavage on gestation days 12 to 21; after delivery, dams received test material in diet; after weaning, 6 male offspring (F_1; no more than 2 males/litter) were fed test material in diet until postnatal day 112; control group received corn oil and basal diet. F_1 body weights and feed consumption assessed every other week until study end; on postnatal day 4, anogenital distance was measured and normalized (each animal's measurement was divided by the cube root of body weight), and all F_1 animals were killed on postnatal day 112. At necropsy, tested were removed, weighted and underwent histological evaluation	On postnatal day 4, there were no statistically significant differences in the median body weights between groups of the male pups. The normalized anogenital distance of the Dibutyl Phthalate group pups was significantly decreased ($p < 0.05$) by 10% compared to the control animals. At postnatal day 112 Dibutyl Phthalate exposure had no effect on body weights when compared to the control. The absolute and relative testicular weights of the Dibutyl Phthalate group were lower than those of the control, but the decrease was not statistically significant. On postnatal day 112, testes from control animals showed intact tubules with normal spermatogenesis and mature sperm in the lumen. Dibutyl Phthalate-treated rats had mostly normal-appearing tubules although some Sertoli cell-only-type seminiferous tubules also appeared. Testicular impairment in Dibutyl Phthalate-treated animals resulted in a score of 1.5, significantly ($p < 0.05$) higher than the control animals.	[77]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	corn oil	groups of 3 pregnant Sprague-Dawley rats	500 mg/kg/d	Dams received test material by oral gavage on gestation days 12 - 16 through 20. Dams were sacrificed on gestation days 17 - 21, and fetuses removed by Caesarean section. Additional pups from dams treated gestation days 12 - 21 were killed on postnatal days 1, 2, or 5. Male fetuses were identified by internal sex organ inspection. At gestation days 17 - 19, both testes were left in situ; at gestation days 20 - 21 and postnatal days 1 - 5, the left testis with epididymis was removed for analysis and the right left in situ.	Dibutyl Phthalate exposure produced abnormal morphology of fetal Sertoli cells, including retraction of cytoplasmic processes, convoluted apical membranes, and collapse of the vimentin cytoskeleton. Histopathological examination revealed a decreased number of seminiferous tubule cross-sections per testis, increased interstitial cell aggregates, and a significantly greater incidence of MNG compared to controls, with MNG containing higher numbers of nuclei. The MNG were not apoptotic (TUNEL-negative) but exhibited abnormal mitoses by postnatal day 5. Sertoli–gonocyte contacts were impaired, as shown by loss of cadherin-marked processes, and gonocytes clustered abnormally. While Sertoli cell morphology appeared restored after birth, MNG persisted postnatally and migrated to the basal lamina. Neural cell adhesion molecule expression remained intact in gonocytes, including MNG. Dibutyl Phthalate exposure also reduced fetal testicular testosterone levels, consistent with impaired Leydig cell function.	[78]
Dibutyl Phthalate	corn oil	groups of 8 or 9 pregnant Sprague-Dawley rats	0 or 500 mg/kg bw every second day starting at gestation day 14.5	Dams received test material on gestation days 14.5 to postnatal day 6 via oral gavage. Corn oil was also subcutaneously injected on gestation days 14.5 and 16.5. Pups were culled on gestation days 10, 24, and 90, and surviving pups were weaned on gestation day 21. Anogenital distance was measured on all pups on postnatal days 2 and 10. Trunk blood samples were collected on postnatal days 10, 24, and 90 and tail vein blood was collected on postnatal days 30, 35, 40, 45, and 55. Testosterone, insulin-like 3 (INSL3), and luteinizing hormone levels were measured. Testes were collected from the male offspring and underwent RNA analysis and Leydig cell counting.	Dams and offspring exhibited no adverse effects. Dams in the control and treatment group delivered exclusively live pups, although there was some subsequent perinatal mortality and testicular dysgenesis. At postnatal day 10, Dibutyl Phthalate-treated male pups had a reduced anogenital distance when compared to the controls. Male offspring from the Dibutyl Phthalate treatment group had relatively small testes at postnatal days 10 and 24, though larger testes at postnatal day 90. When compared to controls, there were no differences in Leydig cell counts or in testosterone or luteinizing hormone levels. Analysis of circulating INSL3 showed that up to postnatal day 40, values tended to be higher in the Dibutyl Phthalate-treated groups compared to controls. For the *Star*, *Hsd11b1,* and *Hsd17b3* gene products, there was an effect of Dibutyl Phthalate treatment at postnatal day 10, and a trend also at this time for the side-chain cleavage enzyme gene, *Cyp11a1*. A trend was also evident for Dibutyl Phthalate treatment for *Insl3* gene transcripts at postnatal day 24,but no effects were observed for any transcript at postnatal day 90.	[61]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate, 99.8% pure	corn oil	groups of 4 pregnant Sprague-Dawley rats	0, 10, 30, 50, or 100 mg/kg/d	Pregnant rats received test material via gavage on gestation days 12 - 21. Offspring were weighed and sexed at birth and litters reduced to 4 males and 4 females per dam, with a mother not given further treatments after gestation day 21. Pups were removed from dams after weaning at postnatal day 21. All animals were weighed at birth, and at weeks 5, 7, 9, 14, and 17 of age. At each time point, 1 male from each dose group was killed, and tested were removed for analysis.	Male pups all dose group had similar body weights with no apparent differences in litter size or sex ratio compared to that of the control group. At postnatal weeks 5, 7, 9, 14, and 17, testicular weights for the 10, 30, and 50 mg/kg/d dose groups were similar to that for the control, but the 100 mg/kg/d dose group had a statistically significant decrease in testicular weight after 9 wk of age. The number of Leydig cells in the 100 mg/kg/d dose group was significantly higher than that of the control at 9, 14, and 17 wk; all other dose groups at all ages were similar to that of the control. Compared to the control, distinctive morphological changes were observed in the 100 mg/kg/d dose group. At 5 - 7 wk of age, the 100 mg/kg dose group had abundant smooth endoplasmic reticulum with a complicated arrangement of non-dilated cisternae and little stripped rough endoplasmic reticulum. The amount of smooth endoplasmic reticulum in Leydig cells was significantly decreased at 9 - 14 wk of age, and smooth endoplasmic reticulum were not observed at 17 wk of age. Mitochondrial swelling was not encountered, and the Golgi apparatus displayed no observable change. From 5 - 17 wk of age, the 10, 30, and 50 mg/kg/d dose groups had testosterone and luteinizing hormone levels similar to the control, while the 100 mg/kg/d dose group had significantly lower testosterone levels. The luteinizing hormone level of the 100 mg/kg/d dose group was significantly lower at 5 and 7 wk of age and significantly higher at 9 - 17 wk of age compared to that of the control.	[80]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate; > 98% pure	feed	groups of 6-8 pregnant CD(SD)IGS rats	0, 20, 200, 2000, or 10,000 ppm	Developmental study to assess toxicity of Dibutyl Phthalate exposure from late gestation through lactation; dams received test material on gestation day 15 to postnatal day 21. Body weight and feed intake of all dams were recorded at gestation days 15 and 20 and postnatal days 2, 10 and 21. Methods and results of the female offspring are described in the section above (Effects in Female Reproductive Organs – Oral). On postnatal day 2, the numbers, weights, and anogenital distances of the neonates were recorded, and on postnatal day 3, each litter was culled randomly to 4 males (when possible). On postnatal day 14, male pups were inspected for presence and numbers of nipples/ areolae. Until postnatal day 21, body weights of pups were recorded. Prepubertal necropsy was conducted in groups of 8 males at postnatal day 21 to evaluate weights and histopathology of endocrine-linked organs. All male pups were examined for preputial separation from postnatal day 35 until each animal acquired this developmental landmark. At postnatal weeks 11 and 20, groups of 8 - 10 male offspring were subjected to organ weight measurement and histopathological examination of endocrine-linked organs. Male offspring were killed on the first day of each time point.	Male offspring from the 10,000 ppm dose group had a decreased neonatal anogenital distance and retention of nipples at postnatal day 14. At postnatal day 21, reduction of testicular spermatocyte development was evident from 20 ppm, as well as mammary gland changes at low incidence. At this time point, population changes of pituitary hormone-immunoreactive cells were observed at 10,000 ppm with a similar pattern of increase in the percentages of luteinizing hormone-positive and decrease in follicle-stimulating hormone and prolactin producing cells. At adult stage necropsy, testicular lesions appeared to be very faint in most cases, but degeneration and atrophy of mammary gland alveoli were observed in males from 20 ppm. Although without clear monotonic dose-dependence, relative pituitary weights were increased with the intermediate doses in males at postnatal week 11. The proportion of follicle-stimulating hormone-positive cells in the pituitaries at postnatal week 11 was increased at 10,000 ppm.	[63]
Dibutyl Phthalate	corn oil	groups of 10 pregnant Wistar rats	0 or 100 mg/kg/d	Developmental study to assess effects of Dibutyl Phthalate exposure on the prostate of male offspring; dams received test material from gestation day 12 until postnatal day 21. On postnatal day 90, male offspring were weighed and killed; blood was collected for hormone assays and the prostate and testes were removed for analyses	Body weights at birth and at postnatal day 90, as well as the absolute and relative weights of the ventral prostate, did not differ significantly between the control and the treated group. Some animals in the treated group had collagen fibrils accumulated adjacent to the epithelium. Disseminated inflammatory infiltrate in the stroma, associated or not with epithelial dysplasia and prostatic intraepithelial neoplasia, was observed in the Dibutyl Phthalate-treated group. Serum and testicular testosterone levels were comparable between groups. In the treated group, the relative proportions and stromal compartments were increased, while the luminal compartment was decreased. Increases in androgen receptor expression, proliferation index and metalloproteinase 9 activity were noted in the treated group.	[82]
Dibutyl Phthalate and Diethyl Phthalate, each 99% pure	corn oil	groups of female Sprague-Dawley rats; 4 rats/dose group for Dibutyl Phthalate and 4-5 rats/dose group for Diethyl Phthalate (control had 9)	0, 33, 50, 100, 300, or 600 mg/kg/d for Dibutyl Phthalate; 0, 100, 300, 600, or 900 mg/kg/d for Diethyl Phthalate	Pregnant rats received test materials via gavage on gestation days 8-18 and killed after the last dosing; uteri from dams were removed and number of live and dead fetuses and resorptions were counted; male fetuses had testes removed for analysis of testosterone production	No adverse effects were observed in dams exposed to Dibutyl Phthalate. Dibutyl Phthalate did not significantly increase the rat of fetal death at any dose level. Total resorptions and fetal mortality were significantly increased ($p < 0.05$) in the 600 mg/kg/d Diethyl Phthalate group, but not in the 900 mg/kg/d dose group. Fetal testicular testosterone production was significantly reduced ($p < 0.001$) at doses of 300 mg/kg/d or higher for Dibutyl Phthalate; however, Diethyl Phthalate did not affect testosterone productions at any dose level	[81]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
A mixture of 5 phthalates, including Dibutyl Phthalate (99% pure)	corn oil	groups of 4 - 8 female Sprague-Dawley rats	0, 5, 10, 20, 40, 60, 80, or 100%; 300 mg/kg/d Dibutyl Phthalate at the top dose; total mixture contained 1300 mg of 5 phthalates (dose ratio was 3:3:3:3:1, with 1 being dipentyl phthalate)	Pregnant rats were dosed via gavage on gestation days 8 – 18; rats were killed after the last dosing; uteri from dams were removed and number of live and dead fetuses and resorptions were counted; male fetuses had testes removed for analysis of testosterone production.	The phthalate mixture containing Dibutyl Phthalate significantly reduced maternal body weight gain at 40% of the top dose (containing 120 mg/kg/d Dibutyl Phthalate) and greater; no other adverse effects were noted in the dams. A significant increase in fetal mortality was observed at 40% of top dose and above. Testosterone production was reduced in a dose-additive manner starting at 20% of top dose (containing 60 mg/kg/d Dibutyl Phthalate) and above. Phthalate-induced underdevelopment of the testes was observed at 100% of the top dose.	[81]
Dibutyl Phthalate	corn oil	groups of 20 male Sprague-Dawley rats	0, 100, 250, or 500 mg/kg/d	Rats received test material via gavage for 21 d. The rats were killed on day 24 and the testis and epididymis were removed for analysis. HPLC was used to detect Dibutyl Phthalate in the testis and expression of expression of H3 histone was measured with immunohistochemistry and immunofluorescence. Morphological changes were performed with optical microscope or transmission electron microscope (TEM). Semen quality was also assessed.	Residuary Dibutyl Phthalate in the testes was increased in a dose-dependent manner, and was significantly increased in the dose groups when compared to the control ($p < 0.05$). When compared to the control group, the microscopic structure of seminiferous tubule, spermatogonial cell, or spermatic cell in Dibutyl Phthalate groups was dramatically changed. In HE staining analysis, the separation between basement membrane of seminiferous tubule and Leydig was observed. In TEM analysis, karyopyknosis, mitochondrial pyknosis, enlarged vacuoles, karyorrhexis, mitochondrial decay and chromatin dissolution were found in spermatogonial cell or spermatic cell. The average optical density, positive negative area, and fluorescent intensity of H3 histone expression were significantly different between control group and each Dibutyl Phthalate group ($p < 0.05$). The quality of sperm in epididymis in the Dibutyl Phthalate groups was significantly reduced when comparing to the control ($p < 0.05$).	[79]
Dibutyl Phthalate	corn oil	groups of 6 male albino rats	0, 200, 400, or 600 mg/kg/d	Adult rats received Dibutyl Phthalate via gavage for 15 d. Blood samples were collected 24 h after the last dose. The rats were killed and the testes were excised and weighed. The cauda epididymides were used for analysis of sperm count and motility assay. One testis from each group was used for histopathological exam. Remaining testes were homogenized for enzyme and other biochemical assays.	Testicular weight was significantly decreased at each dose tested when compared to control group. Sperm count and motility were significantly decreased in a dose-dependent manner. Serum follicle-stimulating hormone, testosterone levels, and testicular lactate dehydrogenase activity were significantly decreased at all doses tested. Serum total antioxidant capacity and the activities of testicular antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione reductase were significantly decreased at all doses tested when compared to control group. Testes of the 200 mg/kg dose group showed degeneration with absence of spermatogenesis in some seminiferous. In the 400 mg/kg dose group, degeneration was also observed with absence of spermatogenesis and sperms from most of the seminiferous tubules. Necrosis of some of the seminiferous tubules was observed in the 600 mg/kg dose group.	[84]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate	corn oil	groups of 10 male Sprague-Dawley rats	250 mg/kg	Rats were exposed to test material, BaP (5 mg/kg), or both substances via gavage for 4, 8, or 12 wk; animals were observed for toxic effects during exposure; at the end of the dosing period, blood was collected and the testes and epididymides were removed and weighed and studied for histopathology and sperm evaluation.	No clinical signs of toxicity were observed during the experiment; feed consumption levels were similar between treatment and vehicle groups; weekly body weights increased with exposure time, but there were no significant changes between groups. No adverse effects were observed in the relative weights of the testes and epididymides. Sperm count increased significantly ($p<0.05$) in the Dibutyl Phthalate and combined treatment groups at 4 wk, but were at control levels at 8 and 12 wk. Abnormal sperm ratio following an ascending trend in Dibutyl Phthalate treated rats, but this was not significantly different from the control. A significant increase ($p< 0.05$) was observed in serum T level from Dibutyl Phthalate exposure for 12 wk; combined exposure produced lower serum T levels than the Dibutyl Phthalate exposure. Vacuolization of Sertoli cells was observed in Dibutyl Phthalate-exposed rats after 12 wk. Combined exposure induced significant decreases in SOD and GSH-Px activities in the testis at 8 wk compared with control ($p < 0.05$). CAT activities significantly decreased following the Dibutyl Phthalate exposure after 8 wk ($p < 0.05$). Combined treatment resulted in a significant elevation in CAT activities at 12 wk control ($p < 0.05$).	[85]
Dibutyl Phthalate, purity not reported	corn oil	groups of 5 male Sprague–Dawley rats	0 or 500 mg/kg bw/d	Rats received treatment by gavage once daily for 3 wk.	Dibutyl Phthalate exposure caused a significant reduction in testes weight ($p < 0.05$), a decrease in the proportion of the germ cell layer in the cross-section of the seminiferous tubules, and a decrease of the sperm density in the lumen of epididymis tissues ($p < 0.05$). Dibutyl Phthalate exposure resulted in significantly increased MDA, Fe^{2+}, and GSSG levels and decreased GSH in testes homogenates ($p < 0.05$), and significant serum testosterone decrease ($p < 0.05$). Immunohistochemistry and q-PCR of testes tissue showed that the expression of PRDX6 and SP1 was upregulated.	[67]
Dibutyl Phthalate; > 99% pure	feed	50 male and 50 female B6C3F1/N mice	0, 1000, 3000, or 10,000 ppm, equivalent to 105 - 112, 329 - 347, and 1306 - 1393 mg/kg bw/d, respectively	2-yr carcinogenicity study (see the Carcinogenicity Studies section for full details); body weights and feed consumption measured, clinical observations made through study period, and complete necropsies and microscopic examinations performed on all mice at study end.	No gross lesions observed in male reproductive tract, but significantly ($p < 0.05$) increased incidences of germinal epithelium degeneration in the testes and exfoliated germ cells in the epididymal duct were observed microscopically, mainly in the 10,000 ppm dose group	[55]
Diethyl Phthalate	feed	groups of male Wistar rats; no further details available	0 or 2000 mg/kg/d (2%)	Rats received test material in the diet for 7 d. Testosterone levels were measured in testes and serum, and testes were examined histopathologically; no further details available	Lower levels of testosterone were measured in the testes and serum of the treated animals, but no testicular damage was observed by microscopic examination	[6]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate, monobutyl phthalate, monomethyl phthalate, monomethyl phthalate, and phthalic acid	corn oil	groups of 6 male Sprague-Dawley rats	250 mg/kg bw/d for monoesters and phthalic acid; 500 mg/kg bw/d for diesters	Rats received test materials orally via gavage for 4 wk; control group received corn oil only; (non-DART methods described in the repeated-dose table above); testes and epididymis were weighted at necropsy; and sperm was collected for sperm count analysis and motility analysis.	Testes weights were significantly reduced in the Dibutyl Phthalate group compared to the control. Dibutyl Phthalate, monobutyl phthalate, and monoethyl phthalate significantly lowered sperm counts and sperm motility of epididymal sperm, detected by a change in the sperm motion parameters.	[40]
Dibutyl Phthalate, 99% pure	corn oil	groups of 3 - 6 pregnant Wistar rats	0 or 500 mg/kg bw/d	Dams received the test material by gavage on gestation days 13.5 - 20.5 or 19.5 - 20.5. Male fetuses were collected on gestation day 21.5; testicular testosterone, cytochrome P450scc enzyme expression, histology, and Leydig-cell aggregation were evaluated.	Both short- and long- term exposure substantially decreased intratesticular testosterone concentrations and cytochrome P450scc expression and induced Leydig-cell aggregation. The decrease in testosterone following the shorter regimen was at least as great as that following the longer regimen, whereas Leydig-cell aggregation was more extensive following the longer exposure regimen.	[90]
Dibutyl Phthalate，99% pure; monobutyl phthalate	DMSO suspended in honey	Neonatal male common marmosets: 5 co-twin pairs for the 14-d monobutyl phthalate study; groups of 4 for the 14-d Dibutyl Phthalate study; groups of 6 - 9 for the acute monobutyl phthalate study	500 mg/kg (single dose) or 500 mg/kg bw/d (14 d)	Beginning at 4 d of age, one twin received monobutyl phthalate and the other received vehicle daily for 14 d; four non-co-twin marmosets received Dibutyl Phthalate for 14 d; in the acute study, males 2 - 7 d of age received a single oral dose of monobutyl phthalate, and blood was collected 5 h later.	After 14 d of monobutyl phthalate exposure, plasma testosterone was unchanged, but Leydig-cell volume per testis was significantly increased compared with co-twin controls ($p = 0.011$). A similar increase after Dibutyl Phthalate treatment ($1.26 \pm 0.17\%$ vs. $0.65 \pm 0.26\%$ in controls) was not statistically significant. A single dose of monobutyl phthalate significantly decreased plasma testosterone after 5 h (1.36 ± 0.23 vs. 2.75 ± 0.55 ng/ml; $p = 0.019$). The authors considered the increased Leydig-cell volume consistent with initial inhibition of steroidogenesis followed by compensatory Leydig-cell hyperplasia/hypertrophy.	[90]
Dibutyl Phthalate	corn oil	Groups of 6 pregnant BALB/c mice	0, 50, 250, or 500 mg/kg bw/d	Dams received test material by gavage from gestation day 12.5 until birth. Testes of male offspring were examined on postnatal day 22 by histology, electron microscopy, a biotin permeability assay, immunofluorescence, and protein-expression analyses. Supporting in vitro experiments used primary mouse Sertoli cells and TM4 cells exposed to 0.1 or 1 mM monobutyl phthalate.	At 50 mg/kg bw/d, histology was not overtly altered, but increased actin-filament bundles, increased full-length occludin, and reduced activated matrix metalloproteinases were consistent with accelerated tight-junction/blood-testis-barrier formation. At ≥ 250 mg/kg bw/d, dislocated immature germ cells, deranged cell layers, tight-junction disassembly, and vacuoles were observed; full-length occludin was reduced at 500 mg/kg/d. In vitro, 0.1 mM monobutyl phthalate increased transepithelial electrical resistance and reduced MMP-2 expression and occludin degradation through down-regulation of NF-κB/COX-2/PGE2 signaling.	[91]

Table 7. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
			OTHER ROUTES			
Dibutyl Phthalate	25% (v/v) tetraglycol, 25% (v/v) polyethylene glycol 300, 25% (v/v) kolliphor EL, 15% (v/v) ethanol, and 10% (v/v) 1,2-propanediol	groups of 4 male C57BL/6J mice	2.5 mg/kg/d	Mice were exposed to control, Dibutyl Phthalate, diethylhexyl phthalate (2.5 mg/kg/d), or a mixture for 40 d via surgically implanted osmotic pumps; caudal epididymal spermatozoa were extracted and analyzed for motility; sperm phosphorylation of protein kinase A substrates and tyrosine phosphorylation were also analyzed; in vitro fertilization was used to evaluate sperm fertilizing capacity.	No significant difference in sperm motility and fertilization potential was observed. Abnormal sperm morphology was observed in all phthalate exposures, particularly in the mixture group. Significant differences in sperm concentration were observed between control and exposed groups. Protein phosphorylation of protein kinase A substrates was decreased in the in mixture group, but no significant changes in protein tyrosine phosphorylation were observed in any of the treatment groups. Assessment of the reproductive functionality did not reveal significant effects on in vitro fertilization and early embryo development rates, but showed wide variability in the mixture group.	[86]
Dibutyl Phthalate	corn oil	groups of 12 neonatal male Sprague-Dawley rats	0, 5, 10, or 20 mg/animal	Dibutyl Phthalate was injected subcutaneously in rats from postnatal day 5 to 14. Animals were observed for clinical signs of toxicity and body weights were measured. Animals were killed on postnatal day 31 or 42, and testes, epididymis, seminal vesicles, ventral prostate, LABC, and Cowper's glands were weighed. The expressions of androgen receptor, estrogen receptors, and steroidogenic factor-1 (SF-1) were also examined in the testes.	No clinical signs of toxicity were observed to any rats in the treatment groups. In the 20 mg dose group, significantly reduced body weight was observed on postnatal days 29 and 31, but changes recovered to control levels by postnatal day 42. No alterations in testicular descent were observed in any treated rats; however, significantly reduced testes, seminal vesicles, LABC, and Cowper's glands weights were observed in the 20 mg dose group when compared to the controls. No significant difference was observed in serum testosterone levels with the treated groups and the controls. In the 20 mg dose group, a marked decreased in seminiferous tubule and Leydig cell hyperplasia were observed. Epithelial cell height in the proximal vas deferens was reduced in the 20 mg dose group and epithelial cell proliferation was observed. At postnatal day 31, the 20 mg dose group had significantly reduced expression of androgen receptor and significantly increased estrogen and SF-1 expression.	[87]
			OTHER			
Dibutyl Phthalate, > 99% pure	DMSO	*Danio rerio*; 6 fish per tank; 3 replicates per dose	11, 113, or 1133 µg/l	Study to investigate the effects of Dibutyl Phthalate alone or with diisobutyl phthalate (10, 103, or 1038 µg/l) on male reproduction and to explore potential toxicological mechanisms. Adult zebrafish were exposed to Dibutyl Phthalate, diisobutyl phthalate, or a mix for 30 d and the effects on plasma hormone secretion, testis histology, and transcriptomics were examined. Chemical exposure was performed in accordance with OECD TG 229. Test included control and solvent control (0.05% DMSO) groups	Highest concentrations of the exposure to the mixture caused greater imbalance testosterone to estradiol ratio and more severe structural damage to testis than exposure to just Dibutyl Phthalate. These effects were consistent with the testis transcriptome analysis for which 4570 genes were differentially expressed in the mixture exposure, while 2795 genes were differentially expressed in Dibutyl Phthalate. KEGG pathway analysis showed that both single and combined exposure of Dibutyl Phthalate and diisobutyl phthalate could affect cytokine-cytokine receptor interaction. The difference was that combined exposure could also affect steroid hormone synthesis, extracellular matrix receptor interaction, retinol metabolism, and PPAR signaling pathways.	[89]

Table 8. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
IN VITRO						
Gene Mutation						
Dibutyl Phthalate	not reported	10 - 2000 µg/plate	*S. typhimurium* strains TA100 and TA1535	Ames test; with and without metabolic activation	Not genotoxic	5
Diethyl Phthalate	DMSO	up to 5000 µg/plate	*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E.coli* strain WP2 uvrA	Ames test in accordance with OECD TG 471, with and without metabolic activation	Not genotoxic	6
Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate	DMSO	100-2000 µg/plate	*S. typhimurium strains* TA98, TA100, TA1535, TA1537, TA1538, TA2637	Ames test, with and without metabolic activation	Dibutyl Phthalate and Diethyl Phthalate were mildly genotoxic in strains TA100 and TA1535 without metabolic activation in a dose-dependent manner. Dimethyl Phthalate was mildly genotoxic in TA1535, and after normalization for cytotoxicity, was mildly genotoxic in TA100 without metabolic activation. Genotoxicity was not observed with metabolic activation in these strains with these test materials.	93
Diethyl Phthalate	DMSO	up to 771 µg/ml	mouse L5178Y TK +/- lymphoma cells	Gene mutation assay in accordance with OECD TG 476; with and without metabolic activation	Not genotoxic	6
Chromosomal Damage						
Dibutyl Phthalate	not reported	0, 30, 50, or 80 µM	cultured bovine lymphocytes	Micronucleus test; cells incubated with test material for 24 h	Genotoxic; a significant ($p < 0.001$) effect of induction of micronuclei and binuclei observed at all 3 test concentrations compared to the negative control group	94
Dibutyl Phthalate	DMSO	10, 15, or 30 µM	CHO cells	Micronucleus test; cells were treated with test material for 24 h	Dibutyl Phthalate had a non-linear induction of micronuclei percentage at all 3 concentrations, with 15 µM causing the highest percentage of micronuclei (4.90%, $p < 0.001$) compared with 10 µM (3.36% $p < 0.05$) and 30 µM (3.66%, $p < 0.01$).	95
Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate	methanol and ethanol	0.05 or 0.2 ppm for each phthalate	TK6 human lymphoblast cells	MicroFlow® micronucleus assay; the assay also evaluated the genotoxicity of 42 perfumes that contained 5 phthalates including Dibutyl Phthalate (mean concentration 0.032 ppm), Diethyl Phthalate (mean concentration 1673 ppm), and Dimethyl Phthalate (mean concentration 32.25 ppm)	The percent frequency of micronuclei was more than twofold higher in the cells treated with the perfumes than those treated with the vehicle controls, but the differences were not significant ($p > 0.05$). Percent frequency of micronuclei by each of the 5 phthalates regardless of their concentration (0.05 or 0.2 ppm), did not differ significantly from those induced by the perfumes. No significant differences in percent frequency of micronuclei between the low and high concentrations of Diethyl Phthalate and Dimethyl Phthalate; the percent frequency of micronuclei for Dibutyl Phthalate was significantly lower at 0.2 than 0.05 ppm ($p < 0.001$).	15
Dibutyl Phthalate	DMSO	10, 15, or 30 µM	CHO cells	Chromosome aberration test; cells were treated with test material for 24 h	Dibutyl Phthalate caused a higher occurrence of chromosomal aberrations at all tested concentration when compared to the vehicle control. Aberrations were predominantly with gaps in both chromatids and chromosomes. Effects occurred in a non-linear fashion with highest clastogenic effects observed at 15 µM	95
Diethyl Phthalate	DMSO	up to 1780 µg/ml	human lymphocytes	Chromosome aberration test in accordance with OECD TG 473; with and without metabolic activation	Not genotoxic	6

Table 8. **Genotoxicity studies**

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
				DNA Strand Breaks		
Dibutyl Phthalate	DMSO	0 or 354 µmol/ml	human mucosal cells or human peripheral lymphocytes	Comet assay; cells were incubated with test material for 60 min. N-methyl-N-nitro-N-nitrosoguanidine served as positive control	Genotoxicity was observed in both cell types. Positive control yielded expected results.	[96]
Dibutyl Phthalate	not reported	0, 30, 50, or 80 µM	cultured bovine lymphocytes	Alkaline comet assay; cells incubated with test material for 24 h	Genotoxic; dose-dependent DNA damage observed	[94]
Dibutyl Phthalate and monobutyl phthalate	ethanol	0.1 - 10 µg/ml	human peripheral blood mononuclear cells	Alkaline comet assay; cells incubated with test materials for 24 h	Genotoxic; a statistically significant increase in DNA damage was induced by Dibutyl Phthalate started at 0.5 µg/ml; tail intensity was evaluated to be 7.2%. At 10 µg/ml Dibutyl Phthalate, DNA damage was significant, with a tail intensity of 32%. For monobutyl phthalate, the first statistically significant effects were observed at 1 µg/ml (tail intensity was 8%); DNA damage at 10 µg/ml was about half that of Dibutyl Phthalate.	[97]
				A plasmid relaxation assay, study of DNA purine and pyrimidine oxidation, studies of total reactive oxygen species and hydroxyl radical levels, and repair kinetics of DNA damage from Dibutyl Phthalate and monobutyl phthalate exposures were also performed	Neither Dibutyl Phthalate or monobutyl phthalate were capable of creating adducts with DNA. Dibutyl Phthalate and monobutyl phthalate more strongly oxidized purines than pyrimidines. An increase of total reactive oxygen species level was observed with both phthalates, but hydroxyl radical was generated mostly by Dibutyl Phthalate. The human peripheral blood mononuclear cells exposed to Dibutyl Phthalate could not completely repair DNA strand breaks during postincubtation; however, the cells could completely repair strand breaks from exposure to monobutyl phthalate.	
Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate	methanol and ethanol	0.05 or 0.2 ppm for each phthalate	TK6 human lymphoblast cells	Alkaline comet assay; the assay also evaluated the genotoxicity of 42 perfumes that contained 5 phthalates including Dibutyl Phthalate (mean concentration 0.032 ppm), Diethyl Phthalate (mean concentration 1673 ppm), and Dimethyl Phthalate (mean concentration 32.25 ppm)	The assay indicated that most of the perfumes caused DNA strand breaks. The average tail moment in the cells treated with perfume (55.05) was significantly higher than the tail moments for the negative and vehicle controls ($p < 0.01$), but not significantly different from the tail moments for each of the 5 phthalates ($p > 0.05$), regardless of the concentration. The extent of DNA damage by Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate was significantly ($p < 0.001$, except for Dimethyl at $p < 0.01$) higher (approximately twofold) at 0.2 than 0.05 ppm. DNA damage did not differ significantly between cells treated with either the low or high phthalate concentrations and cells treated with perfumes	[15]

Table 8. **Genotoxicity studies**

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
				IN VIVO		
				DNA Strand Breaks		
Dibutyl Phthalate, 99% pure	olive oil	0 or 500 mg/kg bw/d	One-month-old male Wistar rats from F1, F2, and F3 generations; n = 6/group/generation	Alkaline comet assay; F0 dams received test material orally on gestation days 6 - 21 and throughout lactation. Exposure at 500 mg/kg bw/d continued in the F1- F3 generations. Cells from cerebral cortex, cerebellum, hippocampus, medulla oblongata, and spinal cord were evaluated in an alkaline comet assay. Cell viability, DNA/RNA content, superoxide dismutase, catalase, and malondialdehyde were also evaluated.	Positive for DNA strand breaks. Olive tail moment, tail DNA percentage, and tail length were significantly increased in cells isolated from the cerebral cortex, cerebellum, hippocampus, medulla oblongata, and spinal cord of F1, F2, and F3 males; the magnitude of DNA damage was generally greatest in F3 animals. Cell viability did not differ significantly from controls. DNA and RNA contents and superoxide dismutase and catalase activities were decreased in several neural regions, whereas malondialdehyde levels were increased, indicating increased lipid peroxidation and accompanying oxidative stress.	[98]

Table 9. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
IRRITATION						
ANIMAL						
Dibutyl Phthalate	undiluted	undiluted	2 male and 1 female Vienna White rabbits	Dermal irritation study performed in accordance with OECD TG 404; rabbits received 0.5 ml of test material on shaved skin on the back or flank with an area of 2.5 cm^2; test sites were semi-occluded, washed after 4 h with lutrol and lutrol/water 1:1, and observed for 72 h	Not irritating; very slight (grade 1) erythema was observed in 2 anils after 4 and 24 h. Reactions were completely reversible after 48 h.	[5]
Diethyl Phthalate	undiluted	undiluted	3 albino rabbits; sex not reported	Dermal irritation study; rabbits received 0.5 ml of test material on shaved and intact or abraded skin; test sites were 2 cm^2 and occluded for 24 h; observation period was 72 h and evaluations for skin reactions were periods at the end of the 24 h exposure and again 48 h later	Not irritating; irritation scores were 0 for all rabbits	[6]
SENSITIZATION						
ANIMAL						
Dibutyl Phthalate	olive oil DAB 9	Intradermal induction: 5% Topical induction: 75% Challenge: 50%	20 female Pirbright-Hartley guinea pigs	GPMT performed in accordance with OCED TG 406; induction with test material intradermally with Freund's adjuvant; topical induction 1 wk later on test site 2 cm x 4 cm that was occluded; after 2 wk rest period, challenge was performed with two 24-h patches (2 cm^2) applied a week apart	Not sensitizing; slight erythema was observed in 2 animals that received Dibutyl Phthalate after the first challenge patch 48 48 h after application. No reactions to the test material were observed in the second challenge. One reaction was observed in a control animal during the first challenge only.	[5]
Diethyl Phthalate	acetone, water, and ethanol; no further details available	up to 100%	Groups of 6 to 8 male and female Himalayan white spotted guinea pigs	Open epicutaneous test, Draize test, maximization test, and Fruend's complete adjuvant test; no further details available	Not sensitizing; no further details available	[6]
Diethyl Phthalate	acetone: olive oil (4:1 v/v)	25, 50, or 100% v/v	groups of 4 female CBA/Ca mice	LLNA in accordance with OECD TG 429	Not sensitizing at any concentration tested; SI were 1.0, 1.3, and 1.5 for 25, 50, and 100%, respectively	[6]
Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate	acetone	1:1 v/v with acetone; no further details provided	groups of female CD-1 mice, no further details provided	Ear swelling test to determine the adjuvant effect of phthalate esters on contact hypersensitivity; mice were epicutaneously sensitized with FITC in acetone with a phthalate ester; sensitization was evaluated after challenge with FITC; draining lymph node cells obtained 24 h after sensitization were examined for FITC fluorescence by flow cytometry; FITC-positive cells were characterized with anti-CD11c and anti-CD11b by 3-color flow cytometry; left ear of mice was treated with 20 µl of acetone/Dibutyl Phthalate alone as control	Mice sensitized with FITC in acetone containing Dibutyl Phthalate had strong enhancement of ear-swelling response; Diethyl Phthalate and Dimethyl Phthalate were less effective but produced some enhancement. With sensitization in the presence of Dibutyl Phthalate, the number of FITC-positive dendritic cells was increased in draining lymph nodes; no significant increase in FITC-positive cell number in the draining lymph nodes was observed with Diethyl Phthalate or Dimethyl Phthalate	[142]

Table 10. Concentration of use (2025) and exposure by FDA product category – Diethyl Phthalate

Product Category/Type of cosmetics exposure	Daily Exposure by Product Category (mg/d)	Retention factor[181]	MaximumConcentration of Use[13]	Daily Exposure Based on the Highest Use Concentration (mg/d)	Note
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	4810	0.1	0.1%	0.481	Estimated exposure to facial cleanser (lathering and non-lathering) at the 90th percentile[182]
Face and neck products (not spray) Leave-on	3500	1	0.15%	5.25	Exposure amount of face cream/lotion applied[183]

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