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

Status:	Draft Tentative Amended Report for Panel Review
Release Date:	September 11, 2026
Panel Meeting Date:	October 5-6, 2026

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Bruce A. Brod, M.D., M.H.C.I., F.A.A.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel member involved in this assessment: David E. Cohen, M.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR, and Jinqiu Zhu, Ph.D., DABT, ERT, DCST, CIR Toxicologist.

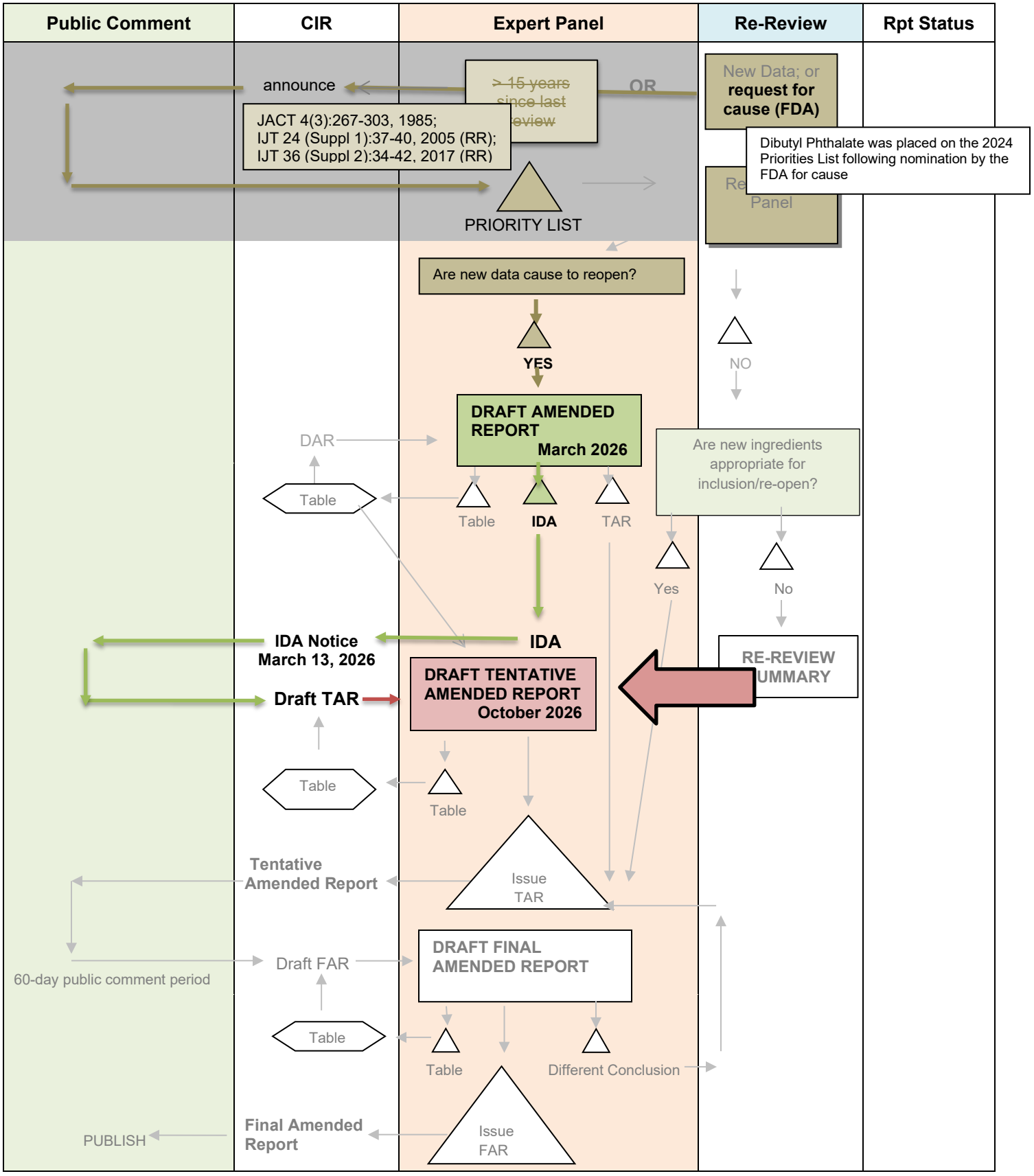
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RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate

MEETING October 2026



COSMETIC
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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR
Jinqiu Zhu, Ph.D., Toxicologist, CIR
Date: September 11, 2026
Subject: Amended Safety Assessment of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate as Used in Cosmetics

Enclosed is the Draft Tentative Amended Report on the Safety Assessment of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate as Used in Cosmetics. (It is identified as *report_Phthalates_102026* in the pdf document). This report was reopened after Dibutyl Phthalate was placed on the 2024 Priorities List following nomination by the FDA for cause. At the March 2026 meeting, the Panel issued an Insufficient Data Announcement (IDA). The additional data needed to determine the safety of these ingredients are:

- Clarification on the type of current uses and maximum concentration of use for Dibutyl Phthalate
- Maximum concentration of use for Dimethyl Phthalate

Since this IDA was issued, CIR has received no additional unpublished data. Comments received from the Council prior to the March 2026 meeting have been addressed (*PCPCcomments_Phthalates_102026* and *response-PCPCcomments_Phthalates_102026*). An updated literature search yielded numerous additional studies on developmental and reproductive toxicity, neurobehavioral effects, and other relevant endpoints. Most of the additional studies have been summarized and highlighted in this current draft of the report. A few of the developmental and reproductive studies were determined to be purely cumulative in nature and were not summarized in the report; however, these studies were cited with a brief description. ***As there is a potential for more cumulative studies to be discovered in future literature updates, does the Panel want these studies to be incorporated fully into the report, or should CIR staff continue to present these purely cumulative studies in brief with a citation?***

Supporting documents for this report package include a flow chart (*flow_Phthalates_102026*), report history (*history_Phthalates_102026*), a search strategy (*search_Phthalates_102026*), a data profile (*dataprofile_Phthalates_102026*), transcripts from the most recent meetings at which Phthalates has been discussed (*transcripts_Phthalates_102026*), the minutes from all the meetings at which Phthalates were discussed during the original reviews (*originalminutes_Phthalates_102026*), the original report (*originalreport1985_Phthalates_102026*), the first and second re-review summaries (*rereview2005_Phthalates_102026* and *rereview2017_Phthalates_102026*), and the 2002 re-review draft (*RRdata_Phthalates_102026*).

The Panel should carefully consider and discuss the data (or lack thereof), and issue a Tentative Amended Report with a safe, safe with qualifications, insufficient data, unsafe, or split conclusion, and identify any additional items for inclusion in the Discussion.

For your recollection:

Data need	Received?	Notes
Clarification on the type of current uses and maximum concentration of use for Dibutyl Phthalate	No	
Maximum concentration of use for Dimethyl Phthalate	No	

Phthalates History

1985– The CIR Final Report on the Safety Assessment of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate was published in the *Journal of the American College of Toxicology*. The Panel concluded that these ingredients are "safe for topical application in the present practices of use and concentration in cosmetic."

2005 – A re-review summary was published reaffirming the 1985 report's conclusion.

2017 – A re-review summary was published reaffirming the 1985 report's conclusion. Prior to the publication, in 2012, the Panel had deliberated studies concerning endocrine disruption and diabetes for Dibutyl Phthalate, Diethyl, Dimethyl, Butyl Benzyl Phthalate. However, the Panel chose not to reopen and published their discussion as a re-review summary.

June 2023 – CIR received communication from members of the FDA nominating Dibutyl Phthalate to the 2024 Priority List, for-cause. The Panel agreed to this request.

March 2024 - The Panel discussed the strategy of the preparation of the Draft Amended Report for Dibutyl Phthalate. The Panel determined that Diethyl Phthalate and Dimethyl Phthalate should be included in the safety assessment as much of the published literature involves data on these ingredients together; however, the Panel stressed that any use, exposure, or risk data from one ingredient could not be read-across to the other ingredients in the report, citing in part very different use conditions from one ingredient to the next. The Panel also offered guidance on the presentation of the data, especially data relating to endocrine effect, in the updated safety assessment. Additionally, the Panel discussed the current regulatory status of Dibutyl Phthalate in the European Union and in several US states. The Panel requested clarification on the status of these regulations, and the data that support such.

June 2025 – In a strategy memo, the Panel was asked:

- Does the Panel, or any other stakeholder, have a particular expert in these areas they would like to invite to give a presentation on these DART and endocrine studies?
    - The Panel responded in the affirmative and provided potential experts to invite.
- Does the Panel support the idea of having Dimethyl Phthalate in a separate re-review proposal document or would the Panel prefer that this ingredient stay in the safety assessment with Dibutyl Phthalate and Diethyl Phthalate?
    - The Panel determined to keep these ingredients together in one report.

March 2026 - The Panel issued an IDA for Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate. The additional data needed to determine the safety of these ingredients are:

- Clarification on the type of current uses and maximum concentration of use for Dibutyl Phthalate
- Maximum concentration of use for Dimethyl Phthalate

Phthalates Data Profile* – October 2026 – Christina Burnett																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Dibutyl Phthalate	X O	O	O	X	X O	X O		X O	X	O	X O	O		X O	X O		X	X O		X O	O		X O	O	O	X	X O	X O	X O
Diethyl Phthalate	X O	O	O	X	X O	X O	O	X O	X	O	X O		X O	X O	X O		O			X O	O		X O	O	O		O		
Dimethyl Phthalate	O	O	O	X	X O	O	O	X O		O	X O		O	X O	X O		O			O	O		X O	O			O		

* "X" indicates that new data were available in a category for the ingredient. "O" indicates data were reported in the original safety assessment.

Phthalates

Ingredient	CAS #	InfoB	SciFind	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Dibutyl Phthalate	84-74-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Diethyl Phthalate	84-66-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Dimethyl Phthalate	131-11-3	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy

PubMed
From 1999 to present:
(Dibutyl Phthalate) OR (84-74-2[EC/RN Number]) = 2849 hits
(Diethyl Phthalate) OR (84-66-2[EC/RN Number]) = 1239 hits
(Dimethyl Phthalate) OR (131-11-3[EC/RN Number]) = 822 hits

ECHA
Entry for CAS # 84-74-2, 84-66-2, and 131-11-3 resulted in finding dossiers for Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate, respectively. Pertinent data not found in the original report is summarized in the amended report.

Typical Search Terms

- INCI names
- CAS numbers
- chemical/technical names
- additional terms will be used as appropriate

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- Connected Papers - https://www.connectedpapers.com/
- DeepDyve - https://www.deepdyve.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees_en https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

Personal Care Products Council

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Jaap Venema, Ph.D.
EVP and Chief Scientist – Personal Care Products Council
Industry Liaison to the CIR Expert Panel

DATE: March 6, 2026

SUBJECT: Draft Amended Report: Amended Safety Assessment of Dibutyl Phthalate, Diethyl Phthalate and Dimethyl Phthalate as Used in Cosmetics (March 2026 meeting draft)

The Personal Care Products Council respectfully submits the following comments on the draft amended report, Amended Safety Assessment of Dibutyl Phthalate, Diethyl Phthalate and Dimethyl Phthalate as Used in Cosmetics.

Key Issues

From 2004-2010 FDA conducted three surveys of phthalates in cosmetics (described at https://www.fda.gov/cosmetics/cosmetic-ingredients/phthalates-cosmetics). They concluded "We learned from these surveys that use of phthalates in cosmetics decreased considerably from 2004 to 2010." If these surveys are not described in a previously published CIR report or re-review summary, they should be described in this CIR report. Although these surveys may not reflect current use concentrations, they may provide useful context to the biomonitoring studies (e.g., exposure to Dibutyl Phthalate in manicurists published in 2008 [reference 122 in the CIR report] included in this CIR report.

The CIR re-review published in 2005 includes a risk assessment calculation for Dibutyl Phthalate. This risk assessment and a description of why the point of departure of 331 mg/kg/day from a dietary rat study was selected should be mentioned in this CIR report. It would be helpful if the study used for the point of departure (Ema, M., E. Miyawaki, and K. Kawashima. 1998. Further evaluation of developmental toxicity of di-n-butyl phthalate following administration during late pregnancy in rats. Toxicol. Lett. 98:87–93.) was described in the text of the Developmental and Reproductive Toxicity Section and clearly identified in Table 6.

Additional Considerations

Chemical Properties – Please correct: "Chemical and properties" (delete "and")

Cosmetic Use – To be consistent with PCPC's recommended policy on tattoo preparations, please delete the study on Dibutyl Phthalate in black tattoo inks (reference 15).

Please reorganize the last paragraph so that the information on Dibutyl Phthalate is presented in one paragraph and the information on Diethyl and Dimethyl Phthalates is presented in another paragraph.

Non-Cosmetic Use – Please correct: "Phahalte"

Non-Cosmetic Use, Summary - Rather than saying that Diethyl Phthalate is a plasticizer in food packaging, it would be more accurate to state that it is permitted to be used as a plasticizer in food packaging.

Dermal Penetration, In Vitro – It would be helpful if reference 30 on Diethyl and Dibutyl Phthalate penetration was presented after the study on Dibutyl Phthalate penetration (reference 28), and the study on Dimethyl Phthalate (reference 29) presented last. In the description of reference 30, it is not clear what is meant by "absorption factor". Is this the amount found in the receptor fluid, or the amount in the receptor fluid plus the amount collected in the cell layers?

Dermal Penetration, Animal – Please correct: "systemic absorbed" ("systemic" should be "systemically")

Absorption, Distribution, Metabolism and Excretion, Oral, old report summary – There are two studies in the Oral section in which oral exposure was compared to intravenous exposure. These studies should be in a separate subsection "Oral and Intravenous".

Absorption, Distribution, Metabolism and Excretion, Parenteral, old report summary – Please rearrange this paragraph so that the two studies on Dibutyl Phthalate are presented together.

Acute, old report summaries – This section would be easier to read if different paragraphs were created for the information on Dibutyl Phthalate and the information on Diethyl and Dimethyl Phthalates.

Acute; Summary – Please state the duration of the inhalation studies (often 4 hours).

Short-Term – Since the effects observed in reference 39 were primarily in the Dibutyl Phthalate treated rats, please summarize this study in a separate paragraph after the studies on Dibutyl Phthalate.

Please correct: "Livers weights" (delete "s" in "Livers")

Chronic, old report summary – Please correct: "No hematological effects on hematological parameters" (delete "hematological effects"). It would be helpful if the study on Dimethyl

Phthalate was presented in a separate paragraph.

Embryotoxic and Teratogenic Effects – This section would benefit from more paragraphs separating the study descriptions, e.g., one paragraph for the Xenopus studies, another for the zebrafish studies, and another for the rodent studies.

Effects in Female Reproductive Organs – Since the dose levels used in reference 50 were unusual (10 and 100 µg/kg/day possible relevant human exposure levels and 1000 mg/kg/day a dose more likely to be used in a toxicology study), saying "doses up to 1000 mg/kg/day" does not adequately describe this study. Please state all of the doses used when describing this study.

Effects in Male Reproductive Organs – Please revise: "which can directly bound to the PRDX6 promoter and transcriptionally activated its expression"

After the paragraph on in vitro studies, this section should be more clearly divided into studies in male offspring of treated females, and studies in which males were treated directly. Currently, this part of the section starts with "Multiple oral studies have investigated the effects of Dibutyl Phthalate in the male rodents (with multiple references some that include Diethyl or Dimethyl Phthalate). Perhaps this should be modified to: "Multiple oral studies have investigated the effects of phthalates on male offspring of females treated with phthalates during gestation" (with only the references concerning gestational exposure). The paragraph on males directly treated with phthalates should then start with an introductory sentence such as: "Additional oral studies have investigated the effects of phthalates on male reproductive organs in male rodents treated directly" (with only the references concerning direct treatment).

Reference 39 is a study in which male rats were directly exposed to Dibutyl Phthalate. It is in a paragraph with studies that used gestational exposure. Reference 39 should be moved with the other studies of direct exposure of male rats.

Genotoxicity, In Vitro, old report summary – It is not clear what study the following refers to as the previous information is a summary of multiple studies: "In the same study using the same concentrations of Diethyl Phthalate and Dimethyl Phthalate…."

Genotoxicity – Were there really two studies in cultured bovine lymphocytes at concentrations up to 80 µM?

Carcinogenicity, Oral – In the last paragraph, it would be helpful to indicate that the "pars distalis" refers to the pituitary.

Tumor Promotion – Please correct: "diemthylbenzanthrancene" (should be "dimethylbenzanthracene")

In Vitro Cell Transformation – Please correct: "MCR-10A cells" (should be MCF-10A cells as stated earlier in the paragraph)

It would be helpful if all the studies on breast cell lines were presented together.

Effects on Gene Expression – The title of reference 87 indicates that the biomarkers were measured in the brain. If this is correct, this should be added to the description of this study. Since there is an overlap of authors, and since the exposures were the same, perhaps there was only one zebrafish study with reference 86 reporting the results in the liver, and reference 87 reporting the results in the brain.

Please correct: "Groups of 5 mice/treatment mice received" (delete the second "mice")

Endocrine Effects, old report summary – Please revise: "in an estrogen receptor binding assay using rat uterine cytosol was acquired the uterine tissue of 10-wk-old Wistar rats."

In the last paragraph, it is not clear if the primary islet β cells were exposed in vitro, or if they were isolated from the exposed rats. If the exposure was in vitro, the exposure concentration/dose should be stated, and this part of the study should be in the in vitro subsection.

Insulin Effects, In Vitro – Please correct: "was using to assess" ("using" should be "used")

Immunotoxicity; Dermal Sensitization – One mouse-ear swelling test in which mice were sensitized with FITC is in the dermal sensitization section while multiple studies looking at the mechanism of primarily Dibutyl Phthalate enhancing the response are in the Immunotoxicity section. Perhaps all of these studies should be presented together (or at least the sensitization section should refer back to the Immunotoxicity section where the mechanistic studies are described).

Immunotoxicity – What concentration of Diethyl Phthalate had relative weak activity on FITC-positive dendritic cell migration?

Case Reports, old report summary – Please correct: "withing" (delete "g")

Occupational Exposure; Summary – Please correct: "across shirt" ("shirt" should be "shift")

Other Clinical Studies, old report summary – Please check the number of subjects used in the study of semen samples from college students. The summary of this study says 5 students, but that seems too small for the results described, e.g., plotting Dibutyl Phthalate concentrations vs sperm density resulting in two clusters of points.

The study in which semen samples were exposed to phthalates should be moved to the Developmental Reproductive Toxicity section (add to Table 6) where other studies of human sperm exposure are summarized.

Phthalate Exposure and Neurodevelopment – Please revise "exposure of phthalates to 30 preterm neonates" (more likely that the neonates were exposed to phthalates).

Are the units (µg/l/µmol/l) used in the description of reference 130 correct?

How many women were included in the study of women in Illinois (reference 131)?

Summary – In the paragraph about the EU opinions, please correct “200321” (delete “21” which is probably the reference number).

Please start a new paragraph for the developmental and reproductive toxicity studies completed in rodents.

Please clearly identify the studies completed in male offspring of treated pregnant female rats and in male rats treated directly with phthalates (separate paragraphs would be helpful).

Please state that the markers were measured in the brains of zebrafish in the second zebrafish study (although they may have been the same fish as the study in which liver markers were measured).

In the Summary, please indicate who described the key elements for Dibutyl Phthalate-induced male reproductive toxicity using the AOP framework.

In the description of the murine FITC-induced contact hypersensitivity model, please correct: “as an in murine FITC-induced contact hypersensitivity” (delete “an”)

Please add “urinary” to “The median [urinary] concentration of Dibutyl Phthalate metabolites in electronic waste processing workers”

Table 3 – Please revise Table 3 to be consistent with the memo from PCPC concerning tattoo preparations (memo dated February 26, 2026).

Phthalates – October 2026 – Christina Burnett	
Comment Submitter: Jaap Venema, Ph.D.; Personal Care Products Council **Date of Submission:** March 6, 2026	
Comment	**Response/Action**
Key Issue: From 2004-2010 FDA conducted three surveys of phthalates in cosmetics (described at https://www.fda.gov/cosmetics/cosmetic-ingredients/phthalates-cosmetics). They concluded "We learned from these surveys that use of phthalates in cosmetics decreased considerably from 2004 to 2010." If these surveys are not described in a previously published CIR report or re-review summary, they should be described in this CIR report. Although these surveys may not reflect current use concentrations, they may provide useful context to the biomonitoring studies (e.g., exposure to Dibutyl Phthalate in manicurists published in 2008 [reference 122 in the CIR report] included in this CIR report.	Not included per direction of Dr. Heldreth.
Key Issue: The CIR re-review published in 2005 includes a risk assessment calculation for Dibutyl Phthalate. This risk assessment and a description of why the point of departure of 331 mg/kg/day from a dietary rat study was selected should be mentioned in this CIR report. It would be helpful if the study used for the point of departure (Ema, M., E. Miyawaki, and K. Kawashima. 1998. Further evaluation of developmental toxicity of di-n-butyl phthalate following administration during late pregnancy in rats. Toxicol. Lett. 98:87–93.) was described in the text of the Developmental and Reproductive Toxicity Section and clearly identified in Table 6.	Not included per direction of Dr. Heldreth. A current concentration of use is needed.
Chemical Properties – Please correct: "Chemical and properties" (delete "and")	Corrected.
Cosmetic Use – To be consistent with PCPC's recommended policy on tattoo preparations, please delete the study on Dibutyl Phthalate in black tattoo inks (reference 15). Please reorganize the last paragraph so that the information on Dibutyl Phthalate is presented in one paragraph and the information on Diethyl and Dimethyl Phthalates is presented in another paragraph.	Study deleted. Reorganized.
Non-Cosmetic Use – Please correct: "Phahalte"	Corrected.
Non-Cosmetic Use, Summary - Rather than saying that Diethyl Phthalate is a plasticizer in food packaging, it would be more accurate to state that it is permitted to be used as a plasticizer in food packaging.	Suggest edit accepted.
Dermal Penetration, In Vitro – It would be helpful if reference 30 on Diethyl and Dibutyl Phthalate penetration was presented after the study on Dibutyl Phthalate penetration (reference 28), and the study on Dimethyl Phthalate (reference 29) presented last. In the description of reference 30, it is not clear what is meant by "absorption factor". Is this the amount found in the receptor fluid, or the amount in the receptor fluid plus the amount collected in the cell layers?	Paragraphs rearranged. Absorption factor values included both the fraction recovered in the receptor fluid and that retained in the cell layer.
Dermal Penetration, Animal – Please correct: "systemic absorbed" ("systemic" should be "systemically")	Corrected.
Absorption, Distribution, Metabolism and Excretion, Oral, old report summary – There are two studies in the Oral section in which oral exposure was compared to intravenous exposure. These studies should be in a separate subsection "Oral and Intravenous".	New section created and paragraphs moved.
Absorption, Distribution, Metabolism and Excretion, Parenteral, old report summary – Please rearrange this paragraph so that the two studies on Dibutyl Phthalate are presented together.	Rearranged.
Acute, old report summaries – This section would be easier to read if different paragraphs were created for the information on Dibutyl Phthalate and the information on Diethyl and Dimethyl Phthalates.	Section reorganized.
Acute; Summary – Please state the duration of the inhalation studies (often 4 hours).	Durations added.

Phthalates – October 2026 – Christina Burnett	
Comment Submitter: Jaap Venema, Ph.D.; Personal Care Products Council **Date of Submission:** March 6, 2026	
Comment	**Response/Action**
Short-Term – Since the effects observed in reference 39 were primarily in the Dibutyl Phthalate treated rats, please summarize this study in a separate paragraph after the studies on Dibutyl Phthalate. Please correct: "Livers weights" (delete "s" in "Livers")	Section reorganized. Corrected.
Chronic, old report summary – Please correct: "No hematological effects on hematological parameters" (delete "hematological effects"). It would be helpful if the study on Dimethyl Phthalate was presented in a separate paragraph.	Corrected. New paragraph formed.
Embryotoxic and Teratogenic Effects – This section would benefit from more paragraphs separating the study descriptions, e.g., one paragraph for the Xenopus studies, another for the zebrafish studies, and another for the rodent studies.	Section revised.
Effects in Female Reproductive Organs – Since the dose levels used in reference 50 were unusual (10 and 100 µg/kg/day possible relevant human exposure levels and 1000 mg/kg/day a dose more likely to be used in a toxicology study), saying "doses up to 1000 mg/kg/day" does not adequately describe this study. Please state all of the doses used when describing this study.	Sentence revised with detail to concentrations.
Effects in Male Reproductive Organs – Please revise: "which can directly bound to the PRDX6 promoter and transcriptionally activated its expression" After the paragraph on in vitro studies, this section should be more clearly divided into studies in male offspring of treated females, and studies in which males were treated directly. Currently, this part of the section starts with "Multiple oral studies have investigated the effects of Dibutyl Phthalate in the male rodents (with multiple references some that include Diethyl or Dimethyl Phthalate). Perhaps this should be modified to: "Multiple oral studies have investigated the effects of phthalates on male offspring of females treated with phthalates during gestation" (with only the references concerning gestational exposure). The paragraph on males directly treated with phthalates should then start with an introductory sentence such as: "Additional oral studies have investigated the effects of phthalates on male reproductive organs in male rodents treated directly" (with only the references concerning direct treatment). Reference 39 is a study in which male rats were directly exposed to Dibutyl Phthalate. It is in a paragraph with studies that used gestational exposure. Reference 39 should be moved with the other studies of direct exposure of male rats.	Revised. Suggested edits accepted. Study moved.
Genotoxicity, In Vitro, old report summary – It is not clear what study the following refers to as the previous information is a summary of multiple studies: "In the same study using the same concentrations of Diethyl Phthalate and Dimethyl Phthalate…."	Sentence revised.
Genotoxicity – Were there really two studies in cultured bovine lymphocytes at concentrations up to 80 µM?	Citations corrected.
Carcinogenicity, Oral – In the last paragraph, it would be helpful to indicate that the "pars distalis" refers to the pituitary.	Edit accepted.
Tumor Promotion – Please correct: "diemthylbenzanthrancene" (should be "dimethylbenzanthracene")	Corrected.
In Vitro Cell Transformation – Please correct: "MCR-10A cells" (should be MCF-10A cells as stated earlier in the paragraph) It would be helpful if all the studies on breast cell lines were presented together.	Corrected. Studies moved.

Phthalates – October 2026 – Christina Burnett	
Comment Submitter: Jaap Venema, Ph.D.; Personal Care Products Council **Date of Submission:** March 6, 2026	
Comment	**Response/Action**
Effects on Gene Expression – The title of reference 87 indicates that the biomarkers were measured in the brain. If this is correct, this should be added to the description of this study. Since there is an overlap of authors, and since the exposures were the same, perhaps there was only one zebrafish study with reference 86 reporting the results in the liver, and reference 87 reporting the results in the brain. Please correct: "Groups of 5 mice/treatment mice received" (delete the second "mice")	Clarification made. Corrected.
Endocrine Effects, old report summary – Please revise: "in an estrogen receptor binding assay using rat uterine cytosol was acquired the uterine tissue of 10-wk-old Wistar rats." In the last paragraph, it is not clear if the primary islet β cells were exposed in vitro, or if they were isolated from the exposed rats. If the exposure was in vitro, the exposure concentration/dose should be stated, and this part of the study should be in the in vitro subsection.	Sentence revised. Paragraph revised with additional detail.
Insulin Effects, In Vitro – Please correct: "was using to assess" ("using" should be "used")	Corrected.
Immunotoxicity; Dermal Sensitization – One mouse-ear swelling test in which mice were sensitized with FITC is in the dermal sensitization section while multiple studies looking at the mechanism of primarily Dibutyl Phthalate enhancing the response are in the Immunotoxicity section. Perhaps all of these studies should be presented together (or at least the sensitization section should refer back to the Immunotoxicity section where the mechanistic studies are described).	A sentence was added to the Dermal Irritation and Sensitization section to refer to the Immunotoxicity Section.
Immunotoxicity – What concentration of Diethyl Phthalate had relative weak activity on FITC-positive dendritic cell migration?	Concentration not specified. Diethyl Phthalate was tested at 1:1 in acetone.
Case Reports, old report summary – Please correct: "withing" (delete "g")	Corrected.
Occupational Exposure; Summary – Please correct: "across shirt" ("shirt" should be "shift")	Corrected.
Other Clinical Studies, old report summary – Please check the number of subjects used in the study of semen samples from college students. The summary of this study says 5 students, but that seems too small for the results described, e.g., plotting Dibutyl Phthalate concentrations vs sperm density resulting in two clusters of points. The study in which semen samples were exposed to phthalates should be moved to the Developmental Reproductive Toxicity section (add to Table 6) where other studies of human sperm exposure are summarized.	5 groups of men (number in each group not reported). Moved.
Phthalate Exposure and Neurodevelopment – Please revise "exposure of phthalates to 30 preterm neonates" (more likely that the neonates were exposed to phthalates). Are the units (µg/l/µmol/l) used in the description of reference 130 correct? How many women were included in the study of women in Illinois (reference 131)?	Revised. Yes, as written in original reference. 481. Sentence updated.

Phthalates – October 2026 – Christina Burnett	
Comment Submitter: Jaap Venema, Ph.D.; Personal Care Products Council **Date of Submission:** March 6, 2026	
Comment	**Response/Action**
Summary – In the paragraph about the EU opinions, please correct "200321" (delete "21" which is probably the reference number).	Corrected.
Please start a new paragraph for the developmental and reproductive toxicity studies completed in rodents.	Paragraph returns made.
Please clearly identify the studies completed in male offspring of treated pregnant female rats and in male rats treated directly with phthalates (separate paragraphs would be helpful).	Clarifications and paragraph editing performed.
Please state that the markers were measured in the brains of zebrafish in the second zebrafish study (although they may have been the same fish as the study in which liver markers were measured).	Edit made.
In the Summary, please indicate who described the key elements for Dibutyl Phthalate-induced male reproductive toxicity using the AOP framework.	Added "by the US EPA" to sentence.
In the description of the murine FITC-induced contact hypersensitivity model, please correct: "as an in murine FITC-induced contact hypersensitivity" (delete "an")	Corrected.
Please add "urinary" to "The median [urinary] concentration of Dibutyl Phthalate metabolites in electronic waste processing workers"	Added.
Table 3 – Please revise Table 3 to be consistent with the memo from PCPC concerning tattoo preparations (memo dated February 26, 2026).	Table revised.

JUNE 2023 MEETING – REQUEST TO REOPEN FOR CAUSE

Belsito Team – June 12, 2023

DR. BELSITO: Priorities. So basically, we've just been asked to prioritize -- that's in admin, right?

DR. SNYDER: Yeah.

DR. KLAASSEN: Yes.

DR. BELSITO: So, since our March meeting we received communication from the FDA nominating ingredients for cause, specifically Toluene and Dibutyl Phthalate. So, we're going to be doing accelerated re-reviews on those. And then there was something here that I just want Monice or someone to clarify. So, it basically said that instead of just doing a re-review summary, we're going to fully open this or something?

MS. FIUME: So, are you talking about Toluene?

DR. BELSITO: Yeah.

DR. SNYDER: We never reviewed it before.

MS. FIUME: Well, it is on our list of items to be re-reviewed. It's currently on Christina's docket. Right, you have Toluene?

DR. BELSITO: Right. We reviewed both of them before.

MS. BURNETT: I think so. I don't know.

DR. SNYDER: Oh, that's the TPO. I was talking about TPO. Yeah.

DR. BELSITO: Right.

DR. SNYDER: I'm sorry, TPO is what I was talking about.

MS. FIUME: Right. TPO is the only on. Dibutyl Phthalate was just re-reviewed in 2017.

DR. BELSITO: Right.

MS. FIUME: But Toluene was scheduled for consideration for re-review this year, so you will be seeing that soon.

DR. BELSITO: Right. But it says, "The CIR will present the panel with a draft amended report on this ingredient instead of an abbreviated re-review document."

MS. FIUME: Okay. So instead of getting the table that you have been --

DR. BELSITO: Right. We are actually going to get a written document?

MS. FIUME: Assuming that you were going to accept FDA's request to reopen it.

DR. BELSITO: I think if FDA comes to us with a request for cause, we have to -- I don't know -- yeah.

MS. FIUME: Which is why you'll get an actual report person versus do you want to reopen? Here's the table of data that we found and then -- just taking that step out.

DR. BELSITO: Right, okay. So, we're going to -- yes, we're reopening Dibutyl Phthalate and Toluene for cause. And I think the third ingredient -- I mean, this is the type of stuff that I want to see happening. Something's going on in Europe, there's a concern about this material for reproductive toxicity, we need to be looking at it, number one. Number two, we've never even reviewed it. So, yes, I personally would like it added to the 2024 priority list.

DR. SNYDER: Agreed.

DR. KLAASSEN: It would be interesting to know why they wanted these first two chemicals. We don't -- why they want us to do Dibutyl Phthalate?

DR. BELSITO: Because it's a huge issue in endocrine disruption --

DR. KLAASSEN: Right, right.

DR. BELSITO: -- and --

DR. KLAASSEN: But I don't think there's any new data since the last time we did it, but maybe there is. And how about Toluene? I mean, I'm not against doing it, I'm just wondering. It'd be nice if they said why.

MS. FIUME: So, I'm looking at the memo and the email that was originally sent on March 20th, it's PDF Page 26. It just says that they're proposing it.

DR. BELSITO: Yeah. This is from Prashiela.

DR. KLAASSEN: Yeah. It says nothing really.

DR. BELSITO: Right.

MS. FIUME: Sorry, Priya has Toluene. So, Priya will be bringing that back probably in September.

MS. BURNETT: And Phthalates.

MS. FIUME: Yeah.

DR. BELSITO: I mean, both of them have gotten a lot of press, you know, bad press.

DR. KLAASSEN: Yeah, I know about the phthalates always do.

DR. BELSITO: Well, Toluene for carcinogenicity.

DR. RETTIE: So, the phthalates are the less (inaudible) issues, right?

DR. BELSITO: Right. I'm surprised that they are supposedly only one reported use because they used to be used in a lot of nail enamels. But I guess now everyone's using acrylic, so I don't know.

DR. KLAASSEN: Well, let's do them.

DR. SNYDER: Been there, done that.

DR. BELSITO: They're also used in a lot of fragranced products to hold the fragrance on the skin as a fixative, I think.

MS. KOWCZ: No.

DR. BELSITO: No?

MS. EISENMANN: Diethyl.

MS. KOWCZ: The Diethyl.

DR. BELSITO: Yeah, diethyl. Okay.

Cohen Team – June 12, 2023

DR. COHEN: All right. Now we're going to Priorities. Okay, for the 2024, draft priorities, we asked for propolis to be accelerated. Two other ingredients were initially proposed and were removed from the list and it was determined that cannabidiol should be reviewed singly.

The others are listed here, some with pretty high frequencies of use reported. Any comments on this? I mean, I don't know if we're going to have a really in depth conversation about this, are we?

DR. HELDRETH: I think that the main point was that FDA had actually asked for three additions to our prioritization. Two of these are request for accelerated rereviews, so Toluene and the Dibutyl Phthalate.

Now Toluene was actually already in our in-house pipeline. We were already working on it, so that one's definitely coming back your way. Dibutyl Phthalate, we haven't started working on yet. But now that FDA has requested it, we've went ahead and added it, unless the Panel has an objection to accelerating that be reviewed.

So, the only real question, I think, for the panel is do they want to add this Trimethybenzoyl Diphenylphosphine Oxide to the prioritization list for next year?

DR. SLAGA: I think we should accelerate it.

DR. COHEN: Yeah. That's a question to the Panel. We should add them.

DR. TILTON: Yeah, I agree.

DR. ROSS: New data. I agree.

DR. HELDRETH: Okay. That's easy.

DR. ROSS: Bart, could I ask you, what was the reason for -- or maybe you don't know -- why FDA nominated Toluene and the Dibutyl Phthalate? Was there a specific reason?

DR. HELDRETH: Prashiela stepped out?

DR. ANSELL: Our FDA person just --

DR. COHEN: We can ask her when she comes back.

DR. ROSS: Ah, okay.

DR. COHEN: These are plastics, the phthalates, right?

DR. HELDRETH: Plasticizer, yeah.

DR. ROSS: Yeah, they're phthalates. Toluene is a little different.

DR. COHEN: Yeah, Toluene is going to be a bit different.

DR. HELDRETH: Well, we've looked at the phthalates before.

DR. ROSS: Yeah.

DR. BERGFELD: And there's a lot of endocrine disruption with that group.

DR. COHEN: So, it's interesting. In 2017, the panel reaffirmed it, so this would be a real short cycle.

DR. HELDRETH: Right.

DR. COHEN: Prashiela, a question. No, no, no, it's okay. For the priority list, the FDA nominated some items, one was Toluene. Do you know why Toluene was nominated?

DR. MANGA: I'm going to have to get back to you on that one. Let me take a quick look at what we --

DR. COHEN: And the phthalates, the dibutyl phthalate?

DR. MANGA: I think there's just a lot of interest in phthalates right now. It's come up quite a bit. The Toluene is being used in a lot of nail products.

DR. ANSELL: Historically.

DR. MANGA: Historically.

DR. COHEN: Are you talking about the Toluene sulfonamide resins or just Toluene?

DR. ANSELL: No Toluene is a diluent.

DR. ROSS: I think Toluene is being reviewed quite a bit at IARC on its own, but also in connection with Benzene.

DR. ANSELL: Right. Also not used anymore, so.

DR. ROSS: Yes.

DR. ANSELL: But we fully support accelerating anything FDA ask us to.

DR. COHEN: We're good. Yeah. So are we.

DR. HELDRETH: Which is a question, I just wondered why they --

DR. BERGFELD: Actually, we really like it when they ask.

DR. ANSELL: Yes. More than support it, encourage it.

DR. MANGA: We appreciate that.

DR. COHEN: No, it's nice we're being paid attention to. And the other one was -- Annex 3 was a little more self-explanatory.

DR. BERGFELD: What was that?

DR. COHEN: The Trimethybenzoyl Diphenylphosphine Oxide.

DR. ROSS: Yeah it's more data. Yeah.

DR. HELDRETH: Yeah, it looks like there may be some repro concerns with that one.

DR. COHEN: Some? I didn't hear what you said.

DR. HELDRETH: Repro -- DART issues with that ingredient.

DR. COHEN: Repro. Okay. All right, so I think we're aligned on the priorities.

DR. BERGFELD: I think when we present this, it would be nice if you, the FDA, presented the reasons for bringing them forth.

DR. COHEN: Just like a sentence.

DR. BERGFELD: It would be very nice.

Full Panel – June 13, 2023

DR. BELSITO: So the FDA has asked us to move Toluene and Phthalates up for cause. And I would agree with doing that. And also, it was brought to our attention that a material that we haven't reviewed, trimethylbenzoyl dimethyl phosphine oxide, is being looked at by the European Chemical Agency, ECHA. And they're very concerned about the safety of this. It's a substance of very high concern (SVHC), and I think we should move that up on our Priority List as well.

And I think this is the type of thing that needs to be done, where we're monitoring what other safety organizations are looking at, perhaps, flagging ingredients that we weren't aware of. And we should continue to do this type of thing.

DR. BERGFELD: Any comments, Dr. Cohen?

DR. COHEN: No, I thought we might had heard from the FDA a little more why they were nominated.

DR. BERGFELD: Jan, do you want to talk about the nominations?

DR. HELDRETH: We also have Dr. Manga online.

DR. BERGFELD: Manga too?

DR. HELDRETH: She had to return to the office.

DR. MANGA: Hi, this is Prashiela. So these three ingredients came up because we've had a couple of inquiries about these being used in nails -- I'm sorry, I'm getting a bit of feedback from the room.

DR. BERGFELD: We can hear you.

DR. MANGA: So these ingredients have been noted particularly for the use in nail products. And that was why we were interested. And then, as Don mentioned, at least for the TPO, that is coming up as a new ingredient. We were concerned that it be reviewed given the other reviews that are going on.

Toluene is now one of the California Department of Toxic Substances Control products that effective January 1, 2023, nail products containing Toluene will become priority products. And, so, we felt that this was also one that needed to be looked at once again.

In terms of Dibutyl Phthalate, this is one which was included when FDA amended the food-additive regulations, to no longer provide for 25 plasticizers in various foods contact applications. They did this because the uses were abandoned, but given that this one was included in these amendments, we felt that it would be timely for CIR to review it as well.

DR. BERGFELD: Thank you very much. We're really appreciative of the FDA coming in and suggesting these particular ingredients.

DR. MANGA: Sure. Can I just make one other announcement quickly, please? Dr. Jannavi Srinivasan is there in person today, she was there yesterday. She will be serving as the FDA liaison when either Linda or I are not available. I just wanted to let you know that she'll be representing FDA when we're not available. Thank you.

DR. HELDRETH: Thank you, Prashiela.

DR. BERGFELD: Thank you.

MARCH 2024 MEETING – STRATEGY MEMO #1

Belsito Team – March 28, 2024

DR. BELSITO: Here we've got comments from Women's Voice of the Earth. That's under an Admin doc, right?

MS. BURNETT: Mm-hmm.

MS. FIUME: Yes.

DR. BELSITO: Yeah, Admin Dibutyl Phthalate. Women's Voices of the Earth, regarding this document, sent a lot of information about various state legislatures banning dibutyl phthalate and some of them also banning dimethyl and diethyl phthalate. The first question I had for you all is should we include these legislations that have passed and/or pending in our report? I didn't think we should. Comments? Allan? Paul? Curt?

DR. SNYDER: Yeah, my comment is similar to the one you just made, Don. We just need to report on the science. All of the other stuff is just kind of extraneous. I mean, we're aware of it and we try to see what their justification is if there's any science to support it, but that's -- I think we largely -- don't include that in our reports.

DR. BELSITO: Right. Curt and Allan, you're okay with that?

DR. KLAASSEN: I was just going to mention, we've often noted what's happening in Europe, for example, in our reports. I don't think we've ever said what was happening in various states. That's kind of a small difference maybe. Why do we say what's happening in Europe, like, this is banned in Europe? We will often say that, or, it's in this category in Europe. I don't feel strongly. I would just like to kind of point out a little bit of a --

MS. BURNETT: The European legislation usually has the scientific opinion attached. The Panel uses that to review what caused Europe to arrive at their conclusions. Whereas, the state legislations might not necessarily have science reports to go with it.

DR. RETTIE: All right. When we can append a citation to European material, then I think that's useful. State by state here, that would be very unwieldy unless we have a general acknowledgment that this is a contemporary issue of concern here.

DR. BELSITO: Although for toluene, I'll point out, we do talk about the California legislation.

MS. BURNETT: Right.

DR. BELSITO: We're going to need to be consistent here.

MS. BURNETT: With using -- and I think we did this in preparing the strategy memo, we did look at the California product database as they have concentrations listed that are not necessarily reported when we do the concentration of use survey. In that respect, we did use the California website to glean some numbers. I don't remember. I don't think in the memo that we put anything about the current status of being banned in California. I can't remember exactly. I do know we did use some of the numbers from the database.

DR. BELSITO: Yeah. When we get to that document, then, we'll have to be sure that we're not referencing California limits if our decision is not to reference state limits since they're not necessarily science-based. Is everyone happy with that?

DR. RETTIE: Yeah.

DR. BELSITO: Curt, Paul, Allan?

DR. SNYDER: Yes, I'm fine with that approach.

DR. RETTIE: Yes. Seems good.

DR. KLAASSEN: Fine.

DR. BELSITO: Okay. Then moving on with the other -- so without a use concentration for dibutyl phthalate in particular, and given the state regulations, the question is how would we handle this? My proposal is, first of all, that we keep dibutyl and dimethyl in this report, not just do diethyl phthalate because I don't think you can just do one as a stand-alone, and that we assume, as we typically have done, that the highest concentration that we're reporting is the limit for all of them. The concentration that we'll report for diethyl phthalate will be the maximum concentration in that product category for DMP and DBP. Is everyone hap- --

DR. SNYDER: Don?

DR. BELSITO: What?

DR. SNYDER: I'm sorry. I'm sorry. Sorry. My notes say that we had zero uses reported. We had one use at 0.1 percent in a foot powder. Is that correct?

MS. BURNETT: Diethyl phthalate has one reported use.

DR. SNYDER: Is that the foot powder?

MS. BURNETT: Yes.

DR. SNYDER: Yeah.

MS. BURNETT: No. That one is a hand and body skin product. The concentration of use, yes, is in the foot powder. There's one use reported in the VCRP and one concentration of use -- oh, two. No, there's several concentrations of use reported. The maximum is 0.12 in a foot powder. I'm sorry.

DR. SNYDER: Yeah. Okay, that's what I thought.

MS. BURNETT: Dr. Belsito, could you back up? You said you were leaning towards including dimethyl phthalate, but not including diethyl? So it'd just be two of the three?

DR. BELSITO: No, no. I'm in favor of including all three.

MS. BURNETT: All three? Okay, thank you.

DR. BELSITO: Paul? Curt? Allan?

DR. SNYDER: Yes, I'm fine with that.

DR. RETTIE: Yeah, that's fine. The ethyl and methyl are not in Annex 2 in Europe. The butyl is. Then there's a dozen of others, of the longer chain ones that are in Annex 2. I was kind of curious about how they split that out, but I'm fine with looking at all three.

DR. BELSITO: Curt, are you fine with keeping all three?

DR. KLAASSEN: Yeah, I guess. It's more complete that way and the way we usually look at these, although I was thinking, as far as economy of work, if we would just look at the endocrine and reproductive effects of dibutyl phthalate, just look at that aspect of it, which is really the essence of the concern. I think that's kind of not the way we usually do things, so I think if it's best if we probably stick the three of them together and do them again and again and again.

DR. BELSITO: Okay. Just to remind everyone, basically, this was an FDA request only for dibutyl phthalate. I think that it's hard for us to assess dibutyl phthalate without including data from diethyl phthalate in particular.

MS. BURNETT: Right.

DR. BELSITO: I would just go with all three.

MS. BURNETT: Yep. Much of the published literature is either a combination of two of them or all three or even more than that.

DR. BELSITO: Okay. Then there were a couple of questions. We've resolved the first about including all three. Then there was a question about do we have any preference how to organize all of the studies on endocrine reproductive and developmental effects? Should the endocrine studies be subheaded under Developmental and Reproductive Toxicity? Or should the studies be classified in the stand-alone section? I thought they should be subheadings of DART. Anyone else have thoughts on that?

DR. SNYDER: I think it can go either way. The writer needs to see how it's easiest for them to organize it.

DR. BELSITO: Christina, so then it's going back to you. What are your recommendations?

MS. BURNETT: I think subheadings under DART make more sense because the way some of the studies that I've seen so far have been set up, you'll start off as a traditional DART. Then they'll focus in on testicular development or the anal-genital distance, so it will be like a two-part study. Previous iterations of this report, the data was kind of patch-worked throughout the report. For me, it was hard to follow in order to summarize it. I feel that everything under DART makes sense and it is interrelated. Again, I'll defer to you.

DR. SNYDER: Well, I think it's how you think you can organize it best for us to review.

MS. BURNETT: Okay.

DR. SNYDER: I think you summarized it there. I'm fine with that.

MS. BURNETT: Right. It's going to be -- I assume, since there's so much data, that tables would be very helpful. It would be subheaded tables under DART. I mean, obviously, whatever I put together, we'll review at the next iteration with all the data, and you can tell me whether it worked or not.

DR. BELSITO: Okay. And then the quest- --

DR. SNYDER: I have faith in you, Christina. I have faith in you.

MS. BURNETT: Thank you.

MS. FIUME: We all do.

DR. SNYDER: Yes.

MS. BURNETT: I will say that I am enlisting help with Jinqiu on this one because some of it's a little over my head this time.

DR. SNYDER: I just want to know how you draw the short straw all the time on getting these difficult ones.

MS. BURNETT: I don't know. Then with all this new data, there's a lot of zebra fish studies and different methodologies that we have not included before. Since we are interested in alternative methodology, I am not -- I mean, I get the gist of these zebra fish studies, but I don't know what the important information of methodology and outcomes are.

DR. BELSITO: I've never heard of that use of zebra fish, so I actually did a -- not a deep dive, but a medium dive into zebra fish and DART. To summarize, what I got was the high structural similarities of the reproductive axis of zebra fish as

compared to humans has resulted in zebra fish often being utilized as the model in the assessment of reproductive toxicity of environmental chemicals, including herbicides.

MS. BURNETT: Right.

DR. BELSITO: A lot of the zebra fish studies were done by the EPA. Without seeing the data, I think we can use the zebra fish data for DART endpoint, but open it up to other members of my panel.

DR. RETTIE: Yeah, I'd be curious to hear -- I was just curious to hear what Paul and Curt had to say about zebra fish studies for DART. I don't know too much about that.

DR. SNYDER: Well, I think it's been a pretty much high level (audio skip) way to look for a signal. Now, they're -- because the model is showing some utility, it's being applied in other instances. I think we're obligated to have a look-see --

MS. BURNETT: Okay.

DR. SNYDER: -- and see if it matches up with other data. We have a lot of data. It's not like we're only going to rely on these non-mammalian studies.

MS. BURNETT: Right.

DR. SNYDER: If it collaborates what we already have, I think we need to look at some of it, yeah.

MS. BURNETT: Okay. Should I just focus on the zebra fish studies that give DART data? Or are there any other endpoints that they're possibly are useful or just ignore it, just focus on the DART?

DR. BELSITO: I would just do DART data.

DR. SNYDER: Yeah, just endocrine effects, yes.

MS. BURNETT: Okay.

MS. FIUME: Paul, just to take that --

DR. BELSITO: Curt, you're okay with the zebra?

MS. FIUME: Oh, this is Monice. I was --

DR. KLAASSEN: Yes, that's fine.

MS. FIUME: I was just going to say, to take it a step further than for generally all reports, if we find studies on zebra fish, we should bring them into the documents for the Panel to take a look at?

DR. SNYDER: Yeah, well, I think so, especially if it's the ingredient --

DR. BELSITO: Yeah.

DR. SNYDER: -- yeah, for repro, yeah.

MS. FIUME: Okay. Thank you.

DR. SNYDER: Do you agree with that, Don?

DR. BELSITO: Yes.

MS. BURNETT: Yeah. If we -- not to skip back to the previous report, but in the pyrogallol table, I do know that there were catfish studies, which were new. We've not included catfish studies. In this case they were acute and short-term toxicity studies, not DART studies.

DR. SNYDER: Yeah, that's a different issue for me. Those are different, yeah.

MS. BURNETT: Okay. I'll include if for now. Then you can tell me, once you see that first draft, whether you like it or not.

DR. BELSITO: Yeah.

DR. SNYDER: Sure.

DR. BELSITO: Also, my recommendation for particularly the DART data since there's so much of it out there is to keep as much in tablative format and limit the text.

MS. BURNETT: Okay.

DR. BELSITO: Basically, refer us to a table.

MS. BURNETT: Okay. Yep.

DR. BELSITO: Not say too much in the text.

MS. BURNETT: Okay. That kind of makes some things easier.

DR. BELSITO: On PDF page 3, where you say, "For further consideration, state CIR staff have calculated the dermal exposure level of diethyl phthalate from various product categories based on a Council survey," --

MS. BURNETT: Yes, that was --

DR. BELSITO: -- my question is why --

MS. BURNETT: I'm sorry. Go ahead.

DR. BELSITO: Why are we using the survey when we have California data suggesting a higher use? Shouldn't we be taking a more conservative approach and using the highest level reported in the California data.

MS. BURNETT: I believe Jinqiu Zhu did calculate a second one using that. If you go past all the calculations on PDF 4, he did add on a sentence saying, "In light of maximum use concentration reported to the California product database, the calculated MOS 86,206 for the bath products at one percent --

DR. BELSITO: Okay.

MS. BURNETT: -- and 10,489 for skincare products at 0.2 percent."

DR. BELSITO: Yeah, I know, but that's sort of -- I mean, to me, it's sort of a little -- I mean, it's important, but it -- I almost would rather not have to make that statement --

MS. BURNETT: Right.

DR. BELSITO: -- and basically say at the beginning, up front, that we have a level from the Council survey. However, we had information from California of higher use in these product categories. As a conservative estimate, we're using this higher level of use.

MS. BURNETT: Okay.

DR. BELSITO: Then go through all the data --

MS. BURNETT: All right.

DR. BELSITO: -- rather than just adding a sentence at the end.

MS. BURNETT: Okay. We'll flip it. We'll recalculate it and show the calculations using the California data.

DR. BELSITO: Right.

MS. BURNETT: Okay. Yeah.

DR. BELSITO: Then the next one was, does the Panel prefer to request survey data on additional phthalates and perform further calculations? This survey was just on diethyl phthalate, right?

MS. BURNETT: Part of this calculation that Jinqiu Zhu recreated was from the re-review from 20- -- that was published in 2005. That's the same calculation, I think, was in there or was similar to that that the Panel put into the re-review summary.

DR. BELSITO: Okay.

MS. BURNETT: Some of the assumptions and stuff were based on what was done then. It might not be what is currently done in our margin of safety calculation. Jinqiu Zhu can update that if you like.

DR. BELSITO: Yeah.

DR. KLAASSEN: I would like to emphasize again, I did not like to see five significant values for 28,846.

MS. BURNETT: Okay.

DR. KLAASSEN: Let's change that to at least 28,800.

MS. BURNETT: Okay.

DR. RETTIE: Three significant figures.

MS. FIUME: Right, Christina, because we have the survey data for those other two phthalates.

MS. BURNETT: Yes, we already --

MS. FIUME: Yeah.

MS. BURNETT: Yes. We already -- or Council provided that a little while ago. We could ask them, I suppose, if there's any updates to it.

MS. FIUME: It was part of the 2023 survey, correct?

MS. BURNETT: Yeah.

MS. FIUME: Yeah.

MS. BURNETT: It's probably as up-to-date as we're going to get.

MS. FIUME: Because was that sentence to ask were there any other additional phthalates? Or is it still referring to the two we're adding in?

MS. BURNETT: I think, internally, we had that discussion about the -- because the very last re-review included benzyl butyl phthalate -- or butyl benzyl phthalate. We're not including that in this query because it's not quite structurally the same.

MS. FIUME: Okay.

MS. BURNETT: That might have been a -- we might not have updated it from that decision.

DR. BELSITO: Okay, yeah. I mean, I think we should just do the phthalates we're reviewing, not other phthalates.

MS. BURNETT: Right.

DR. BELSITO: Okay. We're clear on where we're going on this, Christina?

MS. BURNETT: I believe so.

DR. KLAASSEN: There's a lot of data out there.

MS. BURNETT: Oh, I know. I also have two large archive boxes sitting in my house from the previous reviews with all the data in that too, so, yeah, there's a lot.

DR. BELSITO: Okay. Should be fun.

MS. BURNETT: Mm.

DR. BELSITO: Any other comments on phthalates for Christina before we move on?

DR. SNYDER: None for me.

DR. RETTIE: All good.

DR. KLAASSEN: I'm good.

MS. BURNETT: Thank you.

DR. BELSITO: Okay.

MS. BURNETT: Thank you.

DR. BELSITO: Okie doke.

Cohen Team – March 28, 2024

DR. COHEN: Okay. The last one is dibutyl phthalate. So, the FDA requested dibutyl phthalate -- for us to look at this -- and we got a number of questions back from the staff that we can go through on dibutyl phthalate, but before I open it, I just wanted some clarification because it seems like we could spend a lot of time on this. Is it true, did PCPC agree on banning dibutyl phthalate for the Maryland House Bill 643 that banned dibutyl phthalate amongst a series of other chemicals on Bill 643?

And the reason I'm asking is, like, it's like a patient that's DNR. You don't start doing cat scans and biopsies on it when there's a DNR order on it. So, if PCPC has signed a DNR order on dibutyl phthalate do we go through the exercise of reviewing this a great deal?

DR. EISENMANN: We are no longer supporting this ingredient, yes.

DR. COHEN: Right. And I saw the questions to us that we need to be impartial, and we need to look at the scientific data and to what end? Why are we doing this? Because I did pull the PCPC note to the Maryland legislature, and I looked at the House Bill 643 and it's like they don't support the product anymore. Why can't we just indicate that we're no longer -- use not supported at that point. I know there's more to it than that but that's my visceral response.

DR. EISENMANN: No, I thought that it would be nice to have another category for re-reviews that said something like publish some kind of a statement that says this ingredient is no longer in use. This is a historical, we are no longer going to -- so in other words, your old conclusions don't stand anymore because it's hard when you've published reports, what to do the

original reports that you're not going to review it anymore. You're not going to make a determination whether it's safe or not and you're just not going to review it anymore because it's not used. But there isn't a process yet for you guys to do that.

DR. BERGFELD: We could send a note to the steering committee and request that that be considered, and we can put this on hold.

DR. COHEN: Table it. There's a lot of very important questions from the CIR staff here and a lot of detail and there's a margin of safety. But, again, I don't want us to spin our wheels for no reason. It would be nice to have another category where our recommendations are withdrawn, and we will not re-review. No longer supporting use.

DR. HELDRETH: So, would we want to make it similar to an insufficient data conclusion that's based on zero use where we eventually are saying use not supported? Because, I mean, we're not saying unsafe, we're just saying we don't have enough information to support the safety of this.

DR. EISENMANN: Well, I'm not sure you're reviewing the safety anymore. I mean, we're not asking you to look at the new data, you're just -- it's not used anymore.

DR. HELDRETH: And that's the same situation with insufficient data, zero use.

DR. COHEN: But there's a lot of data on this.

DR. EISENMANN: Right. There's a lot of data on it and one thing -- we really should not use data on diethyl phthalate to estimate exposure for dibutyl phthalate. The uses for dibutyl phthalate were totally different than how diethyl phthalate was ever used so you really don't really have any use information to base anything. You should not use the dye -- I think that was one of the questions.

DR. COHEN: Yes. It is one of the questions.

DR. HELDRETH: Right. So, we have a lot of data, but we are insufficient for use and concentration data.

DR. EISENMANN: Correct. Which is why I hate to see you spend a lot of time on it because you're never going to get that information because they're not using it anymore.

DR. HELDRETH: Right.

DR. EISENMANN: So, there's nothing to hang your hat. There's no exposure to hang your hat on for this ingredient anymore.

DR. ROSS: It's not unusual that we would look at ingredients that we don't have concentrations on but what is very unusual with this one is that it's not being supported anymore, which is now is common sense.

DR. COHEN: That's the point.

DR. ROSS: Yeah.

DR. COHEN: Look, we just finished one that has one use and we're going to go through the whole exercise, right? And so, in this case we can't say there's no data. There's an abundance of data and we would review that. So, if we went out with an IDA, what's our IDA? We want concentration of use and use, we don't get it, but then we still have this massive report to review at the next iteration. So, I just didn't want to -- that's why I pulled the PCPC letter and the House Bill because I thought this was very different.

And I don't think we need to sort of fall on our sword that we can review the data scientifically for absolutely no practical purpose.

DR. HELDRETH: Right. Right. So, then would the request be to the Steering Committee that when we get things like this in front of us the Panel can simply say the use of this is not supported. I mean, it goes into that same bucket. And then, if somebody decides later, ten years from now, I want to use dibutyl phthalate then they're left with the impression well, I need to provide data and information to the Panel to reopen that. I mean, it's the same kind of situation.

DR. BERGFELD: I think we would have to ask the Steering Committee for specific declaration here.

DR. COHEN: Yeah.

DR. HELDRETH: Right.

DR. BERGFELD: And have it call it something specific and then have a mechanism developed of what would be said and what would be retracted. So, I think we have to just table this until we get an answer in the Steering Committee if that would be allowable.

DR. HELDRETH: Yeah.

DR. ROSS: It's certainly interesting points from WVE with all the pending state legislature actions that's compounded. Maybe you want to bring in those at some point, too. But anyway, I totally agree with your proposed way forward, Wilma, I think it's a good way to go.

DR. COHEN: And the WVE was instrumental in the way we adjudicated this one in my opinion because I went and looked at all those links, the state laws, and so good. So, we're going to table this.

DR. HELDRETH: Can I ask since we have FDA representatives here, would it be useful for our Steering Committee to agree to and our Panel to eventually issue that the use is not supported for this because FDA was the one that asked for us to look at dibutyl phthalate again. So, I want to know, is this responsive in the way FDA was hoping for if we moved forward with that?

DR. MANGA: Bart, I'd probably have to take this back and maybe I can give you an answer tomorrow.

DR. HELDRETH: Yeah. That would be great. We want to have good customer service. You asked for this and that's why we're looking at this.

DR. MANGA: No, I appreciate that, and I appreciate that you guys took this up. So, yeah, happy to provide you with an answer tomorrow.

DR. HELDRETH: Wonderful. Thank you, Prasheila.

DR. MANGA: Sure.

DR. COHEN: Okay.

DR. BERGFELD: Could I ask Bart a question?

DR. HELDRETH: Sure.

DR. BERGFELD: Bart, I don't have at home the Steering Committee's sort of document that we have. I wonder if you could maybe copy the part where we might fit this in and just include it in some kind of mailing tonight?

DR. HELDRETH: Oh, you mean get this out to the Steering Committee tonight?

DR. BERGFELD: No, not tonight. To the Panel where this might fit. It's quite a document that gives us great guidance and we'd be looking at some kind of report that allows it to go as use not supported where that would fit. What that'd be called and where that would fit.

DR. HELDRETH: So, you mean the excerpt from the CIR procedures? Yeah, yeah. I can put something like that together and I can even present it in my report to the Panel tomorrow if you would prefer that.

DR. BERGFELD: That'd be great. That would really be great because it gives just another mechanism to do with something like this.

DR. HELDRETH: Okay. I'll do that.

DR. COHEN: There used to be something in the resource about the types of conclusions we had, I remember.

DR. BERGFELD: Yeah, it's documented in pages. I just don't have it here to look at.

DR. COHEN: Yeah, no. I just couldn't find it immediately. Okay.

DR. HELDRETH: Yeah, our conclusion types are safe as used, safe with qualifications, unsafe, and then there's insufficient data that breaks it into two pieces currently. If there are uses and we get no data response from two years of a final safety assessment, that's use not supported. If there are no reported uses after two years on an insufficient data conclusion then that's converted to zero use. And when we get a zero use one, we never look at that again unless somebody brings us data to say reopen it.

DR. BERGFELD: So, it falls somewhere under that category.

DR. HELDRETH: Probably. Probably. I mean, I think that's what we would bring to the Steering Committee. Do you want to use this exactly or do you want another separate procedure that fits right next to this in a similar fashion?

DR. COHEN: That would go under -- that would mean that we're bringing this to a draft report and issuing an IDA, right?

DR. BERGFELD: Not necessarily.

DR. HELDRETH: No. I mean, ideally this could be brought to you as one of these abbreviated re-review documents like we just did for pyrogallol. You could say, hey, nobody's using this. There's no support for this. Why should we move forward with it? And if the Steering Committee provides us with a process to go forward that'd be the end of it. You'd just kick it into that bucket, and we wouldn't think about it again until somebody came forward and said, hey, hey. I'm using this and I have data.

DR. COHEN: So, under the current mechanism what could we do? What could we say, there's no use --

DR. HELDRETH: Yeah. At the current mechanism with just those conclusions that we have available for us, we would have to trudge through to a final report with an insufficient data conclusion, wait two years --

DR. COHEN: No, no. Forget it.

DR. HELDRETH: Right. Right. So maybe we can draw a new pathway on that little flow chart we brought up earlier. It goes right from that bucket right from the abbreviated re-review.

DR. COHEN: Okay. I heard the read across discussion, at least, the back half of it, and there's no point in reiterating it now and it'll already be discussed tomorrow. Is there anything --

DR. BERGFELD: Did you want me to summarize that I have here before me?

DR. COHEN: Sure, sure.

DR. BERGFELD: Okay. The read across working group met. It's composed now -- (audio skip).

DR. COHEN: We lost you. We lost your audio for a second. For a second.

DR. BERGFELD: Okay. I said the read across working group met and the new chair will be Allan Rettie. Members of the working group is Curt Klassen, David Ross, and Susan Tilton. Ad Hoc is Don Bjerke. Consultant to be invited is Dan Lieber. The movement or the summary of what they will -- examination of the need for a read across to collect the CIR past read across documents and see how we've handled those. And three, look at the read across documents that Europe and the reach group uses.

And four, if the CIR has a read across document presently, which is a living document that will ultimately have some changes to it. So that's what we did.

DR. COHEN: Nice. It seemed like that thing could've gone on for hours, but it was more succinct. All right, so are we calling it a day?

DR. BERGFELD: We are.

Full Panel – March 29, 2024

DR. BELSITO: Yeah. So, this was brought up for cause by the FDA for an earlier review and fast tracked. And several questions have been posed to us. First, does the panel want to include, along with dibutyl phthalate, diethyl and dimethyl phthalate in our expedited review. And my team felt we should include these because it would be difficult to evaluate dibutyl phthalate in the absence of information from the other phthalates.

Did we have any preference on how to organize the myriad of endocrine and DART studies, and we felt that it should be as much as possible in a tabular form rather than in written form. Whether we would accept studies from zebrafish. And in my review, actually the zebrafish have been said to have high structural similarities with the reproductive axis of humans and they're utilized as a model so we would accept that.

Apparently, there were some studies on catfish that we decided we would not accept. And that's where we were to include diethyl and dimethyl phthalate along with a dibutyl phthalate re-review and go from there.

DR. COHEN: So, Don, we took a completely different view on this. Ultimately, we thought we would table this issue, primarily because dibutyl phthalate has no reported uses. In March of 2021, the PCPC wrote a letter in favor of Maryland House Bill 643 banning dibutyl phthalate. And so, we have no uses, we have no support by industry for the use of the product, and we thought that taking this memorandum forward to do a really comprehensive report on this would be a complete waste of time.

And so, we thought if we table dibutyl phthalate until Cosmetic Direct comes around, perhaps, to see if there's any uses, we can consider doing that but in the face of it not being used, we wanted to just spare the workload in just not doing this.

DR. BELSITO: I guess my only concern, and I'll let Bart and/or Monice, and FDA comment. I mean, this came to us as a request from the FDA.

DR. COHEN: We asked them about it, and we were hoping to get a response today about that.

DR. BELSITO: I just feel like we've been asked to do it. And then to turn around and say we're going to table it, is probably not the right thing. And I guess this is now the time to raise the discussion that was pointed out at the beginning of our meeting, as to if there are no reported uses for a material it goes into a new category. So perhaps we should have that discussion now.

And if this were to go forward, you know, that MoCRA effort for mandatory is supposably July. But we probably wouldn't get this back until September or December anyway. In which case, at that point, hopefully, if it were to come back in December, we would have definitive information via the MoCRA regulations of, you know, is there existing use out there. If we table it, then we're no closer to getting it on the agenda in September or December.

DR. BERGFELD: Comment, David?

DR. COHEN: Well I was hoping to get a comment from FDA on what they felt about moving this along. Someone has raised their hand, I just can't tell who it is.

DR. MANGA: Hey, David, it's Prashiela. Hey, good morning, everyone. So, FDA would be okay with tabling it. Just to give you some perspective of where we are with Cosmetics Direct, as was mentioned, July is the deadline. We're still working on the system. As you know, it was an unfunded mandate so we're working in a resource-limited situation. We are developing our backend systems, and we hope to have that up and running as soon as possible. So, I can't really give you a timeline on when we'd be able to get you the data. That's sort of where we are.

In terms of tabling it, we'd be okay with that. If in terms of putting it into a new category, we'd be interested to hear what that new category or moving it into sort of a hold status, what that would look like. And I'd be happy to comment once we've had that discussion.

DR. BERGFELD: Bart, can you add to this?

DR. HELDRETH: Sure, absolutely. So, as I discussed in my Director's report, there was some discussion on the Cohen team about petitioning the CIR Steering Committee to create a new category for re-reviews in general, not just the dibutyl phthalate. But re-reviews in general where the evidence in front of the panel demonstrates that it's not in use, and essentially discontinued use in the U.S. since the last time they reviewed it. And the idea being that this is, essentially, insufficient data points. And if that's not available, and it seems like listening to industry it looks like it will not become available, then should the panel really carry on with the whole process of opening up a draft report and going through all the way to a final report.

Or could the Steering Committee approve a new category for such incidences, where a re-review summary would come out with a new conclusion of use not supported. The verbiage, of course, can be different than that, but the idea is that we don't have the data to support the safety because we don't know how it's used and what concentration it's used at.

DR. COHEN: Don, I mean, so I haven't seen before where the PCPC has been supporting a ban on a product, and of course no uses, and we would go ahead and deliberate on this. For what endpoint? You know, the industry themselves have supported the Maryland ban.

DR. BERGFELD: Don, can you respond?

DR. BELSITO: Yeah. I mean, I guess, my only response to that is it would've been nice, I think, for PCPC to at least notify the Panel that they were going to do that.

DR. BERGFELD: Well, I think they did it before we even started on this.

DR. COHEN: This is from 2021.

DR. BELSITO: No, I understand. As a member of the Expert Panel for Cosmetic Ingredient Safety, I just think it would be nice if we were all informed when PCPC is going to issue statements in support of banning cosmetic products that we, as a panel, had previously said were safe as used.

DR. BERGFELD: We can write another letter requesting that in the future. Mm-hmm. I think we need to do that officially. It's an expectation that we have.

DR. COHEN: That's a good point, Don.

DR. BELSITO: Beyond that, if the FDA who brought this petition to us is okay with us tabling it, I think it's fine. On the other hand I think that it's something that, if anything, we need to give our imprimatur to the actions that PCPC took and bring this up. Regardless, I think that diethyl phthalate is still used and there's a lot of controversy about that. And so I think that the phthalates in general should undergo an expedited review.

And I would just like to move it forward to look to getting it on the December agenda. Hopefully by that time we'll have -- Cosmetic Direct will know exactly how many of these phthalates are being currently used and what the current concentrations are, and we can come out with an informed decision.

DR. COHEN: Yeah.

DR. BERGFELD: So, what I'm hearing you say is you don't like the word table? You would rather have the word--

DR. BELSITO: Yeah, because that puts it in limbo and we're no further along.

DR. BERGFELD: Yeah.

DR. COHEN: Well, but we're tabling it pending perhaps a new category. And I certainly support what you're saying about looking at the other phthalates, as long as it didn't contain dibutyl phthalates in it at this point. Because it's just going to create a tremendous amount of work for no useful end product.

DR. BELSITO: Okay. I mean, Paul, Curt?

DR. SNYDER: I think we have to keep in mind largely what we do is for the manufacturers, and we have a report out there that says it's safe as used. And that's not withstanding an issue. So even if we table it, they're going to defer to the last report, and it says safe as used. So, I think, I'm not quite certain, it has to be very clear as to why we're tabling.

As usual, I'm supportive of Don and I don't like to leave these things hanging. Let's just keep moving along and get them off our plate instead of piling our plate up with all these loose ends. And if we can move it along, I think we should do that.

DR. BERGFELD: Well, we can postpone until we have the Cosmetic Direct information. And supposedly, we might have some of that in December.

DR. COHEN: So, Paul, I certainly understand what you're saying, but if we move this along the staff now starts writing a draft report on this, right? So that I don't want to do. Number two, what we're looking to have is use not supported which resends the old one, right, we just can't do that right now.

DR. SNYDER: I guess I'd be more supportive of that if we knew it was going to be in a relatively short timeline. I just don't want this to linger out there and then kind of get lost in the weeds and then -- you know what I mean? We've got to just bring these things to fruition.

DR. BERGFELD: Bart?

DR. HELDRETH: Since, instead of having a re-review/abbreviated summary with a question to open or not or a draft report that we're proceeding in the process here. Since we have only a strategy memo here, I think maybe I'm hearing that the Panel has some consensus that if it came back in December, with the all likelihood of Cosmetics Direct being able to provide us with some more sound numbers on frequency of use at the very least, that that would be good timing.

So, we could just agree to -- if it is truly the Panel's consensus to adding these two other phthalates, adding the endocrine and DART end tables, looking at the zebrafish and not looking to the catfish studies, and including that Cosmetic Direct data that you would see a draft amended report on these three ingredients in December. So, we don't have to worry about tabling or not, it's just a matter of that will be the timing of it.

DR. ROSS: Bart, could I ask a question? How quickly do you think we'd get an answer back on the new category not supported? I mean, would that be next week, next month, three months' time? I mean, what's the timeline?

DR. HELDRETH: You're talking about the petition to the Steering Committee?

DR. ROSS: Yeah.

DR. HELDRETH: So, first we'd have to schedule a meeting of the Steering Committee which doesn't always happen very quickly. And I was hoping to have a meeting of that full Steering Committee instead of just posing a question on them. So, yeah, that would likely be somewhere around the September meeting, I would guess, we would be able to get something scheduled.

DR. ROSS: Okay.

DR. BERGFELD: I think it's two-prong here. We're going to go forward with this other category with the Steering Committee, that's a given. The other part is do we go and update this draft, as proposed by Bart, and nothing extra, and put it on the December schedule for review? Thinking that we will get the Cosmetic Direct information about use and frequency, and perhaps at that time we'll also have this other category that we could possibly use.

DR. BELSITO: I mean, first of all, we don't even need that other category by December, because it's going to be our first go around, right? And then we're going to have another 60 days comment period. So, if we start this in December, my best guestimate is we're not going to be signing off on this until June of 2025.

DR. BERGFELD: Probably.

DR. COHEN: So, I'm just going through this mind exercise. We get Cosmetic Direct back and there's some uses there. We have PCPC supporting a ban of the product that some states have enacted already. And we're going to go ahead and issue a safe as used if we --

DR. BELSITO: We don't know what we're going to issue yet, David. We've got to look at all the new endocrine data. Right now, we have a safe as used conclusion for dibutyl phthalate.

DR. COHEN: I understand that. I understand. I'm just looking at the practical aspects of this.

DR. BELSITO: We'll have to struggle that when we look at the data, right? We haven't looked at all the data yet.

DR. COHEN: The question is, is the assembly of the data for dibutyl phthalate a reasonable thing to have the staff and us do? That's what I'm --

DR. BELSITO: Yes. Because just for the reason you said, PCPC has supported the ban and yet our current document says it's safe as used.

DR. COHEN: That's why if we have another option, we can finish this without all that.

DR. BELSITO: We can't exercise that as an option without reviewing the data.

DR. ROSS: You don't have that category yet anyway.

DR. COHEN: We don't.

DR. ROSS: Yeah. So that's the issue. I mean, you could exercise (audio skip) .

DR. BELSITO: We don't have the absolute data that it's not used.

DR. ROSS: It's not supported. The use isn't supported, I guess, that's a different conclusion as you pointed out.

DR. COHEN: Right. So right now, we have zero uses for dibutyl phthalate and dimethyl phthalate.

DR. BERGFELD: Other than the workload that you're concerned about, what is the other issue here?

DR. COHEN: Well, number one, the workload is not an insignificant issue with an item like this. And two, we go through the exercise and regardless of what we find on this -- if we find that we can support its use, it seems silly to me, right, that we would support its use in a situation where industry has recommended a ban. Okay. And so that's incongruous to me.

DR. BELSITO: How do you know we're going to support the use, David? We've got to look at the new data. We have to look to see what the concentration of the use is. Right now, it's a voluntary system. And we can't assume it's zero just because someone hasn't voluntarily reported that they're using it.

DR. COHEN: Okay.

DR. ROSS: This gets back to the mandatory reporting. We're sort of winding ourselves around the axel again here with respect to coming back to that same point. But if we're not going to have that -- it sounded like we're not going to have that, necessarily, even in December.

DR. COHEN: Can I go back to --

DR. BERGFELD: We will have why the PCPC banned it, they should be giving us that information. What did they put together to make that decision? What was the information?

DR. COHEN: That would be helpful, but I've been sort of countered with precedent issues at this meeting today. And so, can you give me a precedent issue where industry has supported a ban on a product, and we've gone ahead and decided to adjudicate it and come up with a decision on it.

DR. SNYDER: My only comment to that, is we're the expert Panel for the safety of cosmetic ingredients, we're the driver. And so we need to do what we think is best in our expert opinion in moving these ingredients along and what stays and how we do it. I think we sometimes lose track of that, and we get influenced by all these outsiders and things. But we, ultimately -- the Panel -- needs to decide what we think is in the best interest for the safety of these ingredients for the consumer.

DR. ROSS: Yeah. I agree with that.

DR. COHEN: I agree with that.

DR. BERGFELD: We still need to know the data or the information regarding the PCPC statement, why they did it and what drove it. We just need to know. And we need a letter to them saying when they do such things they should at least announce it to us and give us this information.

I see we're sort of at an impasse right now and I think this is only a strategy type of discussion. So, Bart, what do you think the strategy is?

DR. HELDRETH: Yeah. Since it's a strategy memo it really just comes down to a consensus of the Panel for us to move forward. I think what I'm hearing is that we should proceed with a number of things. We should proceed with waiting, to some extent, until we get the Cosmetic Direct data to come in and see if there are uses or not. And once that information is available, proceed with a draft amended report for dibutyl phthalate/dimethyl phthalate/diethyl phthalate, including some of the data and categorization of the data as was questioned in the strategy memo.

But then at the same time, also, petitioning the Steering Committee about the future use of a new conclusion category for re-reviews of ingredients that are not in use. And a letter to industry requesting some explanation of why they were requesting this ban in the state of Maryland, and to communicate with this Panel about future motions in such directions.

DR. COHEN: Yeah.

DR. BELSITO: I agree.

DR. BERGFELD: I think that's right on. Anyone disagree with that?

DR. ROSS: Just some editorial addition there, Bart. You know, scientific rationale for these bans, which it would allow us to come to some balanced opinion when we actually see all of the data. In our team meetings yesterday, just getting back to one issue, there was some discussion and I think it came from PCPC, I may be wrong there. But to not include the additional phthalates. But that's where we have concentration of use data. So I had, actually, in my notes to include them, but there was some discussions there not to include them.

DR. EISENMANN: The discussion is not to use the use --

MS. KOWCZ: in read across.

DR. EISENMANN: Not to use the use information for diethyl for dibutyl because historically dibutyl was used very differently.

DR. ROSS: So, it's not, not include them -- using the double negative. It's just --

MS. KOWCZ: Yes, we just wanted to make sure that it's just the singular ingredient, Dr. Ross.

DR. ROSS: Okay.

MS. KOWCZ: And I just wanted to reiterate real quick that a lot of the times the decisions, as you've said, are not scientifically based. The bans are -- a lot of times we fight for not having ingredients banned because the science speaks otherwise. But we lose the battle, and so we'll definitely take that back to our internal staff and we'll definitely try to do better on the legislative piece. Because, Bart, we've also not had a Steering Committee in, I think, two years, so I think we're definitely due for that. And so maybe this is a good time to discuss that as well.

We are very open with what we can and cannot share. And a lot of times, as you said, Dr. Ross, the bans are not based on science. We're not happy about them either. Just wanted to share that point. Thank you for letting me say that.

DR. ROSS: Excellent comments. And I think we'd like to see the science behind those bans. And since I have to wade through a lot of those data with respect to the endocrine, I think for me it would be best as a separate section just after the DART. Many, but not all of the endocrine effects are repro. So, you do have separate ones.

I think it's best to make it a separate section. And I think it was Paul commented, or Don commented, that they wanted to see that in tabular form, and I'm totally in agreement with that. It's an easier read. So.

DR. BERGFELD: All right. I think we've done our duty here. I think we're going to move on now.

JUNE 2025 MEETING – STRATEGY MEMO #2

Belsito Team – June 9, 2025

DR. SNYDER: All right. We'll move on to the Phthalates. The Phthalates were brought up in the 2024 Priorities List in the 1985 report. They were safe for topical application. In 2002, we reaffirmed that conclusion. In 2012, we looked at it, we did not reopen.

In 2024, due to a large number of new studies with repro and developmental effects, endocrine effects, we've now opened this up. And it's on Admin Page 9 of 19. The question is, do we want to have Dimethyl Phthalate as a separate re-review proposal document, or would you prefer to have this ingredient in a safety assessment with Dibutyl and Diethyl Phthalate? I think that's the question, right?

DR. BELSITO: Well, no, there was no reported uses, right? The question was whether to drop it?

DR. SNYDER: Drop "it" as in which one, the Dimethyl?

DR. BELSITO: Yeah. Wasn't that the question?

DR. HELDRETH: Yeah, the idea was, since we're here looking at these again, and there's no reported uses for the Dimethyl phthalate, and it seems like this is something that's being abandoned altogether, why not just kick it out into a use not supported re-review summary?

DR. BELSITO: My comment on that, Bart, was, if we can read across, why are we getting rid of it? Because number one, with MoCRA, we know that not all companies have to report, right? So it could be being used, just not reported. And number two, that with the VCRP, it was all voluntary, so it could be being used.

So, we really don't know that it's not used. We just don't have reports in the RLD that it's used.

DR. SNYDER: True. So, how do you want to handle then?

DR. BELSITO: I wanted to keep it in unless you thought we can't read across.

DR. RETTIE: I think we can read across from Dimethyl to Diethyl, at least. So, it should be fine for read across.

DR. SNYDER: Okay.

DR. BELSITO: And, Christina, you were asking for an expert. I have the perfect person; he's in the Netherlands. That would be expensive to bring him over, but he might do it virtually.

His name is Aldert, not Albert, A-L-D-E-R-T, Piersma, P-I-E-R-S-M-A. He's in Utrecht, Netherlands. And his, email, if you want it, is Aldert H. Piersma, so, alderthpiersma@gmail.com. He's the repro endocrine expert on the RIFM Panel as well. And if you do a lit search on him, he's done a huge number of publications on phthalates.

DR. SNYDER: Okay.

MS. BURNETT: Thank you.

DR. BELSITO: And I think what we're asking him is to comment on the validity of whatever studies we're being presented with. Because all of these endocrine disruptions, so many of them, the systems aren't really -- what's the word I want -- representative for humans. The doses are extraordinarily high. He would be the person who would be able to comment on these studies that we're going to get. Because I think it's going to overwhelm us.

DR. SNYDER: I think that's a great idea.

DR. KOWCZ: We also have an alternate, if you're interested. We have Dr. George Daston, who has also presented to the Panel before, from Procter & Gamble.

DR. SNYDER: What's it -- spell his name?

DR. KOWCZ: George Daston. D as in Daryl, A-S, as in Sam, T as in Tom, O, N as in Nancy. You know, if Aldert can't make it, or if for some reason the timing doesn't work, we also have George Daston. He has presented, Paul, numerous times.

DR. BELSITO: He would be a lot cheaper to have in person.

DR. KLAASSEN: I have two people as well from the United States. Paul Foster, who has done a lot of this work. And in fact he's done it with Earl Gray. I think they have been -- they're both very recently retired. They've done a lot of work with these phthalates. Anyhow, that's a couple of other names, so you got plenty of names.

DR. SNYDER: Okay.

DR. BELSITO: Well, maybe it would be good to have -- I mean, since we have three people in the US, to have them actually see if they can physically come to the Panel because we've seen the issues with virtual presentations. But, to have two of them so we get more than one person's ideas. And, of course, if they both agree, that makes it very much easier for us, right?

DR. SNYDER: Well, I like the idea of having George come if he's presented to us before. Because he would have a history with us and -- yeah.

DR. KOWCZ: He does have a history with you.

DR. HELDRETH: Is George not in the EU right now, too?

DR. KOWCZ: I'm sorry?

DR. HELDRETH: I thought George was in the EU as well.

DR. KOWCZ: No.

DR. HELDRETH: No? Okay. That must have been --

DR. EISENMANN: I gave him the dates, and your next two meetings are virtual. Of those two meetings, the December one would work for him.

DR. SNYDER: Okay.

DR. BELSITO: Oh, okay.

DR. SNYDER: All right.

DR. BELSITO: Do we want to see if one of Curt's gentlemen or Aldert?

DR. SNYDER: I think so. I like the idea of getting two experts.

DR. BELSITO: Or if Albert could make the virtual meeting in December?

DR. SNYDER: I think so. I think that's a good -- we'll just -- and if we only get one, then we get one. But if we can get two, that's fine, right? Virtual, it's not that -- we're not flying anybody out or anything, so let's do it.

DR. KLAASSEN: I mean, this topic keeps bouncing up, not just with the phthalates, but in general. And it would be good to have two or three people talk about it and see what they say. That's my feeling. But I don't know how many of these would even be willing to do it, and what their schedules are, so whatever.

DR. RETTIE: A point of education for me is, has this Panel used the EPA's screening program for endocrine disruptors before? Is that something you've considered? They have a whole pile of assays that I found out: receptor bindings, steroidogenesis, thyroid function, all that stuff.

DR. SNYDER: No.

DR. RETTIE: We haven't typically looked at that before?

DR. SNYDER: No.

DR. RETTIE: Okay.

DR. SNYDER: Again, this is a little bit like the prostaglandins, it's a very complex ingredient. But I think that's why it's important that we get the experts, like we had today, to provide us some basis. So, I think that's a good plan.

DR. KLAASSEN: You know, in regard to the structure activity relationship of these chemicals, it is known that some of these phthalates have quite different toxicity than others. And, is it smart coming in to putting them all in one category? Or, I guess I'm thinking maybe after we hear these experts, we make that decision at that time, if we should do it as one category or three.

I mean, if you know already -- let's just say, as an example, in a group of compounds -- that one is really known to do this one thing, and the other six compounds in the group is absolutely known not to do that one thing, would you put them in a group or not?

DR. BELSITO: No. No, but I think you're spot on, Curt. That's what we ask these experts. Can these phthalates be grouped in terms of potential endocrine disruption?

DR. KLAASSEN: Okay.

DR. RETTIE: And isn't it also known that the monoesters are at least as toxic as the diesters, maybe more so? So, it could be a metabolic component there, a bit of SAR. I don't know that field.

DR. SNYDER: We can get clarification of all those questions.

DR. RETTIE: Yeah.

DR. HELDRETH: Yeah, and if it turns out that it doesn't seem like they fit together, like Dr. Klaassen is proposing, then we can split it out at that point.

DR. SNYDER: Perfect. All right. Good discussion.

Cohen Team – June 9, 2025

DR. DAVID COHEN: All right. Dibutyl Phthalate was placed on the 2024 Priorities list following nomination by the FDA for cause due to restrictions imposed on uses of plasticizers in food-contact applications.

We first published a Final Report on the safety assessment of Dibutyl Phthalate, Dimethyl Phthalate and Diethyl Phthalate in 1985, and concluded that these ingredients are safe for topical application in the present practices of use and concentrations in cosmetics. On re-review in 2002, the Panel reaffirmed the original conclusion as published in 2005.

In December of 2012, the Panel deliberated on studies separately concerning endocrine disruption and diabetes for Dibutyl Phthalate, Diethyl Phthalate, Dimethyl Phthalate and Butyl Benzyl Phthalate. However, the Panel chose not to reopen the safety assessment of these ingredients and published their decision as a re-review summary in 2017.

In 2024, the CIR staff initiated a literature review. There's a lot of data out there. And the questions to us were, "Does the Panel or any other stakeholder have a particular expert in these areas they would like to invite to give a presentation on these DART and endocrine studies?" Anybody know anyone?

DR. EISENMANN: I have a comment. George Daston of Procter & Gamble would be happy to come and talk to you.

DR. DAVID COHEN: Who is it, again?

DR. EISENMANN: George Daston. He's talked to you before. He would be available for the December meeting, if that works out.

DR. DAVID COHEN: Okay. And the CIR noted that in 2024, RLD and the 2025 concentration of use by the Council indicate no current uses for Dimethyl Phthalate. The 2023 VCRP also reported no uses. Twelve uses were reported to the VCRP, and the maximum concentration of use reported was 2 percent in hairspray in 2001.

So, does the Panel support the idea of having Dimethyl Phthalate in a separate re-reviewed proposed document? Or would the Panel prefer to have this ingredient in the safety assessment with Dibutyl and Diethyl Phthalate?

So, if we keep it together, we could come to split conclusions. There's no problem with that. I just thought, if we have them as a separate report, we're kind of duplicating our efforts by going into this. Was there another thought behind this? Or you're just using a use not supported?

MS. FIUME: Use not supported, yes. It wouldn't be duplicating efforts; it would be use not supported.

DR. DAVID COHEN: Okay.

MS. FIUME: So it would be a re-review summary.

DR. DAVID COHEN: So you just spit it out and it goes to use not supported?

MS. FIUME: And it would be a re-review summary, right, Christina? Is that what we discussed when we discussed that? So it would probably be not reopened because it would be use not supported, like some of our re-review summaries today.

DR. BERGFELD: How long does it have to be not used? Is there a time period on it, or just when you do the survey they say, "not used," and you're done?

MS. FIUME: I don't know. I don't think there has been a time period on it so far. Because then if they --

DR. DAVID COHEN: You want to take a break? You want to take a two-minute break?

DR. ROSS: Sure, go ahead.

DR. DAVID COHEN: We could --

MS. FIUME: Yeah. I was going to say because if it goes use not supported, and then someone wants to use it, they can petition the Panel saying that they are using it and want it looked at.

DR. DAVID COHEN: So, look, from that perspective, I spoke too soon, right? So, let's split it so the actual review of the other two is limited. We agree? You guys alright with that?

DR. BERGFELD: I agree.

DR. TILTON: I agree.

DR. DAVID COHEN: So, the Phthalate discussion tomorrow is basically just answering these questions, correct?

MS. FIUME: Yes. It's to provide some guidance for Christina because there's so much information out there.

DR. DAVID COHEN: Yeah, it's going to be quite a report.

DR. DAVID COHEN: When do you think that hits the docket?

MS. BURNETT: Well, we're hoping to have the speaker in the same time that the Panel looks at it the first time. And if we go with PCPC's recommendation, that earliest would be December.

Full Panel – June 10, 2025

DR. DAVID COHEN: Right. Dibutyl Phthalate was placed on the 2024 priorities list following nomination by the FDA for cause, due to restrictions imposed on the use of plasticizers and food contact applications. The Panel first published on Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate in 1985 with the conclusion of safe.

In 2002, it was reviewed and the Panel reaffirmed the original conclusion. In 2012, the Panel deliberated studies concerning endocrine disruption and diabetes for Dibutyl Phthalate, Diethyl, Dimethyl, Butyl Benzyl Phthalate. However, the Panel chose not to reopen and published their discussion as a re-review summary in 2017.

This is going to come up, many of the studies have expanded. Mechanisms of action since 2024, a new literature search was sent. So we have mechanisms of action, gene expression and other specialized areas. And it may be beneficial for us to have a talk on DART and endocrine studies.

Does the Panel or any stakeholders have any opinions about who to invite regarding DART and endocrine studies? One suggestion from the PCPC was George Daston from P&G, who would be available. There were two other people that I found

that might be able to. One was Linda Birnbaum who is the Director of the NIEHS. Another was Dr. Swan from Mount Sinai. But I'm not sure that she may provide the information we need in this particular setting.

DR. BELSITO: Yeah, our team had discussed this. Yeah, I think George is fine but he is industry. It would be nice to get a non-industry person, get two opinions.

DR. SNYDER: We also liked George because he's presented to the Panel before, so he's familiar with our issues. So I think George and one additional person, that's what we kind of concluded.

DR. BELSITO: I had recommended a guy from Netherlands by the name of Aldert Piersma, who is actually the DART endocrine person on the RIFM panel. And if you look him up, he's done a lot of work on phthalates.

DR. ROSS: He sounds great, Don.

DR. SAM COHEN: Yeah, I think that one would be good. I would not have Dr. Birnbaum, because she has not done any work on this. And was the director of NIEHS, but was dismissed from that. She really has no experience with phthalates other than hand waving.

DR. DAVID COHEN: Okay.

DR. SNYDER: So I think we would suggest you go forward with George and Aldert.

DR. BERGFELD: Are you okay with that, Bart?

DR. KLAASSEN: I would like to also suggest two other excellent people who have worked on this area. One is Paul Foster and the other is Earl Grey.

DR. BELSITO: Are they academics, Kurt?

DR. KLAASSEN: They are government. They were at EPA.

DR. BELSITO: Okay.

DR. KLAASSEN: Or NIEHS, I can't remember for sure which one.

DR. ROSS: Yeah, Paul has done a lot with NTP as well, yeah.

DR. BERGFELD: Do you need anything else?

DR. HELDRETH: I can reach out. I've heard already that George is available for December. I'll reach out to the others and see what their availability is and then we can go from there.

DR. BERGFELD: Would you have them virtually or live or both? Whatever?

DR. BELSITO: We're virtual in December.

DR. HELDRETH: We're virtual in December. And that'll make things easy if we end up bringing in Dr. Piersma since he's in the Netherlands.

DR. BELSITO: Bart, your mic.

DR. HELDRETH: Yeah, being virtual will also make things easy for Dr. Piersma if we're going to have him do a presentation from the Netherlands.

DR. BELSITO: Yeah.

DR. BERGFELD: Okay. Anything else to discuss on the Phthalates, other than --

DR. BELSITO: Yeah. There was a second question about splitting Dimethyl Phthalate out into a separate re-review, and we didn't really think it was necessary, unless someone has a reason.

DR. DAVID COHEN: Our original motion was to split it and then do use not supported. However, in some late, breaking information from FDA, the RLD has three reported uses. So, we're not going to split them.

DR. SNYDER: We agree with that.

DR. BERGFELD: Okay. Any other questions regarding this strategy? Seeing none, then we're going to move on.

MARCH 2026 MEETING – DRAFT AMENDED REPORT

Presentation by Dr. George Daston – March 12, 2026

https://www.cir-safety.org/sites/default/files/Daston_2026_phthalatescir1225.pdf

Belsito Team – March 12, 2026

DR. BELSITO: Okay, so we're taxed with doing phthalates first, so we'll get the worst out first. Dibutyl Phthalate was placed on the 2024 Priority list by the FDA for cause. We first published a safety assessment on Dibutyl, Dimethyl, Diethyl Phthalate in '85, and concluded the ingredients are safe for topical application in the present practice of use in cosmetics. We reaffirmed that in 2005. In 2012, we deliberated on studies concerning endocrine disruption in diabetes and Dibutyl Phthalate, Diethyl and Dimethyl Phthalate, and Butyl Benzyl Phthalate, and we chose not to reopen the assessment at that time.

We have data from that original report and from re-review evaluated by the Panel in 2005. And we've got updated frequency of use data and of course tons of new data, particularly concentrating on DART effects. And then we got that wonderful presentation from Dr. Daston.

We did get a Wave 2 on this from Weaving Voices for Health & Justice, I think, is the new name -- formerly Women's Voices for the Earth -- pointing out an issue primarily with our absorption data that we use in a margin of calculation, referencing a paper by Hu et al., which I forwarded to you.

My summary on that is it's really mathematically based, and the authors even conclude that their data needs to be further confirmed by additional studies. But in the end, I think even if we accept that absorption of 54 percent, or whatever it was, when you look at our margin of exposure it's well within a safe margin of exposure.

But I look to comments from my team about the Hu paper and how that could influence our safety depending upon how we assess it. I'll open up that question to Allan, Curt, and Paul.

DR. RETTIE: Okay. I think we'll have more questions around the MOE in the big Panel meeting. I didn't send you any of my comments on the Hu paper. I was just struck by the lack of a metabolism component in it. So the 25 percent dermal absorption that they came down on for DEP would be influenced, presumably, by that.

I think we are using 5.5 percent in this MOE calculation. And maybe it doesn't need to be much higher than that, but I'd like to hear what my MOE experts have to say on that.

DR. BELSITO: So you want to hear what David Ross has to say? Curt, Paul, no comments?

DR. SNYDER: Yeah, I don't have any input on that regard. My only comment about that paper was that I was a little bit surprised, and I wanted to have a discussion or see what everybody else thought about how the fact that that paper said that the systemically available, at 25 percent, was greater versus the oral exposure, which I wouldn't have predicted. Would you have predicted that, Curt? That the systemic exposure through dermal would be higher than the oral? That surprised me a little bit, because we use oral very significantly, don't we, in our assessments sometimes?

DR. BELSITO: Yeah, I agree, Paul. I thought that was, you know, counterintuitive.

DR. SNYDER: Yeah, I really wanted to have a discussion about that, because we really, really sometimes only have oral and, you know, how much is systemically available oral, and we use that. And so that's the only comment I had in that regard to that paper, which I would have not predicted that.

DR. KLAASSEN: I agree with you a hundred percent, as that makes no sense, that how skin absorption can be better than oral. I mean, I've never, ever, ever seen that before. And it's not -- let's say it's just not logical from what we know about science right now. So I don't -- I tried to understand how they might have gotten to that false conclusion, but I didn't.

DR. BELSITO: Yeah, I mean, particularly with our chronic tox studies it seems that the oral is significantly greater effects than the dermal. Paul, I just wanted your opinion on these liver neoplasms in mice and pituitary adenomas in rats. It's on PDF Page 65.

DR. SNYDER: Sorry, I'm not seeing that on Page 65 in my document.

DR. BELSITO: It's under the Carcinogenicity Studies.

DR. SNYDER: For Phthalates?

DR. BELSITO: Dermal. It says, "This is from the old report: increased incidence of liver neoplasms." First paragraph in the Carcinogenicity Studies.

DR. SNYDER: What page is that on?

DR. BELSITO: Six -- 65 PDF.

DR. SNYDER: For the Dibutyl and the Diethyl and Dimethyl?

DR. BELSITO: Diethyl at 0, 9, 18, or 37 mics, in acetone.

DR. SNYDER: It didn't ping me to be anything outside of --

DR. BELSITO: Yeah, they said it was equivocal because the incidence of hepatic neoplasms was within historical range. And there's no clear dose response --

DR. SNYDER: Yeah, these are such common tumors that really the historical data is very relevant, so. I didn't highlight it, so I would agree with that.

DR. BELSITO: Okay. And, you know, I mean, I think the bulk of the new data we got was on DBP. And as we heard from George, I mean, that's the one that does have endocrine effects on males.

Christina, just a question for you. This is on PDF Page 69, Evaluation of Mechanistic Evidence for Reproductive Toxicity. The title of that section suggests to me that they assessed a possible mechanism that is not discussed there. I mean, George talked about steroidogenesis, in terms of the ligaments, did they go into that mechanism there in that paper or?

MS. BURNETT: I am unsure. I believe this paper was summarized by Jinqiu. And I don't think -- Jinqiu, are you in this session?

DR. ZHU: Yes. I think the paper is focused on adverse outcome pathway. So, like, we -- it's kind of related to the mechanistic event for the DART.

DR. BELSITO: Okay. But, I mean, what we have here, the three conclusions: fetal rats more sensitive, Dibutyl Phthalate androgen-independent adverse outcomes, that are conserved across mammalian models, and Dibutyl Phthalate–induced anti-androgenic effects are conserved in different mammalian. But that, to me, is not mechanistic; that's just observed, right? I mean, again, I just -- when I saw that, I thought there was going to be, you know, the type of explanation that George had given on how this can affect development of the testes and descent of the testes.

MS. BURNETT: Okay. We could rename the section here.

DR. BELSITO: Yeah. So, I mean, I think the data that we have, and the -- I just want to look at the margin of exposure before I discuss what I think. Is the conclusions that we reach is -- where is the margin of exposure here? It was in Table 10, right?

MS. BURNETT: PDF Page 79 has the summary. I believe Table 10 is the information. Yeah, the details.

DR. BELSITO: Yeah, I want the details. So that's -- because that -- there's just so much here. I think that's the critical thing, right? For us is the margin of exposure here. Okay. This was a facial cleanser. Yeah. Okay. And it was 79 that had it, Christina, you said?

MS. BURNETT: The summary, yeah.

DR. BELSITO: And we used for our NOAEL -- we used an oral NOAEL, right, from a 16-week study?

DR. ZHU: Yes.

DR. BELSITO: Why didn't we use the NOAEL from a repro study?

DR. ZHU: We can, of course, because that's for the conservative -- you know, the repeated dose toxicity study has the lower NOAEL value. So we choose to use the lowest value for the calculation. But if the Panel, you know, care about the DART endpoint, we can change the NOAEL value to, like, 200, to recalculate it.

DR. BELSITO: Okay. So, the repro NOAEL would be 1,600 milligrams, and the oral was 200 milligrams?

DR. ZHU: Yeah, 200. It's on page 79.

DR. BELSITO: I mean, I think that's fine, but maybe in the Discussion, since it's really -- you know, people are always talking about the reproductive effects, we could point out that we used the oral because it was a more conservative estimate.

DR. ZHU: Sure.

DR. BELSITO: And, I mean, at the end of the day with all the data we have, I think we can say that Diethyl Phthalate is safe, and we can't say anything about Dimethyl or Dibutyl because we don't have concentrations of use to do a margin of exposure. I mean, that was my conclusion to all of this.

DR. SNYDER: I would agree with that, Don.

DR. BELSITO: Curt? Is Curt there? Allan?

DR. RETTIE: Yeah, I'm here. I agree with that.

DR. KLAASSEN: I'm okay with that.

DR. BELSITO: Okay. So, for discussion points, I think, we have DEP is okay with margin of exposure calculations. We used the oral because it was more conservative than the repro. The insufficiency, because we can't do margin of exposure if we don't have concentration of use. I think we need to address the DART, which we do by margin of exposure.

The only other thing that -- looking through the epi studies -- that I wondered whether we should address are these neurocognitive effects. That was the biggest issue, I think, in the epidemiologic studies, and it was also something that the formerly Women's Voices for the Earth, Alexandra Scranton, had brought up in her letter to us.

So, I think we can address the endocrine, but what about the neurocognitive? Do we say anything about it, or what are the other discussion points we need to bring in?

DR. SNYDER: Well, I guess we could just discuss them if there were. But it's out of my expertise how to interpret that.

DR. BELSITO: But then how can we say that it's safe if we can't -- I do think that there are some areas in there, the neurocognitive -- the epi. You know, I think the biggest thing, perhaps, that we could say it's on PDF Page 81, where they actually did a meta-analysis of all those studies, and they concluded that there was no consistent pattern linking prenatal or early-life thyroid exposure with adverse neurodevelopmental outcomes.

So I think that meta-analysis -- we can bring up, that there are reports, you know, of various neurocognitive abnormalities, issues that have been reported with intrauterine and early-life exposures to phthalates, but in this meta-analysis there was no consistent pattern linking them.

DR. SNYDER: Well, in the last section there, where it says the authors noted that inconsistent findings may reflect methodological limitations.

DR. BELSITO: Right.

DR. SNYDER: I think we can wordsmith something, Don.

DR. BELSITO: Yeah, I mean, I think essentially we can take that conclusion from the meta-analysis and just point out that we're aware of these issues that have been reported in various aspects of neurocognitive behavior, but that -- and, you know, I -- well, in a meta-analysis of the epidemiological data, there were no consistent patterns linking prenatal, early-life phthalate exposure with adverse neurodevelopment outcomes. And that this could reflect methodological limitations, including exposure misclassifications, et cetera, et cetera. I mean, essentially take their conclusion.

Because I think the two big issues, again, that Alexandra Scranton raised, other than whether our absorption was right -- but even if you take the 50 percent, our margin of exposure is still fine -- is the neurocognitive and the endocrine. And clearly, as George pointed out, DEP and DMP are not really having any significant endocrine and developmental effects. It's when you get above C4 or higher.

So, Dibutyl -- but again, even Dibutyl, we know that there's a hazard, but we are tasked with risk, right? And we can't really assess risk without a concentration of use. So, I thought for both DBP and DMP, they were insufficient for concentration of use. Otherwise, we have tons of data.

DR. SNYDER: I fully support your approach, Don, as you proposed it.

DR. BELSITO: Any other issues that should be discussed in the Discussion other than endocrine and neurocognitive?

DR. SNYDER: I didn't have any.

DR. KLAASSEN: Why don't we have -- or can we get concentrations of use? It seems kind of strange that we don't have concentration of use.

DR. BELSITO: There are two products for each of them, I think, right? DMP has two and DBP has two. And Carol went out and she surveyed industry, and they did not report any concentrations of use.

DR. EISENMANN: Our members are not using them anymore.

DR. BELSITO: Right?

DR. KLAASSEN: Okay. They have such bad publicity that companies are just removing them.

DR. BELSITO: Yeah. And as you saw, even -- I mean, BEP is a functional ingredient in fragrances, right? It's not a fragrance. As George pointed out, it's used to hold the fragrance on the skin so it lasts longer. When we looked at it before, it was at 15 percent. Now it's, what, down to 0.15 percent? There's a significant decrease in the use throughout all industries.

DR. EISENMANN: One thing I would suggest be added to the report, if it hasn't been summarized in other CIR reports: the FDA did surveys of cosmetics and what phthalates they contained, 2004 to 2010 time period, and that has never been captured in a CIR report that I can recall. Over that time period, the FDA saw decreasing levels of phthalates in products. I know it's

older data, but it still might be useful to inform some of the epi studies that were done over that time period that are included in the report.

DR. RETTIE: There was a minor question brought up about number of uses from the RLD data in Table 3. Did that get resolved? 284 and 339 didn't seem to gel.

DR. BELSITO: What PDF page are you on, Allan?

DR. RETTIE: I'm on Table 3 -- see where that is.

DR. BELSITO: Okay. Table 3 is, like, starts on PDF Page 92. And what was the issue, Allan?

DR. RETTIE: I thought it was raised by Women's Voices for the Earth in their most recent one.

DR. BELSITO: Well, that's their misunderstanding of RLD. And we responded to that in our letter. You know, it's the way these are reported that the numbers don't jive.

DR. RETTIE: Okay.

DR. BELSITO: Okay. So, what I have here is that DEP is safe, DMP and DBP are insufficient for concentration of use, and that the Discussion will really resolve around the margin of exposure for DEP, in terms of the endocrine/reproductive effects and the reported epi studies on neurocognitive effects, and when looked at in a meta-analysis they were found not to -- well, whatever their conclusion was: inconsistent findings, et cetera. Anything else to this?

DR. SNYDER: Not from me.

MS. BURNETT: Because this is the first time the Panel has seen this, as a Draft Amended Report, this will then go out as an Insufficient Data Announcement for the concentrations of use for Dimethyl and Dibutyl Phthalate.

DR. BELSITO: Right. I think that's fine. I don't think we're going to get anything, but give someone who reported that to FDA a chance to give us their concentrations. Okey-doke.

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DR. BERGFELD: So, that brings us up to a big document that we have to review, the Phthalates. And we've had a big discussion on this today, and we did open it up. And it's been reviewed in the past, as you all know, and we've been stuck on the reproductive and developmental toxicity, specifically for Dibutyl. We heard today that Ethyl probably is okay, and maybe Methyl, even though it's absorbed well.

I'm not going to go over all that was discussed, except that it sounded like it was Butyl that was the problem. And we know that the European group has Annexed II that one, and it has also been stated as a fetal toxin. So, what should we do with this particular group of ingredients? How should we move? Sam?

DR. SAM COHEN: I think that, given what we heard this morning from George, we really can't recommend safe use for Butyl, and given the fact the Europeans have banned it as well. The other two, though, I think, are fine. And given what George mentioned this morning, that the margin of safety is at least two orders of magnitude different between that and Butyl, I think we're okay.

DR. BERGFELD: David?

DR. ROSS: Yeah, when you compare these three, obviously Dibutyl is a bad actor with its effects on male reproduction. And it is interesting; I just want to go back to a little bit of the discussion of our transcripts here, you'll see that in the transcripts. And we have some of these statements in the actual dossier, in the document.

So, we've got, as early as 2012, the FDA issued guidance for industry on limiting the use of Dibutyl in drug and biological products, recommending that the use of this ingredient be avoided if an alternative is available. In our transcripts, and I don't have a first-hand knowledge of this, but PCPC apparently supported a, we're no longer supporting this ingredient, and supported a ban on Dibutyl phthalate.

DR. SAM COHEN: Yeah.

DR. ROSS: And so, we were left with, historically, the use of Dibutyl in manicuring preparations, but that's gone. Now we have two uses of Dibutyl, which I heard from Christina, I think, that this was in liquid wound dressings. Is that right, Christina?

MS. BURNETT: That's what the RLD raw data says, yes.

DR. ROSS: Okay. And so, they're going to be leachable, right, in liquid wound dressings.

DR. BERGFELD: Absolutely.

DR. ROSS: There's a whole paper here on leachable phthalates, so I agree with Sam. Sam, you can probably get down to a NOAEL. You can't probably say there's no concentration that is unsafe in that sense, because you can find NOAELs in the literature, whether it's trace amounts.

DR. SAM COHEN: Yeah. Right.

DR. ROSS: But, I wouldn't support use of this, and I personally, and if the Panel went that way, could we say something that -- you know, given the potent effects of Dibutyl on male reproduction, depending on what the Panel says tomorrow, the Panel would be in agreement with FDA and PCPC, and recommends that Dibutyl not be used in cosmetics where safer alternatives are available.

Now, I don't know if that's the sort of statement that we would make in these dossiers, and I'm going to open that up to Wilma and Monice. How are we going to deal with this? I mean, clearly it's the bad actor, clearly we don't want it, but how are we going to deal with it?

DR. BERGFELD: Well, we're going to have to call it insufficient or unsafe, is what we're going to have to do. We're not going to be able to put FDA in there. We're not going to really be able to cite PCPC, so we're going to have to base it on what we have as data.

So, right now it's insufficient for a level of safety. Susan, do you have a response?

DR. TILTON: Well, I was going to ask -- yeah. So, I guess we have these two reported uses -- thank you, Dave, I didn't know how they were being used -- but we have no concentration of use. But because of those two reported uses -- I mean, a lot of times if an ingredient's not being used, we can come to a conclusion of use not supported. Is that right?

MS. FIUME: If it's not being used, it would go to zero-use category. Use not supported is for ingredients that are insufficient, and after two years the conclusion changes to use not supported. If the data are insufficient, and there are no reported uses, it goes to zero-use as the conclusion.

DR. ROSS: Monice, even though we -- you know, what you've heard here with Sam and myself, we don't want it, at least, and FDA doesn't want it, PCPC doesn't want it in products where safer alternatives exist, we can't say a use not supported? That's for a totally different category?

MS. FIUME: Right. So, if it was insufficient data -- but it does have two uses. So, I guess we need to find out from FDA, being that they're called -- like the liquid bandage. Are those truly correct usages? And if it is, and the data are insufficient, after two years the conclusion will change to use not supported. There's a two-year window for people to submit reasons why it should be safe or otherwise. But if there are no uses, and it's insufficient, it automatically goes to the zero-use category.

DR. ROSS: The insufficiency we have right now is concentration of use, right? Anyone else got any others apart from -- you know, once we get concentration of use, presumably, we'd have to do some sort of risk assessment and MOE calculation.

Given the NOAELs that Dr. Daston was talking about this morning, they were even much lower than the ones I was coming up with, and I thought mine were low. I think there's a long list of safer alternatives, and my sense of this is it's probably not what you want in there, but that's not what we get to review. Right?

DR. BERGFELD: Well, I think you can ask for use concentrations, but FDA is going to have to call in here and take some responsibility for telling us if this is a treatment -- a medical treatment for a wound, or is it considered cosmetic? And the second thing is, it is possible that the potential for endocrine disruption is high. And unless we have greater data that it doesn't do it at certain concentrations, we cannot move on it, so it would be insufficient.

DR. ROSS: No, I like that summary, yeah.

MS. FIUME: I was going to ask, the concentration of use data that were submitted were done about a year ago, so you have the 2025 concentration of use. But beyond that, and based on what you had noted, David, for the NOAELs, is there a safe concentration? Because in the past, even though you didn't have concentration of use you have, I believe, ruled that something was unsafe regardless of the concentration. Or do you think there is a safe concentration, and therefore the data would be insufficient?

DR. ROSS: Well, I think that was a hair dye, and it was cosmogenesis. I think Sam proposed that, I think.

DR. SAM COHEN: Right. Right.

DR. ROSS: And so, that's more of a --

DR. SAM COHEN: But here we actually have a NOAEL.

DR. ROSS: You've got a NOAEL for the trace amount, I think.

DR. SAM COHEN: Yeah, 1.5. You mentioned 5 this morning, and he gave us the reference of 1.5, so it's not zero.

DR. ROSS: I mean, there's even one in our -- you know, trace amounts in fragrances from SCCS.

DR. TILTON: Right.

DR. ROSS: So, I mean, I think, you can't say that -- I mean, I'd like to say it, but you can't say there is no unsafe level of this. Some NOAELs exist. And so, therefore, we have to deal with these two uses.

DR. TILTON: I agree with that, because we've had to address the impurities in the report.

DR. BERGFELD: So, we've come to a conclusion that the Ethyl and Methyl are okay to go forward, but the Butyl not. And the Butyl is insufficient or unsafe.

DR. TILTON: Well, the Methyl would also be insufficient because there is no concentration of use.

DR. BERGFELD: Yes, no concentration of use. Actually, only the Ethyl is used.

DR. ROSS: And I think you could probably read across to the Methyl, actually, based on physicochemical data; but you couldn't read across to the Dibutyl. And also from the toxicological endpoint, you can see that it's massively different, the endpoints, for Dibutyl versus Diethyl and Dimethyl, so I think the read-across would be difficult there.

But, yeah, I think you would need the insufficiency for concentrations of use on the Dimethyl. And then I would just -- Wilma, you mentioned a little earlier an excellent point there that I hadn't really considered. The liquid wound dressings, is that a therapeutic use or is it a cosmetic use? Can you just flesh that out a bit? No pun intended.

DR. BERGFELD: Well, I don't know how the FDA would consider that. For me, it's a medical use.

DR. SAM COHEN: Yeah, that's the way I would consider it.

DR. ROSS: And then this would drop to zero uses, Monice, and we would --

DR. BERGFELD: Yeah. So, we do have to have clarification of how they interpret wound dressings. And I guess the bottom line is, is it something the public can do on their own? Or is it something a medical advisor advises? Maybe they make the distinction that way, other than the actual action of the ingredient.

And so, we need clarification, tomorrow, from the FDA on the use. Maybe we should pre-warn them that we're going to ask them that question, and they can have an answer.

MS. FIUME: I think FDA is on the line. Is that correct?

DR. XIE: Yes, I finally was able to unmute. I was not able to do it before. Thank you. I appreciate that. So, we can come back to you about it. We do note that this is in the registration and listing, so somebody has responded to us as cosmetic use. We will have to come back to you on how we're going to see it. So thank you, Wilma, for giving the heads up, we'll have an answer tomorrow. Thank you.

DR. BERGFELD: Thank you so much. So, as we stand now, if I could summarize, and please interrupt me, we're going to go out for insufficient on Methyl for concentrations of use, or are we going to do read across?

DR. TILTON: Do we read across for concentration?

DR. BERGFELD: Yeah.

DR. TILTON: I think the discussion was we could read across, if we needed to, for any of the toxicity data, but I think the report is pretty -- I think we have a lot of data.

DR. ROSS: I think you should probably go insufficient for concentration of use, try and get that.

DR. BERGFELD: Okay, I have that down, but I just wanted to make clarity on that one. And then, right now, we're going to get FDA clarification on wound dressing for the Dibutyl. And if they're going to consider it cosmetic, then we're insufficient for -- or not? Are we going to use the NOAEL?

DR. ROSS: Well, you're insufficient for concentration on the Dibutyl.

DR. BERGFELD: Okay. How about the NOAEL?

DR. ROSS: Well, you don't have a NOAEL for the concen- --

DR. BERGFELD: But I thought we had -- we had something this morning.

DR. ROSS: You've got NOAELs in the literature, but you don't have a NOAEL for how it's being used in cosmetics, in our report.

DR. BERGFELD: Okay.

DR. ROSS: I think if you go insufficient, depending on what the FDA says, of course, and at that point if you get concentration data, we'll have to deal with it at that point. If you don't get concentration data after two years, it goes to use not supported, right, Monice?

MS. FIUME: Right. If those are cosmetic uses, it'll go to use not supported.

DR. ROSS: But, again, we've got something here that's a potent reproductive toxin. And even though we're going out with the same insufficiency for Dimethyl and Dibutyl, these things are not in the same category.

MS. FIUME: The Dimethyl, if you don't get the concentration of use but you don't have the same toxicity data, the Discussion can probably be crafted to explain why it's safe if it's used in the same practices of use as Diethyl. Is that correct?

DR. ROSS: Correct.

MS. FIUME: Sort of like we did with the hydrolyzed. And then the other explained why the insufficient doesn't count.

DR. ROSS: Correct. Correct. And I think that's an easy explanation for why Dibutyl doesn't carry across. It's one, physicochemical, but, secondly, it's toxicological endpoints. The endpoints of concern are very, very different.

DR. BERGFELD: And so, the question would be, also, why wouldn't you go there directly?

DR. ROSS: You could. And I suspect that's probably what we're going to hear tomorrow in the discussion.

DR. BERGFELD: Yeah, I think so. I think if you can do that, that's what we would do.

DR. TILTON: Yeah, I think that was my question earlier, is that do we -- I don't want to say read across on concentration -- but we can come to a conclusion based on how Diethyl is being utilized, and then that would be our conclusion.

DR. ROSS: Yeah, I'd be okay with that. I mean, you could read across from Diethyl to Dimethyl, I see no reasons. I'm just looking at my logP's, my solubilities and, again, the endpoints we have. We've got a ton of data in this dossier. And, yeah, I think I'd be okay with that.

DR. BERGFELD: So, with that kind of discussion, then we would be going safe Ethyl and Methyl, and insufficient for Dibutyl for concentration first, and clarification by FDA. Is that what we've done?

DR. ROSS: Yeah. And we are -- well, we have the FDA's position in our dossier already. So, I think that's good to have in there. Of course, we don't have the PCPC position in there, but I think that's appropriate that we don't have it in there.

DR. BERGFELD: Well, we have to ask if that is appropriate to put them in there?

DR. ROSS: Correct. Yeah. We don't want to put that in there without their agreement. Yeah.

DR. BERGFELD: Yeah. I'm going to -- FDA is on. Are you okay with us entering FDA's previous request?

DR. XIE: Hi, Wilma. My understanding, from what David is saying, is that is for use in devices, right? The FDA position? When you say FDA, that was the specific one, correct, David?

DR. ROSS: It was in our text, I think.

DR. SAM COHEN: Yeah.

MS. BURNETT: PDF Page 54.

DR. ROSS: Oh, you were ahead of me.

MS. BURNETT: Under Non-cosmetic Use? Is that?

DR. ROSS: Probably, yeah.

DR. XIE: Recollection from what you said was its limited use, as FDA said, for drugs and biologics.

DR. ROSS: Page 54, was that right?

MS. BURNETT: Page 54, last paragraph, under Non-cosmetic Use.

DR. ROSS: Yeah, okay. "As an excipient in regulated drug and biologic products. Recommending that the use of this ingredient be avoided if an alternative is available." So, is that purely devices or -- it doesn't sound like it.

DR. XIE: Sorry, not devices, drugs and biologics. That's what I think it's specific to. So, if that is what FDA has said for those uses, I think that would be fine. We haven't said anything about cosmetics.

DR. ROSS: No. That's the text we have in there right now. So, I think that's okay.

DR. BERGFELD: I don't think we can bring that forward into our Discussion, though, unless we clarify it wasn't cosmetic.

DR. ROSS: No, they're different uses. I don't think it sounds like it's a cosmetic use.

DR. BERGFELD: No, I don't think you can use it there. And if you -- we could ask PCPC -- is Jay on with us today? Or who is from PCPC?

MS. FIUME: Jay is no longer PCPC.

DR. BERGFELD: Okay, I saw him up here on the list this morning.

MS. FIUME: Yeah, he is here on his own accord. Let me see who might be on from PCPC. Kathy? Can you weigh in? Or Kim?

DR. NORMAN: Monice, I'm here, would you just repeat the request?

MS. FIUME: David or Wilma, would you like to repeat the request for PCPC?

DR. ROSS: It was basically whether PCPC was supporting the use of this product, Kim. And I think Carol had commented last time that you're no longer supporting use of that product, but I may have misunderstood that.

DR. NORMAN: Yeah, that's my understanding as well, that we're not supporting.

DR. BERGFELD: Is there a document for that? Or is that just a statement made somewhere?

DR. NORMAN: Let me follow up on that, if we have that actually documented.

DR. BERGFELD: Because if there is, we can cite it.

DR. DAVID COHEN: Which chemical are we on?

DR. BERGFELD: Phthalates.

DR. ROSS: Phthalates. This was the PCPC not supporting use, and we were wondering if that was a written document and that was an official position that we could cite in our dossier, or whether it was just a statement somewhere.

DR. DAVID COHEN: Did we discuss a conclusion?

DR. BERGFELD: Yes, we did. If I might tell you, at this point in time, Diethyl, Dimethyl would be safe. And taking Dimethyl into the Discussion and talking about read-across and why we would apply some of the information on Ethyl to Methyl. Dibutyl, requesting concentration of use. And also FDA clarification -- oh, we did have a clarification, we don't need that anymore.

FDA use is device, so we will not quote that. But we wanted clarification of the wound healing. Is wound healing a medical device, or is wound healing -- excuse me. So, we need to have FDA tell us if it's a medical therapy or if it's a cosmetic therapy, wound healing. So that's where we stood.

DR. DAVID COHEN: Why would it be a medical?

DR. BERGFELD: Well, it's wound healing. It's an open wound. You're applying this to an open wound. Is that a cosmetic?

MS. BURNETT: Someone has their hand up.

DR. SRINIVASAN: Jannavi here, FDA. We see it as a medical device, that is how we would treat that.

DR. BERGFELD: So we have no uses.

DR. SRINIVASAN: Yeah. Thank you.

DR. SAM COHEN: So, then there's no uses cosmetically.

DR. ROSS: Now, this is a different story, yeah.

DR. DAVID COHEN: Are we talking about Dibutyl?

DR. ROSS: The dibutyl, yeah. Basically, the bad actor of the three that we're considering, as we heard this morning from Dr. Daston. And so, we previously had it wasn't supported, we were trying to get safer alternatives for these two uses that we had. Now we have no uses, so it's a different analysis, I think.

So, Monice, this would go to zero uses. And give me the category again.

MS. FIUME: That's the category. It's zero uses. Because it's insufficient, and it has no reported use, the category it becomes is called zero use.

DR. DAVID COHEN: So we can't conclude use not supported?

MS. FIUME: Use not supported happens when an ingredient has reported either frequency or concentration of use, was found insufficient, and then nothing was received within two years, then it goes to use not supported.

DR. ROSS: I tried to go down the road, David, of the potent male reproductive effects of this compound, and FDA on record as saying safer alternatives should be used where possible. PCPC don't support it, therefore, if the Panel agreed, maybe CIR could say that the use is not supported. But that argument didn't seem to carry any traction with the definitions we have to operate in. Is that correct, Monice?

MS. FIUME: I believe so, but Bart would probably be able to clarify this tomorrow, because I can't remember if it was based mostly on chemistry. But when formaldehyde was being reviewed in the light of Brazilian blowouts, I don't know -- I have to look at how the unsafe use was clarified, based on the information that was there versus if it was just the Panel's expertise saying we do not feel this can be used safely. But I have to look back at that.

DR. DAVID COHEN: Sam, is there a concentration of use that you feel is safe for this product, for Dibutyl?

DR. SAM COHEN: Well, we heard this morning that there was a NOAEL of 1.5 milligrams per kilogram, so we could base it on that.

DR. ROSS: There is a safe level, David, if you want to go with a NOAEL. It's not like a carcinogen argument, perhaps, where you have nothing, zero is safe. We had this discussion as well. Sam opened it up by suggesting that he wanted to go unsafe on this. And I would agree, but it's just getting to that point, really. So, how do we get there?

DR. DAVID COHEN: So, Monice, with zero use, what kind of -- well, of course, everyone has freedom to operate any way they wish, but how do you execute as industry on a no uses? What does that mean? Like, I could do whatever I want? What does that translate to?

MS. FIUME: my understanding is how it would translate is that if someone wanted to now use that ingredient, they would have to prove safety, because CIR has not been able -- did not -- the data were insufficient. Use not supported carries more weight in the words because we know it's being used. But they both -- anytime something has been insufficient it's not like it's free for industry to use because there's no conclusion on it. That conclusion is basically saying, we can't say it's safe.

And the only reason it goes to zero use is because no one is using it and we're not expecting to receive data. So that is what the difference has been. Because in the past the Science and Support Committee has asked that the CIR, or the Panel, not even review ingredients that don't have use because no one's going to submit the data.

This is a different situation because it's a re-review, you can't ignore it, so something has to be said about it. But it basically means that if industry wants to use it, they need to show that it's safe.

DR. TILTON: I mean, the alternative to reporting the NOAEL is that we would then be reporting a safe use concentration, as opposed to saying it's not safe, right? And I don't know if we necessarily want that to be the conclusion either.

DR. ROSS: Correct.

DR. DAVID COHEN: But this is a Draft Amended Report; it comes back again to us?

MS. BURNETT: Yes, this is your first look.

DR. DAVID COHEN: So, if we had an IDA for concentration of use, despite the no uses, we got no response, it goes to an Insufficient Data Conclusion. Would the final then, since we didn't get any data, be use not supported? Or would it just sort of stay as a split conclusion, Insufficient Data Conclusion, then they have two years to put it in? But we're affirmatively saying -- we're not saying no uses; we're saying there's no data. It's a slightly different conclusion, isn't it?

MS. FIUME: Not really. You're saying there's no data, but there are no reported uses. So, according to the procedures, it goes to zero use. And I believe that's immediate; I don't think there is a two-year window for that.

DR. ROSS: Monice, you know, in the formaldehyde conclusion, if you had in the expert opinion of the Panel, the use of this material has potentially potent male reproductive hazards and safer alternatives are available, and in the Panel's opinion -- and this is purely hypothetical because we have to have the discussion tomorrow -- the material is unsafe. Is that a way of getting there?

MS. FIUME: Getting there to calling it unsafe?

DR. ROSS: Calling it unsafe, you know, where safer alternatives exist.

DR. DAVID COHEN: Not recommended for use.

DR. ROSS: Not recommended? That's probably a better terminology. Thank you.

MS. FIUME: That's not one of our conclusions, so I don't know where that would be put. Again, in formaldehyde it was cancer, again, right? It was cancer endpoints.

DR. ROSS: Yeah.

MS. FIUME: And the one paragraph says, "The Panel emphasized that a large body of data has demonstrated that formaldehyde gas exposure can cause MPCs. Although debate is ongoing regarding the dose-response relationship for the induction of MPCs, the Panel maintained its view that formaldehyde gas can produce such cancers at high doses." I'm trying to see how they got to calling it unsafe completely.

DR. ROSS: Because NOAELs exist, I don't think we'd get to unsafe.

DR. SAM COHEN: Right.

DR. ROSS: But I think you could get to, as David just pointed out, what I was saying, not supported, but you could get to not recommended where safer alternatives exist.

MS. FIUME: So, I guess an option is -- I don't think you can overcome the conclusion, right, when we have standard conclusions based on our procedures. But your Discussion could emphasize that the Panel wasn't comfortable with its use regardless. You know, wordsmith it however you would need to.

The Discussion could say what your concerns are. But, unfortunately, the conclusion -- and who knows, if Don is having the same feelings, he was a proponent on the format -- well, Dr. Shank really led the charge on it. Because if I recall correctly, it was the same situation where the Panel had the gut feeling that this is not safe and should not be used in cosmetics, the data weren't one hundred percent there, and they were able to formulate a Discussion. And they went ahead with the unsafe conclusion for use in the hair straighteners.

So, it was this -- right, Wilma, do you recall, like, the data weren't one hundred percent there, but it was the Panel's expertise saying, we're not comfortable with not saying this is unsafe?

DR. BERGFELD: I don't remember in detail, but that was the essence of our discussion, yeah.

DR. ROSS: Well, this may be another candidate for that sort of discussion; we'd like to hear opinions.

DR. BERGFELD: We can go back and look at that discussion and see how we posed it. Yeah.

DR. ROSS: I mean, Sam proposed that. I mean, Susan, is that your thought also, or?

DR. TILTON: Yes. It seems like there are no uses; there's no concentration of use. It's going to end up being in this zero-use category of a conclusion. But we should be able to summarize the data that we have in the Discussion.

DR. ROSS: And say what?

DR. BERGFELD: I agree totally. We need to put it in Endocrine Disruptor Concentrations.

DR. TILTON: Well, it would be nice if we could relate back to the PCPC recommendation, or another recommendation, about not recommended.

DR. BERGFELD: It shouldn't be PCPC; it should be some other source.

DR. ROSS: Well, you've got an FDA statement. I think if PCPC comes in with a supportive statement, that's up to them. But we know it's potent, this testicular dysgenesis syndrome, with very low, very low levels. And we can have that discussion, Monice, that you just sort of talked about if the rest of the Panel agrees, they may not. And get to, maybe, the conclusion, use isn't recommended when safer alternatives exist, or something like that.

DR. TILTON: In a way, it's all sort of hypothetical, right? We don't have specific uses that we're trying to describe. Specific products, specific concentration of use.

DR. BERGFELD: We don't have a need for protection here.

DR. ROSS: But you might have in the future.

DR. BERGFELD: In the industry.

DR. TILTON: Yeah. But that's why I think the Discussion is important.

DR. SAM COHEN: Couldn't we include all of this in the Discussion with the details of things? And then, Monice, how do we state it, that since there's no use it's not an issue? And it just disappears now. You don't have to wait the two years.

MS. FIUME: Right? That's my understanding. To go to "use not supported," it's after two years.

DR. SAM COHEN: How do we phrase that since there's no use now?

MS. FIUME: The Conclusion would stay that it's classified as no reported use, that it's a zero-use category. I mean, I think you would still say the data are insufficient, but the classification on the website would be zero-use.

DR. DAVID COHEN: Wait, so we go with an IDA and a zero-use at the same time?

DR. BERGFELD: Not go with an IDA.

MS. FIUME: So, the IDA was originally for concentration of use, which you now know is not needed. But an IDA was going to go out -- is an IDA going out for Dimethyl?

DR. BERGFELD: No, they decided to do that in the Discussion. We have no IDA.

MS. FIUME: Discussion. So, yes, you would issue a Draft Tentative Amended Report.

DR. BERGFELD: We're going out as safe for Ethyl and Methyl, with a discussion on Methyl and a read-across, and no uses for Dibutyl.

MS. FIUME: I was going to say, I'd like to check with Bart because it's been handled different ways. It was, recently, where we -- I think it would go out as saying insufficient. And then, once it goes final, it gets sort of reclassified into the no-use category.

But if you go out insufficient, you have to have reasons for the insufficiency.

DR. BERGFELD: That seems like an unneeded step.

MS. FIUME: And it's hard because this is a re-review. So, if we have no choice -- there is no choice but to continue the review of it. I think it's a policy discussion that, rather than try and make a decision now, I would have to get with Bart, after the meeting, to see how we go forward with it. You know, just because it is so different than what we've done, so I don't want to say something and misspeak. I do see Jannavi has her hand up.

DR. SRINIVASAN: Thank you, Monice. Looking closely -- I'm sorry I'm taking this back to the conversation about dressing. The way I'm reading it is it's under hair preparations. So, I just want to make sure it says tonics. That's what I'm trying to, sort of, like, narrow down, just to make sure we're on the same page. It's under hair preparations, non-coloring, tonics, dressings, other hair grooming aids. I'm not sure where you got the wound dressing, but this table is saying something different. I'm talking Dibutyl, sorry.

DR. BERGFELD: Yeah, yeah.

DR. SAM COHEN: So, then we're back to two uses.

DR. SRINIVASAN: Yeah. Sorry.

DR. BERGFELD: That's okay.

DR. SRINIVASAN: Sorry, Hong pointed that out to me. She's in the audience, too. Thank you, Hong.

DR. DAVID COHEN: Yeah, just know, if you search the word "wound," it doesn't come up in this PDF at all.

MS. BURNETT: Okay. So, the data that we looked at had it categorized as that? That was the data we received last year, or the snapshot that we got from last year. So, it may have updated. Is that how I understand it? So, it's not a liquid wound dressing, it is a hair tonic?

DR. SRINIVASAN: I will have to come back to you on that, Christina, if that got updated, if somebody put that in, you know, in a renewal or something. I'll have to come back to you on that.

MS. BURNETT: Okay. Yeah.

DR. SRINIVASAN: Thank you. Yeah, this is what we're seeing on the 2025 uses. Yep.

MS. BURNETT: Okay.

DR. SRINIVASAN: Thank you.

MS. FIUME: Yeah, because we have it under "other preparations," is what we received. So, yeah, a clarification, I think, is needed all around.

MS. BURNETT: Yes.

DR. ROSS: And then whichever way we go, Monice, can we flesh out the discussion perhaps to say, that use is not recommended where safer alternatives -- you know, putting in everything we've had in this discussion today. Can we get to that point?

MS. FIUME: I think you can. I think, you know, you guys are -- all of you are very good at crafting your points into well-spoken Discussion items, and it can definitely be put in there, and your concerns listed.

DR. ROSS: Thank you.

DR. DAVID COHEN: So, are we back to not no -- well, not a double negative -- we're not using "no uses" because we may have uses; and then we're back to an IDA for concentration of use. Is that right?

DR. BERGFELD: Right. Depending on the clarification by FDA.

DR. DAVID COHEN: Yeah, but in the table --

DR. BERGFELD: We have to clarify it. No, I agree with you. I just said that when we asked FDA earlier, they came back with it was a -- we asked them for wound dressing, whether it was a medical treatment or a cosmetic, because that's what we were told, it was a wound dressing. Now we need clarification: what is the other, and so we can move forward.

DR. DAVID COHEN: I think they were answering the question in regard to if it's a wound dressing, it's going to be a medical device; but if it's not a wound dressing, and it's a tonic or a liquid --

DR. BERGFELD: It's something else. We need a concentration of use, yeah.

DR. DAVID COHEN: Yeah.

MS. FIUME: And we would also need that use information because that is different than what we have in our files. So, we would need something to reference.

DR. BERGFELD: Going back in summary, David, because I know you got this, but safe for Diethyl and Methyl, and maybe insufficient for Dibutyl, and concentration of use depending on clarification of the two other uses for Dibutyl.

DR. ROSS: And expansion of the Discussion.

DR. BERGFELD: And David wants an expansion of the Discussion, yep.

MS. FIUME: And I believe the whole Discussion needs to be built because this is just at the draft stage. So, any other Discussion items to be included, I'm sure Christina would appreciate.

DR. BERGFELD: I'm wondering if the group couldn't consider some of Dr. Daston's information in the Discussion, especially how each of these is used and then, obviously, the reproductive tox data for Dibutyl. And maybe at the place to put the NOAELs, even though they don't apply to cosmetics.

DR. SAM COHEN: And we need that reference from George.

DR. ROSS: Yeah.

DR. BERGFELD: And if we put the FDA and PCPC stuff into the Discussion, we just need a reference for it, I think.

DR. ROSS: And, Christina, I'll send you another reference, which was --

DR. BERGFELD: 27, I think, was the FDA's reference. I don't know what the PCPC was.

DR. ROSS: I'll send Christina another reference on structure activity for development and reproductive toxicity in males, which has some really useful NOAELs in there for this testicular dysgenesis syndrome.

MS. BURNETT: Okay, thank you.

DR. BERGFELD: Okay.

DR. DAVID COHEN: The idea of it not being an androgen blocker, but sort of prevents androgen production is kind of interesting.

DR. BERGFELD: I thought really interesting.

DR. ROSS: Yeah.

Full Panel – March 13, 2026

DR. BELSITO: Dibutyl Phthalate was placed on the '24 Priorities list following nominations by the FDA for cause. We first published a Final Report on the safety assessment of Dibutyl, Dimethyl, Diethyl Phthalate in '85, concluded the ingredients are safe for topical application and present practice of use and concentration of uses in cosmetics. In 2002, we reaffirmed the original conclusion that was published in 2005.

In 2012, we deliberated on studies separately concerning endocrine disruption in diabetes and Dibutyl Phthalate, Diethyl, Dimethyl Phthalate, and Butyl Benzyl Phthalate; however, the Panel chose not to reopen the safety assessment on the ingredients and published our Discussion as a re-review summary in 2017.

The data from the original report from the re-reviews have been included, and we have updated RLD data. And we looked at this, quite an extensive amount of data, particularly on Dibutyl Phthalate. In terms of our conclusion, there'll be lots of discussion, I know. We concluded that -- where is my conclusion? Anyway, off the top of my head, Diethyl Phthalate is safe as

used, and Dimethyl and Dibutyl Phthalate are insufficient for concentration of use since we can't derive a margin of exposure without knowing the concentration.

DR. DAVID COHEN: I think I'm inclined to second. I think -- wait, so you cleared Diethyl, but not Dimethyl?

DR. BELSITO: Yes. Well, Dimethyl we can't do a margin of exposure because we don't have a concentration.

DR. DAVID COHEN: Hold on one second.

DR. BELSITO: It's used in two products. Both Dimethyl and Dibutyl are used in two products and we have no concentration of use for either.

DR. ROSS: Correct. And, you know, I guess we felt that we had two options with Dibutyl. I'll get to that in a second, I suppose. And with Dimethyl, you're right, we've got two uses, we have no concentration. And so, you either tie it to the same concentration as Diethyl or you go insufficient.

And so, I guess it's those two options. But I don't have any issues with the Dimethyl insufficiency if you want to go that route. I mean, that's fine with me.

DR. DAVID COHEN: Yeah, I was going to second his motion on that.

DR. ROSS: Okay.

DR. DAVID COHEN: I'd rather go with that level of safety, so second.

DR. BELSITO: Because I think if we get into assuming the same concentration, then we're going to get into the whole issue of Dibutyl. And I'm very nervous about going ahead with Dibutyl, particularly given the paper we received last night, where we don't really have a NOAEL, we have a LOAEL. And I think it just opens a whole can of worms for two reported ingredients for which we have no concentration of use for the methyl.

DR. ROSS: Well, I agree. I mean, we had long discussions on Dibutyl. I think fundamentally they're very different with respect to the male reproductive toxicity. But again no issues, Don, in going insufficient on the Dimethyl. No issues there at all.

DR. DAVID COHEN: But one second; I just wanted to make sure we knew what the IDA was before we go into the conversation.

DR. BELSITO: Just concentration of use.

DR. DAVID COHEN: For both?

DR. BELSITO: For both.

DR. DAVID COHEN: Okay, we're aligned with that. And I'm sorry, I didn't mean to cut you off, but I know David has some comments about absorption, and the MOE, and some other items.

DR. BELSITO: Oh, yeah, there's lots of discussion here.

DR. DAVID COHEN: Yeah, yeah.

DR. BELSITO: I just gave the conclusion, and then usually we open it up for discussion after a conclusion, right?

DR. DAVID COHEN: Okay. Sorry, David, you could proceed.

DR. ROSS: Can we have some discussion now? Yeah?

DR. DAVID COHEN: Yes.

DR. ROSS: Yeah, that's what I thought. Yeah, so on the Dibutyl, yeah, I would agree that one option is to just call it insufficient and go out and ask for its use concentrations, because you're right we can't do an MOE if we don't have a use concentration.

The other issue we discussed long and hard, was we have wording in our dossier that the FDA had guidance for industry on limiting the use of Dibutyl in drugs and biological products, recommending the use of this ingredient be avoided if alternatives were available. PCPC is on record as not supporting the use of this ingredient. And I wondered where we were as a Panel, given the potent male reproductive toxicity of Dibutyl Phthalate, of making the statement that we agreed with those comments and use wasn't recommended when safer alternatives were available.

I don't know if that's the sort of statement you would put in here. With the Dibutyl, we know that we can get NOAELs. I mean, there are no NOAELs in the literature, whether they're trace amounts. So we can't really say that it's unsafe at any level because we know we have some NOAELs there. How could we make that statement? And I just wanted your input into that, the more experienced Panel members, if we make such statements in these dossiers or not.

DR. BELSITO: Well, I mean, we're scientists, right -- or we're supposed to be. And we could calculate a margin of exposure at which Dibutyl Phthalate might be considered safe. But we can't do that without concentration of use.

I think the male reproductive toxicity of Dibutyl Phthalate will go into the Discussion as will what George pointed out, when you get to C4 or lower you're not seeing that, right? I mean, there's a clear dichotomy in the effects. I think that should not be excluded. I think it should be pointed out that Diethyl and Dimethyl, their NOAELs for reproductive DART endocrine, are extremely high, whereas Dibutyl that's not the case. And that's where you see issues, Dibutyl and higher; Butyl Benzyl, you know, Diethyl- -- but yeah.

I mean, PCPC and FDA can go on record recommending it not be used if alternatives are available, I think we would agree with that. But I don't think we can come to a conclusion that they shouldn't be used. We can conclude that we can't conclude because we don't have concentrations.

DR. ROSS: And I'm fine with that. When we get the concentrations we can do an MOE easily.

DR. BELSITO: Right.

DR. ROSS: And come up with a value. That's not an issue. I just felt that this is a -- to me at least -- it's a lock-solid case of we would rather not have these in products where safer alternatives exist. And do we make that sort of statement? And is that something you've seen before?

DR. BELSITO: That would be an opinion statement from us and not one that's scientifically valid, so I don't think so. But the reality of Dibutyl Phthalate is its primary use was in nail enamels to prevent chipping, to add plasticity. And nail enamels have all been replaced by acrylates, so we'll have this discussion when acrylates come up for re-review in terms of their safety.

But, you know, back in the 70s and 80s and 90s, you looked at any of the quote-unquote formaldehyde-free nail polishes, or even most nail polishes, they had Dibutyl Phthalate. Now, if you can read the labels -- I can't anymore without a magnifying lens -- you'll find that phthalates aren't in virtually any manicuring preparations. Effectively, they've been removed from the market.

DR. SNYDER: Yeah, I was going to say, this is a little bit like the previous one, where we don't really want to hold up a report which gets the most important information out there that DEP is safe as used. And, according to Carol, that the DBP and the DMP are no longer being used, so companies are never going to give us the concentration of use. It would just hold everything up. I think this is, again, just a clean way for us just to get it out there so that the consumers can understand.

DR. ROSS: Yeah, Paul, we discussed that. And I guess we had two uses cropping up for Dibutyl, as Don said, right? And we bounced around what they were, and apparently, they're hair tonics. Isn't that right? Isn't that what we heard yesterday? Confirmation?

DR. BERGFELD: I don't think that they have said what it was yet. FDA needs to tell us.

MS. FIUME: Yeah, they were going to confirm, because that's what they thought it was yesterday.

DR. SRINIVASAN: I can speak to that. We did go back and look at it, at the data we had provided to CIR. It looks like the two uses that were discussed in Cohen's group were not Dibutyl; they were for hair preparations, tonics, dressing and other hair grooming aids, and that was actually Diethyl.

We went back and looked at the table. I just wanted to let folks know, this is the homework we did last night. So that was not Dibutyl. I know we had a lot of discussion yesterday about Dibutyl being the culprit, so that was Diethyl.

And the dressing is under hair preparations, and so it was not, as yesterday was discussed, as wound dressing, it's under hair preparations. It's more under tonics, dressing and hair grooming aids.

DR. DAVID COHEN: So that was not for Dibutyl? So do we have now no uses?

DR. SRINIVASAN: I think the table still had two uses, but unknown under Dibutyl, if I remember correctly.

DR. DAVID COHEN: I've got to go back now.

DR. ROSS: Yeah, I think we need some clarification on that. I mean, we can still use --

DR. DAVID COHEN: Well, it changes the IDA.

DR. ROSS: Yeah, we can still use Don's --

DR. DAVID COHEN: No, it changes the IDA. I just want to make sure.

DR. ROSS: That does change the IDA. Yeah, it would be "use not supported," right, because there's no uses?

DR. DAVID COHEN: So it just says "other" under Dibutyl. Okay. If there are two unknown uses, then the IDA can hold, right?

DR. BERGFELD: Well, we need their clarification of the uses.

DR. ROSS: I agree with Wilma. I mean, do we have two uses or do we have zero uses? Because it totally changes the conclusion here.

DR. BERGFELD: But that can be put in the IDA.

DR. DAVID COHEN: I'm looking. Dibutyl in Table 3 shows two unknown exposure type.

DR. BELSITO: Yeah, other preparations, unknown exposure type is what we have currently in Table 3.

DR. BERGFELD: Right.

DR. BELSITO: So, Jannavi, are you saying that that's incorrect and those two reports under unknown are actually Diethyl?

DR. SRINIVASAN: No, Don, that's not what I'm saying.

DR. DAVID COHEN: No.

DR. SRINIVASAN: So there's two separate things going on. Yesterday's discussion there was two uses that we were told was Dibutyl under dressing. And the discussion was, is this a wound dressing, would that be cosmetic or device? So, that was a clarification we were asked.

And I'm coming back and saying the dressing for that is not under Dibutyl, it's actually under Diethyl; and the dressing is under hair preparations, and not as, quote-unquote, "wound dressing." That's the clarification.

DR. BELSITO: Oh, thank you. So, where did the Cohen team even get that to discuss, because I'm not getting that from Table 3 at all?

DR. TILTON: I think the discussion was about the two unknowns and what those might be.

DR. DAVID COHEN: I think we crossed paths. But I came into that conversation --

DR. ROSS: Christina's got a comment, yeah.

MS. BURNETT: In response to a question Dr. Ross posed before the meeting, we looked at the raw RLD data to determine what those two other uses are. And in the raw data that we were provided by FDA, it claimed that the two uses were in liquid wound dressings. We had that discussion yesterday in Cohen's team, where we did not have that in Belsito's team.

DR. HELDRETH: Right, and those are for Dibutyl.

DR. BELSITO: Right. And so, now we're being told that those two uses were not wound dressing, but they're still Dibutyl. Is that correct?

DR. DAVID COHEN: No, they're Diethyl. They're Diethyl.

DR. ROSS: The wound dressings are, but there are still two uses for Dibutyl, unknown, as Don has just pointed out.

DR. DAVID COHEN: Right. There's no wound dressings.

DR. BELSITO: No, I understand. But there are two uses that are unknown for Dibutyl. Is that correct?

MS. BURNETT: Yes. That we still need clarification on what those two other uses are, from FDA.

DR. HELDRETH: Yeah, I mean, I'm looking right at the raw data, and I don't want to say the names of companies or the names of products, because I don't think that's fair or appropriate, but the names of both of these products include the terms "liquid wound dressing" for the Dibutyl products that we included in the table.

DR. ROSS: Wow. So, we're back to liquid wound dressings, which would be leachable, and that's a bit of a worry.

DR. HELDRETH: And that's not a cosmetic.

DR. ROSS: It's not a cosmetic, no. So we shouldn't actually be looking at that.

DR. HELDRETH: I mean, ultimately, I think that means that these two uses are not cosmetic uses as far as the Panel's purview.

DR. DAVID COHEN: So, you mean Table 3 --

DR. BELSITO: But I thought Jannavi just told us they weren't -- the Dibutyl was not wound dressings, it was Diethyl. Is that not correct?

DR. SRINIVASAN: Sorry, Don. So there was a lot of discussion on the other team; there was a lot of discussions. One thing is the two unknowns from what Christina is saying, and what Bart is saying, they are liquid wound dressing. That is what you

see as the raw data. We clarified yesterday that wound dressing would not be a cosmetic. That's one piece, right? Whether it's Dibutyl, Diethyl, whatever, it's wound dressing. So that is one thing.

The other clarification I was trying to make was the conversation we had about dressing and if that would be wound dressing across the board. We went back and looked, and the Diethyl dressing is actually under hair preparations. So there's two separate dressing conversations that happened yesterday. I hope that clarifies somewhat.

DR. DAVID COHEN: It clarifies it now, but it wasn't -- that is not how we concluded yesterday. We finished up by saying that wound dressings were not wound dressings, they were hair preparations. But it seems new to me that the Dibutyl, two other uses, are liquid wound dressings. That, to my memory, is not how we concluded yesterday.

DR. ROSS: If they are liquid wound dressings they are not in our purview, changes the conclusion. So we do need -- I think -- I'm still a little confused. Do we have any cosmetic uses for Dibutyl Phthalate or not?

DR. HELDRETH: No.

DR. DAVID COHEN: So that changes Don's conclusion.

DR. ROSS: Yeah.

DR. DAVID COHEN: Because then it becomes zero uses for Dibutyl, and that puts this into a more immediate suspension, doesn't it?

DR. BELSITO: No.

DR. DAVID COHEN: Why? It's no uses now.

DR. BELSITO: Right, but it doesn't put it into a suspension. Just remind us, Bart, what the issue is with no uses. We just simply don't review it, correct?

DR. HELDRETH: No, we review it. You've already started the review on it. But once this report goes final, the conclusion -- I'm proposing -- it would be insufficient data because you don't have frequency of use, concentration of use and what have you, and insufficient data, zero use. And because there's no reported uses, that would immediately be commuted to use-not-supported.

DR. DAVID COHEN: It doesn't have a two-year window, correct?

DR. HELDRETH: That's right. The two-year window is only there when there are reported uses. That way manufacturers have time to come back and say, wait, we have the data, we can help you figure this out.

DR. DAVID COHEN: That's what I was meaning, Don, that no use goes immediate.

DR. ROSS: I think that's what we were getting at yesterday, trying to figure out if there were any uses, and if this could go use-not-supported. I think we got there in the end.

DR. DAVID COHEN: Well, we're not concluding use-not-supported; we're concluding zero uses, which administratively becomes --

DR. BELSITO: But I'm still confused by what Jannavi said. Jannavi, can you clarify whether in fact, if we eliminate wound dressings, there are zero uses for Dibutyl?

DR. SRINIVASAN: Based on the data that was provided for this review, that would be correct. But keep in mind that was from 2025, the registration and listing data. That data keeps changing. So, based on what you have there, your conclusion would be correct.

DR. ROSS: Well, good. Thank you very much.

DR. DAVID COHEN: That makes things much different and, I think, much clearer and much easier. Bart, if the 2026 RLD had a use, does that trigger a response?

DR. HELDRETH: Not on our part, no.

DR. DAVID COHEN: So, it's a non-supported use by industry?

DR. HELDRETH: That's right. Once the Panel makes a final conclusion on it for insufficient data, or for unsafe, whether it's zero use or there are uses out there, it goes off to sleep in a grave unless somebody comes to resurrect it from a company saying, hey, I've got data, let's fix this, we can move forward. Otherwise, it's done. We won't ever re-review it again. It's gone.

DR. DAVID COHEN: Don, would you consider -- well, and the 2025 data is the most recent data. I mean, we could wait ad infinitum for something to change, but the data in front of us that is clearly articulated in the record right now is that there's no cosmetic uses for it, and so we go to zero uses. Are you okay amending your conclusion, Don?

DR. BELSITO: I don't know how that conclusion gets amended, right? We're saying that Dibutyl is insufficient for concentration of use and uses?

DR. DAVID COHEN: No.

DR. BELSITO: Bart, what's the correct language when we suddenly have no uses, but there's also an issue with concentration of use?

DR. HELDRETH: So, at this point right now all you would need to do is say that, for a conclusion, is that for the Dibutyl the data are insufficient.

DR. BELSITO: Yeah, that's what we've said in our conclusion.

DR. HELDRETH: The classification of zero use --

DR. BELSITO: That Diethyl is safe as use, and for Dibutyl and Dimethyl it's insufficient. And in the Discussion the insufficiency is concentration of use. And I guess now for Dibutyl would be uses in cosmetic products. Christina?

MS. BURNETT: Yes. So, this is the first review of this as a Draft Amended Report. An IDA will still have to go out procedurally. You would be issuing an IDA for concentration of use and use.

DR. BELSITO: Right.

MS. BURNETT: For Dibutyl, and concentration of use for Dimethyl.

DR. BELSITO: Correct.

DR. DAVID COHEN: I certainly understand that method. But we come on draft reports to conclusions, right, even though they're draft and they come back as draft tentative. Look, if we have no IDA in a draft report, we issue a draft conclusion.

DR. HELDRETH: Correct. If we have no data needs, the Panel can issue a tentative report directly from the draft stage.

DR. DAVID COHEN: Why wouldn't the conclusion for dibutyl be no uses?

DR. BELSITO: Well, we give industry a chance to come back and show us that they are using it in some product. I mean, you know, we look at the information and we have additional data needs, then it has to go out again, right? If we don't have additional data needs, it can jump a step.

DR. HELDRETH: Right. If we have data needs here at the draft stage, whether it's amended or not, our SOP is to go with an IDA, give industry, or the public, or anyone an opportunity to submit those data before we move on with it. So, we usually only move on directly from a draft report to a tentative report, with a conclusion, when we don't have data needs.

DR. BERGFELD: Don, I missed -- because of connections -- what your motion actually was. Can we repeat that?

DR. BELSITO: Diethyl Phthalate is safe as used. Dimethyl and Dibutyl are insufficient. And in the Discussion, the insufficiencies now for Dimethyl would be concentration of use, and for Dibutyl would be use in cosmetics and concentration of use.

DR. BERGFELD: David? Can you go along with that?

DR. DAVID COHEN: I still am hung up on why the conclusion is -- we're asking for concentration of use when we have clarification that there are no uses.

DR. ROSS: I was just going to say the same thing. And, Bart, I wanted a question for you, is have we ever gone to zero uses without going out previously with an insufficiency to ask for uses? Have we ever gone straight, immediately, to zero uses?

DR. HELDRETH: I mean, no. Whenever there's a data insufficiency at the draft report stage, whether it be concentration of use or use in general, or some specific tox endpoint that we don't feel comfortable that we have enough data for, we always go to an insufficient data announcement to give everybody a fair opportunity to reply.

DR. ROSS: (Inaudible).

DR. HELDRETH: For instance here -- this isn't the case, this is just a hypothetical. It's very possible that even though nobody is reporting frequency of use for this ingredient to the FDA's RLD, someone may still be using it and come forward with a concentration of use. And then we will consider it, as a group, to actually be in use.

I mean, I know we have the quote-unquote, "mandatory" reporting with MoCRA, but remember that there are caveats to that. If you've got a manufacturer that's making less than a million dollars in profit, they wouldn't have to report it to the RLD.

So, to be fair to all stakeholders and manufacturers out there, and the general public, we try to put out an IDA at the draft report stage. It doesn't mean we're not going to end up at insufficient data later on, but we need to provide the opportunity. I think Jannavi has had her hand up for a while.

DR. BELSITO: And to follow up on Bart's comment, a lot of these nail polishes, et cetera, are made by companies that probably don't fall under the MoCRA registration requirements.

DR. SRINIVASAN: I just had a clarification, Bart. The raw data, just so that I can follow up, you said was liquid wound dressing? Is that what it was?

DR. HELDRETH: Yeah. I have two here that represent the two that we had in the quote-unquote, "unknown" category. And each one has a trade name for the product. And the first one, after the trade name, says "waterproof liquid wound dressing," and the other one just says, "liquid wound dressing." And both of them are comprised with alcohol, nitrocellulose, Dibutyl Phthalate. And then the last three ingredients in each one vary slightly.

DR. DAVID COHEN: While David and I voiced our opinions about it, I think, because the final disposition of Dibutyl looks like it might go to zero uses ultimately, it gives every opportunity for industry to declare itself on this. So, I'm going to second Don's motion.

DR. BERGFELD: Okay.

DR. DAVID COHEN: It'll just go through another iteration of this, that's all. It's sort of a second call, right? Even though the first call is supposed to be sufficient, here's a second call.

DR. BELSITO: Yes.

DR. BERGFELD: All right. Any other discussion on other things, other than the concentrations? Don?

DR. BELSITO: Just for discussion, and first of all, the question whether we include it is WVHJ, I think, their two major points were that we had absorption incorrect. And they referred to this Hu paper that I sent around, and the neurocognitive effects on children.

In terms of the Hu paper, if we discuss it at all, I think there are a number of limitations. It's a mathematically modeled study that doesn't look into parent hydrolysis to the monoester in the skin. And we know that 98 percent of it is hydrolyzed in the skin. We have that data.

They don't have blood data for model calibration validation for air to skin exposure, and they even say that they need further model validation based on the blood data. They predicted, based upon their mathematical model, that skin absorption would be greater than PO absorption, which doesn't make sense to any of us, suggesting the model has some flaws potentially.

And they even acknowledged that there was medium uncertainty for personal care product exposures, and high uncertainty for air exposure in their model. And that these uncertainties were due to dermal absorption and to individual variabilities of physiological parameters and the fact they have no idea of unbounded EPM plasma. There are a lot of issues that would make using that absorption level not reasonable for us and to go with what we have, which was like a 5 percent level.

In terms of the neurocognitive, if we discuss that, we have the data from the meta-analysis, which essentially shows that there's no consistent neurocognitive effects seen when you look across all the studies. So, I think that answers the major issues that the WVHJ had. The question, before I go on to other discussion issues, are whether we reference the Hu paper and whether we handle the Hu and the neurocognitive in our Discussion?

DR. ROSS: Yeah, good questions. I think the Hu paper, I looked at that and I agree with your comments. I think it is probably overestimating absorption because it's not taking into account hydrolysis. I agree with you there, Don.

Even before we had those comments from WVHJ, I had commented to Jinqiu, he had done that MOE right by the book, it's very accurate, it's right on, with an absorption value of 5.5 percent. But generally I felt that looked a little low to me. Keratinocytes was something like 50 percent, animal studies was 60 percent.

I felt you could take care of these different absorption studies by an extra sentence in the risk assessment, by saying if you did a Tier 1 risk assessment with 100 percent absorption --

DR. BELSITO: It's still safe.

DR. ROSS: Yeah, it's still safe. You'd still have an MOE well over a thousand. You wouldn't even have to calc- -- you could just put an extra sentence in there. But I thank WVHJ for that because --

DR. BELSITO: I like that, actually, because I have that comment. You could go with 100 percent and still be safe as used.

DR. ROSS: Yeah. No, I think that's what we should add in the Risk Assessment section. And I have no problems bringing in the Hu paper. The neurodevelopment issues comments on that from our esteemed team?

DR. BELSITO: Yeah, they brought in study after study that looked at potential associations with ADHD, autism, other neurocognitive issues. We capture a lot of that already, and do we bring in their studies as well? But the bottom line, in our Discussion, is the meta-analysis of all these studies where there's no consistent neurocognitive effects seen.

DR. ROSS: Agreed. Yeah.

DR. DAVID COHEN: I agree. too.

DR. BERGFELD: I don't believe that has to come into the Discussion unless you think it's a big issue. That could just go into text.

DR. BELSITO: I mean, it was a big issue for them, so I think it's a big issue among -- I mean, the big issues among consumers, I think, are the endocrine effects and neurocognitive effects. Those are the big issues with Phthalates, so I thought we should discuss the neurocognitive effects.

DR. BERGFELD: I'm sorry, David, what'd you say? You agreed?

DR. DAVID COHEN: I agree. In the Discussion you're talking about, Don, right?

DR. BELSITO: Yeah.

DR. BERGFELD: Right.

DR. DAVID COHEN: Yeah, I agree. They're just too important and too top of mind right now.

DR. ROSS: I agree with that, yeah.

DR. BERGFELD: So, you're going to put it also into the text, under the Possible Risk, in the Meta-analysis area?

DR. BELSITO: It's there.

DR. BERGFELD: I know, but are you going to put your extra sentence in there?

DR. BELSITO: In the Discussion. You know, something to the effect of various neurocognitive abnormalities have been reported in association with the Phthalates; however, a meta-analysis looking across all of them found no consistent findings that would suggest an association, or something to that effect.

DR. BERGFELD: Well, we can look at that and see how it looks. That sounds a little bit wordy for the Discussion, but it would be nice with the Meta-analysis material.

DR. DAVID COHEN: Yeah, but this is a very important report.

DR. BERGFELD: I don't mind it. If you want to do it, go ahead. Okay, so we've had a discussion --

DR. BELSITO: A few more discussion points.

DR. BERGFELD: Okay. You got more? Okay.

DR. BELSITO: There's a section that says, Evaluation of Mechanistic Evidence for Reproductive Toxicity. The title suggests they're looking at a possible mechanism, but there's no mechanism, as George explained to us, with the dissolution of ligaments and the descent of the testes, et cetera, in that. So, that title needs to be changed. It's not an evaluation of mechanistic evidence.

And, basically, fetal rats were more sensitive than other rodents, Dibutyl Phthalate-induced antigen-independent adverse outcomes, and Dibutyl Phthalate-induced anti-antigenic effects. So, probably The Effects of Dibutyl Phthalate on Reproduction, or something like that, but not mechanistic.

DR. ROSS: That was the EPA, right, Don, I think, wasn't it?

DR. BELSITO: Yeah, it was the EPA one.

DR. ROSS: Yeah, okay.

DR. BELSITO: And then, on PDF Page 78, above Environmental Exposures, "Semen parameters in the men with no Dibutyl Phthalate in the baseline decreased after exposure to the Dibutyl Phthalate…." "This continued to decrease further even after crossback to mesalamine without Dibutyl Phthalate." That whole thing made no sense to me.

DR. DAVID COHEN: What PDF was that?

DR. BELSITO: PDF Page 78. The crossover-crossback perspective study, the results made no sense to me. And I thought we had enough information on effects of DVP on sperm, we could just eliminate that.

DR. BERGFELD: How did you plan to attack the endocrine effects in the Discussion?

DR. BELSITO: The endocrine effects?

DR. BERGFELD: Yeah.

DR. BELSITO: I mean, I think it's pretty clear that Dimethyl, Dibutyl -- Dimethyl, Diethyl have endocrine effects at exorbitantly high levels that aren't relevant to cosmetics, and that the endocrine effects become more apparent when you go above C4.

DR. ROSS: There's a lot of literature on that. I think that can be supported.

DR. BELSITO: Yeah. It's just this crossover-crossback study. It made no sense to me. And I don't think it adds any additional data information; it could be dropped out.

DR. BERGFELD: Okay.

DR. DAVID COHEN: I don't know if that answered Wilma's question, though.

DR. BERGFELD: No, it didn't.

DR. BELSITO: I think I answered Wilma's question that, basically, the endocrine effects are seen when you get above C4.

DR. DAVID COHEN: Are you going to put that in the Discussion?

DR. BELSITO: Yes.

DR. DAVID COHEN: Yeah, okay. I wasn't clear on that. I'm sorry.

DR. BELSITO: We haven't gotten to that. I'm just going through the report and pointing out things.

DR. BERGFELD: Okay. Well, since you pointed out the neuroendocrine, or the neuro- -- I thought you had a --

DR. BELSITO: Well, I wanted to deal with Women's Voices.

DR. BERGFELD: Oh, first, I see.

DR. BELSITO: So, yeah, in the Discussion, I think the two important things are the endocrine and the neurocognitive. And, again, the endocrine, when you get above C4, is when you begin to have potential issues. But we have a lot of data on Dibutyl Phthalate. In fact, most of the new data in this report was on Dibutyl.

So, we have data that theoretically could allow us to come back with a safe-as-use conclusion, right, if we knew the concentration, just based on being a scientific panel, right, we know that, the use and the concentration of use, which we don't have, that's why it's insufficient.

And with the DEP and the absorption, I think David Ross' point about adding a little sentence that even assuming 100 percent absorption, this would still be safe as used, is important to put into the Discussion as well. But we have incredibly high margins of safety with DEP. And that was it.

DR. DAVID COHEN: This will come back in September? Is that right?

DR. SAM COHEN: Don, just a minor correction on what you're stating. Is that it's not above C4, it's at or above C4.

DR. BELSITO: Right, at or above C4. Correct. Sorry.

DR. BERGFELD: It's nice to put all this together now because we can deal with it a little bit more quickly when we see the return information.

So, we have the question that's been proposed, and the conclusion that going out as insufficient as stated. Does anyone have anything else to add to this, either in the Conclusion and/or the Discussion, or into the text, at this point in time? Seeing none, I'm going to call the question. All those against? All those sustaining? It passes. It moves forward as insufficient.

JULY 25-26, 1983 PANEL MEETING – FIRST PUBLIC REVIEW

Full Panel

The following conclusion of the report was unanimously approved:

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

Dr. Hoffman suggested mentioning in the Introduction that this review dose not include the carcinogenic ingredient Di(2-ethylhexyl) Phthalate (DEHP).

Dr. Bergfeld requested the clinical data be expanded in the text of the report, and Dr. Hoffman suggested reference be made to the Russian article L.E. Milkov in the clinical section of the report.

Subject to minor revisions, the document will be announced as a Tentative Report for a 90-day comment period.

JUNE 18-19, 2002 MEETING – FIRST RE-REVIEW

Full Panel

Dr. Belsito said that his Team determined that the available data (summarized in the Draft Report) that have been identified in the published literature since the Panel's Final Report on Dibutyl Phthalate was published in 1985 warrant a decision by the Panel to reopen its original safety assessment.

Dr. Belsito said that the concern at this point relates to the anti-androgen effect of Dibutyl Phthalate, and that the Panel needs to do a thorough risk analysis to determine the extent to which its use in cosmetic products (primarily in nail products) contributes to the potential body load of Dibutyl Phthalate. He added that the Panel needs to examine how cosmetics containing this ingredient are used, what the likely absorption would be, and then do a risk assessment on that.

The Panel voted unanimously in favor of reopening the Final Report on the safety of Dibutyl Phthalate in cosmetics.

Regarding the Panel's deliberations on the Phthalates in Teams, Dr. Andersen recalled discussions of additional data that are under development. He said that it is his expectation that, as the studies are completed, the data will be provided to CIR and incorporated into the next draft (Scientific Literature Review) of the CIR report on Dibutyl, Diethyl, and Dimethyl Phthalates.

NOVEMBER 18-19, 2002 MEETING – DRAFT AMENDED REPORT

Full Panel

At the June 18-19, 2002 Panel meeting, the Panel voted unanimously in favor of reopening the Final Report on the safety of Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate in cosmetics. This decision was made after reviewing data that have entered the published literature since the Final Report was published in 1985 with the following conclusion: On the basis of the available data, the Panel concludes that Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate are safe for topical application in the present practices of use and concentration in cosmetics.

Dr. Marks said that after discussing the anti-androgen effect of Dibutyl Phthalate in great detail and doing a risk analysis (particularly, a margin of safety evaluation), his Team determined that the Panel's original conclusion on Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate should not be changed and, thus, that the Panel's original safety assessment should not be reopened. However, it was agreed that the conclusion should be edited as follows to reflect the current wording of CIR's conclusions: Based on the available information included in this report, the CIR Expert Panel concludes that Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate are safe for use in cosmetic products in the present practices of use and concentration.

In light of his Team's decision, Dr. Marks added that there should be a lengthy discussion of all of the new data that have been presented and a statement indicating the basis for reaffirmation of the Panel's original conclusion, taking into consideration the no-effect of Dibutyl Phthalate, in particular, the exposure from cosmetics, and the margin of safety that has been devised. Dr. Marks said that his Team determined that there is a significant margin of safety in relation to reaffirming the Panel's original conclusion.

Dr. Bergfeld gave each Panel member an opportunity to comment.

Dr. Slaga said that the data used for the calculations adds a tremendous margin of safety, without taking into consideration that Dimethyl, Diethyl, and Dibutyl Phthalates have different potencies (i.e., ranging from essentially no activity to active [Dibutyl Phthalate]). Therefore, he added that the total cosmetic exposure is diluted by the potency of these Phthalates, Dibutyl Phthalate being the one for which, chemically (because it is used in nail products), its reaction does not make it available as

much as the calculations would indicate. Dr. Slaga also said that greater exposure is associated with Diethyl Phthalate, but, that its activity is weaker, thereby causing another dilution and resulting in a greater degree of safety.

Dr. Katz said that it is common knowledge at FDA that cosmetics represent one area in which Phthalates are used, and that the issue of Phthalates and exposure, as expressed during an earlier discussion, will be addressed on a broader perspective. Relative to FDA's review of some of the data, she added that FDA has also been unable to find the burden of proof or show that there is now a safety issue regarding the use of Phthalates in cosmetics.

Dr. Katz stated that FDA will review the CIR report on Phthalates in more detail, in an effort to identify any additional information that will be useful in further discussions within the agency.

Rachel Weintraub commented on data provided by Dr. McEwen at yesterday's Team meetings, specifically, the potential levels of Phthalates in humans. She said that there appear to be inherent limitations to this analysis, considering that all of the products that contain Phthalates are not known. Ms. Weintraub added that this is supported by a report provided by Health Care Without Harm, which discloses many other products containing Phthalates that the Panel had not been aware of.

Ms. Weintraub noted that Dr. McEwen's analysis includes a number of products (i.e., hair spray, deodorant, perfume, and nail polish), but does not include others which may contain Phthalates in the fragrances or flavors that are not indicated on the label (e.g., shampoos and body lotions).

Regarding yesterday's Team discussion (Dr. Mark's Team), Ms. Weintraub recalled comments on the additive effects of DBP and DEHP (source: poster by Foster et al.). She stressed the importance of including all of the data discussing the additive effects, rather than use of the poster by Foster et al. only.

Dr. McEwen said that the memorandum to CIR that he completed this morning addresses the aggregate exposure, as presented in a document from Health Care Without Harm, taking the most conservative NOEL and the 95th percentile maximally exposed subset of the population. This includes all DBP exposure, because it is a biomeasurement of that population. Dr. McEwen stated that the memorandum to CIR will be available to the public.

Dr. McEwen expressed industry's appreciation of information on Phthalates from the public. He said that the documents provided (particularly, the study on exposures from various products) helped him arrive at a decision as to the probable human exposure to Phthalates.

Dr. Bergfeld asked if anyone in the audience wished to make a brief statement.

Charlotte Brody, with Health Care Without Harm, expressed disappointment over the fact that her small, non-profit organization, in collaboration with two other small groups, had to present the cosmetics industry with data on Phthalate levels in its own products. She said that there should be more transparency in terms of which products contain Phthalates, and that this information needs to be made available.

Ms. Brody added that it is difficult for her to respond to Dr. McEwen's use of Health Care Without Harm's study, considering that his information was presented this morning. She said that this is not her idea of a public process. Similarly, she expressed disappointment over the fact that, in her opinion, calculations (based on industry's data on exposure) done in Teams yesterday will not be made available to everyone.

Ms. Brody asked the Panel to consider the question of whether or not the population would be better off with less or more Phthalate exposure. She said that the message to industry should be that less Phthalates in cosmetics would be better than more.

The Panel unanimously concluded that the Final Report on Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate should not be reopened and that the Panel's original conclusion should not be changed.

In summary, the Panel compared the current uses and consumer exposures with the available safety test data, and concluded that not only are exposures low compared to levels shown to produce adverse effects in animals, but that there was a high margin of safety between exposures and levels demonstrated to produce no-observable-effects in animal tests. Therefore, the Panel concluded that these ingredients are safe for use in cosmetic formulations in the current practices and concentrations of use, and that there was no need to reopen the safety assessment. This conclusion, an extensive presentation of the new scientific studies and other data considered by the Panel, and the rationale for the decision will be included in CIR 's Annual Review, which presents the rationale for decisions not to reopen prior safety assessments.

Dr. Bergfeld asked that the Panel have an opportunity to review the Annual Review prior to its announcement.

Dr. Andersen said that the Annual Review will be made available to the Panel prior to its announcement.

FEBRUARY 6-7, 2003 MEETING – FIRST RE-REVIEW SUMMARY

Full Panel

A CIR Final Report with the following conclusion was published in 1985: On the basis of the available data, the Panel concludes that Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate are safe for topical application in the present practices of use and concentration in cosmetics. Since this conclusion was issued, many additional studies have appeared in the scientific literature. These studies, along with current frequency of use and use concentration data, were considered by the CIR Expert Panel.

On November 19, 2002, the CIR Expert Panel announced its decision to not reopen the Final Safety Assessment on the Phthalates and asked that a summary of the newly available data and a discussion of the issues be prepared for the Panel's review.

Dr. Bergfeld stated that the Panel has been provided with the re-review summary and discussion on the Phthalates, to be published in the Annual Review, for review at this Panel meeting.

Dr. Andersen noted that this request was made because of the understanding that documentation of the decision not to reopen (i.e., the summary for inclusion in the Annual Review) the Final Report on Phthalates would be different in comparison with other decisions not to reopen that have been made. He said that this is based on the need to present and discuss a large amount of new information on the Phthalates.

Dr. Andersen also stated that the Panel now has an opportunity to comment on the summary and discussion that have been prepared.

Dr. Snyder said that the Panel's reason for not reopening the Final Report should be stated in the first paragraph of the summary.

In response to Dr. Snyder's comment, Dr. Andersen proposed the following statement: Based on its consideration of the data discussed below, the Panel decided not to reopen this safety assessment.

DECEMBER 10, 2012 MEETING – SECOND RE-REVIEW/STRATEGY MEMO

Dr. Belsito's Team

DR. BELSITO: Right. Exactly. Thank you. Okay. We have time for a few more here. So let's at least start the phthalate discussion. And I guess since Alan is here and he had a chance to review this guidance for industry document and it was just sitting here when I got here and I haven't even seen it yet, basically this came from the FDA Center for Drug Evaluation and Research.

DR. ANDERSEN: That's correct.

DR. BELSITO: So, not from cosmetics.

DR. ANDERSEN: Right.

DR. BELSITO: And their recommendation is in drugs to do what?

DR. ANDERSEN: Phthalates in particular dibutyl phthalate and diethylhexyl phthalate have uses as excipients in drugs, and because there are alternatives to perform those excipient functions at the Center for Drug Evaluation and Research is recommending that the two phthalates not be used. And the science behind it is nothing different than what you have reviewed. There's really no new data. What's different is that the Center for Drugs Evaluation and Research has applied the precautionary principle and not a risk assessment. What you guys have done is a risk assessment with large margins of safety vis a vis cosmetics. So, I would argue that this doesn't have any real impact on the phthalate question, but you needed to know it exists.

DR. BELSITO: Okay.

DR. BRESLAWEC: Dr. Belsito? I might point out that the FDA guidance specifically states that the recommendations in this guidance do not address the use of DBP or DEHB in other types of FDA regulated products.

DR. BELSITO: Well, diethylhexyl phthalate is not a cosmetic ingredient anyway, right? I mean, because we deal with what, dibutyl, diethyl, and --

DR. ANDERSEN: Butyl benzyl.

DR. BELSITO: Butyl benzyl.

DR. ANDERSEN: Yeah, that's correct but I think that sentence in the guidance document was written more for the Center for Devices and Radiological Health, which does have diethylhexyl phthalate as a cross to bear because of its use in tubing to keep it flexible.

DR. BELSITO: Okay. So anyway, what we're presented here were three different studies. One done on a South Bronx population of children looking at phthalates in airway inflammation as measured by nitric oxide, and then two studies looking at diabetic populations. One from Uppsala, Sweden and the other based upon the NHANES data in the United States.

I thought the phthalates and airway inflammation -- I mean, they were able to draw some lines but when I looked at the scattergrams it looked like it was all over the place and I had a real hard time making any sense of it.

The diabetic studies -- I'm not a statistician and I was just sort of overwhelmed with the statistical analysis of these studies, but also impressed that there did seem to be somewhat of a correlation with urinary phthalates and diabetes. And then a possible explanation for this, you know, based upon the nuclear praxisome proliferating activity and the fact that there are drugs that target that to treat diabetes.

So, I don't know if that's cause for re-opening because I'm not sure that phthalates from cosmetic preparations are absorbed to a level where that will reasonably occur. Also, they were looking at phthalates that aren't used in cosmetics, so I really thought that when I was looking at those levels of non-cosmetic phthalates that there were other sources that were likely more important if this was even real, which I'm not sure it is. But there were sources of phthalate exposure such as freezing plastic water bottles or microwaving in plastic whatever that probably were more important than what we were seeing in terms of exposure from cosmetics.

But that was my own personal view, so I open it up to people who know more about phthalates and diabetes and airway inflammation than I do.

DR. SNYDER: Who would that be? (Laughter) Yeah, I read the data on all these. I thought they both did a good bit of addressing the study limitations of both studies. I still think that the big missing link is what's the underlying mechanism. You know, we have these associations but how does that link that they're related, I think is one issue.

I did want to know that we did not have -- I didn't understand why we did not have the Gaithera, 2004 reference was not in our document, in our report previously. But I thought that we had addressed the issues related to -- in the old report we did address the metabolism issues and the enzyme systems as it pertained to the diabetes report. So, I thought we had addressed some of that even though it wasn't directly linked maybe at the time to diabetes, but we were already aware of those issues about the enzyme systems regarding the metabolism of the phthalates.

So, I wasn't all that concerned about the new data set in regards to what we already know about the phthalates.

DR. LIEBLER: I think the papers establish that there is a relationship between the parameters measured and nothing more. I -- you know, for example between exhaled nitric oxide and urinary phthalate metabolites. There is a statistically significant relationship that may or may not be biologically significant, and so I felt that way about all three of these papers. Essentially there are three variations on the same type of study.

And so I said, okay, well if we did use this to re- open what the heck would we do once we did that? Because we can't interpret these studies at any level mechanistically that would inform our evaluation of our prior conclusion. So for that reason I felt that we really can't use these studies to really re-evaluate our conclusion because they provide really no mechanistic insight as to whether or not there's any causal relationship between these ubiquitous environmental contaminants and the disease states -- either airway disease or diabetes.

So I felt if we did re-open it we'd have no place to go and we wouldn't really end up being able to change our conclusion. So for that reason, I suggest we do not re-open.

DR. KLASSEN: I'll basically second what Dan said. You know, these are -- these three studies kind of show a weak association. You know, the data is not that impressive. You know, they are statistical associations. I mean, we know that the -- what the phthalates do biologically, and that's been covered before.

I question that it is a biological significance, or even if it might be reproduce-able in another study. So, there are many explanations for why these associations might occur and to suspect that they really are important is premature at this stage. And I think we should not re-open, because of these papers or anything else.

We also have another paper, I guess, on our desk this morning in regard to the sulfation with the phthalates that we might want to address.

DR. BELSITO: I didn't see that.

DR. KLASSEN: It had to do with sulfation.

DR. BELSITO: Oh, that was part of the child/infant report.

DR. KLASSEN: Yeah, might use it two different ways.

DR. BELSITO: Oh, okay. So we acknowledge the papers, we've read them and don't want to do anything with them. So, Alan, how do we communicate to the public that we did this?

I mean, were we being asked to potentially re-open this on the basis of this data? I thought this was like a panel FYI.

DR. ANDERSEN: Well, it's a panel FYI but you do need to make the decision if the information crosses the threshold to re-open.

DR. BELSITO: Okay.

DR. ANDERSEN: By saying that it doesn't, we'll capture that in the minutes of the meeting and it will become a matter of public record.

DR. BELSITO: Okay.

DR. BERGFELD: But also, it will be in our record and the annual report, will it not? That which we have not re-opened and the reasons given.

SPEAKER: Yes.

DR. BELSITO: I guess the only thing that I have a slight discrepancy with is, you know, when we say, you know, we have no mechanistic clue. I would agree with the airway inflammation but, you know, I thought the argument for the peroxisome proliferator receptors in diabetes was pretty cogent. So, I think they offered a potential mechanism there, so I think we have to come up more with then just a statement we don't think there's any mechanism that helps us.

You know, my point was that they were looking at levels of phthalates that aren't used in cosmetics and in fact the -- as I interpreted the data, the burden from cosmetic use was insignificant compared to burdens from other exposures, particularly given the levels of non-cosmetic phthalates that were found in the urine. So, that was my point. Not that, you know, these were interesting articles. You know, there was an association, there was a potentially plausible explanation for a link. The issue was, we don't think the exposure is largely from cosmetics, we think the cosmetic exposure is negligible and that -- number one, and number two there needs to be further investigations. You know, there were limitations in the study, as the authors readily acknowledge. These studies had limitations, yadda, yadda, yadda.

But to simply say there was no mechanistic explanation for airways, I would agree. But for diabetes, I would have a little bit of a pause.

DR. SNYDER: I agree. I think they did address, and very well -- they said this could potentially be a plausible mechanism, but they even conclude themselves that their data set is insufficient at this time to conclude that that is a mechanism action that further studies are required.

I think it's the same conclusion that we would come up with if they hadn't written that, or that's what we're saying, is that we are aware of these associations but at this time there's no data to support they're nothing more than associations.

DR. BELSITO: I'm sure you can wordsmith it.

DR. ANDERSEN: Message received.

Dr. Marks' Team

DR. MARKS: Okay. Talking about phthalates. So, this morning we found this. The guidance for industry limiting the use of certain phthalates as expediencing CDER regulated products. In 2005, the panel decided not to reopen the phthalates particularly at that time we were focused on the possible endocrine disrupter development issues.

We have some new studies. One, an airway study and a couple on diabetes. Alan's summary states the issues well. Is there any reason to reopen?

DR. SLAGA: Do not reopen.

DR. SHANK: Well, those epidemiological studies are not a cause to reopen. I'm not too sure how to handle this. I haven't read it yet. The CDER regulation but if this applies to drugs is it also going to apply to cosmetics eventually.

DR. ANDERSEN: Very specifically not.

DR. SHANK: Okay.

DR. ANDERSEN: Not the medical devices, not the cosmetics, it's dibutylphthalate, diethylhexophthalate as used as excipients in drugs only.

DR. SHANK: Okay. Thank you.

DR. ANDERSEN: I just didn't want to hide it since it came out last Friday. It seemed timely.

DR. SHANK: Yes. Okay. Then I would say don't reopen.

DR. MARKS: Ron Hill, not reopen? Yeah, okay. Now, in terms of the discussion and the re-review.

DR. SLAGA: Well, we definitely have to discuss the epi studies but as Ron pointed out the early association, there's no way to come up with a concentration to relate to cosmetic. There is concern in my eyes that some of these phthalates may have effect on people who are gamma receptors. Which the gamma ones are the ones that the diabetes drugs, the glitazone class of compounds are effective agonists. And then also the alpha, there's a number of different types of fibrates that interrelate to this too that I just think we just discuss that and that's all we have to do.

DR. ANSELL: Well, this is not a re-review. This was three specific papers which questioned whether this should jump out of cycle.

DR. SLAGA: Yeah.

DR. ANSELL: So, I don't know that, I mean that's of course an issue and we can reopen based on --

DR. SLAGA: No, no. I didn't say reopen. This is in a re-review summary.

DR. ANSELL: But this isn't a re-review, right?

DR. MARKS: It's a thick document for not being a re-review.

DR. ANSELL: I thought this was -- I'm sorry. Maybe I'm out -- I thought this was brought forward specifically to assess three papers that we became aware of.

DR. ANDERSEN: That's correct. It's -- you have the option of reopening based on these new data but the minutes of the meeting could be an adequate summary of the basis --

DR. SLAGA: Right.

DR. ANDERSEN: -- for a decision to not reopen it. Like you did at the last meeting with respect to parabens. You said, sorry Charlie, there's not enough new information here to support reopening it. And our previous conclusion is still okay. And that's what I would -- if you choose not to reopen, that's what I'd do here.

DR. MARKS: Leave the minutes and let it stand and not actually publish a re-review summary.

DR. ANDERSEN: Yeah, I think if we did otherwise we'd be publishing a re-review every time somebody published something.

DR. MARKS: Right.

DR. ANDERSEN: No, thank you.

DR. ANSELL: It would make the threshold to look at a paper that came through so onerous that we might not want to do that.

DR. ANDERSEN: Right. Giving you the option of saying, fiddlesticks, this is important. We'd better reopen this. That is what --

DR. MARKS: And so, in the minutes we'll capture that you've already said that, Alan, and Ron Shank is --

DR. ANDERSEN: Yeah and we'd make the note that it specifically excludes any relevance to cosmetics.

DR. MARKS: Okay. So, we'll not reopen phthalates once again and we'll just capture that in the minutes and the biggest three epidemiologic studies don't warrant reopening. Okay.

Full Panel

DR. BELSITO: This is part of our ongoing surveillance of chemicals that have caught the public's attention in various ways. And this was triggered by two reports, one suggesting a linkage between urinary phthalate monofunctional metabolites and airway inflammation as measured by nitric oxide in children from the South Bronx. It was a rather interesting paper with statistical correlation, but when you looked at the individual points, they were really all over the board. And some of the higher phthalate levels were phthalates that are not used in cosmetics, suggesting that exposures were from sources other than cosmetic exposures. And the mechanism of action was speculated, and the authors actually went through great pains to point out the various limitations to their study and what would need to be done.

Then there were two studies on diabetics, one from Sweden and one part that was taken from the U.S. in the Haney study. And again they linked phthalate levels to diabetes, and they specifically subclassified the types of diabetes depending upon phthalate exposure, hypothesizing in effect on nuclear peroxisome proliferating activity receptors because apparently there are diabetic drugs that act via that mechanism. But again --

DR. SLAGA: Antidiabetic.

DR. BELSITO: Antidiabetic, yes, thank you for the correction. But again, despite the statistical linkages, again many of the phthalates were those that aren't used in cosmetic products. And while we took these under advice for lack of a better word, we didn't really feel a need to reopen the phthalate document because of them.

DR. MARKS: Second.

DR. BERGFELD: So a motion has been made and seconded not to reopen. Any other discussion?

DR. MARKS: Our team wanted to know how we would capture this discussion since phthalates are a hot topic and we felt that the minutes would be adequate.

DR. BERGFELD: Alan, can you respond to that? How this will be recorded? It seems to me we have a mechanism in the annual report that appears in The Journal.

DR. ANDERSEN: I think we have traditionally summarized all decisions to not reopen safety assessments and while we haven't published one recently, they are prepared for inclusion in The Journal. I don't know what our track record is at this point in terms of self-initiated re-reviews. That's what I went through was certainly true for the -- as Dr. Belsito referred to a minute ago -- the 15-year mandated re-reviews. Those for sure are done. It's really the Panel's call as to whether this decision is just captured in the meeting minutes or whether a full re-review summary is prepared for publication. In the most recent example, when the Panel reviewed parabens earlier this year, you did not ask for a re-review summary. You just determined to not reopen it based on the handful of new studies that were available.

Procedurally my concern is a new CIR re-review publication every time a paper comes out; that's procedurally a question. But if that's the Panel's desire, we can go in that direction.

DR. BERGFELD: Jim?

DR. MARKS: As I said, our team felt it could be captured in the minutes. Just precisely for what you said, Alan, is that every time a new study came out if we did a re- review summary, it would just become quite burdensome and probably not add much to the safety of these ingredients.

DR. BERGFELD: I'd like to ask a question. Are the minutes available to the public because this, as Don mentioned, is a hot item now and our response would be important?

DR. ANDERSEN: The answer is yes, they are. The post-meeting announcement that will include all of the details of this discussion will be -- if we follow our current practices and procedures -- be online and available by this Friday. So everybody will have -- any interested party will have ample opportunity to see what we did.

DR. BERGFELD: Don?

DR. BELSITO: I guess the only other comment I would make, and you can take it or leave it, would be that, for instance, if you go in to check CIR status and you were to type phthalate, it would come up with our original report and our re-review. It may also be nice when we've taken a special look, even though we've determined not to reopen, not to issue a report, if you typed in phthalates, when it came up it said "discussed at Panel meeting" -- or something to that effect -- "see minutes." And you can hit on it and it links to the minutes. So someone could look and see not only did we re- review phthalates in whatever year, but in December of 2012 we took a look at these three specific papers and decided that for the reasons reflected in the minutes not to go back and reopen the report.

DR. MARKS: That was a question I was going to ask Kevin. Is this searchable? Will it appear?

DR. ANDERSEN: Speaking on Kevin's behalf, I think the answer is it's searchable if we make the extra effort to make it searchable and if there's nothing automatic about it. But I think given Don's comments, message received.

DR. BERGFELD: I'd like to make a comment. Why is it that we cannot do a document, but just not present it for publication but to present it to the linkage in addition to the minutes? I mean it's not -- it's a one-pager usually that updates with the appropriate references, and you can prepare it for the Website, but not particularly for publication..

DR. MARKS: I guess the minutes really capture it the way Don reviewed it, and if we have three more studies that appear in the next six months and we review those studies again, do we do another page document? Pretty soon it'll become a boilerplate as to these three new studies, these two new studies, these five new studies didn't cause us to reopen. So I think it's captured in the minutes as long as the minutes are searchable as Don suggested. I think that's really important so it'll direct you to the meetings of this discussion right today.

DR. HILL: As long as we know that that key word would result in hitting the post-meeting summary. I mean Kevin will know what's needed there to make that happen. To me that would be sufficient except under a circumstance where we really had the need to put together some sort of a paper. There might be conditions, and I'm not sure this would be one of them, but that's just my opinion.

DR. SNYDER: I have a more general comment. I'm less concerned about what we do with the data, just that we're aware of the data. So I'm curious as to what triggered us to be aware of those three publications. And so do we have or is it so to speak on the radar of a particular writer, parabens, the phthalates, the hot button topics, because I think we need to be kept abreast of the current publications. Now whether they rise to the level that we want to consider a reopening or not, I think we should be not waiting 15 years before we ever look at phthalates or parabens again with it being such a public awareness of those ingredients. And I think we need to -- so I'm more concerned about what are we doing to trigger looking for new data on those ingredients.

DR. BERGFELD: Alan?

DR. ANDERSEN: I wish I could give you a presentation of what triggers that happening. It's in the eye of the beholder with respect to the paraben studies that we looked at earlier this year. It just seemed very obvious that we needed to look at that. So it's really between me and the rest of CIR staff flagging that something is important to bring forward. I don't have anything more than we know it when we see it.

DR. BELSITO: And I think that we need to be vigilant in our own various worlds. I mean I get -- a lot of the phthalate issues get directed to Dan and me because we now sit on the RIFM Panel and phthalates are obviously very important to the fragrance industry. And I think -- and then as a dermatologist I have various links that come up to issues related to concerns of chemicals that are in derm products, and paraben is the largest or is the most frequently used preservative in cosmetics. So I think we all need to take our own level of expertise. I'm sure we're all on various Web links that send us information important to what we do. And when we see something coming across about a cosmetic ingredient, we head it back to Alan.

DR. BERGFELD: Halyna, can you respond to how the Council might identify these particular hot button items?

DR. BRESLAWEC: Well, as you can all imagine, we monitor the safety information and concerns about all cosmetic ingredients pretty vigorously. A lot of times it's something we pick up in the press and we decide needs to come to CIR. We've asked CIR for review and opinion. A lot of times our CIR Science and Support Committee will bring something to the table and also the SRTC, the top toxicologists from all the companies who meet on a regular basis, will bring something up. So we've made a practice of bringing articles and issues that raise concerns about ingredients that CIR has reviewed. We make a practice of bringing that to CIR for their review and assessment.

DR. BERGFELD: So basically you're the alert person for us officially?

DR. BRESLAWEC: From our perspective, absolutely, but I just historically know that FDA's done this and that individual members of the Panel have also raised concerns.

DR. BERGFELD: Don?

DR. BELSITO: And the other Website that I follow to find out the latest breaking news, whether it be Internet roar or a scientific publication, is the Environmental Working Group because you can ensure that it pops up very quickly on their site if there are any concerns about cosmetic products..

DR. BERGFELD: Dan?

DR. LIEBLER: One practical point about having, for example, phthalates, this instance of our discussion of phthalates to show up on the Website. But I don't think we necessarily need is for every time phthalates gets mentioned in a transcript incidentally, for example, to pop up in a search. But more along the lines of perhaps the threshold would be if it's on the agenda, there should be an entry and that will probably cover all of these instances then.

DR. ANDERSEN: Well I'm glad you said that because that increases the likelihood that Kevin won't shoot me.

DR. LIEBLER: Especially because I'm right between you and Kevin.

DR. ANDERSEN: But I should -- we've had a lot of discussion about who is watching what's going on in the scientific literature, and I think any interested party should feel empowered to let CIR know if something has appeared that deserves CIR's attention. There's just no reason for there to be any barrier to that happening. So yes, we're vigilant, and we have people who have a self-interest in being vigilant. We have the scientific expertise on the Panel that picks stuff up. All that's great, but anybody ought to be able to raise a red flag and have us pay attention to it.

DR. BERGFELD: We think that we need to vote on this. I'm just surveying if we voted. The conversation went on so long here. But I call for the motion. The motion I believe is not to reopen and to add -- and the discussion led to how we were going to record and link why we did not reopen for the public and our own interest. So if there's no more discussion, let me call for the vote not to reopen. Unanimous. Thank you.

MARCH 18-19, 2013 PANEL MEETING – SECOND RE-REVIEW SUMMARY

Full Panel

Dr. Bergfeld was very pleased with the summary and discussion, and noted that this report sets the precedent for creating this type of document for controversial ingredients that are considered for re-review in the future. She added that the Panel should routinely have an opportunity to review all re-review summaries and discussions before they are published in the Annual Review.

Dr. Andersen said that the re-review summaries could be routinely included on the meeting agenda, for the Panel's comments, prior to publication in the Annual Review. He noted that the public already has an opportunity to comment on these summaries, because the Panel's re-review decisions are announced to the public and a 90-day comment period is observed.

The re-review summary and discussion on Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate were approved by the Panel, and Dr. Andersen noted that the announcement of this decision will be followed by a 90-day public comment period.

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

Status: Draft Tentative Amended Report for Panel Review
Release Date: September 11, 2026
Panel Meeting Date: October 5-6, 2026

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Bruce A. Brod, M.D., M.H.C.I., F.A.A.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel member involved in this assessment: David E. Cohen, M.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR, and Jinqiu Zhu, Ph.D., DABT, ERT, DCST, CIR Toxicologist.

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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
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

DRAFT 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 formualtions. Diethyl Phthalate is also reported to function as a denaturant. The Panel reviewed the available data to determine the safety of these ingredients. The Panel issued an amended report…[TBD].

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.

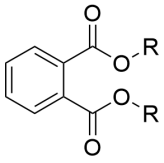


Figure 1. Phthalates, wherein both R substituents are either butyl, ethyl, or methyl.

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 (Table 3).[11,12] Additionally, the RLD reported Dibutyl Phthalate is used in 2 “other” preparations and Dimethyl Phthalate is used in 2 skin care preparations. 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.[14] 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.[15]

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.

Following the passage of MoCRA, the FDA created a number of new product categories, including “(17) Other preparations (i.e., those preparations that do not fit another category)” (Category 17). However, 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 safety assessment conclusion does not apply thereon.

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.[16] 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.[17] 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.[18] Dibutyl Phthalate has been classified as a Category 2 toxic substance to reproduction that "may cause harm to the unborn child".

Diethyl Phthalate and Dimethyl Phthalate are not restricted from use in any way under the rules governing cosmetic products in the European Union.[17] In 2002, the SCCNFP opined that the safety profile for Diethyl Phthalate supported its use in cosmetic products;[19] this opinion was reaffirmed by the SCCNFP in 2003[20] and the Scientific Committee on Consumer Products (SCCP) in 2007.[21]

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 a 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.[22] 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.[23] 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).[23]

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.[24] 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) and is an authorized denaturant (27CFR§21.71, 21.72, 21.106, and 21.151). The European Food Safety Authority established a tolerable daily intake for Dibutyl Phthalate at 0.01 mg/kg bw/d based on reproductive effects.[25]

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/cm^2/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/cm^2/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 cm^2), an infinite dose of [^{14}C]Dibutyl Phthalate (50 mg/cm^2) 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 ±1 µg/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.[26] 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.[27] 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).[28] 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/cm²/h across nude mouse skin and 26.9 ± 6.82 and 11.9 ± 3.20 nmol/cm²/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.[29] 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.[26] [^{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.[30] 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.[31] 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.[32] 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.[33,34] 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.[33]

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).[35] 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.[36] 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.[37] 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.[38] 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).[39] 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.[40-44] 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.[45]

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.[46] 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.[47,48]

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.[49] 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).[50] 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.[51] 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.[52] 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.[53,54] 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.[54] In another feed study, anogenital distance was significantly decreased in male F_1 and F_2 offspring in the 10,000 ppm dose groups.[53] 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.[6,55] 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. [56] 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.[57] 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.[58] 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.[59] 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.[60] 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.[61] 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.[62] 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.[63] 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.[64] 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.[65] 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.[66] 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.[67] 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.[68] 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.[69] 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.[70]

Multiple oral studies have investigated the effects of phthalates on male offspring of female rodents treated during gestation.[60,62,71-82] 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.[71] 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.[60,72-79] 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.[77] 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.[80] 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.[62] 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.[81] 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.[80] 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.[6,39,54,66,83,84] 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.[83] 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.[84] 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.[66] 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.[54] 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.[39] 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.[85] 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.[86]

In additional studies, 100 mg/kg Dibutyl Phthalate injected into chicken eggs significantly reduced hatching and increased late hatching when compared to controls.[87] 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.[88] 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.[89] 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.[90] 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.[91] 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.[92] 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.[93] 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.[94] 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 ($p < 0.001$).[14] 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.[93,95] 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.[96]

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.[97] 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.[54] 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.[54] 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 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.[8,98] 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).[99] 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.[100] 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.[101] 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).[102] 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).[103] 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.[104] 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.[105] 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.[30] 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.[106] 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.[107] 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.[107] 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.[108] 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.[109] 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 1×10^{-4}, 3×10^{-3}, and 7×10^{-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 1.17×10^{-3}M and 1.53×10^{-3} M, respectively. Dimethyl Phthalate did not completely inhibit outgrowth at concentrations less than or equal to 3.05×10^{-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 1.35×10^{-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 3.1×10^{-2}, 6.3×10^{-2}, and 7.7×10^{-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.[93] 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.[69] 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.[110] 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.[111] 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.[112] 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.[113] 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.[114] 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.[114]

A 28-d oral gavage study in male Kunming mice (8 per group) was conducted with Dibutyl Phthalate at 50 mg/kg/d.[115] 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.[115] 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).[116] 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.[117] 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.[118] 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.[119] 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.[120] 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.[121] 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.[122] 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.[123] 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.[124] 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.[125] 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.[126] 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.[127] 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.[128] 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.[129] 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.[129]

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.[130] 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.[131] 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.[132] 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.[133] 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.[134] 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%).[135] 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.[136] 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.[137] 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.[138] 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.[37] 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.[139] 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.[140] 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).[141] 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.[142] 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.[143] 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.[144] 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.[145] 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.[146] 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.[147] 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.[148] 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.[21] 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,[149] 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.

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. All values exceed the safety threshold of 100, indicating an adequate margin of safety.

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)[6,55]

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

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

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.[150] 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.[151] 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% confidence interval 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% confidence interval 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.[152]

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.[153] 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.[154] 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.[155] 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.[156] 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.[157] 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.[158] 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.[159]

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.[160] 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.[161] 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).[162] 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.[163] 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).[164] 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.[165] 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.[69] 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.[166] 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% confidence interval (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.[167] 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.[168] 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.[169] 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.[170] 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.[171] 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.[172] 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.[173] Of the 130 mothers studied, 62 were cryptorchid males infants 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 ($r = –0.22$, $p = 0.033$).

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 and Dimethyl Phthalate is used in 2 skin care preparations. 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. Diethyl Phthalate and Dimethyl Phthalate are not restricted from use in any way under the rules governing cosmetic products in the European Union. 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 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 and is an authorized denaturant.

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 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 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. In an oral study, carcinogenesis was not observed in rats that were fed Dibutyl Phthalate for 18-mo or longer (no further details provided).

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 μg/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.

DRAFT DISCUSSION

[Note: This Discussion is in the draft form, and changes will be made following the Panel meeting.]

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 noted a lack of relevant safety data, specifically maximum concentration of use data for Dibutyl Phthalate and Dimethyl Phthalate. The Panel issued an Insufficient Data Announcement following the March 2026 Panel meeting: these data needs remain unmet. In order to come to a conclusion of safety for these ingredients, the following additional data are needed:

- Clarification on the type of current uses and maximum concentration of use for Dibutyl Phthalate
- Maximum concentration of use for 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 a concentration of use.

The Panel noted the increased incidences of liver adenoma and carcinomas in 2-yr dermal carcinogenicity study; however, the tumors described are common in mice and rats and thus did not concern with the Panel. 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.

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 safety assessment conclusion does not apply thereon.

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; concentration not reported). Limited inhalation toxicity data were available. However, the Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. 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. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. 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

To be determined.

TABLES

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

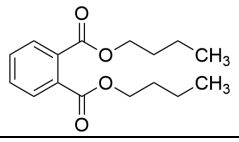
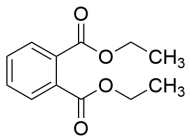
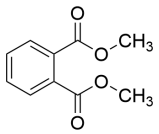
Ingredient & CAS No.	Definition	Function(s)
Dibutyl Phthalate 84-74-2	Dibutyl Phthalate is the aromatic diester of butyl alcohol and phthalic acid. It conforms to the following structure: 	Fragrance ingredient; plasticizer; solvent
Diethyl Phthalate 84-66-2	Diethyl Phthalate is the aromatic diester of 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) (mm Hg @ 20ºC)	1.1 7.5×10^{-5}	[2] [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	[2,19]
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) (mm Hg@ 25ºC)	14 0.0021	[2] [6]
Melting Point (ºC)	-40.5 -60	[2,19] [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} (@ 41ºC)	2.47 2.2	[19] [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 7.5×10^{-4}	[2] [7]
Melting Point (ºC)	5.5 0.36	[2] [7]
Boiling Point (ºC)	282 283	[2] [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*	**2**	**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)	2	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		

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)
Other Preparations (i.e., those that do not fit another category)	2	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)
[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.	[36]

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.	[37]

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.	[38]

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	[39]

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.*	8,174
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.	[46]

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.	[47]
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.	[48]
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.	[49]

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	[50]
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	[51]

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	[52]
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.	[54]

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.	[53]
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.	[6,55]

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.	[56]
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.	[57]

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.	[57]
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 *Igf2* ($p = 0.997$); 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 *Igf1r* mRNA and primordial and primary follicle numbers in 100 µg/kg/d mice, but not in the other doses	[58]

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.	[59]
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.	[60]
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.	[61]
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.	[61]

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.	[62]
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.	[63]

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.	64
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.	65
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.	66

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.	67
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.	68
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.	69
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.	70
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.	89

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.	[71]
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.	[72]

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.	[73]
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.	[74]

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.	[75]
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.	[76]

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.	[77]
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.	[60]

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.	[79]

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.	[62]
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.	[81]
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	[80]

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.	[80]
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 ($p < 0.05$).	[78]
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.	[83]

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 ($p < 0.05$).	[84]
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.	[66]
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	[54]
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.	[39]
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.	[89]
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.	[89]
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.	[90]

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.	[85]
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.	[86]
			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.	[88]

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.	92
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	93
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%, $p < 0.01$).	94
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 ($p < 0.001$).	14
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	94
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.	[95]
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	[93]
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.	[96]
				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	[14]

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.	[97]

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	141

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[175]	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[176]
Face and neck products (not spray) Leave-on	3500	1	0.15%	5.25	Exposure amount of face cream/lotion applied[177]

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7

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

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

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

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

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

INTRODUCTION

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

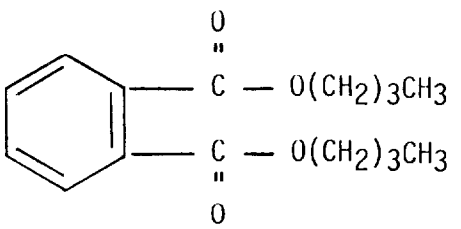
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CHEMICAL AND PHYSICAL PROPERTIES

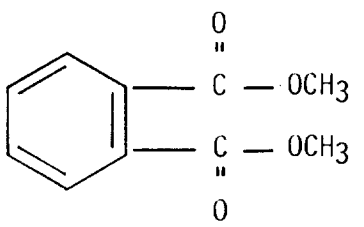
Structure

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

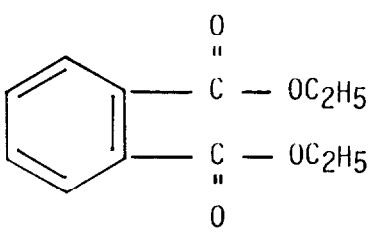
Dibutyl Phthalate



Dimethyl Phthalate



Diethyl Phthalate



Properties

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

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TABLE 1. Chemical and Physical Properties

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

Reactivity

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

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

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

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

Methods of Manufacture and Impurities

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

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

Analytical Methods

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

USE

Purpose in Cosmetics

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

Scope and Extent of Use in Cosmetics

Product types and the number of product formulations containing DBP,

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

Surfaces to which Commonly Applied

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

Frequency and Duration of Application

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

Potential Interactions with Other Cosmetic Ingredients

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

Noncosmetic Uses

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

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

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TABLE 2. Product Formulation Data[39]

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

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

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

GENERAL BIOLOGY

Microbial Metabolism and Toxicity

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

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

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

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

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

In Vitro Cell Toxicity

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

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

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

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

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

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

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

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

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

Effects on Enzymes

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

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

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

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

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

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ANIMAL TOXICOLOGY

Oral Studies

Acute Toxicity

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

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

Subchronic and Chronic Toxicity

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

TABLE 3. Acute Oral Toxicity

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

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

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

Dermal Studies

Acute Toxicity

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

Subchronic and Chronic Toxicity

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

Primary Irritation

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

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

Sensitization

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

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Intradermal Irritation

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

Eye Irritation

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

Inhalation Studies

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

Intraperitoneal Studies

Acute Toxicity

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

Subchronic and Chronic Toxicity

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

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TABLE 4. Subchronic and Chronic Oral Toxicity

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

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

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TABLE 5. Rabbit Eye Irritation

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

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

Other Studies

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

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

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

SPECIAL STUDIES

Animal Reproduction and Teratology

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

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TABLE 6. Acute Parenteral Toxicity

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

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

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

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

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

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

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

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

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

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

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

Mutagenesis

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

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

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

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

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

Carcinogenesis

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

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

CLINICAL ASSESSMENT OF SAFETY

Dermal Studies

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

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

Other Studies

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

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

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

SUMMARY

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

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

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

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TABLE 8. Skin Irritation and Sensitization

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DISCUSSION

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

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

CONCLUSION

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

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DIBUTYL PHTHALATE, DIETHYL PHTHALATE, AND DIMETHYL PHTHALATE

A safety assessment of Dibutyl Phthalate (DBP), Diethyl Phthalate (DEP), and Dimethyl Phthalate (DMP) was published in 1985 with the conclusion that these ingredients "are safe for topical application in the present practices of use and concentrations in cosmetics" (Elder 1985). Since then many additional studies have appeared in the scientific literature. These studies, along with the updated information in Table 8 regarding uses and use concentrations, were considered by the CIR Expert Panel. Based on its consideration of the data discussed below, the Panel decided not to reopen this safety assessment.

DBP, DEP, and DMP are phthalate diesters that are used in cosmetics as plasticizers, solvents and fragrance ingredients in a wide variety of cosmetic product types. DEP is also used as a denaturant. DBP is found primarily in nail care products (at concentrations up to 15%) and in some hair care formulations (up to 0.1%). DEP is found in certain bath preparations, fragrance products, deodorants, lotions, and other skin care products. The highest reported concentration of use of DEP is 11% in perfumes. DMP is an ingredient in some hair care products, including aerosol fixatives. The reported maximum concentration of use of DMP in cosmetics is 2% in aerosol hair sprays. Table 8 provides the frequency and concentration of use as a function of product type.

Recent studies document that DBP, DEP, and DMP all absorb readily through the skin and through the gastrointestinal (GI) tract. Once absorbed, most short-chain phthalate diesters are hydrolyzed to the corresponding monoester and alcohol. The phthalates and their metabolites distribute to most tissues, and cross the placenta, but they do not accumulate in any specific tissue type. Phthalates are quickly eliminated in the urine, usually as the corresponding monoester or its glucuronide conjugate. However, humans and primates metabolize longer-chain diester phthalates (e.g., DEHP) into the glucuronide-conjugated monoester forms to a much larger extent than do rats. Also, rats excrete three to four times more free unconjugated MBP than do hamsters given similar doses of DBP or MBP, possibly due to greater testicular β-glucuronidase activity in rats than in hamsters. Phthalates undergo some enterohepatic cycling, and some phthalate is eliminated in the feces.

New data on acute and short-term toxicity were consistent with previously available data.

In a NTP study, DBP, DEP, and DMP were not found to be dermal irritants or sensitizers, confirming previous data using human and animal subjects.

Although previous data had identified that orally administered (in feed or by gavage) DBP and its metabolite MBP have reproductive and developmental effects in rodents, with impaired male development being the most sensitive effect, newly available data provided additional demonstration of such effects.

When pregnant rats and mice were exposed to 1.0% DBP in powdered feed throughout gestation, the pregnancy outcome showed reductions in fertility, number of pups per litter, number of live pups, and body weights of pups. Adult male rats exposed to 1.0% DBP showed signs of liver and kidney toxicity and reduced weights of the prostate, testes, and seminal vesicles. Pregnant rats exposed to 2% DBP in feed throughout pregnancy had a higher incidence of preimplantation loss and resorptions, and no male pups were born alive. Exposure to 1% or 2% DBP in feed only during the latter half of gestation did not show the preimplantation loss and resorption rate seen in rats exposed throughout pregnancy. However, the increased survivability of these fetuses allowed the morphological defects of developing fetuses to be observed. These defects included reduced body weights in both sexes at 2% DBP, reduced anogenital distance and undescended testes in male fetuses at 1% and 2% DBP, and increased incidence of cleft palate and fused sternebrae. Adverse fetal effects were not seen in this study in a 0.5% DBP feed group, or at 331 mg/kg/day, based on average food consumption.

Oral intubation (gavage) of DBP in rats during gestation produced similar effects to those seen in the feeding studies described above. Pregnant rats given oral doses of approximately 0.63 to 0.75 g/kg/day and higher on certain gestation days produced litters with higher incidences of fetal toxicity and malformations. Exposure to DBP on gestation days 7 through 9 or on days 13 through 15 results in increased incidence of skeletal malformations such as cleft palate, fused sternebrae, and vertebral anomalies, as well as dilatation of the renal pelvis and undescended testes. However, exposure to DBP on gestation days 10 through 12 did not produce these effects, suggesting that DBP teratogenicity may be age dependent. Prenatal exposure to MBP appears to produce fetotoxicity and teratogenicity similar to DBP, following the same patterns of age-dependent sensitivity and dose efficacy. This supports the proposal that it is the monoester metabolite that produces the developmental toxicity of DBP and other phthalates.

DEP fed to mice at concentrations up to 2.5% (calculated to be 3.64 g/kg/day) in a continuous breeding protocol produced no effects of DEP on fertility or pregnancy outcome in the F_0 generation. F_1 male mice of the 2.5% DEP group had enlarged prostates and reduced sperm counts, but sperm motility and morphology were not affected. The F_2 generation showed no treatment-related differences between DEP and control groups. Pregnant rats fed up to 5.0% DEP mixed in feed on gestation days 6 through 15 produced no treatment-related alterations in fetal viability or development.

Repeated dermal application of 2 ml/kg up to 50% DEP to pregnant rabbits on gestation days 6 through 18 did not produce maternal or fetal toxicity or affect fetal development.

DMP was not fetotoxic or teratogenic when administered dermally (in rats) or orally (in rats and mice) during gestation.

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TABLE 8
Historical and current cosmetic product uses and concentrations for Dibutyl, Diethyl, and Dimethyl Phthalate

Product category	1981 use (Elder 1985)	2001 use (FDA 2001)	1981 concentrations (FDA 1981) (%)	2001 concentrations (CTFA 2001a, 2001b, 2001c) (%)
		Dibutyl Phthalate		
Perfumes	—	—	—	38–890 ppm**
Hair sprays	—	—	—	55–160 ppm**
Shampoos (noncoloring)	—	—	—	0.007
Hair preparations (other noncoloring)	3	—	>0.1–1	—
Hair-coloring preparations (other)	3	—	>0.1–1	0.1
Aftershave lotions	3	—	>0.1–1	—
Hair bleaches	—	—	—	0.1
Makeup (other)	1	—	>0.1–1	0.5
Nail basecoats and undercoats	36	32	>1–10	1–6; 15*
Nail creams and lotions	—	2	—	5
Nail extenders	—	—	—	1; 1*
Nail polish and enamel	522	88	≤25	0.5–15; 15*
Nail polish and enamel removers	3	—	0.1–25	2
Nail care preparations (other)	14	25	≤25	5–7; 6*
Underarm deodorants	—	—	—	140–200 ppm**
Personal cleanliness products (other)	5	3	>1–5	—
Total uses/ranges for Dibutyl Phthalate	**590**	**150**	**0.1–25**	**0.0038–15**
		Diethyl Phthalate		
Baby shampoos	—	—	—	0.03
Baby lotions, oils, powders, and creams	—	—	—	0.00003
Baby products (other)	—	—	—	0.05
Bath oils, tablets, and salts	3	1	≤5	—
Bubble baths	—	—	—	0.06
Bath preparations (other)	2	2	≤0.1	0.008–0.09
Colognes and toilet waters	19	24	≤5	0.2–2
Perfumes	23	7	≤50	1–11
Powders	1	5	>0.1–1	—
Sachets	3	2	>0.01–5	—
Other fragrance preparations	2	11	>0.1–50	0.01–1 67–28,000 ppm**
Hair conditioners	—	—	—	0.1–0.2
Hair sprays (aerosol fixatives)	5	—	>0.1–5	0.4 17–1500 ppm**
Shampoos (noncoloring)	—	—	—	0.0008–0.2
Hair tonics, dressings, etc.	—	1	—	14–220 ppm**
Wave sets	1	—	>0.1–1	—
Face powders	—	—	—	0.4
Eye shadow	1	—	≤0.1	—
Eyebrow pencil	—	—	—	0.007
Mascara	—	—	—	0.007–0.07
Eye makeup preparations (other)	—	—	—	0.07
Foundations	—	—	—	0.3
Makeup (other)	—	—	—	0.0003
Nail polish and enamel	—	—	—	0.1
Nail polish and enamel remover	1	—	>1–5	—

(Continued on next page)

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TABLE 8
Historical and current cosmetic product uses and concentrations for Dibutyl, Diethyl, and Dimethyl Phthalate *(Continued)*

Product category	1981 use (Elder 1985)	2001 use (FDA 2001)	1981 concentrations (FDA 1981) (%)	2001 concentrations (CTFA 2001a, 2001b, 2001c) (%)
Nail care preparations (other)	—	—	—	0.2
Bath soaps and detergents	1	—	>0.1–1	2
Underarm deodorants	—	4	—	0.3–1 20–3300 ppm**
Feminine hygiene deodorants	—	—	—	0.4
Other personal cleanliness products	—	—	—	1
Aftershave lotion	3	4	>0.1–1	0.5–2
Shaving cream (aerosol, brushless, and lather)	—	—	—	0.001
Other shaving preparation products	—	—	—	1
Skin-cleansing creams, lotions, liquids and pads	—	—	—	0.0002
Face and neck skin care preparations	1***	—	≤0.1***	0.3
Body and hand skin care preparations		2		0.008–0.5 26–190 ppm**
Foot powders and sprays	—	—	—	1
Night skin care preparations	—	—	—	0.0004
Paste masks (mud packs)	—	1	—	0.1
Skin fresheners	—	4	—	0.1–0.9
Skin care preparations (other)	1	5	>0.1–1	0.00003–0.9
Total uses/ranges for Diethyl Phthalate	**67**	**73**	**≤ 0.1–50**	**0.00003–2**
	Dimethyl Phthalate			
Hair conditioners	2	—	>0.1–1	—
Hair sprays (aerosol fixatives)	—	8	—	0.00002–2
Hair rinses	1	—	>0.1–1	—
Shampoos (noncoloring)	—	—	—	0.00002
Hair tonics, dressings, etc.	2	—	>0.1–5	—
Wave sets	2	—	>0.1–1	—
Hair preparations (other noncoloring)	4	3	>0.1–1	—
Hair color sprays (aerosol)	—	1	—	—
Blushers	—	—	—	0.00008
Face powders	—	—	—	0.00008
Foundations	—	—	—	0.005
Bath soaps and detergents	—	—	—	0.004
Underarm deodorants	—	—	—	33 ppm**–0.2
Aftershave lotions	—	—	—	0.2
Total use/ranges for Dimethyl Phthalate	**11**	**12**	**>0.1–5**	**.00002–2**

*Maximum concentrations reported by Nail Manufacturers Council (NMC 2001).
**Concentrations found in off-the-shelf products (Houlihan et al. 2002).
***These categories were combined when the original safety assessment was performed and are now separate categories.

Exposure to some phthalates has been shown to cause impairments of normal male development in rodents. The documented male-specific effects of phthalates include malformations of the epididymis and vas deferens, undescended testes, hypospadias, retention of thoracic nipples, and reduced anogenital distance. DEP and DMP did not cause the dramatic effects on male development seen with longer-chain dialkyl phthalates. Many studies have reviewed the mechanisms of the male-targeted toxicity of phthalates. DBP, DEP, and DMP have weak or no binding affinity for the estrogen receptor and do not affect estrogen-regulated developmental endpoints. An antiandrogenic mechanism has been proposed, but many studies show that these phthalates do not bind with androgen receptors, either. However, phthalate esters inhibit the synthesis of testosterone, which is an important

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hormone in normal development in males. DBP has also been shown to inhibit the action of Müllerian Inhibiting Substance produced by Sertoli cells.

DBP, DEP, and DMP previously had been screened for mutagenicity in the Ames bacterial reverse mutation assay with no mutagenic potential found. Additional data were available reporting that DBP caused an increase in the number of TA100 revertants in the absence but not in the presence of S9 rat liver fraction. DEP caused increases in the numbers of TA100 and TA1535 revertants, but this effect was also eliminated by the presence of S9. DMP caused an increase in the number of TA1535 revertants, but S9 prevented the effect. Overall, DBP, DEP, and DMP continue to have little genotoxic potential. One study on males of subfertile couples examined the relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay which is said to measure at least two aspects of DNA integrity. Neither the monobutyl form of DBP nor DMP had a significant association with comet assay parameters, and a significant association with the monobutyl form of DEP was seen only with one measure of DNA integrity.

Phthalates are a matter of concern for those responsible for public health and have been (and continue to be) reviewed by many government and international organizations. Phthalates are ubiquitous in the modern environment. The monoester metabolites sof phthalates have been detected in the urine of an adult reference population and in the urine of young children in a small pilot study.

The Centers for Disease Control and Prevention (2003) found that the urinary concentrations of the monoester metabolites of DBP and DEP in 2536 Americans were similar or slightly lower than those reported in a preliminary study of 289 adults (Blount et al. 2000). Environmental exposure to phthalates and other endocrine disruptors have been proposed to be linked to an increased incidence of hypospadias in humans. The developmental effects of phthalates seen in rodents raise questions about the potential for human health risks. However, these effects seen in rodents are at much higher exposure levels than humans are likely to encounter, and they are subject to the species differences in the metabolism of phthalate diesters. The estimated median exposure levels of DEP and DBP are 57 μg/kg/day and 7 μg/kg/day, respectively, while the U.S. EPA reference doses (RfD) for DEP and DBP are 800 μg/kg/day and 100 μg/kg/day, respectively. Thus, the human exposure is well below the safety limits set by the U.S. EPA. Even the median exposure levels of the highest-exposed group (women aged 20 to 40 years) are well below the RfDs. Exposure levels were not available for DMP.

Scientific committees with the governments of the United States and the European Union have evaluated the human risks of DBP and DEP and expressed minimal to no concern over consumer exposure to these compounds (NTP Center for the Evaluation of Risks to Human Reproduction 2000; Netherlands Organization for Applied Scientific Research and National Institute of Public Health and the Environment 2000; Scientific Committee on Cosmetic Products and Non-Food Products 2002).

As in the original safety assessment of these phthalate diesters in 1984, the primary safety issue regarding phthalate esters in this re-review is antiandrogenic activity and the potential effects on male development. The CIR Expert Panel noted that the free monoester metabolite appears to be the active agent in phthalate diester toxicity. Of the three compounds reviewed in this safety assessment, Dibutyl Phthalate raised the most concern.

The Panel reviewed the numerous studies that describe the developmental toxicity of DBP in rodents. The Panel noted that the no observed adverse effect level (NOAEL) of DBP in a gavage study was 50 mg/kg/day (Mylchreest et al. 2000). However, a feeding study reported a NOAEL of 331 mg/kg/day (Ema et al. 1998). Overall, the Panel felt that feeding studies better represent the type of exposure that humans would receive from cosmetics than do gavage studies, but agreed that a worst-case NOAEL of 50 mg/kg/day should be considered.

The Panel considered a Margin of Safety (MOS) approach to assess the risk of DBP exposure to human users of cosmetics based on calculated exposures and the animal developmental toxicity data. Exposure calculations were based on ingredient concentration of use in cosmetic products (CTFA, 2001a, 2001b, 2001c; Houlihan et al. 2002), extent of cosmetic use survey data (Environ Corporation 1985; CTFA 2002b), and dermal (Mint et al. 1994) and subungual penetration data (Jackson Research Association 2002). A conservative approach to penetration was used; i.e., an estimate of approximately 5% absorption of DEP in human skin was considered to be a conservative estimate of DBP absorption, because data suggest that DEP is more readily absorbed in rat skin than DBP (Scott et al. 1987). The Panel used an estimated consumer body weight of 60 kg.

The expected exposure was calculated as follows:

Nail Basecoat or Polish

- 280 mg/application to 10 fingernails (Environ Corporation 1985)
- 15% maximum DBP in nail basecoats and polish (CTFA 2001a, 2001b, 2001c; Houlihan et al. 2002)
- 8.5% penetration through nail in 14 days (Jackson Research Association 2002)

280 mg/day $\times$ 15% $\times$ 8.5%/14 days = 0.255 mg/day/60 kg = 4.25 μg/kg/day and 4.25 μg/kg/day $\times$ 2 (for fingers and toes) = 8.5 μg/kg/day.

Hair Spray

- 5 g/day hair spray use (CTFA, 2002)
- 160 μg/g DBP in hair spray (Houlihan et al. 2002)
- 20% skin contact, from CTFA maximum worst case
- 5% skin absorption (Mint et al. 1994)

5 g/day $\times$ 160 μg/g $\times$ 20% $\times$ 5% = 8 μg/day/60 kg = 0.14 μg/kg/day.

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Deodorant

- 0.52 g/day deodorant use (Environ Corporation 1984)
- 200 μg/g DBP in deodorant (Houlihan et al. 2002)
- 5% skin absorption (Mint et al. 1994)

0.52 g/day $\times$ 200 μg/g $\times$ 5% = 5.2 μg/day/60 kg = 0.09 μg/kg/day.

Perfume

- 0.53 g/day perfume use (CTFA 2002)
- 890 μg/g DBP in perfume (Houlihan et al. 2002)
- 5% skin absorption (Mint et al. 1994)

0.53 g/day $\times$ 890 μg/g $\times$ 5% = 24 μg/day/60 kg = 0.4 μg/kg/day.

Total Exposure

- Sum of each of the separate exposures

8.5 μg/kg/day + 0.14 μg/kg/day + 0.09 μg/kg/day + 0.4 μg/kg/day = 9.13 μg/kg/day

The calculated estimated exposure level of DBP from the concurrent use of multiple cosmetic products was 9.13 μg/kg/day. This value is within the reported range of total human exposure to DBP from all sources in women, 32 μg/kg/day (upper 95th percentile for women of reproductive age) to 6.5 μg/kg/day (upper 95th percentile for rest of group). Therefore, the Panel accepted 9.13 μg/kg/day as a not unreasonable approximation of DBP exposure from cosmetic products.

The Panel calculated the MOS of DBP by dividing the NOAEL of 331 mg/kg/day (from a feeding study) by the expected exposure of 9.13 μg/kg/day, yielding an MOS of 36,254. If the more conservative NOAEL of 50 mg/kg/day (from a gavage study) is used, the MOS is 5476. The Panel also noted that both NOAEL figures were obtained from rat studies, and detoxification metabolism of DBP is faster in humans than in rats.

The Panel acknowledged the use of DBP, DEP, and DMP in hair sprays. The effects of inhaled aerosols depend on the specific chemical species, the concentration, the duration of exposure, and site of deposition (Jensen and O'Brien 1993) within the respiratory system. Particle size is the most important factor affecting the location of deposition. The mean aerodynamic diameter of pump hair spray particles is approximately 80 μm, and the diameter of anhydrous hair spray particles is 60 to 80 μm. Typically less than 1% are below 10 μm which is the upper limit for respirable particles (Bowen 1999). Based on the particle size, DBP, DEP, and DMP would not be respirable in formulation. Therefore, exposure of the lung by inhalation was not considered likely.

Based on the available information included in this report, the CIR Expert Panel concluded that Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate are safe for use in cosmetic products in the present practices of use and concentrations.

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DIMETHICONE COPOLYOL

A safety assessment of Dimethicone Copolyol was published in 1982 with the conclusion that this ingredient "is safe as a cosmetic ingredient in the present practices of use and concentration" (Elder 1982). New studies, along with updated information regarding types and concentrations of use, were considered by the CIR Expert Panel. The Panel determined to not reopen this safety assessment.

Dimethicone Copolyol as an ingredient name has been deleted from the *International Cosmetic Ingredient Dictionary and Handbook* and replaced with several ingredients that adhere to the former definition of Dimethicone Copolyol as "a polymer of dimethylsiloxane with polyoxyethylene and/or polyoxypropylene side chains" (Pepe et al. 2002).

The new ingredients that are described by this definition include: Dimethicone PEG-7 Phosphate, Dimethicone PEG-10 Phosphate, Dimethicone PEG/PPG-7/4 Phosphate, Dimethicone PEG/PPG-12/4 Phosphate, Dimethicone PEG/PPG-20/23 Benzoate, Dimethicone PEG-8 Benzoate, Dimethicone PEG-6 Acetate, Dimethicone PEG-8 Adipate, PEG-3 Dimethicone, PEG-9 Dimethicone, PEG/PPG-20/29 Dimethicone,

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Dibutyl, Dimethyl, and Diethyl Phthalate and Butyl Benzyl Phthalate

F. Alan Andersen[1]

Keywords
phthalates, safety, cosmetics

Conclusion

In a 1985 safety assessment of dibutyl, diethyl, and dimethyl phthalate, the Cosmetic Ingredient Review Expert Panel stated that these ingredients are safe for use in cosmetics in the present practice of use and concentration.[1] Subsequently, in 2005, the Panel conducted an extensive rereview of the newly available studies since that assessment, confirmed the decision, and determined to not reopen that report.[2] In 1992, butyl benzyl phthalate was found safe in the present practice of use and concentration.[3] The Panel reviewed studies performed since that assessment as well as updated the use and concentration data in 2007 and confirmed that conclusion.[4] In 2012, the Panel reviewed 3 new studies on phthalates published in 2012 and confirmed that dibutyl, dimethyl, and diethyl phthalate and butyl benzyl phthalate are safe in cosmetics in the present practices of use and concentration. The Panel did not reopen the safety assessment.

Discussion

The Panel reviewed new studies that focused on the potential for endocrine disruption/reproductive and developmental toxicity on dibutyl, dimethyl, and diethyl phthalate and butyl benzyl phthalate. One study of children aged 5 to 9, who were part of a Manhattan-Bronx cohort, revealed detectable, although varied, levels of phthalates in the urine of all 244 study participants.[5] Higher levels of both diethyl phthalate and butyl benzyl phthalate were associated with airway inflammation.

Two studies addressed diabetes and phthalates. In 1 study, there were 1,015 men and women 70 years of age from Uppsala, Sweden.[6] One sample per participant was collected from 2001 to 2004 and analyzed 5 to 8 years later. In this study, blood levels for dimethyl phthalate, diethyl phthalate, diisobutyl phthalate, and diethylhexyl phthalate were measured and correlated with measures of insulin resistance and poor insulin secretion in nondiabetic participants.

In the second study, urinary concentrations of phthalate metabolites measured by the Centers for Disease Control and Prevention and self-reported diabetes in 2,350 women aged 20 to <80 participating in the National Health and Nutrition Examination Survey (NHANES) (2001-2008) were used.[7] 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.

The Panel noted that all of these studies identified associations between phthalate metabolites and either diabetes or airway inflammation. Such studies did not suggest a causal link between phthalates and any adverse outcome. The possibility that phthalate metabolites may impact peroxisome proliferation pathways was suggested in the diabetes studies, but that mechanism is not established as a mode of action. The Panel agreed that that there is a need for further study of the reported association between phthalates exposures and diabetes and to investigate possible causal links.

Declaration of Conflicting Interests

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PINK BOOK 1

DIBUTYL PHTHALATE GROUP

CIR Expert Panel Meeting
November 18-19, 2002

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INTRODUCTION

The CIR Expert Panel issued a Final Report of the Safety Assessment of Dibutyl Phthalate, Dimethyl Phthalate, and Diethyl Phthalate on January 13, 1984. Based on the review of information available at that time the Panel concluded that "these compounds are safe for topical application in the present practices of use and concentration in cosmetics." As part of a self-initiated process, CIR reviewed new data available since the original safety assessment was completed. Significant among these new data were reproductive and developmental toxicity data. These new data prompted CIR to initiate a re-review of this ingredient. According to CIR procedures, the first step in a formal re-review is to present all the available data in a Scientific Literature Review and offer a 90-day public comment period during which any interested party may comment and/or submit additional data. This report is that Scientific Literature Review.

Included are **previously available data** (i.e., data in the original report) and data that have become available since the Panel's original report was prepared (**newly available data**). Because the monoester forms of these phthalates are believed to be important biologically active metabolites (see for example, Foster et al., 2000), data on Monobutyl Phthalate (MBP), Monoethyl Phthalate (MEP), and Monomethyl Phthalate (MMP) are presented when available.

Phthalate esters are ubiquitous in the modern environment, found in clothing textiles, packaged foods, and even in household dust (Pfordt & Bruns-Weller, 1999). DBP was found in low concentrations in the tissues of wild fish and birds as well as in plants, soils, and waterways in Canada (Environment Canada, 1994).

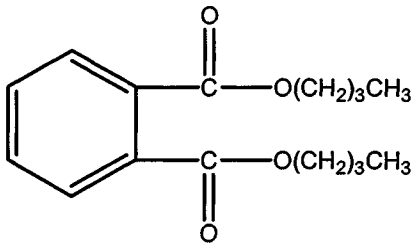
CHEMISTRY

DEFINITION AND STRUCTURE

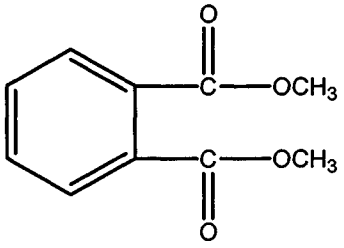
Previously Available Data

Dibutyl Phthalate (CAS No. 84-74-2), Dimethyl Phthalate (CAS No. 131-11-3), or Diethyl Phthalate (CAS No. 84-66-2) are dialkyl phthalates. DBP, DMP, and DEP are the aromatic diesters of butyl, methyl, and ethyl alcohol, respectively, and phthalic acid. The chemical formulas of these alkyl phthalates are as follows (Estrin, 1977):

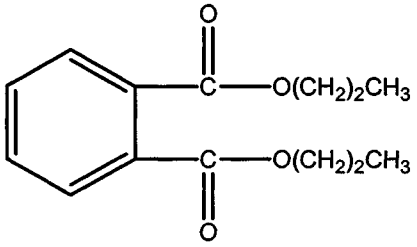
DIBUTYL PHTHALATE



DIMETHYL PHTHALATE



DIETHYL PHTHALATE



PHYSICAL AND CHEMICAL PROPERTIES

Previously Available Data

DBP, DMP, DEP are colorless, oily liquids, soluble in alcohol, ether, and other common organic solvents, and almost insoluble in water. DMP is insoluble in petroleum ether and other paraffin hydrocarbons. DBP is odorless. DMP and DEP have no to slight odors and DEP has a bitter, disagreeable taste (CTFA, 1981a; 1981b; 1981c; Greenberg & Lester, 1954; Hawley, 1971; Windholz, 1976). DBP is soluble in a solution simulating human sweat (an aqueous solution containing 2.5g sodium phosphate, 0.2 g triolein, and 2 drops Tween 85/1) and its solubility in this solution increases with an increase in pH (Ito et al., 1979). Chemical and physical properties of DBP, DMP, and DEP are presented in Table 1.

Table 1. Chemical and Physical Properties of DBP, DMP, and DEP.

Property		DBP	DMP	DEP	Reference
Molecular Weight		287.34	194.19	222.23	Windholz, 1976
Specific Gravity at:					
	14/4°C			1.232	Windholz, 1976
	15.6/15.6°C		1.196		Windholz, 1976
	20°C	1.0459, 1.0465			Windholz, 1976
	20/20°C		1.94		Windholz, 1976
	20/20°C	1.047, 1.049			CTFA, 1981a
	20/20°C		~1.19		CTFA, 1981b
	20/20°C			~1.12	CTFA, 1981c
	20/20°C	1.0484			Hawley, 1971
	25/25°C		1.189	1.12	Hawley, 1971
	25/25°C		1.189		Windholz, 1976
Boiling Point (°C) at:					
	760 mm Hg		283.7		Windholz, 1976
	400 mm Hg		257.8		Windholz, 1976
	200 mm Hg		232.7		Windholz, 1976
	100 mm Hg		210		Windholz, 1976
	60 mm Hg		194		Windholz, 1976
	40 mm Hg		182.8		Windholz, 1976
	20 mm Hg		164		Windholz, 1976
	10 mm Hg		147.6		Windholz, 1976
	5 mm Hg		131.8		Windholz, 1976
	1.0 mm Hg		100.3		Windholz, 1976
	not specified	340	282	295	Greenberg & Lester, 1954
	not specified	340	282	298	Hawley, 1971
	not specified	340		295	Windholz, 1976
Melting Point (°C)		-35		-40.5	Hawley, 1971
			5.5		Windholz, 1976
Vapor Pressure (mm Hg) at :					
	20°C		< 0.1	<0.001 torr	Hawley, 1971;SCCNFP, 2001
	20°C		< 0.01		Windholz, 1976
	25°C	1.4×10^{-5}		1.65×10^{-3}	CTFA, 2002; Sciences International, 1995
	150°C	1.1			Hawley, 1971
	163°C			14	Hawley, 1971
	182°C			30	Hawley, 1971
	295°C			734	Hawley, 1971
Refractive Index at :					
	14°C			1.5049	Windholz, 1976
	20°C	1.49	1.5168		Windholz, 1976
	25°C	1.4915	1.5138	1.5002	Hawley, 1971

REACTIVITY

Previously Available Data

The alkaline hydrolysis products of phthalates are mono- and diacids. The second-order alkaline hydrolysis rate constants in water at 30°C are 1.0×10^{-2}, 6.9×10^{-2}, and 2.5×10^{-2} M^{-1} sec^{1} for DBP, DMP, and DEP respectively. Acid hydrolysis is generally slower than alkaline hydrolysis and neutral hydrolysis is generally too slow to be detected (Wolfe et al., 1980). DBP is stable in solutions with near neutral pH (CTFA, 1981a).

The products of the thermal decomposition at 250 to 500°C of DBP are 1-butene, butanol, phthalic anhydride and small amounts of benzoic acid, butyl benzoate, phthalic acid, and monobutyl phthalate (Saido et al., 1981). The major products in the pyrolysis at 730°C of DBP are isobutene, butene, and propylene (Hagen, 1966).

DBP can be degraded by radiolysis. The major product of a 1 ppm aqueous DBP solution at pH 7 after a dose of 3×10^{4} rad of gamma radiation is monobutyl phthalate (Tezuka et al., 1978).

METHOD OF MANUFACTURE

Previously Available Data

DBP, DMP, and DEP are produced industrially by the reaction of phthalic anhydride with butyl alcohol, methyl alcohol, and ethyl alcohol, respectively (Autian, 1973; Hawley, 1971; Windholz, 1976). The Cosmetic, Toiletry, and Fragrance Association (CTFA) also reported that DBP is manufactured by the esterification of phthalic anhydride with an excess of n-butyl alcohol. Vacuum stripping removes the unreacted –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 DMP and DEP are proprietary information (CTFA, 1981a).

IMPURITIES

Previously Available Data

DEP may contain DMP or ethyl methyl phthalate as impurities (CTFA, 1981b; 1981c). DMP and DEP, for use in cosmetics, should contain minimums of 99 % DMP and DEP, respectively, as determined by gas-liquid chromatography (CTFA, 1981b; 1981c; Estrin, 1974; Japan Cosmetic Industry Association [JCIA],

1967).

Newly Available Data

Peakall (1975) reported that the purity of manufactured phthalate esters is between 99.70 and 99.97 %. The main impurities are isophthalic acid, terephthalic acid, and maleic anhydride.

ANALYTICAL METHODS

Previously Available Data

Qualitative and quantitative determinations of the phthalate esters are made by gravimetric procedure (JCIA, 1967; Persiani & Cukor, 1975; Senzel, 1977), titrimetric analysis (JCIA, 1967), spectrophotometric methods (Druyan, 1972; Rapaport et al., 1972), spectrophotofluorometric analysis (Druyan, 1980), the isotope dilution technique (St. John et al., 1979; Schwartz et al., 1979), gas-liquid chromatography (CTFA, 1981b; 1981c; Ramsey et al., 1980; Takeshita et al., 1977), gas chromatography (Austern et al., 1975; Schouten et al., 1975; Tirosh et al., 1974), gas chromatography-mass spectrometry (Bouwer et al., 1981; Shinohara et al., 1981), gas chromatography with flame ionization (Schouten et al., 1975), vibration spectroscopy (Rezaev et al., 1975), IR spectroscopy (Estrin, 1974; Neergaard et al., 1975; Persiani & Cukor, 1975; Senzel, 1977; Weast, 1978), UV spectroscopy (Mori, 1976; Schouten et al., 1979; Weast, 1978), and NMR spectroscopy (Neergaard et al., 1975; Weast, 1978).

USE

COSMETIC

Newly Available Data/Previously Available Data

Currently, DBP is used in cosmetics as a fragrance ingredient, as a solvent, and as a plasticizer; DEP functions in cosmetics as a denaturant, fragrance ingredient, plasticizer, and solvent; and, DMP is used in cosmetics as a fragrance ingredient, plasticizer, and solvent (Pepe et al., 2002).

Table 2 summarizes the 1981 and 2001 frequency of use and 1981 concentration of use data for DBP, DMP, and DEP as reported to the Food and Drug Administration (FDA) (FDA, 1981; 2001), current concentration of use data supplied by CTFA (2001a; 2001b; 2001c), and maximum concentrations of use as reported by the Nail Manufacturers Council (NMC) (NMC, 2001).

Most cosmetic uses of DBP involve nail care. Other uses include shampoos, hair coloring and

bleaching preparations, and some unspecified makeup preparations. The 1984 Final Report described DBP use in hair sprays and other aerosol products, but the most recent use data supplied by CTFA suggest that DBP is no longer used in such products (CTFA, 2000; CTFA, 2001a).

The types of cosmetic formulations containing DEP reported to the FDA and CTFA (Table 2) include nail products, bath preparations, eye makeup, fragrance products, deodorants, shaving, lotions, hair care products, and other skin care preparations.

According to data reported to the FDA, DMP is an ingredient in some makeups, bath soaps, deodorants, and hair care products, including aerosols.

Table 2. Current and historical concentration of use data for Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate

	1981 Data		2001 Data		
Product Category	No. of formulations containing ingredient (FDA, 1981)	Concentration range in cosmetic products (FDA, 1981)	No. of formulations containing ingredient (FDA, 2001)	Max. Conc. reported to CTFA (CTFA, 2001a; 2001b; 2001c)	Max. Conc. reported by Nail Manuf. Council. (NMC, 2001)
		Dibutyl Phthalate			
Other manicuring preparations	14	≤25 %	25	5 - 7 %	6 %
Nail polish and enamel removers	3	0.1 - 25 %	-	2 %	-
Nail creams and lotions	-	-	2	5 %	-
Nail extenders	-	-	-	1 %	1 %
Nail polish and enamel	522	≤25 %	88	0.5 - 15 %	15 %
Basecoats and undercoats (manicuring preparations)	36	>1 - 10 %	32	1 - 6 %	15 %
Other makeup preparations	1	>0.1 - 1 %	-	0.5 %	-
Other hair preparations (noncoloring)	3	>0.1 - 1 %	-	-	-
Other hair coloring preparations	3	>0.1 - 1 %	-	0.1 %	-
Aftershave lotions	3	>0.1 - 1 %	-	-	-
Hair bleaches	-	-	-	0.1 %	-
Shampoos (noncoloring)	-	-	-	0.007 %	-
Other personal cleanliness products	5	>1 - 5 %	3	-	-
Total frequency of use for DBP	590		150		
		Diethyl Phthalate			
Bath oils, tablets, and salts	3	≤5 %	1	-	-
Other bath preparations	2	≤0.1 %	2	-	-
Baby shampoos	-	-	-	0.03 %	-
Baby lotions, oils, powders, and creams	-	-	-	0.00003 %	-
Other baby products	-	-	-	0.05 %	-

Product Category	1981 Data: No. of formulations containing ingredient (FDA, 1981)	1981 Data: Concentration range in cosmetic products (FDA, 1981)	2001 Data: No. of formulations containing ingredient (FDA, 2001)	2001 Data: Max. Conc. reported to CTFA (CTFA, 2001a; 2001b; 2001c)	2001 Data: Max. Conc. reported by Nail Manuf. Council. (NMC, 2001)
Diethyl Phthalate (continued)					
Bubble baths	-	-	-	0.06 %	-
Other bath preparations	-	-	-	0.008 - 0.09 %	-
Shampoos (noncoloring)	-	-	-	0.0008 - 0.2 %	-
Bath soaps and detergents	1	>0.1 - 1 %	-	2 %	-
Hair conditioners	-	-	-	0.1 - 0.2 %	-
Hair sprays (aerosol fixatives)	5	>0.1 - 5 %	-	0.4 %	-
Wave sets	1	>0.1 - 1 %			
Face powders	-	-	-	0.4 %	-
Eye shadow	1	≤0.1 %			
Eyebrow pencil	-	-	-	0.007 %	-
Mascara	-	-	-	0.007 - 0.07 %	-
Other eye makeup preparations	-	-	-	0.07 %	-
Foundations	-	-	-	0.3 %	-
Lipstick	-	-	-	0.00005 %	-
Other makeup preparations	-	-	-	0.0003 %	-
Nail polish and enamel remover	1	>1 - 5 %			
Nail polish and enamel	-	-	-	0.1 %	-
Other manicuring preparations	-	-	-	0.2 %	-
Colognes and toilet waters	19	≤5 %	24	0.2 - 2 %	-
Perfumes	23	≤50 %	7	1 -11 %	-
Powders	1	>0.1 - 1 %	5	-	-
Sachets	3	>0.01 - 5 %	2	-	-
Other fragrance preparations	2	>0.1 - 50 %	11	0.01 - 1 %	-
Tonic, dressings, and other hair grooming aids	-	-	1	-	-
Deodorants	-	-	4	0.3 - 1 %	-
Feminine hygiene deodorants	-	-	-	0.4 %	-
Other personal cleanliness products	-	-	-	1 %	-
Aftershave lotion	3	>0.1 - 1 %	4	0.5 - 2 %	-
Shaving cream (aerosol, brushless, and lather)	-	-	-	0.001 %	-
Other shaving preparation products	-	-	-	1 %	-
Skin cleansing (cold creams, cleansing, lotions, liquids and pads)	-	-	-	0.0002 %	-
Face and neck creams, lotions, powders, and sprays (excluding shaving preparations)	1	≤0.1 %	-	0.3 %	-
Body and hand creams, lotions, powders, and sprays	-	-	2	0.008 - 0.5 %	-

Product Category	1981 Data: No. of formulations containing ingredient (FDA, 1981)	1981 Data: Concentration range in cosmetic products (FDA, 1981)	2001 Data: No. of formulations containing ingredient (FDA, 2001)	2001 Data: Max. Conc. reported to CTFA (CTFA, 2001a; 2001b; 2001c)	2001 Data: Max. Conc. reported by Nail Manuf. Council. (NMC, 2001)
Diethyl Phthalate (continued)					
Foot powders and sprays	-	-	-	1 %	-
Night creams, lotions, powders, and sprays (excluding shaving preparations)	-	-	-	0.0004 %	-
Paste masks (mud packs)	-	-	1	0.1 %	-
Skin fresheners	-	-	4	0.1 - 0.9 %	-
Other skin care preparations	1	>0.1 - 1 %	5	0.00003 - 0.9 %	-
Total frequency of use for DEP	67		73		
Dimethyl Phthalate					
Hair sprays (aerosol fixatives)	-	-	8	0.00002 - 2 %	-
Other hair preparations	4	>0.1 - 1 %	3	-	-
Hair color sprays (aerosol)	-	-	1	-	-
Shampoos (noncoloring)	-	-	-	0.00002 %	-
Hair conditioners	2	>0.1 - 1 %	-	-	-
Tonics, dressings, and other hair grooming aids	2	>0.1 - 5 %	-	-	-
Hair rinses	1	>0.1 - 1 %	-	-	-
Wave sets	2	>0.1 - 1 %	-	-	-
Blushers (all types)	-	-	-	0.00008 %	-
Face powders	-	-	-	0.00008 %	-
Foundations	-	-	-	0.005 %	-
Bath soaps and detergents	-	-	-	0.004 %	-
Deodorants (underarm)	-	-	-	0.2%	-
Aftershave lotions	-	-	-	0.2 %	-
Total frequency of use for DMP reported to FDA	11		12		

The environmental and public health organizations Environmental Working Group, Coming Clean, and Health Care Without Harm contracted a laboratory to analyze 72 name-brand, off-the-shelf cosmetic products for the presence of phthalate compounds (Houlihan et al., 2002). Of the 72 products analyzed, 52 showed the presence of phthalate esters in varying concentrations. None of the 72 products listed phthalate compounds as ingredients on the labels. Table 3 lists the respective maximum concentrations of DBP, DEP, and DMP found in the cosmetic product types. The method of analysis was not reported.

Table 3. Concentration Ranges of Dibutyl Phthalate, Diethyl Phthalate, and Dimethyl Phthalate Found in Off-the-Shelf Cosmetic Products (Houlihan et al., 2002)

Product Category	Dibutyl Phthalate	Diethyl Phthalate	Dimethyl Phthalate
Fragrance	38 - 890 ppm	67 - 28,000 ppm	-
Deodorant	140 - 200 ppm	20 - 3300 ppm	33 ppm
Hair Spray	55 - 160 ppm	17 - 1500 pmm	-
Hair Gel	-	14 - 220 ppm	-
Hand and Body Lotions	-	26 - 190 ppm	-
Hair Mousse	-	38 - 75 ppm	-

The use of DBP, DEP, and DMP in cosmetic products is not restricted in Japan (Ministry of Health, Labor and Welfare [MHLW], 2001a & 2001b) or in Europe (European Commission, 2002). The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food consumer products. The SCCNFP (2002) reviewed the safety of DEP is cosmetic products and concluded that "the safety profile of Diethyl-phthalate supports its use in cosmetic products at current levels." This opinion was partly based on data indicating maximum cosmetic concentrations of DEP of 50 % in perfumes and other fragrance preparations. Additionally, the SCCNFP did "not recommend any specific warnings or restrictions under the currently proposed conditions of use."

NON-COSMETIC

Previously Available Data

DBP, DMP, and DEP are used as solvents and plasticizers for nitrocellulose, cellulose acetate, and cellulose acetate-butyrate compositions. They are used in the manufacture of varnishes and plastics and in insecticides and insect repellents. DBP is used as a plasticizer in explosives and elastomers, such as polyvinyl, as a textile lubricating agent, as a resin solvent, and in safety glass, printing inks, paper coatings, and adhesives. DMP is used as a camphor substitute in the manufacture of celluloid, as a wetting agent, and as an alcohol denaturant (Hawley, 1971; Windholz, 1976).

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

be used as a catalyst and crosslinking agent for epoxy resins, and DEP may be used as a plasticizer, at no specific concentration limits, in the resinous and polymeric coatings of the food-contact surface of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food. DBP may be used in coatings of containers having a capacity of ≥ 1000 gallons and intended for repeated use with alcoholic beverages of less than or equal to 8 % alcohol by volume (21 CFR 175.300, 175.320). There are no concentration limits for the use of DBP as a component of the uncoated or coated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packaging, processing, preparing, treating, packing, transporting, or holding aqueous and fatty foods (21 CFR 176.170). DBP may be used in the base sheet or coating of cellophane used in packaging food, but total phthalates must not exceed 5 % by weight of the finished cellophane (21 CFR 177.1 200). DBP and DMP may be used, at no specific concentration limits, as solvents for inhibitors, accelerators, and catalysts in crosslinked polyester resins used as articles or components of articles intended for repeated use in contact with food (21 CFR 177.2420). DBP may be used as a plasticizer in rubber articles intended for repeated use in producing, manufacturing, packing, processing, preparing, transporting, or holding food. Total DBP may not exceed 30 % by weight of the rubber product (21 CFR 177.2600). There is no concentration limit for the use of DMP in semirigid and rigid acrylic and modified acrylic plastics used as articles intended for use in contact with food (21 CFR 177.1010). There is no limit in the amount of DEP that may be used in surface lubricants used in the manufacture of metallic articles that contact food (21 CFR 1 78.3910). DEP may be used as a plasticizer in the manufacturer of food-packaging materials with no specific limits. This DEP will not be considered a "food additive" if of good commercial grade, suitable for association with food, and used in accordance with good manufacturing practice; the amount of DEP that migrates into food as a result of its use in food-packaging materials should not be intended to accomplish any physical or technical effect in the food itself and should be reduced to the least amount reasonably possible (21 CFR 181.22, 181.27).

Newly Available Data

DBP and other phthalates are primarily used as a plasticizers in flexible polyvinyl chloride and in cellulose plastics (NTP-CERHR, 2000; Blount et al., 2000). Additional applications include uses as solvents, lubricants, fixatives, and detergents (Blount et al, 2000). DBP is used in the manufacture of medical products including denture base materials, and ocular prosthetics (Khaliq et al., 1992; Lygre et al., 1995; Ow

et al., 1997). Other products containing DBP include plastic wraps and containers for food, paints, plastic toys and inks (Blount et al., 2000; Castle et al., 1988; Moura et al., 1994).

Twenty-six million pounds of plastics containing DEP were produced in the United States in 1988. In addition to many plastic consumer products and plastic-coated materials, DEP is found in packaging for food and drug products. DEP is a plasticizer in a commercial coating for pharmaceutical preparations (Karmin & Mayor, 1991).

DMP is used as an insect repellant and, with permethrin, as a fabric treatment on tents to protect campers from mosquitoes (Schreck, 1991).

BIOLOGICAL PROPERTIES

MICROBIAL INTERACTION

Previously Available Data

A variety of bacteria can utilize DBP or DMP as a carbon source. The corresponding monoesters, phthalic acid, and protocatechuic acid are intermediates in the degradation of these chemicals (Engelhardt et al., 1975; Perez et al., 1976).

The growth of *Pseudomonas aeruginose* was not inhibited by concentrations of up to 1000 ppm DMP. A 1500 ppm solution slightly inhibited the growth of the organism. After a 24-hour incubation, the concentration of a 98 ppm DMP solution decreased to 88 ppm, suggesting some bacterial utilization of the compound (Perez et al., 1976). The concentration of neutralized DEP that inhibited the multiplication of *Ps. putida* was greater than 400 ppm (Bringmann & Kuehn, 1980). The minimum inhibitory concentration of a 10 % solution of DEP in 95 % ethanol was 1000 ppm for *Corynebacterium* sp. and greater than 1000 ppm for *Staphylococcus aureus* and Escherichia coli (Morris et al., 1979).

The growth of the blue-green alga, *Microcystis aeruginosa* was inhibited by 100 to 300 ppm of DMP and suppressed for three days by 400 ppm DMP. After four days, cellular lysis was observed in the 400 ppm DMP culture. Concentrations of DMP from 500 ppm to 800 ppm completely destroyed the cells within 72 hours (Lawrence, 1978; Ruiz & Brown, 1976). Neutralized DEP inhibited the multiplication of M. aeruginosa at a concentration of 15 ppm and inhibited the multiplication of the green alga, *Scenedesmus quadricauda*, at a concentration of 10 ppm (Bringmann & Keuhn, 1978; 1980).

A 10 ppm solution of DBP in phosphate buffer at pH 7 decreased the percent survival of the yeast,

Saccharomyces cervisiae, throughout a 48-hour incubation; a 20 ppm solution was even more toxic (Shahin & von Borstel, 1977). The minimum inhibitory concentration of a 10 % (w/v) solution of DEP in 95 % ethanol was 500 ppm for the fungus, *Candida albicans* (Morris et al., 1979).

A concentration of 50 ppm of DBP completely inhibited the growth of cells of the protozoan, *Tetrahymena pyriformis*. Other phthalate esters were inhibitory as well (Yoshizawa et al., 1977). A concentration of 1000 ppm of DMP markedly inhibited the growth rate of *T. pyriformis* (Lawrence, 1978; Gutierrez et al., 1976). Neutralized DEP inhibited the multiplication of the flagellate protozoan, *Entosiphon sulcatum* at a concentration of 19 ppm (Bringmann & Keuhn, 1980).

IN VITRO CELL TOXICITY

Animal Cells

Previously Available Data

Calley et al. (1966) investigated the toxicity to mouse fibroblasts using the cell overlay method. Pads containing 0.05 ml of a 50 mg/ml emulsion of the phthalates were placed on the agar surface (2.5 mg phthalates/pad), and the cells were observed for 48 hours. DMP and DEP were toxic to the cells and DBP was not.

Nematollahi et al. (1967) reported that all three phthalates were toxic in a 24-hour incubation of mouse fibroblastic cells.

Guess & Haberman (1968) incubated mouse fibroblastic cells for 24 hours with paper discs containing pure DMP and DEP or saline solutions saturated with DMP and DEP at pH 6. Only the pure DMP was toxic to the cells.

Dillingham and Autian et al. (1973) produced dose-response curves and the ID_{50} for the mouse fibroblast cultures, defined as the dose required to inhibit growth by 50 %, was determined for the phthalates. The ID_{50}'s for DBP, DMP, and DEP were 1×10^{-4}, 7×10^{-3}, and 3×10^{-3} mole/L, respectively.

DMP was highly toxic to a replicating mouse fibroblastic cell culture when they were undergoing significant protein turnover (Autian, 1973). A radioactively labeled amino acid mixture (14C) was added to the cultures, and the radioactivity was followed over a 96-hour incubation. Cells were relatively insensitive to growth inhibition by DMP, as measured by uptake of radioactivity, for the first 24 hours. However, between 24 and 96 hours, the uptake of radioactivity decreased continuously.

Kasuya (1976) investigated the effects of DBP, DMP, and DEP on the outgrowth of nerve fibers and fibroblasts in primary cultures of rat cerebellum were investigated. The phthalates were added directly to the nutrient media. DBP and DEP completely inhibited outgrowth at concentrations greater than or equal to 1 .17 x 10^{-3} and 1.53 x 10^{-3} M, respectively. DMP did not completely inhibit outgrowth at concentrations less than or equal to 3.05 x 10^{-3} M.

Oser et al. (1977) studied the response of mouse fibroblastic cells to 1, 5, 10, and 50 % suspensions of DBP, DMP, and DEP. All the suspensions were toxic except the 1 and 5 % suspensions of DBP. In cell suspensions with DBP and DEP, the cellular ATP concentrations decreased over a 6-hour incubation.

Newly Available Data

Kim et al. (2002) extracted limb bud cells from embryonic rats (gestation day 12.5) and exposed *in vitro* to a range of concentrations of DBP or MBP for 96 hours. 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 DBP. MBP was less potent, with respective IC_{50} values for cytotoxicity and cell differentiation at 307.24 µg/ml and 142.61 µg/ml. Cotreatment with antioxidants, such as catalase or vitamin E, protected the cells from DBP cytotoxicity and reduced the DBP-inhibition of cell differentiation. However, antioxidants did not have a protective effect in MBP-exposed cells. The authors suggested that the cytotoxic effects of DBP may be due to oxidative stress.

Human Cells

Previously Available Data

Guess and Haberman (1968) studied the effects of DBP, DMP, and DEP on human amnion and KB human cancer cells in culture. All three compounds killed and lysed the cells (concentrations not available). Saline solutions saturated with DMP and DEP at pH 6 did not cause hemolysis of human erythrocytes.

Jones et al. (1975) reported that the ID_{50} of DBP, defined as the concentration that caused 50 % growth inhibition, for human diploid cell strain WI-85 was 1.35 x 10^{-4} M.

Suzuki & Matsumoto (1980) studied human embryonic lung cell cultures after the addition of 40 µg/ml of DBP to the culture medium. DBP inhibited cell growth and caused morphological changes in the cells, the appearance of lipid drops in the cytoplasm, and the accumulation of triacylglycerol in the cytosol.

Thelestam et al. (1980) found that DBP and DEP were inactive in a test in which the extent of membrane damage in human lung fibroblasts was determined by measuring the amount of a radioactively labeled cytoplasmic marker released into the media.

Ekwall et al. (1982) incubated HeLa cells for 7 days after the addition of DBP, DMP, and DEP to the culture medium. The 7-day IC_{50}s, the geometrical mean values between the totally inhibitory concentrations and the maximal completely noninjurious ones, were 3.1×10^{-2} M for DBP, 7.7×10^{-2} M for DMP, and 6.3×10^{-2} M for DEP.

EFFECTS ON ENZYMES

Previously Available Data

Phthalate esters have a variety of different effects on mammalian enzymes, both *in vivo* and *in vitro*. DBP and DMP affect drug-metabolizing enzymes in mammalian liver. Single-dose intraperitoneal administration of 3.05 ml/kg of DBP and 3.6 ml/kg of DMP to rats inhibited the activity of hepatic aminopyrine N-demethylase and aniline hydroxylase and had no effect on glucose-6-phosphatase, NADPH-cytochrome c reductase, and tyrosine aminotransferase activity. The activities of these enzymes were not decreased when the phthalates were administered intraperitoneally every day for 7 days (Seth et al., 1981; Seth, 1982). Results of another study indicated that DBP weakly enhanced the activity of aminopyrine N-demethylase from rat hepatic 10,000 x g supernatant (Tanaka et al., 1978). The oral administration of 5 mmol/kg per day of DBP for 6 days to male rats increased the hepatic cytochrome P-450, had no effects on glutathione-S-transferase activity or the monooxygenase activities dependent on cytochrome P-450, increased the epoxide hydratase activity, and increased the conjugation of o-aminophenol and 4-methylumbelliferone with glucuronic acid. Rat liver incubated *in vitro* with 2×10^{-3} M DBP had no effect on epoxide hydratase or glutathione-S-transferase activities, decreased the monooxygenase activities, and decreased the conjugation of o-aminophenol and 4-methylumbelliferone with glucuronic acid (Aitio & Parkki, 1978).

DBP, DMP, and DEP inhibited mitochondrial respiration. Concentrations of 5×10^{-5} to 1×10^{-3} M of the phthalates inhibited the respiration of isolated mitochondria from rat liver primarily by uncoupling oxidative phosphorylation rather than by inhibiting electron transport or energy transfer (Inouye et al., 1978; Melnick & Schiller, 1982). Other researchers using the same concentrations have suggested that the contrary is probably true; the phthalates inhibited electron transport or energy transfer (Ohyama, 1976). In

some studies, DBP and DMP inhibited the activities of succinate dehydrogenase and ATPase, enzymes of the rat liver inner mitochondrial membrane, after intraperitoneal administration, and in *in vitro* assays at concentrations of 1 x 10^{-4} to 1.5 x 10^{-3} M (Melnick & Schiller, 1982; Srivastava et al., 1977; Tanaka et al., 1978). DBP stimulated ATPase activity and induced swelling of rat liver mitochondria (Ohyama, 1977).

Administration of 0.7 % DBP or 0.5 % DMP in the diet of male rats for 21 days increased hepatic weights and reduced serum cholesterol concentrations. Acetate incorporation into triglycerides and the steryl ester plus squalene and mevalonate incorporation into squalene plus sterols in liver minces were inhibited by dietary DBP. These results were not observed with DMP. DMP administration resulted in a decrease in total hepatic cholesterol and lipid. This was not observed with DBP (Bell et al., 1978). The intraperitoneal administration of 20 mg/kg/day of DBP to mice for 16 days did not significantly lower serum cholesterol but did lower serum triglycerides. DBP, at a concentration of 2.5 x 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 (Hall et al., 1981). A 5 x 10^{-6} M concentration of DBP and DEP inhibited *in vitro* human blood lecithin/cholesterol acyltransferase. DMP, at the same concentration, inhibited the enzyme slightly (Lagente et al., 1979).

DBP elevated the activities of mouse and rat serum lactate dehydrogenase, glutamic oxalacetic transaminase, and glutamic-pyruvate transaminase (Miyahara et al., 1973; Nishiyama et al., 1977; Sajiki, 1978). DBP increased the activity of alkaline phosphatase in mice but had no effect on this enzyme in rats (Nishiyama et al., 1977).

Newly Available Data

Melnick & Schiller (1985) reported that DBP and MBP inhibit mitochondrial respiration by uncoupling energy-dependent processes and inhibiting succinate dehydrogenase activity, causing a diminished energy supply, altered ion transport, and mitochondrial swelling. They stated that these altered processes interfere with ATP energy production.

Tavares & Vine et al. (1985) reported that DBP inhibited the metabolism of arachidonic acid by cyclooxygenase and lipoxygenase in rat peritoneal leukocytes. Aliquots of peritoneal leukocytes from rats were pre-incubated with 0, 1, or 10, or 100 µg/ml DBP. The cells were then incubated with 1.7 nM radiolabeled arachidonic acid for 5 min at 37°C. Reactions were stopped with methanol and formic acid. Metabolites of arachidonic acid were measured by silica gel thin layer chromatography and scintillation.

Cells pre-incubated with DBP showed reduced concentrations of prostaglandins, LTB_4, 5-HETE, 11-HETE, and 15-HETE. DBP and other phthalates have some structural resemblance to prostaglandins, which may explain their inhibitory activity in enzymes involved in arachidonic acid metabolism.

Murakami et al. (1986a) evaluated the effects of DBP on mitochondrial enzymes in rat liver. Male Wistar rats were fed a diet containing 5% DBP for 35 to 45 days. Body weights were reduced in the DBP group. DBP-treated rats had increased liver, kidney, and spleen organ weights and reduced testicular weights ($p < 0.01$), compared to control rats. Respiration in liver mitochondria and succinate dehydrogenase and pyruvate dehydrogenase activities were reduced in DBP-treated rats. In *in vitro* preparations of liver cells, less than 100 µM DBP inhibited respiration, and 150 µM DBP inhibited dehydrogenase activity.

Walseth & Nilsen (1984) reported that inhalation of DBP (0.5, 2.5, or 7.0 ppm) for five days dose-dependently decreased lung microsomal cytochrome P-450, and was accompanied by a reduction in *n*-hexane and benzo(a)pyrene metabolism. Liver cytochrome P-450 was not affected, but *n*-hexane and benzo(a)pyrene metabolism in liver was increased.

These authors (Walseth and Nilsen, 1986) also demonstrated that DBP is a weak inducer of liver cytochrome P-450. Male Sprague-Dawley rats were dosed by oral gavage with 0, 0.01, 0.1, or 1.0 mmol/kg DBP for five days. Livers and lungs were weighed, and microsomal enzyme assays were performed. Cytochrome P-450 and NADPH-cytochrome-c-reductase activities in the liver were increased at the lowest DBP dose, and the microsomal enzyme activity did not return to normal after a four-week recovery period. Liver microsomal metabolism of n-hexane was increased by DBP, but the increased n-hexane metabolism did not persist. Metabolism of benzo(a)pyrene was not affected by DBP.

Okita and Okita (1992) reported that induction of the cytochrome P450-mediated fatty acid ω-hydroxylation system is one of the responses of the liver to the presence of peroxisome proliferators. Oral intake of DEP (5.4 mmol/kg/day for three days) caused a slight (~1.6 fold) increase and DBP (3 mmol/kg/day) caused a five-fold increase in the activity of hepatic laurate 12-hydroxylase. Likewise, palmitoyl-CoA oxidase activity was only slightly increased by DEP and increased six-fold in animals dosed with DBP. These data raise little concern for DEP as a peroxisome proliferator, but the authors suggest that some concern is warranted for DBP and some other phthalate esters (Okita & Okita, 1992).

Citing studies by Batala et al. (1996, 1997), David et al. (1999), and Lington et al. (1999) regarding

phthalates as peroxisome proliferators, McKee (2000) notes that Di(2-ethylhexyl) Phthalate (DEHP) and Diisononyl Phthalate (DINP) may induce peroxisome proliferation in rodents. However, DEHP and DINP have been shown not to be peroxisome proliferators in primates (Hall et al., 1999; Kurata et al., 1998; Pugh et al., 2000; McKee, 2000).

ANTI-CANCER PROPERTIES

Newly Available Data

Chu-Tse et al. (1993) demonstrated that exposure to 50 µg/ml DBP can suppress the growth of HL-60 (a leukemia cell line) cells. Human bone marrow granulocyte/macrophage progenitor (CFU-GM) and human myeloid leukemia (L-CFU) assays were used to show that DBP has no effect on normal bone marrow cell growth, but growth of leukemic cells was dose- and time-dependently reduced in vitro.

Kageyama et al. (1994) found that DBP at a concentration of 50 µM inhibited the incorporation of [^{3}H]-thymidine into the DNA of Ehrlich *ascites* tumor cells and inhibited cell proliferation. This inhibition was synergistically enhanced by hyperthermia at 42°C for 1 hour. DEP and DMP did not show these inhibitory effects. The authors proposed the use of certain phthalate diesters in combination with hyperthermia as a cancer treatment.

According to Lisheng et al. (1996b), the ability of DBP to suppress leukemic cell growth may work through an apoptotic mechanism. Lisheng et al. (1996a) proposed DBP as a clinical treatment for leukemia.

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

GENERAL

Previously Available Data

Smith (1953) stated that DBP was hydrolyzed by crude pancreatic lipase solution.

Newly Available Data

According to Kluwe (1982), most dialkyl phthalate esters display similar basic pharmacokinetics in mammalian species after oral ingestion. At least 90 % of orally ingested DBP is absorbed from the gastrointestinal tract. Once absorbed, DBP distributes to the liver, adipose, kidney, muscle, and some other tissues, but it does 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 DBP metabolites are found in the feces, but they have been detected in the bile at levels up to 44% of the DBP dose given to rats (60 mg/kg orally), suggesting extensive enterohepatic cycling (Kluwe, 1982).

Studies of diethylhexyl phthalate suggest that the biological half-life of diester phthalates is < 3 hours and of monoester phthalates is approximately 12 hours (Liss et al., 1985; Dirven et al., 1993; Albro, 1986).

Foster et al (2000) suggested that primates and humans metabolize phthalate diesters to the corresponding free monoesters to a lesser extent than do rats. This comparison was based on studies of the metabolism of di(2-ethylhexyl) phthalate (DEHP) in marmosets (Rhodes et al, 1986), African green monkeys (Albro et al., 1981), and humans (Schmid & Sclatter, 1985).

Albro et al (1981) gave African green monkeys an intravenous (IV) dose of ^{14}C-labeled DEHP and measured the urinary metabolites by HPLC, gas chromatography, and mass spectroscopy. Approximately 80 % of the urinary metabolites were excreted in the form of glucuronide conjugates. The authors noted that by contrast, rat urinary metabolites of DEHP are primarily excreted unconjugated.

Rhodes et al (1986) compared the metabolism of [^{14}C]-DEHP in marmosets (*Callithrix jacchus*) and Wistar rats. Rats (n = 10/sex) and marmosets (n = 5/sex) were given daily oral doses of 2000 mg/kg [^{14}C]-DEHP in corn oil for 14 days. A different set of male marmosets were given daily intraperitoneal doses of 1000mg/kg [^{14}C]-DEHP in corn oil for 14 days. Animals were housed in metabolism cages for complete collection of urine and feces. Urine and feces samples were collected for 24-hour periods following dosing on days 5 and 14. Radioactivity of the samples was scintillation counting. The marmoset excreted 2 % of the DEHP dose in the urine, compared to 50 % in the rat. Approximately 50 to 75 % of the DEHP dose was excreted by the marmoset in feces. The rat excreted less than 50% of the DEHP dose in feces. After the 14th day, the tissues of the marmoset contained 1/5 to 1/10 the levels of DEHP metabolites as seen in the rat tissues. The researchers also studied the pharmacokinetics of a single dose of [^{14}C]-DEHP in the marmoset by various routes of adminstration: 100 mg/kg IV, 1000 mg/k IP, and 100 or 2000 mg/kg oral. Urine and feces were collected at 24-hour intervals for seven days. After the seventh day, animals were killed, and the tissues were analyzed for radioactivity. The cumulative excretion of DEHP metabolites in the urine and feces following IV dosing was approximately 40 and 20 % of the administered dose, respectively.

The cumulative excretion of DEHP metabolites in the urine and feces following IP dosing was approximately 10 and 4 % of the administered dose, respectively. The remaining 86 % of the IP dose remained in the peritoneal cavity after seven days. Seven days after the 2000 and 100 mg/kg oral doses, 84 and 50 % of the dose was eliminated in the feces, respectively, and 4 and 30 % in the urine, respectively. Of the small amounts of DEHP metabolites that remained in the tissues, the majority was found in the lungs of animals dosed IV or IP, whereas the testes were the tissue of greatest concentration of DEHP metabolites in orally dosed marmosets.

In an additional study, Rhodes et al. (1986) gave male marmosets a single oral dose of 0.25 mmole/kg of either [^{14}C]-DEHP or [^{14}C]-MEHP and used GC and GC-MS to measure the urinary metabolites excreted in the first eight hours after dosing. Approximately 17 % of the DEHP dose and 24 % of the MEHP dose was recovered in the urine within eight hours after dosing. Of the total metabolites recovered in urine, 21 and 68 % were conjugated in the DEHP- and MEHP-dosed animals, respectively. The amount of free and conjugated MEHP in the urine of DEHP-dosed animals came to 2.02 and 0.87 % of the administered dose. The remainder of the metabolites were ω- and ω-1 oxidation products of MEHP, of which approximately 21 % were conjugated.

Scmid and Schlatter (1985) gave an oral dose of 30 mg DEHP to each of two healthy adult male volunteers with body weights of 61 to 71 kg. The urine of these volunteers was collected every six hours for 48 hours. Aliquots of the urine samples were hydrolyzed with β-glucuronidase and analyzed by gas-liquid chromatography-mass spectrometry. Samples of non-hydrolyzed urine were also analyzed. The mass chromatogram of the urinary metabolites of DEHP revealed MEHP and several variations of MEHP with modifications to the 2-ethylhexyl moeity. In the non-hydrolyzed urine, 65 to 66.4 % of the DEHP metabolites was conjugated.

ANIMAL STUDIES

Previously Available Data

Autian (1973) studied the absorption of DEP through the skin of rabbits. Labeled DEP (^{14}C) was applied topically, and the application sites were covered with cotton patches. Analysis of urine indicated that approximately 9 % of the radioactivity was excreted after 24 hours, 14 % after 48 hours, and 16 to 20 % within 72 hours. After 3 days of topical exposure, tissue distribution was determined by autoradiography. Radioactivity was detected in the lung, heart, liver, kidney, gonads, spleen, and brain. It was not detected in

the skin and subdermal fatty tissue at the site of application.

Albro & Moore (1974) administered DBP by gavage to male rats in two doses of 0.2 ml 24 hours apart. Urine was collected for 48 hours after the first dose, and DBP and its metabolites were quantitated. A total of 24.6 % of the phthalate moiety was recovered in the urine. The recovered phthalate moiety consisted of 89.8 % monobutyl phthalate (MBP), 2.7 % phthalic acid (PA), 0.4 % intact DBP, and four other metabolites in very small amounts. The researchers suggested that DBP was metabolized by hydrolysis of one ester bond and both terminal and subterminal oxidation of the remaining alkyl chain. The resulting primary and secondary alcohols were, presumably, further oxidized to acids and ketones, respectively. DMP was administered by gavage to male rats in a single dose of 0.1 ml, and the urine was collected for 24 hours. A total of 44.6 % of the phthalate moiety was recovered in the urine, and it consisted of 77.5 % monomethyl phthalate, 14.4 % PA, and 8.1 % intact DMP. DMP appeared to be metabolized only by hydrolysis of one or both ester groups.

Singh et al. (1975) administered labeled DEP (^{14}C) intravenously to pregnant rats on Day 5 or Day 10 of gestation. Diester and/or metabolic products were present in maternal blood, fetal tissue, amniotic fluid, and placentas after Day 8 or Day 11, respectively, and throughout gestation.

Yamaguchi et al. (1976) administered male mice labeled DBP (^{14}C) orally or intravenously. The radioactivity accumulated in the liver and kidney within 6 hours of oral administration and within 1 hour of intravenous administration. The radioactivity was rapidly excreted in the urine and feces.

Lake et al. (1977) incubated labeled DBP, DMP, and DEP (^{14}C) with rat, ferret, and baboon hepatic postmitochondrial supernatant and with intestinal-mucosal cell homogenates. All of the diesters were hydrolyzed by cell homogenates. They were all hydrolyzed by all the preparations, and greater than 90 % of the total metabolite formed was the corresponding monoester. Baboon liver preparations hydrolyzed the diesters faster than rat liver preparations; ferret liver preparations were the least active. Baboon intestinal-mucosal cell preparations hydrolyzed the diesters faster than rat intestinal-mucosal cell preparations, and ferret intestinal-mucosal cell preparations were the least active (Lake et al., 1976). Hepatic preparations from humans also catalyzed the monohydrolysis of DBP, DMP, and DEP. The toxic effects of phthalates administered orally may depend on the properties of the corresponding monoesters and/or alcohols.

Kaneshima et al. (1978) incubated DBP and DMP, in concentrations of 0.4 mg/ml with rat liver and kidney homogenates at 37°C. DBP and DMP almost completely disappeared after 2 hours of incubation

with rat liver homogenates. The action of rat kidney homogenates was slower; however, approximately 90 % of the DBP and 95 % of the DMP disappeared during a 5-hour incubation. The phthalates were found not to be degraded spontaneously under these experimental conditions.

Kaneshima et al. (1978) also administered 500 mg/kg dose of labeled (^{14}C) DBP in ethanol by gastric intubation to male rats and the bile was collected every hour for 6 hours. Six hours after oral administration of DBP, 4.5 % of the radioactivity was recovered in the bile. Five hours after intravenous injection of DBP, 10 % of the radioactivity was detected in the bile. DBP bile metabolites included MBP, intact DBP, PA, an MBP glucuronide, and traces of other glucuronides. A small amount of DBP appears to be absorbed unaltered from the intestine, and the excretion of DBP through the biliary route has a role in its metabolic fate.

Okada and Tamemasa (1978) reported that DBP interacted with DNA *in vitro*, but after oral administration of labeled DBP (^{14}C) to mice, no radioactivity was recovered from hepatic DNA. DBP and its metabolites appeared not to be transported into the nuclei.

Tanaka et al. (1978) administered labeled DBP (^{14}C) orally in dimethyl sulfoxide in a dose of 60 mg/kg or intravenously in saline in a dose of 10 mg/kg to male rats. Urine and feces were collected, and the amount of radioactivity excreted was determined. The percentage of administered radioactivity excreted varied from 81.4 to 97.7 % in the urine and from 1.0 to 8.2 % in the feces in the first 24 hours after oral or intravenous administration of DBP. Several rats were killed, and tissue distribution of radioactivity was determined. Brain, heart, liver, lung, spleen, muscle, adipose, stomach, prostate, and thymus tissues, blood, and the intestinal contents were examined 24 hours after oral or intravenous administration of DBP. Very little radioactivity was recovered. The elimination of DBP from tissues and organs was rapid, and no organ had any significant affinity for accumulation. Rats were given labeled DBP (^{14}C) orally, and bile was collected. From 27.6 to 52.8 % of radioactivity was excreted in the bile within 24 hours after oral administration of DBP. Since more radioactivity was excreted in the bile than in the feces, there was apparently absorption of DBP and its metabolites from rat intestine. Urinary metabolites were identified in male rats, male hamsters, and male guinea pigs given a single oral dose of 60 mg/kg DBP. All 24 hour urine samples contained MBP as the major product, intact DBP, PA, MBP glucuronide, and two other MBP oxidation products. The hamster urine contained an additional oxidation product. The livers from rats were

examined 1 hour after intravenous dosing of DBP, and the data obtained indicated that DBP was rapidly hydrolyzed to MBP by the microsomal fraction. No PA was detected. The bile contained MBP and intact DBP but not PA. Since PA was detected in the urine, it was suggested that its formation must occur at other sites than the liver. It was concluded that the hydrolysis of DBP to MBP occurred in the liver, that there was enterohepatic circulation of DBP and its metabolites and good absorption from the intestine, and that MBP was the main metabolite of DBP and was primarily excreted in urine.

White et al. (1980) studied the *in vitro* intestinal absorption of DBP and DMP using an everted gut-sac preparation from the rat small intestine. In one experiment with DBP, S,S,S,-tributylphosphoro-trithioate (DEF), administered orally before gut-sac preparation, was used as an esterase inhibitor. Most of the DBP and DMP was hydrolyzed to the corresponding monoester before crossing the intestinal mucosa. Only 4.5 % of the DBP and 18.8 % of the DMP crossed the intestine intact. Inhibition of mucosal esterases by DEF reduced the amount of DBP hydrolyzed to MBP. Approximately the same amount of intact DBP was absorbed by the intestine with and without DEF, and DEF did not affect MBP absorption. Intestinal absorption of these compounds may be controlled by the hydrolysis of DBP to MBP.

Kozumbo (1982) reported that a homogenate of rat epidermis metabolized DMP at approximately 1.5 % of the rate of the metabolism of DMP by a homogenate of rat liver. The homogenates were compared on a mg wet weight basis. DMP was bound to the epidermis in quantities seven to eight times greater than those in which it was bound to an equal dry weight of hepatic tissue.

Newly Available Data

Small et al. (1973) applied radiolabeled DEP to circles (diameter 25 mm) on the shaved backs of three female albino rabbits. DEP doses were approximately 299 to 569 mmoles per rabbit. The exposure sites were occluded with aluminum foil. Blood samples were collected at 1, 24, 48, 72, and 96 hours after application. Urine and feces were collected at 24, 48, 72, and 96 hours. At 96 hours after application, the rabbits were killed and tissues from the vital organs were collected. Detection of DEP in the biological samples was conducted by liquid scintillation counting. The results of the study suggest that DEP is rapidly absorbed through the skin, widely distributed to tissues, and actively excreted. Approximately 50 % of the applied DEP was excreted in four days. The authors estimated that it would take approximately one month to eliminate the entire DEP dose applied. There was evidence of higher deposition of DEP in the adrenal tissue and kidneys than in other tissues.

Williams and Blanchfield (1975) performed a study of the kinetics of DBP after oral administration. Single doses of 0.27 g/kg or 2.31 g/kg of DBP-7-^{14}C were given by oral intubation to Wistar rats. Urine and feces were collected at 4, 8, 24, and 48 hrs after dosing. At the same time points, selected animals were killed, and their tissues and organs were weighed and analyzed for metabolites of DBP-7-^{14}C. By 48 hours after dosing, 80 to 90% of the DBP-7-^{14}C administered had been excreted in urine or feces as phthalic acid (PA), MBP, mono(3-hydroxybutyl) phthalate, or mono(4-hydroxybutyl) phthalate. Less than 0.001% of the dose was found in the tissues at 48 hours. The radioactivity was cleared more rapidly from the body in the lower dose group (actual rates not reported). Furthermore, rats fed a diet of 1% DBP for 12 weeks did not accumulate DBP or its monoester metabolites in the tissues or organs.

According to White et al. (1983), upon oral administration, DBP is absorbed in the intestines of rats mostly as its monoester Monobutyl Phthalate (MBP). 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]DBP (doses 6, 10, 14, 25, and 30 g/kg) in rats. Blood concentrations of circulating [^{14}C]MBP were not measured. Four hours after a single dose of 837 mg/kg [^{14}C]DBP, 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 MBP in the lumen.

Elsisi et al. (1989) examined the dermal absorption and distribution/excretion fate of radiolabeled phthalates in an *in vivo* study. [^{14}C]DBP, [^{14}C]DEP, or [^{14}C]DMP (157 µmol/kg for each phthalate) was applied to a 1.3 cm diameter patch of shaved skin on the backs of F344 rats. The dosing site was covered with a perforated cap to contain the radiolabeled phthalate at the dosing site. Rats were then placed in a metabolic cage that allowed separate collection of urine and feces which were collected every 24 hours for seven days. Phthalate excretion was measured by scintillation analysis of ^{14}C in the excreta. ^{14}C was excreted in the urine and feces at a nearly constant rate of 10 to 12% of the [^{14}C]DBP dose per 24 hours throughout the seven days of collection. Approximately 24 times as much ^{14}C was excreted in the urine as in feces. For [^{14}C]DEP, 24 % was excreted in the first 24 hours, then slowed to 11 % in the second 24 hours, and slowed to 5 % every 24 hours for the remaining period of measurement. The rate of [^{14}C]DMP excretion was a relatively constant 6 to 7.5 % per 24 hours over the entire seven days. Rats were killed on the seventh day, and various tissues and organs as well as the skin at the dosing site and the cap were analyzed by scintillation counting for the presence of ^{14}C. The mean percentages (± SD) of the [^{14}C]DBP,

[^{14}C]DEP, and [^{14}C]DMP doses were distributed in various tissues as shown in Table 4. Most of the absorbed phthalate had been excreted in the urine and feces. Very little was retained in the tissues.

Table 4. Percentage (mean ± SD) and location of phthalate dose (^{14}C equivalents) at seven days following a single application of radiolabeled phthalates to the skin of rats (Elsisi et al., 1989).

Tissue	Percentage of dose (mean ± SD)		
	DMP	DEP	DBP
Adipose Tissue	0.3 ± 0.3	0.03 ± 0.03	0.41 ± 0.07
Muscle	0.6 ± 0.7	0.14 ± 0.07	1.1 ± 0.2
Skin	0.4 ± 0.4	0.06 ± 0.02	1.4 ± 1.0
Skin of Dose Site	19 ± 23	34 ± 33.2	33 ± 2
Other Tissues	< 0.5	< 0.5	< 0.5
Plastic Cap	5 ± 0.3	4.8 ± 3.2	2.2 ± 2
Urine & Feces*	~39	~49	~60
Total Recovery	66 ± 26	74 ± 21	100 ± 3

*Urine & feces data for the seven-day total recovery of radioactivity were estimated from a line graph, as the specific figures were not presented in the reference.

Foster et al. (1983) compared the urinary metabolites of rats and hamsters after exposure to DBP or MBP. In both rats and hamsters, the major urinary metabolite of DBP or MBP was MBP-glucuronide. However, the levels of unconjugated MBP in rat urine were three- to fourfold 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 MBP-glucuronide to free MBP, this difference in enzyme activity between species may contribute to the quantitative differences in urinary metabolites and to the male developmental toxicity of DBP and MBP described in the REPRODUCTIVE AND DEVELOPMENTAL TOXICITY section later in this report.

Saillenfait et al. (1998) reported a study in which pregnant Sprague-Dawley rats were given a single oral dose of radiolabeled DBP (up to 3 g/kg) on gestation day (GD) 14. Tissues from the dams and embryos were analyzed for DBP and its metabolites (MBP and MBP-glucuronide) for several time points up to 48 hours after dosing. The percent of administered dose in the placenta and embryonic tissue peaked at 0.3% and 0.15%, respectively, at two hours after dosing. By 48 hours after dose administration no detectable phthalates were found in these tissues nor in the uterus, ovaries, or amniotic fluid. Although DBP was shown

to cross the placenta into fetal tissues, neither DBP nor its metabolites accumulated in the fetus, placenta or in maternal tissues.

Payan et al. (2001) conducted a study in which Sprague-Dawley rats were given a single intravenous injection of 1 or 10 mg/kg [^{14}C]DBP. After dosing, the animals were placed in individual metabolic cages for collection of urine and feces for 72 hours. Arterial blood was collected at 10 seconds and 72 hours after dose administration. Biological samples were analyzed by HPLC and/or liquid scintillation counting to determine the presence of DBP and its metabolites. The main route of excretion of ^{14}C was in urine (85 % of the administered dose), followed by feces (9 to 10 % of the administered dose). More than 95 % of the ^{14}C in urine was excreted within the first 24 hours. Seventy-two hours after the dose administration, 0.6 to 2.0 % of the administered dose remained in the exanguinated rat carcass, and 1.9 to 2.3 % remained in the blood. Unchanged DBP was undetectable in the urine. MBP in urine accounted for 22 ± 3.7 % of the administered dose. MBP-glucuronide in urine accounted for 34.6 ± 3.6 % of the administered dose.

In another experiment in this report, circular rings (10 cm^2) were glued to the shaved backs of male Sprague-Dawley rats. Twenty-four hours later, 10 µl/cm^2 neat [^{14}C]DBP was applied to the area inside the ring, and the dosing area was covered with a perforated plastic cap. Groups of 3 to 8 rats were killed by bleeding of the abdominal aorta at several time points between 0.5 to 72 hours after application of the [^{14}C]DBP. After the animal was dead, the dosing area was washed five times with ethanol. The areas within and surrounding the ring were separately digested in KOH for detection of radiation by liquid scintillation analysis. Blood, urine and feces were collected for analysis by HPLC and/or liquid scintillation analysis. DBP penetrated the skin rapidly. Thirty minutes after application, 20 % of the applied dose had entered the skin. Within the first 8 hours, the absorption flux was 43 µg/cm^2/h. Between 8 and 48 hours, the rate of absorption flux increased to 156 µg/cm^2/hr. At the 24- and 72-hour time points, the [^{14}C]DBP that absorbed into the skin within the ring had radially diffused into the surrounding stratum corneum and/or epidermis, which the authors described as a reservoir for DBP. DBP was hydrolyzed by skin esterase before reaching systemic circulation. At all time points, unchanged DBP accounted for 2 % of the plasma radioactivity, whereas MBP and MBP-glucuronide combined accounted for 61 to 85 % of the plasma radioactivity. The mean ratio of plasma MBP to MBP-glucuronide was 1.3 (SD = 0.5, n = 33). In the urine, MBP and MBP-glucuronide together accounted for 50 to 66 % of the urinary radioactivity, with no significant correlation to time. The mean ratio of MBP to MBP-glucuronide in urine was 0.73 (SD = 0.08, n = 25). Over the maximum 72-hour

exposure, the total urinary excretions of MBP and MBP-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 DBP absorption rates as the males. Male hairless rats had a higher percutaneous absorption of DBP (75 % of the applied dose). The urinary excretion rate of [^{14}C]DBP in hairless rats was 2.5-fold higher than that of the haired rats (Payan et al., 2001).

HUMAN STUDIES

Previously Available Data

Phthalates are ubiquitous in the environment, and human exposure is likely. DBP was found in normal and diseased kidneys (Overturf et al., 1978; 1979), adipose tissue at autopsy (Mes et al., 1974), in the blood of pregnant women, and in umbilical cords (Yagi et al., 1977). Possible routes of exposure to phthalates for humans are by oral or dermal contact, inhalation, or as a result of the use of medical devices, such as blood storage bags (Lawrence, 1978).

DMP was absorbed through human skin, and some of its metabolites were detected in human urine (Gleiberman et al., 1978).

Newly Available Data

Anderson et al. (2001) gave groups of eight volunteers a single oral dose of 255 (low dose) or 510 µg (high dose) isotope-labeled DBP (weights of volunteers not reported). Twenty-four hours after the doses were given, the MBP content in the subjects' urine was determined by liquid chromatography-mass spectroscopy following hydrolysis of the glucuronide conjugate. Subjects in the low and high dose groups respectively excreted 64 and 73 % of the DBP dose given as MBP in the first 24 hours. The amount of conjugated MBP was not measured.

Waliszewski and Szymczyński (1989) obtained semen samples from 58 men and analyzed for phthalates by gas-liquid chromatography. These samples were found to contain concentrations of DBP, DMP, and DEP at 0.89, 0.22, and 0.40 mg/kg, respectively. The phthalates apparently entered the body through general environmental exposure.

Blount et al. (2000) reported a study done by the Centers for Disease Control and Prevention (CDC) in which phthalate monoesters were measured in urine samples from 289 adult humans aged 20 to 60 years old. The analyses were performed after the samples were treated with β-glucuronidase to release the

phthalate monoesters from the glucuronide conjugates. The authors reported finding high concentrations of MEP (mean = 345 ng/ml, max = 16200 ng/ml) and MBP (mean = 41.5 ng/ ml, max = 4670 ng/ml). Women of child-bearing age, 20 to 40 years old, were found to have higher urinary MBP 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.

A detailed demographic analysis of the CDC phthalate exposure data by Koo et al. (2002) found that people with a high school education or less have higher urinary output of DBP and butylbenzyl phthalate metabolites, compared to those with education levels beyond high school.

In a pilot study by Brock et al. (2002), urine samples were collected from 19 young children (17 Hispanic, 2 Caucasian; 14 boys, 5 girls; ages 11.8 to 16.5 months). The samples were analyzed for the presence of phthalate monoesters after deconjugation by β-glucuronidase. MEP and MBP were detected in the children's urine samples at respective mean (± SD) concentrations of 184.1 ± 246.9 ng/ml and 117.4 ± 287.6 ng/ml. The levels of MBP fall into the 75th to 100th percentile of the levels found in adult urine in the previously described study by Blount et al (2000). 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 (Brock et al., 2002).

Hoppin et al. (2002) conducted a study to determine the reproducibility of detecting urinary phthalate metabolites in first morning urine samples. Forty-six women, ages 35-49, residing in the Washington, D.C., area in 1996 - 1997 provided first morning urine samples on two consecutive weekdays. The samples were analyzed for detection of seven phthalate monoesters using high-pressure liquid chromatography followed by tandem mass spectrometry on a triple quadrapole instrument using atmospheric pressure chemical ionization. Of the seven phthalate monoesters sought, four were detected in samples from all subjects. The mean concentrations of MBP was 78 ng/ml (range 0.7 - 251 mg/ml); MEP 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.

IN VITRO STUDIES

Previously Available Data

Rowland et al. (1977) incubated DBP, DMP, and DEP, in concentrations of 1 mg/ml, for 16 hours at 37°C with the contents of rat stomach, small intestines, or cecum or with suspensions of human feces. The phthalates were metabolized rapidly to the corresponding monoesters when incubated with the contents of rat small intestine. Metabolism was slower in the presence of rat cecal contents and only DMP was metabolized to any extent by rat stomach contents. Human feces were almost inactive in metabolizing the phthalates; DBP and DMP were metabolized faster than DEP. The intestinal contents of younger male rats metabolized DBP and DMP at a slower rate than intestinal contents from more mature male rats. Among adults, intestinal contents from male rats metabolized DBP at a faster rate than intestinal contents from female rats. The monoesters were the only products of metabolism; complete hydrolysis to PA did not occur. It may be significant toxicologically that there is a good correlation between rate of phthalate hydrolysis and the acute oral toxicity to rats that is reported in the literature. The more rapidly hydrolyzed phthalate esters are more toxic. In another experiment, rat intestinal contents were incubated at 37°C for 90 minutes or centrifuged or filtered before addition of DMP. Preincubation reduced the ability of the small intestine contents to degrade DMP. The enzymes involved in DMP metabolism appeared to be labile *in vitro*. Both centrifugation and filtration reduced the rate of DMP hydrolysis. The effect of antibiotics was studied by adding antibiotics to the incubation mixture or to the intestinal contents during the 90-minute preincubation period. The antibiotics used in the experiments were antibacterial enzymes. They had no effect on the rate of metabolism of DMP by small intestine contents, suggesting that the involved enzymes are not bacterial and more probably are mammalian in origin. Mucosal cell enzymes may be involved in DMP metabolism. The low rate of phthalate hydrolysis by rat cecal contents and human feces might be explained by the presence of a low number of active intestinal mucosal cells.

Newly Available Data

Scott et al. (1987) measured dermal absorption rates of four undiluted phthalate esters in *in vitro* preparations of human and rat skin. Sheets of epidermis were prepared from the abdomen of human cadavers and from the skin on the dorsal region of euthanized Wistar rats. Permeability constants (Kp) of the epidermal samples to tritiated water were assessed to reject damaged samples. The diffusion of DMP,

DEP, and DBP across the epidermal samples was measured for various exposure periods. Table 5 summarizes the absorption rates of the three phthalate esters. For all phthalate esters the absorption rate was greater in rat skin than in human skin.

Table 5. Absorption rate data of phthalate diesters through human and rat skin (Scott et al., 1987)

	Permeability constant[a] ($\times 10^{-3}$cm/hr)		Steady-state absorption rate[a] (μg/cm^2/hr)	
Chemical	Human	Rat	Human	Rat
DMP	3.32 ± 0.54	34.50 ± 3.51	30.51 ± 06.43	410.55 ± 41.77
	n = 4	*n* = 10	*n* = 14	*n* = 10
DEP	1.14 ± 0.10	37.00 ± 8.3	12.75 ± 1.12	413.67 ± 92.79
	n = 11	*n* = 11	*n* = 11	*n* = 11
DBP	0.23 ± 0.06	8.95 ± 0.09	2.40 ± 0.63	93.35 ± 0.94
	n = 15	*n* = 8	*n* = 15	*n* = 9

[a] Permeability constant and steady-state absorption rates are mean ± SE; *n* = sample size

Mint et al. (1994) determined the percutaneous absorption of DEP in *in vitro* cultures of shaved rat skin and of human breast skin (removed during surgery). Absorption through the rat skin was rapid and extensive. There was an initial lag for the first eight hours during which the absorption was 1.7%. From eight to 72 hours 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 and 72 hours was 0.094 mg/cm^2/hr (occluded) and 0.103 mg/cm^2/hr (unoccluded). The percutaneous absorption of DEP through human skin was slow and incomplete. The initial lag time was 10 hours. At 8 hours 0.1 % had absorbed in occluded skin and 0.2 % in unoccluded skin. At 72 hours 3.9 and 4.8 % of applied DEP had absorbed in occluded and unoccluded skin, respectively. The mean rate of absorption of DEP through human breast skin between 12 and 72 hours was 0.011 mg/cm^2/hr (occluded) and 0.014 mg/cm^2/hr (unoccluded). In this *in vitro* model, the rate of absorption of DEP through rat skin was significantly ($p < 0.05$) faster than through human skin.

Newly Available Data

Payan et al. (2001) assessed the percutaneous absorption of DBP in an *in vitro* model with static diffusion cells using full-thickness skin from haired (n = 9) and hairless (n = 9) male Sprague-Dawley rats. Four circular skin sections (1.76 cm^2) were obtained from each rat and placed in diffusion cells. Skin sections in the diffusion cells were maintained in a simulated physiological bath at 36°C. The integrity of the

skin samples and of the diffusion cell system was confirmed prior to application of DBP. An infinite dose of [^{14}C]-DBP (50 mg/cm^2) was applied to the stratum corneum side of the skin samples for 24 hours. Aliquots (200 µl) of the receptor fluid on the dermis side of the skin sample were collected periodically over the 24-hour period. Receptor fluid samples were analyzed by liquid scintillation counting and HPLC for the presence of DBP and its metabolites (MBP and MBP-glucuronide). The percutaneous absorption fluxes of DBP across the skin sections of haired and hairless male rats were 26 ± 1 and 39 ± 1µg/cm^2/h, respectively. All of the radioactivity contained in the receptor fluid had the same HPLC retention time as MBP. Addition of an esterase inhibitor caused a reduction of 99% of the absorption flux of ^{14}C, and MBP was barely detectable in the receptor fluid (Payan et al., 2001).

Tokunaga et al. (2001) studied the *in vitro* permeation of DEP through abdominal guinea pig skin. A sample of skin was mounted into a Franz-type diffusion cell. The donor side of the skin was pre-treated for two hours at 32°C with one of the following surfactant solutions: 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 % DEP was added to the donor side for 16 to 24 hours at 32°C. A control condition consisted of DEP treatment without surfactant pre-treatment. In additional experiments, the skin samples were treated with 0.05 % DEP in a mixture with each of the respective surfactants described above, without surfactant pre-treatment. The amount of DEP permeated to the receptor side was determined by HPLC. Skins pre-treated with 10 mM sodium dodecyl sulfate, 10 mM benzalkonium chloride, or 0.5 % polyoxyethylene (10) oleyl ether had respective permeation rates that were 190, 174, and 110 % of the permeation rate of the control condition. The authors suggested that damage of the stratum corneum by surfactants increased the transdermal flux of DEP. Co-treatment of DEP with 10 mM sodium dodecyl sulfate, 10 mM benzalkonium chloride, or 0.5 % polyoxyethylene (10) oleyl ether caused DEP permeation rates of 82.0, 105.7, or 99.5 % of the control (no surfactant) DEP treatment. The cumulative amount of DEP permeation through guinea pig skin without surfactant at 24 hours was 21.8 µg/cm^2.

ANIMAL TOXICOLOGY

ACUTE TOXICITY

Previously Available Data

The acute oral toxicity of DBP, DMP, and DEP was studied in rats(Draize et al., 1948; Gesler, 1973;

Homrowki & Nikonorow, 1959; Krauskopf, 1973; Lehman, 1955; Nikonorow et al., 1973; Radeva & Dinoeva, 1966; Smith, 1953), mice (Draize et al., 1948; Miyahara et al., 1973; Omori, 1976; Sajiki et al., 1979; Yamada et al., 1975), rabbits (Clayton & Clayton, 1981; Draize et al., 1948), guinea pigs, and chicks (Draize et al., 1948) (Table 6).

Table 6. Acute Oral Toxicity

Material Tested	Method	Species	LD50	Comments	Reference
DBP	-	Rats	23.0 g/kg	-	Gesler, 1973; Radeva & Dinoeva, 1966
DBP	-	Rats	12.5 g/kg	-	Homrowski & Nikonorow, 1959; Nikonorow et al., 1973
DBP	-	Rats	14.95 g/kg	-	Komarova, 1979
		Mice	9 g/kg		
DBP	Animals were observed for 7 days following DBP administration	Male mice	>20 ml/lg	-	Miyahara et al., 1973; Omori, 1976
DBP	Oral administration of 200 mg DBP to 10 mice	Mice	-	6/10 of the mice died within 7 hours.	Sajiki et al., 1979
undiluted DBP	Oral administration of 4, 8, 16, and 32 g/kg DBP to 3, 9, 6, and 6 rats, respectively	Rats	~8 g/kg	0/3, 4/9, 6/6, and 6/6 rats died, respectively. The 4 g/kg dose had no effect on growth, the 8 g/kg dose slightly inhibited growth, and the 16 and 32 g/kg dose groups succumbed too quickly to exhibit significant changes in growth.	Smith, 1953
DBP	Animals were observed for 7 days following DBP administration	Male mice	Between 14.8 and 17 g/kg	-	Omori, 1976; Yamada et al., 1975
DBP	Animals were observed for 11 days after dosing.	Rats	>7.5 g/kg	No clinical sign of toxicity	Phthalate Esters Panel, American Chemistry Council, 1982a
undiluted DMP	40 rats, 80 guinea pigs, and 120 chicks were fasted prior to DMP administration. 110 mice and 80 rabbits were not fasted. Animals were observed for 6 days following DMP administration. DMP was given to 10 animals/dose.	Rats	6.9 ml/kg	-	Draize et al., 1948
		Mice	7.2 ml/kg		
		Rabbits	4.4 ml/kg		
		Guinea Pigs	2.4 ml/kg		
		Chicks	8.5 ml/kg		
DMP	-	Mice	6281 mg/kg	-	Plasterer et al., 1985
DEP	-	Rabbits	1.0 g/kg	-	Clayton & Clayton
DEP	-	Rats	8.2 ml/kg	-	Krauskopf, 1973
DEP	-	Rats	9 g/kg	-	Kamrin & Mayor, 1991
Nail polish, 9 % DBP	5 male and 5 female animals/dose were fasted 16 hours prior to oral intubation and were observed for 14 days after.	Rats	>5 ml/kg	No signs of gross pathology on necropsy of rats receiving 5 ml/kg.	Biodynamics, 1976a
Nail preparation 6 % DBP	Preparation administered by oral intubation to 10 animals	Rats	> 5 g/kg	"Nontoxic."	CTFA, 1977a

The LD_{50} for rats administered DBP orally ranged from approximately 8 g/kg to 23.0 g/kg. The LD_{50} value for rats administered DMP orally was 6.9 ml/kg. In the Hodge and Sterner(1949) classification of single-dose oral toxicity for rats, DBP and DMP would be classified as practically nontoxic to relatively harmless and as practically non-toxic, respectively.

The acute oral toxicity of two nail preparations, one containing 9 % DBP and one containing 6 % DBP, was studied in rats (Biodynamics, 1976a; CTFA, 1977a). Both preparations were practically nontoxic.

Acute intraperitoneal toxicity of DBP, DMP, and DEP was studied in mice (Calley et al., 1966; Guess & Haberman, 1968; Karel et al., 1947; Lawrence et al., 1975) and in rats (Singh et al., 1972) (Table 7). The acute intraperitoneal LD_{50}s for rats for DBP, DMP, and DEP were 3.05 ml/kg, 3.38 ml/kg, and 5.06 ml/kg, respectively.

Table 7. Acute Parenteral Toxicity

Material Tested	Method	Number and Species of Animals	LD50	Comments	Reference
DBP	Single i.p. injection of 4 dose levels ranging from 0.5 to 16 g/kg	Mice	4.00 mg/kg	-	Calley et al., 1966
DBP	Single i.p. injection. Pathological changes observed up to 72 hours. Deaths recorded for 7 hours.	Female mice	14.9 mmoles/kg	Pulmonary congestion, edema and petechial hemorrhage, toxic reaction in spleen, and renal tubular degeneration observed after 72 hours.	Karel et al., 1947
DBP	Undiluted DBP administered and animals observed 7 days for deaths; 2 ml/kg administered i.p., 2 mice killed at days 1, 2, 4, 7, and, and heart, lung, kidneys, spleen, liver, pancreas, and bowel examined histologically for irritation.	10 male mice /dose to determine LD50; 10 mice of unspecified sex for histological evaluation.	3.57 g/kg (3.41 ml/kg)	No evidence of significant intraperitoneal irritation.	Lawrence et al., 1975
DBP	Animals observed for 7 days after i.p. injection.	Female rats	3.05 ml/kg	-	Singh et al., 1972
DMP	Single i.p. injection of 4 doses ranging from 0.5 to 16 g/kg.	Mice	1.58	-	Calley et al., 1966
DMP	Single i.p. injection of saline saturated with DMP. 25 ml/kg DMP.	Mice	-	No deaths observed	Guess & Haberman, 1968
DMP	Single i.p. injection. Pathological changes observed up to 72 hours. Deaths recorded for 7 days.	Female mice	18.8 mmoles/kg	Pulmonary congestion and atelectasis, toxic reaction in spleen and lymph nodes, and renal tubular necrosis observed after 72 hours.	Karel et al., 1947

Table 7 (continued). Acute Parenteral Toxicity

Material Tested	Method	Number and Species of Animals	LD50	Comments	Reference
DMP	Undiluted DMP administered and animals observed 7 days for deaths; 2 ml/kg administered i.p., 2 mice killed at days 1, 2, 4, 7, and 10, and heart, lungs, kidneys, liver, pancreas, and bowel examined histologically for irritation.	10 male mice/dose to determine LD50; 10 mice of unspecified sex for histological evaluation.	3.98 g/kg (3.35 ml/kg)	No evidence of significant intraperitoneal irritation.	Lawrence et al., 1975
DMP	Animals observed for 7 days after i.p. injection.	Female rats	3.38 ml/kg	-	Singh et al., 1972
DEP	Single i.p. injection of 4 doses ranging from 0.5 to 16 g/kg.	Mice	2.83 g/kg	-	Calley et al., 1966
DEP	Single i.p. injection of saline saturated with DEP. 25 ml/kg DEP.	Mice	-	No deaths occurred	Guess & Haberman, 1968
DEP	Single i.p. injection. Pathological changes observed up to 72 hours. Deaths recorded for 4 days.	Female mice	12.4 mmoles/kg	Pulmonary congestion, edema and petechial hemorrhage, toxic reaction in spleen, and renal tubular degeneration observed after 72 hours.	Karel et al., 1947
DEP	Undiluted DMP administered and animals observed 7 days for deaths; 2 ml/kg administered i.p., 2 mice killed at days 1, 2, 4, 7, and 10, and heart, lungs, kidneys, liver, pancreas, and bowel examined histologically for irritation.	Female rats	3.22 g/kg (2.87 ml/kg)	No evidence of significant intraperitoneal irritation.	Lawrence et al., 1975.
DEP	Animals observed for 7 days after i.p. injection.	Female Rats	5.06 ml/kg	-	Singh et al., 1972

The acute intravenous LD_{50} of DBP to male mice was 0.72 g/kg (Miyahara et al., 1973; Omori, 1976). DEP, in a 3 % acacia suspension, was administered to an anesthetized rabbit through the jugular vein. The DEP was administered in repeated doses of 50 mg/kg to a total dose of 650 mg/kg (time between doses was not given). The first six doses caused a transient fall in blood pressure. The total dose of 650 mg/kg did not cause death or significant change in the animal. Five doses of the 3 % acacia vehicle did not produce any blood pressure changes (Calley et al., 1966). A 0.25 ml/kg dose of DEP in saline was injected slowly into the femoral vein of a dog. At first, respiration was stimulated and then it was paralyzed. The intravenous administration of 0.5 ml of DEP into a rabbit ear vein caused convulsions "similar to those produced by strychnine" within a few minutes. The symptoms "soon" disappeared and the rabbit appeared normal. A larger dose was fatal to rabbits by causing paralysis of respiration (Blickensdorfer et al., 1930).

The intramuscular administration of DBP in a dose of 4 g/kg to 3 rats and 8 g/kg to 3 rats did not result in any deaths, and there was no effect on the growth of the rats (Smith, 1953).

The subcutaneous LD_{50} of DEP to guinea pigs was greater than or equal to 3 g/kg (Clayton & Clayton, 1981; Lawrence, 1978).

Newly Available Data

The Approximate Lethal Dose of commercial grade DBP administered orally to male albino rats was found to be more than 7500 mg/kg. There were no clinical signs of toxicity and no pathological findings eleven days after dosing (Phthalate Esters Panel, American Chemistry Council, 1982a).

The oral LD_{50} for DEP in rats (strain not specified) was 9 g/kg (Kamrin & Mayor, 1991). The respective oral and dermal LD_{50}'s for DEP were determined to be >5.0 ml/kg and >10 ml/kg in adult albino rats (Consumer Product Testing, 1978a; 1978b). Plasterer et al. (1985) reported that the oral LD_{50} for DMP in female CD-1 mice was 6281 mg/kg.

SHORT-TERM TOXICITY (<3 mos)

ORAL

Previously Available Data

DBP, DMP, and DEP in corn oil were administered by oral intubation for 4 days to groups of 12 rats in doses of 7.2 mmol/kg per day (approximately 2.0 g/kg per day DBP, 1.4 g/kg per day DMP, and 1.6 g/kg per day DEP). There were no significant changes in food intake or body weight. DMP and DEP administration did not result in significant changes in testes weight, no testicular atrophy was observed, and urinary zinc excretion was unaffected. Administration of DBP decreased weight of testes and produced severe atrophy of the seminiferous tubules. Most of the tubules had complete loss of spermatocytes and spermatids. DBP administration was accompanied by an increase in the urinary excretion of zinc, and there was a decrease in the zinc content of testes on an absolute and relative weight basis (Cater et al, 1977; Foster et al, 1980; Gangolli, 1982). The administration of zinc, concurrently with DBP, provided substantial protection against DBP-produced testicular damage (Cater et al., 1977). DBP produced testicular atrophy in the rat, mouse, guinea pig, and ferret, but not in the hamster after oral administration in a dose of 2.0 g/kg per day for 10 days (Gangolli et al., 1982).

Table 8. Subchronic and Chronic Oral Toxicity

Material Tested	Dose & Vehicle	Length of Study	Number and Species of Animals	Results	Reference
DBP	0.03, 0.3, and 1.0 % in feed	14 days	Mice	Reduced body weight in males at 1.0% DBP. Increased liver weights in males and females at 1.0% DBP.	Lamb et al., 1997b
DBP and MBP	0.5 and 5 % DBP or MBP in feed	34 to 36 days	Rats	Increased liver, kidney, and spleen weights and liver necrosis at 5% MBP or 5% DBP.	Murakami et al., 1986b
DBP	1 ml/kg in oil 2 times a week	6 weeks	Rats	No adverse effects reported	Bornmann et al., 1956; Shibko & Blumenthal, 1973
DBP	20 mg/kg	11 weeks and 3 days	Rats Mice	Leukocytosis was observed in rats. Mouse growth was inhibited.	Komarova, 1979
DBP	0.12 and 1.2 g/kg/day in olive oil	3 months	10 male and 10 female rats/dose, 40 control rats given only olive oil.	1/10 rats from the high dose group died. No specific cause of death was determined. Both DBP doses produced a statically significant increase in the animals' mean liver weight. No histologic evidence of any pathologic changes were found in the liver, kidneys, and spleen.	Nikonorow et al., 1973
DBP	400, 2000, and 10000 ppm DBP in drinking water	3 months	Rats	No effects seen at 400 and 2000 ppm levels. At 10000 ppm, increase in liver weights in both sexes.	BASF, 1992
DBP	2.5 mg/kg/day	6 months	Rats	No adverse effects were observed.	Maslenko, 1968; Krauskopf, 1973
DBP	0.125 % in feed	1 year	20 male and 20 female rats in dosed and in control groups	6/40 rats from the dosed group died. No specific cause of death was determined. No "remarkable" alterations were observed upon gross and histologic examination of liver, kidneys, and spleen of dosed rats.	Nikonorow et al., 1973
DBP	0.01, 0.05, 0.25, and 1.25 % in feed	1 year	10 rats/dose, 10 control rats	At 0.25 % in the diet or lower, there was no effect on growth or survival. At 1.25 % in the diet, 5/10 rats died during the first week. The remaining rats gained weight as did the controls. No rats exhibited significant changes in the number or distribution of elements in the peripheral blood or specific gross pathologic changes.	Smith, 1953
DBP	1 ml/kg in oil 2 times a week	1½ years	Rats	No pathologic changes observed. No effects on hematologic parameters or on organ weights.	Bornmann et al., 1956; Daniel, 1978; Shibko & Blumenthal, 1973
DMP	2.0, 4.0, and 8.0 % in feed	2 years	10 female rats/dose	2.0 % in the feed had no effect on growth. 4.0 and 8.0 % had a slight but significant effect on growth. Chronic nephritis seen in rats on 8.0 % in diet. Mortality rates were not different from those for control rats.	Lehman, 1955

Material Tested	Dose & Vehicle	Length of Study	Number and Species of Animals	Results	Reference
DEP	3 ml/kg/day	8 days	Rabbits	The rabbits appeared normal for the 8 days and for 2 weeks afterwards. "Temporary distress" was observed after DEP administration.	Blickensdorfer & Templeton, 1930
DEP	0.2, 1.0, and 5.0 % in feed	2, 6, and 16 weeks	5 male and 5 female rats in dosed and control groups on diet for 2 and 6 weeks. 6 rats of each sex, litter mate-paired, in 5.0 % diet and control groups for 16 weeks. 15 rats of each sex in dosed and control groups on diet for 16 weeks.	No changes in behavioral patterns or clinical signs of toxicity were observed. Both sexes on 5.0 % feed and females on 1.0 % feed consumed less food and gained less weight than the controls. There was a pattern of reduction in absolute weight and an increase in relative weight of the brain, spleen, heart, kidneys, adrenal glands, gonads, and pituitary of rats on the 5.0 % diet. A pattern of increases in absolute and relative weights was observed in livers and various parts of the G.I. tract in these rats. Both liver and kidneys were enlarged but histologically normal.	Brown et al., 1978

Newly Available Data

Six rats were given repeated oral doses of 7500 mg/kg DBP (commercial grade). Two rats died within 20 to 40 hours after the second dosing. Two additional rats died within two hours after the fourth dosing. The two surviving rats received ten doses, but showed clinical signs of toxicity and weight loss. The surviving rats had completely recovered ten days after the last treatment (Phthalate Esters Panel, American Chemistry Council, 1982a).

Male and female Swiss CD-1 mice were exposed to DBP at concentrations of 0, 0.03, 0.3, or 1.0% in powdered feed for 14 days. Body weights were reduced ($p < 0.05$) in male mice in the 1.0% DBP exposure group. Liver weights were increased ($p < 0.05$) in male and female mice in the 1.0% DBP group. There were no effects on food or water consumption, mortality rate, or incidence of clinical signs due to DBP exposure (Lamb et al., 1997b).

Male Wistar rats were fed a powdered diet containing 0, 0.5, or 5% DBP or MBP for 34 to 36 days. Liver enzyme activities, serum chemistry, and histology were evaluated. Relative weights of liver, kidney, and spleen were increased in rats exposed to 5% DBP and 5% MBP. Succinate dehydrogenase and pyruvate dehydrogenase activities in liver mitochondria were reduced in rats exposed to 5% DBP or 0.5% MBP or 5% MBP. Glutamate dehydrogenase was not affected by DBP or MBP. Serum chemistry analysis

revealed increases in ALP, GOT, GPT, and CPK in rats exposed to 5% DBP. Serum globulin was reduced, and A/G was increased in both doses of DBP and MBP. Both doses of MBP decreased TG and uric acid levels. DBP and MBP at 5% produced increased liver necrosis (single cells and zonal) and hypospermatogenesis. Microscopic examination of hepatocytes revealed that the 5% dose of DBP and MBP caused increased peroxisomes, lysosomes, and mitochondria. Increased mitochondrial swelling and decreased liver glycogen were seen in 5% MBP-treated rats (Murakami et al., 1986b).

DERMAL

Previously Available Data

DBP and DMP were tested for long-term dermal toxicity by applying 0.5, 1.0, 2.0, and 4.0 ml/kg per day for 90 days to the clipped, intact skin of rabbits. The chemicals were applied to approximately 10 % of the body surface. The subchronic dermal LD_{50} of DBP to rabbits was greater than 4 ml/kg per day for 90 days. DBP was slightly irritating to skin and very irritating to rabbit penile mucosa. A slight dermatitis was observed, and in the 4 ml/kg dosed rabbits, slight renal damage (not further described) was observed (Lehman, 1955). The subchronic dermal LD_{50} of DMP to rabbits was also greater than 4 ml/kg per day for 90 days. No skin irritation or dermatitis was observed, although DMP was irritating to rabbit penile mucosa. Pulmonary edema and slight renal damage were observed in the rabbits that died during the study. Rabbit survivors had varying degrees of nephritis (not further described) at the two highest doses (Draize et al., 1948).

Newly Available Data

NTP (1995b) conducted a study of the dermal effects of DEP in rats and mice. F344/N rats were dermally exposed to 0, 46, 92, 184, or 369 µg neat DEP (n = 10 rats per sex per group), five days per week for four weeks. Animals were weighed and observed for signs of toxicity throughout the study. All animals survived to study termination, at which time all rats were killed for necropsy. There was no evidence of dermal toxicity and no adverse clinical signs. Food consumption and weight gain were unaffected by DEP treatment. The relative liver weights and relative kidney weights of males and females in the 369 µg group and of the females of the 184 µg group were greater than those of control animals. No other adverse effects of DEP were observed.

$B6C3F_1$ mice were dermally exposed to 0, 15, 31, 62, or 123 µg neat DEP (n = 10 mice per sex per

group), five days per week for four weeks. Animals were weighed and observed for signs of toxicity throughout the study. One control animal died prior to study termination, at which time all rats were killed for necropsy. There was no evidence of dermal toxicity and no adverse clinical signs. Food consumption and weight gain were unaffected by DEP treatment. The absolute and relative liver weights females in the 31 and 62 µg group were greater than those of control animals. No other adverse effects of DEP were observed (NTP, 1995b).

INHALATION

Previously Available Data

Male rats were exposed to 1.5 mg/m^3 of DBP vapor for 6 hours per day and 6 days per week for approximately 1 month. There were no significant effects on body or organ weights when the rats were compared to controls. No significant toxic effects were observed (Nishiyama et al., 1977). Rats were exposed to 0.5 mg/m^3 and 50 mg/m^3 of DBP mist for 6 hours per day for 6 months. Rats exposed to either concentration had smaller weight gains and greater brain and lung weights than control rats. The higher concentration had a greater effect than the lower concentration (Kawano, 1980).

Newly Available Data

BASF (1999) exposed Wistar rats to 0, 1, 5, or 500 mg/m^3 aerosolized DBP by inhalation for six hours per day, five days per week for four weeks (n = 5 rats per sex per group). Particle size of the aerosolized DBP ranged from 1.5 to 2.0 µM. Body weights, food consumption, and clinical observations were observed throughout the study. Behavioral toxicity was measured by a functional observational battery and motor activity after the four-week exposure period. Ophthamological examinations were performed prior to and after the study period. There were no significant treatment-related effects of the DBP inhalation exposure.

In what appears to be an early, unaudited report of this same study, BASF (no date) reported that rats were exposed to DBP by inhalation six hours per day, five days a week for four weeks a total of 20 6-hour exposures). Inhalation was managed by a head and nose exposure apparatus to which animals were acclimated with regular air prior to the DBP exposures. DBP was aerosolized to particles between 1.5 and 1.9 µM in size (a size highly respirable for rats) and delivered to the animals by positive pressure to the head and nose exposure apparatus. DBP exposure levels were 0 (air control), 1, 5, 50, and 500 mg/m^3 (n =

5/sex/dose level). Dose levels were confirmed by gas chromatography of air samples taken during the exposures. The health effects of DBP inhalation were monitored in terms of clinical observations, ophthamological examination, food and water consumption, body weight, functional observational battery, and motor activity. Blood and urine were collected for analysis. At the end of the study, the animals were killed for necropsy and histopathological examination. There were no treatment-related deviations from control animals in any of the parameters of toxicity investigated. The No Observed Effect Concentration for inhalation exposure to aerosolized DBP was the highest concentration tested in this study, 500 mg/m^3.

INTRAPERITONEAL

Previously Available Data

DEP was administered intraperitoneally in a dose of 2 ml/kg per day to rabbits for 8 days. "Temporary distress" was observed during and after administration. There was no paralysis or other abnormal effect. The intraperitoneal administration of 1.5 ml/kg per day of DEP to guinea pigs for 8 days did not result in any permanent ill effects during or after the experiment (Blickensdorfer et al., 1930). A DEP emulsion was administered intraperitoneally in a dose of 125 mg/kg per day for 6 weeks to 20 to 30 mice. There was slight retardation in weight gain and some evidence of peritonitis. The organ:body weight ratios for liver, heart, lungs, kidneys, spleen, and testes of treated mice were not different from the control mice ratios. No abnormal hematological patterns were observed (Calley et al., 1966).

A series of doses of DBP, DMP, and DEP was injected intraperitoneally into groups of 10 male mice 5 days a week. The apparent LD_{50} was calculated each week until it remained constant for 3 weeks; this was the chronic LD_{50}. DBP, DMP, and DEP reached chronic LD_{50}s in 25, 18, and 14 weeks, respectively. The chronic LD_{50} values were 0.85 ml/kg per day 5 days a week for DBP, 1.18 ml/kg per day 5 days a week for DMP, and 1.39 ml/kg per day 5 days a week for DEP (Lawrence et al., 1975).

SUBCHRONIC TOXICITY

DIBUTYL PHTHALATE

Previously Available Data

Long-term oral toxicity of DBP, DMP, and DEP was studied in rats (Bornmann et al., 1956; Brown et al., 1978; Daniel, 1978; Komarova, 1979; Krauskopf, 1973; Lehman, 1955; Maslenko, 1968; Nikonorow et al., 1973; Shibko & Blumenthal, 1973; Smith, 1953), mice (Komovara, 1979), and rabbits (Blickensdorfer & Templeton, 1930) (Table 7). Except at dietary concentrations of 1.25 % DBP for 1 year, 8.0 % DMP for 2

years, and 5.0 % DEP for 16 weeks, the phthalates were relatively nontoxic to rats in subchronic and chronic oral tests.

Newly Available Data

BASF (1992) reported results of a study in which Wistar rats were fed diet containing 0, 400, 2000, or 10,000 ppm DBP for three months (n = 10 rats/sex/dose level). Prior to, during, and after the dosing period each rat was evaluated for clinical chemistry and hematology, urinalysis, and behavioral performance. Animals were checked daily for general health. Body weights were measured weekly. Eye examinations were performed in the control and 10000 ppm groups prior to dosing and near the end of the study. At the end of the dosing period animals were subject to gross necropsy and histopathological examinations. Based on food consumption data, the DBP exposure levels for the 400, 2000, and 10,000 ppm treatments were 30, 152, and 752 mg/kg/day, respectively. No treatment-related effects were seen in the 400 and 2000 ppm groups. In the 10000 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 hematocytes of both sexes (BASF, 1992).

NTP (1995a) fed six-week-old F344/N rats powdered diet containing 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm DBP for 13 weeks. Throughout the dosing period animals were observed for clinical observations and weighed, and food consumption was monitored. After the last dose blood samples were collected for hematology and clinical chemistry and animals were killed for necropsy and histopathological examination of tissues. All animals survived to study completion. Male and female animals in the 40,000 ppm DBP group were emaciated at the end of the 13-week dosing period. No other clinical observations were reported. The lowest observed effect levels for the remarkable findings of this study are summarized in Table 9.

Table 9. Summary of observations with lowest observed effect level (LOEL) for a 13-week feed study of DBP using F344/N rats (NTP, 1995a).

Observations	LOEL in ppm (sex)
Reduced final BW & BW gain	10000 (m), 20000 (f)
Reduced food consumption	40000 (m,f)
Increased liver and kidney wts.	5000 (m), 10000 (f)
Reduced testes wts.	20000 (m)
Reduced hematocrit	20000 (m)
Reduced hemoglobin	5000 (m)
Increased platelets	5000 (m)
Reduced cholesterol	20000 (m, f)
Reduced triglycerides	2500 (m), 10000 (f)
Increased Palmitoyl-CoA oxidase	5000 (m, f)
Reduced serum testosterone	20000 (m)
Reduced zinc in testes, serum	20000 (m), 40000 (m)
Increased incidence cytoplasmic alteration in the liver	10000 (m, f)
Increased incidence of germinal epithelium atrophy	10000 (m)

Six-week-old B6C3F$_1$ mice were fed powdered diet containing 0, 1250, 2500, 5000, 10,000, or 20,000 ppm DBP for 13 weeks. Throughout the dosing period animals were observed for clinical observations and weighed. Food consumption was monitored. After the last dose blood samples were collected for hematology and clinical chemistry and animals were killed for necropsy and histopathological examination of tissues. All animals survived to study completion. There were no clinical signs attributed to the DBP exposures. The lowest observed effect levels for the remarkable findings of this study are summarized in Table 10. Many of the observations of the 13-week feed study in F344/N rats (previous study in this report) were not seen in the mice of this study which used essentially the same study design. For example, effects of DBP on hematology, clinical chemistry, and testicular effects were less apparent in mice. Most notably, serum testosterone levels increased rather than decreased in the DBP-exposed mice in this study. The average exposure of DBP (mg/kg/day) for corresponding dose levels over the 13-week period was much greater in the mice than in the rats. Species/strain differences in sensitivity may explain some of the different observations (NTP, 1995a).

Table 10. Summary of observations with lowest observed effect level (LOEL) for a 13-week feeding study of DBP using B6C3F$_1$ mice (NTP, 1995a).

Observations	LOEL in ppm (sex)
Reduced final BW & BW gain	5000 (m), 20000 (f)
Increased relative kidney weight	2500 (f)
Increased relative liver weight	5000 (m, f)
Reduced hematocrit	20000 (f)
Increased serum testosterone	1250 (m)
Increased zinc in testes	5000 (m)
Increased incidence cytoplasmic alteration in the liver	10000 (m), 20000 (f)

DIETHYL PHTHALATE

Newly Available Data

The subchronic toxicity of DEP in combination with ethanol (EtOH) was evaluated in young Sprague-Dawley Rats. Twenty-four rats were divided into four treatment groups of six to be exposed to DEP or EtOH in the drinking water for 120 days. The groups were Control, 5%(v/v) EtOH, 50 ppm (w/v) DEP, and 5%(v/v) EtOH + 50 ppm (w/v) DEP. Animals were weighed weekly, and food and water consumption rates were monitored throughout the study. After the treatments ended all rats were killed and blood was collected for analysis of serum enzyme activities. The livers were examined, weighed, and then homogenized for enzyme and protein analysis. The serum and liver biochemical parameters measured were aspartate aminotransferase (AST), alanine aminotransferase (ALT), succinate dehydrogenase (SDH), acid phosphatase (ACP), alkaline phosphatase (ALP), liver glycogen, triglycerides (TG), cholesterol, and lipid peroxidation (LP). No significant changes were seen in the body weights, liver weights, daily water consumption, and serum glucose levels of any treatment groups. Table 10 shows the significant increases (↑) and decreases (↓) of measured serum and liver parameters, compared to controls. (• = no difference; n/a = not applicable). Generally, DEP caused changes in enzymes and biochemical endpoints in the liver and serum that were not affected by the presence of EtOH, thus there was no interaction between EtOH and DEP at these doses, but DEP alone appears to cause toxic injury to the liver, under these treatment conditions (Sonde et al., 2000).

Table 11. Effects of DEP and EtOH on serum and liver enzymes in rats (Sonde et al., 2000).

Treatment	Tissue	AST	ALT	SDH	ACP	ALP	liver glycogen	TG	cholesterol	LP
50 ppm DEP	liver	↓	↓	↑	↑	•	↑	↑	↑	↑
	serum	↑	↑	•	↑	↑	no data	↓	↑	no data
50 ppm DEP + 5% EtOH	liver	↓	↓	↑	↑	•	↑	↑	↑	↑
	serum	↑	↑	•	↑	↑	no data	↓	↑	no data
5% EtOH	liver	•	•	•	•	•	•	↑	•	•
	serum	•	•	↑	↓	•	•	↓	•	no data

CHRONIC TOXICITY

Chronic (2-year) toxicity studies on DBP, DMP, and DEP are described in the Carcinogenicity section of this report.

OCULAR IRRITATION

Previously Available Data

The eye irritation potential of DBP, DMP and DEP was studied in rabbits (Carpenter & Smith, 1946; Draize et al., 1944; Lawrence et al., 1975). The eye irritation potential of nail preparations containing 9 % DBP and 6 % DBP also was investigated (Biodynamics, 1976b; CTFA, 1977a). These studies are summarized in Table 12. Overall, DBP, DEP, and nail preparations containing DBP were relatively nonirritating to the rabbit eye. With long contact time, undiluted DMP may be injurious to the eyes of rabbits.

Newly Available Data

A test material containing 12.5 % DEP was tested for ocular irritation in rabbits using the Draize method. Three healthy albino rabbits each were given on instillation of 0.1 ml of the test material in the right eye. The left eye was left untreated and used as a control for comparison. Twenty-four hours later and again on the seventh day, both eyes of each rabbit were scored according to the Draize scale of ocular lesions. Instillation of the test material did not produce corneal opacity or iris congestion. However, a severe conjunctival irritation did occur, including chemosis and discharge (Leberco Laboratories, 1963).

Table 12. Rabbit Eye Irritation

Material Tested	Method	Results	Reference
DBP	Undiluted DBP instilled into eyes. Eyes examined at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 24.0, and 48.0 hours post-instillation.	No grossly observable irritation at any examination time.	Lawrence et al., 1975
DMP	0.5 ml undiluted DMP applied to corneal center while eyelids are retracted. Lids released after 1 minute. Eye injury scored on a scale of 0 to 20 points after 18 to 24 hours.	Injury score was > 0.1 and < 0.5. 5.0 is the level representative of severe injury; necrosis visible after staining and covering ~75 % of the surface of the eye.	Carpenter & Smith, 1946.
DMP	0.1 ml undiluted DMP instilled into the conjunctival sac of the eyes. Injury scored on a scale of 0 to 110 points after 1 and 24 hours.	Score was 3.3 after 1 hour and 2.2 after 24 hours.	Draize et al., 1944
DMP	Undiluted DMP instilled into eyes. Eyes examined at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 24.0, and 48.0 hours post-instillation.	No grossly observable irritation at any examination time.	Lawrence et al., 1975
DEP	0.1 ml undiluted DEP instilled into the conjunctival sac of the eyes. Injury scored on a scale of 0 to 110 points after 1 and 24 hours.	Score was 3.2 after 1 hour and 1.5 after 24 hours.	Draize et al., 1944
DEP	Undiluted DEP instilled into eyes. Eyes examined at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 24.0, and 48.0 hours post-instillation.	No grossly observable irritation at any examination time.	Lawrence et al., 1975
Nail polish, 9 % DBP	0.1 ml instilled into the conjunctival sac of one eye of 9 rabbits. Lids held together for 1 sec. In 3 rabbits, treated eye treated eye washed at 30 sec. with 20 ml water. Score on a scale of 0 to 110 at 24, 48, and 72 hours and 4 and 7 days post-instillation.	Unwashed eyes' average score were 11.3, 9.7, 6.8, 4.8, and 0.5 and washed eyes' average scores were 8.3, 7.7, 4.0, 2.7, and 0.3 at 24, 48, and 72 hours and 4 and 7 days post-instillation, respectively.	Biodynamics, 1976b
Nail preparation, 6 % DBP	0.1 ml instilled into conjunctival sac of one eye of 6 rabbits. Lids held together for 1 sec. Ocular reactions recorded at 24, 48, and 72 hours.	No positives for conjunctival redness or chemosis, keratitis, or iritis. "Nonirritating."	CTFA, 1977a

DERMAL IRRITATION & SENSITIZATION

Previously Available Data

Draize et al. (1948) reported a study in which the acute dermal toxicity of DMP to rabbits was determined by placing DMP in contact with the clipped skin and holding it in place with a rubber cuff. The rabbits were exposed for 24 hours and then observed for 2 weeks. The acute dermal LD_{50} of DMP to rabbits was greater than 10 ml/kg.

Lehman (1955) applied DBP and DMP to the clipped, intact, and abraded skin of 3 rabbits. The rabbits were exposed to 0.5 ml of the chemicals for 24 hours with an occluded patch. DBP caused "very

slight irritation." DMP was not irritating except in molting areas and the Primary Irritation Index (PII) was 0.7. This author also reported no evidence of sensitization in rabbits receiving daily topical applications of DBP and DMP at doses of up to 4.0 ml/kg per day for 90 days (Lehman, 1955).

Guess and Haberman (1968) measured the intradermal irritation of phthalates to rabbits by injecting the phthalates into the skin of the shaven backs. A trypan blue solution was injected into the marginal ear vein, and the extravasated trypan blue at the injection site was used as a measure of the extent of the inflammatory response. In one study, 0.2 ml of 100 mg/ml phthalate emulsions was injected. DBP gave a mild inflammatory response after 10 minutes and a moderate response after 26 minutes. A rapid and marked inflammatory response to DMP and DEP was noted (Calley et al., 1966). Other researchers used cottonseed oil as a diluent for DBP, DMP, and DEP. DBP was not irritating, but DMP and DEP produced a significant degree of irritation (Lawrence et al., 1975). In another study, saline solutions saturated with the phthalates were administered. No response was observed to DMP and DEP.

Srivastava et al. (1978) studied the effect of DMP on primary irritation in rabbits induced by formalin using a pill box device. Pill boxes were affixed to shaved rabbit skin, and 0.1 ml of a 20 % solution of formalin ("as the primary irritant") was painted onto the skin and allowed to dry. Discs containing 0.2 ml of DMP were placed in the pill box and the box was closed. A 0.25 ml volume of a 0.5 % sterile Evans blue solution was injected intravenously. After 18 hours, the blue color at the pill box sites was evaluated and correlated with irritancy. Ten to 15 separate observations were made. DMP had an irritation score of 0.8 on a scale of 0 to 3; DMP was less than slightly irritating.

Newly Available Data

The E. I. du Pont de Nemours and Company (1946) stated that DMP as an insect repellant ingredient was found to be negative for tests of dermal irritation and sensitization using an unspecified "standard technique for making such tests." The company later reported a study in which one drop (0.05 ml) of DMP was applied to the intact skin of nine male albino guinea pigs. All animals were negative for primary irritation. Ten different guinea pigs were given nine 0.05 ml treatments on abraded skin over a three-week period. After a two-week rest period, animals were challenged with DMP applications on intact and abraded skin. There were no signs of edema or erythema at any time during the test (E. I. du Pont de Nemours and Company, 1970).

van Beek (1974) reported that undiluted DEP was found to cause slight to moderate skin irritation in

a primary irritation test using 12 albino rabbits. DEP (0.5 ml) was applied to intact or abraded skin and occluded under a gauze patch for 24 hours. Sites were scored for signs of primary irritation at 24 and 72 hours after application. In both abraded and intact skin, undiluted DEP induced scaliness or very slight incrustation.

Consumer Product testing (1978c) exposed twelve male guinea pigs to 0.5 ml of a 50 % DEP solution on a 3" x 3" area of shaved dorsal skin, occluded for six hours, nine times over a 21 day period. Two challenge applications (one on the induction site, one on an untreated site) were performed after a 14-day rest period. Challenge sites were scored for signs of sensitization 24 hours later. The 50 % DEP solution did not induce any signs of local or systemic sensitization.

The Phthalate Esters Panel, American Chemistry Council (1982a) reported a study in which commercial grade DBP was applied to a shaven area between the shoulders of two female albino rabbits. One rabbit received a dermal dose of 8 g/kg, and the other 16 g/kg. Both rabbits showed a loss of appetite and were inactive for a day after treatment. At the dosing site there was moderate inflamation which subsided after two days. There were no significant pathological signs at necropsy 18 to 22 days after treatment.

This group also reported a study in which an experimental shampoo formulation containing 1 % DEP was topically applied to the shaved backs of three male and three female New Zealand rabbits. The dose was 2 ml of the shampoo per kg body weight applied to an area approximately 10 % of the total surface area of the animal. The shampoo was described as containing 1 % DEP, but the other ingredients were not disclosed. A control group received 2 ml/kg water. Doses were applied daily for 20 days over a period of 28 days. Treatments were not washed off between dosing applications. The only dose-related effects observed was moderate primary irritation and fissuring of the test-site after the first treatment. Reducing the concentration of the treatment to 0.05 ml shampoo per kg body weight allowed the skin to return to normal by the conclusion of the study. No signs of systemic toxicity were observed (Phthalate Esters Panel, American Chemistry Council, 1982b).

Proctor & Gamble Co. (1984) reported a study in which pregnant New Zealand White rabbits received a daily application of 2 ml/kg 0, 5, 15, or 50 % DEP on a 10 x 10 cm shaved area of dorso-lumbar skin on gestation days 6 through 18 (n = 12/dose level). The vehicle was 5 % carboxymethylcellulose. Animals were restrained to prevent oral ingestion of the test material. The treatment application remained on

the skin uncovered for seven hours before being washed off with tepid water. The dosing sites were periodically graded according to the Draize skin reaction scoring system. Half of the animals in the 15 % DEP group showed slight erythema and individuals showed slight edema and slight atonia in the treated skin area after six treatment days and only slight desquamation. The maternal and fetal toxicity data of this study is presented in the Reproductive and Developmental Effects section later in this report.

RIFM (1985) reported a study in which 0.5 ml DEP was applied to the shaved skin of the flank of four healthy female New Zealand White rabbits. The DEP was left on the test site semi-occluded for 4 hours, at which time the exposure site was cleaned and scored for signs of irritation immediately. Additional scoring was done at 24, 48, 72, and 168 hours after the initial application. Very slight edema was observed in one animal at 48 hours. Otherwise, all other observations showed no signs of edema or erythema. A related previous study using the same methodology in three rabbits resulted in an observation of very slight erythema in two rabbits at four hours, but no other reactions were observed (RIFM, 1984).

Inveresk Research International (1990) used DEP as a vehicle control in a 2-week dose range finding study for four phenyl ether compounds. Ten male and 10 female rats received dermal applications of 2 ml/kg/day DEP on clipped dorsal skin. The treatment site was semi-occluded for six hours per day for 14 days. The treatment site was cleaned and scored for signs of irritation before and after each exposure period. The DEP exposure sites showed erythema and slight desquamation, and histological examination revealed very mile epidermal thickening and slight hyperkeratosis.

Bagley et al. (1996) used rabbit skin to test the skin irritation potential of 176 compounds, one of which was DEP. Two 0.5 ml samples of 99.7% DEP from different sources were each tested on three rabbits. The DEP was applied to the flank of each rabbit under semi-occlusive patches. At 24, 48, and 72 hours after application, the dose sites were evaluated for erythema/eschar formation and edema on scales of 0 to 4, with 0 being no observed effect and 4 being a severe effect. The data were reported as Primary Irritation Index (PII), in which PII = ((∑erythema grades for all animal at 24, 48, and 72 hours) + (∑edema grades for all animal at 24, 48, and 72 hours))÷(3 x number of animals). Essentially the PII is the mean of all scores recorded for a sample. The PII for a compound can be used to compare the irritant potentials of multiple compounds. For the two samples of DEP the PII scores were 0.0 and 0.17, meaning DEP was not an irritant.

REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

Previously Available Data

Singh et al. (1972) administered DBP in doses of 2 and 4 ml/kg, DMP in doses of 0.5, 1, and 2 ml/kg, and saline in a dose of 4 ml/kg intraperitoneally on Days 3, 6, and 9 of gestation to groups of 5 pregnant female rats. Day 1 of gestation was the day sperm were found in vaginal smears. Five control rats survived, and four of those implanted. Five and four rats survived, and four and three implanted, respectively, in the 2 and 4 ml/kg DBP groups. DBP administration resulted in a 50 % reduction in the number of pups weaned per litter. Two male pups, one from each of two litters in the 2 ml/kg DBP group, had no eyes. In the 0.5, 1, and 2 ml/kg DMP groups, 5, 2, and 5 rats survived, and 4, 1, and 5 implanted, respectively. The numbers of pups weaned were not significantly different from the controls (Peters & Cook, 1973). In another study in which Day 1 of gestation was the day after sperm were found in vaginal smears, groups of 5 pregnant female rats were administered DBP, DMP, and DEP intraperitoneally, in doses of l/3, l/5, and l/10 of a previously determined acute intraperitoneal LD_{50} (3.05 ml/kg for DBP, 3.4 ml/kg for DMP, and 5.06 ml/kg for DEP), on Days 5, 10, and 15 of gestation (Table 13). Control rats were untreated or were administered distilled water, normal saline, or cottonseed oil. The rats were killed on Day 20, 1 day before expected parturition. Phthalate administration did not interfere with fertility, as reflected by corpora lutea:implantation site ratios. However, there were significant effects on embryonic and/or fetal development. The average weights of the fetuses from the treated groups and those administered saline were significantly lower than the average weight of the fetuses from the untreated controls. The investigator normally selected 30 to 50 % of the fetuses for visualization of skeletal abnormalities. There was a significantly higher number of skeletal abnormalities in the fetuses from the test group as compared to the controls. 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.

Table 13. Embryotoxicity and Teratogenic Effects of Phthalates (Singh et al., 1972)

Treatment Groups	Volume injected[a] (ml/kg)	Number of Corpora Lutea	Number of Resorptions[b]	Number of Dead Fetuses[b]	Number of Live Fetuses[b]	Number of Gross Abnormal-ities[c]	Number of Skeletal Abnormalities[d]
Untreated Controls	None	60	0	0	59 (100)	0	0
Distilled Water	10	59	4 (6.8)	0	55 (93.2)	0	0
Normal Saline	10	62	7 (11.5)	0	54 (88.5)	1 (1.9)	4 (14.3)
Cottonseed Oil	10.00	5954	4 (6.8)	0	55 (93.2)	1 (1.8)	3 (10.7)
	5.00		3 (6.4)		44 (93.6)	0	0
DBP	1.017	64	23 (36.5)	0	40 (63.5)	0	8 (33.3)
	0.610	56	2 (3.6)		53 (96.4)		7 (24.1)
	0.305	56	4 (7.3)		51 (92.7)		6 (20.7)
DMP	0.125	55	17 (32.1)	5 (9.4)	31 (58.5)	4 (11.1)	9 (75.0)
	0.675	55	0	1 (1.9)	52 (98.1)	4 (7.5)	6 (35.3)
	0.338	65	21 (33.3)	0	42 (66.7)	4 (9.5)	4 (25.0)
DEP	1.686	57	2 (3.6)	0	54 (96.4)	0	13 (81.3)
	1.012	59	0		57 (100.0)		8 (47.1)
	0.506	65	28 (44.4)		35 (55.6)		5 (26.3)

[a] 5 pregnant female rats injected i.p. on days 5, 10, and 15 of gestation and killed on day 20.
[b] Numbers in parentheses are percent values based on total number of implantations.
[c] Numbers in parentheses are percent values based on total number of viable and nonviable fetuses.
[d] Numbers in parentheses are percent values based on total number of stained fetuses (Generally 30 -50 % of the fetuses were stained.

Nikonorow et al. (1973) administered DBP in the feed to pregnant mice throughout gestation, and the mice were killed on Day 18. Day 0 of gestation was the day on which a vaginal plug was found. DBP was administered in five dietary concentrations from 80 to 2100 mg/kg. Implantation was not affected, but resorptions and fetal deaths increased with dosage. Maternal weight gain was depressed at the higher dosages and was due to increased embryonic or fetal death. Two of three live fetuses from the 2100 mg/kg DBP group had neural tube defects. Ossification was depressed, but malformation and resorption rates and fetal weights were not significantly affected by DBP administration up to 350 mg/kg/day (Shiota & Nishimura, 1982). In another study, 120 and 600 mg/day of DBP in olive oil were administered by gavage to groups of 10 female rats for approximately 3 months prior to their being mated. Additional groups of female rats received the same doses for 21 days following fertilization. The uteri and fetuses from all the rats were removed on Day 21 of gestation. Fetuses from treated and control rats did not differ significantly in number of sternum ossification foci, in development of the bones of the base of the skull or in the paws of the front and hind extremities, and in rib fusion. The administration of DBP before gestation did not cause any significant changes in other measured parameters. Administration of DBP to pregnant rats did result in lower placental weights, and fetal weights were significantly lower in the high DBP dose group. There were 4, 2, and 22 resorptions in the control, 120, and 600 mg/day DBP groups, respectively.

The dietary administration of DBP, in doses of 10 and 100 mg/kg per day, to two mouse strains for three generations increased the formation of renal cysts in the F_1 and F_2 generations (Omori, 1976; Onda et al., 1974). In another three-generation reproduction study, female rats were dosed daily for 6 weeks with 50 % DBP solution in oil, at a dose of 1 ml/kg, and then were paired with untreated males. The offspring were bred to produce two additional generations; it is not known whether the second and third generations were dosed with DBP. No impairment of reproductive performance was noted. Development, growth, and fertility were normal for all three generations (Bornmann et al., 1956; Daniel, 1978; Shibko & Blumenthal, 1973).

DIBUTYL PHTHALATE

CONTINUOUS BREEDING PROTOCOL

Newly Available Data

Lamb et al. (1987) examined the reproductive toxicity of DBP in mice in a continuous breeding protocol. Male-female pairs of six-week-old COBS Crl:CD-1, (IC)BR mice were randomly assigned to a control group (n = 40 per sex) or one of three DBP dosing groups (n = 20 per sex per group). Mice were exposed to 0, 0.03, 0.3, or 1.0% DBP in powdered feed for a 7-day premating period and then assigned to mating pairs within the dosing groups where they cohabitated for 98 consecutive days. During this period newborn pups were collected within 12 hours 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 98^{th} day of cohabitation mating pairs were separated for 21 days while DBP exposure in the diet continued. During this 21-day period, any remaining litters were delivered and kept for at least 21 days. 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% DBP treatment, compared to control. The lower DBP doses did not affect these parameters. Live pup weight was not different from control in any DBP treatment groups.

At the end of the continuous breeding phase, mice in the high dose (1% DBP) group were re-paired for crossover mating, to create three groups of pairs: control male x control female; control male x 1% DBP female; or 1% DBP male x control female. The offspring of the crossover pairs were analyzed, and the parents were necropsied. Pups that were born to control female x 1% DBP 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 DBP females were mated with control males. Organ weights and histological results were recorded

for all mated control and 1% DBP-exposed mice. In male mice of the 1% DBP 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% DBP 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% DBP, compared to controls (Lamb et al., 1987).

A similar continuous breeding protocol for DBP was performed in rats (NTP, 1991 and Wine et al., 1997). Male-female pairs of VAF Crl:CD BR outbred Sprague-Dawley albino rats were assigned to a control group (n = 40/sex) or to one of three DBP dosing groups (n = 20/sex/group). Breeding pairs were exposed to DBP in powdered feed at dry concentrations of 0, 0.1, 0.5, or 1.0 %. Body weights and clinical signs of toxicity in the mating pairs (F_0) were monitored. The pups from the first four litters from each mating pair were counted, weighed, sexed, killed and discarded without necropsy. The number of litters per pair was not different from control in any of the DBP treatment groups. The number of live pups per litter and pup weights was significantly reduced ($p < 0.05$) in all DBP treatments, compared to control. Live pup weights were significantly reduced ($p < 0.05$) in the 0.5 and 1.0 % DBP groups, compared to control. The fifth litter was kept alive to become the next generation (F_1) of breeding pairs. The F_0 rats were then re-paired for crossover mating between control and 1 % DBP rats. Crossover pairs were as follows: control male x control female; 1 % DBP male x control female; and control male x 1 % DBP female (n = 20 pairs/crossover group). The pups from the crossover pairs were counted, weighed, sexed, killed and discarded without necropsy. The pregnancy rate, fertility, 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 % DBP female group, compared to those in the control male x control female group.

After crossover mating was completed, the estrous cycles of the F_0 females were examined by vaginal lavage for 12 days, and all F_0 rats were killed and necropsied. Endpoints measured were body weights, organ weights, and histopathology. Additionally, sperm motility, sperm count, and sperm morphology were measured for male F_0 rats. Body weight, liver weight, and kidney weight were reduced ($p < 0.05$) in 1 % DBP female F_0 rats, compared to controls. In 1 % DBP 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 DBP treatment in F_0 males at the doses tested.

The F_1 rats were treated with the same concentrations of DBP as their parents. At sexual maturity,

F_1 rats were assigned to mating pairs with non-sibling rats within the same treatment group. The pups from the F_1 mating pairs were counted, weighed, sexed, killed and discarded without necropsy. The F_1 rats were then killed and subjected to the same endpoint measurements as the F_0 rats. 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 % DBP F_1 mating pairs. Live pup weights were reduced ($p < 0.05$) in all DBP concentrations tested (down to 0.1%), compared to controls. Necropsy of F_1 rats revealed reduced ($p < 0.05$) body weights for both males and females in the 1 % DBP treatment. In the 1% DBP 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 DBP treatments, the total spermatid heads per testis and per g testis were reduced ($p < 0.05$) in the 1% DBP treatment, compared to control.

FEEDING/ORAL GAVAGE STUDIES

Newly Available Data

Cummings and Gray (1987) investigated the influence of maternal effects of DBP on fetotoxicity. Daily vaginal smears of female Holtzman rats were evaluated to ensure regular estrous cycles. Rats that showed two consecutive 4- or 5-day cycles were included in the study. Some females were paired with males for regular breeding. Day 0 of pregnancy was defined by the presence of sperm in vaginal smears. Other females were subjected to artificial stimulation of the cervix by a glass rod during proestrus or estrus in order to induce a pseudopregnancy. Day 0 of pseudopregnancy was defined as the last day of vaginal cornification. Deciduomata were induced surgically to simulate implantation of a blastocyst in pseudopregnant rats. DBP was diluted in sesame oil to dosing concentrations. All rats were dosed by oral gavage on day 1 through day 8. Pregnant rats received DBP doses of 0, 250, 500, 1000, or 2000 mg/kg/day (n = 6 per treatment). Pseudopregnant rats received DBP doses of 0, 500, 1000, or 2000 mg/kg/day (n = 6 to 8 per treatment). On day 9 rats were killed, and blood was collected. Uteri and ovaries were dissected and cleaned of adjacent tissues and weighed. DBP at doses up to 2000 mg/kg/day did not significantly affect whole body weights, uterine weights, ovarian weights, or serum progesterone levels in the pregnant or pseudopregnant rats. In pregnant rats, there was no effect of DBP dosing on the number of implantations. The authors concluded that any fetotoxic effects of short-term DBP exposure are due to DBP 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 DBP.

The teratogenicity of DBP in rats was evaluated by Ema et al. (1993). Pregnant Wistar rats were dosed with DBP (0, 0.5, 0.63, 0.75, or 1.0 g/kg/day; n = 11 or 12 rats per dose group) by oral gavage on days 7 through 15 of gestation. The vehicle was olive oil, and the control group received only olive oil on gestation days 7 through 15. The pregnant rats were killed on gestation day 20 and fetuses were removed and examined. Maternal body weights and food consumption were the same in all groups before DBP dosing began. 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 food 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 DBP. The number resorptions, dead fetuses, and postimplantation 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. The body weights of live fetuses were reduced ($p < 0.05$) in the 0.63 and 0.75 g/kg groups. The reduced live litter size and fetus weights possibly account for the reduced maternal body weights seen in late gestation in the higher dose groups. Table 14 summarizes the findings of morphological examinations of fetuses (Ema et al., 1993).

In a related study, Ema et al. (1994) divided the dosing schedule to expose pregnant rats to DBP by daily oral gavage either on gestation days 7 to 9, 10 to 12, or 13 to 15. DBP doses were 0, 0.75, 1.0, or 1.5 g/kg (n = 10 or 11 rats per exposure condition). As in the above study, dams were killed on gestation day 20, and fetuses were examined. 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 Ema et al., 1993 (Table 14), however there was a higher incidence of skeletal malformations in the vertebral column and ribs. Of the three 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 (Ema et al., 1994).

Table 14. Malformations in fetuses of rats given DBP daily by oral gavage on gestation days 7-15 (Ema et al., 1993)

Malformation	DBP Dose (g/kg)			
	0	0.5	0.63	0.75
External malformations				
No. fetuses (litters) examined	118(11)	92 (9)	73(10)	10(2)
No. fetuses (litters) with malformations	0	0	7(3)	6(2)*
No. Fetuses (litters) with Cleft palate	0	0	7(3)	6(2)
Skeletal malformations				
No. fetuses (litters) examined	87(11)	61(9)	48(10)	7(2)
No. fetuses (litters) with malformations	1(1)	1(1)	7(4)	1(1)
No. Fetuses (litters) with:				
Fusion of cervical vertebral arches	1(1)	1(1)	2(2)	1(1)
Absence of cervical vertebral arches	0	0	1(1)	0
Fusion of thoracic vertebral bodies	0	0	1(1)	0
Fusion of sternebrae	0	0	4(1)	0
Internal malformations				
No. fetuses (litters) examined	40(11)	31(9)	25(10)	3(2)
No. fetuses (litters) with malformations	0	2(2)	2(2)	1(1)
No. Fetuses (litters) with:				
Dilation of renal pelvis	0	2(2)	1(1)	1(1)
Undescended testis	0	0	1(1)	1(1)

* Significantly different from control value ($p < 0.05$).

In a very similar study, pregnant rats were dosed by daily oral gavage with 0, 0.75, 1.0, or 1.25 g/kg DBP on gestation days 7 to 9, 10 to 12, or 13 to15 (n = 11 or 13 rats per exposure condition). Dams were killed for fetal examinations on gestation day 20. There was a significant increase in postimplantation loss at all DBP doses in animals dose in all three 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 DBP were observed in animals dosed on days 10 to 12. Increased incidence of cleft palate and fused sternebrae were seen at all DBP doses tested in animals exposed on days 13 to 15. As seen in Ema et al. (1994) above, fetuses were more vulnerable to DBP-induced malformations on days 7 to 9 and on days 13 to 15 than on days 10 to 12 (Ema et al., 1995a).

Ema et al. (1997b) conducted a study in which DBP (2 %) was mixed into powdered feed given ad libitum to pregnant rats from gestation days 0 through 20 (n = 10 rats per group). Dams were killed, necropsied, and fetuses were examined on gestation day 20. Some rats were killed for examination on gestation days 7, 9, or 11. Body weight gain and food consumption were reduced in dams exposed to 2 %

DBP. Number of implantations and number of corpora lutea were not affected by DBP, but postimplantation loss and number of resorptions per litter were increased, and number of live fetuses was reduced in the DBP exposure group. There were no live male fetuses in the DBP group. Due to high fetal death, malformations were not observed. Ovarian weight, uterine weight, and serum progesterone were reduced in the DBP treated dams on gestation day 11.

In further work by these authors (Ema et al., 1998), DBP mixed into powdered feed at concentrations of 0, 0.5, 1.0, or 2.0 % was given ad libitum to pregnant rats on gestation days 11 to 21 (n = 10 or 15 rats per dose group). Dams were killed for fetal and maternal examinations on day 21. Body weight gain and food consumption were reduced in dams exposed to 1 and 2% DBP. There was no effect of DBP on fetal survival, number of resorptions, or sex ratio of live fetuses. Fetal body weights in both sexes were reduced in the 2% DBP group. Anogenital distance was reduced in male fetuses in the 1% and 2% DBP groups. Cleft palate and fused sternebrae were observed in the 2%DBP group. Undescended testes was seen in the 1% and 2% DBP groups.

NTP (1995a) reported the results of a study in which pregnant F344/N rats were exposed to 0 or 10000 ppm DBP mixed in powdered feed from the beginning of gestation through lactation. As the pups began to eat solid food, they were exposed to the same feed conditions as their mothers until they were eight weeks old at which time perinatal DBP exposure stopped. The number of live pups born was not different between the control and 10000 ppm DBP perinatal groups. However, by the first day after parturition the survivability of DBP-exposed pups was significantly lower than that of control pups and remained so through weaning. Pup body weights were also lower in the 10000 ppm DBP perinatal group from the time of birth through weaning. At postnatal week 13 selected rats that had been perinatally exposed to 10000 ppm DBP were divided into dosing groups to receive 0, 2500, 5000, 10000, 20000, or 40000 ppm DBP mixed in powdered feed for 13 weeks (n = 10/sex/dose). An additional control group did not receive any DBP in feed at any time (n = 10/sex). Animals were monitored for clinical signs, body weights, and food consumption throughout the study. At the end of the 13-week dosing period, animals were killed for necropsy and histopathological examinations. Special analyses included serum and testis zinc and testosterone concentrations, sperm morphology and vaginal cytology, and liver peroxisome and enlargement analysis. All rats survived the 13-week dosing portion of the study. The mean body weights of control animals who had not been exposed to DBP perinatally were higher than all animals that had been

dosed perinatally. Female rats that had been exposed to DBP perinatally but not during the 13-week 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 20000 or 40000 ppm DBP as adults remained less than those unexposed controls at the end of the study. Males in the 40000 ppm DBP group lost weight during the study. All male and female animals in the 40000 ppm DBP 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 to DBP perinatally but not during the 13-week dosing period had lower testicular weights than unexposed control males. No other organ weights differed between these two groups. Table 15 lists findings in this study with the lowest observable effect levels for each observation. The observations noted are in comparison to the control animals that had been exposed to DBP perinatally. All other organ weight differences were considered a function of body weight differences. 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 affects on the estrous cycles of the females in this NTP study.

Table 15. Summary of observations with lowest observed effect level (LOEL) for a 13-week feed study of DBP using F344/N rats that had been perinatally exposed to DBP (NTP, 1995a).

Observations	LOEL in ppm (sex)
Reduced final BW & BW gain	2500 (m), 20000 (f)
Reduced food consumption	40000 (m)
Increased relative liver weight	10000 (m, f)
Increased relative kidney weight	10000 (m), 2500 (f)
Reduced relative testes weight	20000 (m)
Reduced hematocrit	10000 (m), 40000 (f)
Reduced hemoglobin	10000 (m), 40000 (f)
Increased platelets	10000 (m)
Reduced cholesterol	10000 (m, f)
Reduced triglycerides	10000 (m), 20000 (f)
Increased Palmitoyl-CoA oxidase	5000 (m), 10000 (f)
Reduced serum testosterone	10000
Reduced zinc in testes, serum	40000, 20000
Increased incidence cytoplasmic alteration in the liver	10000 (m, f)
Increased incidence of germinal epithelium atrophy	10000 (m)
Reduced epididymal weight	2500 (m)
Hypospermia of the epididymis	20000 (m)

Ema et al. (1997a) conducted a study in which pregnant rats were given a single oral dose of 0 or 1500 mg/kg DBP on one day between gestation day 6 through 16 (n = 10 to 12 rats per exposure condition). Dams were killed, and fetal examinations were conducted on gestation day 20. There was a significant increase in postimplantation 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.

Ema et al. (2000a) administered DBP at daily doses of 0, 1000, or 1500 mg/kg by oral gavage to pregnant rats on gestation days 12 to 14, 15 to 17, or 18 to 20 (n = 10 to 13 rats per exposure condition). Dams were killed for fetal and maternal examinations on day 21. Body weight gain and food consumption were reduced in dams exposed to 1000 or 1500 mg/kg DBP in all dosing schedules. Litters exposed to 1500 mg/kg DBP 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 postimplantation 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 to DBP in all treatment schedules.

Ema et al. (2000b) administered DBP by oral gavage to pregnant or pseudopregnant rats on day 0 through day 8. DBP doses were 0, 250, 500, 750, 1000, 1250, and 1500 mg/kg (n = 10 to 13 rats per exposure condition). Pregnant rats were killed on day 20 for maternal necropsy and fetal examinations. Pseudopregnant rats were killed and necropsied on day 9. Body weight gain and food consumption were reduced in dams exposed 500 mg/kg and higher doses of DBP. Increases in preimplantation loss were seen in doses of 1250 and 1500 mg/kg DBP. The number of resorptions and dead fetuses per litter was 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 DBP, but both male and female fetal weights were reduced at all DBP doses tested. Body weight gain and food

consumption were reduced in pseudopregnant rats exposed 750 mg/kg and higher doses of DBP. In the pseudopregnant rats ovarian weight, number of corpora lutea, and serum estradiol were not affected by DBP, but uterine weight was reduced at doses of 750 mg/kg and higher. Serum progesterone was reduced in pseudopregnant rats only at the 1500 mg/kg dose.

DIBUTYL AND MONOBUTYL PHTHALATE

FEEDING/ORAL GAVAGE STUDIES

Newly Available Data

Saillenfait et al. (2001) reported a study in which pregnant Sprague-Dawley rats were given a single oral administration of 0, 1.8, 3.6, 5.4, or 7.2 mmol/kg of DBP or MBP on day 10 of gestation (n = 11 to 15 rats per exposure condition). The vehicles for DBP and MBP were olive oil and ammonium chloride, respectively. On day 12 (48 hours after dosing) the pregnant rats were killed and necropsied, and the uteri were removed. Implantation sites were counted, and viable embryos (those with a beating heart) were examined microscopically for growth and morphological development. Eleven to 15 litters were examined per dose group. Embryos exposed to DBP 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 four-fold. The observations in the higher groups 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 DBP treatment. The embryos exposed to MBP 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 MBP doses. In additional experiments, rats treated with 0, 5.4, or 7.2 mmol/kg MBP on Day 10 were killed on Day 12, 13, 14, or 18 (n = 6 to 12 rats per exposure condition), and the rate of non-viability of fetuses on each day was determined. On Day 12, the survivability of embryos was similar across the groups. On Days 13 and 14 there was an increase in the rate of non-viable fetuses (no heartbeat). On Day 18 surviving MBP-exposed fetuses exhibited eye anomalies, absent digits, misshapen lower jaws, enlargement of the pericardium and/or cardiac tube, and brain anomalies.

MONOBUTYL PHTHALATE

FEEDING/ORAL GAVAGE STUDIES

Newly Available Data

Ema et al. (1995b) dosed pregnant Wistar rats daily with 0, 250, 500, or 650 mg/kg MBP by gastric intubation on gestation days 7 to 15, the period of fetal organogenesis (n = 11 to 15 rats per dose group). The vehicle used was ammonium chloride. Maternal body weights and food consumption were recorded daily. On gestation day 20 all dams were killed. Fetuses were removed by cesarian section and examined for developmental markers. Maternal body weight gain and food consumption rates were reduced in the 500 and 650 mg/kg doses ($p < 0.01$). Fetal effects seen at the 500 and 650 mg/kg doses included increased postimplantation loss (resorptions and dead fetuses), reduced number of live fetuses, and lower body weights ($p < 0.01$). Examination of the fetuses revealed an increased incidence of external malformations (cleft palate), skeletal malformations of the cervical and thoracic vertebral arches and sternebrae, and internal malformations (dilatation of the renal pelvis) at the 500 and 650 mg/kg doses ($p < 0.05$). Developmental effects were not seen at the 250 mg/kg dose. The authors note that the developmental consequences of prenatal MBP exposure are very similar to those of prenatal DBP exposure, and they suggest that MBP may be the active agent in DBP developmental toxicity.

In order to determine age-specific sensitivity to the developmental effects of MBP seen in the previous study, Ema et al. (1996) dosed pregnant Wistar rats daily with 0, 500, 625, or 750 mg/kg MBP by oral intubation on different dosing schedules to determine the critical periods of exposure for the developmental toxicity of MBP. The dosing schedules were gestation days 7 to 9, 10 to 12, or 13 to 15. The vehicle was ammonium chloride. Maternal body weights and food consumption were recorded daily. On gestation day 20 all dams were killed. Fetuses were removed by cesarian section and examined for developmental markers. Maternal body weight gain and food consumption were reduced during the periods that dams were dosed. For the 750 mg/kg group, dams dosed on gestation days 10-12 and 13-15 showed continued reductions in food consumption and weight gain through gestation day 20. The percentage of postimplantation loss was increased in the 625 and 750 mg/kg dose group at all dosing schedules ($p < 0.01$) and was as high as 95% at 750 mg/kg dose of animals exposed on gestation days 13 to 15. Body weights of live fetuses were most reduced by MBP at all doses in those exposed on gestation days 7 to 9 ($p < 0.05$). This observation may be skewed by the high rates of postimplantation loss in the latter dosing schedules.

Examination of the fetuses revealed that cleft palate developed upon exposure to 625 and 750 mg/kg MBP during gestation days 13 to 15 ($p < 0.01$). Skeletal malformations of the cervical vertebral arches occurred at all doses during gestation days 7 to 9, and fusion of the sternebrae was seen at the two higher doses exposed on gestation days 13 to 15 ($p < 0.05$). It was concluded that deviant development induced by MBP varies with the developmental stage at the time of administration. The authors noted that these age-dependent developmental effects of MBP exposure correspond to the pattern seen in the effects of DBP seen in Ema et al. (1994; 1995a) and that this supports the notion that the metabolite MBP is the active agent in the developmental toxicity of DBP.

Ema and Miyawaki (2001a) conducted a study in which pregnant Wistar rats were orally dosed with 0, 250, 500, or 750 mg/kg MBP on gestation days 15 through 17 (n = 16 rats per dose group). The rats were killed on gestation day 21 when pregnancy outcome and fetal development were recorded. Maternal food consumption was reduced in the 750 mg/kg MBP dose group, and maternal body weight gain was reduced in the 500 and 750 mg/kg MBP groups. Fetal observations with LOELs are listed in Table 16. Male sexual development was impaired by MBP, female development was not. These findings support the case for MBP inducing some of the anti-androgenic effects of DBP.

Ema and Miyawaki (2001b) dosed pregnant and pseudopregnant Wistar rats orally with 0, 250, 500, 750 or 1000 mg/kg MBP on days 0 through 8 of pregnancy or pseudopregnancy (n = 16 rats per exposure condition). Pregnant rats were killed on gestation day 20, and pregnancy outcome was determined. Pseudopregnant rats were killed on day 9 for measurement of uterine weights as an index of decidualization. MBP at 1000 mg/kg caused increases on the incidence of preimplantation loss. Uterine decidualization in pseudopregnant rats was decreased in the 1000 mg/kg MBP dose group. The authors concluded that early embryonic loss due to MBP exposure is mediated by suppression of uterine decidualization.

Table 16. Summary of observations with lowest observed effect level (LOEL) for fetal developmental effects of MBP exposure during late pregnancy (Ema & Miyawaki, 2001a).

Observations on Gestation Day 21	LOEL
Reduced maternal food consumption on gestation days 15-18	750 mg/kg
Reduced maternal body weight gain on gestation days 15-18	500 mg/kg
Increased number of resorptions and dead fetuses per litter	500 mg/kg
% postimplantation loss per litter	500 mg/kg
Reduced body weights of male fetuses	750 mg/kg
Reduced body weights of female fetuses	750 mg/kg
Increased number of male fetuses with undescended testes	250 mg/kg
Reduced anogenital (AGD) distance of male fetuses	250 mg/kg
Reduced ratio of AGD/body weight	250 mg/kg
Reduced ratio of AGD/cube root of body weight	250 mg/kg

IN VITRO STUDIES

Newly Available Data

Saillenfait et al. (2001) incubated whole, day 10 rat embryos without antibiotics in cell culture flasks containing growth medium with inactivated rat serum. MBP was added to the culture medium at a concentration of 0, 0.5, 1.0, 1.5, 2.0, 2.5, or 5 mM. The embryos were incubated in culture at 37°C for 46 hours. Embryos with a beating heart were considered viable and examined microscopically for growth and morphological development. There was a decrease 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 the 5 mM concentration level 20% of the embryos were non-viable, and those surviving exhibited severe dysmorphogenesis.

DIETHYL PHTHALATE

CONTINUOUS BREEDING PROTOCOL

Newly Available Data

Lamb et al. (1997a) tested the reproductive toxicity of DEP in the standard Continuous Breeding design using Swiss CD-1 mice. DEP was mixed in the diet at concentrations of 0, 0.25, 1.25, and 2.5 %. Food consumption was not affected by the presence of DEP, and the calculated consumption rates of DEP

were 0.34, 1.77, and 3.64 g/ kg/day. There were no effects of DEP on fertility or reproductive outcome of the F_0 generation. F_1 mice from the control and high dose group were mated within their treatment groups (not cross-mated between groups). There were no treatment-related differences between the treated and control groups of the F_2 generation. The F_1 mice were killed and necropsied. 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 DEP and neither were testicular, epididymal, and seminal vesicle weights.

FEEDING STUDY

Newly Available Data

Field et al. (1993) fed pregnant Sprague-Dawley rats diet containing 0, 0.25, 2.5, or 5.0 % DEP on gestation days 6 to 15 (n = 26 to 32 rats per dose group). The four treatment groups provide daily doses of approximately 0, 0.20, 1.91, and 3.21 g/kg, respectively. Dams were weighed, and their food and water consumption were monitored throughout the gestation period. On gestation day 20 the rats were killed and subjected to necropsy, and the fetuses were removed and evaluated for viability and developmental malformations. The high dose (5 % DEP) group had reduced body weight gain during the dosing period. However, food and water consumption were reduced in the 5 % DEP group during dosing, so the effect on body weight gain may have been due to palatability of the DEP in the feed. There was no effect of DEP on any parameter of fetal viability or development, except for an increased incidence of supernumerary ribs in the 5 % DEP group.

DERMAL STUDY

Newly Available Data

The Proctor & Gamble Co. (1984) reported a study in which pregnant New Zealand White rabbits were exposed to DEP dermally and examined for maternal and fetal toxicity and teratogenesis. Rabbits in each group (n = 12/dose level) received a daily application of 2 ml/kg 0, 5, 15, or 50 % DEP on a 10 x 10 cm shaved area of dorso-lumbar skin on gestation days 6 through 18. The vehicle was 5 % carboxymethylcellulose. Animals were restrained to prevent ingestion of the test material. The treatment application remained on the skin uncovered for seven hours before being washed off with tepid water. Body weights were recorded daily for accurate dosing. The dosing sites were periodically graded according to the Draize skin reaction scoring system (dermal irritation data from this study is covered in the Dermal Irritation

& Sensitization section earlier in this report). Throughout the study the animals were observed for clinical evidence of toxicity and subjected to behavioral tests for neurological toxicity. The rabbits were killed on gestation day 29 and subjected to necropsy. The uterus was removed and weighed, and markers of fetal survivability were recorded. Fetuses were examined for external, skeletal, and visceral development. 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 food 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 % DEP group had a higher number of early resorptions, but this did not provide a statistical significance between treatment conditions. Fetal examinations revealed one fetus with acranie and malformations of the ribs and lumbar and coccygeal vertebrae, and one fetus showed hernia umbilicalis with prolapse of abdominal organs and incurved ribs. Both malformed fetuses were in the 50 % DEP treatment group. However in the discussion of this study it was concluded that these were not treatment-related effects, based on the historical occurrence of these malformations. Based on this study, it was concluded that repeated dermal application of 2 ml/kg up to 50 % DEP to pregnant rabbits did not produce maternal or fetal toxicity or affect fetal development.

DIMETHYL PHTHALATE

FEEDING/ORAL GAVAGE STUDIES

Newly Available Data

Plasterer et al. (1985) exposed pregnant CD-1 mice to 3500 mg/kg DMP (n = 36 rats) or corn oil (vehicle control; n = 40 rats) by oral gavage daily on gestation days 7 through 15. Offspring mice were examined externally and weighed 12 to 24 hours after birth and again on postnatal day 3. There were no signs of maternal toxicity or changes in weight gain due to DMP treatment. There were no DMP-related effects on fetal survivability, litter size, litter weight, or physical development of the fetus. Under these exposure conditions DMP produced no observed fetotoxic or teratogenic effects.

Field et al. (1993) fed pregnant Sprague-Dawley rats diet containing 0, 0.25, 1.0, or 5.0 % DMP on gestation days 6 to 15 (n = 25 to 30 rats per dose group). The four treatment groups provide daily doses of approximately 0, 0.20, 0.84, and 3.57 g/kg, respectively. Dams were weighed, and their food and water consumption were monitored throughout the gestation period. On gestation day 20 the rats were killed and subjected to necropsy, and the fetuses were removed and evaluated for viability and developmental

malformations. The high dose (5 % DMP) group had increased maternal liver weights and reduced body weight gain. However, food and water consumption were reduced in the 5 % DMP group during dosing, so the effect on body weight gain may have been due to palatability of the DMP in the feed. There was no effect of DMP on any parameter of fetal viability or development.

DERMAL STUDY

Hansen and Meyer (1989) examined the teratogenic potential of dermally applied DMP. Female outbred Mol:Wis rats were mated at 11 weeks of age. Doses of 0.5, 1.0, or 2.0 ml DMP/kg body weight was applied to the shaved skin of the pregnant rats and occluded with a porous dressing covered with aluminum foil. Control groups were treated similarly with 0.5 ml paraffin oil/kg body weight (n = 15 to 25 rats per dose group). The DMP or paraffin oil was on the skin for two hours per day on gestation days 6 to 15 or 1 to 20. On gestation day 21, the rats were killed and examined. Fetal survivability and postimplantation loss were calculated. The fetuses were removed and examined for external, visceral, and skeletal malformations. There were no significant changes in fetal development or survivability between control animals and those exposed to the prenatal dermal application of DMP. Table 17 summarizes the reproductive and developmental studies reviewed above.

Table 17. Summary of Reproductive and Developmental Toxicity Studies for DBP, MBP, DEP, and DMP.

Chemical	Route of Exposure	Species	Study Design	Results	Reference
DBP	Feed	Mouse	Continuous Breeding Protocol. Doses 0, 0.03, 0.3, or 1.0% DBP mixed in feed. F_0 generation mice were exposed to DBP 7 days prior to and throughout 98-day cohabitation period. Litters born during cohabitation were counted, sexed, measured, and discarded. At end of 98-day period, mating pairs were separated for 21 days, and any pups born were kept alive for 21 days. F_0 mice were then crossover mated between control and high groups (control male x control female, control male x 1% DBP female, and 1% DBP male x control female). Crossover offspring were counted, sexed, measured, and discarded. F_0 were killed for necropsy and histological evaluation.	Litters of 1% DBP pairs showed reduced fertility, number pairs per litter, and proportion of pups born alive, compared to controls. Other doses had litter parameters similar to controls. Litters of crossover pairs showed pups born to control female x 1% DBP male pairs were similar to pups of two control parents; pups born to control males x 1% DBP females had reduced fertility, number of live pups per litter, and live pup weight. F_0 males showed reduced total body weight in 1% DBP group. No treatment-related effects on organ weights or sperm morphology, motility, or concentration. F_0 females showed increased liver weights and reduced uterine weights in 1% DBP group.	Lamb et al., 1987

Chemical	Route of Exposure	Species	Study Design	Results	Reference
DBP	Feed	Rats	Continuous Breeding Protocol. Doses 0, 0.1, 0.5, or 1.0% DBP mixed in feed. F_0 generation rats were exposed to DBP 7 days prior to and throughout 122-day cohabitation period. The first four litters born during cohabitation were counted, sexed, measured, and discarded. The fifth litter per mating pair (F_1 generation) was kept alive to become the next breeding generation. After the F_1 pups were weaned, F_0 rats were re-paired to mate as crossover pairs (control male x control female, control male x 1% DBP female, and 1% DBP male x control female). The offspring of the crossover mating were counted, sexed, measured, and discarded. F_0 rats were then killed for necropsy and histological evaluation. F_1 rats were fed diets consistent with their respective parents' treatments. The offspring of F_1 rats were counted, sexed, measured, and discarded. F_1 rats were then killed for necropsy and histological evaluation.	Litters in all DBP-dosed groups had fewer live pups per litter. Pups of 0.5 and 1% DBP groups had lower body weights. Litters of control male x 1% DBP female crossover pairs had reduced live pup weights. No other litter data from crossover pairing were different from controls. F_0 males of the 1% DBP group showed reduced liver and kidney weights. No treatment-related effects on organ weights or sperm morphology, motility, or concentration. F_0 females of the 1% DBP group showed reduced body, liver, and kidney weights. Litters of F_1 rats showed reduced mating index, pregnancy index, and fertility index in the 1% DBP group. Live pup weights were reduced in all DBP-treated groups. F_1 males of the 1% DBP group showed reduced body weights, reduced prostate, seminal vesicle, and testes weights, and increased liver and kidney weights. F_1 females of the 1% DBP group had reduced body weights.	Wine et al., 1997; NTP, 1991
DBP	Feed	Rats	Exposed pregnant dams to 0 or 10000 ppm DBP mixed in feed from gestation day 0 through postnatal week 8. Beginning on postnatal week 13, rats of the 10000 ppm group above were exposed to 0, 2500, 5000, 10000, 20000, or 40000 ppm DBP mixed in feed until postnatal week 26, at which time animals were killed for necropsy and histological evaluation.	Males exposed to 10000 ppm DBP perinatally had lower testicular weights, compared to non-treated controls. Males of all DBP doses had reduced body weights, compared to control rats that had been exposed to DBP perinatally and compared to non-treated controls. Females of all DBP doses had reduced body weights, compared to control rats that had been exposed to DBP perinatally. Females of the 20000 and 40000 ppm DBP groups had reduced body weights, compared to non-treated controls Males and females of the 20000 and 40000 ppm DBP groups were emaciated by the end of the study and had lower food consumption than controls. Observations on DBP-related effects included reduced epididymis weight (LOEL = 2500 ppm); increased liver and kidney weights, reduced serum testosterone, and increased incidence of germinal epithelium atrophy (LOEL = 10000 ppm); reduced testes weight and hypospermia of the epididymis (LOEL = 20000 ppm). See Table 7 (page 33) in report for other observations.	NTP, 1995a
DBP	Feed	Rats	Exposed pregnant dams to 0 or 2% DBP mixed in feed on gestation days 0 through 20. Necropsy of dams and examination of fetuses on gestation day 20.	Reduced body weight gain and food consumption in 2% DBP dams. Reduced ovarian weight, uterine weight, and serum progesterone in 2% DBP dams.	Ema et al.,1997b

Chemical	Route of Exposure	Species	Study Design	Results	Reference
				Litter parameters showed increased postimplantation loss, reduced number of live fetuses, and no live male fetuses in the 2% DBP group.	
DBP	Feed	Rats	Exposed pregnant dams to 0, 0.5, 1.0, or 2.0% DBP mixed in feed on gestation days 11 through 21. Necropsy of dams and examination of fetuses on gestation day 21.	Reduced body weight gain and food consumption in 1 and 2% DBP dams. No effect of DBP on fetal survival, number of resorptions, or sex ratio of live fetuses. Reduced fetal body weights of both sexes in 2% DBP. Reduced anogenital distance and undescended tested in male fetuses of 1 and 2% DBP groups. Cleft palate and fused sternebrae observed in 2% DBP fetuses.	Ema et al., 1998
DBP	Oral Gavage	Rats	Exposed pregnant dams to 0, 250, 500, 1000, or 2000 mg/kg/day DBP on gestation days 1 through 8. Exposed pseudopregnant rats to 0, 500, 1000, or 2000 mg/kg/day DBP on days 1 through 8 after induction of pseudopregnancy. One day after the last dose, rats were killed, blood was collected, and the ovaries and uteri were dissected and analyzed.	No effects of DBP on body weights, uterine weights, ovarian weight, or serum progesterone levels in the pregnant or pseudopregnant rats. No dose-related effects on number of implantations in pregnant rats. Authors' conclusion: fetotoxicity of short-term DBP exposure is not a result of maternal toxicity, and DBP did no affect the viability of preimplantation embryos.	Cummings & Gray, 1987
DBP	Oral Gavage	Rats	Exposed pregnant and pseudopregnant rats to 0, 250, 500, 750, 1000, 1250, or 1500 mg/kg/day DBP on days 0 through 8. Pseudopregnant rats were killed for necropsy on day 9. Pregnant rats were killed on gestation day 20 for maternal necropsy and fetal examinations.	Pseudopregnant rats dosed with DBP had reduced food consumption, body weight gain, and uterine weight (LOEL = 750 mg/kg), and reduced serum progesterone (LOEL = 1500 mg/kg). There were no DBP dose-related effects on uterine weight, number of corpora lutea, or serum estradiol. Pregnant rats dosed with DBP had reduced food consumption and food weight gain (LOEL = 500 mg/kg), reduced number of live fetuses per litter (LOEL = 750 mg/kg), increased number of resorptions and dead fetuses per litter (LOEL = 1000 mg/kg), and increases in preimplantation loss (LOEL = 1250 mg/kg). Fetal sex ratios were not affected by DBP, but male and female fetal body weights were reduced (LOEL = 250 mg/kg).	Ema et al., 2000b
DBP	Oral Gavage	Rats	Exposed pregnant rats to 0, 0.5, 0.63, 0.75, or 1.0 g/kg/day DBP on gestation days 7 through 15. Dams were killed on gestation day 20, and fetuses were examined.	Pregnant rats dosed with DBP showed reductions in maternal body weight gain and food consumption in the 0.75 and 1.0 g/kg groups. Litters of dosed rats showed increased numbers of resorptions, dead fetuses, and postimplantation loss (LOEL = 0.63 g/kg); reduced numbers of live fetuses (LOEL = 0.63 g/kg); increased number of litters totally resorbed (LOEL = 0.75 g/kg); and increased numbers of fetuses with malformations* (LOEL = 0.75 g/kg). Zero live fetuses were found in the 1.0 g/kg group.	Ema et al., 1993

Chemical	Route of Exposure	Species	Study Design	Results	Reference
				The incidences of specific malformations were not statistically significant. See Table 14 for details of malformations.	
DBP	Oral Gavage	Rats	Exposed pregnant rats to 0, 0.75, 1.0, or 1.5 g/kg/day DBP on one of three dosing schedules: gestation days (GD) 7 to 9, GD 10 to 12, or GD 13 to 15. Dams were killed on gestation day 20, and fetuses were examined.	There were no live fetuses on GD20 in the 1.5 g/kg DBP groups of all three dosing schedules. GD 7 to 9: Increased incidence of fusion and/or absence of cervical vertebral arches, fusion and/or absence of thoracic vertebral arches and/or bodies, and fusion or absence of ribs (LOEL = 0.75 g/kg). GD 10 to 12: No statistically significant increase in incidence of any malformation. GD 13 to 15: Increased incidence of cleft palate and fusion of sternebrae (LOEL = 0.75 g/kg	Ema et al., 1994
DBP	Oral Gavage	Rats	Exposed pregnant rats to 0, 0.75, 1.0, or 1.25 g/kg/day DBP on one of three dosing schedules: GD 7 to 9, GD 10 to 12, or GD 13 to 15. Dams were killed on gestation day 20, and fetuses were examined.	There was an increase in postimplantation loss at doses in all three dosing schedules. GD 7 to 9: Decreased numbers of live fetuses per litter, increased postimplantation loss, and reduced male and female fetal body weights (LOEL = 0.75g/kg). Increased incidence of fusion and/or absence of cervical vertebral arches, fusion and/or absence of thoracic vertebral arches and/or bodies, and fusion or absence of ribs (LOEL = 0.75 g/kg). Increased incidence of fusion and/or absence of lumbar vertebral arches (LOEL = 1.25 g/kg). GD 10 to 12: Decreased numbers litters, numbers of live fetuses per litter, increased postimplantation loss, and reduced female fetal body weights (LOEL = 0.75g/kg). Reduced male fetal body weights (LOEL = 1.25 g/kg). Increased proportion of male to female live fetuses (LOEL = 1.0 g/kg). No statistically significant increase in incidence of any malformation. GD 13 to 15: Increased incidence of postimplantation loss (LOEL = 0.75 g/kg). Decreased numbers of live fetuses per litter (LOEL = 1.0 g/kg). Increased incidences of cleft palate and fusion of sternebrae (LOEL = 0.75 g/kg)	Ema et al., 1995a

Chemical	Route of Exposure	Species	Study Design	Results	Reference
DBP	Oral Gavage	Rats	Exposed pregnant rats to 0, 1000, or 1500 mg/kg DBP on one of three dosing schedules: GD 12 to 14, GD 15 to 17, or GD 18 to 20. Subjects in the GD 15 to 17 schedule had an additional dose group of 500 mg/kg/day. Dams were killed on gestation day 21, and fetuses were examined.	Reduced body weight gain and food consumption in dams of both DBP-dosed groups in all dosing schedules. GD 12 to 14: Increased number of total litters absorbed and number of resorptions per litter, decreased number of live fetuses per litter, and reduced number of male fetuses per litter (LOEL = 1500 mg/kg). Reduced male and female fetal body weights (LOEL = 1000 mg/kg). GD 15 to 17: Increased number of resorptions per litter, decreased number of live fetuses per litter, and reduced male and female fetal body weights (LOEL = 1500 mg/kg). Reduced number of male fetuses per litter (LOEL = 500 mg/kg). GD 18 to 20: Reduced male and female fetal body weights (LOEL = 1000 mg/kg).	Ema et al., 2000a
DBP	Oral Gavage	Rats	Exposed pregnant rats to a single dose of 0 or 1500 mg/kg DBP on one day between GD 6 and 16. Dams were killed on gestation day 20, and fetuses were examined.	The single dose of 1500 mg/kg DBP on GD 6, 8, 9, 10, 12, 13, 14, 15, or 16 caused an increase in postimplantation loss. Cleft palate and fused sternebrae were observed in those fetuses dosed on GD 15. Malformations of the cervical and thoracic vertebrae were seen in fetuses dosed on GD 8 or 9. Dilatation of the renal pelvis was seen in fetuses dosed on GD 9.	Ema et al., 1997a
DBP and MBP	Oral Gavage	Rats	Exposed pregnant rats to a single dose of 0, 1.8, 3.6, 5.4, or 7.2 mmol/kg DBP or MBP on GD 10. Dams were killed on gestation day 12, and uteri and viable embryos were examined.	Observations of DBP-induced impairments of embryonic development were seen in reduced crown-rump length and somite number (LOEL = 1.8 mmol/kg), and reduced head length (LOEL = 3.6 mmol/kg). Additional impairments in nervous and skeletal development were observed with dose-dependent severity. Observations of MBP-induced impairments of embryonic development were seen in reduced crown-rump length and head length (LOEL = 5.4 mmol/kg). Additional impairments in nervous and skeletal development were observed with dose-dependent severity.	Saillenfait et al., 2001
MBP	Oral Gavage	Rats	Exposed pregnant rats to a single dose of 0, 5.4, or 7.2 mmol/kg MBP on GD 10. Dams were killed on gestation day 12, 13, 14, or 18, and embryos/fetuses were examined for viability and morphology.	On GD 12 survivability was similar between both MBP and control treatments. On GD 13 and 14 there was an increase of non-viable fetuses. On GD 18 fetuses showed nervous, skeletal, and cardiac abnormalities.	Saillenfait et al., 2001
MBP	Oral Gavage	Rats	Exposed pregnant and pseudopregnant rats to 0, 250, 750, 1000 mg/kg/day MBP on days 0 through 8. Pseudopregnant rats were killed for necropsy on day 9. Pregnant rats were killed on gestation day 20 for determination of pregnancy outcome.	Pseudopregnant rats dosed with 1000 mg/kg DBP had decreased uterine decidualization. Pregnant dams dosed with 1000 mg/kg DBP had an increased incidence of preimplantation loss. The authors concluded that early embryonic loss due to MBP exposure is mediated by suppression of uterine decidualization.	Ema & Miyawaki, 2001b

Chemical	Route of Exposure	Species	Study Design	Results	Reference
MBP	Oral Gavage	Rats	Exposed pregnant rats to 0, 250, 500, or 650 mg/kg/day MBP on GD 7 to 15. Dams were killed on gestation day 20, and fetuses were examined.	Pregnant rats dosed with MBP showed reductions in maternal body weight gain and food consumption (LOEL = 500 mg/kg). Litters of MBP-dosed dams showed increased postimplantation loss, reduced number of live fetuses, reduced fetal body weights (LOEL = 500 mg/kg). Fetuses exposed to MBP also showed an increased incidence of cleft palate and vertebral malformations (LOEL = 500 mg/kg)	Ema et al., 1995b
MBP	Oral Gavage	Rats	Exposed pregnant rats to 0, 500, 625, or 750 mg/kg/day MBP on one of three dosing schedules: GD 7 to 9, GD 10 to 12, or GD 13 to 15. Dams were killed on gestation day 20, and fetuses were examined.	GD 7 to 9: Increased postimplantation loss per litter, decreased number of live fetuses per litter (LOEL = 750 mg/kg). Reduced live fetal body weights (LOEL = 500 mg/kg). Increased incidence of cleft palate (LOEL = 625 mg/kg). Increased incidence of fusion and/or absence of cervical vertebral arches (LOEL = 500 mg/kg). GD 10 to 12: Increased postimplantation loss per litter, decreased number of live fetuses per litter (LOEL = 625 mg/kg). Reduced live male fetal body weights (LOEL = 500 mg/kg). Reduced live female fetal body weights (LOEL = 750 mg/kg). No malformations observed.	Ema et al., 1996
				GD 13 to 15: Increased postimplantation loss per litter, decreased number of live fetuses per litter (LOEL = 625 mg/kg). Increased incidence of cleft palate and fusion of sternebrae (LOEL = 625 mg/kg).	Ema et al., 1996
MBP	Oral Gavage	Rats	Exposed pregnant rats to 0, 250, 500, or 750 mg/kg/day MBP on GD 15 to 17. Dams were killed on gestation day 21, and fetuses were examined.	Pregnant rats dosed with MBP showed reductions in maternal body weight gain (LOEL = 500 mg/kg) and food consumption (LOEL = 750 mg/kg). MBP-exposed fetuses exhibited the following developmental effects: Reduced absolute and relative anogenital distance and increased number of males with undescended testes (LOEL = 250 mg/kg). Increased number of resorptions per litter and postimplantation loss (LOEL = 500 mg/kg). Reduced body weights of male and female fetuses (LOEL = 750 mg/kg). See Table 15 for details.	Ema & Miyawaki, 2001a
MBP	In vitro	Rat embryo	Day 10 rat embryos grown in culture exposed to 0, 0.5, 1.0, 1.5, 2.0, 2.5, or 5.0 mM MBP for 46 hours and examined for morphological development.	MBP-exposed embryos had reduced head length (LOEL = 1 mM) and reduced yolk sac diameter, crown-rump length, and somite number (LOEL = 1.5 mM). In the 2 mM and higher concentrations, 100% of live embryos had developmental defects of the nervous and/or skeletal systems.	Saillenfait et al., 2001

Chemical	Route of Exposure	Species	Study Design	Results	Reference
DEP	Feed	Mice	Continuous Breeding Protocol. Doses 0, 0.25, 1.25, 2.5 % DEP mixed in feed. F_0 generation mice were mated within dose groups. F_1 generation mice of the 2.5 % MBP and control groups were mated within their treatment groups. F_2 generation mice were examined and discarded.	Male and female F_1 mice of the 2.5 % DEP group had reduced adult body weights and increased liver weights. Male F_1 mice had increased prostate size and reduced sperm counts. There were no DEP-related effects observed in the F_2 mice.	Lamb et al., 1997a
DEP	Feed	Rats	Exposed pregnant rats to 0, 0.25, 2.5, 5.0 % DEP mixed in feed on GD 6 to 15. Dams were killed on gestation day 20, and fetuses were examined.	Dams exposed to 5% DEP had reduced body weight gain and food and water consumption during the dosing period. No DEP-related effects were observed in fetuses.	Field et al., 1993
DEP	Dermal	Rabbits	Exposed pregnant rabbits to a daily application of 2 ml/kg of 0, 5, 15, or 50% DEP on a 10 x 10 shaved dorso-lumbar skin on GD 6 through 18. Does were observed for clinical signs of toxicity, and neurobehavioral evaluations. Dosing sites were scored daily according to Draize scale. Does were killed on gestation day 29, and fetuses were examined.	No clinical signs of DEP-induced toxicity or neurobehavioral effects. No DEP-related effects on maternal body weight gain or water or food consumption. No effects of DEP exposure on number of live fetuses, sex ratio, fetal body weights, or incidence of dead fetuses. No treatment-related effects on fetal development.	Proctor & Gamble Co., 1984
DMP	Feed	Rats	Exposed pregnant rats to 0, 0.25, 1.0, 5.0 % DMP mixed in feed on GD 6 to 15. Dams were killed on gestation day 20, and fetuses were examined.	Dams exposed to 5% DMP had reduced body weight gain and food and water consumption during the dosing period. No DMP-related effects were observed in fetuses.	Field et al., 1993
DMP	Oral Gavage	Mice	Exposed pregnant rats to 0 or 3500 mg/kg/day DMP on GD 7 through 15. Pups were examined and weighed on postnatal days 1 and 3.	No signs of DMP-related maternal toxicity. No DMP-related effects on offspring survivability, litter size, litter weight, or physical development of the pups.	Plasterer et al., 1985
DMP	Dermal	Rats	Exposed pregnant rats to 0, 0.5, 1.0, or 2.0 ml/kg DMP by application to shaved skin and occluded with a porous dressing for 2 hrs per day on GD 6 to 15 or on GD 1 to 20. Dams were killed on gestation day 21, and fetuses were examined.	No signs of DMP-related maternal toxicity. No DMP-related effects were observed in fetal survivability or development.	Hansen & Meyer, 1989

PHTHALATES

THELARCHE

Newly Available Data

Colón et al. (2000) reported a high incidence of premature breast development (thelarche) in young girls of Puerto Rico. Serum samples from 41 thelarche patients and 35 control subjects in Puerto Rico were analyzed by gas chromatography/mass spectrometry for detection of phthalate esters and other possible contaminants. Thelarche patients are defined as girls younger than eight years who had growth of mammary tissue without other manifestations of puberty. No pesticides or pesticide metabolites were detected in the samples tested. DBP, DEP, DMP, were detected in the serum of thelarche patients but not

the control subjects. DEHP was found in both control and thelarche subjects' sera. Dioctyl phthalate was found only in the serum of control subjects. There was no statistically significant evidence that the premature breast development was a consequence of phthalate exposure. The authors concluded, "The findings of this study cannot be interpreted as the cause of premature thelarche in Puerto Rico girls at present. It may be well that the etiology of the various manifestations of premature development (including thelarche) on this island are multifactorial."

MALE-SPECIFIC REPRODUCTIVE EFFECTS

GENERAL ESTROGENIC EFFECTS

Newly Available Data

Predicated on reproductive and developmental effects of DBP on male animals, Harris et al. (1997) assessed the estrogenic effects of several phthalate esters in a recombinant yeast assay and in estrogen-responsive human breast cancer cells. DBP and DEP were shown to have extremely weak estrogenic activity, and MBP had no estrogenic activity.

Milligan et al. (1998a) reported that DBP was shown to have no estrogenic activity in an *in vivo* mammalian assay to test for xenoestrogens.

Zacharewski et al. (1998) evaluated the estrogenic potential of phthalate esters using *in vivo* and *in vitro* assays. In the *in vitro* assays, 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 DBP was 36% of the response to 10 nM 17-β-estradiol, suggesting a weak estrogenic activity. In the *in vivo* uterotrophic assay, immature ovariectomized Sprague-Dawley rats were given 0, 20, 200, or 2000 mg/kg/day DBP by oral gavage for four days (n = 10 rats per group). On the day after the fourth dose, all animals were killed and the uteri were weighed. The uterine weights of DBP-exposed animals did not differ from those of control animals. In a vaginal cell cornification assay, mature ovariectomized female Sprague-Dawley rats were given 0, 20, 200, or 2000 mg/kg/day DBP by oral gavage for four days. 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 DBP-treated rats were similar to those of control rats. Thus, in these

assays, DBP showed weak estrogenic activity *in vitro* and none in vivo.

Unilever (1999) used a recombinant yeast assay to determine the estrogenic potential of DEP. In this assay, 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). 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 EC50 of 3.2×10^{-11} M. Spectrophotometry of the medium treated with DEP at concentrations up to 10^{-3} M did not differ from that of negative control and untreated cultures. DEP apparently did not activate hER in this test system.

Lovekamp and Davis (2001) reported that MBP, MEP, and MMP at concentrations up to 400 µM did not affect estradiol production in primary cultures of rat ovarian granulosa cells, whereas mono-(2-ethylhexyl) phthalate (MEHP) at 100 and 200 µM significantly reduced estradiol production. Furthermore, they stated that MBP at 100 µM failed to affect aromatase RNA and aromatase protein levels in cultured granulosa cells, while MEHP dramatically reduced these parameters.

EFFECTS ON ENDOCRINE RECEPTORS

Newly Available Data

Monsanto Europe (no date) suggested that phthalates do not interact with estrogen receptors because MBP did not promote growth of the uterus in 20- to 22-day-old female rats at oral doses of 0, 10, 100, 500, or 1000 mg/kg/day for three days (n = 6 rats per dose group). A positive control compound, 0.5 µg/rat/day estradiol benzoate, did cause an increase in uterine weight.

DBP 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 (Japanese Plasticizer Industrial Association, 1997). DBP at 1.0×10^{-3} M had weak binding to estrogen receptors in rat uterine tissue, however it showed no estrogenic activity (Japanese Plasticizer Industrial Association, 1998).

Milligan et al. (1998b) reported that DBP did not interact with estrogen receptors as determined in three binding assays involving rat, human, and rainbow trout estrogen binding proteins.

Unilever (1999) evaluated DEP in an estrogen receptor binding assay. Rat uterine cytosol was acquired from the supernatant of the ground and centrifuged uterine tissue of ten-week-old Wistar rats. β-estradiol (10^{-6} to 10^{-13} M) was used as a positive control in a saturation binding assay, and caffeine (10^{-3} to 10^{-7} M) was used as a negative control. DEP concentrations used were 10^{-3} to 10^{-7} M. In the assay, DEP 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. Binding of the H^3-E_2 to estrogen receptors were measured by scintillation counting. The more H^3-E_2 detected, the less binding by the potential competitor. In this assay the β-estradiol reduced the binding of H^3-E_2 to the estrogen receptors with a EC_{50} of 1.3×10^{-3} M. Caffeine and DEP at concentration up to 10^{-3} M did not reduce H^3-E_2 binding in this assay.

Blair et al. (2000) found that DBP, DEP, and DMP each had no binding affinity for the estrogen receptor and failed to prevent [3H]-estradiol from binding to estrogen receptors obtained from the uteri of NCTR:SDN outbred rats.

Jury et al. (2000) reported that sex hormone binding globulin (SHBG) has a high binding affinity for sex steroids such as estradiol. In a protein binding assay, several phthalates were evaluated for their potential for binding to SHBG in competition with radiolabeled H^3-E_2. 200 µM DBP or 200 µM MEP failed to displace appreciable amounts of H^3-E_2. Thus, DBP and MEP did not bind to SHBG.

Paganetto et al. (2000) evaluated the ability of DBP (100 µM), MBP (10 µM), DEP (100 µM), and MEP (10 µM) to inhibit the binding of known ligands to endocrine receptors *in vitro*. The ligands were all radiolabeled with [^{3}H] so that binding to their respective receptors could be measured. Healthy human uterus and prostate tissues were used as sources of endocrine receptors. DBP, MBP, DEP, and MEP did not significantly inhibit the binding of known ligands to the estrogen, progesterone, testosterone, or retinoic acid (uterus and prostate) receptors.

UTEROTROPHIC EFFECTS

Newly Available Data

Unilever (1999) reported the results of a uterotrophic assay conducted to determine the estrogenic potential of DEP, immature female Wistar rats received a daily oral dose of 0, 50, 150, or 500 mg/kg DEP or 0.4 mg/kg β-estradiol as a positive control (n = 10 rats per group) for three consecutive days. The vehicle was peanut oil. Body weights and detailed clinical observations were recorded daily. Approximately 24 hours after the third dose, all of the animals were killed, and the 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 DEP were similar to those in the control group. Using uterine weight as the endpoint, DEP did not exhibit estrogenic activity in the immature rat at doses of up to

500 mg/kg.

Gray et al. (1999) found that DBP failed to induce estrogenic responses in a uterotrophic assay. Ovariectomized rats were exposed to estrodiol benzoate (10 µg, s.c.) or DBP (200 or 400 mg/kg/day, s.c. or 1000 mg/kg/day orally) for two days. On the third day, the rats were given 0.5 mg progesterone s.c. Six to 8 hours after the progesterone injection, the females were paired with a sexually active male for observation of lordosis bahavior. After the lordosis behavioral observations, the females were killed and their uteri were cleaned of mesenteric fat and weighed. In this protocol, estrogenic compounds typically induce a uterotrophic response and lordosis behavior. The DBP treatments did not affect uterine weights or lordosis behavior, compared to control (oil) treatment. The estradiol group showed significant increases in these parameters.

TESTICULAR EFFECTS

Newly Available Data

Gray et al. (1982) stated that there are species differences in the sensitivity of DBP-induced testicular toxicity. DBP dissolved in corn oil, sodium salt of MBP in aqueous solution, or vehicle control was given orally to male Sprague-Dawley rats (n = 6 per group), TO mice (n = 10 per group), Dunkin-Hartley guinea pigs (n = 5 per group), and DSN Syrian hamsters (n = 8 per group) for seven to nine days. Histological examinations of the testes were conducted in each species after DBP or MBP exposure. DBP (2000 mg/kg) produced severe atrophy in the seminiferous tubules of rats and guinea pigs, with milder effects in mice. Hamsters' testes were not affected by DBP. In additional experiments, male rats were exposed to 2000 mg/kg/day DBP for nine days or to 800 mg/kg/day MBP for five days. Each treatment produced a significant reduction ($p < 0.001$) in testicular weight, compared to control treatments. Histologically, the DBP- and MBP-treated rats showed > 90 % tubular atrophy in the testes. However, neither 3000 mg/kg/day DBP nor 1600 mg/kg/day MBP for nine days produced any changes in testicular weight in hamsters. While absorption of DBP and MBP is similar in rats and hamsters, the metabolism of MBP may be different between the species.

Murature et al. (1987) collected semen samples from healthy college students (number of subjects = 5). The samples were analyzed by gas chromatography-mass spectrometry for concentrations of DMP, DBP, and DEHP, for which the respective average concentrations were 10 ± 1.5, 59.0 ± 6.5, and 15.0 ± 2.2 % at the level of 10 part per million. Only the diesters were measured, not the monoesters or glucuronide

cunjugates. Phthalate exposures were presumably from ambient levels in the environment. The concentrations of DBP were compared to the sperm densities of the semen samples. The plot of DBP concentration vs. sperm density gave two clusters of points. The two clusters were surmised to represent the subjects' differing abilities to metabolize DBP. For those with a lesser ability to metabolize DBP, the average DBP concentration was 129 ppb, the correlation coefficient between sperm density and DBP concentration was -0.4, and the slope of the regression line was -0.7. The subjects with apparent greater ability to metabolize DBP had an average semen concentration of DBP of 51 ppb. The correlation coefficient between sperm density and DBP 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 DBP concentration.

Fukuoka et al. (1989) investigated possible mechanisms of testicular atrophy induced by DBP in rats were investigated in a series of experiments. Repeated oral doses of DBP at 8.6 mmol/kg/day for seven consecutive days in 28 Wistar rats caused 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.

In a series of studies (Fukuoka et al., 1990; Zhou et al., 1990; Fukuoka et al., 1993) it was reported that, after a single dose of 8.6 mmol/kg DBP in 27 male Wistar rats, the same histological and biochemical events as those in Fukuoka et al. (1989) were seen. Additionally, a single dose of DBP causes increases 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. It was proposed that DBP-induced testicular toxicity is caused by a shortage of energy fuels or by anoxia.

Srivastava et al. (1990b) characterized the testicular effects of DBP in five-week-old male Wistar rats. DBP was administered by oral gavage at doses of 0, 250, 500, and 1000 mg/kg/day for 15 days (n = 6 rats per dose group). Animals were weighed daily. On day 16 rats were killed and testes were promptly

weighed and prepared for histopathological examination and enzyme activity assessments. There were no unscheduled deaths in this study, but body weights and testicular weights were reduced in the 500 and 1000 mg/kg doses. Testicular sorbitol dehydrogenase activity was reduced by 500 and 1000 mg/kg DBP. The activity of testicular acid phosphatase was reduced at all DBP doses. Glucose-6-phosphate dehydrogenase, β-glucuronidase, and γ-glutamyl transpeptidase in the testes showed increased activities at all DBP doses tested. Histopathological examination of the testes revealed dose-dependent damage to the seminiferous tubules. Spermatocytes in the 1000 mg/kg DBP group showed pycnotic nuclei. The authors stated that these findings were consistent with a similar study (Srivastava et al, 1990a).

Fredericsson et al. (1993) collected semen samples were collected from "normal" men. The samples were maintained in culture and exposed to various ranges of concentrations of DBP, DMP, or DEP for 0, 2, or 18 hours. After the phthalate exposures, 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 control sperm. The respective EC_{25} values for 0, 2, and 18 hours of DBP exposure on sperm motility were 1, 30, and 8 mM. The EC_{25} values for 0, 2, and 18 hours of DEP exposure on sperm motility were >3.3, 2.5, 0.7mM, respectively. The respective EC_{25} values for 0, 2, and 18 hours of DBP exposure on sperm motility were >0.94, >.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 DBP was the most potent of the three phthalate diesters in inhibiting these parameters. DEHP was also tested and was more potent than DBP in inhibiting sperm motility.

Jones et al. (1993) reported a study in which three male Wistar rats were dosed with 2 g/kg DEP by oral gavage once a day for two consecutive days. Three control rats received an oral dose of the vehicle corn oil. All rats were killed 24 hours after the second dose. The bodies were perfused with a fixative solution, and the testes were removed, sliced, and stained for histological examination. DEP produced no change in seminiferous tubular structure or Leydig cell morphology. However, the Leydig cells of DEP-treated rats showed ultrastructural changes, specifically swelling of the mitochondria and vesiculation and focal dilatation of the smooth endoplasmic reticulum.

These same authors also exposed cell cultures of Wistar rat Leydig cells to 1000 µM Monoethyl Phthalate (MEP, the primary DEP metabolite) or DMSO vehicle for two to three hours. Some cells were

exposed to leutinizing hormone to test for Leydig cell functional integrity, as measured by testosterone output. Cells were fixed and processed for histopathological evaluation. MEP did not affect testosterone output or induce any structural changes in Leydig cell morphology or ultrastructure (Jones et al., 1993).

Imajima et al. (1997) conducted a study in which MBP was administered to seven pregnant female Wistar-King A rats at a daily 3 ml gavage dose of 0.3 g/d dissolved in sesame oil from gestation days 15 to 18. The rats' body weights were 280 to 300 g. Seven pregnant control animals received the vehicle sesame oil on the same dosing schedule. On gestation day 20, three MBP-treated and three control rats were killed for cesarian section. Male fetuses were killed and fixed for examination of the abdomen. The degree of testicular descent was measured relative to the bladder neck. The testes of control fetuses were in the lower abdominal cavity near the bladder neck. The testes of MBP-treated fetuses were relatively high in the abdominal cavity, with some near the kidneys. The remaining pregnant dams were allowed to deliver and the male offspring were killed on postnatal day 30 or 40. The young males were examined to determine the position of the testes and frequency of cryptorchidism (failure of the testes to descend into the scrotum). The testes of 30- and 40-day-old rats from the control group (n = 15) were all located in the scrotum, and there was a 0 % incidence of cryptorchidism. Twenty-two of the 26 MBP-treated males at postnatal day 30 or 40 showed cryptorchidism (14 unilateral and eight bilateral undescended testes), an 84.6 % incidence. The majority (86.7 %) of the undescended testes were in the abdominal cavity, while the remaining were at the external inguinal ring. Prenatal exposure to MBP interfered with the testicular descent of male offspring. The authors speculated that this interference may be due to a disturbance of the Sertoli cells which secrete Müllerian-inhibiting substance, a regulator of transabdominal descent of the testes.

Gray et al. (2000) reported that DEP failed to affect sexual differentiation in developing male rats that were exposed perinatally. DEP (750 mg/kg/day) was administered by oral gavage to five pregnant Sprague-Dawley rats from gestation day 14 through postnatal day 3. The vehicle control was corn oil (n = 9 rats in control group). Maternal weight gain, litter size, pup weight, and sex ratio of pup were not affected by the DEP treatment. No measures of male development were affected by perinatal DEP exposure. Such measures included the weights of the testes and other reproductive tissues, anogenital distance, and incidence of males with areolas. These measures of male development were dramatically affected (feminized) by similar exposure to other phthalate compounds such as DEHP and butyl-benzyl phthalate.

Gray et al. (2000) also reported that DMP failed to affect sexual differentiation in developing male

rats that were exposed perinatally. DMP (750 mg/kg/day) was administered by oral gavage to five pregnant Sprague-Dawley rats from gestation day 14 through postnatal day 3. The vehicle control was corn oil (n = 9 rats in control group). Maternal weight gain, litter size, pup weight, and sex ratio of pup were not affected by the DMP treatment. No measures of male development were affected by perinatal DMP exposure. Such measures included the weights of the testes and other reproductive tissues, anogenital distance, and incidence of males with areolas.

Ohanti et al. (2000) reported that DBP at concentrations of 0, 0.1, 1, and 10 μM dose-dependently impaired the genital development of genetically male tadpoles of *Rana rugosa*. Effects of DBP were similar to the effects seen in male tadpoles exposed to 17β-estradiol (E_2), but the DBP was 1000-fold less potent than E_2 in these effects.

Mylchreest et al. (2002) gave pregnant rats 0 or 500 mg/kg/day DBP in corn oil by oral gavage on gestation days 12 to 21 (n = rats per dose group). On gestation days 16-21, DBP caused focal hyperplasia of Leydig cells in male fetuses. At gestation day 21, DBP-exposed fetuses showed testicular atrophy and seminiferous cords were enlarged. DBP-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.

EFFECTS ON GENE EXPRESSION

Newly Available Data

In pursuit of the question of the mechanism of the anti-androgenic effects of DBP, Shultz et al. (2001) investigated the altered genetic expressions profiles in fetal male rats exposed to 500 mg/kg/day DBP via daily gavage on gestation days 12 to 21. Corn oil was used as a negative control, and the antiandrogen flutamide (50 mg/kg/day) 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). DBP reduced the expression of the steroidogenic enzymes 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 DBP group. DBP exposure also caused an increase in the expression of the cell survival proteins testosterone-repressed prostate messenger-2 (TRPM-2) and bcl-2, which may be involved in DBP-induced Leydig cell hyperplasia. A reduction in c-kit (stem cell factor receptor) in the DBP group may contribute to gonocyte degeneration.

MECHANISM OF ACTION

Newly Available Data

Mylchreest et al. (1998) investigated the male-targeted teratogenicity of DBP in order to consider possible endocrine mechanisms. Pregnant CD rats were exposed to 0, 250, 500, or 750 mg/kg/day throughout pregnancy and lactation until postnatal day 20 (n = 10 per dose group). The number of live pups per litter was decreased at the 750 mg/kg/day DBP dose, probably due to maternal effects. Anogenital distance was reduced in male (but not female) pups exposed to 500 and 750 mg/kg/day DBP. Other effects observed in male DBP-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 DBP exposure. It was proposed that DBP's male-specific developmental effects are an anti-androgenic rather than estrogenic.

To support the idea that DBP induces male-developmental effects through anti-androgenic effects, Mylchreest et al. (1999) compared the teratogenicity of DBP to that of flutamide, a known androgen antagonist. Pregnant Sprague-Dawley outbred CD rats were treated with DBP (100, 250, 500 mg/kg/day; n = 10 rats per dose group) or flutamide (100 mg/kg/day; n = 5 rats) or corn oil (control; n = 10 rats) on gestation days 12-21, the period of male reproductive tract development. Male pups were killed and examined either on postnatal day 60 or after sexual maturity, postnatal day 100 to 105. Female pups were killed and examined on postnatal day 20 to 30. Pup examinations focused on the reproductive tissues. An immunohistological evaluation for androgen receptor expression was performed. DBP 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 DBP and flutamide effects. For example, there was some variation in the sensitivities of androgen-dependent tissues between

DBP and flutamide. Inguinoscrotal descent, an androgen-dependent event was impaired by flutamide and not DBP, whereas transabdominal descent (an androgen-independent event) was affected by DBP and not by flutamide. Other androgen-independent events were affected by DBP, as well. Thus, it was proposed that DBP has anti-androgenic effects without interacting directly with the androgen receptor.

Pregnant CD rats were exposed to DBP via oral gavage on gestation days 12 to 21. The exposure levels were 0, 0.5, 5, 50, or 100 mg/kg/day (n=19 - 20 per group) or 500 mg/kg/day (n=11). Beginning on the day of delivery (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 days, and females were killed for necropsy on postnatal day 80 ± 5 days. There were no effects of DBP treatment on rate of pup survival at birth. On postnatal day 14, there was an increase in female pup weights at 5 mg/kg/day DBP and higher doses, 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/day DBP 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; semineferous tubule degradation; focal interstitial cell hyperplasia; and interstitial cell adenoma. Male pups of the 100 and 500 mg/kg/day DBP 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/day DBP represented the lowest observed adverse effect level for DBP in this study. The no-observed-adverse-effect-level (NOAEL) for DBP in this study is 50 mg/kg/day (Mylchreest et al., 2000).

Gray et al. (1999) gave eight pregnant Sprague-Dawley rats daily oral doses of 500 mg/kg from gestation day 14 to postnatal day 3. Ten control rats received corn oil on the same schedule. At five months of age, the male offspring (50 control, 48 DBP-exposed) were killed and examined externally and internal for indications of sexual development. In DBP-exposed 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, epididymes, 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 DBP-exposed

males. 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 months of age, male offspring were examined. In the DBP-exposed 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 DBP-exposed males. Hypospadias was not seen in DBP-exposed LE rat offspring (Gray et al., 1999).

GENOTOXICITY

Previously Available Data

The mutagenic activity of DBP, DMP, and DEP for *Salmonella typhimurium* mutants depended on the assay protocol. In the standard Ames test, DBP and DEP were negative in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without metabolic activation (Florin et al., 1980; Kawachi et al., 1980; Omori, 1976). DEP was also negative in these strains when using a preincubation protocol (NTP, 1980). In a liquid suspension assay with a 4-hour incubation, DBP, DMP, and DEP were positive in strain TA100 without metabolic activation and negative with metabolic activation (Seed, 1982). In a modified Ames test in which histidine and biotin were incorporated into the bottom agar, DBP, DMP, and DEP were negative with and without metabolic activation in strain TA98, and DBP was negative with and without metabolic activation in strain TA100. DMP and DEP were positive in strain TA100 without metabolic activation, and the response was dose related. They were negative in strain TA100 with metabolic activation (Kozumbo et al., 1982; Rubin et al., 1979). DBP and DEP were not mutagenic to *E. coli* (Omori, 1976; Szybalski, 1958).

DNA repair enzyme-deficient *Bacillus subtilis* and *E. coli* were equally or less sensitive to DBP and DEP than the wild-type bacteria (Kawachi et al., 1980; Omori, 1976). Rosenkranz and Leifer (1980) reported that DBP did not affect either the wild-type or the DNA repair enzyme-deficient *E. coli*. There were no measurable zones of growth inhibition for either strain.

DBP was tested for mutagenic activity by reversion analysis of the yeast, *S. cerevisiae*. DBP had no mutagenic effect on the yeast whether the test was conducted with or without metabolic activation (Shanin & von Borstel, 1977).

The effects of DBP on Chinese hamster cell chromosome aberrations and sister chromatid

exchanges (SCEs) have been investigated in several studies. In one study DBP was negative for chromosome aberrations and SCEs(Kawachi et al., 1980). In another study, the mitotic index was not appreciably decreased when the cells were exposed to DBP in ethanol. A significant increase over the vehicle for the number of SCEs was found, but no dosage effect was found for chromosome aberrations or SCEs (Abe & Sasaki, 1977). DBP in a 0.2 % bovine albumin solution was examined in a third study. The percentages of chromosome aberrations with DBP and with bovine albumin were 6 and 1.8 %, respectively. These results did not conclusively prove that DBP caused chromosome aberrations; DBP was called a suspicious compound by the researchers (Ishidate & Odashima, 1977). DBP, DMP, and DEP, in doses of 0.25 mg/ml, had no effect on chromatid aberrations in human leukocyte cultures compared to controls (Tsuchiya & Hattori, 1976).

Newly Available Data

Agarwal et al. (1985) conducted an Ames bacterial reverse mutagenicity test in several strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538, and TA2637) to evaluate the mutagenic ability of DBP, DEP, and DMP. The phthalate doses tested ranged from 100 to 2000 µg/plate with and without 500 µl S9 metabolic activation fraction. DBP at these concentrations induced no cytotoxicity. There was a slight increase in the number of TA100 revertants in the absence of S9 at all DBP doses. This increase in revertant population was not seen in any other strains, nor was it seen in TA100 where S9 was present. Mutagenicity in TA100 suggests that DBP could be a base-pair substitution mutagen. DEP produced significant increases in the numbers of TA100 and TA1535 revertant colonies. Addition of S9 prevented this effect.

DMP caused a slight reduction in the number of TA100 revertant colonies at the 2000 µg/plate dose. Further analysis of the data at the lower doses suggested that the apparent cytotoxicty of DMP may be masking a mutagenic effect, but this was not conclusive. However, DMP produced dramatic increases in the numbers of TA1535 revertant colonies, an effect that reached a plateau at 500 to 750 µg/plate. The effect of DMP on TA1535 was eliminated when S9 was present in the mix (Agarwal et al., 1985).

A study by Kozumbo and Rubin (1991) confirmed that DMP was mutagenic in TA100 with dose-dependent increases in revertant counts at doses ranging from 500 to 4000 µg/plate in the absence of S9. Adding S9 prevented this effect. DMP's metabolites, MMP and methanol, were not mutagenic to TA100.

In an NTP study (NTP, 1995b), DEP (10 - 10,000µg/plate) and DMP (33 - 6,666 µg/plate) were

both negative for mutagenicity in *Salmonella* strains TA98, TA100, TA1535, and TA1537, with and without S9. And in cultured Chinese hamster ovary (CHO) cells, both DEP and DMP induced sister chromatid exchanges in the presence of but not in the absence of S9. Neither DEP nor DMP induced chromosomal aberrations, with or without S9, in CHO cells.

Barber et al. (2000) tested a series of phthalate esters for mutagenicity in the L5178Y mouse lymphoma assay. DBP (0.015, 0.030, 0.040, 0.050, and 0.060 µl/ml) and DMP (0.20, 0.40, and 0.60 µl/ml) were tested with and without S9 microsomal activation. Both DMP and DBP were found to be mutagenic in this assay in the presence but not in the absence of S9. However, the authors reasoned that it was not the diester or even the monoester that produced the mutagenic effects observed. Rather, the authors reasoned that the alcohols produced from the de-esterification in the presence of S9 produced aldehydes which are known to be muatgenic in this assay.

Kleinsasser et al. (2000) evaluated the genotoxicity of DBP in human mucosal cells from the upper respiratory tract. Human mucosal cells were biopsied from the oropharynx and inferior nasal turbinate during tonsillectomy surgery. Cells were prepared according to the alkaline version of the microgel electrophoresis assay to detect single strand breaks after cell suspensions (5×10^4) were incubated with 354 µmol/ml DBP. There was an increase in single strand breaks in DNA of cells incubated with DBP, compared to control cells incubated in DMSO. Cells of the nasal mucosa were more sensitive to DBP-induced single strand breaks than oropharyngeal epithelia.

CARCINOGENICITY

Previously Available Data

Dernehl, 1973 reported that carcinogenesis was not observed in 18-month or longer DBP feeding studies in rats; and Abe and Sasaki (1977) and Kawachi et al. (1980) reported that DBP was noncarcinogenic, but specific details of experiments are lacking.

In a one-year initiation/promotion study in male Swiss mice, the NTP (1995b) tested DEP and DMP as tumor initiators with and without the known skin tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). DEP and DMP were also tested for the potential as promoters with and without the known skin tumor initiator 7, 12 dimethylbenzanthrancene (DMBA). In these studies, neither DEP nor DMP was able to initiate

skin carcinogenesis when promoted by TPA., nor was DEP or DMP able to promote carcinogenesis in skin initiated by DMBA.

Newly Available Data

F344/N rats were dermally exposed to 0, 123, or 369 µg neat DEP (n = 60 rats per sex per group), five days per week for 103 weeks. Animals were weighed and observed for signs of toxicity throughout the study. Survival of dosed rats during the first 15 months was similar to controls, however survival to two years was reduced in male rats in all groups, 8% survival in control rats and 12 % survival in each of the DEP groups. Mean body weights of male rats in the 369 µg group were slightly less than controls throughout the study. The incidence of anterior pituitary adenoma was higher in all groups of male and female rats than the historical incidence. This may be partially responsible for the reduced survivability of the males in all groups. There were no adverse clinical effects of DEP treatment observed, including no evidence of dermatotoxicity (NTP, 1995b). The NTP concluded that there was no evidence of carcinogenic activity of DEP in male and female F344/N rats.

B6C3F_1 mice were dermally exposed to 0, 9, 18, or 37 µg DEP in 100 µl acetone (n = 60 mice per sex per group), five days per week for 103 weeks. Animals were weighed and observed for signs of toxicity throughout the study. The two-year survivability of dosed mice was similar to that of controls. There were no adverse clinical signs and no signs of dermatotoxicity. Mean body weights were unaffected by DEP treatment. Food consumption of dosed animals was similar to or slightly greater than that of control animals. There was an increase in liver neoplasms observed in dosed male and female mice. The incidence of adenoma or carcinoma (combined) was increased in male mice of the 37 µg DEP group in female mice of the 9 and 15 µg DEP groups but not in the 37 µg DEP group. The increased incidence of liver neoplasms was considered equivocal because the incidence of hepatic neoplasms was withing the historical range and because there was no clear dose-response relationship in females. No other treatment-related findings were observed (NTP, 1995b).

CLINICAL ASSESSMENT OF SAFETY

IRRITATION AND SENSITIZATION

Previously Available Data

Patch tests have been performed on human subjects with the phthalates (Kaaber et al., 1979; Schulsinger & Mollgaard, 1980). The cosmetic industry has conducted studies on the skin irritation, sensitization, and photosensitization of a variety of products containing DBP(CTFA, 1976; 1977B; 1979; 1980a; 1980b; 1981d; Hill Top Research, 1976; Ivy Research Laboratories, 1976) as shown in Table 18. These ingredients were mostly non-irritating (with some reports of minimal irritation), non-sensitizing and non-photosensitizing.

Table 18. Skin Irritation and Sensitization

Material Tested	Concen-tration (%)	Method	Number of Subjects	Results	Reference
Nail polish, 9 % DBP	100	Twenty-one 23-hour patches on same site on back. 1-hour rest. Scored (0-3) 1 hour after patch removal.	2 men, 11 women	Composite total score was 247 (out of possible maximum of 819). "Slightly irritating."	Hill Top Research, 1976
Nail polish, 9 % DBP	100	48-hour occluded patch to back or forearm.	25	No irritation observed.	Ivy Research Laboratories, 1976
Nail polish, 9 % DBP	100	Modification of the maximization test (Kligman, 1966). 5 48-hour occluded induction patches on back or forearm with 24-hour rests in between them; 24-hour sodium lauryl sulfate (SLS) pretreatment before first patch. 10-day rest period. 1-hr SLS pretreatment followed by 48-hour challenge patch; scored at patch removal and 24 hours later.	25	"No instances of contact-sensitization."	Ivy Research Laboratories, 1976
Nail preparatio n, 6 % DBP	100	Prophetic patch test (Schwartz & Peck, 1944). 2 24-hour open and closed patches 10 14 days apart. 1 open patch irradiated for 1 min. at a distance of 12 in. with a Hanovia Tanette Mark i lamp (UV). Scored at patch removal and daily for 5 days thereafter (1+ to 3+).	99	No positive reactions were observed. "Nonirritating, nonsensitizing, nonphtotsensitizing."	CTFA, 1976
Nail preparatio n, 6 % DBP	100	Repeated insult patch test (Shelanski & Shelanski, 1953). 10 24-hour open and closed induction patches 24 hours apart. 2-3 week rest. 48-hour challenge patches. Open and closed patches at inductions 1, 4, 7, and 10 and at challenge were irradiated for 1 min. at a distance of 12 in. with a Hanovia Tanette Mark i lamp (UV). Scored at patch removal and daily for 5 days thereafter (1+ to 3+).	48	Five 1+ and one 2+ reactions to open patches at induction. One reaction to an open patch at UV challenge. "Nonirritating, nonsensitizing, nonphotosensitizing."	CTFA, 1976

Table 18 (continued). Skin Irritation and Sensitization

Material Tested	Concen-tration (%)	Method	Number of Subjects	Results	Reference
Nail preparation, 6 % DBP	100	Controlled use study for 4 weeks. Fingernails and eyes were examined each week (1+ to 3+)	47	No positive reactions were observed. "Nonirritating."	CTFA, 1977
Deodorant, 4.5 % DBP	100	21-day cumulative irritancy test (Phillips et al., 1972). 12 24-hour occluded patches of 0.3 g applied to the back over 21 days. each patch scored at removal (0-4).	1 man, 11 women	Total score calculated on the basis of 10 subjects was 140.8 out of a possible maximum of 840. "Slightly irritating."	CTFA, 1979
DBP	5 % in petrolatum	48 hour closed patch test on back. Readings 48 and 72 hours after patch application.	7 men and 48 women who wore dentures and suffered from "burning mouth syndrome"	No positive reactions.	Kaaber et al., 1979
Deodorant, 4.5 % DBP	100	Modification of the repeated insult patch test (Draize, 1959; Marzulli & Maibach, 1977). 8 48-hour induction patches of 0.2 g on upper arms. 2-week rest. 72-hour challenge at original and new sites. Scored at 48 and 96 hours (0-3).	41	9 reactions of 1 (mild erythema) at induction and 1 equivocal reaction at original site at 96-hour challenge observation. "Not an allergen under conditions of this test."	CTFA, 1980a
Deodorant, 4.5 % DBP	100	Modification of repeated insult patch test (Draize, 1959; Marzulli & Maibach, 1977). 10 24-48 hour occlusive induction patches of 0.2 g ~ 3 times a week. Patches applied to the back. 10-day rest. 72-hour challenge patch. Scored after patch removal (0-4).	159	4 equivocal reactions and 1 score of 1 (erythema) during induction. One equivocal and 1 score of 1 at challenge. "Does not appear to be an allergen under test conditions."	CTFA, 1980b
DBP, DMP, and DEP	All phthalates at 2 % in petrolatum	48 hour closed patch test. Joint study by International Contact Dermatitis Research Group.	1532	1 positive reaction.	Schulsinger & Mollgaard, 1980
Deodorant, 4.5 % DBP	100	Antiperspirant efficacy test, normal use conditions (Federal Register 43: 46694-732, Oct. 10, 1978). Applied 0.5 g/day for 2 days.	43	"No irritation observed" in the axillary region.	CTFA, 1981d
Nail enamel, 5.32 % DBP	0.3 ml	Maximization test. Under occlusive patch on forearm for 5 48-hour periods. 10 day rest, challenge patch for 24 hours	25	No contact sensitization	Ivy Research Laboratories, 1984
DBP	5.0 % (w/w)	Patch test on subjects with allergies to glues and/or plastics.	839	No allergic reaction	Tarvainen, 1995
DBP	5.0 % (w/w)	Patch test on subjects with allergies to glues and/or plastics.	173	No allergic reaction	Kanerva et al., 1997
Nail enamel, 2.74 % DBP	0.2 ml	Repeated insult patch test. Under occlusive patch for 9 24-hour periods. 2 week rest, challenge patch for 24 hours.	105	No contact sensitization	Harrison Research Laboratories, 1998
DBP	5.0 % (w/w)	Patch test on subjects with allergies to glues and/or plastics.	357	No allergic reaction	Kanerva et al., 1999

Newly Available Data

Hill Top Research, Inc. (1968) conducted a study in which undiluted DEP was a vehicle control in a primary irritation patch test on a coded unidentified cosmetic product. Forty-five human subjects were each exposed to 0.5 ml DEP under an occlusive patch for 24 hours. Test sites were scored for signs of primary irritation upon removal of the patch. Little or no primary irritation was observed following application of the DEP vehicle.

Hill Top Research, Inc. (1971) evaluated DEP for the potential to induce dermal irritation or sensitization in a Repeated Insult Patch Test. 0.5 ml of a test material containing DEP (concentration not reported) was applied to the upper arm of each of 37 human subjects and occluded for 24 hours. Applications were repeated nine times, and the exposure sites were scored for irritation each time. After a two-week rest period, subjects were challenged with additional applications of the test material. the challenge sites were scored for sensitization 48 hours after the challenge application. There were no signs of sensitization or irritation to the test material.

Ivy Research Laboratories, Inc. (1973) exposed ten healthy adult male volunteers to DEP (amount not reported) once daily for ten days under occlusion. Exposure sites were scored for primary irritation daily. There were no instances of primary irritation from DEP.

In a study reported by the E. I. du Pont de Nemours and Company (1974), DMP was applied in one-inch square patches to the arms of eight men and the arms or legs of eight women and held in place for six days. The patches were removed for a two-week rest period, and then new patches were applied for two days as a challenge to test for sensitization. The skin under the patches was examined at two and six days after the initial application and upon removal of the challenge patches. The DMP treatments did not produce any skin irritation or sensitization in any of the 16 subjects.

Epstein (1978) reported that DEP produced no allergic reaction in 26 healthy adult volunteers who were exposed to undiluted DEP under occlusive patch for 48 hours five times. The subjects were challenged with an additional 48-hour exposure after a 10 to 14 day rest period. Challenge sites were subsequently scored for signs of sensitization.

Ivy Research Laboratories, Inc. (1984) tested twenty-five healthy adult volunteers in a maximization test to determine the contact sensitizing potential of a nail enamel containing 5.32 % DBP. 0.3 ml of the nail enamel product was applied under an occlusive patch to the same site on the volar aspect of the forearm for five 48-hour periods. Because the material was found to be non-irritating, 1.5 % sodium lauryl sulfate (SLS)

was applied prior to the application of the test material. After a ten-day rest period, a challenge patch of the test material was applied to a different site for 48 hours under an occlusive dressing. The challenge site was pretreated with a 5.0 % SLS. The challenge site was scored for signs of sensitization immediately after removal of the dressing and 24 hours later. There were no instances of contact sensitization from this nail enamel product.

In a study at the Essex Testing Clinic, Inc. (1989), approximately 2 ml of 99.8 % DEP was applied nine times to the skin of 100 human subjects and occluded for 24 hours at a time with 24- to 48-hour rest periods between applications. The patch sites were evaluated for edema and erythema throughout and after the exposure period. One subject showed a barely perceptible erythema which was not considered irritant or allergic in nature. None of the other subjects showed any signs of irritation or sensitization. A follow-up study using the same methods, but with an additional challenge test 10 to 21 days after the ninth regular application. In the challenge 2 ml of DEP was applied to an area of skin that had not been previously exposed to DEP. The site was scored 24 and 48 hours later. Again, DEP did not cause any irritation or sensitization (Essex Testing Clinic, Inc., 1990).

The US Environmental Protection Agency (1994a) reported that DEP did not induce irritant or allergic contact dermatitis in 50 human subjects who were exposed to a 9 Repeated Insult (occlusive) Patch Test. In this test subjects were exposed to 2 ml pure DEP on an area of dorsal skin between the scapula and waist. The treated area was covered with a bandage for 24 hours. The treated area was scored for erythema and other signs of irritation by trained personnel. The procedure was repeated until nine exposures were completed. One subject out of the 50 showed a "barely perceivable redness" after the first exposure. No erythema was seen in that subject after the first exposure, and it was not seen at all in any of the other subjects.

The US Environmental Protection Agency (1994b) reported a similar study using 100 human subjects and the same exposure protocol resulted in only one subject having a "barely perceptible" non-specific patch test response to neat DEP during the challenge phase of the study. None of the other subjects had any response to the patch testing.

Api (1997) evaluated DEP for potential use as a vehicle in a phototoxicity assay using an *in vitro* human skin model, Skin$^{2™}$. In this model, cultured skin cells are exposed to the test compound on an applicator pad overnight in an incubator at 37°C. The next day, the applicator pad was removed, and the

medium was changed. The plates were then either returned to the incubator for an hour, or they were exposed to UVA and UVB radiation for 30 minutes, then placed in the incubator for 30 minutes. The irradiated and non-irradiated cells were then rinsed, transferred to multi-well plates, and incubated overnight. On the next day, cell viability was assessed and compared to blank (untreated) controls. The DEP concentrations used were 10, 30, 50, 70, and 100%. The 30% DEP and higher treatments killed virtually all of the cells in the culture system. The 10% DEP treatment resulted in 70 % viability of the cells. Results were similar between irradiated and non-irradiated cells. However, due to the cytotoxicity that author concluded that DEDP would not be a suitable vehicle for the Skin$^{2™}$ assay.

Hill Top Research, Ltd. (1997a and b) conducted separate human photoallergy studies on unidentified fragrance materials used a vehicle control that contained 75 % ethanol and 25 % DEP. The two studies used 23 and 29 human subjects, respectively. In each study, six repeated 24-hour occluded patch applications of 0.2 ml of the vehicle control solution were performed in three weeks (two applications per week). Patch sited were exposed to UVB/UVA radiation within ten minutes of removal of the test article. After a two-week rest period in which the application site was not exposed to the vehicle solution or to ultraviolet radiation, the subjects were exposed to 0.2 ml of the vehicle solution on three naive sites. Two of the challenge sites were exposed to UVA and UVB radiation within ten minutes of the challenge application. In both studies, the vehicle solution containing 25 % DEP did not show evidence of systemic sensitization or photoallergenicity.

The Harrison Research Laboratories (1998) conducted a study in which a nail enamel product containing 2.74 % DBP was evaluated using the Repeated Insult Patch Test (RIPT). One hundred five human subjects were each exposed to 0.2 ml of the nail enamel for 24 hours under occlusion nine times over a period of three weeks. After a two-week rest period, a new site was challenged with the test material for 24 hours under occlusive patch. The challenge site was scored for signs of dermal sensitization upon removal of the challenge patch and 48, 72, and 96 hours later. The nail enamel product tested (containing 2.74 % DBP) did not induce contact dermal sensitization.

Hill Top Research, Inc. (1998; 1999) conducted two human phototoxicity studies of unidentified fragrance material used a vehicle containing 25% DEP in ethanol. One study consisted of 29 human subjects, the other had 35 subjects. In each study single 24-hour application of duplicate patches was made to naive sites. The dose at each site was 0.3 ml of the 25 % DEP solution. One of the duplicate sites was

exposed to UVB and UVA radiation, while the other site was not exposed to ultraviolet light. The treatment sites were evaluated for signs of irritation at 1, 24, 48, and 72 hours after application of the DEP vehicle. In one study the DEP vehicle did not produce any signs of irritation or phototoxicity. In the other study, the 25 % DEP vehicle showed some evidence of primary irritation but not phototoxicity.

CASE REPORTS

Previously Available Data

McLaughlin (1946) reported that proper treatment of a human corneal burn caused by DMP resulted in healing within 48 hours and no loss of vision.

Cagianut (1954) reported that a chemical worker accidentally swallowed approximately 10 g of DBP. The worker's symptoms included nausea, vomiting, dizziness, headache, pain and irritation in the eyes, conjunctivitis, and toxic nephritis. He recovered completely after 2 weeks.

Milkov et al. (1973) reported on the health status of 147 workers subjected to prolonged occupational exposure to mixtures of phthalate plasticizers (including DBP); many workers had a moderately pronounced toxic polyneuritis.

Newly Available Data

While Wilkinson and Beck (1992) note that sensitization to DBP in cosmetics and pharmaceuticals is rare, they reported a case in which a patient exhibited acute swelling and erythema of the groin and genitals after using a cream used to treat intertrigo. The patient who had previous occupational exposure to epoxy resin was sensitive to DBP in a patch test.

In a patch test study by Tarvainen (1995), 0 out of 839 patients with suspected allergic contact dermatitis to plastic or glue had allergic reactions to 5% DBP.

Kanerva et al. (1997) reported that, in patch tests of humans suspected to have allergic contact dermatitis reactions to plastics and/or glues, 0 out of 173 patients had any allergic response to 5% (w/w) DBP). Kanerva et al. (1999) patch-tested DBP at a concentration of 5.0% (w/w) in human subjects who were suspected of having allergic contact dermatitis reactions to glues and/or plastics due to occupational exposure. Zero out of 357 patients showed an allergic reaction to DBP in a period from 1991 to 1996.

Gall et al. (1999) described an incident in which a patient exhibited anaphylactic shock from a drug capsule containing DBP. Itching, urticaria, and respiratory distress occurred within 20 minutes of

consumption of the capsule. A prick test of the compounds used to make up the capsule coating was conducted on the volar aspect of the arm. Undiluted DBP induced a wheal of 3 cm in diameter which expanded over the entire arm. Prick tests with DBP in 10 healthy volunteers were negative.

OTHER ASSESSMENTS

Newly Available Data

The Agency for Toxic Substances and Disease Registry (ATSDR) determined the Minimal Risk Level (MRL) for several compounds. The MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specific duration of exposure. The MRL for DBP at an intermediate duration oral route was reported to be 0.6 mg/kg/day. The MRL for DEP was 7 mg/kg/day for acute oral exposure and 6 mg/kg/day for intermediate duration oral exposure (Selene et al., 1998).

A 1999 review of DBP data by the Phthalate Esters Panel of the American Chemistry Council concluded that "although DBP has demonstrated adverse effects in rodents, the NOEL levels are very high compared to human exposure levels, indicating that there is little human risk to humans for adverse reproductive or developmental effects" (Phthalate Esters Panel, American Chemistry Council, 1999).

In response to the publication of the CDC data on urine phthalate concentrations presented earlier (Blount et al., 2000), the Phthalate Esters Panel of the American Chemistry Council reported the estimated intake for DEP and DBP to be 57 µg/kg/day and 7 µg/kg/day, respectively. These exposure estimates are well below the EPA reference doses (RfD) of 800 µg/kg/day and 100 µg/kg/day for DEP and DBP, respectively (David, 2000).

Kohn et al. (2000) used the CDC data presented earlier (Blount et al., 2000) to calculate the estimated exposures for the 20- to 40-year-old women. The estimated median and maximum exposures were 13 and 170 µg/kg/day for DEP and 1.7 and 113 µg/kg/day for DBP, respectively.

Baskin et al. (2001) suggested that DBP is among the list of environmental endocrine disruptors that may contribute to a high incidence of hypospadias in the United States, roughly 1 in 125 live male births.

DBP was reviewed in 2000 by the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (CERHR). The CERHR Expert Panel considered the developmental toxicity of DBP based on all possible routes of human exposure. They noted the malformations seen in perinatally exposed

animals, particularly to developing males, and agreed that these effects seem to be through an anti-androgenic rather than estrogenic mechanism. However, in the conclusion of this report the CERHR Expert Panel had "minimal concern about the effects to human development and development of the reproductive system from current estimated exposure to DBP" (NTP-CERHR, 2000).

In January, 2001, a Risk Assessment on DBP was released by the European Commission. This document covered the environmental as well as human health aspects of DBP and included a section on exposure through cosmetics, namely nail care products. The consideration of DBP in nail products focused on inhalation as the primary route of exposure. Using a NOAEC of 509 mg/m^3 and a yearly inhalation exposure estimate for DBP from nail polish of 8.59×10^{-9} mg/m^3, the reviewers decided that the high safety margin of 6×10^6 indicated that there is "no concern for consumers using nail polish containing DBP" (Netherlands Organization for Applied Scientific Research and National Institute of Public health and the Environment, 2001).

The Research Institute for Fragrance Materials (RIFM) reviewed the use and safety of DEP in fragrance and cosmetic products. It was noted that DEP does not appear to raise the concerns of reproductive toxicity and teratogenicity seen with some other phthalate esters. There was a low level of concern for carcinogenic hazard from DEP in the current conditions of use. The RIFM monograph concluded that "the potential for dermal exposure to DEP, based on use data with conservative assumptions about maximum use patterns, is within the levels of exposure deemed safe by other routes of exposure and is not considered to present any significant toxic liability for its current uses as a solvent and vehicle in cosmetic product" (Api, 2001).

The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP; 2002) is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food consumer products. The SCCNFP calculated a margin of safety of DEP in the context of a "worst case" exposure of humans to DEP via cosmetic products. Assuming that a 60 kg person applies 1120 mg DEP (in a cosmetic product) to the skin, and assuming a 5 % absorption through the skin (Mint et al., 1994), the SCCNFP used the following calculation to determine the systemic exposure dose (SED): SED = (1120 mg x 5 %)/60 kg = 0.93 mg/kg. Given a NOAEL of 150 mg/kg (Brown et al., 1978) and the formula, Margin of Safety = NOAEL/SED, the SCCNFP calculated a Margin of Safety of 150 for DEP exposure from cosmetic product exposure. Based on this and other safety data, the SCCNFP concluded

that "the safety profile of Diethyl-phthalate supports its use in cosmetic products at current levels." Additionally, the SCCNFP did "not recommend any specific warnings or restrictions under the currently proposed conditions of use."

SUMMARY

Dibutyl phthalate (DBP), Diethyl Phthalate (DEP), and Dimethyl Phthalate (DMP) were originally reviewed by the CIR Expert Panel in January, 1984, with the conclusion that "these compounds are safe for topical application in the present practices of use and concentrations in cosmetics." This document is intended to update the Panel on the information that has come available after the 1984 Final Report was issued.

DBP, DEP, and DMP are phthalate diesters that are used in cosmetics as plasticizers, solvents and fragrance ingredients. DEP is also used as a denaturant. DBP is found primarily in nail care products (at concentrations up to 15 %) and in some hair care formulations (up to 0.1 %). DEP is found in certain bath preparations, fragrance products, deodorants, lotions, and other skin care products. The highest reported concentration of use of DEP is 11 % in perfumes. DMP is an ingredient in some hair care products, including aerosol fixatives. The reported maximum concentration of use of DMP in cosmetics is 2 % in aerosol hair sprays.

DBP, DEP, and DMP all absorb readily through the skin and through the GI tract. Once absorbed, most short-chain phthalate diesters are hydrolyzed to the corresponding monoester and alcohol. The phthalates and their metabolites distribute to most tissues, and cross the placenta, but they do not accumulate in any tissue type. Phthalates are quickly eliminated in the urine, usually as the corresponding monoester or its glucuronide conjugate. However, humans and primates metabolize longer-chain diester phthalates (e.g., DEHP) into the glucuronide-conjugated monoester forms to a much larger extent than do rats. Also, rats excrete three to four times more free unconjugated MBP than do hamsters given similar doses of DBP or MBP, possibly due to greater testicular β-glucuronidase activity in rats than in hamsters. Phthalates undergo some enterohepatic cycling, and some phthalate is eliminated in the feces.

The Approximate Lethal Dose for orally administered DBP was 7500 mg/kg in rats. Other acute oral toxicity studies have found LD_{50} values for DBP ranging from < 7.5 g/kg to 23 g/kg in rats. The oral LD_{50} values for DEP were 8.2 to 9 g/kg in rats and 1.0 g/kg in rabbits. The oral LD_{50} for DMP was 6281 mg/kg in

female mice.

In short-term oral exposures to DBP or its metabolite, monobutyl phthalate (MBP), the liver was the primary target organ showing biochemical signs of toxicity at 5 % DBP mixed in feed. DBP was moderately toxic to rats when mixed in feed at a concentration of 1.25 % for one year. DMP showed no evidence of toxicity when given to rats by oral gavage, 1 ml/kg twice a week for 1½ years. DEP administered to rats at concentrations of 1 and 5 % in feed for 16 weeks reduced body weight gain and at 5 % produced enlarged livers and kidneys. Rabbits exposed to 3 ml/kg/day DEP for 8 days appeared normal during dosing and for two weeks after. Rats fed 10000 ppm DBP mixed in feed for three months showed biochemical signs of liver toxicity and histopathological signs of testicular toxicity. Rats exposed to 50 ppm DEP in drinking water for 120 days showed biochemical signs of liver toxicity.

No signs of toxicity from DBP inhalation were found in rats exposed to up to 500 mg DBP/m^3 air for 6 hours a day over a period of 20 days. Rats exposed to 1.5 mg/m^3 DBP vapor for 6 hours per day for one month showed no signs of toxicity. Rats exposed to 0.5 and 50 mg/m^3 DBP mist for 6 hours per day for 6 months produced dose-dependent reductions in growth and organ weights.

DBP, DEP, and DMP were not found to be dermal irritants or sensitizers in several studies using human and animal subjects.

Orally-administered (in feed or by gavage) DBP and its metabolite MBP have reproductive and developmental effects in rodents, with impaired male development being the most sensitive effect.

When pregnant rats and mice were exposed to 1.0 % DBP in powdered feed throughout gestation, the pregnancy outcome showed reductions in fertility, number of pups per litter, number of live pups, and body weights of pups. Adult male rats exposed to 1.0 % DBP showed signs of liver and kidney toxicity and reduced weights of the prostate, testes, and seminal vesicles. Pregnant rats exposed to 2 % DBP in feed throughout pregnancy had a high incidence of preimplantation loss and resorptions, and no male pups were born alive. Exposure to 1 or 2 % DBP in feed only during the latter half of gestation did not show the preimplantation loss and resorption rate seen in rats exposed throughout pregnancy. However, the increased survivability of these fetuses allowed the morphological defects of developing fetuses to be observed. These defects included reduced body weights in both sexes at 2 % DBP, reduced anogenital distance and undescended testes in male fetuses at 1 and 2 % DBP, and increased incidence of cleft palate and fused sternebrae.

Oral intubation (gavage) of DBP in rats during gestation produced similar effects to those seen in the feeding studies described above. Pregnant rats given oral doses of approximately 0.63 to 0.75 g/kg/day and higher on certain gestation days produced litters with higher incidences of fetal toxicity and malformations. Exposure to DBP on gestation days 7 through 9 or on days 13 through 15 results in increased incidence of skeletal malformations such as cleft palate, fused sternebrae, and vertebral anomalies, as well as dilatation of the renal pelvis and undescended testes. However, exposure to DBP on gestation days 10 through 12 did not produce these effects, suggesting that DBP teratogenicity may be age-dependent. Prenatal exposure to MBP appears to produce fetotoxicity and teratogenicity similar to DBP, following the same patterns of age-dependent sensitivity and dose efficacy. This supports the proposal that it is the monoester metabolite that produces the developmental toxicity of DBP and other phthalates.

DEP was fed to mice at concentrations up to 2.5 % (calculated to be 3.64 g/kg/day) in a continuous breeding protocol. There were no effects of DEP on fertility or pregnancy outcome in the F_0 generation. F_1 male mice of the 2.5 % DEP group had enlarged prostates and reduced sperm counts, but sperm motility and morphology were not affected. The F_2 generation showed no treatment-related differences between DEP and control groups. Pregnant rats fed up to 5.0 % DEP mixed in feed on gestation days 6 through 15 produced no treatment-related alterations in fetal viability or development.

Repeated dermal application of 2 ml/kg up to 50 % DEP to pregnant rabbits on gestation days 6 through 18 did not produce maternal or fetal toxicity or affect fetal development.

DMP was not fetotoxic or teratogenic when administered dermally (in rats) or orally (in rats and mice) during gestation.

Exposure to some phthalates has been shown to cause impairments of normal male development in rodents. The documented male-specific effects of phthalates include malformations of the epididymis and vas deferens, undescended testes, hypospadias, retention of thoracic nipples, and reduced anogenital distance. DEP and DMP did not cause the dramatic effects on male development seen with longer-chain dialkyl phthalates. Many studies have reviewed the mechanisms of the male-targeted toxicity of phthalates. DBP, DEP, and DMP have weak or no binding affinity for estrogen receptor and do not affect estrogen-regulated developmental endpoints. An anti-androgenic mechanism has been proposed, but many studies show that these phthalates do not bind with androgen receptors, either. However, phthalate esters inhibit the synthesis of testosterone, which is an important hormone in normal male development. DBP has also been

shown to inhibit the action of Müllerian Inhibiting Substance produced by Sertoli cells.

DBP, DEP, and DMP have each been screened for mutagenicity in the Ames bacterial reverse mutation assay. DBP caused an increase in the number of TA100 revertants in the absence but not in the presence of S9 rat liver fraction. DEP caused increases in the numbers of TA100 and TA1535, but this effect was also eliminated by the presence of S9. DMP caused an increase in the number of TA1535 revertants, but S9 prevented the effect.

Phthalates are a matter of concern for those responsible for public health and have been (and continue to be) reviewed by many government and international organizations. Phthaltes are ubiquitous in the modern environment. The monoester metabolites of phthalates have been detected in the urine of an adult reference population and in the urine of young children in a small pilot study. Environmental exposure to phthalates and other endocrine disruptors have been proposed to be linked to an increased incidence of hypospadias in humans. The developmental effects of phthalates seen in rodents raise questions about the potential for human health risks. However, these effects seen in rodents are at much higher exposure levels than humans are likely to encounter, and they are subject to the species differences in the metabolism of phthalates. The estimated median exposure levels of DEP and DBP are 57 µg/kg/day and 7 µg/kg/day, respectively, while the U.S. EPA reference doses (RfD) for DEP and DBP are 800 µg/kg/day and 100 µg/kg/day, respectively. Thus, the human exposure is well below the safety limits set by the U.S. EPA. Even the median exposure levels of the highest-exposed group (women aged 20 to 40 years) are well below the RfD's. Exposure levels were not available for DMP.

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