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Preliminary Materials
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Preliminary Materials for the Integrated Risk Information System (IRIS) Toxicological Review of Diethyl Phthalate (DEP)

[CASRN 84-66-2]

March 2014

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PREFACE

This draft document presents a planning and scoping summary, information on the approaches used to identify pertinent literature and primary studies, results of the literature search, approaches for selection of studies for hazard identification, and presentation of characteristics and information from primary studies in evidence tables and exposure-response arrays for diethyl phthalate (henceforth referred to as DEP) prepared under the auspices of EPA's Integrated Risk Information System (IRIS) Program. This material is being released for public viewing and comment prior to a public meeting, providing an opportunity for the IRIS Program to engage in early discussions with stakeholders and the public on data that may be used to identify adverse health effects and characterize dose-response relationships.

The planning and scoping summary includes information on the uses of DEP, occurrence of DEP in the environment, and the rationale and scope for the development of the assessment. This information is responsive to recommendations in the 2009 National Research Council (NRC) report *Science and Decisions: Advancing Risk Assessment* (NRC, 2009) related to planning and scoping in the risk assessment process.

The preliminary materials are also responsive to the NRC (NRC) 2011 report *Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde* (NRC, 2011). The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that "the committee recognizes that the changes suggested would involve a multi-year process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others." Phase 1 of implementation has focused on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focused on assessments near the end of the development process and close to final posting. Phase 2 of implementation is focused on assessments that are in the beginning stages of assessment development. The IRIS DEP assessment is in Phase 2 and represents a significant advancement in implementing the NRC recommendations. In the development of this assessment many of the recommendations are being implemented in full, while others are being implemented in part. Achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and independent external peer review. Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC, including the development of a standardized approach to describe the strength of evidence for

noncancer effects. On May 16, 2012, EPA announced[1] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

The literature search strategy, which describes the processes for identifying scientific literature, screening studies for consideration, and identifying primary sources of health effects data, is responsive to NRC recommendations regarding the development of a systematic and transparent approach for identifying the primary literature for analysis. The preliminary materials also describe EPA's approach for the selection of primary studies to be included in the evidence tables, as well as the approach for evaluating methodological features of studies that will be considered in the overall evaluation and synthesis of evidence for each health effect. The development of these materials is in response to the NRC recommendation to thoroughly evaluate critical studies with standardized approaches that are formulated and based on the type of research (e.g., observational epidemiology or animal bioassays). In addition, NRC recommendations for standardized presentation of key study data are addressed by the development of the preliminary evidence tables and preliminary exposure-response arrays for primary health effect information.

EPA welcomes all comments on the preliminary materials in this document, including the following:

- the clarity and transparency of the materials;
- the approach for identifying pertinent studies;
- the selection of primary studies for data extraction to preliminary evidence tables and exposure-response arrays;
- any methodological considerations that could affect the interpretation of or confidence in study results; and
- any additional studies published or nearing publication that may provide data for the evaluation of human health hazard or dose-response relationships.

The preliminary evidence tables and exposure-response arrays should be regarded solely as representing the data on each endpoint that have been identified as a result of the draft literature search strategy. They do not reflect any conclusions as to hazard identification or dose-response assessment.

After obtaining public input and conducting additional study evaluation and data integration, EPA will revise these materials to support the hazard identification and dose-response assessment in a draft Toxicological Review that will be made available for public comment.

[1] EPA Announces NAS' Review of IRIS Assessment Development Process. 05/16/2012. http://yosemite.epa.gov/opa/admpress.nsf/0/1ce2a7875daf093485257a000054df54?OpenDocument

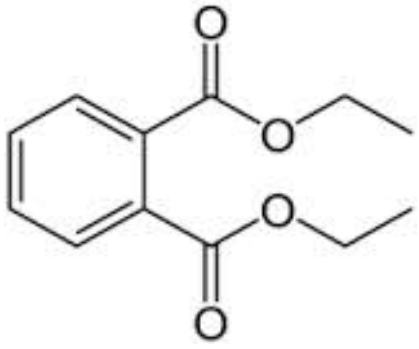
1. PLANNING AND SCOPING SUMMARY

1.1. DEP Chemistry and Uses

In the 1980's United States DEP production was around 20 million pounds per year and in 2005 and 2006 production was between 10 and 50 million pounds.[2,3] It was listed under the EPA's 1990 High Production Volume Challenge Program.[4]

DEP is a colorless liquid with slight aromatic odor. It is soluble in water and slightly volatile. Impurities in technical DEP include isophthalic acid, terephthalic acid and maleic anhydride at levels of less than 1%.[5]

The DEP molecule contains two "ester" chemical groups. Ester chemical groups are generally susceptible to being hydrolyzed by a number of biotic and abiotic processes. Cleaving one DEP ester group leads to the formation of a monoester (monethyl phthalate – MEP) and cleaving both ester groups produces the diacid metabolite/degradate, phthalic acid.[6]



Diethyl Phthalate

($C_{12}H_{14}O_4$; CASRN 84-66-2)

DEP is used to improve the performance and durability of a number of products. As a plasticizer, it is added to plastic polymers to help maintain flexibility. It has been used in a variety of products including plastic films, rubber, tape, toothbrushes, automotive components, tool handles and toys. In addition to plastics, DEP is present in a wide range of personal care products (e.g., cosmetics, perfume, hair spray, nail polish, soap, detergent, and lotions), industrial materials (e.g., rocket propellent, dyes, packaging, sealants and lubricants), and medical products (e.g., enteric

[2] http://www.epa.gov/oppt/chemrtk/hpvis/hazchar/Category_%20Phthalate%20Esters_March%202010.pdf
[3] http://cfpub.epa.gov/iursearch/2006_iur_companyinfo.cfm?chemid=6514&outchem=both
[4] http://www.epa.gov/hpv/pubs/update/hpv_1990.pdf
[5] http://www.atsdr.cdc.gov/toxprofiles/tp73.pdf
[6] http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf

coatings on tablets and in dental impression materials).[7,8] Previous uses as an inert ingredient in pesticide formulations are no longer allowed in the U.S.[9]

1.2. DEP in the Environment

DEP can be released during its production, incorporation into products, product use and disposal in landfills or during incineration. Because DEP is generally added to products, but not covalently bound to them, it can be released from the products during their use. DEP from personal care products is introduced into wastewater during bathing and washing. Consequently, DEP can then be present in the discharge from waste water treatment plants to ambient natural water bodies.[10] In a review of DEP concentration in surface water in North America and Western Europe, the geometric mean concentrations ranged from approximately 0.01 to 0.5 µg/liter.[11] DEP has been detected in 4 to 5% of soil and groundwater samples from sites on the National Priorities List.[12]

DEP has been observed to degrade relatively quickly through biological processes in natural waters and soils.[13] It does not appear to bioaccumulate and has a relatively low propensity to bioconcentrate. Measured environmental half lives in water and soil are on the order of days and are largely dependent upon the quantity of microbial life in the media. In soil, DEP binds weakly to organic matter suggesting that it could leach into groundwater, but rapid biodegradation reduces the leaching potential. Because it is semivolatile, DEP exposed to air can partition into the atmosphere where its half-life is approximately one day.[14]

Humans can be exposed to DEP in a number of settings and through the dermal, oral, and inhalation routes. Exposure to DEP has been documented in occupational, medical, and residential settings,[15] and DEP has been identified as a contaminant of concern in at least 84 Superfund sites.[16] Dermal exposure through the use of personal care products (e.g., cosmetics, shampoo, lotion, etc.) has been identified as an important exposure pathway.[17] Phthalate levels in cosmetics are reported to have declined considerably from 2004 to 2010.[18] Inhalation of indoor air is another pathway of exposure to DEP. DEP can off-gas from materials to which it was added and be inhaled in gas form

[7] http://www.epa.gov/oppt/chemrtk/hpvis/hazchar/Category_%20Phthalate%20Esters_March%202010.pdf
[8] http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf
[9] http://www.gpo.gov/fdsys/pkg/FR-2012-03-14/html/2012-6164.htm
[10] http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+@rel+84-66-2
[11] http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf
[12] http://www.atsdr.cdc.gov/toxprofiles/tp73.pdf
[13] http://www.epa.gov/oppt/chemrtk/hpvis/hazchar/Category_%20Phthalate%20Esters_March%202010.pdf
[14] http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf
[15] http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf
[16] http://cumulis.epa.gov/supercpad/cursites/srchsites.cfm
[17] http://www.nicnas.gov.au/communications/publications/information-sheets/existing-chemical-info-sheets/diethyl-phthalate-dep
[18] http://www.fda.gov/cosmetics/productandingredientsafety/selectedcosmeticingredients/ucm128250.htm

or as DEP bound to airborne dust.[19] Oral exposure can occur through eating food or drinking water containing DEP. Surgical tubing can be a significant source of phthalate exposure to individuals undergoing certain medical treatments, but DEP levels in dialysis tubing and most other types of medical tubing are reportedly relatively small.[20]

The NRC concluded that infants and children may be especially vulnerable to phthalate exposures during critical stages of growth and development because they have higher exposure levels and are exposed through critical developmental stages.[21] An important pathway of exposure identified for children is mouthing toys that contain DEP.[22] Other potentially important routes of exposure to DEP or MEP for young children are consumption of breast milk, hand-to-mouth exposure of DEP-containing house dust, and use of personal care products intended specifically for infants that contain DEP.[23,24,25] DEP's metabolite, MEP, has been detected in 93% of amniotic fluid samples suggesting that fetuses are exposed in the womb and DEP exposure has been documented during many stages of growth and development.[26] Biomonitoring data show young children as having higher exposures for many phthalates, however, a recent nationally representative biomonitoring study from the National Health and Nutritional Examination Survey found consistently lower levels of MEP in the urine of children (6 to 11 years old) than in adults.[27,28] MEP is the major metabolite of DEP and has been measured in a number of biomonitoring studies including analyses based on age, sex and race.[29,30,31]

1.3. Rationale for the Development of the Toxicological Review

The existing IRIS assessment for DEP was last revised in 1993[32] and much research has been conducted on health effects of DEP exposure in the last 20 years, including several epidemiological studies. Given the documented widespread human exposure to DEP, the IRIS Program is developing an assessment of DEP to address multiple needs. Several activities that would benefit from the IRIS assessment of DEP are presented below:

[19] http://www.nap.edu/openbook.php?record_id=12528
[20] http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf
[21] http://www.nap.edu/openbook.php?record_id=12528
[22] http://www.nicnas.gov.au/communications/publications/information-sheets/existing-chemical-info-sheets/diethyl-phthalate-dep
[23] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1367843/
[24] http://dx.plos.org/10.1371/journal.pone.0062442
[25] http://pediatrics.aappublications.org/content/121/2/e260.long
[26] http://www.nap.edu/openbook.php?record_id=12528
[27] http://www.jhsph.edu/research/centers-and-institutes/center-for-excellence-in-environmental-health-tracking/Third_Report.pdf
[28] http://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Sep2013.pdf
[29] http://www.nap.edu/openbook.php?record_id=12528
[30] http://www.cdc.gov/biomonitoring/DEP_BiomonitoringSummary.html
[31] http://www.jhsph.edu/research/centers-and-institutes/center-for-excellence-in-environmental-health-tracking/Third_Report.pdf
[32] http://www.epa.gov/iris/subst/0226.htm

- EPA programs are seeking an updated DEP IRIS assessment for toxicity values needed to conduct risk assessment and define remediation goals.
    - DEP has been identified at more than 80 Superfund sites as a contaminant of concern. Risk assessors and risk managers of Superfund and Corrective Action hazardous waste sites generally rely on IRIS information when it is available to determine remediation goals for contaminants.
    - DEP is a Resource Conservation and Recovery Act (RCRA) listed hazardous constituent and is a widespread environmental contaminant associated with the manufacturing and disposal of plastics. DEP is frequently a RCRA concern in industrial ponds (surface impoundments) and in air around hazardous waste incinerators.
- Under the Safe Drinking Water Act, EPA is required to update its Contaminant Candidate List (CCL) every five years and identify those contaminants that may warrant future regulatory action. EPA uses a multi-step process to evaluate occurrence and health information to determine the substances that are included on the CCL. IRIS Reference Values, cancer dose-response information and cancer descriptors, when they are available, are used to evaluate health effects of potential CCL chemicals. DEP was considered for inclusion on the third CCL (CCL 3) but was not included.[33] DEP was also nominated by the public to be considered for inclusion on the CCL in the future. Revised and updated health effect information would be informative in future CCL determinations regarding DEP.
- Because of children's unique exposure scenarios and potential sensitivities, EPA's Office of Children's Health Protection has identified DEP as a priority and is seeking an IRIS assessment of DEP toxicity.

1.4. General Scope of the Toxicological Review

The Toxicological Review of DEP will consider health effects data for cancer and noncancer endpoints from subchronic and chronic exposures to DEP. Three broad types of studies, if available, will be used to inform human health effects: controlled human exposure, epidemiologic, and experimental studies. Mechanistic or mode of action data will be evaluated and may inform questions of human relevance, susceptibility, and dose-response relationships. Considering the potential uses of IRIS information and potential pathways of exposure, an IRIS assessment of DEP would be expected to incorporate the following, provided that adequate data are available:

- Systematic identification of hazards from long-term exposures
- Analysis of mode of action information, if available
- Dose-response relationships for identified hazards
- Chronic Reference Concentration (RfC)

[33] http://water.epa.gov/scitech/drinkingwater/dws/ccl/upload/Nomination_Summary083109_508_v3.pdf

- Chronic Reference Dose (RfD)
- Cancer assessment and weight of evidence descriptor for oral and inhalation exposure, including dose-response information
- Identification of human populations and developmental stages with potentially greater susceptibility to DEP

The DEP assessment will rely on existing analytical tools and toxicity data and contain qualitative characterizations of uncertainty and variability related to hazard assessment and dose-response relationships. The development process for this assessment will provide opportunities for public comment and dialogue and includes independent external peer review.

2. DRAFT LITERATURE SEARCH AND SCREENING STRATEGY

The NRC (NRC, 2011) recommended that EPA develop a detailed search strategy utilizing a graphical display documenting how initial search findings are narrowed to the final studies that are selected for further evaluation on the basis of inclusion and exclusion criteria. Following these recommendations, a literature search and screening strategy was applied to identify literature related to characterizing the health effects of DEP. This strategy consisted of a search of online scientific databases and other sources, casting a wide net in order to identify all potentially pertinent studies. In subsequent steps, references were screened to exclude papers not pertinent to an assessment of the health effects of DEP, and remaining references were sorted into categories for further evaluation.

The literature search for DEP was conducted in five online scientific databases including PubMed, Web of Science, Toxline, TSCATS2, and Toxcenter. PubMed and Web of Science were most recently searched in August, 2013. The literature search approach, including the search strings and the number of citations identified per database, is presented in Table 2-1.

The computerized database searches were also supplemented by review of online regulatory sources as well as "forward" and "backward" searches of Web of Science for five primary literature sources (Table 2-2). The process for screening the literature search results is presented below and is shown graphically in Figure 2-1:

- After electronically eliminating duplicates from the citations retrieved through the multiple databases, 1,190 unique citations were identified.
- An additional 93 citations were obtained using additional search strategies described in Table 2-2.
- The resulting 1,283 citations were screened using the title, abstract, and/or full text for pertinence to examining the health effects of DEP exposure.
    - A total of 140 references were identified as primary sources of health effects data and were considered for data extraction to evidence tables and exposure-response arrays.
    - A total of 575 references were excluded from further consideration (see Figure 2-1 for exclusion categories).
    - A total of 53 studies were kept for further review. This category includes references that did not provide enough material to evaluate pertinence (e.g., no abstract).
    - A total of 420 references were considered pertinent, but not as primary sources of health effects data (e.g., reviews and editorials, risk assessments, and regulatory documents).

- A total of 95 studies were identified as supporting studies, but not as primary sources of health effects data (e.g., adsorption-distribution-metabolism-excretion [ADME] and mechanistic and genotoxicity studies).

Of the 140 references identified as primary sources of health effects information, 65 were classified as animal toxicity studies. These studies evaluated a health outcome in relation to DEP or the primary metabolite (MEP) and were considered for data extraction to evidence tables. Seventy-five human studies were also identified from the references categorized as primary sources of health effects information. These studies were found using the search strings in Table 2-1. Most human health effects studies for phthalates are not limited to examination of a single phthalate and the names of all of the phthalates examined in a particular study may not appear in the abstract or indexing terms. Thus, in addition to the literature search described above, EPA conducted a targeted literature search using modified search terms to identify human data pertaining to DEP and additional phthalates including dibutyl phthalate (DBP), di-(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP), diisobutyl phthalate (DIBP), and butyl benzyl phthalate (BBP). This search was conducted in the Web of Science, PubMed and ToxNet databases in June, 2013 using keywords and limits described in Table 2-3. The overall study selection strategy and number of references obtained at each stage of screening of this targeted literature search is shown graphically in Figure 2-2. From this targeted search, 61 studies of human health effects were identified and considered for data extraction to evidence tables.

The literature will be regularly monitored for the publication of new studies and a formal updated literature search and screen will be conducted after the IRIS bimonthly public meeting discussing these preliminary materials.

The documentation and results for the literature search can be found on the Health and Environmental Research Online (HERO) website[34] (http://hero.epa.gov/DEP and http://hero.epa.gov/phthalates-human studies).

[34]HERO (Health and Environmental Research On-line) is a database of scientific studies and other references used to develop EPA's risk assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 300,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

It is important to note that the HERO database will be regularly updated as additional references are identified during assessment development. Therefore, the numbers of references (by tag) displayed on the HERO webpage for DEP may not match the numbers of references identified in Figures 2-1 and 2-2 (current through March 2014).

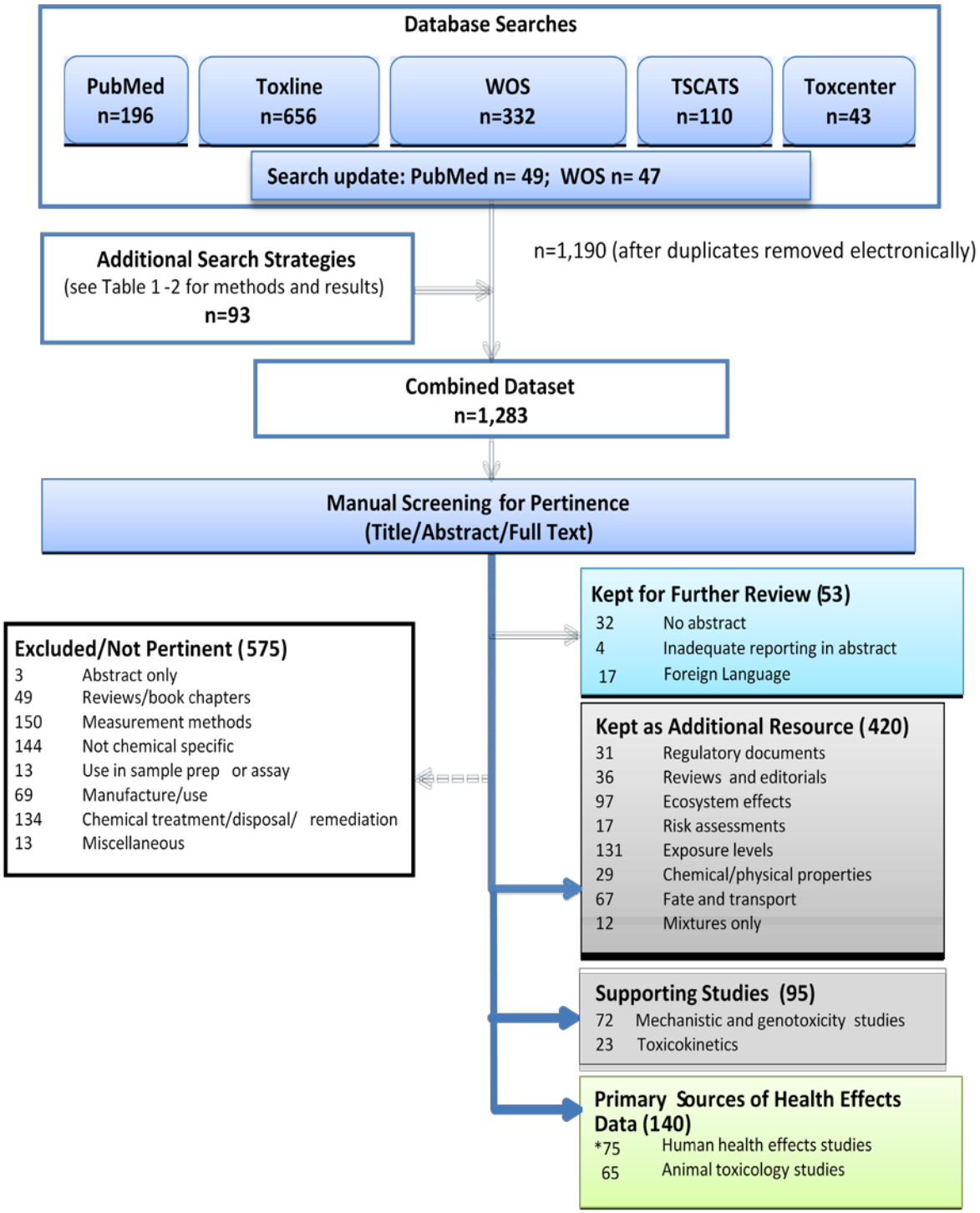
Table 2-1. Summary of detailed search strategies for DEP (Pubmed, Toxline, Toxcenter, TSCATS)

Database	Terms	Hits
Initial Strategy		
PubMed 10/31/2012 8/31/13	((("Diethyl o-phthalate"[tw] OR "Diethyl phthalate"[tw] OR "Ethyl phthalate"[tw]) OR (DEP[tw] AND (phthalate[All Fields] OR phthalate/1[All Fields] OR phthalate/2[All Fields] OR phthalate/25[All Fields] OR phthalate/adipate[All Fields] OR phthalate/aged[All Fields] OR phthalate/cellulose[All Fields] OR phthalate/dialkoxyalkyl[All Fields] OR phthalate/ethanol[All Fields] OR phthalate/ferrocene[All Fields] OR phthalate/goethite[All Fields] OR phthalate/kg[All Fields] OR phthalate/mg[All Fields] OR phthalate/ml[All Fields] OR phthalate/naoh[All Fields] OR phthalate/toxicity[All Fields] OR phthalate/water[All Fields] OR phthalate's[All Fields] OR phthalated[All Fields] OR phthalaten[All Fields] OR phthalates[All Fields] OR phthalates/kg/day[All Fields] OR phthalates/toxicity[All Fields] OR phthalates'[All Fields]))) NOT medline[sb]) OR "84-66-2"[EC/RN Number]	200 49
Web of Science 11/1/2012 8/31/13	((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS=chronic OR TS=immun* OR TS=lymph* OR TS=neurotox* OR TS=toxicokin* OR TS=pharmacokin* OR TS=biomarker* OR TS=neurolog* OR TS=subchronic OR TS=pbpk OR TS=epidemiolog* OR TS=acute OR TS=subacute OR TS=ld50)	80 47
	((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS=lc50 OR TS=inhal* OR TS=pulmon* OR TS=nasal OR TS=lung* OR TS=respir* OR TS=occupation* OR TS=workplace OR TS=worker* OR TS=oral OR TS=orally OR TS=ingest* OR TS=gavage OR TS=diet OR TS=diets OR TS=dietary OR TS=drinking OR TS=gastr* OR TS=intestin*)	109
	((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS=gut OR TS=sensitiz* OR TS=abort* OR TS=abnormalit* OR TS=embryo* OR TS=cleft* OR TS=fetus* OR TS=foetus* OR TS=fetal* OR TS=foetal* OR TS=fertil* OR TS=malform* OR TS=ovum OR TS=ova OR TS=ovary OR TS=placenta* OR TS=pregnan*)	60
	((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS=dermal* OR TS=dermis OR TS=skin OR TS=epiderm* OR TS=cutaneous OR TS=carcinog* OR TS=cocarcinog* OR TS=cancer OR TS=precancer OR TS=neoplas* OR TS=tumor* OR TS=tumour* OR TS=oncogen* OR TS=lymphoma* OR TS=carcinom* OR TS=genetox* OR TS=genotox* OR TS=mutagen* OR TS=nephrotox* OR TS=hepatotox* OR TS=endocrin* OR TS=estrogen* OR TS=androgen*)	156

Database	Terms	Hits
	((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS=hormon* OR TS=blood OR TS=serum OR TS=urine OR TS=bone OR TS=bones OR TS=skelet* OR TS=rat OR TS=rats OR TS=mouse)	148
	((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS=mice OR TS=guinea OR TS=muridae OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS=dog OR TS=dogs OR TS=beagle* OR TS=canine OR TS=cats OR TS=feline OR TS=pig OR TS=pigs OR TS=swine OR TS=porcine OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset* OR TS=toxic* OR TS=adverse OR TS=poisoning)	174
	((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS=prenatal OR TS=perinatal OR TS=postnatal OR TS=reproduc* OR TS=steril* OR TS=teratogen* OR TS=sperm* OR TS=neonat* OR TS=newborn* OR TS=development* OR TS=zygote* OR TS=child OR TS=children OR TS=adolescen* OR TS=infant* OR TS=wean* OR TS=offspring OR TS=age)	139
	-omics search	
	2 ((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS="Genomics" OR TS="Proteomics" OR TS="Metabolic Profile" OR TS="Metabolome" OR TS="Metabolomics" OR TS="Microarray" OR TS="Nanoarray")	2
	11 ((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS="Gene expression" OR TS="Transcript expression" OR TS="transcriptomes" OR TS="transcriptome" OR TS="Phenotype" OR TS="Transcription" OR TS="Trans-act*" OR TS="transact*" OR TS="trans act*" OR TS=genetic OR TS="genetics" OR TS="genotype")	11

Database	Terms	Hits
	4 ((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS="Genetic transcription" OR TS="Gene transcription" OR TS="Gene Activation" OR TS="Genetic induction" OR TS="Reverse transcription" OR TS="Transcriptional activation" OR TS="Transcription factors" OR (TS="Biosynthesis" AND (TS=RNA OR TS=DNA)) OR TS="mRNA")	4
	6 ((TS=DEP AND TS=phthalat*) OR (TS="1,2-Benzenedicarboxylic acid, diethyl ester" OR TS="Diethyl 1,2-benzenedicarboxylate" OR TS="Diethyl o-phthalate" OR TS="Diethyl phthalate" OR TS="Di-n-ethyl phthalate" OR TS="Ethyl phthalate" OR TS="Phthalic acid, diethyl ester")) AND (TS="messenger RNA" OR TS="transfer RNA" OR TS="peptide biosynthesis" OR TS="protein biosynthesis" OR TS="protein synthesis" OR TS="RT-PCR" OR TS="RTPCR" OR TS="Reverse Transcriptase Polymerase Chain Reaction" OR TS="DNA sequence")	6
ToxLine 11/1/2012	@OR+("diethyl phthalate"+"unimoll da"+solvanol+"placidol e"+phthalol+"palatinol a"+neantine+"ethyl phthalate"+anozol+@term+@rn+84-66-2)+@not+@org+pubmed+pubdart+crisp	584
	@term+@rn+84-66-2+@AND+@org+tscats	105
TSCATS2, TSCA recent notices 10/31/2012	84-66-2	8 TSCATS2
	84-66-2 (8E OR FYI) TSCA	1 recent notices

Database	Terms	Hits
Toxcenter 3/27/2012 NOTE: took all non caplus items and caplus with synonyms in titles only, sequence"Dupicates were removed; results were date limited to avoid extensive overlap with Toxline	((84-66-2) not (patent/dt OR tscats/fs)) and (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous? OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon?) AND ("1,2-Benzenedicarboxylic acid, 1,2-diethyl ester"/ti OR "1,2-Benzenedicarboxylic acid, diethyl ester"/ti OR Anozol/ti OR "Diethyl 1,2-benzenedicarboxylate"/ti OR "Diethyl o-phenylenediacetate"/ti OR "Diethyl o-phthalate"/ti OR "Diethyl phthalate"/ti OR "Di-n-ethyl phthalate"/ti OR "DPX-F5384"/ti OR "Estol 1550"/ti OR "Ethyl phthalate"/ti OR Neantine/ti OR "o-Benzenedicarboxylic acid diethyl ester"/ti OR "o-Bis(ethoxycarbonyl)benzene"/ti OR "Palatinol A"/ti OR "Phthalic acid, diethyl ester"/ti OR Phthalol/ti OR "Placidol E"/ti OR Solvanol/ti OR (DEP/ti AND (phthalate/ti OR phthalates/ti))	2,526
	–omics search	65
	("Computational biology" OR "Bio-Informatics" OR Bioinformatics OR ("Molecular Biology" AND Computational) OR Informatics OR ("Information Science" AND Medical))	
	Genomics OR Proteomics OR "Metabolic Profile" OR "Metabolome" OR "Metabolomics" OR "Microarray" OR "Nanoarray"	
	"Gene expression" OR "Transcript expression" OR transcriptomes OR transcriptome OR Phenotype OR Transcription OR Trans-act? OR	
	transact? OR trans()act? OR genetic OR genetics OR genotype	
	"Systems biology" OR ("Biological systems" AND (monit? OR data OR analysis))	
	(Genetic transcription OR "Gene transcription" OR "Gene Activation" OR "Genetic induction" OR "Reverse transcription" OR "Transcriptional activation" OR "Transcription factors" OR (Biosynthesis AND (RNA OR DNA)))	
	mRNA OR "messenger RNA" OR "transfer RNA" OR "peptide biosynthesis" OR "protein biosynthesis" OR "protein synthesis" OR RT-PCR OR RTPCR OR "Reverse Transcriptase Polymerase Chain Reaction" OR "DNA	
Merged Reference Set	(duplicates eliminated through electronic screen)	1,190



*This set of 75 studies was not screened in detail. A targeted literature search for epidemiology studies was conducted using modified search terms to identify human data pertaining to DEP and additional phthalates; from this targeted search, 145 primary studies of human health effects were identified, of which 61 examined DEP or its major metabolite, MEP (See Table 2-2 and Figure 2-2). This targeted search was conducted because most human health effects studies for phthalates are not limited to examination of a single phthalate and the names of all of the phthalates examined in a particular study may not appear in the abstract or indexing terms

Figure 2-1. Summary of literature search and screening process for DEP.

1 **Table 2-2. Additional strategies utilized in literature search**

System Used	Selected Key Reference(s) or Sources	Date	Additional References Identified
Manual search of citations from regulatory documents	NICNAS (National Industrial Chemicals Notification and Assessment Scheme). (2008). Existing chemical hazard assessment report. Diethyl phthalate. National Industrial Chemicals Notification and Assessment Scheme.http://www.nicnas.gov.au/Industry/Existing_Chemicals/Phthalate_Hazard_Assessments/DEP%20hazard%20assessment%2030-4-07.pdf.	5/2013	10 citations added
	ATSDR (Agency for Toxic Substances and Disease Registry). (1995). Toxicological profile for diethyl phthalate. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.	5/2013	4 citations added
	WHO (World Health Organization). (2003). Concise International Chemical Assessment Document 52: Diethyl phthalate. Geneva. http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf.	5/2013	2 citations added
Web of Science, forward search	Jones, HB; Garside, DA; Liu, R; et al. (1993) The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo. Exp Mol Pathol 58:179–193.	6/2013	4 citations added
	Shiraishi, K; Miyata, K; Houshuyama, S. (2006) Subacute oral toxicity study of diethylphthalate based on the draft protocol for "Enhanced OECD Test Guideline no. 407". Arch Toxicol. 80: 10-16.	6/2013	0 citations added
	Field, EA; Price, CJ; Sleet, RB; et al. (1993) Developmental toxicity evaluation of diethyl and dimethyl phthalate in rats. Teratology, Jul; 48 (1): 33-44.	6/2013	2 citations added
	Swan SH. (2008). Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environmental Research 108(2): 177-184.	6/2013	10 citations added
	Pereira, C; Mapuskar, K; Rao, CV. (2007) Chronic toxicity of diethyl phthalate--A three generation lactational and gestational exposure study on male Wistar rats. Envir Toxicol and Pharma 23:319–327.	6/2013	0 citations added
Web of Science, backward search	Jones, HB; Garside, DA; Liu, R; et al. (1993) The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo. Exp Mol Pathol 58:179–193.	6/2013	1 citations added
	Shiraishi, K; Miyata, K; Houshuyama, S. (2006) Subacute oral toxicity study of diethylphthalate based on the draft protocol for "Enhanced OECD Test Guideline no. 407". Arch Toxicol. 80: 10-16.	6/2013	0 citations added
	Field, EA; Price, CJ; Sleet, RB; et al. (1993) Developmental toxicity evaluation of diethyl and	6/2013	2 citations added

System Used	Selected Key Reference(s) or Sources	Date	Additional References Identified
	dimethyl phthalate in rats. Teratology, Jul; 48 (1): 33-44.		
	Swan SH. (2008). Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environmental Research 108(2): 177-184.	6/2013	6 citations added
	Pereira, C; Mapuskar, K; Rao, CV. (2007) Chronic toxicity of diethyl phthalate--A three generation lactational and gestational exposure study on male Wistar rats. Envir Toxicol and Pharma 23:319–327.	6/2013	4 citations added
References obtained during the assessment process	DEP references in previous assessment or previously added to the HERO project page		47 citations added
Background Check	Searched a combination of CASRNs and synonyms on the following databases: ATSDR (http://www.atsdr.cdc.gov/substances/index.asp) CalEPA (Office of Environmental Health Hazard Assessment) (http://www.oehha.ca.gov/risk.html) eChemPortal4 (http://www.echemportal.org/echemportal/participant/page.action?pageID=9) EPA Acute Exposure Guideline Levels (http://www.epa.gov/oppt/aegl/pubs/chemlist.htm) EPA – IRISTrack/New Assessments and Reviews5 (http://cfpub.epa.gov/ncea/iristrac/) EPA NSCEP (http://www.epa.gov/ncepihom/) EPA RfD/RfC and CRAVE meeting notes EPA Science Inventory (http://cfpub.epa.gov/si/) Federal Docket (www.regulations.gov) Health Canada First Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/index-eng.php) Health Canada Second Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index-eng.php) IARC (http://monographs.iarc.fr/htdig/search.html) IPCS INCHEM (http://www.inchem.org/) ITER (TERA database) (http://iter.ctcnet.net/publicurl/pub_search_list.cfm) NAS via NAP (http://www.nap.edu/) NCI (http://www.cancer.gov) NCTR (http://www.fda.gov/AboutFDA/CentersOffices/OC/	10/2012	1 citations added

System Used	Selected Key Reference(s) or Sources	Date	Additional References Identified
	OfficeofScientificandMedicalPrograms/NCTR/default.htm) NIEHS (http://www.niehs.nih.gov/) NIOSHTIC 2 (http://www2a.cdc.gov/nioshtic-2/) NTP - RoC, status, results, and management reports (http://NTPsearch.niehs.nih.gov/query.html) WHO assessments – CICADS, EHC (http://www.who.int/ipcs/assessment/en/)		

1

Table 2-3. Summary of search terms: targeted epidemiology search

Database, Search Date	Terms	Hits
PubMed 6/6/2013 No date restriction	(phthalate OR phthalates OR phthalic acid) AND (human OR case-control OR pregnancy OR cohort OR workers OR children OR survey)	Imported: 2,505 After duplicates deleted: 2,482
Web of Science 6/6/2013 No date restriction	(TS="phthalic acid" OR TS="phthalate" OR TS="phthalates") AND (TS="humans" OR TS="human" OR TS="case-control" OR TS="pregnancy" OR TS="cohort" OR TS="workers" OR TS="child" OR TS="children" OR TS="survey")	Imported: 1,840 After duplicates deleted: 1,836
ToxNet 6/6/2013 No date restriction	(phthalate OR phthalates OR phthalic acid) AND (human OR case-control OR pregnancy OR cohort OR workers OR children OR survey)	Imported: 2,505 After duplicates deleted: 2,427
Merged Reference Set	Merged dataset, with duplicates eliminated through electronic screen	4,128

2

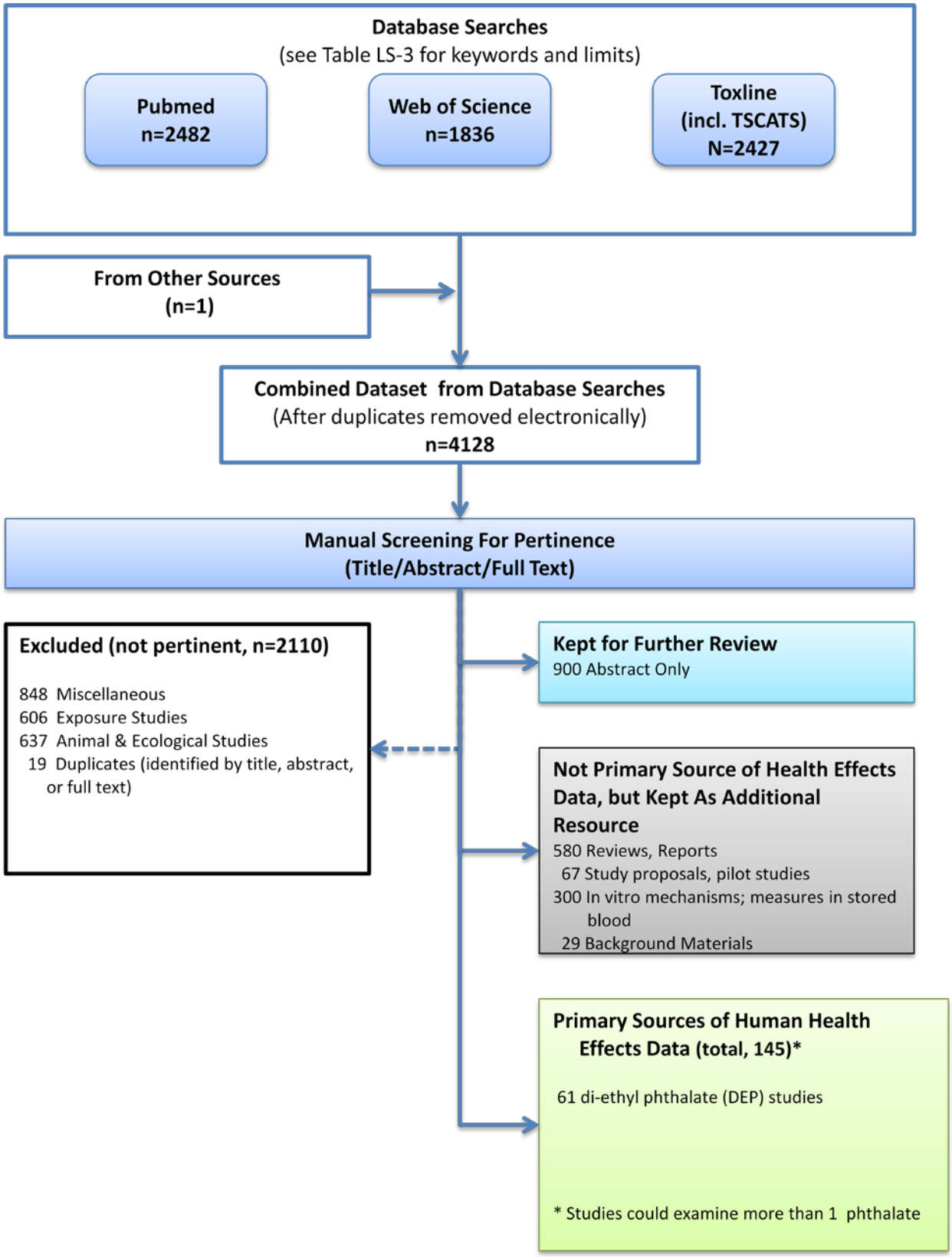


Figure 2-2. Summary of targeted literature search and screening process for epidemiologic studies of DEP.

3. SELECTION OF STUDIES FOR HAZARD IDENTIFICATION

3.1. General Approach

The NRC (NRC, 2011) recommended that after studies are identified for review by utilizing a transparent search strategy, the next step is to summarize the details and findings of the most pertinent studies in evidence tables. The NRC suggested that such tables should provide a link to the references, and include details of the study population and methods and key findings. This approach provides for a systematic and concise presentation of the evidence. The NRC also recommended that the methods and findings should then be evaluated with a standardized approach. The approach that was outlined identified standard issues for the evaluation of epidemiological and experimental animal studies.

In response to the NRC recommendations, each study retained after the literature search and screen is evaluated for aspects of its design or conduct that could affect the interpretation of results and the overall contribution to the synthesis of evidence for determination of hazard potential. Much of the key information for conducting this evaluation can generally be found in the study's methods section and in how the study results are reported. Importantly, this evaluation does not consider study results or more specifically, the direction or magnitude of any reported effects. For example, standard issues for evaluation of experimental animal data identified by the NRC and adopted in this approach include consideration of the species and sex of animals studied, dosing information (dose spacing, dose duration, and route of exposure), endpoints considered, and the relevance of the endpoints to the human endpoints of concern.

To facilitate the evaluation outlined above, evidence tables are constructed that consistently summarize the important information from each study in a standardized tabular format as recommended by the NRC (NRC, 2011). In general, the evidence tables include all studies that inform the overall synthesis of evidence for hazard potential. At this stage, exclusion of studies may unnecessarily narrow subsequent analyses by eliminating information that might later prove useful. Premature exclusion might also give a false sense of the consistency of results across the database of studies by unknowingly reducing the diversity of study results. Thus, at this early stage of study evaluation the goal is to be inclusive.

Even at this early stage, however, a study can be excluded if flaws in its design or conduct are so great that the results would not be considered credible. Such study design flaws are discussed in a number of EPA's guidelines (see http://www.epa.gov/iris/backgrd.html) or

summarized in the draft Preamble to the IRIS Toxicological Review ("Preamble").[35] Examples of these flaws include studies where impurities in the test chemical are so great as to prohibit attribution of the results to the chemical, or studies where concurrent or essential historical control information is lacking. Studies excluded because of fundamental flaws in their design or conduct are not included in evidence tables. Instead, text accompanying the evidence tables lists the reasons that studies were excluded.

The size of the database can influence both the type and number of evaluation criteria that are applied at this early stage. For example, if there are few studies on a health effect, additional evaluation criteria might not be needed, and thus the evidence tables may include all studies without severe flaws. Especially with smaller databases, it is important to consider all studies and not exclude studies unnecessarily. On the other hand, if there are many studies on a health effect (e.g., more than 20), additional criteria could facilitate a more efficient review of the database and help to focus on the more pertinent or stronger studies indicating the potential for hazard. These criteria could be specific to each type of study or a particular endpoint, and may consider factors such as those discussed in EPA's guidelines or summarized in the draft Preamble. Application of such additional criteria could result in initially setting aside some studies and not summarizing them in the evidence tables. Also, there may be situations in which the initial review of the available data will lead to a decision to focus on a particular set of health effects, and to exclude others from further evaluation. This situation could occur, for example, with a chemical with a large database with a few well-developed areas of research, but many other areas that consist of sparse data offering a very limited basis for drawing conclusions regarding hazard. In this case, EPA will focus on the more developed areas of research for hazard identification.

3.2. Selection of Primary Studies for Evidence Tables for DEP

3.2.1. Epidemiologic Studies

The initial review of epidemiologic studies was conducted for those that were retained after the literature was manually screened for pertinence (title, abstract, and/or full text) (Figure 2-2; Primary Sources of Human Health Effects Data).

The epidemiological database is quite broad, covering a large variety of reproductive and chronic disease outcomes in infants, children, and adults. The data for many of these outcomes, however, are sparse (i.e., examined in only one or two studies). To improve the efficiency of the hazard identification process, EPA's evaluation will focus on two sets of studies. The first group consists of outcomes (health effects) from human studies that could correspond to an endpoint that has been examined in experimental animal studies in which either the human or the animal studies provide some indication of potential toxicity. This set includes sexual differentiation effects (e.g.,

[35] See the draft Preamble in the Toxicological Review of Ammonia (revised external review draft) at http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=254524 or in the Toxicological Review of Trimethylbenzenes (revised external review draft) at http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=254525.

anogential distance), pregnancy-related outcomes (e.g., early pregnancy loss, gestational age, birth weight) and male reproductive outcomes (e.g., steroidal and gonadotropin hormone levels, sperm parameters, infertility). The second group consists of outcomes that have not been examined in experimental animal studies, but which include several epidemiological studies conducted in a similar lifestage (e.g., children, adults), with some indication of a potentially positive association. This set includes neurobehavioral effects in children, obesity, and diabetes and insulin resistance. Selection into these groups does not mean that EPA has concluded that a particular health effect represents a hazard for DEP exposure; rather, selection indicates that EPA concluded that a more detailed evaluation of the body of research is warranted (for example, because differing conclusions regarding these effects have been reached in published reviews on these topics).

At the present time, EPA is not planning to conduct additional review of epidemiological studies of DEP in relation to health effects for which there is a lack of evidence of associations. These effects include timing of male puberty, central precocious puberty and general female pubertal development, endometriosis, thyroid hormones (adults), neurological effects in adults, asthma, and cholesterol and other cardiovascular risk factors. EPA is also not planning to conduct additional review of three other health effects (pulmonary function, thyroid hormones (children), and breast cancer), which are each based on a single study. The availability of additional studies on any of these health effects may result in a reevaluation of the need for further review.

3.2.2. Experimental Animal Studies

An initial review was also performed for the experimental animal studies identified in the literature search and screen (Figure 2-1). The DEP experimental animal database consists of studies designed to examine repeat-dose oral toxicity (including chronic, subchronic, and short-term duration studies) and endpoint-specific toxicities (including reproductive and developmental toxicity). In addition, one dermal cancer bioassay is available for DEP. The majority of these studies involved administration of DEP in the diet or via gavage administration. These studies are pertinent to evaluating the health effects of DEP associated with human environmental exposure, and none had severe flaws (as discussed in EPA's guidelines and summarized in the draft Preamble) that would compromise the credibility of their results. Because there are relatively few experimental animal toxicity studies of DEP, these studies are all included in the preliminary evidence tables.

The DEP experimental animal database also includes studies of acute toxicity and ocular and dermal irritation. As these short-duration studies are generally less pertinent for characterizing health hazards associated with chronic exposure, they are not summarized in the preliminary evidence tables. Studies utilizing intraperitoneal exposure also were not summarized in the preliminary evidence tables. Nevertheless, these studies will still be evaluated as possible sources of toxicokinetic or mechanistic information during assessment development. In addition, based on the manual screening for pertinence, Hayashi et al., 2010 (evaluation of a mixture); Lamb et al., 1997 (lack of reporting of any quantitative data); and Field et al., 1993 (data reported in NTP, 1998) were excluded from the evidence tables.

3.3. Preliminary Evidence Tables and Exposure-Response Arrays

Data from the primary studies identified by the approaches outlined above have been extracted and presented in evidence tables (Appendix A). The evidence tables present data from studies related to a specific outcome or endpoint of toxicity. At a minimum, the evidence tables include the relevant information for comparing key study characteristics such as study design, exposure metrics, and dose-response information. Evidence tables will serve as an additional method for presenting and evaluating the suitability of the data to inform hazard identification for DEP during the analysis of hazard potential and utility of the data for dose-response evaluation.

The information in the preliminary evidence tables is also displayed graphically in preliminary exposure-response arrays. In these arrays, a significant effect (indicated by a filled circle) is based on statistical significance.

The complete list of references considered in preparation of these materials can be found on the HERO website at http://hero.epa.gov/DEP and http://hero.epa.gov/phthalates-human studies.

3.4. Study Characteristics That Will Be Considered in the Evaluation and Synthesis of the Primary Studies for DEP

The database of human studies for DEP, as well as phthalates in general, is relatively large. It consists, in large part, of studies conducted at environmental or background levels of exposure, and may play an important role in hazard identification. In this document, the discussion of the evaluation process EPA is using for the human database is developed in more detail than the evaluation process proposed for the animal database.

3.4.1. Epidemiologic Studies

Several considerations will be used in EPA's evaluation of the studies of human health effects of DEP. The general considerations for evaluating issues relating to the study population, exposure, outcomes, confounding, and analysis are outlined in the draft Preamble. These, along with more specific issues pertaining to exposure and outcomes studied, are described below and in Table 3-1.

Study population

The general considerations for evaluating issues related to the study population include adequate documentation of participant recruitment, such as eligibility criteria, participation rates, missing data, loss to follow-up, and general demographic characteristics. This information is used to evaluate the potential for selection bias, as well as to facilitate comparison of results across different study populations. It is important to note that low participation rates, or even different participation rates between exposed and non-exposed or between cases and controls, is not evidence of selection bias. Rather, selection bias arises from a differential pattern of participation with respect to exposure *and* disease, e.g., if people with high exposure and the outcome of interest are more likely to participate than people with low exposure and the outcome.

The available epidemiological studies generally examined metabolites from many different phthalates within the context of research on environmental exposures. Study participants typically do not have knowledge of their exposure to DEP and thus, knowledge of exposure or exposure level is unlikely to result in differential participation with respect to outcomes. However, EPA will consider the possibility that a particular concern about the specific sources of DEP (e.g., perfume and other personal care products) would have motivated people to participate in a study or to continue participation throughout a follow-up period. EPA will also consider indirect ways in which a common factor could contribute both to DEP exposure and to a specific outcome. In the absence of evidence that any of these scenarios is at play, EPA will not consider selection bias attributed to these factors to be a likely limitation of a study.

Exposure measures

The general considerations for evaluating issues relating to exposure include characterization of exposure during the appropriate critical period for the outcomes under study, and use of appropriate ascertainment methods to classify individuals with regards to the exposure.

The simple monoester metabolite MEP is the most commonly measured DEP metabolite in epidemiologic studies. Urine provides an integrated measure of phthalate exposure from all sources. The monoester metabolite is considered the primary biomarker for exposure to the low molecular weight phthalates such as DEP, and has been found in human and animal metabolism studies to account for between 50 and 80% of exposure dose. MEP accounts for an estimated 69% of the urinary excretion of DEP (Anderson et al., 2001). This value is based on human data derived for mono-n-butyl phthalate (MnBP), which (based on animal data) is expected to have very similar toxicokinetics as MEP (Koch et al., 2003; Lake et al., 1977). Given this assumption, EPA considers the use of MEP to be a good proxy for total DEP exposure. The metabolite, rather than the parent compound, is preferred because the parent compound is metabolized very quickly.

Although urine measures are most commonly used in epidemiological studies, measures in other tissues (serum, semen and breast milk) have also been used. In a study of 60 men ages 18 – 26 years, the correlation between MEP levels measured in urine and serum was very strong (Spearman r = 0.92); the correlation between urine and seminal fluid measures was also relatively strong (Spearman r = 0.75) (Frederiksen et al., 2010). Measurement in breast milk is more challenging, with a greater proportion of samples below the limit of detection (Hines et al., 2009; Hogberg et al., 2008). Based on these data, EPA considers MEP measures in serum or semen to be as useful as those in urine, but has greater uncertainty about measures in breast milk (Hanberg et al., 2005).

EPA does not consider the reliance on spot urine samples for exposure estimation and ranking to be a major limitation for epidemiological studies. Urinary phthalate metabolite concentrations peak shortly after exposure (Koch et al., 2012; Taylor et al., 2011; Kluwe, 1982) and urine sampled during this time of peak concentration could lead to artificially high estimates of daily intake, and conversely, measurements made after concentrations have peaked and declined could lead to artificially low intake estimates. Although this variability may affect the accuracy of

an estimated intake for a single individual, studies have demonstrated that on a group level, spot urine samples provide a reasonable approximation of concentrations that would have been observed using full-day urine samples (Christensen et al., 2012) and that a single spot sample was reliable in ranking subjects according to tertile (Teitelbaum et al., 2008). Because of the potential for greater inaccuracy of estimates in the "tails" of the distribution, however, EPA will include additional considerations (e.g., discussion of analysis of residuals, sample size, outliers), when evaluating analyses based on use of MEP as a continuous measure.

Several studies have evaluated the stability of phthalate metabolite concentrations in urine over time. Stability is usually evaluated with the intraclass correlation coefficient (ICC), a measure of the 'between-individual' variance, divided by the total variance (between and within individuals). A higher ICC indicates greater reproducibility (i.e., lower within-person variance). For MEP measures in adults (other than during pregnancy), moderate correlations were seen over a period of 2-7 days (ICC 0.48 and 0.77, respectively in Preau et al., 2010; Hoppin et al., 2002), with slightly lower values seen over a 3 month period (ICC 0.43 and 0.68, respectively, inHauser et al., 2004 and Frederiksen et al., 2013, spot urine samples). Townsend et al., 2013 examined a longer period in an analysis based in the Nurses Health Study, with urine samples collected 1-3 years apart: the ICC in this study was 0.33 for all samples and 0.44 for first-morning samples. Several studies conducted among pregnant women have found similar estimates for stability of urinary measures of MEP. (Cantonwine et al., 2014) reported ICC = 0.23 comparing first trimester to third trimester and approximately 0.5 comparing first to second trimester or second to third trimester. In other studies during pregnancy, covering periods from 4-8 weeks, ICCs ranged 0.3 to 0.6 (Braun et al., 2012; Peck et al., 2010; Adibi et al., 2008). Data for children are sparse: one study evaluated variability in children aged 6-10 years old over a 6 month period (Teitelbaum et al., 2008) and found an ICC of 0.26 (creatinine-adjusted measures). Based on these studies, EPA does not consider the use of a single measurement to be a major limitation in studies in adults in which the measure of exposure is closely aligned with the relevant window(s) of exposure, if known, for the effect under study. EPA has greater uncertainty about measurements taken at a period outside of the relevant time window (e.g., several years after diagnosis, or the difference between first and 3rd pregnancy), and measurements taken in children.

Use of spot urine samples also introduces the issue of identifying the optimal approach to considering urinary volume or dilution in the analysis; options include use of creatinine-adjusted (or specific-gravity adjusted) metabolite concentrations, or use of unadjusted concentrations. For outcomes that are strongly related to factors affecting creatinine levels, such as measures of obesity, creatinine-adjusted exposure measures may produce biased effect estimates. Thus EPA prefers results using unadjusted concentration for these types of outcomes. For other outcomes, EPA does not have a basis for preferring one type of analysis over another.

EPA also considers the distribution of exposure in evaluating individual studies and comparing results among groups of studies. One consideration is the span of exposure levels (i.e., the contrast between "high" and "low"): a study with a very narrow span may not have sufficient variability to detect an effect that would be seen over a broader range. Another consideration is the

absolute level of exposure: different effect estimates may be expected in studies examining different exposure levels.

Primary outcome measures

The general considerations for evaluating issues relating to outcomes include adequate duration of exposure and follow-up in order to evaluate the outcomes of interest, and use of appropriate ascertainment methods to classify individuals with regard to the outcome.

Issues relating to assessment of the specific primary health effects are discussed below and summarized in Table 3-1.

Sexual differentiation

In animal toxicology studies, anogenital distance is a routine marker used to assess endocrine disruption. In the first demonstration of the use of anogential distance as a measure in epidemiological studies, (Salazar-Martinez et al., 2004) reported a low degree of between-observer variability, using a standardized protocol and trained observers. It is important to consider general size in the evaluation of anogenital distance, for example by incorporating birth weight or length. Because of the importance of size and age in the interpretation of this measure, EPA has greater confidence in studies with measures taken at birth rather than over a larger age range.

Cryptorchidism, or undescended testes, can be present at birth (congenital cryptorchidism) or can occur later during infancy and childhood (acquired cryptorchidism). Retractile testes can move back and forth between the scrotum and the abdomen; this condition usually resolves by puberty and is not associated with reproductive or other complications. Classification criteria for cryptorchidism described by (Scorer, 1964) are commonly used in clinical research. EPA will consider the definition used and age range in interpreting studies of cryptorchidism or related outcomes.

Gender-related behaviors have been examined in relation to direct or indirect measures of fetal testosterone levels. This work includes studies of relatively rare genetic conditions (e.g., congenital adrenal hyperplasia and complete androgen insensitivity syndrome), as well as studies focusing on the normal variability seen in the general population (reviewed in Hines, 2006). EPA will consider the assessment tool used to examine gender-related behaviors; details of the assessment method, or references providing this information, should be provided. In addition, validation studies of these tools and the appropriateness of the tool for evaluation in the specific study population (e.g., age range, language) will also be considered.

Pregnancy Outcomes

Gestational age and birth weight have been examined in the DEP epidemiology studies. These variables are sometimes defined as dichotomous outcomes, e.g., low birth weight (defined as < 2500 g) or preterm birth (defined as < 37 weeks gestation). They can also be examined as continuous variables, often in analyses in which preterm or low birthweight births are excluded, so that the focus of the analysis is on variability within the "normal" range. EPA considers both types

of analyses (i.e., dichotomous and continuous) to be informative with respect to hazard identification, but will consider each separately as they address different issues. In the birth cohort studies included in the DEP database, data pertaining to birth weight are generally taken directly from medical records. EPA considers this to be a reliable source. Although more prone to measurement area than birthweight measures, gestational age, estimated from date of last menstrual period from information collected early in pregnancy may provide a more unbiased estimate than measures based on ultrasound (Henriksen et al., 1995).

Pregnancy loss is another pregnancy outcome examined within the DEP database. Pregnancy loss can occur even before a clinically recognized pregnancy. Early (i.e., pre-clinical) pregnancy loss is very common, accounting for approximately 20% of pregnancies (Wilcox et al., 1988); this outcome is based on measurement of human chorionic gonadotropin (hCG). Medical record or interview data can also be used to ascertain losses at later stages of gestation.

Male reproductive outcomes

The details of the laboratory procedures, including information on the basic methods, limit of detection, and coefficient of variation, are important considerations for the hormone assays. Much of the focus of the research on male steroidal and gonadotropin hormones in the DEP database concerns testosterone; one issue with respect to these measures is the estimation method used for free testosterone. Based on the analysis by Vermeulen et al., 1999, EPA will consider estimates based on total testosterone divided by immunoassay-derived sex hormone-binding globulin (SHBG) levels to be a reliable estimate of free testosterone.

The WHO laboratory methods for analysis of sperm counts and semen parameters (see, for example, WHO, 1999) are generally recognized as standards in this field. EPA will consider studies that reference these methods, regardless of which revision used, to be reliable measures.

Infertility is generally defined clinically and for research purposes as the inability to conceive a clinically-recognized pregnancy after 12 months of intercourse of regular frequency without use of contraceptives. With respect to male-mediated infertility, EPA will consider measures based on the reference values derived using WHO standards for sperm concentration and other parameters (Cooper et al., 2010) to be reliable measures.

Neurodevelopmental Outcomes

With respect to neurodevelopmental outcomes, a major consideration is the assessment tool(s) used by the study investigators; details of the assessment method, or references providing this information, should be provided. Validation studies of these tools and the appropriateness of the tools for evaluation in the specific study population (e.g., age range, language) will also be considered.

Obesity

The studies of obesity measures in the DEP database are based on weight, body mass index, or waist circumference using measurements taken as part of the data collection protocol. EPA

considers all of these to be informative measures. Although it does not come up in the set of studies currently available, EPA notes that use of self-reported weight (e.g., report of pre-pregnancy weight) would not be considered as reliable as actual measurements.

Diabetes/insulin resistance

In the DEP database, diabetes has been assessed by a variety of biomarkers of glucose and insulin, and self-report of previous diabetes diagnosis. Oral glucose tolerance testing and glycosolated hemoglobin (HbA1c) are used clinically and in epidemiological research (Selvin et al., 2011). Self-report of prevalent diabetes can have high sensitivity and specificity in comparison to diagnosed diabetes based on validated medical record data (Oksanen et al., 2010; Leikauf and Federman, 2009). The biomarker-based classifications, however, offer an added advantage of being able to include undiagnosed disease. EPA will consider these points in assessing the reliability and validity of the diabetes measures used in the studies. None of the currently available studies assessed diabetes through cause of death data; sensitivity of diabetes assessed using cause of death data is low, even if underlying and other contributing cause of death fields are included (Cheng et al., 2008).

Insulin resistance, a marker of diabetes risk, can be measured using the homeostatic model assessment (HOMA) method, a physiologically-based structural model, using fasting glucose and insulin or C-peptide concentrations. HOMA is a validated tool for the estimation of insulin resistance in epidemiology studies, and requires a single measurement of fasting glucose and insulin (Wallace et al., 2004). Although the mean of three samples taken at 5-minute intervals results in a more precise estimate, insulin resistance estimated using a single baseline measurement is well correlated with that using the mean of three measurements when used to estimate a group mean. Therefore EPA does not consider the use of a single measurement as an input to the HOMA model to be a limitation.

Confounding

The general considerations for evaluating issues relating to potential confounding include consideration of which factors may be potential confounders (i.e., those that are strongly related to both the exposure and the outcome under consideration), and if needed, adequate control for these potential confounders in the study design or analysis.

Potential confounding by other phthalates

EPA does not generally consider lack of adjustment for DEHP (or its metabolites) to limit the interpretation of the observed associations with MEP. This determination is based on data pertaining to associations among urinary metabolites indicating a low correlation between MEP and the DEHP-related metabolites. In an analysis conducted by EPA of 5,109 samples from the 2005 – 2008 National Health and Nutrition Examination Survey (NHANES) participants aged ≥ 6 years, the pairwise Spearman correlation coefficient between MEP and DEHP metabolites (mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-oxohexyl phthalate (MEOHP), or mono-2-

ethyl-carboxypentyl phthalate (MECCP)) ranged from 0.24 to 0.27. These correlations are not unexpected, given differences in the source and route of exposure for DEP compared with the high molecular weight phthalates, which includes DEHP.

The correlations between MEP and metabolites of other low molecular weight phthalates are similar or slightly larger than those seen with DEHP metabolites. In the NHANES analysis described above, the Spearman correlations between MEP and other metabolites were 0.39 for MBP, 0.28 for monobenzyl phthalate (MBzP), 0.36 for monoisobutyl phthalate (MiBP), and 0.20 for monocarboxyisooctyl phthalate (MCOP). Similar results were observed in smaller studies in the published literature (Baird et al., 2010; Itoh et al., 2009; Hauser et al., 2006; Pan et al., 2006). Thus as with the DEHP metabolites, EPA does not consider lack of adjustment for these other phthalate metabolites to be a limitation of a study; an exception would be a situation in which an association with other metabolites was considerably stronger than the association seen with MEP.

Potential confounding by demographic factors

Age and sex are considered important explanatory factors for most types of outcomes measured in epidemiological research. In NHANES data, urinary MEP levels were lower among children ages 6-11 (median 75 µg/L) compared to teenagers and adults (median 150 to 211 µg/L across ages 12-19 through ≥40 years) (Silva et al., 2004). Variability by sex and by race or ethnicity was also observed, with higher levels in women compared with men (median 174 and 154 µg/L, respectively, in women and men) and in non-Hispanic blacks (median 306 µg/L) compared with non-Hispanic whites (median 133 µg/L) or Mexican Americans (median 174 µg/L). Socioeconomic status was not associated with MEP levels in a study using NHANES data (Tyrrell et al., 2013), and in a study in Hmong women living in Wisconsin (Peck et al., 2010). EPA will consider these data in assessing the potential influence of demographic factors on observed effect estimates for DEP.

Potential confounding by other factors

Some of the health effects under consideration may have strong associations with other risk factors. For example, smoking is associated with increased risk of low birth weight and preterm births, and with infertility. Abstinence time is strongly related to sperm concentration measures. In evaluating the potential for confounding by any of these factors, EPA will review evidence pertaining to the strength and direction of its association with DEP (or MEP).

Table 3-1. General and outcome-specific considerations for DEP study evaluation

General Considerations	
Study population	- Study population and setting: geographic area, site, time period, age and sex distribution, other details as needed (may include race/ethnicity, socioeconomic status) - Recruitment process; exclusion and inclusion criteria, knowledge of study hypothesis, knowledge of exposure and outcome - Participation rates: Total eligible, participation at each stage and for final analysis group and denominators used to make these calculations - Length of follow-up, loss to follow-up - Comparability: Participant characteristic data by group, data on non-participants
Exposure	- Tissue (e.g., urine, serum, semen, breast milk) - Limit of detection (LOD) or level of quantitation (LOQ) - Exposure distribution (e.g., central tendency, range), proportion < LOD
Analysis	- Consideration of skewness of exposure and outcome measures - Consideration of influence of "tails" in analysis based on continuous exposure measure - Consideration of values below LOD or LOQ - Consideration of creatinine or other approach to adjust for urine volume - Presentation of quantitative results, rather than statement regarding presence or absence of statistical significance
Outcome-specific Considerations	
Sexual differentiation **Measures**	- Anogenital distance: protocol, training procedures, standardization and inter-rater reliability - Cryptorchidism: definition and criteria
Consideration of confounding	- Anogenital distance: variability by size (e.g., birthweight); temporal trends in DEP exposure if study spans several years and includes a wide age range - Cryptorchidism: preterm birth
Early pregnancy loss **Measures**	- Source of data (e.g., human chorionic gonadotropin, self-report)
Consideration of confounding	- Age, smoking, gravidity
Gestational age **Measures**	- Source of data (e.g., ultrasound or last menstrual period data from early in pregnancy)
Consideration of confounding	- Smoking
Birthweight **Measures**	- Source of data (e.g., medical records, birth certificate)
Consideration of confounding	- Gestational age, pregnancy complications
Steroidal and gonadotropin hormones **Measures**	- Type of assay - Sensitivity/detection limits, coefficient of variation; number of samples below LOD
Consideration of confounding	- Age
Sperm parameters **Measures**	- Type of assay (e.g., WHO protocol)
Consideration of	- Age, smoking, abstinence time (associated with sperm parameters, but if not

confounding	related to MEP levels, would result in increased imprecision, rather than biased estimate)
Infertility **Measures**	- Definition, source of data
Consideration of confounding	- Age, smoking
Neurobehavioral **Measures**	- Standardized assessment tool, validation studies for specific study population (e.g., age group, geographic location) - Blinding of assessor to exposure
Consideration of confounding	- Age, sex, socioeconomic status
Obesity **Measures**	- Source of data (e.g., measures of weight and height, if BMI used; self-report)
Consideration of confounding	- Age, sex, ethnicity
Diabetes and insulin resistance **Measures**	- Source of data (e.g., biomarkers of insulin or glucose, medical records, self-report)
Consideration of confounding	- Age, sex, ethnicity

3.4.2. Experimental Animal Studies

Beyond the initial methodological screening described above in Section 3.2.2, methodological aspects of a study's design and conduct will be considered again in the overall evaluation and synthesis of the pertinent data that will be developed for each health effect. Some general questions that will be considered in evaluating experimental animal studies are presented in Table 3-2. These questions are, for the most part, broadly applicable to all experimental studies.

Table 3-2. Questions and relevant experimental information for evaluation of experimental animal studies

Methodological feature	Question(s) considered	Examples of relevant information extracted
Test animal	Based on the endpoint(s) in question, are concerns raised regarding the suitability of the species, strain, or sex of the test animals on study?	Test animal species, strain, sex
Experimental setup	Are the timing, frequency and duration of exposure, as well as animal age and experimental group allocation procedures/ group size for each endpoint evaluation, appropriate for the assessed endpoint(s)?	Age/lifestage of test animals at exposure and all endpoint testing timepoints Timing and periodicity of exposure and endpoint evaluations; duration of exposure Experimental group allocation procedures and sample size for each experimental group (e.g., animals; litters; dams) at each endpoint evaluation
Exposure	Are the exposure conditions and controls informative and reliable for the endpoint(s) in question, and are they sufficiently specific to the compound of interest?	Test article composition, stability, and vehicle control Exposure administration techniques (e.g.,

Table 3-2. Questions and relevant experimental information for evaluation of experimental animal studies

Methodological feature	Question(s) considered	Examples of relevant information extracted
		route; chamber type) and related controls
Endpoint evaluation procedures	Do the procedures used to evaluate the endpoint(s) in question conform to established protocols, or are they biologically sound? Are they sensitive for examination of the outcome(s) of interest?	Specific methods for assessing the effect(s) of exposure, including related details (e.g., biological matrix or specific region of tissue/organ evaluated) Endpoint evaluation controls, including those put in place to minimize evaluator bias
Outcomes and data reporting	Were data reported for all pre-specified endpoint(s) and study groups, or were any data excluded from presentation/ analyses?	Data presentation for endpoint(s) of interest

Note: "Outcome" refers to findings from an evaluation (e.g., steatosis), whereas "endpoint" refers to the evaluation itself (e.g., liver histopathology).

Evaluation of some specific methodological features identified in Table 3-2, such as exposure, is likely to be relatively independent of outcome. Other methodological features, in particular those related to experimental setup and endpoint evaluation procedures, are generally outcome specific (i.e., reproductive and developmental toxicity). Some specific aspects of study methodology that will be considered in the evaluation and synthesis of the DEP literature include the following:

Test Animals

Evidence indicates that in utero exposure to various phthalates during late gestation elicits a variety of effects in developing male rats termed the "phthalate syndrome" (effects include cryptorchidism; hypospadias; decrease in anogenital distance; delayed preputial separation; agenesis of the prostate, epididymis, and vas deferens; degeneration of the seminiferous epithelium; interstitial cell hyperplasia of the testis; and the retention of thoracic areolas or nipples) (Foster, 2006). However, testing of both sexes (in both developing and adult animals) is preferred because some effects have been observed in adult males and females following exposure to DEP. In addition, there is some evidence that rats may be more sensitive to phthalate syndrome effects compared to mice and that slight differences in strain sensitivity exist for some of these endpoints in rats. However, testing of both sexes is preferred for certain endpoints (including reproductive, neurological, and endocrine) because of possible gender differences (e.g., differences associated with maturation of reproductive hormone systems and cyclicity in females). These methodological features will be further considered in subsequent study evaluation.

Exposure

The majority of studies administered DEP in the diet. Several studies also utilized drinking water or gavage administration of DEP. All dietary studies should verify the homogeneity and stability of the test material in the feed over the course of the study; because DEP is semi-volatile and can partition into the atmosphere when exposed to air, documentation of stability of the test material in the diet will be a consideration.

Outcomes and Data Reporting

In general, experimental animal studies will be compared against traditional assay formats (e.g., those used in guideline studies), with deviations from the protocol evaluated in light of how the deviations could alter interpretation of the outcome in question. Most of the DEP studies fall in the categories of general and reproductive and developmental toxicity studies.

Outcome Specific Considerations

Reproductive and Developmental Endpoints

EPA's Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996a) detail study design parameters that are of particular importance in reproductive toxicity studies. These factors include duration of dosing, length of mating period and number of males and females mated, type of test (single versus multi generation studies), and endpoints evaluated. Test guidelines for the conduct of single- and multigeneration reproduction protocols that have been published by EPA and OECD will be utilized in evaluation of the reproductive and developmental toxicity database for DEP (U.S. EPA, 1996b, 1985; Galbraith et al., 1983; OECD, 1983; U.S. EPA, 1982).

Likewise, EPA's Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991) detail study design parameters that are of particular importance in developmental toxicity studies. Evaluation of developmental endpoints includes studies that typically involve exposure of pregnant animals during critical windows of organogenesis, evaluation of maternal toxicity throughout pregnancy, and examination of dams and uterine contents (U.S. EPA, 1991). Developmental toxicity studies also may evaluate exposures of one to a few days to investigate critical windows of development. The route of exposure in developmental toxicity studies is usually oral, unless the chemical or physical characteristics of the chemical or human exposures indicate another route of administration is more appropriate. Endpoints typically evaluated in developmental toxicity studies include assessment of maternal toxicity, altered survival and growth, morphological development, and functional deficits. A particular consideration in developmental toxicity studies is the selection of a high dose that produces minimal maternal or adult toxicity (i.e., a level that at the least produces marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity, and at the most produces no more than 10% mortality). At doses that cause excessive maternal toxicity (that is, significantly greater than the minimal toxic level), information on developmental effects may be difficult to interpret and of limited value.

A full evaluation of all pertinent studies will be performed as part of the critical review and synthesis of evidence for hazard identification for each of the health endpoints identified in the evidence tables (Appendix A).

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APPENDIX A. PRELIMINARY EVIDENCE TABLES AND EXPOSURE-RESPONSE ARRAYS

A.1. Data Extraction: Preparation of Preliminary Evidence Tables and Exposure-Response Arrays for Primary Studies

Key study design information, including study characteristics that inform the quality of the studies, and results from primary sources of health effects data considered pertinent for evaluating the health effects from chronic exposure to DEP are summarized in preliminary evidence tables (Appendix A). The information in the preliminary evidence tables is also displayed graphically in preliminary exposure-response arrays. In these arrays, a significant effect (indicated by a filled circle) is based on statistical significance.

The complete list of references considered in preparation of these materials can be found on the HERO website at (http://hero.epa.gov/DEP) and (http://hero.epa.gov/phthalates-human studies).

A.2. Liver Effects Evidence Tables and Exposure-Response Array

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results
Liver weight	
(Gray et al., 2000) Rat (Sprague Dawley); 19 female controls and 5 female DEP-treated; adrenal weights were assessed in 17 control litters (n=45 males) and 3 DEP-treated litters (n=12 males) 0, 750 mg/kg-day Gavage GD14-PND3 Note: The litter was the statistical unit of comparison. Body weight was analyzed as a covariate.	No significant changes in absolute or relative liver weight compared to controls were observed
(Moody and Reddy, 1978) Rat (F344); 4 exposed males, 14 control males 0, 2.0% (0, 1753 mg/kg-day) Diet 21 days	Relative liver weight (*percent change compared to control*) 0 | 1753 - | 16%*
(Kwack et al., 2009) Rat (Sprague Dawley); 6 males/group 0, 500 mg/kg-day DEP and 0, 250 mg/kg-day MEP Gavage in corn oil 28 days	Relative liver weight (*percent change compared to control*) 0 | 500 (DEP) | 0 | 250 (MEP) - | 13% | - | 6%
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day Gavage in corn oil 28 days	Relative liver weight (*percent change compared to control*) 0 | 40 | 200 | 1000 Males: - | -2% | -3% | -2% Females: - | -4% | -1% | 3%
(Mapuskar et al., 2007) Mouse (Swiss); 5 females/group 0, 0 (oil control), 10, 25, 50 mg/kg (0, 0 (oil control), 1.25, 3.125, 6.25 mg/kg-day) Diet (DEP dissolved in corn oil) 90 days	No significant changes in absolute or relative liver weight compared to controls were observed (Quantitative data not reported by study authors)

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results				
(Brown et al., 1978) Rat (Sprague Dawley); 5/sex/group 0, 1, 5% Diet 42 days, and 15/sex/group 0, 0.2, 1, 5% in diet (M: 0, 150, 770, 3160 mg/kg-day; F: 0, 150, 750, 3710 mg/kg-day) 112 days	Relative liver weight (*percent change compared to control*)				
	Males	0	150	770	3160
	42 day	-	N/A	15%*	33%
	112 day	-	-3%	3%	33%*
	Females	0	150	750	3710
	42 day	-	N/A	9%	33%*
	112 day	-	6%*	8%*	31%*
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/generation; liver weights measured in 21-24/sex/group (F0 and F1 parental, F1 and F2 weanlings) 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks of dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	Absolute liver weight (*percent change compared to control*)				
	Males	0	40/46	197/222	1016/1150
	F0 parental	-	-5%	-1%	-2%
	F1 parental	-	2%	5%	14%*
	F1 weanling	-	-5%	-2%	-4%
	F2 weanling	-	0%	3%	8%
	Females	0	51/56	255/267	1297/1375
	F0 parental	-	0%	0%	11*%
	F1 parental	-	5%	4%	11*%
	F1 weanling	-	-8%	-6%	-12*%
	F2 weanling	-	1%	4%	8%
	Relative liver weight (*percent change compared to control*)				
	Males	0	40/46	197/222	1016/1150
	F0 parental	-	-3%	-1%	7*%
	F1 parental	-	2%	2%	11*%
	F1 weanling	-	-5*%	-1%	11*%
	F2 weanling	-	0%	3%	16*%
	Females	0	51/56	255/267	1297/1375
	F0 parental	-	-1%	2%	10*%
	F1 parental	-	4%	2%	10*%
	F1 weanling	-	-5%	-3%	9*%
	F2 weanling	-	0%	2%	16*%
(Sonde et al., 2000) Rat (Sprague Dawley); 6 males/group 0, 50 ppm (0, 13.7 mg/kg-day) Drinking water 120 days	No change in absolute or relative liver weight compared to controls (Quantitative data not reported by study authors)				

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results			
(NTP, 1984) Mouse (CD-1); Continuous breeding protocol F0: 40 control and 18-20 breeding pairs/treatment group F1: 20 breeding pairs/group/generation F0: 0, 0.25, 1.25, 2.5 % (0,340,1770, 3640 mg/kg-day) F1: 0, 2.5% (0, 3640 mg/kg-day)[a] Diet F0: 7 days premating + 98 days cohabitation + 21 days segregation (126 days total) F1: in utero and via lactation, and then in the diet through a 7 day mating period at 74±10 days old (females allowed to deliver litters)	Absolute liver weight in F1 parental mice (*percent change compared to control*)			
		0	3640	
	Males	-	3%	
	Females (n=19)	-	15%*	
	Relative liver weight in F1 parental mice (*percent change compared to control*)			
		0	3640	
	Males	-	18*%	
	Females (n=19)	-	28%*	
(Pereira and Rao, 2006) Rat (Wistar); 6 females/group 0, 50 mg/kg diet (0, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	Relative liver weight (*percent change compared to control*)			
	0		2.85	
	-		≈ 8%	
(Pereira et al., 2006) Rat (Wistar); 6 males/group 0, 10, 25, 50 mg/kg diet (0, 0.57, 1.425, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	Relative liver weight[a] (*percent change compared to control*)			
	0	0.57	1.425	2.85
	-	21%*	-9	-13
(Sinkar and Rao, 2007) Rat (Wistar); 8/sex/group 0, 50 ppm (0, 2.5 mg/kg-day) Drinking water 180 days	Study authors did not report a change in absolute or relative liver weight compared to controls (quantitative data not provided)			
(Pereira and Rao, 2007) Rat (Wistar); 6 breeding pairs/group; liver weights measured in 6 pups/sex/group 0, 50 mg/kg diet (0, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 100 days (premating) + 10 days (mating) and through gestation and weaning of the PND 21 male and female pups (150 days total for parental animals)	Absolute liver weight at PND 21 (*percent change compared to control*)			
		0	2.85	
	Males	-	-16%*	
	Females	-	-56%*	
	Relative liver weight at PND 21 (*percent change compared to control*)			
		0	2.85	
	Males	-	31%*	
	Females	-	-42%*	

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results
(Pereira et al., 2007a) Rat (Wistar); Multigenerational study design: 6 breeding pairs/group/generation; liver weights measured in 6 adult males/group/generation F0: 0, 50 mg/kg diet (0, 2.85 mg/kg-day) F1: 0, 25 mg/kg diet (0, 1.425 mg/kg-day) F2: 0, 10 mg/kg diet (0, 0.57 mg/kg-day) Diet (DEP dissolved in corn oil) F0: Adult exposure [150 days: 100 days (premating) + 10 days (mating) and through gestation and weaning] F1, F2: Developmental exposure [GD0 – PND21] and Adult exposure [150 days (see F0 protocol) starting PND 35-40]	Relative liver weight[a] (*percent change compared to control*) F0: 0 / 2.85; F1: 0 / 1.425; F2: 0 / 0.57 Males: - / -9% / - / 34%* / - / 50%*
(NTP, 1995) Mouse ($B_6C_3F_1$); 10/sex/group 0, 12.5, 25, 50, 100 µl/day (5 days/week) (0, 14, 28, 56, 112 mg/day) Dermal (neat) 28 days, and 60/sex/group 0, 7.5, 15, 30 µL/day (5 days/week) (0, 8.4, 16.8, 33.6 mg/day) Dermal (mixed with acetone) 104-105 weeks (liver weights recorded at 15-month interim sacrifice only [9-10/sex/group]) Rat (F344/N); 10/sex/group 0, 37.5, 75, 150, 300 µl/day (5 days/week) (0, 42, 84, 168, 336 mg/day) Dermal (neat) 28 days, and 60/sex/group 0, 100, 300 µl/day (5 days/week) (0, 112, 336 mg/day) Dermal (neat) 104 weeks (liver weights recorded	Absolute liver weight [28 day study] (*percent change compared to control*) Mouse: 0 / 14 / 28 / 56 / 112 Males: - / 4% / 1% / 2% / 2% Females: - / 9% / 15%* / 9% / 14%* Rats: 0 / 42 / 84 / 168 / 336 Males: - / -1% / 0% / 0% / 4% Females: - / 2% / 6% / 6% / 2% Relative liver weight [28 day study] (*percent change compared to control*) Mouse: 0 / 14 / 28 / 56 / 112 Males: - / 2% / 4% / 3% / 3% Females: - / 7% / 9%* / 6% / 10%* Rats: 0 / 42 / 84 / 168 / 336 Males: - / 2% / 3% / 6% / 11%* Females: - / 4% / 5% / 8%* / 7%* Absolute liver weight at 15 months [104 week study] (*percent change compared to control*) Mouse: 0 / 8.4 / 16.8 / 33.6 Males: - / -2% / 1% / 0% Females: - / -8% / -4% / -5% Rat: 0 / 112 / 336 Males: - / 2% / -1% Females: - / 1% / 2% Relative liver weight at 15 months [104 week study] (*percent change compared to control*) Mouse: 0 / 8.4 / 16.8 / 33.6 Males: - / 3% / -1% / 5%

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results				
at 15-month interim sacrifice only [9-10/sex/group])	Females	-	-1%	0%	4%
	Rat	0	112		336
	Males	-	9%		7%
	Females	-	5%		4%
Serum clinical chemistry; liver function					
(Kwack et al., 2009) Rat (Sprague Dawley); 6 males/group 0, 500 mg/kg-day DEP and 0, 250 mg/kg-day MEP Gavage in corn oil 28 days	(*percent change compared to control*)				
		Serum		Serum	
		0	500 (DEP)	0	250 (MEP)
	GOT (ALT)	-	-0.1%	-	14%
	GPT (AST)	-	21%	-	58%
	ALP	-	20%	-	6%
	Glucose	-	14%	-	15%
	Total bilirubin	-	40%	-	30%
	Cholesterol	-	-13%	-	-11%
(Mapuskar et al., 2007) Mouse (Swiss); 5 females/group 0, 0 (oil control), 10, 25, 50 mg/kg (0, 0 (oil control), 1.25, 3.125, 6.25 mg/kg-day) Diet (DEP dissolved in corn oil) 90 days	*(percent change compared to control)*				
		0	1.25	3.125	6.25
	ALT[a] Serum	-	382%*	1131%*	921%*
	AST[a] Serum	-	231%*	523%*	681%*
	ACP[a] Serum	-	44%*	66%*	91%*
	LDH[a] Serum	-	12%	304%*	396%*
	Chol-Esterol[a] Serum	-	-13%*	-53%*	-55%*
	Chol-Esterol[a] Liver	-	126%*	68%*	74%*
	Tri-glycerides[a] Serum	-	47%*	65%*	153%*
	Tri-glycerides[a] Liver	-	1229%*	1371%*	1771%*
	Glycogen[a] Liver	-	29%*	56%*	87%*
(Sonde et al., 2000) Rat (Sprague Dawley); 6 males/group 0, 50 ppm (0, 13.7 mg/kg-day) Drinking water 120 days	*(percent change compared to control)*				
		Serum		Liver	
		0	13.7	0	13.7
	ALT[a]	-	349%*	-	-28%*
	AST[a]	-	323%*	-	-30%*
	ALP[a]	-	245%*	-	-18%
	ACP[a]	-	75%*	-	61%*
	SDH[a]	-	-10%	-	100%*
	Cholesterol[a]	-	2600%*	-	11873%*
	Triglycerides[a]	-	-81%*	-	119%*
	Glycogen[a]	N/A	N/A	-	364%*

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results					
(Pereira and Rao, 2006) Rat (Wistar); 6 females/group 0, 0 (oil control), 50 mg/kg diet (0, 0 (oil control), 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	*(percent change compared to control)*					
			Serum		Liver	
			0	2.85	0	2.85
	ALT[a]		-	286%*	-	119%*
	AST[a]		-	569%*	-	389%*
	ALP[a]		-	-53%*	-	-75%*
	ACP[a]		-	254%*	-	206%*
	LDH[a]		-	225%*	-	182%*
	SDH[a]		-	21%	-	45%
	Glucose[a]		-	1033%*	N/A	N/A
	Glycogen[a]		N/A	N/A	-	29%
	Cholesterol[a]		-	356%*	-	782%*
	Triglycerides[a]		-	250%*	-	41%
(Pereira et al., 2006) Rat (Wistar); 6 males/group 0, 10, 25, 50 mg/kg diet (0, 0.57, 1.425, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	*(percent change compared to control)*					
			0	0.57	1.425	2.85
	ALT[a]	Serum	-	1783%*	1483%*	1592%*
		Liver	-	254%*	192%*	250%*
	AST[a]	Serum	-	498%*	591%*	779%*
		Liver	-	333%*	475%*	676%*
	ACP[a]	Serum	-	310%*	117%*	90%*
		Liver	-	100%*	19%	55%
	LDH[a]	Serum	-	53%*	30%*	38%*
		Liver	-	106%*	67%*	83%*
	Glycogen[a]	Liver	-	40%*	115%*	191%*
	Chol-Esterol[a]	Serum	-	-19%	-90%*	-94%*
		Liver	-	-3%	37%*	176%*
	Tri-Glycerides[a]	Serum	-	141%*	114%*	136%*
		Liver	-	275%*	226%*	234%*

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results				
(Sinkar and Rao, 2007) Rat (Wistar); 8/sex/group 0, 50 ppm (0, 2.5 mg/kg-day) Drinking water 180 days	*(percent change compared to control)*				
		Serum		Liver	
	Males	0	2.5	0	2.5
	ALT[a]	N/A	N/A	-	7%
	AST[a]	-	-5%	N/A	N/A
	ALP[a]	-	4%	-	0%
	ACP[a]	-	-21%*	-	30%*
	LDH[a]	-	-50%*	-	50%
	SDH[a]	-	-50%	-	0%
	Gluta-thione[a]	N/A	N/A	-	-8%
		Serum		Liver	
	Females	0	2.5	0	2.5
	ALT[a]	N/A	N/A	-	0%
	AST[a]	-	4%	N/A	N/A
	ALP[a]	-	0%	-	-29%*
	ACP[a]	-	0%	-	0%
	LDH[a]	-	-21%*	-	0%
	SDH[a]	-	-8%	-	-34%*
	Gluta-thione[a]	N/A	N/A	-	-17%
(Pereira and Rao, 2007) Rat (Wistar); 6 breeding pairs/group; liver function was measured in 6 pups/sex/group 0, 50 mg/kg diet (0, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 100 days (premating) + 10 days (mating) and through gestation and weaning of the PND 21 male and female pups (150 days total for parental animals)	*(percent change compared to control)*				
		Serum		Liver	
	Males	0	2.85	0	2.85
	ALP[a]	-	1300%*	-	-64%*
	ACP[a]	-	379%*	-	321%*
	LDH[a]	-	226%*	-	72%*
		Serum		Liver	
	Females	0	2.85	0	2.85
	ALP[a]	-	1244%*	-	-25%
	ACP[a]	-	463%*	-	382%*
	LDH[a]	-	303%*	-	142%*

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results								
(Pereira et al., 2007a) Rat (Wistar); Multigenerational study design: 6 breeding pairs/group/generation; liver function assessed in 6 adult males/group/generation 0, 50 mg/kg diet (0, 2.85 mg/kg-day) (F0 rats) 0, 25 mg/kg diet (0, 1.425 mg/kg-day) (F1 rats) 0, 10 mg/kg diet (0, 0.57 mg/kg-day) (F2 rats) Diet (DEP dissolved in corn oil) F0: Adult exposure [150 days: 100 days (premating) + 10 days (mating) and through gestation and weaning] F1, F2: Developmental exposure [GD0 – PND21] and Adult exposure [150 days (see F0 protocol) starting PND 35-40]	*(percent change compared to control)*								
			F0 Males		F1 Males		F2 Males		
			0	2.85	0	1.425	0	0.57	
	ALT[a]	Serum	-	213%*	-	1602%*	-	1444%*	
		Liver	-	62%*	-	78%*	-	104%*	
	AST[a]	Serum	-	790%*	-	1673%*	-	1600%*	
		Liver	-	421%*	-	541%*	-	597%*	
	Tri-Glycerides[a]	Serum	-	233%*	-	380%*	-	443%*	
		Liver	-	25%*	-	119%*	-	169%*	
	Chol-esterol[a]	Serum	-	116%*	-	-21%*	-	-94*%	
(NTP, 1995) Rat (F344/N); 10/sex/group 0, 100, 300 µl (5 days/week) (0, 112, 336 mg/day) Dermal 104-105 weeks (clinical chemistry reported from 15-month interim sacrifice only [9-10/sex/group])	*(percent change compared to control)*								
	Males		0		112		336		
	Urea nitrogen		-		5%		3%		
	Creatinine		-		7%		-5%		
	ALP		-		-3%		7%		
	SDH		-		0%		0%		
	Females		0		112		336		
	Urea nitrogen		-		2%		0%		
	Creatinine		-		2%		-7%		
	ALP		-		11%		16%*		
	SDH		-		-10%		-10%		
Hepatic cytochrome (CYP) P450s									
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/generation; heptatic CYPs evaluated in 6 F0 males/group 0, 600, 3000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day) Diet ~98 days (F0 parental males; 14 weeks of dosing during premating and mating)	*(percent change compared to control)*								
			0		40		197		1016
	CYP 1A1		-		0%		0%		0%
	CYP 1A2		-		11%		-3%		-48%
	CYP 2B1		-		25%		-15%		13%
	CYP 3A4		-		12%		-40%		65%*
	CYP 4A1		-		9%		-16%		358%*

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results							
Liver lipid peroxidation[a]								
(Sonde et al., 2000) Rat (Sprague Dawley); 6 males/group 0, 50 ppm (0, 13.7 mg/kg-day) Drinking water 120 days	*(percent change compared to control)*							
	0					13.7		
	-					600%*		
(Pereira and Rao, 2006) Rat (Wistar); 6 females/group 0, 50 mg/kg diet (0, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	*(percent change compared to control)*							
	0					2.85		
	-					3380%*		
(Pereira et al., 2006) Rat (Wistar); 6 males/group 0, 10, 25, 50 mg/kg diet (0, 0.57, 1.425, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	*(percent change compared to control)*							
	0		0.57		1.425		2.85	
	-		725%*		233%*		475%*	
Liver antioxidant systems								
(Pereira and Rao, 2006) Rat (Wistar); 6 females/group 0, 50 mg/kg diet (0, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	*(percent change compared to control)*							
			Liver					
			0				2.85	
	Glutathione[a]		-				-17%*	
	Glutathione reductase[a]		-				-81%*	
(Pereira et al., 2006) Rat (Wistar); 6 males/group 0, 10, 25, 50 mg/kg diet (0, 0.57, 1.425, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	*(percent change compared to control)*							
		Liver						
		0		0.57		1.425		2.85
	Glutathione	-		-62%*		12%		-36%*
(Pereira et al., 2007a) Rat (Wistar); Multigenerational study design: 6 breeding pairs/group/generation; liver antioxidants measured in 6 adult males/group/generation 0, 50 mg/kg diet (0, 2.85 mg/kg-day) (F0 rats) 0, 25 mg/kg diet (0, 1.425 mg/kg-day) (F1 rats) 0, 10 mg/kg diet (0, 0.57 mg/kg-day) (F2 rats)	*(percent change compared to control)*							
		Liver						
		F0 Males		F1 Males		F2 Males		
		0	2.85	0	1.425	0	0.57	

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results							
Diet (DEP dissolved in corn oil) F0: Adult exposure [150 days: 100 days (premating) + 10 days (mating) and through gestation and weaning] F1, F2: Developmental exposure [GD0 – PND21] and Adult exposure [150 days (see F0 protocol) starting PND 35-40]	Glutathione[a]	-	-16%*	-	-60%*	-	-79%*
	Glutathione reductase[a]	-	-66%*	-	-93%*	-	-97%*
Histopathological effects							
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day DEP Gavage in corn oil 28 days	No remarkable observations were noted.						
(Brown et al., 1978) Rat (Sprague Dawley); 5/sex/group 0, 1, 5% (M: 0, 770, 3160 mg/kg-day; F: 0, 750, 3710 mg/kg-day) Diet 42 days, and 15/sex/group 0, 0.2, 1, 5% in diet (M: 0, 150, 770, 3160 mg/kg-day; F: 0, 150, 750, 3710 mg/kg-day) 112 days	No remarkable observations were noted.						
(NTP, 1995) Mouse ($B_6C_3F_1$); 60/sex/group 7.5, 15, 30 µL/day (5 days/week) (0, 8.4, 16.8, 33.6 mg/day) Dermal (mixed with acetone) 104-105 weeks (50/sex/group) Interim sacrifice at 15 months (10/sex/group)	*Incidence of basophilic focus in the liver*						
		0		8.4	16.8		33.6
	Males	0/50		1/50	9/50*		3/50
		0		8.4	16.8		33.6
	Females	2/50		3/51	6/50		2/50

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/generation 0, 600, 3000, 15,000 ppm in the diet (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks of dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	No remarkable observations were noted in the animals that were examined (i.e. control and high dose F0 and F1 parental males and females).
(Mapuskar et al., 2007) Mouse (Swiss); 5 females/group 0, 10, 25, 50 mg/kg (0, 1.25, 3.125, 6.25 mg/kg-day) Diet (DEP dissolved in corn oil) 90 days	Intracellular vacuolations, proliferation of peroxisomes and mitochondria. (Quantitative data not reported by study authors).
(Sinkar and Rao, 2007) Rat (Wistar); 8/sex/group 0, 50 ppm (0, 2.5 mg/kg-day) Drinking water 180 days	Vacuolations in hepatocytes, loss of hepatic architecture, degenerative changes in the centrilobular and periportal areas, and necrotic changes. (Quantitative data not reported by study authors.)
(Pereira and Rao, 2006) Rat (Wistar); 6 females/group 0, 50 mg/kg diet (0, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	Loss of hepatic architecture, granular deposits in hepatocytes and vacuolation in the centrilobular and periportal areas. (Quantitative data not reported by study authors.)
(Pereira et al., 2006) Rat (Wistar); 6 males/group 0, 10, 25, 50 mg/kg diet (0, 0.57, 1.425, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 150 days	Rats in the 0.57 mg/kg-day group, but not the 1.425 or 2.85 mg/kg-day group, showed severe intra- and intercellular vacuolations, loss of hepatic architecture, fatty degeneration in the centrilobular and periportal areas, and increased number of peroxisomes. Rats in the .425 or 2.85 mg/kg-day groups showed granular deposits in the hepatocytes and mild vacuolations in the centrilobular and periportal areas. All groups showed increased mitochondrial proliferation in a dose-dependent manner. (Quantitative data not reported by study authors.)

Table A-1. Evidence pertaining to hepatic effects in animals following exposure to DEP

Reference and Study Design	Results
(Moody and Reddy, 1978) Rat (F344); 4 exposed males, 14 control males 0, 2.0% (0, 1753 mg/kg-day) Diet 21 days	In rats exposed to DEP for 21 days, control animals exhibited a "normal" mitochondria-peroxisome ratio of 5:1 whereas DEP treated rats were found to have a 5:2 ratio.
(Pereira and Rao, 2007) Rat (Wistar); 6 breeding pairs/group; livers examined microscopically in 6 pups/sex/litter 0, 50 mg diet/kg (0, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 100 days (premating) + 10 days (mating) and through gestation and weaning of the PND 21 male and female pups (150 days total for parental animals)	Mild vacuolations in the livers of PND 21 pups. (Quantitative data not reported by study authors.)

*Statistically significant ($p<0.05$) based on analysis of data by study authors.

Percent change compared to control = $\frac{\text{treated value} - \text{control value}}{\text{control value}} \times 100$

[a]Values were digitally extracted from graphically presented data

1

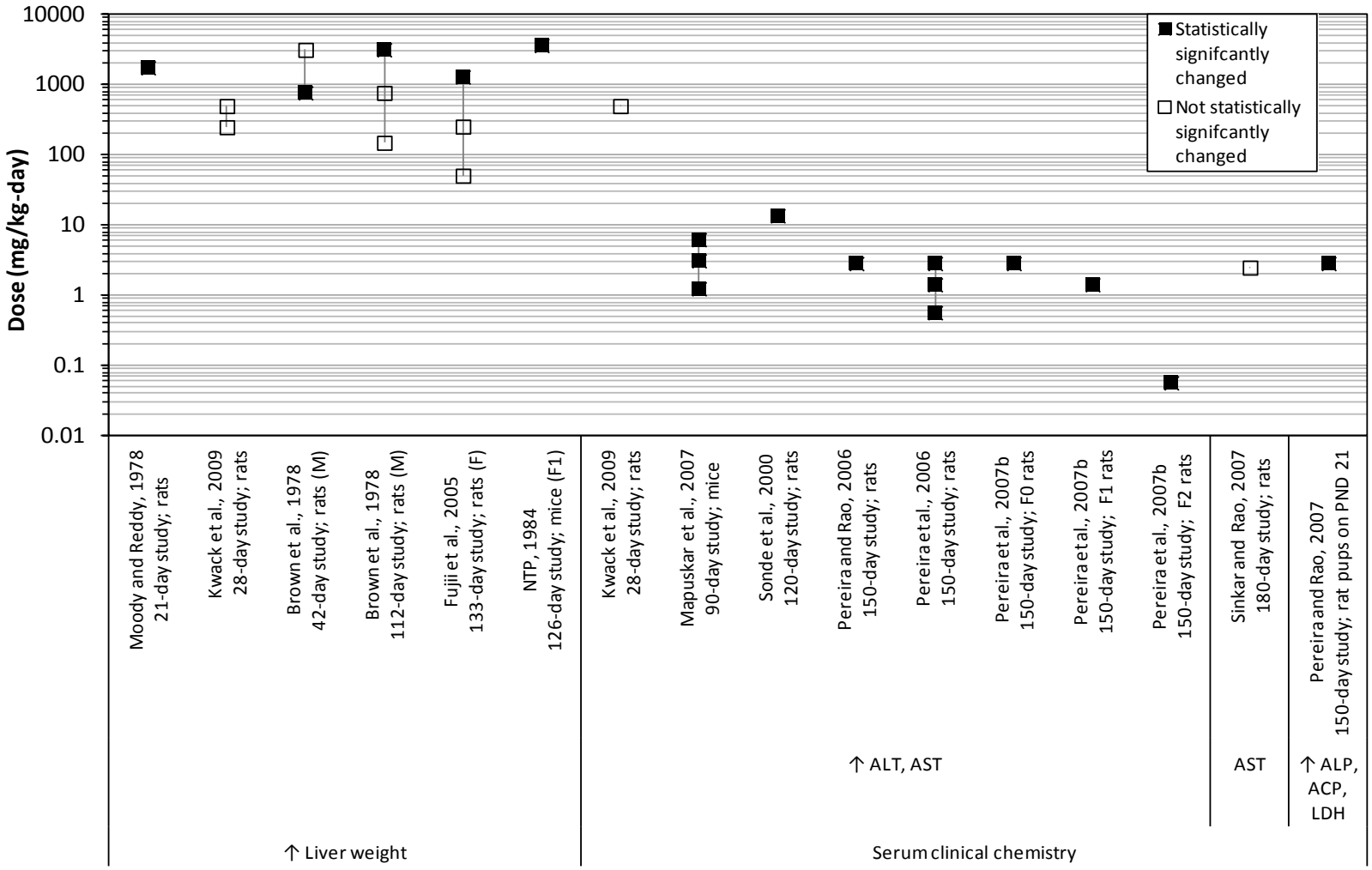

Figure A-1. Exposure-response array of liver effects following oral exposure to DEP

A.3. Reproductive and Developmental Effects Evidence Tables and Exposure-Response Array

Table A-2. Evidence pertaining to mono ethyl phthalate (MEP) and sexual differentiation effects in humans

Reference and Study Design	Results
Anogenital distance (AGD)	
(Suzuki et al., In Press) (Japan) Birth cohort study; 111 male infants (time period not given) **Outcome:** AGD measured 1–3 d after birth (AGD1 to anterior genitalia, mean 45.8 mm, 14.8 mm/kg; AGD2 to posterior genitalia, mean 20.3 mm, 6.6 mm/kg) **Exposure:** Maternal urine sample, mean 29 wks gestation MEP in urine (ng/mL): Median — 75th percentile Unadjusted 7.8 32 SG-adjusted 11 44 **Analysis:** Linear regression considering gestational week, birth order, maternal age, maternal smoking during pregnancy, maternal environmental tobacco smoke exposure, maternal urinary daidzein (soy isoflavone) and equol (a urinary metabolite of daidzein) concentrations and environmental tobacco smoke (smoking status of husbands of non-smoking women) as potential confounders	Association between MEP and AGD measures reported as not statistically significant (quantitative results not reported)
(Swan, 2008) (United States; Minnesota, Missouri, California) Multicenter birth cohort study, 2000–2002; 106 boys, mean age 12.8 mo (0 to 36 mo) **Outcome:** AGD (to posterior genitalia) measured at 0–36 mo (mean 70.4 mm, 7.1 mm/kg) **Exposure:** Maternal urine sample, 3rd trimester MEP in urine (ng/mL): Median — 75th percentile Unadjusted 128 437 **Analysis:** Regression analysis using mixed model adjusting for age and weight percentile **Related references:** **(Swan et al., 2005)** (exposure data)	Percent change in AGD per interquartile increase in MEP concentration(*p*-value): MEP -4.0 (0.005) The association between MEP and AGD was similar in magnitude or slightly smaller than seen between the DEHP metabolites and AGD (percent change per DEHP metabolite -3.9 to -4.5), and slightly larger than seen between MBP, MiBP, or MMP and AGD (percent change -3.2 to -3.6).

Table A-2. Evidence pertaining to mono ethyl phthalate (MEP) and sexual differentiation effects in humans

Reference and Study Design	Results
Cryptorchidism or testicular position	
(Swan, 2008) (United States; Minnesota, Missouri, California) Multicenter birth cohort study, 2000–2002; 106 boys, mean age 12.8 mo (0 to 36 mo) **Outcome:** Incomplete testicular descent assessed at clinical exam (10% prevalence) **Exposure:** Maternal urine sample, 3rd trimester MEP in urine (ng/mL): Median: Unadjusted 128; 75th percentile: 437 **Analysis:** Logistic regression, adjusting for age and weight percentile **Related references:** **(Swan et al., 2005)** (exposure data)	MEP reported as not associated with testicular position (quantitative results not reported)
(Main et al., 2006) (Denmark, Finland) Case-control study within two birth cohorts; n = 130 boys born 1997–2001; 62 3-mo-old boys with cryptorchidism, 68 controls **Outcome:** Cryptorchidism, at birth and/or 3 mo **Exposure:** Breast milk samples collected 1–3 mo of age MEP in breast milk (µg/L), all samples: Median (range) Denmark 0.93 (0.07–33.6) Finland 0.97 (0.25–41.4) **Analysis:** Mann-Whitney U test for comparison of MEP concentrations in boys with and without cryptorchidism	Median MEP in breast milk (µg/L) Controls: 0.976; Cases: 0.898 ($p > 0.40$)
Infant hormone levels	
(Lin et al., 2011) (Taiwan) Birth cohort study; 155 newborn infants (81 boys, 74 girls), born 2000–2001 **Outcome:** Cord blood hormone levels **Exposure:** Maternal urine sample 3rd trimester MEP in urine: Median; 75th perc.; 95th perc. Unadjusted (ng/mL) 35; 61; 241 Cr-adjusted (µg/g Cr) 56; 106; 346 **Analysis:** Pearson correlation analysis and linear regression adjusted for maternal age, BMI, smoking habit, gestational age, parity, and use of contraceptive drugs as potential confounders.	Pearson correlation coefficient (r) and regression coefficient (β), log-MEP (µg/g Cr) and cord blood hormone level r / β Boys Free testosterone (ng/dL) -0.10 / NR Estradiol (pg/mL) 0.02 / -0.02 Free testosterone: estradiol ratio -0.13 / -0.17 Girls Free testosterone (ng/dL) -0.24* / 0.02 Estradiol (pg/mL) 0.01 / NR Free testosterone: estradiol ratio -0.29* / -0.02

Table A-2. Evidence pertaining to mono ethyl phthalate (MEP) and sexual differentiation effects in humans

Reference and Study Design	Results
	NR = not reported *p < 0.01; all other p-values > 0.10 The correlation between MEP and free testosterone in girls was smaller than the correlation between this outcome and the summed DEHP metabolites (r = -0.38); the DEHP association remained in the adjusted regression analysis.
(Main et al., 2006) (Denmark, Finland) Case-control study within two birth cohorts; n = 130 boys born 1997–2001; 62 3-mo-old boys with cryptorchidism, 68 controls (includes 5 preterm cases, 3 preterm controls) **Outcome:** Serum steroidal and gonadotropin hormone levels in infants, samples collected when breast milk samples delivered to hospital **Exposure:** Breast milk samples collected 1–3 mo of age. MEP in breast milk (µg/L), all samples: Median (range) Denmark 0.93 (0.07–33.6) Finland 0.97 (0.25–41.4) **Analysis:** Cases and controls combined for analysis of association between metabolite concentration and hormone analysis using partial Spearman correlation coefficients adjusted for country of birth; hormone ratios evaluated using regression analysis, considering gestational age, weight for gestational age, parity, smoking, diabetes, and country of origin as potential covariates	Spearman correlation coefficient (p-value), MEP (µg/L) and serum hormone level (n = 96 boys) SHBG (nmol/L) 0.323 (0.002) Free testosterone (nmol/L) -0.191 (0.068) Testosterone (nmol/L) -0.010 (0.93) LH (IU/L) 0.185 (0.075) FSH (IU/L) 0.050 (0.63) Adjusted regression coefficient (95% CI), log-MEP and log-hormone level (adjusted for country of origin) SHBG (nmol/L) 1.15 (1.03, 1.28) Free testosterone (nmol/L) 0.86 (0.69, 1.06) LH: free testosterone ratio 1.26 (0.99, 1.60)

Table A-2. Evidence pertaining to mono ethyl phthalate (MEP) and sexual differentiation effects in humans

Reference and Study Design	Results
Gender-related play	
(Swan et al., 2010) (United States) (United States; Minnesota, Missouri, California, Iowa) Multicenter birth cohort study, 2000–2002 and 2002-2005 (Iowa); n = 145, ages 4–7 years; second follow-up study of birth cohort **Outcome:** Gender-specific play based on Pre-School Activities Inventory (24 items completed by parent or caregiver; sub-scores of male-oriented items and female-oriented items and a composite score consisting of male summation minus female summation scores) **Exposure:** Maternal urine sample, 3rd trimester MEP in urine (ng/mL). Distribution not reported for this analysis; EPA assumed similar distribution as seen in Swan et al., 2005 MEP in urine (ng/mL): Median 75th percentile Unadjusted 128 437 **Analysis:** Regression analysis using Generalized Linear Models, considering creatinine, sex and age of child, maternal age, parental education, number of same and opposite sex siblings, ethnicity, clinic location, and parental attitude as potential covariates **Related references: (Swan et al., 2005)** (exposure data)	log-MEP reported as not associated with masculine or composite activity score (quantitative results not reported)

Table A-3. Evidence pertaining to male reproductive effects in humans

Reference and Study Design	Results
Reproductive hormones	
(Joensen et al., 2012)(Denmark) 881 men from the general population, assessed at military conscript exam*, median age 19.1 yrs (5th, 95th percentiles: 18.4, 22.0 yrs), 2007–2009 **Outcome:** Serum steroidal and gonadotropin hormones **Exposure:** Urine sample, collected at same time as serum sample. MEP in urine (ng/mL): Median 95th percentile Unadjusted 78 1,936 **Analysis:** Linear regression considering age, BMI, smoking, alcohol consumption, time of blood sampling, assay type, ethnicity, BMI squared, *in utero* exposure to tobacco smoke, previous or current diseases, recent fever, and recent use of medication as potential covariates. *As reported by (Ravnborg et al., 2011).	Results for individual phthalate metabolites (including MEP) reported as "few significant associations" with free testosterone, estradiol, SHBG, LH, inhibin-B, or FSH (quantitative results not reported); analyses adjusted for age, BMI, smoking, alcohol consumption, and time of blood sampling (and assay type for inhibin-B only).
(Meeker et al., 2009a)(United States; Boston) 425 male partners seen in sub-fertility clinic from 2000–2004; mean age 36 yrs; **Outcome:** Serum steroidal and gonadotropin hormones **Exposure:** Urine sample, collected at same time as serum sample for hormone analysis MEP in urine (ng/mL): Median 75th percentile SG-adjusted 153 518 **Analysis:** Linear regression using untransformed (testosterone, estradiol) or natural logarithm transformed (free androgen index, FSH, LH) hormone levels; considering age, BMI, smoking status, race, previous infertility example, prior ability to impregnate partner, and season and time of sample collection as potential confounders. **Related references: (Duty et al., 2005)**	Regression coefficient (95% CI) for change in hormone with interquartile range (IQR) increase in adjusted MEP concentration (adjusted for age, BMI, smoking, season and time of sample collection) Untransformed hormone level (0.0 = no effect) Testosterone (ng/dL) 8.87 (-7.18, 24.9) Estradiol (pg/mL) 0.71 (-0.97, 2.40) Ln-transformed hormone level (1.0 = no effect) Free androgen index 1.04 (0.99, 1.09) FSH (IU/L) 0.98 (0.91, 1.06) LH (IU/L) 0.98 (0.91, 1.04)
(Jonsson et al., 2005) (Sweden) 234 men ages 18–21 yrs from the general population, assessed at military conscript exam **Outcome:** Serum steroidal and gonadotropin hormones **Exposure:** Urine sample, collected at same time as serum sample MEP in urine: Median 75th percentile Unadjusted (ng/mL) 240 870 Adjusted (nmol/mmol Cr) 83 310	Mean difference (95% CI), highest compared with lowest quartile of MEP (nmol/mmol Cr) Testosterone (nM) -0.3 (-2.3, 1.8) Free testosterone (T/SHBG) 0.06 (-0.05, 0.2) Estradiol (pM) 1.8 (-4.2, 7.7) FSH (IU/L) 0.5 (-0.5, 0.6) LH (IU/L) 0.7 (0.1, 1.2) MEP quartiles: low ≤9.95 and high ≥308 nmol/mmol Cr. Positive difference indicates lower value in highest exposure

Table A-3. Evidence pertaining to male reproductive effects in humans

Reference and Study Design	Results
Analysis: Mean difference between high and low quartiles	quartile
Sperm parameters	
(Joensen et al., 2012) (Denmark) 881 men from the general population, assessed at military conscript exam*, median age 19.1 yrs (5th, 95th percentiles: 18.4, 22.0 yrs), 2007–2009 **Outcome:** Semen analysis **Exposure:** Urine sample, collected at same time as semen sample MEP in urine (ng/mL): Median 95th percentile Unadjusted 78 1,936 **Analysis:** Linear regression, considering age, BMI, smoking, alcohol consumption, ethnicity, BMI squared, *in utero* exposure to tobacco smoke, previous or current diseases, recent fever, recent use of medication, abstinence time, and time from ejaculation to analysis as potential covariates.	Results for individual phthalate metabolites (including MEP) reported as "few significant associations" with sperm volume, count, or percentage progressively motile sperm (quantitative results not reported; analyses adjusted for abstinence time [volume, concentration, and count] or time from ejaculation to analysis [progressively motile], percent of morphologically normal sperm was left unadjusted).
(Liu et al., In Press) (China) 97 male partners seen in sub-fertility clinic 2009–2010; mean age 32 yrs **Outcome:** Semen analysis; results dichotomized above and below WHO reference values; n = 43 with normal semen parameters **Exposure:** Urine sample, collected at same time as semen sample MEP in urine: Median 66th percentile Unadjusted (ng/mL) 12.6 21.3 Cr-adjusted (µg/g Cr) 15.2 28.5 **Analysis:** Logistic regression, adjusting for age, BMI, abstinence time, smoking, alcohol use, and education	OR (95% CI) by tertile of MEP (adjusted for age, BMI, abstinence time, smoking, alcohol use) MEP Tertile | Sperm concentration <20 × 10^6/mL (n = 11) | Sperm motility <50% motile (n = 34) | Semen volume <2 mL (n = 15) 1 (low) | 1.0 (referent) | 1.0 (referent) | 1.0 (referent) 2 | 1.4 (0.2, 8.8) | 0.7 (0.2, 1.9) | 0.2 (0.1, 1.2) 3 (high) | 1.5 (0.2, 9.6) | 0.4 (0.1, 1.2) | 0.8 (0.2, 3.0) (trend *p*) | (0.66) | (0.10) | (0.78)

Table A-3. Evidence pertaining to male reproductive effects in humans

Reference and Study Design	Results
(Pant et al., 2008) (India) 300 male partners (n = 100 fertile, 200 infertile) seen in obstetrics and gynecology department from both urban and rural areas; mean age 29 yrs; time period not reported **Outcome:** Semen analysis **Exposure:** Semen sample DEP in semen (µg/mL), mean ± SE: Fertile / Infertile Rural areas 0.64 ± 0.24 / 1.13 ± 0.11 Urban areas 0.74 ± 0.04 / 3.11 ± 0.26 **Analysis:** Pearson correlation analysis	Pearson correlation coefficient between semen DEP and sperm parameter: r Sperm concentration (× 10^6/mL) -0.19* Sperm motility (%) 0.03 Morphology (percent abnormal) -0.02 DNA fragmentation index (chromatin integrity) 0.07 *p < 0.05; all other p-values > 0.05 The correlation between DEP and sperm concentration was similar to or slightly smaller that the correlation between this outcome and DBP (r=-0.20) or DEHP (r = -0.25).
(Hauser et al., 2007) (United States; Boston) n = 379 male partners seen in sub-fertility clinic 2000–2004; mean age 36 yrs **Outcome:** Sperm DNA damage assessed by neutral comet assay **Exposure:** Urine sample, collected at same time as semen sample MEP in urine (ng/mL): Median / 75th perc. / 95th perc. SG-adjusted 154 / 513 / 2,030 **Analysis:** Linear regression, considering age, abstinence time, smoking status, and race as potential covariates **Related reference: (Duty et al., 2003b)**	Regression coefficient (95% CI) for DNA damage associated with interquartile range increase in ln-MEP (adjusted for age and smoking status) Comet extent (µm) / Tail distribution (µm) / %DNA tail 6.06 (0.941, 12.3) / 2.72 (0.46, 5.00) / -0.26 (-2.52, 2.02)
(Hauser et al., 2006) (United States; Boston) n = 443 male partners seen in sub-fertility clinic 2000–2004; mean age 36 yrs **Outcome:** Semen analysis; results dichotomized above and below WHO reference values **Exposure:** Urine sample, collected at same time as semen sample MEP in urine (ng/mL): Median / 75th perc. / 95th perc. SG-adjusted 158 / 535 / 2,214 **Analysis:** Logistic regression, considering age, race, BMI, abstinence time, and smoking as potential covariates **Related references: (Duty et al., 2004); (Duty et al., 2003a)**; (Hauser et al., 2005)	OR (95% CI) by quartile of MEP (ng/mL) (adjusted for age, abstinence time, and smoking; comparison group = 210 men without deficiencies on any of these three parameters) MEP quartile / Sperm concentration < 20 × 10^6/mL / Sperm motility < 50% motile / Sperm morphology < 4% normal 1 (low) 1.0 (referent) / 1.0 (referent) / 1.0 (referent) 2 1.5 (0.7, 3.6) / 1.1 (0.6, 1.9) / 0.8 (0.4, 1.6) 3 1.0 (0.4,2.5) / 0.8 (0.5, 1.5) / 0.7 (0.3, 1.3) 4 (high) 1.2 (0.5, 3.0) / 1.0 (0.6, 1.8) / 0.5 (0.3, 1.1) (trend p) (0.94) / (0.84) / (0.07) OR (95% CI) for sperm motion parameters by quartile of MEP (ng/mL) (adjusted for age, smoking and abstinence time) MEP (ng/mL) quartile / Straight line velocity (µm/s) / Curvilinear velocity (µm/s) / Linearity (%)

Table A-3. Evidence pertaining to male reproductive effects in humans

Reference and Study Design	Results
	1 (low) 1.0 (referent) 1.0 (referent) 1.0 (referent) 2 0.02 (-2.66, 2.70) -0.28 (-4.82, 4.25) 0.34 (-1.55, 2.23) 3 0.81 (-1.92, 3.55) -0.47 (-5.09, 4.16) 1.67 (-0.25, 3.60) 4 (high) 2.11 (-0.61, 4.83) 4.48 (-0.13, 9.08) -0.31 (-2.23, 1.61) (trend *p*) 0.10 0.07 0.93 MEP quartile cut points: 8.7–58.7, 59.6–157.6, 157.9–534.3, 535.0–11,371 ng/mL No interaction with polychlorinated biphenyls (PCBs)
(Zhang et al., 2006) (China) 52 men seen in Shanghai Institute of Planned Parenthood Research in 2002; mean age 32 yrs **Outcome:** Semen analysis **Exposure:** Semen samples Mean (range) DEP (mg/L) 0.47 (0.13–1.32) **Analysis:** Spearman correlation analysis	Spearman correlation coefficient (*p*-value), semen DEP (mg/L) and sperm parameter: Sperm density (× 10^6/mL) -0.25 (0.15) Sperm livability (%) -0.13 (0.45) Sperm rate of malformations (%) 0.19 (0.28)

Table A-3. Evidence pertaining to male reproductive effects in humans

Reference and Study Design	Results
(Jonsson et al., 2005)(Sweden) 234 men ages 18–21 yrs from the general population, assessed at military conscript exam **Outcome:** Semen analysis **Exposure:** Urine sample, collected at same time as semen sample MEP in urine: Median 75th percentile Unadjusted (ng/mL) 240 870 Adjusted (nmol/mmol Cr) 83 310 **Analysis:** Mean difference between high and low quartiles	Mean difference (95% CI), highest (≥308 nmol/mmol Cr) compared with lowest (≤9.95 nmol/mmol Cr) quartile MEP (Positive difference indicates lower value in highest exposure quartile) Sperm concentration (× 10^6/mL) 5.0 (-15, 25) Sperm motility (%) -0.4 (-6.4, 5.6) Sperm damage (chromatin integrity) 0.8 (-2.8, 4.4)
Infertility	
(Tranfo et al., 2012) (Italy) Case-control study; n = 56 infertile couples from assisted reproduction center, n = 56 fertile couples (parents of one or more children, living in same area); mean age 39–40 yrs in both groups; time period not reported **Outcome:** Primary or secondary infertility as assessed by WHO criteria (cause attributed to males in 8/56 couples) **Exposure:** Urine sample MEP in urine, fertile couples: Median 95th percentile Cr-adjusted (µg/g Cr) 52 651 **Analysis:** Mann-Whitney test for comparison of MEP concentrations by group	MEP concentration in urine (µg/g Cr) in fertile and infertile couples Fertile Infertile *p*-value Median 52 199 <0.001 95th percentile 651 2507 Sex-stratified comparison was also significant for men and for women ($p < 0.001$, quantitative results not reported). The case-control difference in MEP was the largest of the 4 phthalate metabolites examined; differences were also seen with MnBP, and to a lesser extent with MBzP and the summation of MEHP + MEHHP.
(Pant et al., 2008) (India) Case-control study; n = 100 fertile, 200 infertile men visiting obstetrics and gynecology department from both urban and rural areas; mean age 29 yrs; time period not reported **Outcome:** Infertility based on female partners who had not conceived after 1-yr unprotected intercourse and who had no diagnosed fertility disorder **Exposure:** Semen sample DEP in semen (µg/mL), mean ± SE: Fertile Infertile Rural areas 0.64 ± 0.24 1.13 ± 0.11 Urban areas 0.74 ± 0.04 3.11 ± 0.26 **Analysis:** Two-way ANOVA for difference in DEP concentrations between fertile and infertile with rural/urban as additional variable	DEP concentration in semen (µg/mL), mean ± SE, in fertile and infertile men Rural: Fertile (n = 40) Infertile (n = 88) 0.64 ± 0.24 1.13 ± 0.11 Urban: Fertile (n = 60) Infertile (n = 112) 0.74 ± 0.04 3.11 ± 0.26* *$p < 0.05$ The case-control difference in DEP was similar or smaller than the difference seen with DBP, DEHP, or DMP.

Table A-4. Evidence pertaining to male reproductive effects in animals

Reference and Study Design	Results				
Serum Hormone levels					
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/generation; reproductive hormones measured in 6 F0 males/group 0, 600, 3000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day) Diet ~98 days (F0 parental males; 14 weeks dosing during premating and mating)	*(percent change compared to control)*				
		0	40	197	1016
	Testosterone	-	-28%	-80%*	-50%*
	Progesterone	-	36%	125%	16%
(Pereira et al., 2008a) Rat (Wistar); 6 males/group 0, 0 (oil control), 10, 25, 50 mg/kg (0, 0 (oil control), 0.57, 1.425, 2.85 mg/kg-day Diet (DEP dissolved in corn oil) 150 days	*(percent change compared to oil control)*				
		0	0.57	1.425	2.85
	Testosterone[b]	-	-35%*	-43%*	-62%*
	Androstenedione[b]	-	-28%*	-43%*	-32%*
(Yamasaki et al., 2005) Rat (Sprague-Dawley); Multigenerational study design; 0, 600, 3,000, 15,000 ppm Diet 10 weeks prior to mating (3 weeks in F1 parents), during mating, gestation, delivery, and lactation (males dosed up to autopsy)	A decrease in levels of serum testosterone was observed at 3,000 and 15,000 ppm. (Quantitative data not reported by study authors)				

Table A-4. Evidence pertaining to male reproductive effects in animals

Reference and Study Design	Results				
Anogenital distance (AGD)					
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/generation; AGD measured in 22-24 litters/group/generation 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks of dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	*(percent change compared to control)*				
	Males	0	40/46	197/222	1016/1150
	F1 males at PND 0	-	1%	4%	-3%
	F1 males at PND 4	-	-4%	-2%	-2%
	F2 males at PND 0	-	-1%	0%	1%
	F2 males at PND 4	-	-2%	-1%	0%
Reproductive organ weight					
(Gray et al., 2000) Rat (Sprague Dawley); 19 female controls and 5 female DEP-treated; adrenal weights were assessed in 17 control litters (n=45 males) and 3 DEP-treated litters (n=12 males) 0, 750 mg/kg-day Gavage GD14-PND3 Note: The litter was the statistical unit of comparison. Body weight was analyzed as a covariate.	Absolute weights *(percent change compared to control)*				
		0	750		
	testes weight	-	-3%		
	seminal vesicles	-	-12%		
	epididymis (paired)	-	-5%		
(Kwack et al., 2009) Rat (Sprague Dawley); 6 males/group 0, 500 mg/kg-day DEP and 0, 250 mg/kg-day MEP Gavage in corn oil 28 days	Relative weights *(percent change compared to control)*				
		0	500 (DEP)	0	250 (MEP)
	Testis weight (paired)	-	-9%	-	-7%
	Epididymis weight (left)	-	4%	-	-3%

Table A-4. Evidence pertaining to male reproductive effects in animals

Reference and Study Design	Results				
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day Gavage in corn oil 28 days	Relative weights (*percent change compared to controls*)				
		0	40	200	1000
	testes	-	6%	6%	11%
	epididymes	-	9%	9%	9%
(Brown et al., 1978) Rat (Sprague Dawley); 5/sex/group 0, 1, 5% (M: 0,770, 3160 mg/kg-day; F: 0, 750, 3710 mg/kg-day) Diet 42 days, and 15/sex/group 0, 0.2, 1, 5% (M: 0, 150, 770, 3160 mg/kg-day; F: 0, 150, 750, 3710 mg/kg-day) Diet 112 days	Relative gonad weight (percent change compared to control)				
	Males	0	150	770	3160
	42 day study	-	N/A	9%	43%*
	112 day study	-	-3%	0%	29%*
(Pereira et al., 2008a) Rat (Wistar); 6 males/group 0, 0 (oil control), 10, 25, 50 mg/kg diet (0, 0 (oil control), 0.57, 1.425, 2.85 mg/kg-day Diet (DEP dissolved in corn oil) 150 days	Absolute testis weight *(percent change compared to control)*				
	0	0.57	1.425	2.85	
	-	-18%*	-23%*	-28%*	
	Absolute epididymis weight *(percent change compared to oil control)*				
	0	0.57	1.425	2.85	
	-	-22%*	-35%*	-43%*	
(Pereira et al., 2007b) Rat (Wistar); 6/sex/group 0, 50 (F0) (0, 2.85 mg/kg-day) 0, 25 (F1) (0, 1.425 mg/kg-day) Diet 150 days/generation]	Absolute testis weight *(percent change compared to control)*				
	F0 parental males		F1 adult males		
	0	2.85	0	1.425	
	-	-3%	-	-8%	

Table A-4. Evidence pertaining to male reproductive effects in animals

Reference and Study Design	Results				
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/generation; reproductive organs weighed in 21-24 males/group (F0 and F1 parental, F1 and F2 weanlings) 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	Absolute testis weight *(percent change compared to control)*				
		0	40/46	197/222	1016/1150
	F0 parental	-	-3%	1%	3%
	F1 parental	-	-1%	-1%	-2%
	F1 weanling	-	7%	0%	-11%
	F2 weanling	-	0%	0%	-6%
	Relative testis weight *(percent change compared to control)*				
		0	40/46	197/222	1016/1150
	F0 parental	-	0%	0%	0%
	F1 parental	-	-2%	-3%	-3%
	F1 weanling	-	6%	0%	2%
	F2 weanling	-	0%	2%	4%
	Absolute epididymis weight *(percent change compared to control)*				
		0	40/46	197/222	1016/1150
	F0 parental	-	-5%	-1%	-5%*
	F1 parental	-	0%	3%	1%
	F1 weanling	-	-3%	-2%	-9%
	F2 weanling	-	0%	1%	-6%
	Relative epididymis weight *(percent change compared to control)*				
		0	40/46	197/222	1016/1150
	F0 parental	-	0%	0%	0%
	F1 parental	-	0%	5%	0%
	F1 weanling	-	-3%	-1%	5%
	F2 weanling	-	-1%	0%	-1%
	Absolute prostate weight *(percent change compared to control)*				
		0	40/46	197/222	1016/1150
	F0 parental	-	3%	13%	8%
	F1 parental	-	3%	4%	-5%
	F1 weanling	-	0%	0%	-20%*
	F2 weanling	-	0%	0%	-12%
	Relative prostate weight *(percent change compared to control)*				
		0	40/46	197/222	1016/1150
	F0 parental	-	5%	12%	12%
	F1 parental	-	5%	2%	-6%
	F1 weanling	-	2%	2%	-6%
	F2 weanling	-	0%	0%	-6%
	Absolute seminal vesicle weight *(percent change compared to control)*				
		0	40/46	197/222	1016/1150
	F0 parental	-	0%	3%	-2%
	F1 parental	-	-4%	1%	-5%
	F1 weanling	-	0%	0%	0%
	F2 weanling	-	-5%	-5%	-10%
	Relative seminal vesicles *(percent change compared to control)*				
		0	40/46	197/222	1016/1150
	F0 parental	-	0%	3%	3%
	F1 parental	-	-3%	0%	-6%
	F1 weanling	-	4%	4%	17%
	F2 weanling	-	-9%	-4%	-4%

Table A-4. Evidence pertaining to male reproductive effects in animals

Reference and Study Design	Results					
(NTP, 1995) Mouse ($B_6C_3F_1$); 10/sex/group 0, 12.5, 25, 50, 100 µl/day (5 days/week) (0, 14, 28, 56, 112 mg/day) Dermal (neat) 28 days, and Rat (F344/N); 10/sex/group 0, 37.5, 75, 150, 300 µl (5 days/week) (0, 42, 84, 168, 336 mg/day) Dermal (neat) 28 days	Absolute testis (right) weight *(percent change compared to control)*					
		0	14	28	56	112
	Mouse	-	-3%	-3%	0%	-2%
		0	42	84	168	336
	Rat	-	-3%	-2%	-2%	-2%
	Relative testis (right) weight *(percent change compared to control)*					
		0	14	28	56	112
	Mouse	-	-5%	-1%	-1%	-1%
		0	42	84	168	336
	Rat	-	0%	3%	3%	5%
(NTP, 1984) Mouse (CD-1); Continuous breeding protocol F0: 40 control and 18-20 breeding pairs/treatment group F1: 20 breeding pairs/group/generation F0: 0, 0.25, 1.25, 2.5 % (0, 438, 875, 1750, 4375 mg/kg-d) F1: 0, 2.5% (0, 3640 mg/kg-day) Diet F0: 7 days premating + 98 days cohabitation + 21 days segregation (126 days total) F1: in utero + lactation, and then in the diet through a 7 day mating period at 74±10 days old (F1 females were allowed to deliver litters)	Absolute weights in F1 parental males *(percent change compared to control)*					
			0		3640	
	Testis		-		-8%	
	Epididymis		-		-9*	
	Prostate		-		32%*	
	Seminal vesicles		-		-11%	
	Relative weights in F1 parental males *(percent change compared to control)*					
			0		3640	
	Testis		-		1%	
	Epididymis		-		1%	
	Prostate		-		32%*	
	Seminal vesicles		-		-4%	
Testicular lipid peroxidation						
(Pereira et al., 2008a) Rat (Wistar); 6 males/group 0, 0 (oil control), 10, 25, 50 mg/kg (0, 0 (oil control), 0.57, 1.425, 2.85 mg/kg-day Diet (DEP dissolved in oil) 150 days	*(percent change compared to control)*					
	0		0.57	1.425		2.85
	Testis[b]-		130%*	215%*		285%*

Table A-4. Evidence pertaining to male reproductive effects in animals

Reference and Study Design	Results				
Effects on sperm					
(Kwack et al., 2009) Rat (Sprague Dawley); 6 males/group 0, 500 mg/kg-day DEP and 0, 250 mg/kg-day MEP Gavage in corn oil 28 days	Sperm parameters *(percent change compared to control)*				
		0	500 (DEP)	0	250 (MEP)
	No. of sperm (× 10^6/g right cauda epididymis)	-	-16%	-	-41%*
	Motility (%)	-	-25%	-	-56%*
	Sperm LIN (%)	-	-18%	-	10%
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day DEP Gavage in corn oil 28 days	Dose-related effects on sperm (morphology, count) were not observed. (Quantitative data not reported by study authors)				
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/ generation; sperm parameters assessed in 23-24 parental males/group/generation 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 46, 222, 1150 mg/kg-day in F1 males) Diet ~98 days for F0 and F1 parental males (14 weeks dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	Sperm parameters *(percent change compared to control)*				
	F0 parental males	0	40	197	1016
	No. of sperm (× 10^6)				
	Per testis	-	-8%	-7%	-6%
	Per gram testis	-	-9%	-7%	-2%
	Per cauda epididymis	-	-8%	2%	-3%
	Per gram cauda Epididymis	-	-4%	0%	3%
	Motility (%)	-	-4%	1%	1%
	Abnormal sperm (%)	-	[a]633%	73%*	-5%
	Tailless sperm (%)	-	[a]733%	85%*	-7%
	F1 parental males	0	46	222	1150
	No. of sperm (× 10^6)				
	Per testis	-	-2%	-1%	-4%
	Per gram testis	-	0%	2%	-2%
	Per cauda epididymis	-	-5%	-5%	-2%
	Per gram cauda Epididymis	-	-4%	-5%	-2%
	Motility (%)	-	-2%	-3%	-1%
	Abnormal sperm rate (%)	-	38%	118%*	153%*
	Tailless sperm rate (%)	-	29%	116%*	141%*

Table A-4. Evidence pertaining to male reproductive effects in animals

Reference and Study Design	Results		
(NTP, 1984) Mouse (CD-1); Continuous breeding protocol F0: 40 control and 18-20 breeding pairs/treatment group F1: 20 breeding pairs/group/generation F0: 0, 0.25, 1.25, 2.5 % (0, 438, 875, 1750, 4375 mg/kg-d) F1: 0, 2.5% (0, 3640 mg/kg-day) Diet F0: 7 days premating + 98 days cohabitation + 21 days segregation (126 days total) F1: in utero + lactation, and then in the diet through a 7 day mating period at 74±10 days old (F1 females were allowed to deliver litters)	Sperm parameters *(percent change compared to control)* in F1 parental males		
		0	3640
	No. of sperm (× 10^3/mg caudal tissue)	-	-30%*
	Motility (%)	-	-4%
	Abnormal sperm (%)	-	65%
	Tailless sperm (%)	-	0%

*Statistically significant ($p<0.05$) based on analysis of data by study authors.

[a] Large standard deviation reported

Percent change compared to control = $\frac{\text{treated value} - \text{control value}}{\text{control value}} \times 100$

[b] Values used to derive these results were digitally extracted from bar graphs within the publication.

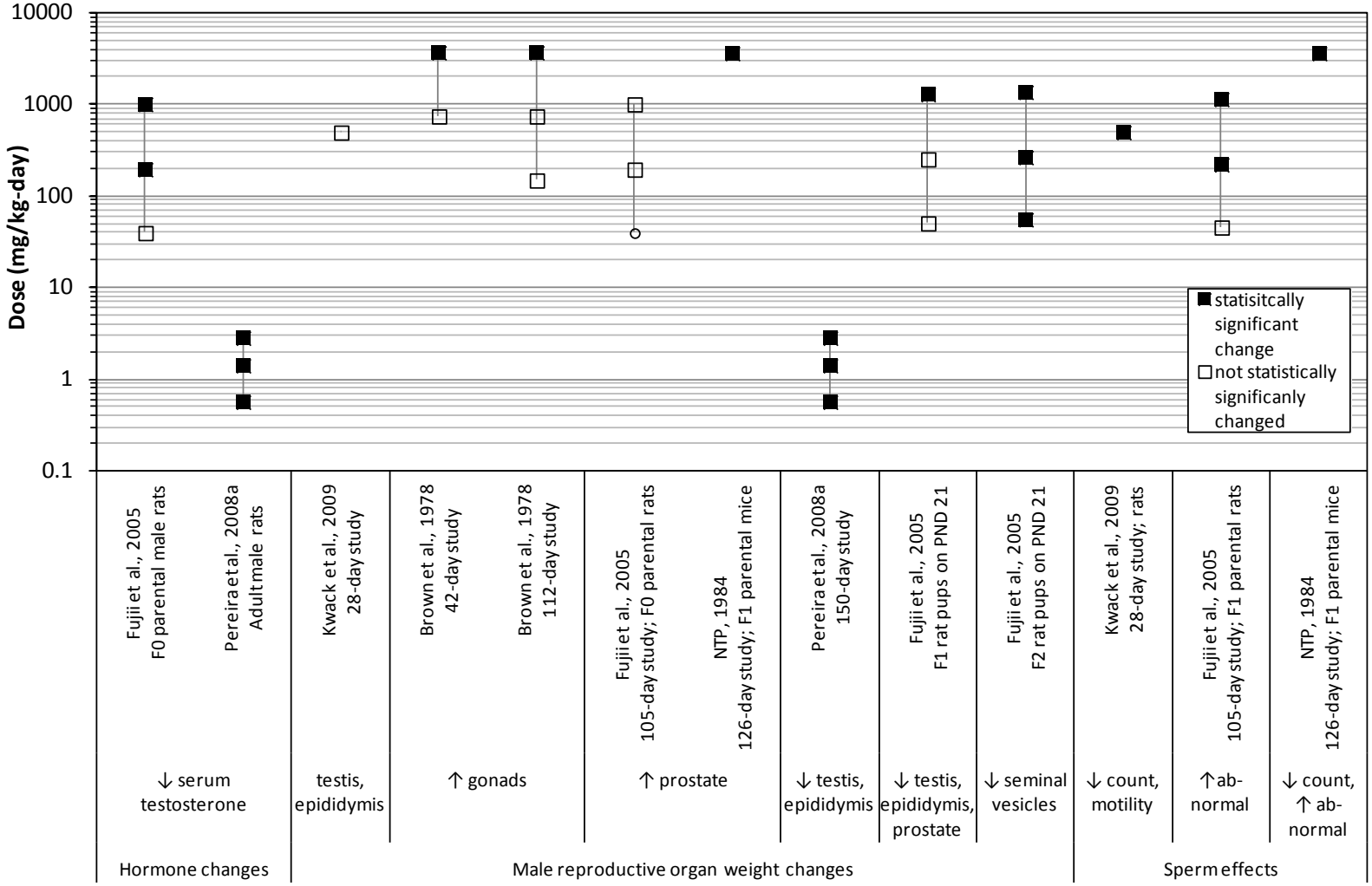

Figure A-2. Exposure-response array of male reproductive effects following exposure to DEP

Table A-5. Evidence pertaining to MEP and the timing of male puberty in humans

Reference and Study Design	Results
(Mieritz et al., 2012) (Denmark) Nested case-control study in cohort of 555 boys, 6–19 yrs old, participating in the COPENHAGEN Puberty Study, 2006–2008; 38 boys with pubertal gynecomastia and 190 age-matched controls. **Outcome:** Anthropometry, pubertal stage (pubic hair and genital development), presence of gynecomastia, and serum testosterone **Exposure:** Urine sample, collected at clinical evaluation MEP in urine (ng/mL): Median 95th percentile Group 3 36.24 263.9 (boys without gynecomastia, all ages) **Analysis:** Two-tailed Mann–Whitney U-test for comparisons between groups; linear regression with age adjustment for association with serum testosterone; probit analysis with phthalate concentrations divided in quartiles for analysis of puberty timing.	MEP concentration (ng/mL) by group Group 1 (n = 38) / Group 2 (n = 189) / Group 3 (n = 517) Median 38.70 / 37.95 / 36.24 95th percentile 314.3 / 359.9 / 263.9 Group 1 = boys with palpable gynecomastia Group 2 = boys without palpable gynecomastia (age-matched) Group 3 = boys without palpable gynecomastia (all ages) No association between MEP concentration and timing of puberty or serum testosterone level (quantitative results not reported).

Table A-6. Evidence pertaining to MEP and the timing of female puberty in humans

Reference and Study Design	Results
Central precocious puberty and premature thelarche	
(Frederiksen et al., 2012) (Denmark) Case-control study, n = 24 girls with precocious puberty (n = 13 with central precocious puberty, n = 6 with early normal puberty, n = 5 with premature thelarche) recruited from outpatient clinic 2008–2009 and n = 184* age-matched controls from COPENHAGEN Puberty Study cohort, recruited from high schools 2006–2008 **Outcome:** Precocious puberty, early normal puberty, or premature thelarche, defined based on clinical standards **Exposure:** Urine sample (child's), collected at clinical evaluation MEP in urine (ng/mL), controls: Median (range) Unadjusted 38 (4.8–1,649) **Analysis:** MEP concentrations in cases and controls compared with Mann-Whitney U test * Study reports number of controls inconsistently; text reports 164 controls, while Table 4 reports 184.	Median (range) MEP (ng/mL) in cases and controls: Controls: 38 (4.8–1,649) Precocious puberty: 30 (12–371) (*p*-value): (>0.05)
(Lomenick et al., 2010) (United States, Ohio and Kentucky) Case-control study; n = 28 girls with central precocious puberty, n = 28 age- and race-matched controls; recruited from pediatric endocrinology clinic 2005–2008; mean age 7 yrs **Outcome:** Central precocious puberty defined based on clinical standards (appearance of physical characteristics of puberty before 8 yrs of age, with laboratory confirmation of central origin of breast development) ; no cases had received medical treatment prior to urine sample collection **Exposure:** Urine sample (child's), collected at clinical evaluation MEP in urine of controls Mean ± SE Unadjusted (ng/mL) 253 ± 58 Cr-adjusted (µg/g Cr) 244 ± 51 **Analysis:** MEP concentrations in cases and controls compared with Wilcoxon rank-sum test	Mean ± SE MEP in cases and controls: Unadjusted (ng/mL): Controls 253 ± 58; Central precocious puberty 139 ± 24; (*p*-value) (0.40) Cr-adjusted (µg/g Cr): Controls 244 ± 51; Central precocious puberty 165 ± 26; (*p*-value) (0.38)

Table A-6. Evidence pertaining to MEP and the timing of female puberty in humans

Reference and Study Design	Results
Pubertal development (general population)	
(Frederiksen et al., 2012) (Denmark) 725 healthy girls ages 5.6–19.1 yrs from COPENHAGEN Puberty Study cohort, recruited from high schools during 2006–2008 **Outcome:** Stage of breast or pubic hair development; Serum steroid and gonadotropin hormones **Exposure:** Urine sample (child's), collected at time of pubertal stage assessment MEP in urine (ng/mL), all 725 participants: Median 95th percentile Unadjusted 39 262 **Analysis:** Probit analysis, results verified using Pool-Adjacent-Violators algorithm.	Mean age (95% CI) (yrs) at entry into breast stage 2 or pubic hair stage 2, by quartile of MEP: MEP quartile / Breast stage 2 (n = 394) / Pubic hair stage 2 (n not reported) 1 (low) 9.94 (9.47, 10.42) 10.95 (10.66, 11.25) 2 10.08 (9.59, 10.57) 11.22 (10.91, 11.53) 3 9.89 (9.40, 10.37) 11.20 (10.91, 11.50) 4 (high) 9.83 (9.30, 10.37) 11.13 (10.82, 11.46) Levels of FSH, LH, estradiol, testosterone were similar across MEP exposure groups (quantitative results not reported)

Table A-7. Evidence pertaining to MEP and gynecological conditions in humans

Reference and Study Design	Results
Endometriosis and leiomyomas	
(Buck Louis et al., 2013) (California and Utah, United States) Matched cohort study*; n = 473 women undergoing laparoscopy or laparotomy and 127 population age- and residence-matched referents, aged 18–44 yrs (2007–2009) **Outcome:** Endometriosis confirmed by surgery (operative cohort) or MRI (population cohort) **Exposure:** Urine sample MEP in urine (ng/mL), Cr-adjusted : Geometric mean (95% CI) Operative cohort-Endometriosis 107.2 (88.73, 129.4) Operative cohort-Controls 109.6 (93.64, 128.3) Population cohort-Endometriosis 152.0 (59.11, 390.8) Population cohort-Controls 138.2 (107.1, 178.4) **Analysis:** Student's t-test or Wilcoxon test for continuous data; logistic regression, adjusting for age, BMI, and creatinine; sensitivity analyses conducted restricting cohort to endometriosis stages 3 and 4 diagnoses or visually and histologically confirmed endometriosis, and referent group consisting of women with postoperative diagnosis of normal pelvis *Confirmed cases of endometriosis matched to women without endometriosis within each cohort: operative cohort, 190 cases, 283 controls; population cohort: 14 cases, 127 controls.	OR (95% CI) for endometriosis per unit increase in ln-MEP, by cohort (adjusted for age, BMI, and creatinine) Operative cohort 1.01 (0.82, 1.24) Population cohort 1.07 (0.56, 2.04) Adjusted OR (95% CI) for endometriosis per unit increase in ln-MEP in operative cohort (sensitivity analysis): Endometriosis stage 3 and 4 (n = 339) 1.04 (0.75, 1.43) Visual/histological confirmed endometriosis (n = 473) 1.04 (0.78, 1.39) Comparison with women with postoperative diagnosis normal pelvis (n = 320) 1.05 (0.81, 1.35) Note: Concentrations were log transformed and rescaled by their SDs for analysis.
(Huang et al., 2010) (Taiwan) Case-control study, n = 28 endometriosis cases, n = 36 leiomyoma cases, n = 16 adenomyosis cases, and n = 29 controls; mean ages ~38, 41, and 36 yrs, respectively; recruited from laparotomy patients in medical center, 2005–2007 **Outcome:** Clinical diagnosis of endometriosis, leiomyoma, or adenomyosis confirmed by pathology **Exposure:** Urine sample MEP in urine) Median (range) Unadjusted (ng/mL) Cr-adjusted(µg/g Cr) Control 37.2 (10.6–396.2) 71.4 (5.6–373.3) Endometriosis 31.6 (13.4–712.9) 58.0 (13.4–422.3) Leiomyoma 28.5 (6.7–705.9) 103.7 (11.2–519.0) Adenomyosis 33.8 (9.7–96.8) 53.4 (13.4–147.7) **Analysis:** Logistic regression, considering age, BMI, and GSTM1 polymorphism as covariates.	OR (95% CI) for case status by MEP above compared with below the median (for endometriosis, adjusted for GSTM1 polymorphism and BMI; for leiomyomas and adenomyosis, adjusted for GSTM1 polymorphism and age) Endometriosis Leiomyomas Adenomyosis 0.66 (0.21, 2.09) 1.32 (0.44, 3.96) 1.08 (0.26, 4.57)

Table A-7. Evidence pertaining to MEP and gynecological conditions in humans

Reference and Study Design	Results
(Weuve et al., 2010) (United States, NHANES) Case-control study of 1,227 female participants in the 1999–2004 NHANES, ages 20–54 yrs; n = 87 endometriosis cases, n = 151 leiomyomata cases, and n = 1,020 controls; mean age ~36 yrs **Outcome:** Self-reported diagnosis of endometriosis or leiomyomata; median time since diagnosis, 9 yrs **Exposure:** Urine sample, collected at time of survey MEP in urine (ng/mg Cr): Geometric mean (SE) Endometriosis cases 207 (27.5) Leiomyomata cases 210 (21.9) Controls 220 (14.1) **Analysis:** Logistic regression, adjusting for variables shown in results column	OR (95% CI) for gynecological condition by quartile of MEP (ng/mg Cr) (adjusted for age, race/ethnicity, age at menarche, current pregnancy status and current breast-feeding status) MEP Quartile / Endometriosis / Leiomyomata 1 (low) 1.0 (referent) 1.0 (referent) 2 0.89 (0.44, 1.82) 0.72 (0.35, 1.46) 3 1.13 (0.56, 2.27) 1.29 (0.74, 2.25) 4 (high) 1.12 (0.53, 2.35) 0.85 (0.47, 1.54) (trend *p*) (0.6) (0.9)
(Itoh et al., 2009) (Japan) Case-control study, n = 57 endometriosis patients, n = 80 controls; all seeking evaluation for infertility **Outcome:** Clinical diagnosis of endometriosis (American Fertility Society stages II-IV) by laparoscopy; controls were stages 0–1 **Exposure:** Urine sample Unadjusted MEP in urine (µg/L): Median / 75th percentile Controls 21.4 53.2 Cases 39.6 74.9 Cr-adjusted MEP in urine (µg/g Cr): Median / 75th percentile Controls 11.2 24.7 Cases 18.9 37.7 **Analysis:** Logistic regression, adjusting for menstrual regularity and average menstrual cycle length; Jonkheere Terpstra trend test for concentration by stage.	OR for endometriosis by MEP (µg/g Cr) above compared with below the median (adjusted for menstrual regularity and average menstrual cycle length) OR (95% CI) = 1.72 (0.81, 3.68) Median MEP in urine by stage of endometriosis: Endometriosis stage / Unadjusted (µg/L) / Cr-adjusted (µg/g Cr) 0 20.3 10.5 I 28.5 16.1 II 49.1 19.1 III 44.9 17.6 IV 31.2 16.1 (trend *p)* (0.09) (0.23)

Table A-8. Evidence pertaining to MEP and neurobehavioral and neurodevelopmental effects in infants and children

Reference and Study Design	Results
Attention and executive function in pre-school and school-aged children	
(Engel, 2010) (United States, New York City) Birth cohort study, n = 177 children from original birth cohort studied by Engel et al. (2009), 54% boys, three follow-up exams at ages 4.5–5.5, 6–6.5, 7–9 yrs **Outcome:** Behavior assessed by maternal reporting on Behavior Rating Inventory of Executive Function (BRIEF) and Behavior Assessment System for Children—Parent Rating Scales (BASC-PRS) **Exposure:** Maternal urine sample, 25–40 wks gestation* Median 75th percentile MEP (µg/L)* 386 1,025 Sum LMW (µM/L) 1.88 4.59 (sum of MBP, MEP, MiBP, and MMP) **Analysis:** Generalized linear regression model, adjusting for variables shown in results column. Other (no-specified) variables were considered). * MEP concentrations not reported in (Engel et al., 2010); values reported here are from an earlier analysis of this cohort described in (Engel et al., 2009)	Regression coefficient for change in behavioral score per unit increase in ln-phthalate level (µM/L) in boys (adjusted for race, educational level and marital status of the primary caretaker, and urinary creatinine) MEP / Low molecular weight phthalate sum Clinical scales (higher score = more problem behaviors) Aggression 0.91 1.24* Anxiety 0.79 0.78 Attention problems 1.28* 1.29* Atypicality 0.74 0.95 Conduct problems 1.85* 2.40* Depression 0.97* 1.18* Hyperactivity 0.83 1.03 Somatization 0.11 0.36 Withdrawal 0.44 0.46 Adaptive scales (lower score = more problem behaviors Adaptability -0.97* -1.08* Leadership -0.84 -0.88 Social skills -0.97 -1.04 Composite scales (higher score = more problem behaviors) Externalizing problems 1.33* 1.75* Internalizing problems 0.80 0.99 Adaptive skills -0.79 -0.98 Behavioral Symptoms Index 1.32* 1.55* BRIEF Scores (higher score = worse executive functioning) Behavioral regulation index 0.89 1.13 Metacognition index 0.89 1.05 Global executive composite score 1.02 1.23* *$p \leq 0.05$ Study authors reported there were few significant

Table A-8. Evidence pertaining to MEP and neurobehavioral and neurodevelopmental effects in infants and children

Reference and Study Design	Results
	associations between phthalate concentration and behavior among girls (quantitative results not reported)
Social function in pre-school and school-aged children	
(Miodovnik et al., 2011) (United States, New York City) Birth cohort study, n = 137, ages 7–9 yrs, Mt Sinai Children's Environmental Health study (enrolled 1998–2002) **Outcome:** Social functioning based on maternal reporting on Social Responsiveness Scale (SRS) (5 domains) **Exposure:** maternal urine sample, 25–40 wks gestation Phthalates in urine (µg/L): MEP: Median 372, 75th percentile 964 Low molecular weight phthalate metabolites: Median 419, 75th percentile 1,015 Low molecular weight phthalate metabolites include MMP, MEP, MiBP, and MBP. See Engel et al. (2008) for data pertaining to individual metabolite levels in the Mt. Sinai Children's Environmental Health cohort. **Analysis:** Generalized linear regression model,, considering maternal age, IQ, marital status, education, and urinary creatinine, and child's sex, race, and age as potential covariates	Regression coefficient (95% CI) for change in social functioning score per unit increase in ln-MEP (µg/L) (adjusted for child race, sex, caretaker marital status, urinary creatinine) Total SRS: 1.38 (0.23, 2.53) Cognition: 1.28 (0.10, 2.47) Communication: 1.67 (0.44, 2.90) Mannerisms: 0.77 (-0.46, 2.00) Motivation: 0.77 (-0.28, 1.83) Awareness: 1.10 (0.06, 2.14)

Table A-9. Evidence pertaining to MEP and pregnancy outcomes in humans

Reference and Study Design	Results
Preterm birth (<37 wks)	
(Meeker et al., 2009b) (Mexico) Nested case-control study in birth cohort, n = 30 preterm births, n = 30 term births, recruited during pregnancy, 2001–2003. **Outcome:** Preterm birth (<37 wks gestation), determined using maternal recall of last menstrual period **Exposure:** Maternal urine sample, third trimester MEP in urine, unadjusted (µg/L): Median; 75th percentile Term births 108 224 Preterm births 171 437 MEP in urine, SG-adjusted (µg/L): Median; 75th percentile Term births 134 284 Preterm births 182 340 MEP in urine, Cr-adjusted (µg/g Cr): Median; 75th percentile Term births 186 401 Preterm births 232 396 **Analysis:** Logistic regression, considering maternal age, pre-pregnancy BMI, parity, education, marital status, infant's sex, and gestational age at urine sample as potential covariates.	OR (95% CI) for preterm birth by MEP above compared with below the median (adjusted for marital status, maternal education, infant sex and gestational age at time of urine sample) Unadjusted (µg/L) 2.3 (0.7, 7.3) SG-adjusted (µg/L) 1.3 (0.4, 4.2) Cr-adjusted (µg/g Cr) 1.3 (0.4, 4.1)
Low birth weight (<2,500 g)	
(Zhang et al., 2009) (Shanghai, China) Nested case-control study in birth cohort; n = 88 low birth weight infants, n = 113 controls, born 2005–2006 **Outcome:** Low birth weight defined as <2,500 g among infants born ≥37 wks gestation. **Exposure:** Cord blood sample DEP in cord blood (mg/L): Median; 75th percentile Controls 2.0 2.4 Cases 1.6 2.0 **Analysis:** Conditional logistic regression, considering gestational age, pregnancy complications, exposure to tobacco smoke, socioeconomic level, and pre-pregnancy BMI as potential covariates.	OR for low birth weight by quartile of DEP in cord blood (mg/L) (adjusted for gestational age, smoking, socioeconomic level, pre-pregnancy BMI, and other phthalates) DEP Quartile; OR (95% CI) 1 (low) 1.0 (referent) 2 1.28 (0.92, 1.65) 3 0.69 (0.30, 1.57) 4 (high) 1.11 (0.43, 2.28) (trend *p)* (0.38)

Table A-9. Evidence pertaining to MEP and pregnancy outcomes in humans

Reference and Study Design	Results
Birth weight, birth length, head circumference and gestational age	
(Philippat et al., 2012) (France) Nested case-control study in two birth cohort studies of male genital malformations (EDEN and PELAGIE mother-child cohorts); n = 287 (72 cases with undescended testis or hypospadias and 215 matched controls); 2002–2006 **Outcome:** Standard clinical measurements at birth **Exposure:** Maternal urine sample, collected between 6 and 19 (PELAGIE) or between 24 and 30 (EDEN) weeks gestation MEP in urine (ng/mL): Median 95th percentile Measured 110 983 Standardized* 105 727 **Analysis:** Cases and controls combined for this analysis; weighted linear regression using tertiles or ln-transformed urine concentrations, adjusting for variables shown in results column. Analysis by tertiles for evaluation of possible non-monotonic relationship. Analyses corrected for oversampling of malformation cases. *Standardized for sampling conditions and gestational age at collection	Regression coefficient (95% CI) for change in birth outcome by MEP tertile and per unit change in ln-MEP (standardized, ng/mL) (adjusted for gestational duration, maternal pre-pregnancy weight and height, maternal smoking, maternal education, parity, recruitment center, urine creatinine, mode of delivery as potential covariate; head circumference model also adjusted for mode of delivery) MEP tertile (µg/L) | Birth weight (g) | Birth length (cm) | Head circumference (cm) 1 (<113.8) | 0 (referent) | 0 (referent) | 0 (referent) 2 (113.8–275.7) | 46 (-102, 194) | 0.5 (-0.2, 1.1) | 0.2 (-0.3, 0.7) 3 (≥ 275.7) | -14 (-162, 133) | 0.0 (-0.6, 0.7) | 0.4 (-0.1, 1.0) (trend *p*-value) | (0.60) | (0.58) | (0.14) Ln (MEP) | 3 (-51, 70) | 0.0 (-0.3, 0.2) | 0.1 (-0.2, 0.3)
(Suzuki et al., 2010) (Japan) Birth cohort study; n = 149 births; 2 were preterm (<37 wks); mothers recruited during pregnancy 2005–2008 **Outcome:** Standard clinical measurements at birth **Exposure:** Maternal urine sample, gestation wk 9–40 (mean ± SD = 29 ± 8 wk) MEP in urine: Median 75th percentile Unadjusted (ng/mL) 6.01 16.7 Cr-adjusted (mg/g Cr) 7.73 19.9 **Analysis:** Pearson's correlation analysis for individual metabolites	Pearson's correlation coefficient (p-value) between MEP (mg/g Cr) and birth outcome: Birth weight (g) -0.118 (>0.05) Birth length (cm) -0.014 (>0.05) Head circumference (cm) -0.021 (>0.05) Gestational age (wks) -0.028 (>0.05)

Table A-9. Evidence pertaining to MEP and pregnancy outcomes in humans

Reference and Study Design	Results
(Wolff et al., 2008) (United States, New York City) Birth cohort study (Mt Sinai Children's Environmental Health study); n = 382 singleton live births without medical complications, mothers recruited during pregnancy, 1998–2002. **Outcome:** Standard clinical measurements at birth **Exposure:** Maternal urine sample, third trimester MEP in urine (ng/mL): Median; 75th percentile Unadjusted: 380; 1,010 **Analysis:** Linear regression, adjusting for variables shown in results column.	Regression coefficient (95% CI) for change in birth outcome with unit increase in ln-MEP (ng/mL) (adjusted for race/ethnicity, infant sex, gestational age at delivery, ln-creatinine, prenatal smoking, pre-pregnancy BMI, maternal education, and marital status) Birth weight (g): 9.0 (-20, 38) Birth length (cm): 0.05 (-0.11, 0.21) Head circumference (cm): 0.12 (0.01, 0.23) Gestational age (wks): 0.11 (-0.01, 0.22) Restricted to observations with creatinine ≥20 mg/dL The association between MEP and gestational age AGD was slightly smaller than seen between MEHP and gestational age (Beta = 0.15).
Early pregnancy loss	
(Toft et al.) (Denmark) Cohort study of couples planning first pregnancy; n = 48 women with pregnancy loss and n = 80 with pregnancies ending in a live birth; recruited during pregnancy, 1992–1994 **Outcome:** Any pregnancy loss (n=48), early (subclinical) embryonal loss (pregnancy identified by elevation in human chorionic gonadotropin; n = 32) or clinically-identified pregnancy loss (n = 16) **Exposure:** Urine samples (one conception cycle, one preconception cycle) MEP in urine (ng/mL): Mean; Maximum Live birth: 406; 2,783 Pregnancy loss: 378; 2,766 **Analysis:** Logistic regression, adjusting for variables shown in results column	OR (95% CI) for any pregnancy loss by tertile MEP (ng/mL) in the preconception cycle or conception cycle (adjusted for age, BMI, smoking, alcohol and caffeine intake, and MEP in the other cycle) MEP Tertile; Pre-conception Cycle; Conception Cycle 1 (low): 1.0 (referent); 1.0 (referent) 2: 0.93 (0.35, 2.50); 1.51 (0.57, 3.98) 3 (high): 0.81 (0.31, 2.09); 1.98 (0.74, 5.34) OR (95% CI) for types of pregnancy loss by tertile MEP (ng/mL) in the conception cycle (adjusted for age, BMI, smoking, alcohol and caffeine intake, and MEP in the preconception cycle) MEP Tertile; Subclinical pregnancy loss; Clinical pregnancy loss 1 (low): 1.0 (referent); 1.0 (referent) 2: 1.29 (0.43, 3.83); 2.19 (0.42, 11.5) 3 (high): 1.13 (0.36, 3.59); 4.63 (0.92, 23.3) The magnitude of the association between MEP and clinical pregnancy loss was larger than that seen with MBP, MBzP, or the DEHP metabolites.

Table A-10. Evidence pertaining to female reproductive effects in animals

Reference and Study Design	Results					
Fertility and birth outcomes						
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/generation 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks of dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	No. of implantations *(percent change compared to control)*					
		0	51/56	255/267	1297/1375	
	F0 parental females	-	2%	1%	1%	
	F1 parental females	-	0%	4%	3%	
	Fertility Index *(percent change compared to control)*					
		0	51/56	255/267	1297/1375	
	F0 parental females	-	0%	4%	0%	
	F1 parental females	-	0%	0%	0%	
	Gestation length (days) *(percent change compared to control)*					
		0	51/56	255/267	1297/1375	
	F0 parental females	-	0%	0%	-1%	
	F1 parental females	-	0%	0%	-1%*	
	No. of pups delivered *(percent change compared to control)*					
		0	51/56	255/267	1297/1375	
	F0 parental females	-	-1%	1%	1%	
	F1 parental females	-	4%	7%	2%	
(Hardin et al., 1987) Mouse (CD-1); 50 dams/group 0, 4500 mg/kg-day Gavage in corn oil GD 6-GD 13	*(percent change compared to control)*					
		0			4500	
	No. of live pups/litter	-			0%	
	Birth weight	-			-6%	
	Surviving pups					
		0			4500	
	Percent survival	99.4			95.7	
(Howdeshell et al., 2008) Rat (Sprague Dawley); 3-5 dams/ treatment group and 9 control dams 0 (vehicle control), 100, 300, 600, 900 mg/kg-day Gavage in corn oil GD 8-GD 18	*(percent change compared to control)*					
		0	100	300	600	900
	No. of implantations	-	5%	3%	4%	13%
	No. of live fetuses	-	7%	5%	-6%	16%
	Total resorptions	-	-100%	-100%	325%*	-100%
	Fetal mortality (%)	-	2.9	0	11.1*	0

Table A-10. Evidence pertaining to female reproductive effects in animals

Reference and Study Design	Results				
(U.S. EPA, 1994) Rabbit (NZW); 12 dams/group 0 (untreated) 5, 15, 50% (w/w) DEP dissolved in 0.5% carboxymethylcellulose for a total application volume of 2 ml/kg body weight/day applied directly to skin (10 X 10 cm) in the dorsolumbar region GD 6-GD 18		0	5	15	50
	Gestation index %	100	100	100	100
	Still birth index %	0	1	0	0
	Resorption index %	8.9	2.5	1.3	14.9
	Post implantation loss index(%)	8.9	3.7	1.3	14.9
	Corora lutea per dam (percent change compared to control)	-	-6.4	-15.8	5.6
(Hazleton Laboratories, 1983) Mouse (CD-1); 50 dams/dose; timed-pregnant females 0 (corn oil), 4,500 mg/kg-day Gavage GD 7-GD 14	*(percent change compared to control)*				
	F0 females		0		4,500
	Reproductive index (%)		97		94
	No. females – viable litter		-		-3%
	No. females – pregnant		-		0%
	No. live pups per litter	PPD1	-		-2%
		PPD3			-10%
	pup litter wt/litter	PPD1	-		-6%
		PPD3	-		-5%
(NTP, 1984) Mouse (CD-1); Continuous breeding protocol F0: 40 control and 18-20 breeding pairs/treatment group F1: 20 breeding pairs/group/generation F0: 0, 0.25, 1.25, 2.5 % (0,340,1770, 3640 mg/kg-day) F1: 0, 2.5% (0, 3640 mg/kg-day) Diet F0: 7 days premating + 98 days cohabitation + 21 days segregation (126 days total) F1: in utero + lactation, and then in the diet through a 7 day mating period at 74±10 days old (F1 females were allowed to deliver litters)	*(percent change compared to control)*				
	F0 females	0	340	1770	3640
	No. of live pups/litter	-	23%*	14%	3%
	Live pup weight	-	-2%	-2%	1%
	F1 females	0		3640	
	No. of live pups/litter	-		-14%*	
	Fertility index (%)	95		95	
	Live pup weight	-		-3%	
(NTP, 1988) Rat (Sprague Dawley); 31-32 dams/group; reproductive endpoints reported for dams with litters (27-32 litters/group) 0, 0.25, 2.5, 5% (0, 198, 1909, 3214 mg/kg-day) Diet GD 6 to GD 15	*(percent change compared to control)*				
		0	198	1909	3214
	Corpora lutea per dam	-	4%	-2%	1%
	Implantation sites per litter	-	4%	-1%	2%
	Resorptions per litter	-	5%	13%	-11%

Table A-10. Evidence pertaining to female reproductive effects in animals

Reference and Study Design	Results				
	Percent resorptions per litter	3.8	3.9	4.1	3.1
	Live fetuses per litter	-	4%	-2%	3%
(Singh et al., 1972) Rat (Sprague Dawley); 5 dams/group 0 (untreated), 0.506, 1.012, 1.686 mL/kg (0, 627, 1133, 1888 mg/kg) Intraperitoneal injections GD5, 10, and 15 (termination on GD 20) Note: Statistical analysis was not conducted by study authors for this endpoint		0	627	1133	1888
	No. of corpora lutea	60	65	59	57
	No. of resorptions	0	28	0	2
	No. of live fetuses	59	35	57	54
Anogenital distance					
(Fujii et al., 2005) Rat (Sprague Dawley), Multigenerational study design: 24 breeding pairs/group/generation; AGD measured in 21-24 litters/group/generation 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	*(percent change compared to control)*				
	Females	0	51/56	255/267	1297/1375
	F1 pups at PND 0	-	-5%	-5%	1%
	F1 pups at PND 4	-	-3%	-2%	-1%
	F2 pups at PND 0	-	-2%	0%	-1%
	F2 pups at PND 4	-	-1%	-1%	-2%
Reproductive organ weights					
(Fujii et al., 2005) Rat (Sprague Dawley), Multigenerational study design: 24 breeding pairs/group/generation; reproductive organs weighed in 21-24 females/group (F0 and F1 parental, F1 and F2 weanlings) 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males;	Absolute ovary weight *(percent change compared to control)*				
		0	51/56	255/267	1297/1375
	F0 parental	-	-4%	-10%	-5%
	F1 parental	-	1%	2%	4%
	F1 weanling	-	4%	-8%	-4%
	F2 weanling	-	0%	0%	-4%
	Relative ovary weight *(percent change compared to control)*				
		0	51/56	255/267	1297/1375
	F0 parental	-	-5%	-8%	-5%
	F1 parental	-	0%	0%	2%

Table A-10. Evidence pertaining to female reproductive effects in animals

Reference and Study Design	Results				
0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	F1 weanling	-	7%	-3%	17%
	F2 weanling	-	-3%	-3%	0%
	Absolute uterus weight *(percent change compared to control)*				
		0	51/56	255/267	1297/1375
	F0 parental	-	2%	4%	-4%
	F1 parental	-	4%	7%	-1%
	F1 weanling	-	3%	7%	-22%*
	F2 weanling	-	-11%	-17%	-27%*
	Relative uterus weight *(percent change compared to control)*				
		0	51/56	255/267	1297/1375
	F0 parental	-	0%	6%	-3%
	F1 parental	-	4%	4%	0%
	F1 weanling	-	5%	9%	-5%
	F2 weanling	-	-12%	-17%	-20%*
(Pereira et al., 2007b) Rat (Wistar); 6/sex/group 0, 50 (F0) (0, 2.85 mg/kg-day) 0, 25 (F1) (0, 1.425 mg/kg-day) Diet 150 days/generation	Absolute ovary weight *(percent compared to control)*				
	F0 parental females		F1 adult females		
	0	2.85	0	1.425	
	-	40%*	-	23%*	
(NTP, 1984) Mouse (CD-1); Continuous breeding protocol F0: 40 control and 18-20 breeding pairs/treatment group F1: 20 breeding pairs/group F0: 0, 0.25, 1.25, 2.5 % (0,340,1770, 3640 mg/kg-day) F1: 0, 2.5% (0, 3640 mg/kg-day) Diet F0: 7 days premating + 98 days cohabitation + 21 days segregation (126 days total) F1: in utero + lactation, and then in the diet through a 7 day mating period at 74±10 days old (F1 females were allowed to deliver litters)	Ovary weight in F1 parental females *(percent change compared to control)*				
		0		3640	
	Absolute	-		-3%	
	Relative	-		3%	
	Uterus weight in F1 parental females *(percent change compared to control)*				
		0		3640	
	Absolute	-		-4%	
	Relative	-		-4%	
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day Gavage in corn oil 28 days	Relative weights (percent change compared to control)				
		0	40	200	1000
	Ovary	-	0%	3%	8%
	Uterus	-	-6%	-6%	6%

*Statistically significant ($p<0.05$) based on analysis of data by study authors.
Percent change compared to control = $\frac{\text{treated value} - \text{control value}}{\text{control value}} \times 100$

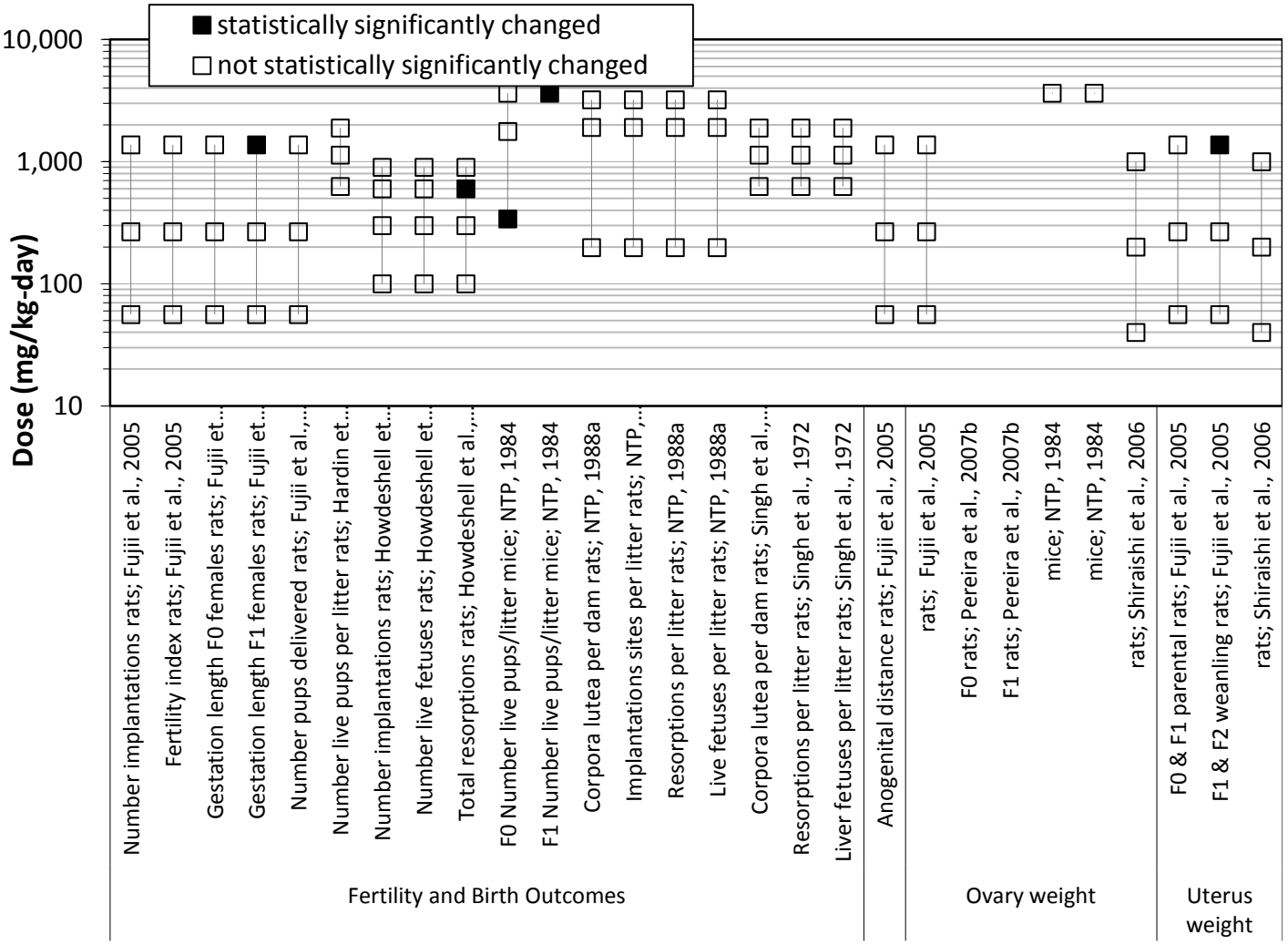

Figure A-3. Exposure-response array of female reproductive effects following exposure to DEP

Table A-11. Evidence pertaining to developmental effects in animals

Reference and Study Design	Results				
Skeletal variations					
(NTP, 1988) Rat (Sprague Dawley); 31-32 dams/group; 27-32 litters/group 0, 0.25, 2.5, 5% (0, 198, 1909, 3214 mg/kg-day) Diet GD 6 to GD 15		0	198	1909	3214
	External malformations per litter	0	0.03	0	0.06
	Visceral malformations per litter	0.11	0	0	0.13
	Skeletal malformations per litter	0	0.07	0.07	0
	Fetuses malformed per litter	0.11	0.07	0.07	0.19
		0	198	1909	3214
	Percent litters with extra rib (male and female fetuses)	-44	39	47	74*
(Singh et al., 1972) Rat (Sprague Dawley); 5 dams/group 0 (untreated), 0.506, 1.012, 1.686 mL/kg (0, 627, 1133, 1888 mg/kg) Intraperitoneal injections on GD5, 10, and 15 (termination on GD 20) Note: Statistical analysis was not conducted by study authors for this endpoint		0	627	1133	1888
	No. of skeletal abnormalities	0	5	8	13
(U.S. EPA, 1994) Rabbit (NZW); 12 dams/group 0 (untreated) 5, 15, 50% (w/w) DEP dissolved in 0.5% carboxymethylcellulose for a total application volume of 2 ml/kg body weight/day applied directly to skin (10 X 10 cm) in the dorsolumbar region GD 6-GD 18		0	5	15	50
	Malformation index (%)	0	0	0	2.2
Fetal body weight					
(Singh et al., 1972) Rat (Sprague Dawley); 5 time-mated females/group 0 (untreated), 0.506, 1.012, 1.686 mL/kg (0, 627, 1133, 1888 mg/kg) Intraperitoneal injections GD 5, 10, and 15 (termination on GD 20)	Fetal (GD20) body weight *(percent change compared to control)*				
	Male and female fetuses (average weight per group)	0	627	1133	1888
		-	-46%*	-41%*	-41%*

Table A-11. Evidence pertaining to developmental effects in animals

Reference and Study Design	Results				
(NTP, 1988) Rat (Sprague Dawley); 31-32 females (dams)/group; 27-32 litters/group 0, 0.25, 2.5, 5% (0, 198, 1909, 3214 mg/kg-day) Diet GD 6 to GD 15	Fetal body weight *(percent change compared to control)*				
		0	198	1909	3214
	Male and female fetuses (average weight/litter)	-	6%	7%	4%
Postnatal growth					
(Fujii et al., 2005) Rat (Sprague Dawley); Multigenerational study design: 24 breeding pairs/group/generation; pup weight assessed in 21-24 litters/group 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks of dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	Weanling body weight (litter average) *(percent change compared to control)*				
	Males	0	40/46	197/222	1016/1150
	F1 pup	-	-4%	-7%	-18%*
	F2 pup	-	-2%	-4%	-19%*
	Females	0	51/56	255/267	1297/1375
	F1 pup	-	1%	0%	-12%*
	F2 pup	-	1%	0%	-12%*
(Pereira and Rao, 2007) Rat (Wistar); 6 breeding pairs/group; body weight measured in 6 pups/sex/group 0, 50 mg/kg (0, 2.85 mg/kg-day) Diet (DEP dissolved in corn oil) 100 days (premating) + 10 days (mating) and through gestation and weaning of the PND 21 male and female pups (150 days total for parental animals)	Weanling body weight *(percent change compared to control)*				
		0		2.85	
	Males	-		-35%*	
	Females	-		-24%*	

Table A-11. Evidence pertaining to developmental effects in animals

Reference and Study Design	Results		
(NTP, 1984) Mouse (CD-1); Continuous breeding protocol F0: 40 control and 18-20 breeding pairs/treatment group F1: 20 breeding pairs/group F0: 0, 0.25, 1.25, 2.5 % (0,340,1770, 3640 mg/kg-day) F1: 0, 2.5% (0, 3640 mg/kg-day) Diet F0: 7 days premating + 98 days cohabitation + 21 days segregation (126 days total) F1: in utero + lactation, and then in the diet through a 7 day mating period at 74±10 days old (F1 females were allowed to deliver litters) Note: Statistical analysis was not conducted by study authors for this endpoint	Weanling body weight *(percent change compared to control)*		
		0	3640
	Males	-	-25%
	Females	-	-23%

*Statistically significant ($p<0.05$) based on analysis of data by study authors.

Percent change compared to control = $\frac{\text{treated value} - \text{control value}}{\text{control value}} \times 100$

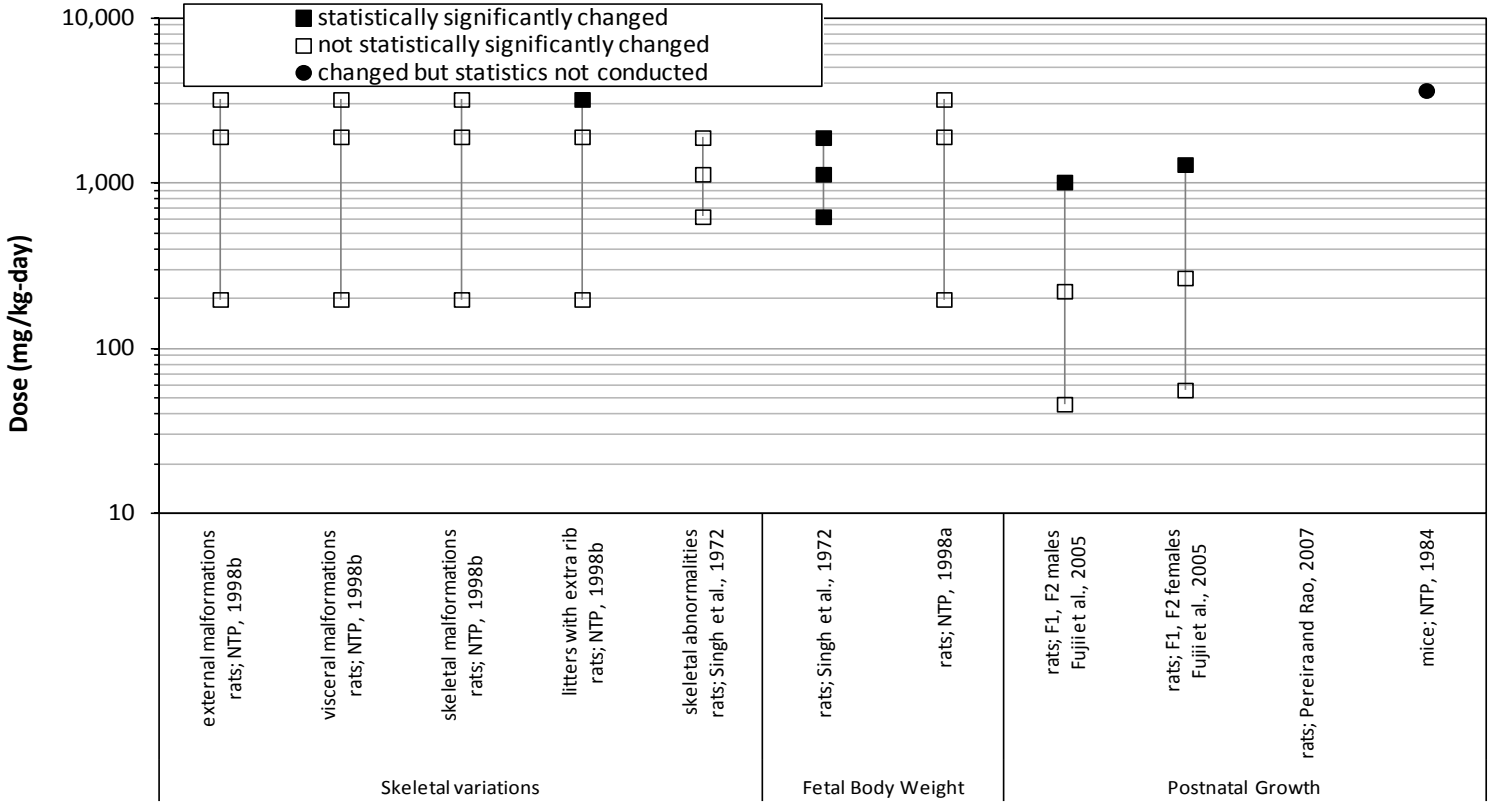

Figure A-4. Exposure response array of developmental effects following exposure to DEP

A.4. Obesity Evidence Tables

Table A-12. Evidence pertaining to MEP and obesity in humans

Reference and Study Design	Results
(Trasande et al., 2013) (United States, NHANES) n = 2,884 participants in the 2003–2008 NHANES, 6–19 yrs old **Outcome:** BMI z-score, obesity (BMI z-score ≥95th percentile), and overweight (BMI z-score ≥85th percentile) (measured) **Exposure:** Urine sample, collected at same time BMI measurement ΣLMW phthalates in urine (µM): Geometric mean Not obese 0.701 Obese 0.855 ΣLMW phthalates = sum of MEP, MBP, and MIBP **Analysis:** Logistic regression for overweight and obese classification; linear regression of BMI z-score as continuous variable; adjusted for variables shown in results column	Full sample results, no association with ln-LMW phthalates: OR or regression coefficient (95% CI) per one unit increase in ΣLMW phthalates (µM) (Model 2 results shown, adjusted for urinary creatinine, sex, poverty-income ratio, parental education, serum cotinine, age, and race/ethnicity, caloric intake and television watching) Overweight OR (95% CI) 1.01 (0.90, 1.13) Obese OR (95% CI) 1.02 (0.90, 1.17) BMI z-score β (95% CI) 0.03 (-0.03, 0.09) Interaction by ethnicity seen, with associations seen between ln-LMW phthalates and each of the obesity measures in blacks, but not in whites or Hispanics. The patterns seen with ΣLMW phthalates were also seen in analyses for MEP. Using same adjustment factors as above, the associations with ln-MEP are: ΣLMW phthalates: Hispanic / White / Black; MEP: Black Overweight OR (95% CI): 0.88 (0.72, 1.08) / 0.97 (0.78, 1.22) / 1.21 (1.05, 1.39) / 1.18 (1.04, 1.34) Obese OR (95% CI): 0.97 (0.83, 1.14) / 0.94 (0.69, 1.29) / 1.22 (1.07, 1.39) / 1.19 (1.05, 1.35) BMI z-score β (95% CI): -0.04 (-0.15, 0.06) / 0.02 (-0.08, 0.12) / 0.09 (0.003, 0.18) / 0.08 (0.01, 0.16)
(Wang et al., 2013) (China) 259 primary and middle school students, 8–15 yrs old, stratified sample from 6 schools, selected based on sex and BMI **Outcome:** BMI, waist circumference (measured) **Exposure:** First morning urine sample, collected at same time BMI measurement MEP in urine (ng/mL): Geometric mean (SE) 15.3 (1.1) **Analysis:** Linear regression, sampling weights applied to adjust for sampling strategy; see results for covariates considered.	Regression coefficient (95% CI) for change in BMI or waist circumference per unit increase in SG-adjusted ln-MEP (adjusted for age and sex in Model 1; plus sum of DEHP, MCHP, sum of DBP and MMP in Model 2) Model 1 β (95% CI) / Model 2 β (95% CI) BMI: 0.025 (0.009, 0.040) / 0.022 (0.005, 0.0040) Waist circumference: 0.020 (0.008, 0.032) / 0.020 (0.006, 0.033) The magnitude of the effect of MEP was similar to that for ∑DBP (BMI: 0.035, WC: 0.023) metabolites, and for MiBP (BMI: 0.027, WC: 0.022)

Table A-12. Evidence pertaining to MEP and obesity in humans

Reference and Study Design	Results
(Lind et al., 2012a) (Sweden) Prospective cohort study, n = 1,016 (507 men, 509 women), age 70 yrs at enrollment, Prospective Investigation of Vasculature in Uppsala Seniors study, 2001–2003. **Outcome:** BMI, waist circumference measured at enrollment; dual energy X-ray absorptiometry (DXA) (n = 890 participated) and MRI of abdominal region (n = 287 randomly selected) 2 yrs later **Exposure:** Serum sample (fasting), collected at baseline MEP in serum (ng/mL): Median 75th percentile Women 11.6 16.8 Men 11.6 18.5 **Analysis:** Linear regression, adjusted for variables shown in results column **Related reference**: (Olsén et al., 2012)reports cross-sectional analysis of BMI from this study population, see Table 14	Regression coefficient (95% CI) for change in body metric per unit increase in ln-MEP (ng/mL) (adjusted for serum cholesterol and triglycerides, education, exercise, and smoking) Outcome — Males β (95% CI) — Females β (95% CI) BMI (kg/m^2) — 0.31 (-0.097, 0.72) — 0.008 (-0.67, 0.69) Waist circumference (cm) — 0.73 (-0.45, 1.9) — -0.80 (-2.4, 0.81) DXA total fat (kg) — 269 (-776, 1315) — -469 (-1,877, 938) MRI visceral adipose tissue (cm^2) — 16 (0.49, 32) — 3.6 (-11, 19)
(Teitelbaum et al., 2012) (United States, New York City) Prospective cohort study, n = 387 Hispanic and black children (80 boys, 307 girls), 6 to 8 yrs at cohort enrollment, Growing Up Healthy Study, 2004–2008 **Outcome:** BMI and waist circumference measured 1 yr after enrollment. Normal weight = BMI <85th percentile (n=2284); overweight = BMI ≥85th percentile (n=578) **Exposure:** Urine sample, collected at enrollment Cr-adjusted phthalates in urine (µg/g Cr), median: MEP ΣLow MWP Boys 152 253.2 Girls 177.7 294 Low molecular weight phthalate metabolites included MEP, MBP, and MiBP. **Analysis:** Linear regression, considering	Full sample results, regression coefficient (95% CI) for change for change in body metric per unit change in ln-MEP (µg/g Cr) (adjusted for creatinine, age, sex, sedentary hours, metabolic equivalent hours, Hispanic ethnicity, caloric intake, season, parental education level) BMI (kg/m^2) — 0.19 (-0.17, 0.55) Waist circumference (cm) — 0.51 (-0.45, 1.46) Among girls, mean measurement by quartile of MEP (µg/g Cr), stratified by weight group (adjusted for same variables as above) Normal weight — Overweight MEP quartile — BMI (kg/m^2) — Waist circumference (cm) — BMI (kg/m^2) — Waist circumference (cm) 1 (low) — 16.3 — 59.9 — 21.3 — 73.4 2 — 16.4 — 60.1 — 21.7 — 73.5 3 — 16.1 — 59.3 — 23.8 — 79.2 4 (high) — 15.9 — 58.7 — 23.5 — 78.8 (trend *p*) — (0.41) — (0.37) — (<0.0001) — (<0.0001)

Table A-12. Evidence pertaining to MEP and obesity in humans

Reference and Study Design	Results
sex, age at baseline, sedentary hours, metabolic equivalent hours, caloric intake, race, ethnicity, season of urine collection, family income, and parent education as potential covariates. Restricted to children with creatinine ≥10 mg/dL.	Interaction between BMI percentile and MEP was significant ($p < 0.05$) in analyses of both BMI and waist circumference in girls.
(Hatch et al., 2008)(United States, NHANES) 4,369 (2,251 males, 2,118 females) participants in the 1999–2002 NHANES, ages 6–80 yrs; separate analyses by sex-age group (ages 6–11, 12–19, 20–59, 60–80) **Outcome:** BMI, waist circumference (measured) **Exposure:** Urine sample, collected at time of obesity measurement MEP in urine (µg/g Cr): Range of geometric means in different age-sex groups = 94–226 Unadjusted geometric means not reported **Analysis:** Linear regression, adjusting for variables shown in results column	Regression coefficient for change in body metric per quartile increase in unadjusted MEP (µg/L), by age (age, creatinine, height, race/ethnicity, socioeconomic status, fat intake, dairy intake, fruit and vegetable intake, physical activity, TV/video and computer use, smoking status, and for women, menopausal status, parity)

MEP Quartile	6–11 yrs β	12–19 yrs β	20–59 yrs β	60–80 yrs β
Waist circumference, males				
1 (low)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
2	–0.75	–1.00	0.85	0.11
3	1.42	–0.30	1.25	1.55
4 (high)	–0.67	–1.20	2.19	1.68
(trend *p*)	(0.99)	(0.64)	(0.11)	(0.21)
Waist circumference, females				
1 (low)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
2	0.74	2.31	0.07	–0.62
3	0.99	2.7	0.46	–1.62
4 (high)	1.05	4.11	2.07	–0.22
(trend *p*)	(0.61)	(0.02)	(0.1)	(0.82)
BMI, males				
1 (low)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
2	-0.29	-0.05	0.36	0.18
3	0.97	0.02	0.47	0.76
4 (high)	-0.02	-0.13	0.82	1.05
(trend *p*)	(0.65)	(0.89)	(0.11)	(0.03)
BMI, females				
1 (low)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)

Table A-12. Evidence pertaining to MEP and obesity in humans

Reference and Study Design	Results				
	2	0.35	0.84	-0.03	-0.03
	3	0.54	1.16	0.10	-0.79
	4 (high)	0.30	1.74	0.92	-0.21
	(trend *p*)	(0.66)	(0.03)	(0.14)	(0.64)
(Stahlhut et al., 2007) (United States, NHANES) 1,451 male participants in the 1999–2002 NHANES; ages >18 yrs; excluded if taking insulin, oral hypoglycemic agents, or sex hormone agonists/antagonists, analyses only) **Outcome:** Waist circumference (measured) **Exposure:** Urine sample, collected at time of obesity measurement MEP in urine (µg/g Cr): Median Cr-adjusted 188.1 **Analysis:** Linear regression, adjusting for variables shown in results column	Adjusted regression coefficient per unit increase in ln-MEP (adjusted for age, age-squared, race/ethnicity, fat intake, calorie intake, physical activity level, smoking exposure based on cotinine, urinary creatinine, glomerular filtration rate, serum ALT, and GGT) β ± SE (*p*–value) Waist circumference (n = 1,292): 0.66 ± 0.31 (0.041) Association with MEP was similar to or smaller than seen for MBP (adjusted Model 2 *Beta* = 0.79) or MBzP (adjusted Model 2 *Beta* = 1.09) Increase in waist circumference began in 3rd quartile of exposure (data shown graphically).				

A.5. Other Systemic Effects Evidence Tables and Exposure-Response Array

Table A-13. Evidence pertaining to MEP and neurological effects in adults

Reference and Study Design	Results
(Shiue, 2013a) (United States, NHANES) 2,287 participants aged ≥50 yrs in the 2003–2004 NHANES **Outcome:** Self-reported status, in the last year: • vision (n=136 "poor", comparison = "fair," "good," and "excellent") • hearing (n=261 "lots of trouble" or "deaf", comparison = "good" and "little trouble") • balance function (n=748 positive response to "dizziness, difficulty with balance, or difficulty with falling") • ear ringing (n=754 positive response to "ringing, roaring, or buzzing" in ear) **Exposure:** Urine sample, collected at time of survey; measured concentrations were not reported. **Analysis:** Logistic regression, adjusting for age, sex, ethnicity, and urinary creatinine. Referent group not defined.	OR (95% CI) for poor status, per unit increase in ln-MEP (adjusted for age, sex, ethnicity, and urinary creatinine) Vision 0.92 (0.70, 1.21) Hearing 1.14 (0.95, 1.37) Balance 0.94 (0.83, 1.06) Ears ringing/roaring/buzzing 0.99 (0.88, 1.12)

Table A-14. Evidence pertaining to MEP and diabetes and measures of insulin resistance in humans

Reference and Study Design	Results
(James-Todd et al., 2012) (United States, NHANES) Case-control study of 2,350 female participants in the 2001–2008 NHANES, ages 20–79 yrs; n=215 cases, 2135 controls. Cross-sectional analysis of insulin resistance measures among women without history of diabetes. **Outcome:** Positive response to, "Other than during pregnancy, have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?"; among women without history of diabetes, fasting blood glucose (FBG) (n=985), homeostasis model assessment-estimated insulin resistance (HOMA) (n=971), glycosolated hemoglobin A1c (n=2092) **Exposure:** Urine sample, collected at time of survey MEP in urine (units not reported): Geometric mean (95% CI) Unadjusted 164.8 (150.5, 180.3) **Analysis:** Logistic regression, adjusting for urinary creatinine, fasting time, age, race/ethnicity, education, poverty status, behavioral factors	OR (95% CI) for diabetes by quartile of MEP (adjusted for urinary creatinine, age, race/ethnicity, education, poverty status, fasting time, total caloric intake, total fat intake, smoking status, and physical activity; little change with additional adjustment for BMI and waist circumference) MEP Quartile 1 (low) 1.0 (referent) 2 0.95 (0.60–1.51) 3 1.09 (0.61–1.96) 4 (high) 0.89 (0.47–1.67) Among women without diabetes, OR (95% CI)] for glucose and insulin parameters by quartile of MEP (Model 1 adjusted for urine creatinine, age, race/ethnicity, education level, poverty status, fasting time, total caloric intake, total fat intake, smoking status, and physical activity; Model 2 also adjusted for BMI and waist circumference) MEP Quartile / Model 1 / Model 2 Fasting glucose (mg/dL) 1 (low) 1.0 (referent) 1.0 (referent) 2 0.95 (-0.94, 2.85) 1.10 (-0.83, 3.04) 3 1.18 (-0.91, 3.27) 0.38 (-1.91, 2.67) 4 (high) -0.03 (-2.16, 2.09) -0.61 (-2.99, 1.78) Ln(HOMA) 1 (low) 1.0 (referent) 1.0 (referent) 2 0.06 (-0.10. 0.14) 0.03 (-0.09, 0.14) 3 0.07(-0.08, 0.23) 0.01(-0.11, 0.14) 4 (high) 0.10 (-0.07, 0.26) -0.04 (-0.17, 0.09) A1c (%) 1 (low) 1.0 (referent) 1.0 (referent) 2 0.01 (-0.04, 0.06) -0.02 (-0.07, 0.02) 3 -0.02 (-0.07, 0.03) -0.03 (-0.07, 0.02) 4 (high) -0.03 (-0.08, 0.02) -0.05 (-0.10, 0.00)

Table A-14. Evidence pertaining to MEP and diabetes and measures of insulin resistance in humans

Reference and Study Design	Results
(Lind et al., 2012b)(Sweden) n = 1,003 (501 men, 502 women), age 70 yrs at enrollment; cross-sectional analysis within the Prospective Investigation of Vasculature in Uppsala Seniors study, 2001–2003. **Outcome:** Diabetes (n=88; history of diabetes or fasting glucose >7.0 mmol/L, mean duration 8.9 years); ratio of fasting proinsulin to insulin; HOMA **Exposure:** Serum sample (fasting), collected at time of clinical assessment MEP in serum (ng/mL): Median 75th percentile Women 11.6 16.8 Men 11.6 18.5 **Analysis:** Logistic regression for diabetes classification; linear regression for continuous outcomes (proinsulin/insulin and HOMA-IR); adjusting for variables shown in results column **Related reference: (Olsén et al., 2012)** presents blood glucose data for this study population; the regression coefficient per unit increase in serum ln-MEP was 0.007 (-0.01, 0.03) (see Table 14)	Diabetes analysis: OR(95% CI) per unit increase in serum ln-MEP (adjusted for sex, serum cholesterol and triglycerides, BMI, smoking, exercise and education) 1.28 (0.97, 1.7) Diabetes analysis: OR (95% CI) by quintile of ln-MEP P (adjusted for sex, serum cholesterol and triglycerides, BMI, smoking, exercise and education) MEP Quintile 1 1.0 (referent) 2 2.25 (1.06, 4.79) 3 2.87 (1.37, 6.03) 4 2.44 (1.14, 5.21) 5 (high) 2.27 (1.08, 4.81) (trend *p*) (0.061) Regression coefficient (95% CI) for insulin measures per unit increase in serum ln-MEP (adjusted for sex, serum cholesterol and triglycerides, BMI, smoking, exercise and education) Proinsulin/insulin -0.05 (-0.097, -0.002) HOMA 0.069 (0.023, 0.116) The magnitude of the association between proinsulin/insulin and MEP was similar to that for two of the other metabolites studied, but in the opposite direction of MEHP and MiBP (0.046 and 0.06, respectively), and much greater compared to MMP (-0.005). The magnitude of the association between HOMA-IR and MEP was greater than that for the other metabolites studied (range: -0.012 to 0.47). The magnitude of the association between prevalent diabetes and MEP was greater than that for MEHP or MiBP, and similar to that for MMP in the highest quintile.

Table A-14. Evidence pertaining to MEP and diabetes and measures of insulin resistance in humans

Reference and Study Design	Results
(Svensson et al., 2011) (Mexico) n=221 women; average age 54 years; healthy controls from a case-control study of breast cancer **Outcome:** Self-reported diabetes **Exposure:** First morning urine samples MEP in urine (µg/g creatinine): Geometric Mean (SD) No diabetes 108.0 (3.4) Diabetes 101.3 (2.7) **Analysis:** Logistic regression, adjusting for creatinine and education (age and waist-height ratio not found to be potential confounders).	OR(95% CI) per unit increase in ln-MEP 1.02 (0.74, 1.39)
(Stahlhut et al., 2007) (United States, NHANES) 1,451 male participants in the 1999–2002 NHANES; ages >18 yrs; excluded if taking insulin, oral hypoglycemic agents, or sex hormone agonists/antagonists, or if not fasting before specimen collection **Outcome:** Homeostatic model assessment (HOMA) **Exposure:** Urine sample, collected at time of survey MEP in urine (µg/g Cr): Median Cr-adjusted 188.1 **Analysis:** Linear regression, considering variables shown in results column	Regression coefficient per unit increase in ln-MEP (Model 1 adjusted for age, age-squared, race/ethnicity, fat intake, calorie intake, physical activity level, smoking exposure based on cotinine, and urinary creatinine; Model 2 also adjusted for glomerular filtration rate, serum ALT, and GGT) Outcome: HOMA (ln) (n = 622) Model 1 β ± SE (*p*–value): 0.056 ± 0.020 (0.008) Model 2 β ± SE (*p*–value): 0.044 ± 0.021 (0.045) Increases in HOMA began in 3rd quartile of exposure (data shown graphically). Association with MEP was similar to or smaller than seen for MBP (adjusted Model 2 *Beta* = 0.043) or MBzP (adjusted Model 2 *Beta* = 0.061)

Table A-15. Evidence pertaining to MEP and thyroid effects in humans

Reference and Study Design	Results
Thyroid hormones and thyroid stimulating hormone	
(Boas et al., 2010)(Denmark) 758 children, who were participants in longitudinal cohort study, examined 2006–2007 at ages 4–9 yrs **Outcome:** Serum thyroid hormone levels (non-fasting sample) **Exposure:** Urine sample (child's), collected same day as serum samples Unadjusted MEP in urine (µg/L): Boys: Median 21, 75th percentile 39 Girls: Median 21, 75th percentile 44 Cr-adjusted MEP in urine (µg/g Cr): Boys: Median 31, 75th percentile 52 Girls: Median 36, 75th percentile 65 **Analysis:** Linear regression, adjusting for sex and age	Regression coefficient (*p*-value) for change in hormone level with unit change in ln-MEP (adjusted for sex and age) (0.0 = no effect) T_3: Unadjusted MEP -0.06 (0.015); Cr-adjusted MEP -0.02 (0.61) Free T_3: Unadjusted MEP -0.13 (0.013); Cr-adjusted MEP 0.00 (0.99) T_4: Unadjusted MEP -1.49 (0.29); Cr-adjusted MEP -1.18 (0.54) Free T_4: Unadjusted MEP -0.01 (0.93); Cr-adjusted MEP -0.07 (0.71) TSH: Unadjusted MEP 0.02 (0.30); Cr-adjusted MEP 0.06 (0.005) IGF-1: Unadjusted MEP -0.01 (0.21); Cr-adjusted MEP -0.01 (0.56) IGFBP-3: Unadjusted MEP 0.00 (0.88); Cr-adjusted MEP 0.02 (0.11) Similar patterns seen in analyses stratified by gender. Units for hormone analyses were not reported in the publication.
(Huang et al., 2007) (Taiwan) 76 pregnant women undergoing amniocentesis due to age >35 yrs or abnormal α-fetoprotein or β-hCG test, 2005–2006 **Outcome:** Serum thyroid hormone levels collected during 2nd trimester **Exposure:** Urine sample, collected same day as serum samples MEP in urine: Unadjusted (ng/mL): Median 28, 75th percentile 52, 95th percentile 2,346 Cr-adjusted (µg/g Cr): Median 68, 75th percentile 205, 95th percentile 4,414 **Analysis:** Spearman correlation analysis; linear regression, adjusting for variables shown in results column	Spearman correlation coefficient between hormone level and MEP T_3 (ng/dL): Unadjusted MEP (ng/mL) -0.019; Cr-adjusted MEP (µg/g Cr) -0.008 T_4 (µg/dL): Unadjusted MEP -0.039; Cr-adjusted MEP -0.021 Free T_4 (ng/dL): Unadjusted MEP 0.017; Cr-adjusted MEP 0.041 TSH (µIU/mL): Unadjusted MEP -0.082; Cr-adjusted MEP -0.107 Adjusted regression coefficient (*p*-value) for change in ln-T_4 with change in ln-MEP (adjusted for age, BMI, gestational age, and other phthalate metabolites - MBP, MEHP, MBzP, MMP): T_4 (nmole/L): 0.013 (0.40) Free T_4 (pmole/L): 0.026 (0.12)

Table A-15. Evidence pertaining to MEP and thyroid effects in humans

Reference and Study Design	Results
(Meeker et al., 2007) (United States, Boston) 408 male partners seen in subfertility clinic during 2000–2004, mean ± SD age 36 ± 5.3 yrs **Outcome:** Serum thyroid hormone levels **Exposure:** Urine sample, collected same day as serum samples MEP in urine (ng/mL): Median; 75th percentile; 95th percentile SG-adjusted: 158; 535; 2,343 **Analysis:** Linear regression, considering age, BMI, smoking status, race, previous examination for infertility, prior impregnation of partner, timing of blood and urine samples, and time of day as potential covariates	Regression coefficient (95% CI) for change in hormone level per IQR change in SG-adjusted MEP (ng/mL, after back-transformation from lnMEP) (adjusted for age, BMI, current smoking, and time of blood sample) Untransformed hormone levels (0.0 = no effect) Total T_3 (ng/mL): 0.018 (-0.009, 0.044) Free T_4 (ng/dL): 0.011(-0.048, 0.026) Ln-transformed hormone levels (1.0 = no effect) TSH (µIU/mL): 0.94 (0.85, 1.03) Adjusted for

Table A-16. Evidence pertaining to MEP and immune effects in humans

Reference and Study Design	Results
Asthma and hypersensitivity conditions	
(Bertelsen et al., In Press) (Norway) 623 children aged 10 yrs participating in the Environment and Childhood Asthma study; children with current asthma over-sampled (2001–2004) **Outcome:** Current asthma (parental report of history of asthma plus ≥1 of the following: dyspnea, chest tightness and/or wheezing in previous 12 mo; use of asthma medications in previous 12 mo; positive exercise challenge test) **Exposure:** First morning urine sample, collected at study examination MEP in urine (µg/L): Median; 75th percentile; 95th percentile Unadjusted 56.7 94.4 360.2 SG-adjusted 56.3 101.1 320.2 **Analysis:** Logistic regression, adjusting for urine specific gravity, sex, parental asthma, and household income	OR (95% CI) for current asthma by quartile of MEP (µg/L) (adjusted for urine specific gravity, sex, parental asthma, and household income) 1: ≤32.6 (ref) 1.0 (referent) 2: >32.6–56.7 0.97 (0.55, 1.7) 3: >56.7–94.4 0.85 (0.47, 1.6) 4: >94.4 0.99 (0.55, 1.8) Increase in odds of current asthma per $\log_{10}$ IQR MEP = 0.98 (0.39, 2.5)
(Just et al., 2012) (United States, New York) 244 children (ages 4.9–9.1 yr) in Columbia Center for Children's Environmental Health birth cohort, 2006–2010 **Outcome:** Measured fractional exhaled nitric oxide (feNO) (1-3 measures per child), measured seroatopy (specific IgE to dust mite, cockroach, or mouse allergens, ≥ 0.35 IU/ml), wheeze within past year or in subsequent year (based on parent report at feNO study visit and at the next study visit), with additional information to model wheezing phenotype **Exposure:** urine sample (child's), collected at time of feNO measurement MEP in urine (ng/mL): Geometric mean (95% CI) Unadjusted 111 (96, 129) **Analysis:** Generalized estimating equation regression models adjusted for variables shown in results column	Adjusted percent difference in feNO per unit increase in ln-MEP (ng/mL) (adjusted for specific gravity, age, sex, race/ethnicity, time of day of feNO collection, and ambient NO; similar results with additional adjustment for seroatopy and MnBP, MBzP, and MEHHP) % Difference (95% CI) *p*-value 6.5 (1.0, 12.4) 0.021 No association between urinary concentration of MEP and incident seroatopy or reported wheeze (quantitative results not reported).

Table A-16. Evidence pertaining to MEP and immune effects in humans

Reference and Study Design	Results
(Kanazawa et al., 2010) (Sapporo, Japan) Cross-sectional study, n = 134 residents (41 dwellings), including 33 reporting at least one symptom and 101 with no reported symptoms **Outcome:** Self-reported "sick house syndrome" symptoms (fatigue; feeling heavy-headed; headache; nausea/dizziness; difficulty concentrating; itching, burning or irritation of the eyes; irritated, stuffy, or runny nose; hoarse, dry throat; cough; dry or flushed facial skin; scaling/itching of the scalp or ears; and dry, itching or red-skinned hands) **Exposure:** Air and dust samples in dwellings DEP in room air (ng/m^3): Median Range Total conc 60.7 22.3–203 DEP in dust (mg/kg): Median Range Multi-surface 0.35 <MDL–6.3 Floor 0.33 <MDL–1.9 **Analysis:** Logistic regression, considering age, gender, history of allergy, time spent at home, moldy odor, condensation as potential covariates	OR (95% CI) for mucosal symptoms per 10-fold increase in DEP concentration (adjusted for age, gender, history of allergy, time spent at home; similar results with additional adjustment for moldy odor and for condensation) Exposure medium — OR (95% CI) Air (ng/m^3) — 0.1 (0.01–0.9) Multi-surface dust (mg/kg) — 0.3 (0.1–0.9) Floor dust (mg/kg) — 0.4 (0.1–1.6)
(Kolarik et al., 2008) (Bulgaria) Nested case-control study; n = 102 cases, 82 controls; ages 2–7 yrs (ALLHOME cohort, n = 4479), 2004–2005. **Outcome:** Eczema, wheezing, or rhinitis (Cases had at least one of these three symptoms). **Exposure:** Surface dust samples from children's bedrooms, DEP in dust (mg/g) Geometric mean (95% CI) All homes 0.35 (0.27, 0.42) **Analysis:** Dust concentrations compared between case and control homes overall, and between cases with specific symptoms in the preceding 12 months and controls, using Mann-Whitney U-test (untransformed data) and Dunnett test (log-transformed data).	Concentration in dust (mg/g dust) Median (mean) in cases — Median (mean) in controls Case status 0.32 (0.68) 0.36 (0.74) Wheezing 0.31 (0.68) 0.36 (0.74) Rhinitis 0.30 (0.66) 0.36 (0.74) Eczema 0.35 (0.70) 0.36 (0.74) $p > 0.3$ in all statistical tests

Table A-16. Evidence pertaining to MEP and immune effects in humans

Reference and Study Design	Results		
(Bornehag et al., 2004) (Sweden) Nested case-control study; n = 198 cases, 202 controls; ages 2–7 yrs (follow-up of Dampness in Buildings and Health cohort, n = 10,852), 2001–2002. **Outcome:** Eczema, wheezing, or rhinitis (Cases report at least two incidents of eczema, or wheezing or rhinitis without a cold, in the preceding year, and at follow-up 1.5 yrs later). **Exposure:** Surface dust samples from children's bedrooms, DEP in dust (mg/g) Median All homes 0.000 **Analysis:** Mann-Whitney U-test for comparing concentrations in all homes; t-test for comparing log-transformed concentrations in homes with concentrations above detection limit	Concentration in dust (mg/g dust)		
		Median, all homes (n = 346)	Geometric mean (95% CI), homes with phthalate > detection limit (n = 175)
	Controls	0.000	0.058 (0.035, 0.097)
	Cases (all)	0.000	0.102 (0.049, 0.211)
	$p > 0.2$ in both tests		

Table A-17. Evidence pertaining to MEP and pulmonary function in humans

Reference and Study Design	Results
(Hoppin et al., 2004) (United States, NHANES) 240 participants in NHANES III (1988-1994); ages 20-60 yrs, only African-American and white participants; excluded if missing information on phthalate levels, pulmonary function, medical or smoking history **Outcome:** FVC, FEV1, PEF, MMEF **Exposure:** Urine sample, collected at time of pulmonary function testing MEP in urine (ng/mL): Mean (SD) Men 323 (6.4) Women 307 (4.9) MEP in urine (µg/g Cr): Men 240 (5.4) Women 321 (4.2) **Analysis:** Linear regression, stratified by sex and adjusted for variables shown in results column.	Regression coefficient for change in pulmonary function measure per interquartile range increase in MEP (608.8 ng/g creatinine) (adjusted for age, age squared, height, BMI, smoking, race) B (SE) Men / Women FVC: -121 (58)* / 37 (50) FEV1: -102 (47)* / 67 (43) PEF: -250 (167) / 86 (124) MMEF: -106 (116) / 162 (95) *p<0.05 Results among non-smokers only showed no significant associations for either men or women

Table A-18. Evidence pertaining to MEP and cardiovascular disease in humans

Reference and Study Design	Results
(Trasande et al., 2013) (United States, NHANES) 2,447 participants in the 2003–2008 NHANES, 8–19 yrs old **Outcome:** Systolic blood pressure (SBP) and diastolic blood pressure (DBP) z-score (based on CDC norms, sex and age); prehypertension (BP≥90th percentile for age/height/sex); fasting serum triglycerides (n=906; high = ≥ 100 mg/dL); nonfasting high density cholesterol (HDL; n=2555; low = < 40 mg/dL)) **Exposure:** Urine sample, collected at time of BMI measurement ΣLMW phthalates in urine (µM): Geometric mean BP<90th percentile 0.817 BP≥90th percentile 1.002 ΣLow MWP = sum of MEP, MBP, and MIBP **Analysis:** Logistic regression for pre-hypertension (BP≥90th percentile) classification; linear regression for SBP and DBP z-score and triglycerides and HDL as continuous variable; all models adjusted for variables shown in results column.	Change in z-score (95% CI) per unit increase in ln-phthalates (adjusted for sex, caloric intake, television watching, poverty:income, parental education, serum cotinine, urinary creatinine, BMI, race/ethnicity, age) ΣLMW phthalates / MEP SBP: 0.03 (-0.02, 0.07) / 0.02 (-0.02, 0.06) DBP: 0.02 (-0.04, 0.07) / 0.02 (-0.03, 0.06) Triglycerides: -0.22 (-4.40, 0.07) / not reported HDL: 0.13 (-0.60, 0.85) / not reported OR (95% CI) for BP≥90th percentile per unit increase in ln-phthalates ΣLMW phthalates / MEP BP≥90th percentile: 1.19 (0.96, 1.47) / 1.20 (1.01, 1.43) High triglycerides: 0.85 (0.71, 1.01) / not reported Low HDL: 1.00 (0.87, 1.15) / not reported Interactions with covariates examined in supplemental analyses; none of these stratified analyses showed a statistically significant association between ΣLow MWP and SBP. The OR for BP≥90th percentile associated with MEP was larger in magnitude than that for other phthalate metabolites studied (ORs ranged from 0.80 to 1.12)
(Shiue, 2013b) (United States, NHANES) Case-control study of 11,010 participants in 2001–2002 NHANES (204 cases, 10,826 controls) and 10,122 participants in 2003–2004 NHANES (212 cases, 9910 controls). Not age-matched; mean age 67 years for cases, 28 years for controls. **Outcome:** Self-reported stroke (definition not described), time since diagnosis not reported **Exposure:** Urine sample, collected at time of survey MEP in urine of controls Mean ± SD 2001–2002 444.12 ± 1,226.73 2003–2004 466.82 ± 1,325.59 **Analysis:** Student's t-test comparing urinary concentrations; logistic regression, adjusting for creatinine, age, sex, smoking, hypertension, cholesterol, BMI, prior cardiovascular disease, binge drinking	MEP concentrations (units not reported) in cases and controls Time period / Cases (mean ± SD) / Controls (mean ± SD) / *p*-value 2001–2002 / 506.46 ± 1,233.80 / 444.12 ± 1,226.73 / 0.745 2003–2004 / 321.20 ± 559.95 / 466.82 ± 1,325.59 / 0.438 OR (95% CI) for stroke and urinary MEP concentrations (units not reported) (adjusted for creatinine, age, and sex; little difference in results seen with additional adjustment for smoking, hypertension, high cholesterol, BMI, prior cardiovascular disease, and binge drinking) Time period / OR (95% CI) 2001–2002 1.00003 (0.99979–1.00027) 2003–2004 0.9998 (0.9993–1.0003)

Table A-18. Evidence pertaining to MEP and cardiovascular disease in humans

Reference and Study Design	Results
(Olsén et al., 2012) (Sweden) 1,016 (507 men, 509 women), age 70 yrs at enrollment, cross-sectional analysis within the Prospective Investigation of Vasculature in Uppsala Seniors study, 2001–2003. **Outcome:** BMI and blood pressure measured at study visit; fasting serum sample for LDL and HDL cholesterol, triglycerides, and glucose; Framingham risk score **Exposure:** Serum sample, collected at time of examination; results not shown **Analysis:** Linear regression, adjusted for the variables shown in results column.	Regression coefficient for change in outcome per unit increase in ln-MEP (adjusted for sex, smoking, diabetes (except for glucose) and the other variables in the table; model for Framingham Risk Score only adjusted for sex) (β [SE]) LDL 0.062 (-0.01, 0.14) HDL -0.007 (-0.03, 0.04) Triglycerides -0.002 (-0.03, 0..04) BMI 0.197 (-0.17, 0.56) SBP -2.35 (-4.31, -0.40) DBP -1.79 (-2.56, -0.82) Glucose 0.007 (-0.01, 0.03) Framingham Risk Score 0.02 (-0.27, 0.32)

Table A-18. Evidence pertaining to MEP and cardiovascular disease in humans

Reference and Study Design	Results
(Lind and Lind, 2011) (Sweden) n=1,016 (507 men, 509 women), age 70 yrs at enrollment, cross-sectional analysis within the Prospective Investigation of Vasculature in Uppsala Seniors study, 2001–2003 **Outcome: C**arotid artery intima media thickness (IMT); grey scale media of the intima media complex (IM-GSM); plaque in carotid artery; **Exposure:** Serum sample (fasting), collected at time of clinical assessment MEP in serum (ng/mL): Median 11.6; 75th percentile 17.5 **Analysis:** Linear regression for continuous outcomes (IMT, IM-GSM) and ordinal logistic regression for number of carotid arteries with plaques (0, 1, 2), adjusted for variables shown in results column	Median IMT by quintile of MEP (adjusted for sex, BMI, fasting blood glucose, SBP, DBP, HDL and LDL cholesterol, triglycerides, smoking, antihypertensive treatment, statin use) MEP Quintile: IMT (Median IMT; p-value); IM-GSM (Median IM-GSM; p-value) 1 (low): 0.87; (referent); 72; Referent 2: 0.87; (0.44); 74; (0.80) 3: 0.89; (0.30); 74; (0.69) 4: 0.86; (0.63); 75; (0.26) 5 (high): 0.87; (0.82); 85; (0.0001) Regression coefficient (β [p-value]) per unit increase in serum MEP (adjusted for sex, BMI, fasting blood glucose, SBP, DBP, HDL and LDL cholesterol, triglycerides, smoking, antihypertensive treatment, statin use) IMT: 4.5 (0.0001) IM-GSM: -0.0032 (0.88) OR for presence of plaques and median value of plaque GSM by quintile of MEP (adjusted for sex, BMI, fasting blood glucose, SBP, DBP, HDL and LDL cholesterol, triglycerides, smoking, antihypertensive treatment, statin use) MEP Quintile: Plaque prevalence (OR; p-value); Plaque GSM (Median; p-value) 1 (low): 1.0; (referent); 68; (referent) 2: 1.24; (0.16); 67; (0.74) 3: 1.07; (0.86); 70; (0.91) 4: 1.35; (0.13); 65; (0.92) 5 (high): 1.54; (0.018); 72; (0.13) Odds ratio or regression coefficient per unit increase in serum MEP Plaque prevalence: OR (95% CI): 1.17 (0.99, 1.39) Plaque GSM: β [p-value: 3.0 (0.19) The regression models did not show evidence of interaction by gender (interaction term p-values ranged from 0.18 to 0.85). The magnitude of the ORs for plaque prevalence for MEP were

Table A-18. Evidence pertaining to MEP and cardiovascular disease in humans

Reference and Study Design	Results
	generally greater than those for the other phthalate metabolites evaluated (ORs for quintile 5 ranged from 0.64 (MiBP) to 1.15 (MMP).

Table A-19. Evidence pertaining to MEP and oxidative stress and inflammation in humans

Reference and Study Design	Results
(Ferguson et al., 2012) (United States, NHANES) 10,026 participants in 1999–2006 NHANES, ages ≥6 yrs **Outcome:** Serum markers of oxidative stress (bilirubin) and inflammation (alkaline phosphatase, ferritin, absolute neutrophil count, and fibrinogen) **Exposure** Urine samples, collected same day as serum samples (data reported in Ferguson et al., 2011) MEP in urine (µg/g Cr): Median: Cr-adjusted 145; 75th percentile: 383; 95th percentile: 1,879 **Analysis:** Linear regression, adjusting for variables shown in results column.	Regression coefficient for percent change in serum marker level per IQR increase in MEP (adjusted for age, sex, race and ethnicity, serum cotinine, poverty index ratio, BMI, and urinary creatinine) Serum marker — β (95% CI) Bilirubin (mg/dL) (n = 7,175) — 1.06 (-0.30, 2.42) Alkaline phosphatase (U/L) (n = 7,176) — -3.33 (-5.05, -1.84) Ferritin, adjusted (n = 5,299) — -5.99 (-9.31, -2.75) Neutrophil count (1,000 cells/µL) (n = 8,331) — -0.83 (-1.72, 0.06) Authors reported no statistically significant association between phthalate metabolites and fibrinogen (quantitative results not reported)
(Ferguson et al., 2011) (United States, NHANES) 10,026 participants in 1999–2006 NHANES, ages ≥6 yrs **Outcome:** Serum markers of oxidative stress (gamma glutamyltransferase; GGT) and inflammation (C-reactive protein; CRP) **Exposure** Urine samples, collected same day as serum samples MEP in urine (µg/g Cr): Median: Cr-adjusted 145; 75th percentile: 383; 95th percentile: 1,879 **Analysis:** Linear regression, considering age, sex, race and ethnicity, poverty index ratio BMI, serum cotinine, alcohol use, education, and urinary creatinine as covariates	Regression coefficient for change in ln-transformed serum marker level per unit increase in ln-MEP (adjusted for age, sex, race and ethnicity, serum cotinine, poverty index ratio, BMI, and urinary creatinine) Serum marker — β (95% CI) — *p*-value GGT (U/L) (n = 7,181) — 0.008 (-0.002, 0.018) — (0.11) CRP (mg/dL) (n = 8,342) — -0.020 (-0.040, 0.0003) — (0.05)

Table A-20. Evidence pertaining to adrenal and pituitary gland effects in animals

Reference and Study Design	Results				
Adrenal gland weight					
(Brown et al., 1978) Rat (Sprague Dawley); 5/sex/group 0, 1, 5% (0, 750, 3710 mg/kg-day) in females; 0, 770, 3160 mg/kg-day in males) Diet 42 days, and 15/sex/group 0, 0.2, 1, 5 % (0, 150, 770, 3160 mg/kg-day in males; 0, 150, 750, 3710 mg/kg-day in females) Diet 112 days.	Relative adrenal gland weight *(percent change compared to control)*				
	Males	0	150	770	3160
	42 day	-	N/A	-14%	8%
	112 day	-	-5%	-3%	17%*
	Females	0	150	750	3710
	42 day	-	N/A	5%	8%
	112 day	-	-3%	3%	12%
(Fujii et al., 2005) Rat (Sprague Dawley), Multigenerational study design: 24 breeding pairs/group/generation; adrenal weight measured in 21-24/sex/group/generation 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	Absolute adrenal gland weight *(percent change compared to control)*				
	Males	0	40/46	197/222	1016/1150
	F0	-	-5%	-9%	12%*
	F1	-	-2%	-7%	-7%*
	F1 pup	-	0	-8%	-12%*
	F2 pup	-	0%	0%	-12%*
	Females	0	51/56	255/267	1297/1375
	F0	-	3%	1%	-4%
	F1	-	3%	-1%	-1%
	F1 pup	-	0	-12%*	-19%*
	F2 pup	-	-4%	-4%	-17%*
	Relative adrenal gland weight *(percent change compared to control)*				
	Males	0	40/46	197/222	1016/1150
	F0	-	-2%	-8%	-8%
	F1	-	0%	-7%	-8%*
	F1 pup	-	0%	-6%	0%
	F2 pup	-	-3%	-3%	-7%
	Females	0	51/56	255/267	1297/1375
	F0	-	1%	2%	-7%
	F1	-	1%	-4%	-3%
	F1 pup	-	3%	-6%	0%
	F2 pup	-	-3%	-3%	-7%

Table A-20. Evidence pertaining to adrenal and pituitary gland effects in animals

Reference and Study Design	Results					
(Gray et al., 2000) Rat (Sprague Dawley); 19 female controls and 5 female DEP-treated; adrenal weights were assessed in 17 control litters (n=45 males) and 3 DEP-treated litters (n=12 males) 0, 750 mg/kg-day Gavage GD14-PND3 Note: The litter was the statistical unit of comparison. Body weight was analyzed as a covariate.	Absolute adrenal gland weight *(percent change compared to compared to control)*					
		0	750			
	Male offspring at 3-5 months of age	-	-13%			
(Kwack et al., 2009) Rat (Sprague Dawley); 6 males/group 0, 500 mg/kg-day DEP 0, 250 mg/kg-day MEP Gavage in corn oil 28 days	Relative adrenal gland weight *(percent change compared to control)*					
		0	500 (DEP)	0	250 (MEP)	
	Males	-	-25%	-	0%	
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day DEP Gavage in corn oil 28 days	Relative adrenal gland weight (*percent change compared to control*)					
		0	40	200	1000	
	Males	-	-7%	-7%	3%	
	Females	-	0	2%	14%*	
Hormonal changes						
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day DEP Gavage in corn oil 28 days	estradiol serum concentration (*percent change compared to control*)					
		0	40	200	1000	
	Males	-	-14%	-22%	-54%*	
	Females	-	19%	23%	34%	
	Dose-dependent changes in T_3, T_4, and TSH were not observed.					

Table A-20. Evidence pertaining to adrenal and pituitary gland effects in animals

Reference and Study Design	Results
Adrenal gland histopathology	
(Pereira et al., 2008b) Rat (Wistar); Multigenerational study design: 6 breeding pairs/group/generation; adrenal glands assessed in 6 adults/group/ generation F0: 0, 50 mg/kg diet (0, 2.85 mg/kg-day) (F0 rats) F1: 0, 25 mg/kg diet (0, 1.425 mg/kg-day) (F1 rats) F2: 0, 10 mg/kg diet (0, 0.57 mg/kg-day) (F2 rats) Diet F0: Adult exposure [150 days: 100 days premating + mating, gestation, and weaning] F1, F2: Developmental exposure [GD0 – PND21] and Adult exposure [150 days (see F0 protocol) starting PND 35-40]	Vacuolations and degeneration of the zona fasciculata region of the adrenal cortex. Severity in males: F0>F1≈F2 Severity in females: F0≈F1<F2 Quantitative data were not reported by the study authors.
(Pereira et al., 2007c) Rat (Wistar); Multigenerational study design adrenal glands assessed in 6 adults/group/ generation F0: 0, 50 mg/kg diet (0, 2.85 mg/kg-day) (F0 rats) F1: 0, 25 mg/kg diet (0, 1.425 mg/kg-day) (F1 rats) Diet F0: Adult exposure [150 days: 100 days premating + mating, gestation, and weaning] F1: Developmental exposure [GD0 – PND21] and Adult exposure [150 days (see F0 protocol) starting PND 35-40	Vacuolations and degeneration of the zona fasciculata region of the adrenal cortex in F0 and F1 rats. No effect on the zona glomerulosa and zona reticularis of the adrenal cortex and medulla region Quantitative data were not reported by the study authors
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day DEP Gavage in corn oil 28 days	Dose-related histopathological changes in the adrenal gland were not observed.

Table A-20. Evidence pertaining to adrenal and pituitary gland effects in animals

Reference and Study Design	Results				
Pituitary weight					
(Gray et al., 2000) Rat (Sprague Dawley); 19 female controls and 5 female DEP-treated; adrenal weights were assessed in 17 control litters (n=45 males) and 3 DEP-treated litters (n=12 males) 0, 750 mg/kg-day Gavage GD14-PND3 Note: The litter was the statistical unit of comparison. Body weight was analyzed as a covariate.	Absolute pituitary gland weight *(percent change compared to compared to control)*				
			0	750	
	Male offspring at 3-5 months of age		-	-5%	
(Brown et al., 1978) Rat (Sprague Dawley); 5/sex/group 0, 1, 5% (0, 750, 3710 mg/kg-day in females; 0,770,3160 mg/kg-day in males Diet 42 days, and 15/sex/group 0, 0.2, 1, 5 % (0, 150, 770, 3160 mg/kg-day in males; 0, 150, 750, 3710 mg/kg-day in females) Diet 112 days.	Relative pituitary gland weight *(percent change compared to control)*				
	Males	0	150	770	3160
	42 day	-	N/A	-10%	-3%
	112 day	-	-5%	6%	19%*
	Females	0	150	750	3710
	42 day	-	N/A	0%	-12%
	112 day	-	-4%	0%	-6%

Table A-20. Evidence pertaining to adrenal and pituitary gland effects in animals

Reference and Study Design	Results				
(Fujii et al., 2005) Rat (Sprague Dawley), Multigenerational study design: 24 breeding pairs/group/generation; adrenal weight measured in 21-24/sex/group/generation 0, 600, 3,000, 15,000 ppm (0, 40, 197, 1016 mg/kg-day in F0 males; 0, 51, 255, 1297 mg/kg-day in F0 females; 0, 46, 222, 1150 mg/kg-day in F1 males; 0, 56, 267, 1375 mg/kg-day in F1 females) Diet ~98 days for F0 and F1 parental males (14 weeks dosing during premating and mating) and ~133 days for F0 and F1 parental females (10 weeks premating, 3 weeks mating, 3 weeks gestation, 3 weeks lactation)	Absolute pituitary gland weight *(percent change compared to control)*				
	Males	0	40/46	197/222	1016/1150
	F0	-	5%	6%	-5%
	F1	-	-1%	-1%	-4%
	F1 pup	-	0%	3%	-3%
	F2 pup	-	3%	3%	-3%
	Females	0	51/56	255/267	1297/1375
	F0	-	-4	-6	-7
	F1	-	0%	3%	-4%
	F1 pup	-	3%	9%	-9%
	F2 pup	-	-6%	-6%	-6%
	Relative pituitary gland weight *(percent change compared to control)*				
	Males	0	40/46	197/222	1016/1150
	F0	-	8%	6	0
	F1	-	-1%	-2%	-5%
	F1 pup	-	2.5%	5%	15%*
	F2 pup	-	3%	3%	3%
	Females	0	51/56	255/267	1297/1375
	F0	-	-5%	-5%	-7%
	F1	-	-2%	0%	-5%
	F1 pup	-	5%	11%	14%
	F2 pup	-	-9%	-9%	0%
(NTP, 1984) Mouse (CD-1); Continuous breeding protocol F0: 40 control and 18-20 breeding pairs/treatment group F1: 20 breeding pairs/group/generation F0: 0, 0.25, 1.25, 2.5 % (0,340,1770, 3640 mg/kg-day) F1: 0, 2.5% (0, 3640 mg/kg-day) Diet F0: 7 days premating + 98 days cohabitation + 21 days segregation (126 days total) F1: in utero + lactation, and then in the diet through a 7 day mating period at 74±10 days old (F1 females were allowed to deliver litters)	Absolute pituitary gland weight in F1 parental mice *(percent change compared to control)*				
		0		3640	
	Males	-		-5%	
	Females	-		-17%*	
	Relative pituitary gland weight in F1 parental mice *(percent change compared to control)*				
		0		3640	
	Males	-		-5%	
	Females	-		-12%*	

Table A-20. Evidence pertaining to adrenal and pituitary gland effects in animals

Reference and Study Design	Results
(Shiraishi et al., 2006) Rat (Sprague-Dawley); 10/sex/group 0, 40, 200, 1,000 mg/kg-day DEP Gavage in corn oil 28 days	Dose-related changes in pituitary weight were not observed.

*Statistically significant ($p<0.05$) based on analysis of data by study authors.

Percent change compared to control = $\frac{\text{treated value} - \text{control value}}{\text{control value}} \times 100$

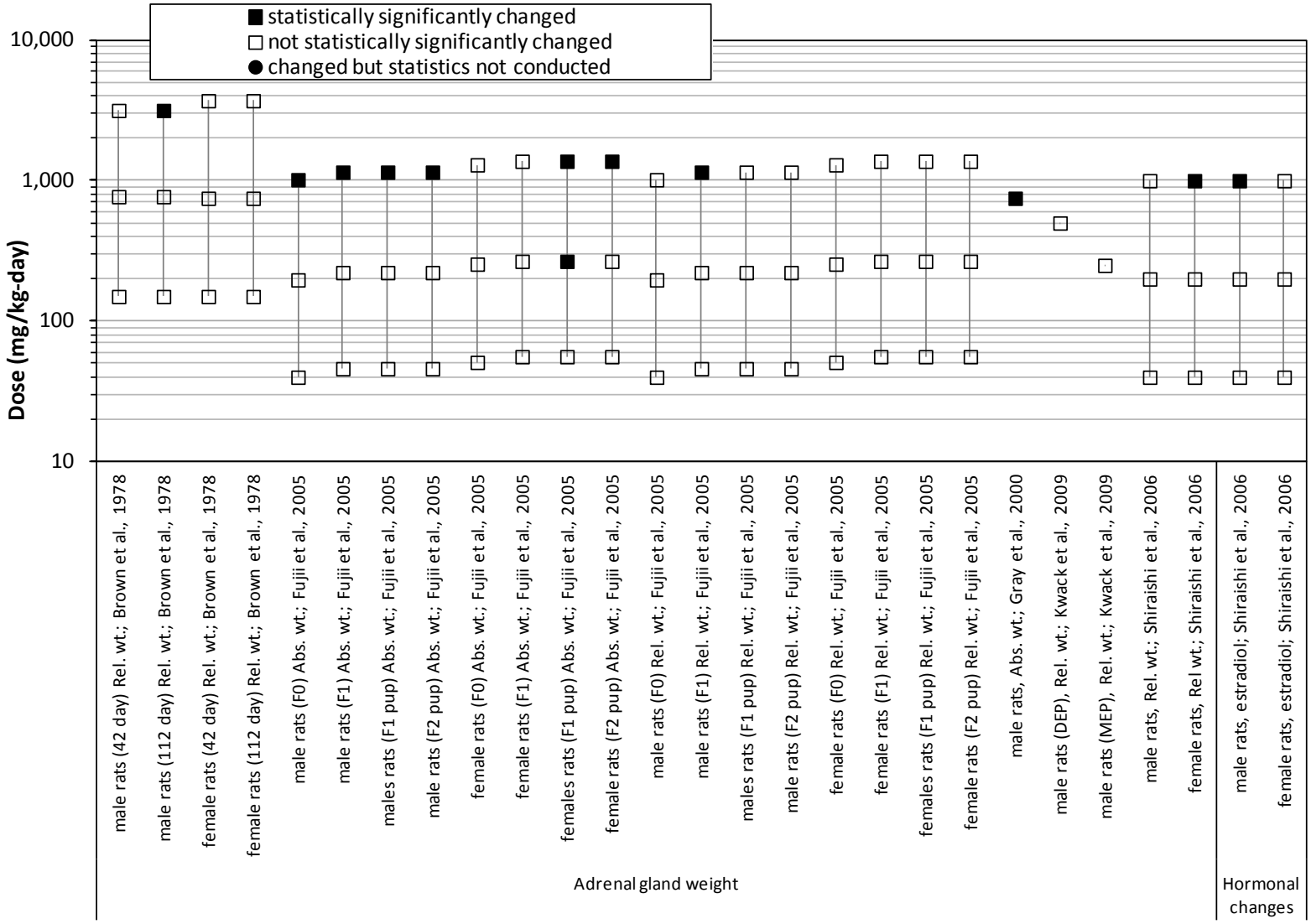

Figure A-5. Exposure-response array of adrenal effects following exposure to DEP

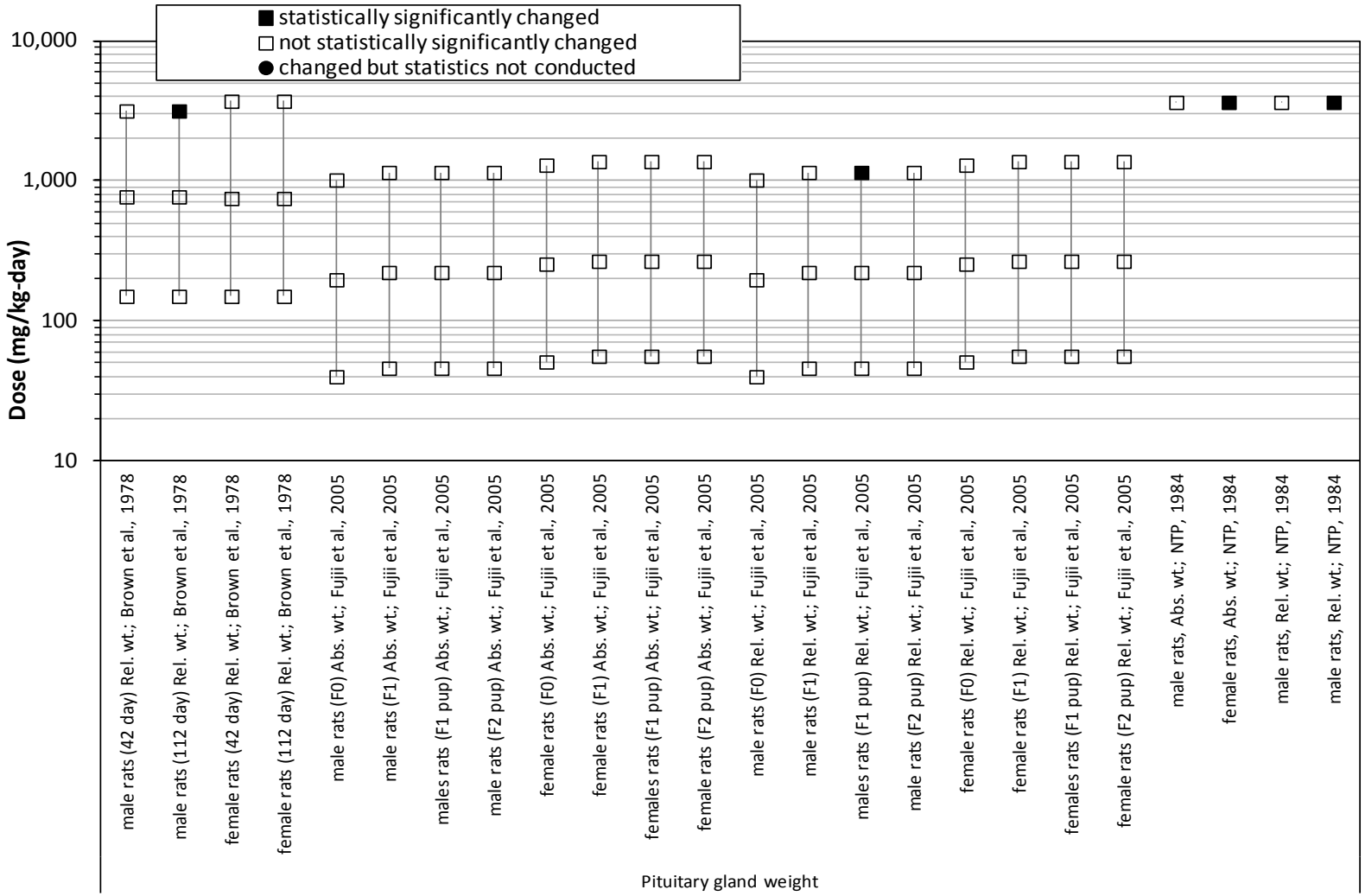

Figure A-6. Exposure-response array of pituitary effects following exposure to DEP

A.6. Carcinogenicity

Table A-21. Evidence pertaining to carcinogenic effects in humans

Reference and Study Design	Results
Breast cancer	
(Lopez-Carrillo et al., 2010) (Mexico) Case-control study, n = 223 hospitalized women, 221 population controls matched by age and residency, ≥18 yrs of age, >1 yr in study area, 2007–2008; mean age 53 years. Participation rates: 94.8% of cases and 99.5% of controls. **Outcome:** Histologically-confirmed breast cancer **Exposure:** Urine sample (for cases, urine collected on average 2 mo after diagnosis, but before treatment) MEP in urine (µg/g Cr): Geometric mean (95% CI) Cases 170 (142, 203) Controls 107 (91, 125) **Analysis:** Logistic regression, considering variables shown in results column	OR (95% CI) for breast cancer, by tertile of MEP (adjusted for current age, age at menarche, parity, menopausal status, and other phthalate metabolites) MEP tertile (µg/g Cr) / Full sample / Pre-menopause / Post-menopause 1 (9.4–56.2): 1.0 (referent) / 1.0 (referent) / 1.0 (referent) 2 (56.2–181.4): 1.42 (0.85–2.38) / 1.84 (0.73, 4.6) / 1.32 (0.69, 2.53) 3 (181.4–18,986): 2.20 (1.33–3.63) / 4.13 (1.6, 10.7) / 1.84 (0.99, 3.42) (trend *p*): (0.003) / (0.060) / (0.060) The association with MEP was larger in magnitude than the association seen with any other phthalate metabolite; MBzP and MCPP were inversely associated with breast cancer risk.

Table A-22. Evidence pertaining to carcinogenic effects in animals

Reference and Study Design	Results				
(NTP, 1995) Mouse ($B_6C_3F_1$); 50/sex/group 0, 7.5, 15, 30 µL /day (0, 8.4, 16.8, 33.6 mg/day) [0, 7.5, 15, 30 µL DEP were dissolved in acetone for a total application volume of 100 µL] and applied to clipped interscapular skin 5x/week Dermal (mixed with acetone) 104-105 weeks	Combined incidence of hepatocellular adenoma or carcinoma				
		0	8.4	16.8	33.6
	Males	9/50	14/50	14/50	18/50*
	Females	7/50	16/51*	19/50*	12/50

*Statistically significant ($p<0.05$) based on analysis of data by study authors.

A.7. Genotoxicity

Table A-23. Evidence pertaining to genotoxicity

Endpoint	Test system	Dose[a]	Results		Test conditions/ comments	Reference
			–S9	+S9[b]		
Prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	10,000 µg/plate	–	– [c]	Preincubation test (20 min). Toxicity observed in duplicate assay at 3.3 mg/plate.	(NTP, 1995)
	S. typhimurium TA98, TA100, TA1535, TA1537	10,000 µg/plate	–	– [c]	Preincubation test (20 min).	(Zeiger et al., 1985; Zeiger et al., 1982)
	S. typhimurium TA98, TA100, TA1535, TA1537 *E. coli* WP2 (*uvr*A)	5000 µg/plate	– (T)	– (T)	Plate incorporation test.	(Dow Corning, 1994)
	S. typhimurium TA98	1000 µg/plate	–	–	No toxicity information reported. High background reversion frequency.	(Kozumbo et al., 1982)
	TA100	1000 µg/plate	± (DR)	–	Statistically significant –S9 at 500 ug/plate, but revertant count <2X negative control.	
	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	50 µg/plate	–	–	Spot test and plate incorporation test.	(Blevins and Taylor, 1982)
	S. typhimurium TA100	1500 µg/plate	+ (DR)	–	Plate incorporation test. Spot tests were all negative. No measure of cytotoxicity.	(Agarwal et al., 1985)
	TA98, TA1535, TA1537, TA1538, TA2637	2000 µg/plate	–	–		
	S. typhimurium TA100	733 µg/mL	– (T)	– (T)	Preincubation test.	(Seed, 1982)
Forward mutation	*S. typhimurium* TA100	733 µg/mL	+ (DR) (T)	– (DR) (T)	8-azaguanine resistance test.	(Seed, 1982)
Mammalian cells						
SCEs	Chinese hamster ovary cells	167 µg/mL	–	+ [c]	Toxicity observed at 750 µg/mL.	(NTP, 1995)
CAs	Chinese hamster ovary cells	324 µg/mL	–	– [c]	Small dose-related increase without S9.	(NTP, 1995)
	Chinese hamster	250 µg/mL	– (T)	ND	Highest dose induced 50%	(Ishidate

	fibroblasts (CHL)				cytotoxicity.	and Odashima, 1977)

+ = positive, ± = equivocal or weakly positive, – = negative, T = cytotoxicity, ND = not determined, SCE = sister chromatid exchange, CA = chromosomal aberration

[a]Lowest effective dose for positive results; highest dose tested for negative results.

[b]Exogenous metabolic activation used; S9 liver fraction from male Sprague-Dawley rats induced with Aroclor 1254 unless otherwise noted.

[c]S9 liver fraction from male Sprague-Dawley rat or Syrian hamster induced with Aroclor 1254.