Abstract

Clear documentation of literature search strategies and analysis methodologies can improve transparency in chemical hazard assessments. We sought to improve clarity for the scientific support for carcinogenic mechanism of action conclusions using a systematic approach to collection, selection, presentation, and evaluation of literature studies. We focused on how di(2-ethylhexyl)phthalate (DEHP) causes liver tumorigenesis, a question critical to interpreting a large and complex database of >3,000 peer-reviewed publications. Relevant mechanisms of hepatic carcinogenesis were identified from published reviews. The literature search strategy included relevant synonyms and wildcards for DEHP and metabolites, mechanisms, and species of interest. Tiered evaluation criteria for study exclusion/inclusion were defined, and applied to the retrieved literature. Manual curation was conducted for mechanisms with large literature databases. Literature trees documented identification, curation and selection of the literature evidence. The selected studies were summarized in evidence tables accompanied by succinct narratives. Primary publications were made available via the Health and Environmental Research Online (http://hero.epa.gov/) database, and identified by evaluation criteria and key terms to permit organized retrieval. This approach effectively manages a large volume of literature, improves appraisal and synthesis of information, and enables analysis of similarity in mechanism of action across studies for the purpose of chemical hazard assessment.

Acknowledgements

So many people have helped me make this possible over the course of the past year that it is hard to know where to start...

First and foremost, I must thank my advisor, Ivan Rusyn, for taking a chance on a jaded ex-academic postdoc who needed a career change. I think things worked out pretty well...

I also owe a debt of gratitude to my mentors at the United States Environmental Protection Agency for making my ORISE fellowship possible—especially Bob Sonawane, Kate Guyton, and my direct mentor Andrew Kraft—for all of their support and encouragement as this project matured...

I could not have asked for a better group of colleagues to work with on the DEHP IRIS assessment—chemical manager Sue Makris, and team members Chris Brinkerhoff, Ghazi Dannan, Yu-Sheng Lin, Raghu Nath, and Deborah Segal. Special thanks to Connie Sickel-Kang for her efforts as well....

Thanks to Ellen Lorang, Gerry Guerevich, and all the HERO staff, especially Rachel Carriere, Ryan Jones, and Sandy Pham for their assistance...

Also thanks to Jackie MacDonald-Gibson for her support and service on my thesis committee. Also thanks for that "H" in risk assessment...but I guess I earned that one...

To all my labmates (past and present) Nour Abdo, Travis Book, Grace Chappell, Sungkyoon Kim, Tanya Kobets, Oksana Kosyk, Bridget O'Brien, Andrew Shapiro, Svitlana Shymonyak, Valerie Soldatow, Myroslav Sypa, Jessica Wignall, and Hong-Sik Yoo: Thanks for the support, laughs, gripes, and all that's in between. I wish everyone

the best of luck and that we all end up where we want to be. Robin—so great to catch up once again after so many years...funny how life brings people around the same way again sometimes...

To Jae, thank you for your unwavering support. I hope Harvard is all you want it to be...but just remember UNC will always have the leg up on public health, hehe. ☺ So when you find me 'round "Bahstahn" and "Hahvahd Yahd" I got braggin' rights on that one, 'k?

To my feline companions of 11.5 years, Target and Zoe, for their unconditional love and companionship, through the best of times and, well, other... You two barf a lot but that's ok.

To my mom and dad...thanks for the Benjamins...☺...I am so fortunate to know I can always count on you...

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AhR—Aryl hydrocarbon receptor

DEHP – Di(2-ethylhexyl)phthalate

CAR—Constitutive androstane receptor

EBTC—Evidence-Based Toxicology Collaboration

GJIC – Gap junction intercellular communication

GRADE--Grading of Recommendations, Assessment, Development, and Evaluation

HERO—Health and Environmental Research Online

IRIS – Integrated Risk Information System

MEHP-- Mono-ethylhexyl phthalate

MeSH—Medical subject headings

MOA—Mode of action

NRC—National Research Council

NTP—National Toxicology Program

PCoA—Palmitoyl-CoA Oxidase activity

Chapter 1

Introduction

Overview. The review of large bodies of scientific literature during the human health risk assessment process requires procurement of quality evidence in a way that is transparent and reproducible, especially with regard to the process of examining and presenting studies key to understanding a chemical's toxicity. Systematic guidelines for this review process should also be applicable to assessments on both cancer and noncancer endpoints. However, recent reports by the National Research Council (NRC) highlight challenges associated with the current literature review and hazard assessment processes (NRC 2010, NRC 2011). In particular, existing assessments do not sufficiently describe the approaches used to search for and evaluate the available literature for a given chemical, which is essential to ensure the best available evidence was included in the assessment. Improving the review process to address the concerns presented above will result in more scientifically sound and standardized assessments with an impact upon regulatory decision-making for human health risks to environmental hazards.

Systematic Reviews and Toxicology. Systematic review is the primary tool of evidence-based medicine, a clinical initiative including "the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients" (Sackett and Rosenberg 1995). The review process is stepwise, framing the question to be addressed, determining how studies will be identified and retrieved, determining how studies will be included or excluded, appraising included studies for quality and absence of bias, and synthesizing data across studies.

Examples of systematic processes that attempt to integrate study results date back over 250 years. James Lind, a fellow of the Royal College of Physicians in Edinburgh, noted that in the treatment of scurvy, a "full and impartial view" of the subject was needed "before the subject could be set in a clear and proper light" (Lind 1753). This observation led Lind to author a document with inquiry into the nature, causes, and cures for the disease. In more modern times, both quantitative and qualitative methods have been proposed to address variations in primary research, and professional initiatives have developed frameworks and standards for conduct of systematic reviews. Readily-available guidelines often referenced are provided by the Cochrane Collaboration, an international network of volunteers that prepare reviews of randomized controlled trials for health interventions, the Campbell Collaboration, a network of professionals that prepare reviews of the effects of social interventions, and the Agency for Healthcare Research and Quality a division of the US Department of Health and Human Services that aims to demonstrate how research results can be used to improve healthcare. Standards for systematic review are also outlined in The Handbook of Research Synthesis and Meta-Analysis (Cooper et. al 2009), and in Summing Up: The Science of Reviewing Research (Light and Pillemer, 1984). Of special interest to toxicologists, the Evidence-Based Toxicology Collaboration ,(http://www.ebtox.com) as well as the Navigation Guide Collaboration (http://coe.ucsf.edu/prhe/naviationguide_strategy.html), engages stakeholders from various professional sectors to produce guidance documents with the overall goal of introducing novel, flexible approaches to chemical hazard and risk assessment that incorporate research syntheses and evidence-based reviews.

Systematic review methodologies that explore the relationship between toxics, adverse health outcomes, and regulatory impacts can also influence and inform policy. The National Academy of Sciences (2010, 2011) has stressed that systematic evaluation

of toxicological studies be used in public health decision-making regarding chemical hazards to ensure that the strongest scientific evidence available is considered in the assessment of an agent of interest. In addition to these recent reports from the NRC, the use of systematic review to understand and present evidence for environmentally-induced disease burden is suggested in a report by the Institute of Medicine (2008) that describes a comprehensive framework for evaluating health effects resulting from chemical exposures incurred during military service. The findings from this review were expected to guide decisions regarding disability benefits rewarded to war veterans with injuries attributable to the chemicals.

Key recommendations for research synthesis and review have emerged from these professional collaborations, advisory panels, handbooks, and other recent reviews with great concordance between entities. An overall theme to emerge has been the establishment of evidence synthesis stages, with evaluative questions addressing specific aspects of studies under consideration for inclusion in the review. Such questions review whether one's sources and search strategies are explicit, if firm editorial or assessment criteria for study inclusion or exclusion are well-defined, and if similar coding of study attributes, such as acceptable sample sizes or number of experimental trials, has been performed. A comprehensive list of twenty questions for research synthesis evaluation was proposed by Harris Cooper (2007), and includes whether appropriate search terms were utilized to mine relevant literature, if selection criteria were applied consistently and reliably across studies deemed fit for inclusion, and if study designs were explicitly defined. Ultimately, presentation of results of the research synthesis should be clear, with complete documentation of applied methodologies included. Graphical or tabular presentation of evidence flows and decision-making processes listing criteria for stepwise choices are new components of data presentation featured in some recent assessments and proposed analysis

frameworks found in the primary literature (Selgrade et al 2012, (Woodruff and Sutton 2011).

A key consideration in evaluating human health risks of chemicals is the human relevance of toxic modes of action observed in animals. Frameworks to evaluate and extrapolate modes-of-action in experimental animals have been proposed for cancer and non-cancer effects (Sonich-Mullin et al. 2001; Boobis et al. 2006; Boobis et al. 2008; Klaunig et al. 2003) (Cohen et al 2003, 2004; Meek et al 2003, Bars et al 2012). Dourson et al. (2008) exemplifies the approach in assessing the evidence for two modes of action (heritable mutagenicity and follicular cell proliferation) for thyroid tumorigenesis from acrylamide. Study results illustrating dose-response relationships for acrylamide exposure and cellular damage endpoints were expressed in graphical and tabular format, with a final table listing key events, related evidence, and plausibility of each mode of action. Brief, bulleted summaries for changes in measured endpoints and correlation between dose-response were incorporated into the overall assessment. However, a major weakness of the analysis was that the authors did not provide any information on sources of supporting data for the modes of action studied. While the report will aid the understanding of where the chemical falls on the gradient of human exposure and the severity of the resulting health effects, the framework must further discuss strategies for mining and selecting the data used in order to maximize their effectiveness in risk assessment. Other limitations of the mode of action framework approach have been identified.(Guyton et al. 2008).

Hazard Identification, Literature Review, and Human Health Risk Assessment. Currently, a four-step assessment paradigm is used to determine the potential health risks of environmental chemical hazards. These steps include 1) hazard assessment and identification, 2) dose-response assessment, 3) exposure assessment, and 4) risk characterization.

As the first step in understanding overall risk, hazard identification is critical for the integrity of the risk assessment process. The current review approach for hazard identification embodies elements of causal inference of relating exposures to outcomes, and elements of Bradford Hill criteria that seek consistency, strength, and specificity of evidence (Hill 1965). Approaches presenting hierarchies for classifying strength of evidence and for reporting meta-analyses are hallmarks of documents that guide public health decision-making (DHHS 2004; Stroup et al. 2000; IOM 2008). Together, these methods warrant consideration throughout the hazard assessment process, with particular emphasis upon explicit documentation of evidence selection.

Modern literature reviews to support hazard assessment must consider the scope and management of search strategies and study inclusion/exclusion criteria (Smith et al. 2011). Search terms should be explicitly defined and related to specific questions addressed by the hazard review, yet should also be broad enough in scope to capture all relevant data. However, the terms should be precise enough to avoid capture of extraneous evidence irrelevant to the agent of interest. Yields of articles from searches should be displayed such that documentation of how literature included in the final review is mined on the basis of explicit inclusion and exclusion criteria. The NRC (2011), in its review of EPA's draft IRIS assessment of formaldehyde, noted that a list of search terms used relevant to the chemical of interest and its health outcomes was not provided. In addition, the IRIS draft lacked a description of search results indicating numbers of articles included or excluded, and of inclusion/exclusion criteria for studies. Other discussions lacking in the draft include advantages and limitations of studies identified in the search and used in the assessment, and how they increase or decrease weight given to epidemiological or experimental results in the overall hazard evaluation (US EPA 2005).

In the interest of clarity and transparency, the NRC (2011) suggested that long

narratives describing study results be replaced with evidence tables that inform and support the conclusions about chemical hazard and the mechanism of action, and that study details, when appropriate, may be placed into appendices. The use of standardized tables for reporting should clarify relevance of the evidence to the chemical of interest, its metabolites, mechanisms of action, species of interest, originality of data, dose-concentration design, replicates and variability, and experimental details and results. Following the tables, short narrative summaries for the lines of evidence presented should eliminate the need for long text descriptions of studies as had been standard in previous IRIS assessments. Graphical tools such as decision trees may also be employed to aid visualization of evidence capture and selection and documentation of inclusion and exclusion of literature. Finally, the literature included in the assessment should also be made available in a publically accessible database such as the US EPA's Health and Environmental Research Online (HERO, http://hero.epa.gov) database.

Di(2-ethylhexyl)phthalate as a case study chemical for a new evidence review process. We chose a case-study chemical with a large, complex, and controversial literature database for its proposed mechanisms of action. Di(2-ethyhexyl)phthalate (DEHP), the most common of the class of phthalate plasticizers, was used as a case study to design and test a framework for identification of mechanistic evidence relevant to liver cancer hazard.

DEHP is produced from the reaction of phthalic anhydride with 2-ethylhexanol. Occupational and environmental exposures to humans are ubiquitous, occurring through use of plastic consumer products and medical devices, food intake, and air and soil-related exposures (Wittassek et al. 2007; Colacino et al. 2010). DEHP is a chemical with an extensive body of literature describing its mechanisms of action in various species (Rusyn and Corton 2012; Caldwell 2012), with conflicting conclusions per its

ability to cause cancer (Klaunig et al. 2003; Guyton et al. 2008; Melnick et al. 1987). Much of the evidence for toxicity of DEHP focuses upon its potential as an endocrine disruptor (Wilson et al. 2008; Gentry et al. 2011), but it is also a well-established rodent liver carcinogen (Melnick et al. 1987; Voss et al. 2005).

Human data on the carcinogenicity of DEHP are sparse and largely inconclusive, with possible associations inferred from other chemicals acting through similar molecular mechanisms (Cummings and Lash 2000; Lash et al. 2000; Ramdhan et al. 2010). Thus, DEHP may elicit carcinogenic effects in humans. Indeed, several human studies have addressed potential hepatotoxicity attributable to DEHP and examined the role of polyvinyl chloride-containing medical devices in DEHP exposures that may increase risk of hepatoblastoma (Reynolds et al. 2004; Maruyama et al. 1999; Oue et al. 2003). However, the majority of human experimental data was observed *in vitro* in cultured human liver cells, which may not replicate responses seen *in vivo*, and do not elicit responses similar to those observed in cultured rodent cells.

Goals of the project. We sought to develop and test a multi-step strategy that would effectively evaluate, synthesize, and report scientific evidence for a chemical with an extensive and controversial body of evidence available through publicly searchable resources. We designed this methodology to provide 1) clear definition of the question of interest's scope and comprehensive, clear searches in public databases, 2) explicit statements of study assessment criteria in the selection of evidence, 3) visual representation and succinct summarization of evidence, and 4) public availability of information used in the assessment. Specifically, we wished to systematically review the liver cancer hazards of DEHP and present evidence to inform its relevance in humans. The components of this strategy include 1) clear specification of a study question; 2) identification of relevant mechanisms of action; 3) definition of a comprehensive search process to capture as much relevant literature as possible; 4) application and illustration

of explicit assessment criteria for study inclusion or exclusion; 5) search and examination of the PubMed database for literature on the consensus mechanisms of action; 6) construction of graphical tools documenting the search and assessment process; 7) presentation of evidence in succinct literature tables and narrative summaries; and 8) organization of evidence in an online database. This strategy enables comprehensive, integrative, and transparent selection and presentation of evidence supporting the consensus mechanisms of action of DEHP. Ultimately, the goal of this work is to explore opportunities to improve clarity and strength of the scientific support presented in human health risk assessments used in regulatory decisions for chemicals that may pose a significant hazard to human health.

Chapter 2

Methodology

The framework for identification of evidence to be included or excluded in the assessment of DEHP toxicity is illustrated in Figure 1 as a flowchart.

2.1 *Specification of the study question.* In Step 1, a clearly specified study question is identified to inform the literature search strategy. For this case study, the question concerns the mechanisms by which the chemical (DEHP) causes carcinogenic hazard in the target tissue (liver). The significance of this study question for assessing the overall hazard of DEHP is based on multiple reports of hepatocarcinogenic hazard of DEHP in two rodent species of both sexes upon chronic exposure (Melnick et al. 1987). Additionally, liver is among the more sensitive target organs of carcinogenicity of DEHP in rodents. Differing conclusions have been reached about the potential relevance of the liver tumor findings in humans. In contrast to the 2000 IARC conclusions regarding the carcinogenic risks of DEHP (IARC 2000), the 2011 IARC Working Group evaluating DEHP concluded that "the human relevance of the molecular events leading to DEHP-induced cancer in several target tissues (e.g., liver and testis) in rats or mice could not be ruled out, resulting in the evaluation of DEHP as a Group 2B agent, rather than Group 3" (Grosse et al. 2011). For these reasons, the question of how DEHP causes liver tumors may be informative for both qualitative and quantitative conclusions in assessing its human health hazards. As for DEHP, study questions for other chemicals with voluminous literature concerning controversial issues relevant to health hazard decision-making may be specified in the course of nomination or evaluation for human health risk assessment. Questions specific to target tissues may be framed

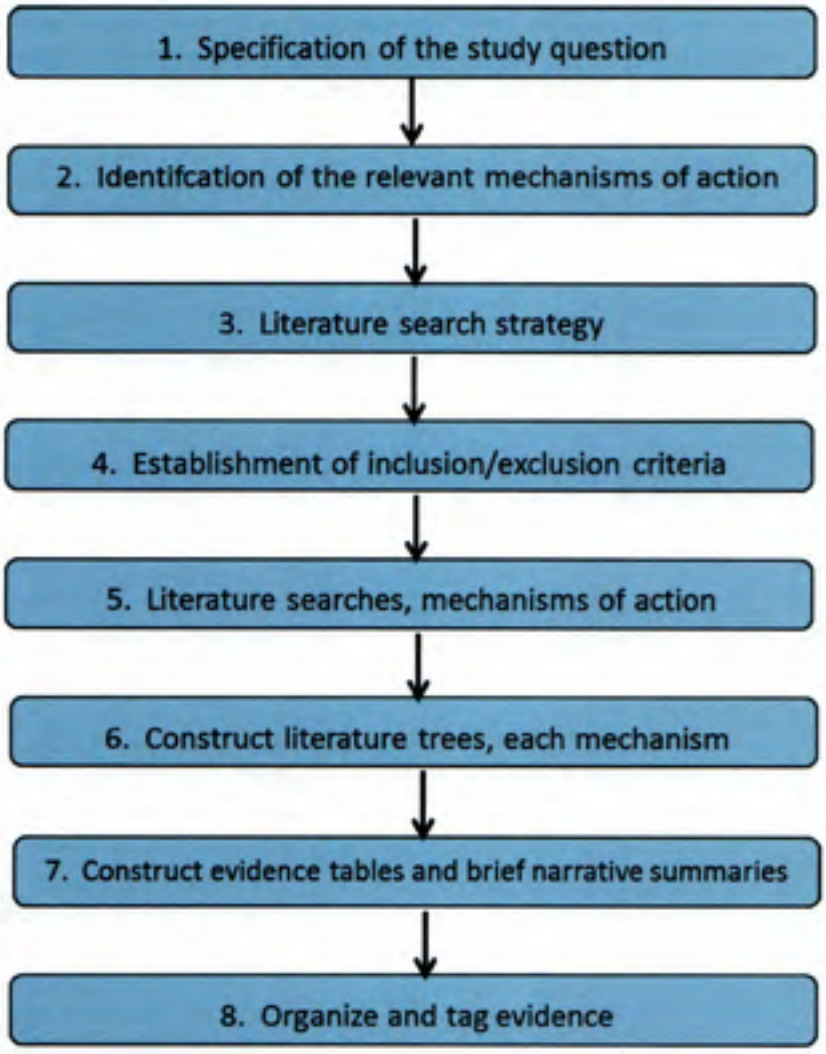


Figure 1. The 8-step systematic framework for literature review for consensus mechanisms of action in this case study focusing on DEHP-induced liver toxicity. This is a stepwise process for evidence identification and assessment, beginning from selection of chemical and target tissue, to construction of evidence tables summarizing literature supporting each mechanism, and tagging of evidence in the HERO database.

based on detection of the chemical in the human body, indications of human health relevance, or indications of environmental contamination (NRC 1991).

2.2. *Identification of relevant mechanisms of action*. Most environmental chemicals are thought to be operating through multiple mechanisms and the challenge of comprehensive review of the complexities in the mechanism of action has been reviewed (Gehlhaus et al. 2011). For the purpose of this work, we constructed an inclusive list of the potential mechanisms of DEHP-induced liver carcinogenesis by using relevant reviews on the mechanisms of liver effects of DEHP (Klaunig et al. 2003; Rusyn and Corton 2012; Caldwell 2012; Guyton et al. 2009; Roberts et al. 2007; Rusyn et al. 2006; Peters et al. 2005) authored by a diverse set of stakeholders (academia, government agencies, and the industry).

2.3. *Literature search strategy*. A search strategy was devised to account for mechanistic studies that may have used both the parent compound and/or its metabolites. In the case of DEHP, many studies have used major metabolites (e.g., mono-ethylhexyl phthalate) to account for rapid hydrolysis of the parent molecule in the gastro-intestinal tract. The initial search was conducted in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) using only "DEHP" as the term, and the number of results, as well as the medical subject heading (MeSH) decompositions, were recorded and dated (Table 1). To acquire mechanistic evidence for metabolites of DEHP, we consulted recent comprehensive studies of DEHP metabolism in humans and other species (Lorber et al. 2010; Lorber et al. 2011; Svensson et al. 2011; Teitelbaum et al. 2012) to create an inclusive string of relevant search terms. The expanded search string was submitted to PubMed, again without any limits or filters, and the number of results and MeSH subheadings recorded. MeSH subheadings were verified for relevance to the search terms entered. If any subheadings deemed irrelevant (e.g., not a known metabolite of DEHP) were noted, they were deleted from the PubMed query

Table 1. Overall search strategy for DEHP, liver, selected mechanisms of action, and species. Search strings, PubMed MeSH query translations, and returned results for DEHP and its metabolites, the target tissue, selected mechanisms of action, and species of interest. Searches performed June 25, 2012.

Level of Search	Search String	MeSH Headings
Chemical	DEHP	"diethylhexyl phthalate"[MeSH Terms] OR ("diethylhexyl"[All Fields] AND "phthalate"[All Fields]) OR "diethylhexyl phthalate"[All Fields] OR "dehp"[All Fields]
	(mono (3-carboxypropyl) OR mcpp OR mono (2-ethylhexyl) OR mono (2-ethyl-5-oxohexyl) OR meohp OR mono (2-ethyl-5-carboxypentyl) OR mecpp OR mono (2-ethyl-5-hydroxyhexyl) OR mehp OR dehp OR 2-ethylhexanol OR (phthalic acid) OR (2-ethyl-5-hydroxyhexyl) OR MEHHP OR (2-carboxymethyl) OR MECPP OR MMHP OR (2-ethyl-5-oxyhexyl))	mono[All Fields] AND 3-carboxypropyl[All Fields] OR mcpp[All Fields] OR mono[All Fields] AND 2-ethylhexyl[All Fields] OR mono[All Fields] AND 2-ethyl-5-oxohexyl[All Fields] OR ("methylphosphite"[Supplementary Concept] OR "methylphosphite"[All Fields] OR "meoph"[All Fields]) OR mono[All Fields] AND 2-ethyl-5-carboxypentyl[All Fields] OR ("2-methyl-5,6-cyclopentapyrimidine"[Supplementary Concept] OR "2-methyl-5,6-cyclopentapyrimidine"[All Fields] OR "mecpp"[All Fields]) OR mono[All Fields] AND 2-ethyl-5-hydroxyhexyl[All Fields] OR ("mono-(2-ethylhexyl)phthalate"[Supplementary Concept] OR "mono-(2-ethylhexyl)phthalate"[All Fields] OR "mehp"[All Fields]) OR ("diethylhexyl phthalate"[MeSH Terms] OR ("diethylhexyl"[All Fields] AND "phthalate"[All Fields]) OR "diethylhexyl phthalate"[All Fields] OR "dehp"[All Fields]) OR ("2-ethylhexanol"[Supplementary Concept] OR "2-ethylhexanol"[All Fields] OR "2 ethylhexanol"[All Fields]) OR ("phthalic acid"[Supplementary Concept] OR "phthalic acid"[All Fields]) OR 2-ethyl-5-hydroxyhexyl[All Fields] OR ("mono-(2-ethylhexyl)phthalate"[Supplementary Concept] OR "mono-(2-ethylhexyl)phthalate"[All Fields] OR "mehp"[All Fields]) OR 2-carboxymethyl[All Fields] OR ("2-methyl-5,6-cyclopentapyrimidine"[Supplementary Concept] OR "2-methyl-5,6-cyclopentapyrimidine"[All Fields] OR "mecpp"[All Fields]) OR ("mono-5-methylhexylphthalate"[Supplementary Concept] OR "mono-5-methylhexylphthalate"[All Fields] OR "mmhp"[All Fields]) OR (2[All Fields] AND ethyl[All Fields] AND 5[All Fields] AND oxyhexyl[All Fields])
Tissue	liver	liver[MeSH Terms] OR "liver"[All Fields]
	(liver OR hepato*)	("liver"[MeSH Terms] OR "liver"[All Fields]) OR (hepato[All Fields] OR ¶...)

¶. Wildcard search for 'hepato*' results in more than 600 variations, hence the search uses only the first 600 variations.

Table 1 (continued)

Level of Search	Search String	MeSH Headings
Mechanism	(peroxisome proliferation)	("peroxisomes"[MeSH Terms] OR "peroxisomes"[All Fields] OR "peroxisome"[All Fields]) AND proliferation[All Fields]
	(peroxis*)	peroxis[All Fields] OR peroxiscan[All Fields] OR peroxisides[All Fields] OR peroxismal[All Fields] OR peroxisme[All Fields] OR peroxismes[All Fields] OR peroxismose[All Fields] OR peroxisom[All Fields] OR peroxisoma[All Fields] OR peroxisomal[All Fields] OR peroxisomal/gloxysomal[All Fields] OR peroxisomal/glyoxysomal[All Fields] OR peroxisomal/mitochondrial[All Fields] OR peroxisomal/myelin[All Fields] OR peroxisomal/nuclear[All Fields] OR peroxisomalbeta[All Fields] OR peroxisomale[All Fields] OR peroxisomalen[All Fields] OR peroxisomales[All Fields] OR peroxisomally[All Fields] OR peroxisomalni[All Fields] OR peroxisomalniho[All Fields] OR peroxisomas[All Fields] OR peroxisombrist[All Fields] OR peroxisome[All Fields] OR peroxisome/mitochondria[All Fields] OR peroxisome/vacuole[All Fields] OR peroxisome'[All Fields] OR peroxisome's[All Fields] OR peroxisomedb[All Fields] OR peroxisomedivision[All Fields] OR peroxisomelike[All Fields] OR peroxisomen[All Fields] OR peroxisomendefekt[All Fields] OR peroxisomeninduktion[All Fields] OR peroxisomenproliferation[All Fields] OR peroxisomenproliferator[All Fields] OR peroxisomeproliferators[All Fields] OR peroxisomers[All Fields] OR peroxisomes[All Fields] OR peroxisomes/cell[All Fields] OR peroxisomes/chemistry[All Fields] OR peroxisomes/classification[All Fields] OR peroxisomes/cm3[All Fields] OR peroxisomes/enzymology[All Fields] OR peroxisomes/genetics[All Fields] OR peroxisomes/glyoxysomes[All Fields] OR peroxisomes/immunology[All Fields] OR peroxisomes/metabolism[All Fields] OR peroxisomes/microbiology[All Fields] OR peroxisomes/mitochondria[All Fields] OR peroxisomes/parasitology[All Fields] OR peroxisomes/pathology[All Fields] OR peroxisomes/physiology[All Fields] OR peroxisomes/secretion[All Fields] OR peroxisomes/ultrasonography[All Fields] OR peroxisomes/ultrastructure[All Fields] OR peroxisomes/virology[All Fields] OR peroxisomes'[All Fields] OR peroxisomial[All Fields] OR peroxisomic[All Fields] OR peroxisomicine[All Fields] OR peroxisomicines[All Fields] OR peroxisomnes[All Fields] OR *(continued)*

Table 1 *(continued)*

Level of Search	Search String	MeSH Headings
Mechanism *(continued)*	(peroxis*) *(continued)*	peroxisomopathies[All Fields] OR peroxisomopathy[All Fields] OR peroxisomotropic[All Fields] OR peroxisomu[All Fields] OR peroxisone[All Fields] OR peroxisones[All Fields] OR peroxisoom[All Fields] OR peroxissoma[All Fields]
	(cell proliferation)	cell proliferation[MeSH Terms] OR ("cell"[All Fields] AND "proliferation"[All Fields]) OR "cell proliferation"[All Fields]
	((cell proliferation) OR (cell growth) OR (dna replication) OR (dna synthesis) OR (replicative dna synthesis) OR mitosis OR (cell division) OR (growth response) OR hyperplasia OR hepatomegaly)	("cell proliferation"[MeSH Terms] OR ("cell"[All Fields] AND "proliferation"[All Fields]) OR "cell proliferation"[All Fields]) OR (("cells"[MeSH Terms] OR "cells"[All Fields] OR "cell"[All Fields]) AND ("growth and development"[Subheading] OR ("growth"[All Fields] AND "development"[All Fields]) OR "growth and development"[All Fields] OR "growth"[All Fields] OR "growth"[MeSH Terms])) OR ("dna replication"[MeSH Terms] OR ("dna"[All Fields] AND "replication"[All Fields]) OR "dna replication"[All Fields]) OR ("dna replication"[MeSH Terms] OR ("dna"[All Fields] AND "replication"[All Fields]) OR "dna replication"[All Fields] OR ("dna"[All Fields] AND "synthesis"[All Fields]) OR "dna synthesis"[All Fields]) OR (replicative[All Fields] AND ("dna replication"[MeSH Terms] OR ("dna"[All Fields] AND "replication"[All Fields]) OR "dna replication"[All Fields] OR ("dna"[All Fields] AND "synthesis"[All Fields]) OR "dna synthesis"[All Fields])) OR ("mitosis"[MeSH Terms] OR "mitosis"[All Fields]) OR ("cell division"[MeSH Terms] OR ("cell"[All Fields] AND "division"[All Fields]) OR "cell division"[All Fields]) OR (("growth and development"[Subheading] OR ("growth"[All Fields] AND "development"[All Fields]) OR "growth and development"[All Fields] OR "growth"[All Fields] OR "growth"[MeSH Terms]) AND response[All Fields]) OR ("hyperplasia"[MeSH Terms] OR "hyperplasia"[All Fields]) OR ("hepatomegaly"[MeSH Terms] OR "hepatomegaly"[All Fields])
	(oxidative stress)	oxidative stress[MeSH Terms] OR ("oxidative"[All Fields] AND "stress"[All Fields]) OR "oxidative stress"[All Fields]
	(oxidative stress OR oxidative OR (reactive oxygen) OR (lipid peroxidation) OR reduced glutathione OR (glutathione peroxidase) OR catalase OR (oxidative DNA damage) OR hydroxyguanosine)	("oxidative stress"[MeSH Terms] OR ("oxidative"[All Fields] AND "stress"[All Fields]) OR "oxidative stress"[All Fields]) OR oxidative[All Fields] OR (reactive[All Fields] AND ("oxygen"[MeSH Terms] OR "oxygen"[All Fields])) OR ("lipid *(continued)*

Table 1 *(continued)*

Level of Search	Search String	MeSH Headings
Mechanism *(continued)*	(oxidative stress OR oxidative OR (reactive oxygen) OR (lipid peroxidation) OR reduced glutathione OR (glutathione peroxidase) OR catalase OR (oxidative DNA damage) OR hydroxyguanosine) *(continued)*	peroxidation"[MeSH Terms] OR ("lipid"[All Fields] AND "peroxidation"[All Fields]) OR "lipid peroxidation"[All Fields]) OR ("glutathione"[MeSH Terms] OR "glutathione"[All Fields] OR ("reduced"[All Fields] AND "glutathione"[All Fields]) OR "reduced glutathione"[All Fields]) OR ("glutathione peroxidase"[MeSH Terms] OR ("glutathione"[All Fields] AND "peroxidase"[All Fields]) OR "glutathione peroxidase"[All Fields]) OR ("catalase"[MeSH Terms] OR "catalase"[All Fields]) OR (oxidative[All Fields] AND ("dna damage"[MeSH Terms] OR ("dna"[All Fields] AND "damage"[All Fields]) OR "dna damage"[All Fields])) OR hydroxyguanosine[All Fields]
Species	(human)	humans[MeSH Terms] OR "humans"[All Fields] OR "human"[All Fields]
	(human OR homo sapiens)	("humans"[MeSH Terms] OR "humans"[All Fields] OR "human"[All Fields]) OR ("humans"[MeSH Terms] OR "humans"[All Fields] OR ("homo"[All Fields] AND "sapiens"[All Fields]) OR "homo sapiens"[All Fields])
	(mouse or rat)	("mice"[MeSH Terms] OR "mice"[All Fields] OR "mouse"[All Fields]) OR rat[All Fields]
	(mouse OR rat OR hamster OR rodent OR murine OR Mus musculus or Rattus)	("mice"[MeSH Terms] OR "mice"[All Fields] OR "mouse"[All Fields]) OR rat[All Fields] OR ("cricetinae"[MeSH Terms] OR "cricetinae"[All Fields] OR "hamster"[All Fields]) OR ("rodentia"[MeSH Terms] OR "rodentia"[All Fields] OR "rodent"[All Fields]) OR ("mice"[MeSH Terms] OR "mice"[All Fields] OR "murine"[All Fields]) OR ("mice"[MeSH Terms] OR "mice"[All Fields] OR ("mus"[All Fields] AND "musculus"[All Fields]) OR "mus musculus"[All Fields]) OR ("rats"[MeSH Terms] OR "rats"[All Fields] OR "rattus"[All Fields])
	(primate)	primates[MeSH Terms] OR "primates"[All Fields] OR "primate"[All Fields]
	(primate or monkey)	("primates"[MeSH Terms] OR "primates"[All Fields] OR "primate"[All Fields]) OR ("haplorhini"[MeSH Terms] OR "haplorhini"[All Fields] OR "monkey"[All Fields])

translation, and the search was repeated with any changes in results recorded.

2.4. *Establishment of inclusion/exclusion criteria.* Table 2 lists tiered criteria by which retrieved literature was screened for inclusion into evidence tables for each consensus mechanism of action. The Tier 1 criteria used in evaluating all of the retrieved literature databases considered whether the publication addressed the chemical, tissue, mechanism, and species of interest, and contained original data. If a particular mechanism of action has been a subject of many studies (e.g., the number of relevant publications exceeding 20), Tier 2 criteria were applied to address study's clarity, completeness, and soundness. For example, greater weight was assigned to studies that employed dose-response designs, included a large number of replicates, reported experimental variability, and provided clear description of the methodology. These inclusion and exclusion criteria were informed by the EPA's summary of general assessment factors on evaluation of scientific and technical information (EPA 2003) and US EPA's *Guidelines for Carcinogen Risk Assessment* (EPA 2005).

2.5. *Literature searches, mechanisms of action.* The search string for DEHP and its metabolites was expanded to incorporate the target tissue (liver), the mechanism, and the relevant species of interest to build a literature database of evidence. Based on the comprehensive reviews (see section 2.2), we selected three types of species, humans, non-human primates, and rodents (rat or mouse), as most relevant for the purpose of constructing the evidence tables for DEHP as a case study. To automate these searches to the greatest extent possible, we determined that the use of wildcards and synonyms for relevant search terms was most effective in identifying relevant literature (Table 1). As described in section 2.3, PubMed results were recorded and dated, including MeSH subheadings from the query.

2.6. *Construction of the literature trees.* In the next step, literature trees were constructed to visualize and document how initial search findings are narrowed to those

Table 2. Evaluation criteria used in the consideration of inclusion and exclusion of evidence for DEHP mechanisms of action in this case study.

Tiers	Inclusion/Exclusion Criteria
Tier 1: Applicability and Utility (Applied to all searches)	1.Did the study evaluate effects of the chemical of interest or its metabolites known to be formed in humans?
	2.Did the study evaluate effects in the tissue (organ) of interest or cells derived from this tissue?
	3.Did the study evaluate cellular, biochemical or molecular effects relevant to the mechanism under consideration?
	4.Did the study include information sufficient to determine what species were studied?
	5.Does the publication include original data?
Tier 2: Clarity, completeness, soundness (Applied to mechanisms with large databases)	6.Did the study employ dose-/concentration-response design?
	7.Does the study contain sufficient number of replicates within each experimental group to evaluate significance of the effect?
	8.Does the study report variability within each experimental group/condition?
	9.To what extent the study clearly and completely describes both the experimental details and results?

considered for inclusion into the evidence tables. Beginning from the highest level of the tree, the chemical of interest, publications that were not relevant to the target tissue, selected mechanism, or species were removed. After excluding reviews, as determined by PubMed, full text references were then retrieved for manual evaluation and inclusion into evidence tables, with Tier 2 assessment criteria applied to large databases. The manual curation of the articles identified most relevant publications that met all of the evaluation to be included into the evidence tables.

2.7. *Construction of the evidence tables.* Evidence tables for each DEHP mechanism of action in liver were constructed and stratified in accordance with the type of an experimental system(s) and other relevant information. Results were grouped according to whether studies were conducted *in vivo* or *in vitro*, and were listed in the table with highest doses first. Studies that tested the relationship of the mechanism to an outcome by evaluating the impact of altering, suppressing or enhancing the mechanism (i.e., in genetically-modified or other experimental models, or in recovery experiments) were presented first. Tables were composed in a format such that information about the study (reference and a hyperlink to the PDF in HERO database), the experimental system or materials used, the number of samples tested, and any relevant treatment periods were included. Marker(s) or endpoint(s) for each mechanism were clearly stated and quantitative information (where available) on the doses used corresponding to the observed changes at that endpoint was provided. Experimental results were expressed as percent change from control. Statistically significant treatment-related changes were denoted with an asterisk, and abbreviations were indicated as footnotes. Based upon results presented in the tables, we then composed brief narrative summaries of the results for each mechanism of action relevant to each species of interest. These summaries included types of effects, species concordance, dose-response relationships, and statistical significance of results.

2.8. *Literature organization in HERO.* In the final step of the framework, primary literature considered in the assessment of DEHP mechanism of action in the liver was uploaded and organized in the HERO (http://hero.epa.gov) database. References were labeled in accordance with both the inclusion and exclusion assessment criteria, as well as other pertinent information, for ease of navigation and searches.

Chapter 3

Results

3.1. Literature search for DEHP and its metabolites. Consistent with the assertion of Smith et al (2011) that a thorough scan of the literature is central to a modern literature review, we constructed comprehensive search strings for DEHP, liver, the consensus mechanisms of action, and species of interest. Table 1 lists literature search terms representative of DEHP, its mechanisms of action, and species of interest. For example, terms in search string for DEHP and its metabolites was concatenated with the Boolean operator "OR" in order to account for studies relevant to the parent compound and its metabolites. To illustrate how expanded search strings using synonyms and wildcards returned more comprehensive results, MeSH subheadings relevant to the input(s) are provided. The majority of subject headings returned were mapped to DEHP or one of its metabolites, and these query translations were also examined to ensure terms that do not appear relevant to DEHP metabolism (e.g., "2-methyl-5,6-cyclopentapyrimidine") are excluded. We also confirmed the exclusion of irrelevant headings did not affect the number or content of search results returned (not shown). Also of note is that MeSH strings were concatenated with the Boolean operators "AND" and "OR", with "OR" distinguishing between subject headings for discrete inputs. "AND" is the default Boolean operator connecting terms appearing side-by-side when input into PubMed, reflected in the MeSH breakdown as well.

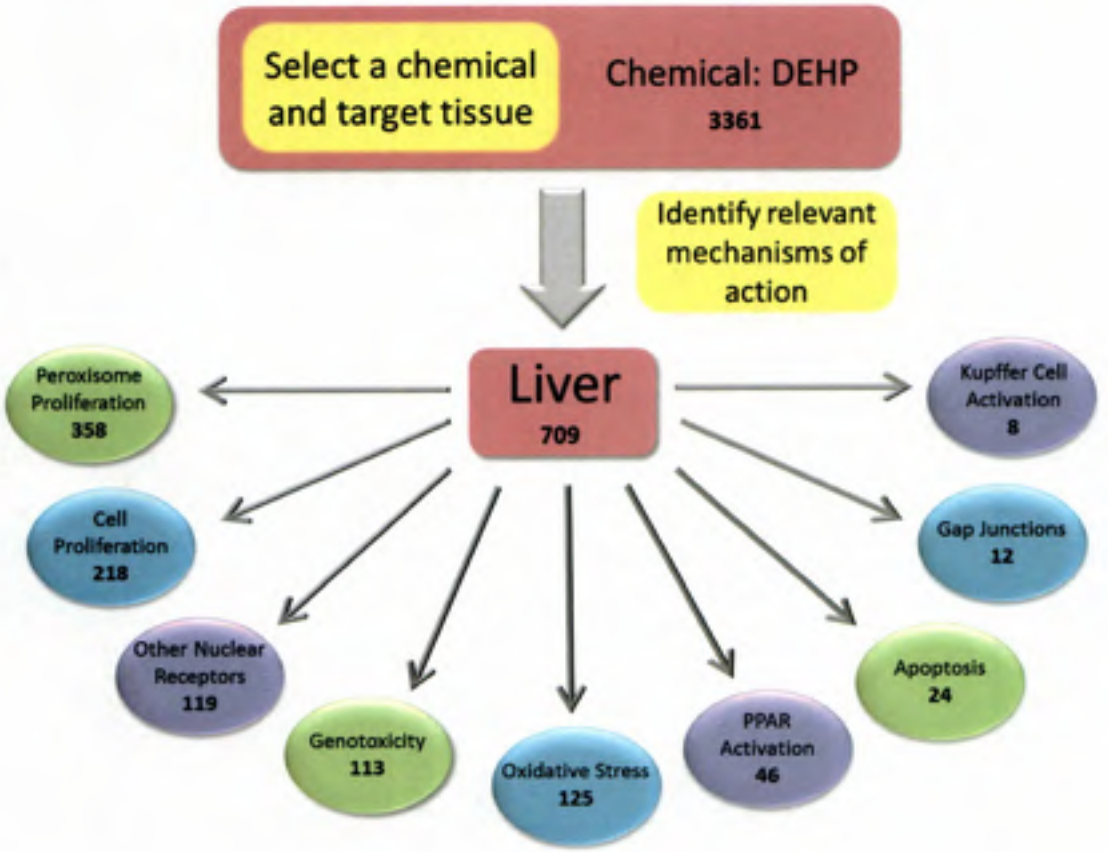
The initial search was performed by entering "DEHP" into PubMed and yielded a return of 2319 references (as of June 25, 2012), with MeSH subheadings all relevant to DEHP and other phthalates. The expanded search string, including metabolites and

relevant chemical abbreviations and prefixes for DEHP-relevant species, when tested in PubMed yielded 3361 results (as of June 25, 2012), an increase of nearly 45%.

3.2. Identification of DEHP mechanisms of action in liver. The survey of seven recent reviews and/or highly cited reviews from diverse stakeholders (Klaunig et al. 2003; Rusyn and Corton 2012; Caldwell 2012; Guyton et al. 2009; Roberts et al. 2007; Rusyn et al. 2006; Peters et al. 2005) was conducted, from which nine consensus mechanisms for DEHP in liver were determined (Figure 2): peroxisome proliferation, cell proliferation, PPAR receptor activation, oxidative stress, nuclear receptor activation, genotoxicity, apoptosis, disruption of gap junction intercellular communication, and Kupffer cell activation. Accordingly, search phrases describing the tissue of interest, liver, and each of the mechanisms were composed and concatenated with the search string devised for DEHP and its metabolites. Query translation anomalies were addressed, and irrelevant MeSH headings were also removed. For instance, because the MeSH query translation for liver did not include the synonym "hepatic" or any variation thereof, the wildcard "hepat*" was included to maximize returns for relevant evidence (Table 1). Similarly, the wildcard "peroxis*" was included because the term "peroxisomal" was not returned in the query translation for "peroxisome proliferation". We also included Latin terms representing genus, species, or both in search strings specific to some species of interest in order to capture the most literature possible (Table 1).

3.3. Application of inclusion/exclusion criteria, construction of literature trees and evidence tables. After collecting literature databases for each mechanism, we subjected each reference to predefined assessment criteria to determine its suitability for inclusion into an evidence table. As representative of a mechanism with a large literature database, the screening of literature for "peroxisome proliferation" is described using the Tier 1 criteria listed in Table 2. Beginning with the 3361 references identified as relevant

Figure 2. Consensus mechanisms of action of DEHP in liver and PubMed search results for sizes of literature databases for each. The numbers represent the quantity of articles in the databases at the level of chemical, tissue, and mechanisms of action.



to DEHP and its metabolites, 2652 were not included in further consideration as they were not relevant to "liver".

To illustrate the process for a mechanism with a large number of publications, the 709 liver-relevant references were screened for the "peroxisome proliferation". Three hundred fifty eight publications passed this filter and were then further sub-divided according to species of interest, which included humans, non-human primates, and rodents (rats or mice). Of these, 74 references were classified to humans, 342 to rodents, and 59 to non-human primates. Studies reporting results for DEHP toxicity in other species were also noted; in our case, a total of four references studying DEHP toxicity in rabbits or fish were returned. Furthermore, 27 reviews, as identified by PubMed, were not considered further in the database from humans, 18 from rodents, and 23 from non-human primates. The resulting numbers of references subject to expert evaluation were therefore 47 for humans, 324 for rodents, and 36 for non-human primates.

Evaluation of the remaining evidence was performed manually by examining the abstracts of each study. Of the 324 publications identified by automated query as containing information on peroxisome proliferation in rodents, 262 were excluded based on the expert evaluation of the study's abstract. The primary reasons for exclusion were lack of evidence regarding DEHP or its metabolites, tissue (liver), species (rodents), or the mechanism of interest. Many were identified as reviews that were not indexed by PubMed. This first manual curation step was essentially a manual reapplication of the Tier 1 assessment criteria. The follow up step was to examine full-text of each article and 19 publications were further excluded leaving 61 publications to be screened using Tier 2 criteria as listed in Table 2. Using full text articles, 43 were excluded, of which 31 were single dose/concentration studies, 8 reported no variability measures for quantitative data, and 4 studies had unclear description of the methodology (i.e., the

strain or sex of the animals used was not identified). At the end of the screening process, 18 unique publications were selected for inclusion into the evidence table for peroxisome proliferation in rodents (Table 3). To visualize the screening process, a literature tree (Figure 3) was drawn documenting how initial search findings are narrowed and where and why literature may be removed from further consideration.

As a representative of a mechanism with an intermediate number of publications in its database, the screening of literature for "peroxisome proliferator-activated receptor" (PPAR) is described. Forty-six publications were identified through automated PubMed query as relevant to "PPAR" and "liver". Of these, 19 were classified to humans, 43 to rodents (mice and rats), and 18 to non-human primates, and all met the established Tier 1 criteria. Studies classified as reviews by PubMed were dropped from the search results, with 8 reports thus not considered for humans, 6 reports not considered for rodents, and 8 reports not considered for non-human primates. As a result, 11, 37, and 10 studies moved forward for manual curation in accordance with Tier 2 assessment criteria. Using full text articles, 10 of 11 were excluded for humans, of which 1 did not contain original data, 5 were not relevant to humans, 3 were not relevant to liver, and 1 did not study PPAR activation. For rodents, 31 of 37 were excluded, of which 2 did not contain original data, 6 were not in liver, 9 did not study PPAR activation, 4 were not specific to rodents, 4 were not quantitative, 3 did not contain sufficient replicates, 2 were not the chemical of interest, and 1 was not in English. In the case of studies retrieved for non-human primates, 6 of 10 were excluded, with 1 not in liver and 5 not specific to primates. At the end of the screening, 1 unique reference for humans, 7 unique references for rodents, and 4 unique references for non-human primates were included in the evidence table for PPAR activation. Tables 4A, 4B, and 4C present evidence collected for PPAR activation in humans, rodents, and non-human primates, respectively, and a literature tree was drawn to illustrate the inclusion/exclusion of

Table 3. Example of an evidence table for the "peroxisome proliferation" mechanism of action in rodents. Results are stratified by *in vivo* cancer bioassay, or chronic or subchronic acute studies, or *in vitro* experimental systems. Endpoints measured and quantitative dose-response information is also presented.

Liver Peroxisome Proliferation: Rodents							
Study Design and Reference	**Endpoint and Assay**	**Results (% Change from Control)**					
In Vivo Chronic Cancer Bioassays							
David et al (1999) Rats (F344), Male and Female Chronic (78 weeks, and 78 weeks followed by 26 weeks of recovery) N = 6/group	Palmitoyl-CoA Oxidase (nmol/min/mg protein), Males		*mg/kg/day DEHP* [1]				
			0	71	357	1786	Recovery
		1 week	0	*nd*	*nd*	255 *	*nd*
		2 weeks	0	*nd*	*nd*	556 *	*nd*
		13 weeks	0	*nd*	*nd*	390 *	*nd*
		104 weeks	0	-29	71 *	257 *	-24
	Palmitoyl-CoA Oxidase (nmol/min/mg protein), Females		*mg/kg/day DEHP*				
			0	71	357	1786	Recovery
		1 week	0	*nd*	*nd*	145 *	*nd*
		2 weeks	0	*nd*	*nd*	396 *	*nd*
		13 weeks	0	*nd*	*nd*	345 *	*nd*
		104 weeks	0	-13	92 *	410 *	16
David et al (1999) Mice ($B6C3F_1$), Male and Female Chronic (78 weeks, and 78 weeks followed by 26 weeks of recovery) N = 6/group	Palmitoyl-CoA Oxidase (nmol/min/mg protein), Males		*mg/kg/day DEHP*				
			0	143	1529	2500	
		1 week	0	28	581	654	
		4 weeks	0	139	894	1002	
		13 weeks	0	7	372	394	
			mg/kg/day DEHP				
			0	14	71	214	857	Recovery
		104 weeks	0	0	115	170	662	11
	Palmitoyl-CoA Oxidase (nmol/min/mg protein), Females		*mg/kg/day DEHP*				
			0	143	1529	2500	
		1 week	0	145 *	1205 *	1344 *	
		4 weeks	0	65 *	527 *	771 *	
		13 weeks	0	34 *	896 *	740 *	
			mg/kg/day DEHP				
			0	14	71	214	857	Recovery
		104 weeks	0	30	309	576 *	1401 *	40

* - statistically significant; 1 -- converted to mg/kg/day from ppm (1 mg/kg = 7 ppm)

Table 3 (continued)

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)				
In Vivo Acute and Subchronic Studies						
Macdonald et al (2001) Mice (Sv/129, F3 generation), 3 males PPARα [1] -null and wild-type PPARα Acute (0, 24 hours)	Peroxisome volume density, electron microscopy		mg/kg/day DEHP			
			0	1150		
		Wild-type	0	157		
		PPARα-null	0	-20		
Lake et al (1984) Rats (Sprague-Dawley), Male Short-term (2 weeks) N =5/group	Palmitoyl-CoA Oxidase (nmol/min/mg protein)		mg/kg DEHP			
		0	25	100	250	1000
		0	-5	150	320	825
	Carnitine Acetyltransferase (nmol/min/mg protein)		mg/kg DEHP			
		0	25	100	250	1000
		0	-5	175	825	1400
	Carnitine Palmitoyltransferase (nmol/min/mg protein)		mg/kg DEHP			
		0	25	100	250	1000
		0	-5	0	5	15
Hinton et al (1986) Rats (Wistar albino), Male 24 hours N = 3-4	Palmitoyl-CoA Oxidase (nmol/min/mg protein), Males		mg/kg DEHP			
		0	50	200	1000	
		0	62 *	57 *	468 *	
	Palmitoyl-CoA Oxidase (nmol/min/mg protein), Females		mg/kg DEHP			
		0	50	200	1000	
		0	37	105 *	450 *	
	Catalase (nmol/min/mg protein), Males		mg/kg DEHP			
		0	50	200	1000	
		0	0	9	22 *	
	Catalase (nmol/min/mg protein), Females		mg/kg DEHP			
		0	50	200	1000	
		0	0	9	37 *	
De Angelo et al (1985) Rats (Sprague-Dawley), Male Long-term (10 weeks) N = 3-5 group	Carnitine Acetyltransferase (mmol/min/mg protein)		mg/kg DEHP			
		0	1000	5000	10000	20000
		0	82	136	255	582

* - statistically significant

Table 3 (continued)

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)
In Vivo Acute and Subchronic Studies (Continued)		
Dostal et al (1987) Rats (Sprague-Dawley), Male Short-term (5 days) N = 13-16	Palmitoyl-CoA Oxidase (nmol/min/mg protein)	*mg/kg/day DEHP* Age (days) 0 10 100 1000 2000 6-10 0 12 195 * 1181 * *nd* 14-18 0 52 * 593 * 2601 * *nd* 16-20 0 *nd* *nd* 1009 * *nd* 21-25 0 6 118 * 1415 * *nd* 42-46 0 *nd* 151 * 1077 * 1403 * 86-90 0 *nd* 298 * 1358 * 1598 *
	Carnitine Acetyltransferase (nmol/min/mg protein)	*mg/kg/day DEHP* Age (days) 0 10 100 1000 2000 6-10 0 38 * 167 * 831 * *nd* 14-18 0 3 679 * 2481 * *nd* 16-20 0 *nd* *nd* 668 * *nd* 21-25 0 28 136 1068 *nd* 42-46 0 *nd* 259 * 1033 * 1258 * 86-90 0 *nd* 340 * 982 * 996 *
Isenberg et al (2000) Rats (F344), male Short-term (1 to 6 weeks) N = 5/group	Peroxisomal Beta-oxidation Activity (% of control)	*mg/kg/day DEHP* 0 500 1000 6000 12000 20000 1 week 0 *nd* *nd* 445 * *nd* 865 * 2 weeks 0 *nd* 127 510 * 663 * 1056 * 4 weeks 0 *nd* *nd* 391 *nd* 1123 * 6 weeks 0 *nd* *nd* 334 *nd* 1908 *
Sharma et al (1988) Rats (Wistar albino), Male Short-term (3 days) N = 6/group	Palmitoyl-CoA Oxidase (nmol/min/mg protein)	*mg/kg DEHP* 0 250 500 750 1000 1250 0 200 300 400 520 560
Lake et al (1986) Rats (Sprague-Dawley), Male Short-term (7 days) N = 5	Palmitoyl-CoA Oxidase (nmol/min/mg protein)	*mg/kg DEHP* 0 50 200 500 0 80 210 560
	Carnitine Acetyltransferase (nmol/min/mg protein)	*mg/kg DEHP* 0 50 200 500 0 150 230 760

Table 3 (continued)

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)
In Vivo Acute and Subchronic Studies (Continued)		
Reddy et al (1986) Rats (F344), Male Short-term (30 days) N = 3-4	Peroxisome Volume Density (% cytoplasmic volume)	*mg/kg DEHP* 0 — 2500 — 5000 — 10000 — 20000 180 — 2800 — 3760 0 — 3000
Ochs et al (1992) Mice ($B6C3F_1$), Male Short-term (5 days) N = 4-5/group	Acyl-CoA Oxidase (nmol/min/mg protein)	*mg/kg DEHP* 0 — 3300 — 6700 — 10000 0 — 222 * — 375 * — 364 * *Cipro, mg/kg DEHP* 0.002, — 0.004, — 0.006, 0 — 3300 — 6700 — 10000 0 — 439 * — 567 * — 593 *
Howarth et al (2001) Rats (Wistar albino), male Short-term (2 weeks) N = 5/group	Palmitoyl-CoA Oxidation (nmol/min/mg protein)	*mg/kg/day DEHP* 1, 0 — 10000 — 1% DnHP 0 — 734 * — 729 *
	Catalase (nmol/min/mg protein)	*mg/kg/day DEHP* 1, 0 — 10000 — 1% DnHP 0 — 28 — 26
Isenberg et al (2000) Mice ($B6C3F_1$), Male Short-term (1 to 6 weeks) N = 5/group	Peroxisomal Beta-oxidation Activity (% of control)	*mg/kg DEHP* 0 — 500 — 6000 2 weeks: 0 — 105 — 1329 * 4 weeks: 0 — 101 — 788 *
In Vitro Bioassays with Primary Hepatocytes		
Goll et al (1999) 1° hepatocytes from male Sprague-Dawley Rats 72 hours N = 3	Acyl-CoA oxidase activity (nmol/min/mg protein)	*µM DEHP* 0 — 100 — 250 — 500 0 — 10 * — 6 — 3
	Carnitine acetyltransferase activity (nmol/min/mg protein)	*µM DEHP* 0 — 100 — 250 — 500 0 — 10 * — 6 — 3

Table 3 (continued)

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)							
In Vitro Bioassays with Primary Hepatocytes (continued)									
Gray et al (1983) 1° hepatocytes from male Sprague-Dawley Rats 48 hours N = 3	Carnitine acetyltransferase activity (% of control)				*µM DEHP*				
		0	10	20	50	100	200	500	1000
		0	20	120	540	900	1300	900	-30
	Carnitine palmitoyltransferase activity (% of control)				*µM DEHP*				
		0	10	20	50	100	200	500	1000
		0	-5	-10	-5	5	0	-5	-70
Mitchell et al (1984) 1° hepatocytes from male Alderley Park Rats 72 hours N = 5 to 6	Palmitoyl-CoA Oxidation (% of control)			*µM MEHP*					
		0	50	100	250	500			
		0	27	375	700	1450			
Gray et al (1982) 1° hepatocytes from male Wistar Rats 48 hours N = 3	Carnitine acetyltransferase activity (% of control)		*µM 2-EH*						
		0	200	1000					
		0	21	843 *					
Hildebrand et al (1999) 1° hepatocytes from male Wistar Rats 144 hours N = not indicated	Carnitine acetyltransferase activity (nmol/min/mg protein)				*µM DEHP*				
		0	1	10	100	1000			
		0	89	229	711	577			
Mitchell et al (1985) 1° hepatocytes from male Alderley Park Rats 72 hours N = 4	Peroxisomal Beta-oxidation Activity (nmol/min/mg protein)				*µM MEHP*				
		0	25	50	100	250	500		
		0	2	28	300	638	700		
Elcombe and Mitchell (1986) 1° hepatocytes from male Alderley Park Rats 72 hours N = 3-8	Peroxisomal Beta-oxidation Activity(nmol/min/mg protein)				*µM MEHP*				
		0	50	100	250	500			
		0	40	325 *	700 *	1450 *			

Table 3 (Continued)

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)
In Vitro Bioassays with Primary Hepatocytes (continued)		
Dirven et al (1993) 1° hepatocytes from male Wistar Rats 72 hours N = 3	Palmitoyl-CoA Oxidation (nmol/min/mg protein)	*µM MEHP* 0 — 25 — 50 — 100 — 300 0 — 100 — 169 * — 215 * — 746 *
Gray et al (Toxicology, 1983) 1° hepatocytes from male Sprague-Dawley Rats 48 hours N = 3-4	Palmitoyl-CoA Oxidation (nmol/min/mg protein)	*µM MEHP or 2-EH* [2] 0 — 200 MEHP — 200 2-EH — 200 MEHP, 200 2-EH 0 — 473 * — 2 — 556 *
	Carnitine acetyltransferase activity (nmol/min/mg protein)	*µM MEHP or 2-EH* 0 — 200 MEHP — 200 2-EH — 200 MEHP, 200 2-EH 0 — 1456 * — 51 — 1674 *

2 - 2-ethylhexanoic acid

Figure 3. Literature tree for the "peroxisome proliferation" mechanism of action. Progression from the initial 3361 identified articles relevant to DEHP or its metabolites to those included in the evidence tables is shown.

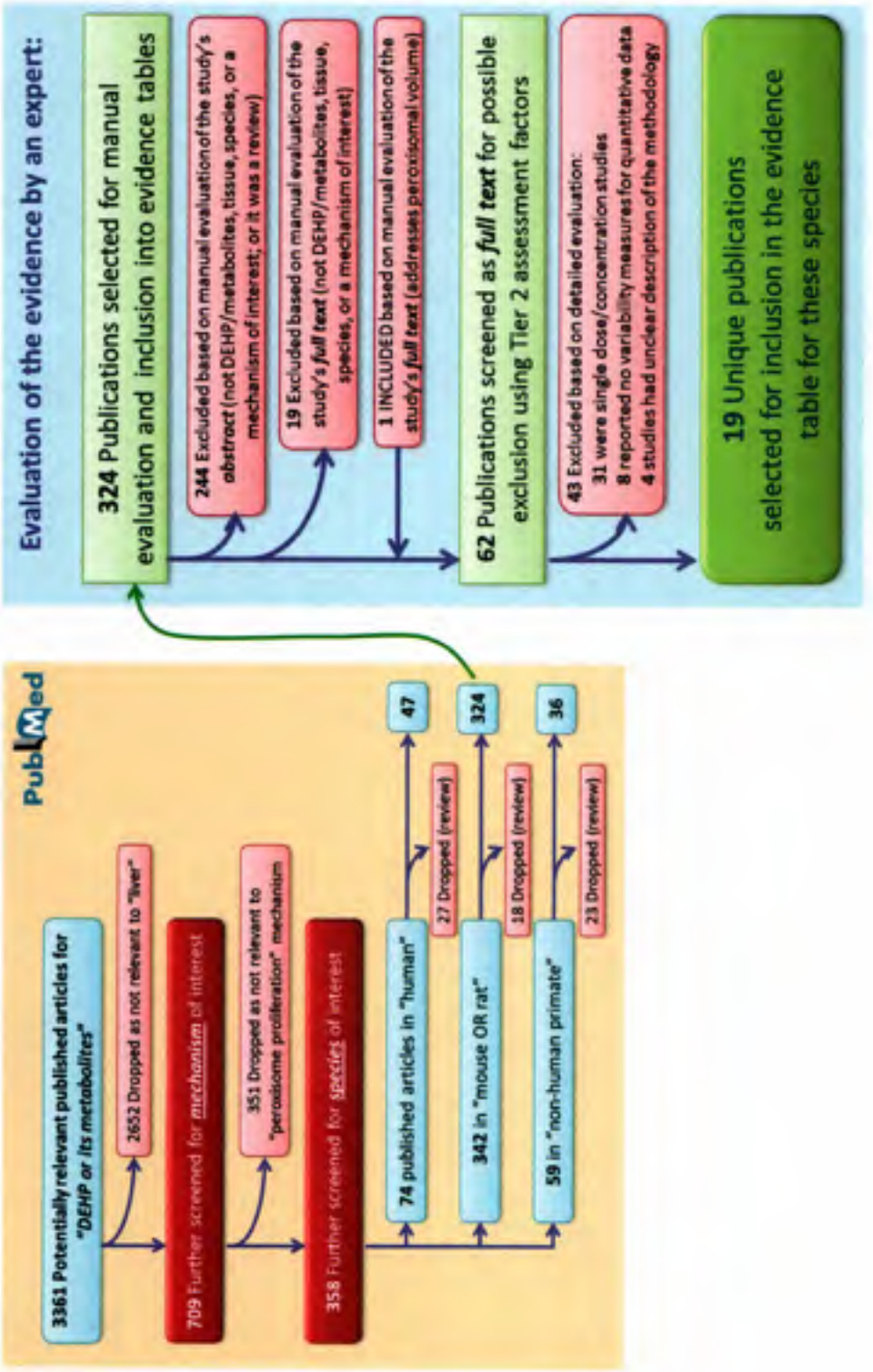


Table 4A. Example of an evidence table for the "peroxisome proliferator activated receptor" (PPAR) mechanism of action. Results for humans (A), rodents (B), and primates (C) are presented. Results are stratified by *in vivo* cancer bioassay, or chronic or subchronic acute studies, or *in vitro* experimental systems. Endpoints measured and quantitative dose-response information is also presented.

Liver PPAR Activation: Humans										
Study Design and Reference	**Endpoint and Assay**	**Results (% Change from Control)**								
In Vivo										
no primary literature evidence										
In Vitro Acute Studies–Transgenic Cell Lines										
Lapinskas et al (2005) Transfected human hepatoma cell line (HepG2, derived from 15 year-old male) N = 3 cultures/group 24 hours	PPAR receptor subtype activation, relative luciferase assay		μM DEHP					μM DEHP		
			0	50				0	50	
		hPPARα	0	100			hPPARα	0	627	
		hPPARβ	0	900			hPPARβ	0	200	
		hPPARγ	0	20			hPPARγ	0	620	
	PPAR receptor subtype activation, relative luciferase assay		μM DEHP							
			0	0.5	1	10	25	50	100	250
		hPPARα	0	-13	40 *	181 *	188 *	186 *	194 *	155 *
		hPPARβ	0	143	180	199	535 *	572 *	665 *	740 *
		hPPARγ	0	380	495	460	1456 *	1760 *	2001 *	1483 *

* statistically significant ($P < 0.05$); 1 -- Peroxisome proliferator activated receptor

Table 4B. Evidence table for the PPAR mechanism of action in rodents.

Liver PPAR Activation (PPAR and PPAR-Dependent Genes): Rodents					
Study Design and Reference	**Endpoint and Assay**	**Results (% Change from Control)**			
In Vivo Chronic and Subchronic Cancer Bioassays					
no primary literature evidence identified					
In Vivo Acute and Subchronic Studies					
Macdonald et al (2001) Mice (Sv/129, F3 generation), 3 males/group PPARα [1] -null and wild-type PPARα Gavage: 0, 1150 mg/kg/day DEHP Acute (0, 24 hours)	Catalase protein expression, as measured by mass spectrometry			mg/kg/day DEHP 0	 1150
			PPARα, wild type	0	480
			PPARα-null [3]	0	0
Feige et al (2010) Mice (C57BL/6J), wild-type and PPARα-null, 6/group Mice ((Sv/129)/(C57BL/6J) mixed) wild-type and PPARβ, 6/group 7 weeks old Diet: 0, 500 mg/kg/day DEHP Subchronic (91 consecutive days)	PPAR-target gene activation, with transcriptonal activity measured by relative luciferase activity			mg/kg/day DEHP 0	 500
		ACOX [2]	PPARα, wild type	0	100 *
			PPARα-null	-60	-59
			PPARβ	0	180 *
			PPARβ-null	-10	100
		MCAD [4]	PPARα, wild type	0	80 *
			PPARα-null	-10	-12
		FABP-1 [5]	PPARα, wild type	0	110 *
			PPARα-null	-25	-30
		FGF-21 [6]	PPARα, wild type	0	50*
			PPARα-null	-80	-84
Evelliard et al (2009) Mice (C57BL/6J), 10 males/group mPPARa-null and wild-type mPPARa 16-22 weeks old Gavage: 0, 20, 200 mg/kg/day DEHP Short-term (3 weeks)	PPAR-target gene transcriptonomic profiles, as measured by cDNA macroarray			mg/kg/day DEHP 0	 200
		Cyp4a10	PPARα, wild type	*nd*	5.4
			PPARα-null	*nd*	0
			Untreated PPARa-null/wt	-3.4	*nd*
		CYP4a12	PPARα, wild type	nd	3
			PPARα-null	nd	0
			Untreated PPARa-null/wt	1.5	*nd*
		CYP4a14	PPARα, wild type	*nd*	7.6
			PPARα-null	*nd*	0
			Untreated PPARa-null/wt	-1.7	*nd*
	PPAR-target gene mRNA, as measured by quantitative real-time PCR			mg/kg/day DEHP 0	 20	 200
		CYP4a14	PPARα, wild type	0	370 *	2800 *
			PPARα-null	0	100	-40
		Vanin1	PPARα, wild type	0	25	410 *
			PPARα-null	0	10	5

* statistically significant ($P < 0.05$); 1 -- Peroxisome proliferator activated receptor; 2 -- Acyl CoA Oxidase; 3 -- relative to control for wild type animals; 4 -- Medium-chain acyl-CoA dehydrogenase; 5 -- Intracellular fatty acid shuttling protein; 6 -- Fibroblast growth factor 21

Table 4B (continued)

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)			
In Vivo Acute and Subchronic Studies (continued)					
Ito et al (2012) Mice (Sv/129), wild-type and hPPARa, 5 males/group 12 weeks old Gavage: 0 ,977, 1953 mg/kg/day DEHP Short-term (2 weeks)	PPAR-target gene mRNA, as measured by quantitiative real-time PCR			mg/kg/day DEHP	
			0	977	1953
		PPARa mPPARα	0	-15	-45
		hPPARα	0	-27	-28
		CYP4a14 mPPARα	0	1233 *	3233 *
		hPPARα	0	-29	47
		MCAD mPPARα	0	-32	79 *
		hPPARα	0	17	42
		VLCAD [7] mPPARα	0	-33	44 *
		hPPARα	0	8	-8
		PH [8] mPPARα	0	-25	565 *
		hPPARα	0	250	325
		PT [9] mPPARα	0	44	277 *
		hPPARα	0	94	69
		Fas mPPARα	0	132	480 *
		hPPARα	0	122 *	-6
		MTP [10] mPPARα	0	-24	172
		hPPARα	0	1440 *	900 *
	PPAR-target gene protein levels, measured by scanning densitometry of immunoblot			mg/kg/day DEHP	
			0	977	1953
		PPARa mPPARα	0	36	0
		hPPARα	0	28	28 *
		MCAD mPPARα	0	43	79
		hPPARα	0	14	43 *
		VLCAD mPPARα	0	381 *	456 *
		hPPARα	0	23 *	40 *
		PH mPPARα	0	413 *	473 *
		hPPARα	0	70 *	89 *
		PT mPPARα	0	144 *	178 *
		hPPARα	0	23 *	23 *
In Vitro					
no primary literature evidence					

* statistically significant ($P < 0.05$); 7 -- Very long chain acyl-CoA dehydrogenase; 8 -- Peroxisomal bifunctional protein; 9 -- Peroxisome thiolase; 10 -- Microsomal triacylglycerol transfer protein

Table 4C. Evidence table for the PPAR mechanism of action in primates.

Liver PPAR Activation: Primates

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)
In Vivo Chronic Studies		
Ito et al (2007) Common marmosets, 3 males/group 3 months old Gavage: 0, 98, 490, 2450 mg DEHP/kg/day Chronic (105 weeks)	PPAR and PPAR-target genes, measured by quantitative real-time PCR	*mg/kg/day DEHP* 0 / 98 / 490 / 2450 PPARα: 0 / -50 / -50 / 0 MCAD [2]: 0 / -44 / -105 / -18 PT [3]: 0 / -30 / 50 / 33
	PPAR-target gene protein levels, measured by scanning densitometry of immunoblot	*mg/kg/day DEHP* 0 / 98 / 490 / 2450 MCAD: 0 / -2 / -16 / 0 PT: 0 / -17 / -17 / -33
In Vitro--transgenic cell systems with murine and human PPAR		
Hurst and Waxman (2003) COS-1 monkey kidney cells, transfected with human and murine PPARα and γ of liver origin N = 3 cultures /group 24 hours	PPAR receptor subtype activation, with transcriptional activity measured by relative luciferase activity	*μM MEHP* 0 / 0.03 / 0.1 / 0.3 / 0.5 / 0.7 / 1 / 2 / 3 / 6 / 10 / 20 mPPARα: 0 / 0 / 10 / 20 / 70 * / 70 * / 70 * / 90 * / 100 * / 130 * / 100 * / 80 * *μM MEHP* 0 / 0.5 / 1 / 2 / 3 / 4 / 10 / 15 / 20 / 30 / 50 hPPARα: 0 / -9 / 0 / 20 / 70 * / 40 * / 70 * / 195 * / 195 * / 275 * / 200 * *μM MEHP* 0 / 0.3 / 1 / 2 / 4 / 8 / 10 / 20 / 50 hPPARγ: 0 / 10 / 10 / 20 / 70 * / 100 * / 70 * / 80 * / 100 * *μM MEHP* 0 / 0.1 / 0.5 / 1 / 5 / 10 / 20 / 30 / 40 / 50 / 60 mPPARγ: 0 / -9 / 0 / 0 / 80 * / 80 * / 150 * / 155 * / 150 * / 155 * / 150 *

* statistically significant ($P < 0.05$); 1-- Peroxisome proliferator receptor subtype alpha; 2 -- Medium-chain acyl-CoA dehydrogenase; 3 -- Peroxisome thiolase

Table 4C (continued)

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)
In Vitro–transgenic cell systems with murine and human PPAR (Continued)		
Shipley and Waxman (2004) COS-1 monkey kidney cells, transfected with mPPARγ of liver origin N = 3 cultures /group 24 hours	PPAR receptor subtype activation, with transcriptonal activity measured by relative luciferase activity	*μM MEHP* 0 / 5 / 20 / 40 mPPARγ: 0 / 25 / 75 / 98
Maloney and Waxman (1999) COS-1 monkey kidney cells, transfected with human and murine PPARα and γ of liver origin N = 3 cultures /group 24 hours	PPAR receptor subtype activaiton, with transcriptonal activity measured by relative luciferase activity	*μM DEHP* 0 / 0.5 / 1 / 2 mPPARα: 0 / -12.5 / -20 / 25 hPPARα: 0 / 17 / 23 / 17 mPPARγ: 0 / 17 / 0 / 3 hPPARγ: 0 / -25 / -23 / -20
		μM DEHP 0 / 0.1 / 0.5 / 1 / 5 / 20 / 50 / 100 mPPARα: 0 / 9 / 109 / 204 / 226 / 248 / nd / nd hPPARα: 0 / -13 / 30 / 85 / 117 * / 183 * / nd / nd mPPARγ: 0 / 10 / nd / 30 / 100 / 160 / 220 / 220 hPPARγ: 0 / 0 / nd / 30 * / 80 * / 240 * / 250 * / 270 *
		μM EHA [4] 0 / 10 / 50 / 100 / 250 / 500 mPPARα: 0 / 40 * / 150 * / 160 * / 520 * / 340 * hPPARα: 0 / 0 / 160 / 200 / 100 / 240 mPPARγ: 0 / -4 / -4 / 80 / 70 / 0 hPPARγ: 0 / 10 / 0 / 10 / 30 / 30
		μM EOH [5] 0 / 100 / 250 / 500 mPPARα: 0 / -100 / 10 / 100 hPPARα: 0 / -10 / 0 / 25 mPPARγ: 0 / -10 / 0 / -5 hPPARγ: 0 / 10 / -5 / 50

4 -- 2-ethylhexanoic acid; 5 -- 2-ethylhexanol

evidence for this mechanism of action (Figure 4).

Representing a mechanism with a small literature database, the screening of literature for "gap junction intercellular communication" (GJIC) is described. In contrast to the database for peroxisome proliferation that consisted of 358 publications across all species of interest, the database for GJIC only consisted of 12 articles (Figure 2). Each reference was assessed using the automated PubMed search through Tier 1 criteria, and a breakdown of the amount of evidence for each species of interest and whether or not the reference contained original data was performed. Of the 709 liver-relevant references, 697 were dropped as not relevant to GJIC. Seven publications relevant to humans, 12 relevant to rodents, and 7 relevant to non-human primates were identified. For each species of interest, 4 reviews were dropped, with 3 human-relevant, 8 rodent-relevant, and 3 non-human primate-relevant references then subject to expert evaluation. Manual evaluation of the full texts of the remaining evidence followed, as was performed for peroxisome proliferation. Two publications were excluded from the "humans category", with one identified as a review and one not species-relevant. Three publications were excluded from the "rodents category", with one identified as a review, and two articles were not-species relevant. Only one publication identified as a review was excluded from the "non-human primates" category. At the end, 1 unique article for humans, 5 for rodents, and 2 for non-human primates were selected for inclusion into the evidence tables for GJIC (Table 5). A literature tree was also drawn illustrating the inclusion and exclusion of evidence for this mechanism of action (Figure 5).

3.4. Short narrative summaries for mechanisms of action. Succinct summaries reporting critical conclusions from the gathered evidence, and capturing details relevant to study design, observed effects, dose-response relationships, and significance of results were developed. Consistent with US EPA guidance (EPA 2005), the strongest mechanistic evidence is provided by studies that experimentally challenge the role of the

Figure 4. Literature tree for the "peroxisome proliferator activated receptor" mechanism of action. Progression from the initial 3361 identified articles relevant to DEHP or its metabolites to those included in the evidence tables is shown.

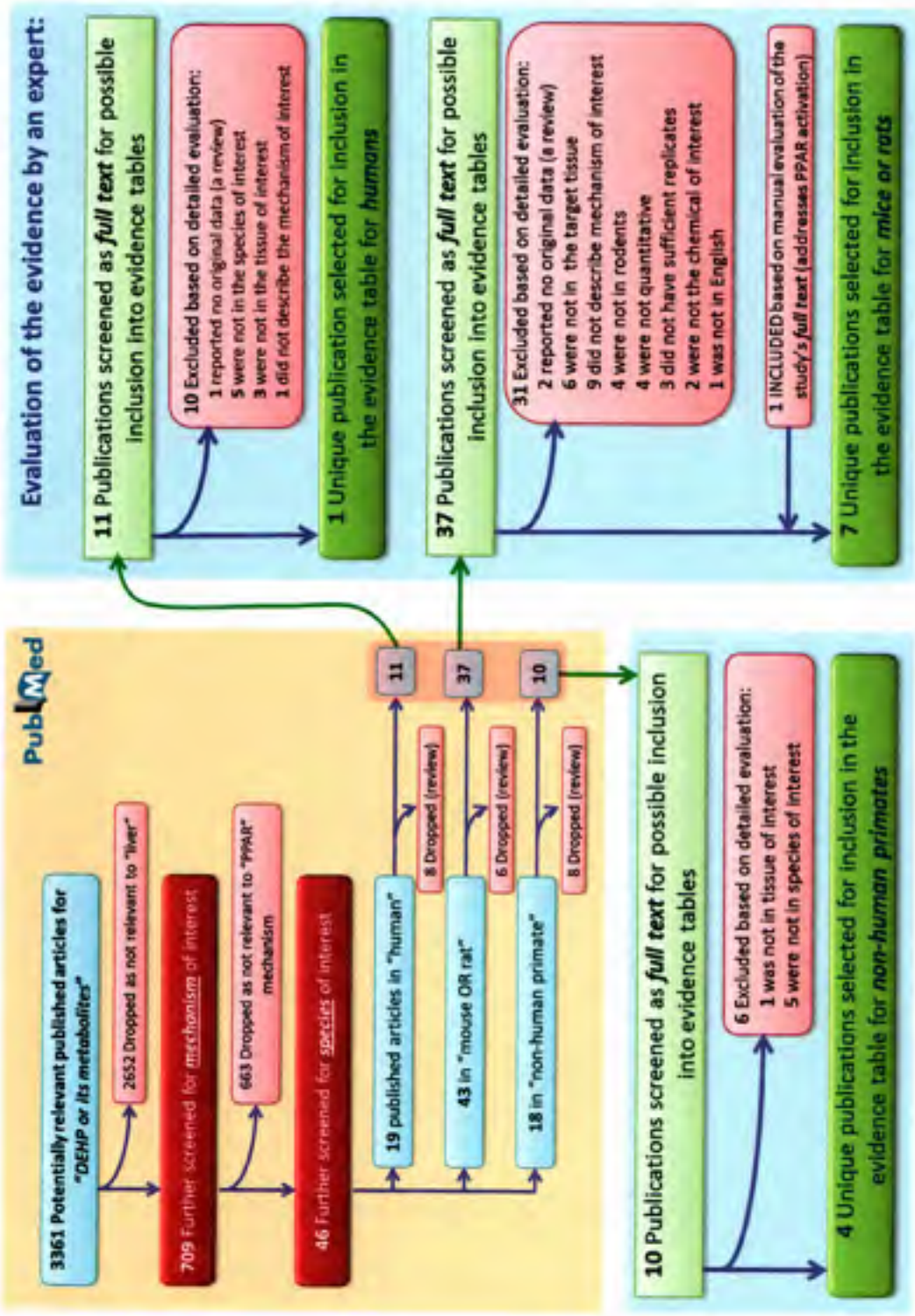


Table 5A. Example of an evidence table for the "gap junction intercellular communication" (GJIC) mechanism of action. Results for humans (A), rodents (B), and primates (C) are presented. Results are stratified by *in vivo* or *in vitro* experimental systems. Endpoints measured and quantitative dose-response information is also presented.

Gap Junction Intra/Intercellular Communication (GJIC): Humans								
Study Design and Reference	**Endpoint and Assay**	**Results (% Change from Control)**						
In Vivo								
no primary literature evidence								
In Vitro								
Kamendulis et al (2002) Human 1° hepatocytes from three males HLEC-04 cells: Human 1° hepatocytes, one male, aged 35 years: SV-40 transformed N = 4 cultures/group 4 or 24 hours	GJIC disruption, in situ dye transfer (ISDT)			*µM MEHP*				
			0	50	100	200	300	500
		1° Hepatocytes, 4 hours	0	-1	-3	*nd*	-2	*nd*
		24 hours	0	-8	-6	*nd*	-7	*nd*
		HLEC-04 cells, 4 hours	0	-8	-6	*nd*	-7	*nd*
		24 hours	0	-7	-6	*nd*	-7	-2

* statistically significant ($P < 0.05$); nd - not determined

Table 5B. Evidence table for GJIC in rodents.

Gap Junction Intra/Intercellular Communication: Rodents		
Study Design and Reference	**Endpoint and Assay**	**Results (% Change from Control)**
In Vivo--Chronic Lifetime Bioassays		
Isenberg et al (2001) Rat (F344, male) N = 5 /group Chronic (18 months) or short-term with recovery (1 or 2 week treatment; 2 or 4 weeks off)	GJIC disruption, in situ dye transfer (ISDT)	mg/kg/day DEHP [1] 0 — 900 Chronic: 0 — -28 mg/kg/day DEHP 0 — 20000 — 2 weeks off — 4 weeks off Short-term: 0 — -40 * — -15 — 5
Isenberg et al (2001) Mouse (B6C3F1, male) N = 5 /group Chronic (18 months)	GJIC disruption, in situ dye transfer (ISDT)	mg/kg/day DEHP 0 — 6000 0 — -30
In Vitro--Primary Hepatocytes		
Kamendulis et al (2002) Rat (F344, male) 1° hepatocytes N = 4 cultures/group 4 or 24 hours	GJIC disruption, in situ dye transfer (ISDT)	µM MEHP 0 — 50 — 100 — 200 — 300 — 500 4 hours: 0 — -7 — -20 * — *nd* — -33 * — *nd* 24 hours: 0 — -70 * — -80 * — *nd* — -99 * — *nd*
Kamendulis et al (2002) Mouse (B6C3F1, female) 1° hepatocytes N = 4 cultures/group 4 or 24 hours	GJIC disruption, in situ dye transfer (ISDT)	µM MEHP 0 — 50 — 100 — 200 — 300 — 500 4 hours: 0 — -12 * — -15 * — *nd* — -26 * — *nd* 24 hours: 0 — -19 * — -24 * — *nd* — -65 * — *nd*
Isenberg et al (2000) Rat (F344, male) 1° hepatocytes N = 5 cultures/group Short-term (1,2,4, or 6 weeks)	GJIC disruption, in situ dye transfer (ISDT)	µM DEHP 0 — 2.5 — 15 — 30 — 51 One week: 0 — *nd* — -38 * — *nd* — -43 * Two weeks: 0 — -5 * — -41 * — -45 * — -45 * Four weeks: 0 — *nd* — -27 * — *nd* — -36 * Six weeks: 0 — *nd* — -27 * — *nd* — -32 *

* - statistically significant; nd - not determined; 1 - converted to mg/kg/day from ppm (1/mg/kg = 7 ppm);
2 - Protein kinase C

Table 5B (continued)

Gap Junction Intra/Intercellular Communication: Rodents

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)				
In Vitro–Primary Hepatocytes (Continued)						
Isenberg et al (2000) Mouse (B6C3F1, male) 1° hepatocyte N = 5 cultures/group Short-term (2 or 4 weeks)	GJIC disruption, in situ dye transfer (ISDT)			*µM DEHP*		
			0	1.3	15	
		Two weeks	0	-20 *	-45 *	
		Four weeks	0	-5 *	-42 *	
Klaunig et al (1988) Mouse (B6C3F1, male) 1° hepatocyte N = 3 cultures/group 8 hours	GJIC disruption, [5-^{3}H] uridine nucleotide transfer			*µM MEHP*		
		0	5	10	50	100
		0	-14 *	-23	-23	*nd*
Leibold et al (1994) Rat (Wistar, male) 1° hepatocytes N = 3 cultures/group 5-8 hours	GJIC disruption, in situ dye transfer (ISDT)				*µM DEHP*	
				0	1.3	15
		With PKC [2] inhibitor		0	-43 *	-90 *
		Without PKC Inhibitor		0	-36 *	-89 *

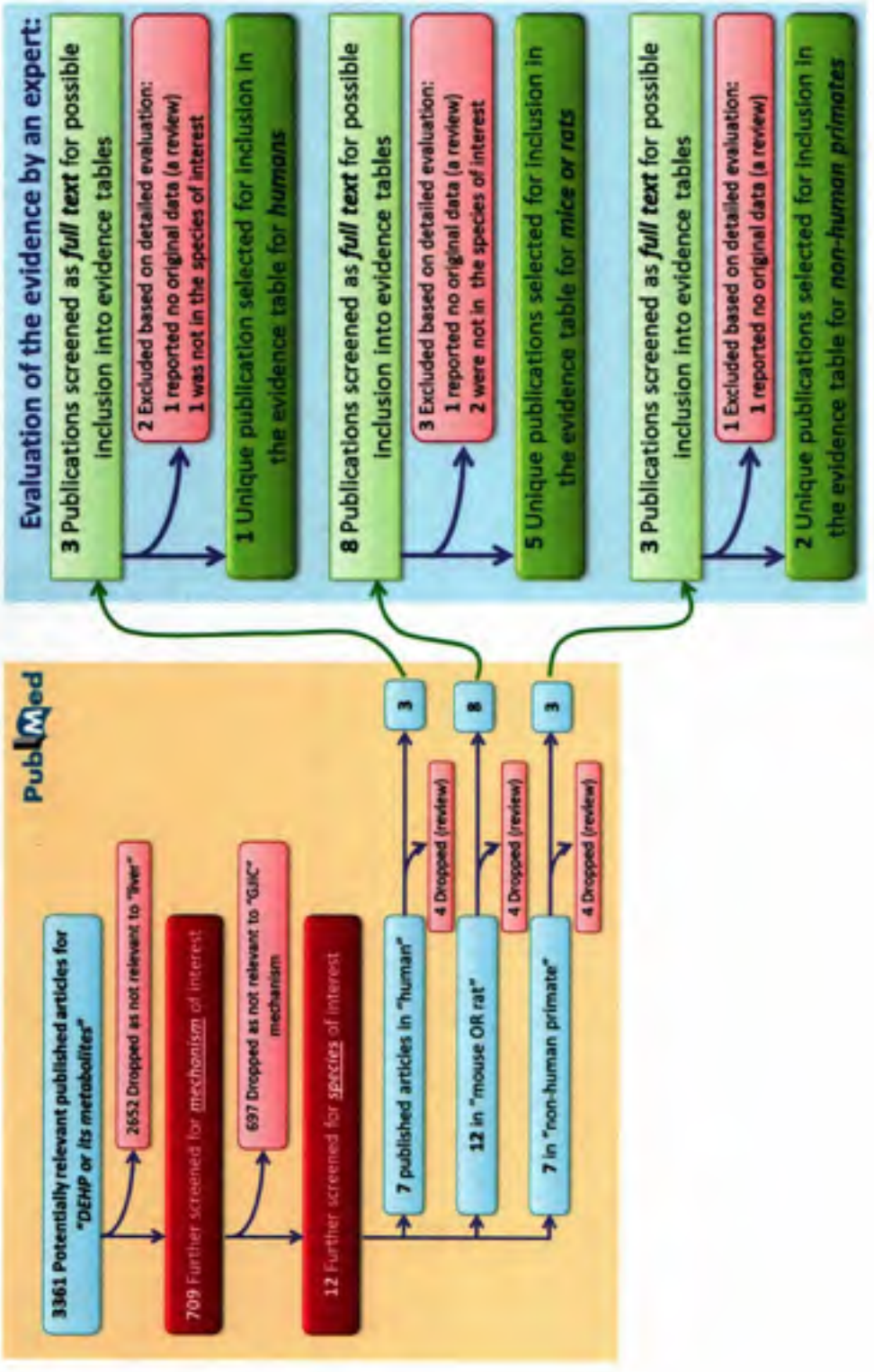
* - statistically significant; nd - not determined; 2 - Protein kinase C

Table 5C. Evidence table for GJIC in non-human primates.

Gap Junction Intra/Intercellular Communication: Primates								
Study Design and Reference	**Endpoint and Assay**	**Results (% Change from Control)**						
In Vivo								
Pugh et al (2000) Cynomolgus monkeys, 4 males/group Short-term (2 weeks) Young adults, ~ 2 yrs of age	GJIC disruption, in situ dye transfer (ISDT)	*mg/kg/day DEHP* 0 0	 500 8					
In Vitro								
Kamendulis et al (2002) Cynomolgus monkey 1° hepatocytes, from five males N = 4 cultures/group 4 or 24 hours	GJIC disruption, in situ dye transfer (ISDT)		*µM MEHP*					
			0	50	100	200	300	500
		4 hours	0	-1	*0*	*nd*	-11	*nd*
		24 hours	0	-3	-2	*nd*	-5	*nd*

nd - not determined

Figure 5. Literature tree for the "gap junction intercellular communication" mechanism of action. Progression from the initial 3361 identified articles relevant to DEHP and its metabolites to those included in the evidence tables is shown.



mechanism in carcinogenesis or other adverse outcomes, in particular by evaluating the impact of altering, suppressing or enhancing the mechanism (*i.e.*, in genetically-modified or other experimental models, or in recovery experiments). The summary of the peroxisome proliferation mechanism addresses this type of evidence. For experimental studies, the species, strain, sex, time and dose/exposure at which mechanistic effects were reported is summarized. An overall conclusion concerning the likelihood that the mechanism is operative in humans is provided. Support for this conclusion is meaningfully strengthened by consistent results in different experimental models, but not by replicate experiments in the same model. The below summaries for peroxisome proliferation and gap junction intercellular communication illustrate aspects of the mechanism evidence synthesis that may inform potential mechanism of action conclusions for DEHP in liver carcinogenesis.

3.4.1. Summary for the peroxisome proliferation mechanism of action of DEHP. Insufficient evidence exists to identify whether DEHP significantly contributes to peroxisome proliferation in humans and non-human primates. In isolated primary human hepatocytes or HepG2 hepatoma cells, no significant effect upon markers of peroxisome proliferation was observed in two studies with DEHP or MEHP, or in one study with MEOHP. Treatment periods in these studies did not exceed 72 hours. In non-human primate livers *in vivo*, one study with DEHP showed a significant increase in peroxisomal volume in livers from male marmosets treated for 91 days at doses of 500 and 2500 mg DEHP/kg/day, and another study reported a significant increase in catalase activity at a dose of 2000 mg DEHP/kg/day for two weeks, also in marmoset liver. No significant effects were observed *in vitro* in primary hepatocytes isolated from male cynomolgous monkeys treated with up to 0.6 mM MEHP, or in male marmosets treated with up to 1 mM MEOHP for 72 hours.

Evidence for dose-dependent DEHP-induced peroxisome proliferation in rodents,

as indicated by changes in peroxisomal volume and enzyme activities, suggests that this mechanism contributes to hepatocarcinogenesis and hepatotoxicity. Two lifetime cancer bioassays showed significant increases in palmitoyl-CoA (PCoA) oxidation activity at the highest dose administered at each time endpoint examined in male and female F344 rats. Catalase and carnitine acetyltransferase activities were also significantly elevated in one of those studies. In female mice, palmitoyl-CoA activity was significantly increased at the end of a chronic lifetime bioassay. Also of importance is the increase in peroxisomal volume noted in one in vivo acute study after treatment with DEHP, and the abolishment of the effect in PPARα-null animals. Significant, dose-dependent increases in PBOX, and PCoA, and carnitine acetyltransferase activity were also observed in other acute and subchronic rodent bioassays in response to DEHP, MEHP, or 2-EH across several different species of rats and mice.

In vitro, significant increases in acyl-CoA and carnitine acetyltransferase activities in primary hepatocytes isolated from different mouse strains were observed as a result of treatment with DEHP. Significant increases in peroxisomal beta oxidation, palmitoyl-CoA, and carnitine acetyltransferase activities were also significantly increased in primary hepatocytes treated with MEHP or 2-ethylhexanol. No cultures were treated for longer than 72 hours, and significant effects showed a dose-response relationship for MEHP and 2-ethylhexanol, but not DEHP. Although changes in endpoints representing peroxisome proliferation *in vivo* and *in vitro* in rodents were consistent and dose-related across sex, species, and rodent strains, the evidence database may not inform how these changes relate to chronic human exposures given results between rodents and humans were not concordant.

3.4.2. Summary for the peroxisome proliferator-activated receptor mechanism of action of DEHP. Limited information suggests that PPAR receptor activation may be a mechanism for effects of DEHP exposure on liver carcinogenesis in humans. Dose-

related increases in PPARα, β, and γ activation were observed following acute treatment with MEHP, the primary metabolite of DEHP, in a single *in vitro* study of immortalized human hepatoma cells. This same study also observed increased PPAR activity (up to 900% over controls) following DEHP treatment, but these effects did not reach statistical significance. The relevance of these observations in immortalized cancer cells to cancer initiation is unclear. Conversely, one chronic *in vivo* study in male marmosets did not observe significant changes in PPARα or PPARα-dependent genes at dose levels from ~100-2500 mg/kg-day; however, due to potential differences in phthalate metabolism between marmosets and humans, the use of this species in the study of DEHP has been questioned (Caldwell 2012).

Clear PPAR-related changes following DEHP exposure have been observed across multiple studies of varied duration in male mice, as well as in a single study of pregnant female mice and in a single study of male rats. Overall, in PPARα-wild type and PPARα-null mice, evidence of significantly increased activation was observed at doses from 119 to 500 mg/kg/day DEHP in wild-type mice, but these effects were greatly reduced or abolished in PPARα-null mice across all endpoints in all studies. *In vitro* evidence also provides evidentiary support that activation of PPAR isoforms by DEHP is likely to occur in humans. In three acute studies by the same research laboratory, MEHP or 2-ethylhexanoic acid treatment resulted in dose-related transactivation of both mouse and human PPAR constructs in a monkey kidney cell line, with murine PPAR exhibiting greater sensitivity. Importantly, these alterations were not observed following DEHP treatment, suggesting that activation of PPAR requires metabolism of DEHP. Given the observation of PPAR-related changes both in rodents and humans, the evidence suggests that PPAR receptor activation may contribute to liver carcinogenesis following DEHP exposure.

3.4.3. Summary for the gap junction intercellular communication (GJIC) mechanism of action of DEHP. Insufficient evidence exists to identify whether GJIC disruption, as measured by *in situ* dye transfer, resulting from DEHP exposure is likely to contribute to hepatocarcinogenesis in humans. No significant *in vitro* effects on GJIC were observed in a single study of human hepatocytes, pooled from three human male livers or in a cell line established from one human male liver. In a single study of non-human primate hepatocytes treated with up to 300 μM MEHP for up to 24 hours pooled from livers of five male cynomolgous monkeys, no significant in vitro effects on GJIC were observed. Similarly, a single dose *in vivo* study in male primates did not observe effects on GJIC following short-term treatment with 500 mg/kg-day DEHP. No human or non-human primate studies evaluated females or exposures longer than two weeks.

Limited information in rodents suggests that GJIC disruption, as measured by ISDT, may be a mechanism for the effects of DEHP on liver carcinogenesis. Decreases in dye transfer of approximately 30% were observed in a single study of male F344 rats and B6C3F$_1$ mice treated with 6000 mg/kg-day DEHP for 18 months. *In vitro*, dose-dependent significant decreases were observed across four studies examining primary hepatocytes derived from male and female B6C3F$_1$ mice, as well as male Wistar and F344 rats, following treatments with DEHP or MEHP from 1.3- 300 μM for up to six weeks. Although decreases in GJIC in rodents were consistent and dose-related across sex, species, and strain, the evidence database was inadequate to inform how these changes translate to chronic human exposures.

Chapter 4

Discussion

The goal of this study was to develop and implement a systematic approach for literature review and identification that would improve transparency of evidence collection and standardization of reporting for human health assessment of chemical hazards. The stepwise approach detailed herein addresses all critical points of guidance presented in recent NRC reports (NRC 2010, 2011). Major benefits include reducing a large literature database into a smaller, more manageable one, allowing for better analysis of the strength of evidence provided by concordance of results across studies and species, strain, sex, dose and time of exposure. The strategy has also proven effective for critical appraisal and synthesis of large amounts of information, and is conducive towards visual data displays that help track capture of results and thus facilitate decision-making processes. The workflow in this process is also iterative such that the results from each previous step are carried forward and built upon in the subsequent steps.

Key Features. A clear, quality-controlled process ensuring standardization of evidence review is necessary for comprehensive hazard evaluation. Key features of our stepwise strategy directly address elements that should be common to modern evidence review that makes use of sophisticated information technology tools, and common to evaluation proposed previously by various stakeholders (refs). Where have significantly improved the evidence evaluation process, especially applicable to human health risk assessments such as conducted by the US EPA IRIS program, is in much more rigorous documentation of each step of our strategy and in the visualization and presentation of

study evaluation.

First, we provide transparent documentation of search terms used to identify literature describing mechanisms of action of DEHP and its metabolites. Incorporating synonyms and wildcards representing critical terms in the PubMed search is necessary in order to capture the most comprehensive results possible. When performing searches for lines of evidence of a particular chemical's mechanism of action in tissues and species of interest, we recognize that some publications may be selected erroneously even though they may not contain experimental data specific to the subject of a search. The search strings do not discern the location of the desired information in the article; therefore, expert evaluation was necessary to determine that original data was captured relevant to DEHP-induced liver toxicity. We posit that while the automation of searches in PubMed is useful to narrow down the vast literature that may be available, subsequent steps must be subject to manual curation by expert-driven evaluation of the evidence. The pre-defined inclusion/exclusion criteria described in Table 2 are then applied by the expert, and articles that do not meet such criteria are excluded from further consideration.

Second, establishment of tiered assessment criteria for inclusion and exclusion of literature allows for transparent and consistent critical evaluation of evidence. These criteria focus on study design and experimental outcomes. By focusing on the experimental information provided in the literature, application of inclusion and exclusion criteria minimizes bias based upon the overall conclusions of the reviewed studies. The tiered approach also effectively reduces large literature databases to manageable ones that include the strongest experimental results for inclusion in evidence tables. Our criteria were largely based on what is used for the evaluation of the epidemiological evidence (NRC 2011), such as characterizing a study population, approaching exposure assessment, identifying outcome and analytical methods, and coding metrics to model

exposure responses. As applied to evaluating mechanistic evidence for DEHP, our criteria included consideration of target tissues, species and sex of animals, whether the study was conducted *in vitro* or *in vivo*, dosing information, and characteristic endpoints of the relevant mechanisms.

Third, visualizations of the evidence inclusion and exclusion in the form of a literature tree comprehensively documents study selection. The progression from the initial 3361 articles identified as relevant to DEHP to those included in the mechanism-based evidence tables is transparent, displaying how search findings are narrowed to articles in the final review selection based upon the pre-defined criteria.

These key features of evidence capture all lead to summarizing findings in evidence tables that eliminate the need for long descriptions of studies for each mechanism. We constructed tables in a manner that provides information about the publication itself, as well as necessary experimental details and methods, endpoints measured, and dose-response relationships, if any. Statistical significance of the findings, if such was observed, was also noted in the tables. Each table was quality controlled with at least two reviewers verifying the information that was included. With the comprehensive, yet succinct, reporting of evidence in tables, short narrative summaries may be easily composed for each mechanism highlighting significant results and dose-response relationships in species of interest. A one to two sentence summary is also included that states the overall relevance of the evidence to DEHP-induced liver effects.

This stepwise process is also potentially applicable to quantitative aspects of chemical hazard assessment, including ranking of evidence and rationale employed in dose-response analysis or in the conduct of cross-chemical or cross-species meta-analyses. We clearly illustrate comparability of study results based upon experimental characteristics rather than outcomes, which is a component of the Navigation Guide

Project supporting evidence-based decision-making in environmental health (Woodruff and Sutton 2011). The NRC (NRC 2011) also suggested that IRIS assessments address which studies are included for derivation of reference values for noncancer endpoints and unit risks for cancer endpoints, and systematic identification of approaches for these endpoints may assure that experimental conditions, study quality, and quantitative outcomes are all in accordance for the referenced body of evidence.

Hazard assessment vs. clinical assessment. The methodology described herein was conceived in the spirit of systematic review as utilized in evidence-based medicine to assess issues with bearing on healthcare questions. Internationally, the Cochrane Collaboration engages clinicians and researchers to perform reviews, and the Agency for Healthcare Research and Quality supports 14 evidence-based practice centers in the United States to conduct reviews related to medical care. The comprehensive reviews produced by these agencies and other stakeholders provide a foundation for clinical decision-making and selection of health policy options, as well as serve as tools for clinicians in the choice of treatment strategies. An additional approach known as GRADE—the Grading of Recommendations, Assessment, Development, and Evaluation—rates the quality and strength of whether or not to administer a particular medical intervention based on trade-offs between benefits, costs, and risks (Montori and Guyatt 2008). GRADE thus provides a qualitative approach to discerning weak versus strong treatment recommendations. Likewise, the tiered assessment criteria defined in this study allow for selection of studies, particularly from large databases, with the most thorough documentation of materials, methods, and statistical significance. In particular, our approach qualitatively discriminates between weaker and stronger experimental design, ensuring that the stronger studies are selected and presented as evidence for DEHP toxicity.

However, clinical evidence and decision contexts differ from those in

environmental health. As discussed in Woodruff and Sutton (2011), clinicians cannot assume similar *in vivo* and/or *in vitro* testing of chemicals of environmental concern as with pharmaceuticals prior to entry into the marketplace and in turn, widespread human exposures. The most rigorous standard for informing clinical risk-benefit decisions is a randomized controlled trial, an analogy for which that measures health risk-benefit is not currently in the environmental hazard assessment paradigm. Benefits of environmental chemicals are not typically measured in the context of human health, and significance of exposures and resulting toxicities will vary depending upon the physical properties of the agent.

To comprehensively assess health effects and clinical intervention strategies for environmental chemical exposures, it is therefore necessary to reconcile these methodological differences between risk assessment and medical science. A process referred to as "Epidemiology-Toxicology" (Adami et al. 2011) presents both epidemiological and toxicological evidence in a causal relationship grid that permits a clear view of the intersection of the data between the two fields. Such an approach can synergize important decisions about human safety and treatment options should a medical treatment be needed after a chemical exposure event. Other frameworks should also clearly illustrate comparability of study results based upon experimental characteristics rather than outcomes, which is a component of both the review process described in this paper and the Navigation Guide Project supporting evidence-based decision-making in environmental health (Woodruff and Sutton 2011). In addition to evidence on toxicity of the agent of interest and less toxic alternatives, the Navigation Guide integrates patient preferences and values in treatment options.

Limitations. There are, however, limitations to the proposed framework. To ensure the transparency and reproducibility of search strategies, we utilized a publicly available database (i.e., PubMed) for identification of the evidence. While

comprehensive and large, this database may miss some studies and thus this approach may need to be extended to other online databases (e.g., Medline, ToxNet, Web of Science). The process of capturing mechanistic information from "grey" literature (e.g., study reports) or restricted-access evidence materials (e.g., proprietary information submitted for regulatory review by various commercial entities) also needs to be developed.

It is important to note that automated searches may overlook useful references or not discern between various types of publication (e.g., review vs. primary article), thus necessitating manual review of all retrieved articles. For example, PubMed does not identify all publications that could be considered reviews, and it is up to an expert assessor of evidence to determine if the article does or does not meet the criteria of containing original data. It is also conceivable an article may contain DEHP-relevant experimental results that are not explicitly described in the study's methods. This highlights the necessity for manual review of an article's full text and figures, with application of inclusion and exclusion criteria to determine the relevance of results. The process should also undergo quality control by at least one additional expert reviewer in order to ensure correct capture and interpretation of data. This measure should ensure that conclusions about the existing evidence across different reviewers are concordant. Another challenge facing this framework is how to engage stakeholders in its adoption for hazard assessments. It should be stressed that this effort is a focused undertaking that optimizes efficiency of literature searching and database management, and can be accomplished by one reviewer in a reasonable amount of time. The standardization of search terms and assessment criteria can also assure stakeholders that all lines of evidence are analyzed using a hierarchic structure.

Perspectives for the future. The multi-step evaluation of the literature database for DEHP exemplifies improved transparency and efficiency for this aspect of hazard

identification and assessment. As the evidence base for many environmentally-relevant chemicals grows rapidly, the review process described here may be amended to incorporate assessment of new experimental strategies and tools, especially as focus shifts to pathway-based approaches for determination of toxicity in humans. The proposed evaluation process described here and those of others could also be translated electronically into a system that integrates the evidence database, inclusion and exclusion criteria, and report outputs that are suitable for inclusion in human health risk assessment. Such an interface would greatly accelerate the time to completion of hazard and dose-response assessments, and may also allow for data sharing between those charged with evaluation of a chemical's adverse effects.

In summary, by employing automated retrieval of literature in PubMed, expert evaluation of articles, and methods of evidence reporting that can be conducted and displayed in a consistent and transparent manner, we have developed a framework that will strengthen the validity of scientific evidence considered in chemical hazard assessments. Transparency of searches and selection criteria for literature, and presentation of evidence will allow for more ease in drawing conclusions about the relevance of a chemical's mechanisms of action that are relevant to toxicity. The resulting deliverables can also be included in various portions of an assessment's a toxicological review, with evidence tables and short narrative summaries in the primary body of the document, and search strategies, literature trees, and assessment criteria included in an appendix. Online resources such as the Health and Environmental Research Online (HERO) database can also be utilized to capture study information, with all of the literature considered in the assessment classified in accordance with inclusion and exclusion criteria. Our approach also allows for expert evaluation of results at every stage, a critical component to conduct and interpretation of the relevance of results to the assessment process. Ultimately, the use of systematic

methods for mechanism of action analysis in health risk assessments is a step toward a unifying framework integrating understanding biological responses at particular exposures. These efforts are expected to make risk assessments more useful for decision-making relevant to the protection of human health.

Chapter 5

Practium Report

For my practicum, I was awarded ORISE fellowship to serve in the US Envrionmental Protection Agency's National Center for Environmental Assessment (NCEA). My project piloted systematic approaches to evaluation and presentation of mechanistic and toxicological evidence in human health risk assessments using DEHP as a representative chemical hazard to humans. This approach was conceived in direct response to advice from the National Research Council in the review of the draft IRIS assessment of formaldehyde, in which improvements per transparency and consistency of evidence selection were suggested for hazard and dose-response assessment in the IRIS assessment process. IRIS assessments are used by the EPA in decisions protecting the public against exposure to chemical hazards. With literature review a critical component of IRIS assessments, it follows that systematic guidelines offered by the evidence-based medicine initiative seem applicable for examination of toxicological evidence for a chemical. However, no such standardized framework is in place for evaluation of toxicity, and thus exists the urgent need to apply systematic review as the foundation of determination of mechanisms of action of chemicals. It is expected that the resulting review will aid selection of environmental health policy options and further strengthen public health decision-making processes with regard to federal regulatory programs, guidelines, and authorities that control environmental health issues, current environmental risk assessment methods, and mechanisms of toxicity in response to an environmental exposure. During the summer and fall of 2012, I worked with the NCEA team current assessing DEHP and the EPA's Health and Environmental Research

Online (HERO) database librarians to develop a systematic evaluation process for review of the mechanistic evidence for DEHP liver carcinogenicity. This process may become an integral part of the current DEHP assessment and will clearly document literature search and analysis methodology supporting mechanism of action conclusions. Specific deliverables for integration into the Toxicological Review for DEHP were identified, along with supplementary information that may become part of an appendix to the IRIS assessment.

The initial collaboratons with NCEA-Washington began in January 2012, with initial project investigations, including the desired structure of the literature review framework and overall search strategy, devised by the end of April 2012. Throughout June 2012-August 2012 while I served my fellowship onsite at NCEA's offices in Arlington, VA, consensus mechanisms of action for DEHP were identified and literature databases gathered for each. In consultation with NCEA's DEHP assessment team, explicit inclusion and exclusion criteria for relevant studies were defined and applied to each database in order to determine the studies that would be included in evidence tables that are part of the IRIS assessment. By the end of August 2012, draft evidence tables for the consensus mechanisms of action were composed. A presentation to the entire IRIS staff describing this systematic approach to mechanism-of-action analysis was given on August 29, 2012. Ongoing efforts expected to last through December 2012 include finalizing the evidence tables and organizing and tagging literature in the EPA's HERO database.

The approaches developed in this practicum project demonstrate clear documentation of search strategies and analysis methodologies for application in chemical hazard assessments, as well as effective management and synthesis of information critical in regulatory decision-making processes applicable to DEHP. Most importantly, these approaches improve transparency and enhance standardization of

evidence gathering, selection, presentation, and evaluation. As an iterative and flexible method, the process could also be applied to ongoing and future health assessments conducted by the US EPA, enhancing the quality of the literature review process and scientific evidence selection in analysis of chemical hazards of critical importance to public health.

Core competencies as defined by the ASPH addressed by this project include application of evidence-based concepts in public health decision-making, and development of an approach stressing transparency of information collection and presentation with input from colleagues from UNC and the USEPA, and other stakeholders. It also demonstrates how information technology may be used to effectively search for and retrieve critical public health data relevant to environmental chemical exposures. In addition to the ASPH core competencies, the project has directly addressed three department-specific competencies, including understanding federal regulatory programs, guidelines and authorities that control environmental health issues, current environmental risk assessment methods, and mechanisms of toxicity in response to an environmental exposure.

References

Adami HO, Berry SC, Breckenridge CB, Smith LL, Swenberg JA, Trichopoulos D, et al. 2011. Toxicology and epidemiology: improving the science with a framework for combining toxicological and epidemiological evidence to establish causal inference. Toxicological sciences : an official journal of the Society of Toxicology 122(2): 223-234.

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, et al. 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical reviews in toxicology 36(10): 781-792.

Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, et al. 2008. IPCS framework for analyzing the relevance of a noncancer mode of action for humans. Critical reviews in toxicology 38(2): 87-96.

Caldwell JC. 2012. DEHP: genotoxicity and potential carcinogenic mechanisms-a review. Mutation Research 751(2): 82-157.

Colacino JA, Harris TR, Schecter A. 2010. Dietary intake is associated with phthalate body burden in a nationally representative sample. Environmental health perspectives 118(7): 998-1003.

Cooper, Harris and others. The Handbook of Research Synthesis and Meta-Analysis. New York: The Russell Sage Foundation, 2009.

Cummings BS, Lash LH. 2000. Metabolism and toxicity of trichloroethylene and S-(1,2-dichlorovinyl)-L-cysteine in freshly isolated human proximal tubular cells. Toxicological sciences : an official journal of the Society of Toxicology 53(2): 458-466.

DHHS (U.S. Department of Health and Human Services). 2004. The Health Consequences of Smoking: A report of the Surgeon General [online]. Available: http://www.cdc.gov/tobacco/data_statistics/sgr/2004/complete_report/index.htm [accessed Oct. 22, 2012].

Drummond MB, Dasenbrook EC, Pitz MW, Murphy DJ, Fan E. 2008. Inhaled corticosteroids in patients with stable chronic obstructive pulmonary disease: a systematic review and meta-analysis. JAMA 300(20): 2407-2416.

EPA (U.S. Environmental Protection Agency). 2003. A Summary of General Assessment Factors for Evaluating the Quality of Scientific and Technical Information [online]. Available: http://www.epa.gov/spc/pdfs/assess2.pdf [accessed Oct. 22, 2012].

EPA (U.S. Environmental Protection Agency). 2005. Guidelines for carcinogen risk assessment [online]. Available: http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF [accessed Oct. 22, 2012].

IOM (Institute of Medicine). 2008. Improving the presumptive disability decision-making process for veterans. Washington, DC: National Academy Press.

Gentry PR, Clewell HJ, 3rd, Clewell R, Campbell J, Van Landingham C, Shipp AM. 2011. Challenges in the application of quantitative approaches in risk assessment: a case study with di-(2-ethylhexyl)phthalate. Critical reviews in toxicology 41 Suppl 2: 1-72.

Guyton KZ, Barone S, Jr., Brown RC, Euling SY, Jinot J, Makris S. 2008. Mode of action frameworks: a critical analysis. Journal of toxicology and environmental health Part B, Critical reviews 11(1): 16-31.

Guyton KZ, Chiu WA, Bateson TF, Jinot J, Scott CS, Brown RC, et al. 2009. A reexamination of the PPAR-alpha activation mode of action as a basis for assessing human cancer risks of environmental contaminants. Environmental Health Perspectives 117(11): 1664-1672.

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, et al. 2003. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Critical Reviews in Toxicology 33(6): 655-780.

Lash LH, Fisher JW, Lipscomb JC, Parker JC. 2000. Metabolism of trichloroethylene. Environmental health perspectives 108 Suppl 2: 177-200.

Light, Richard and David Pillemer. Summing Up: The Science of Reviewing Research. Cambridge, MA: Harvard University Press, 1984.

Lorber M, Angerer J, Koch HM. 2010. A simple pharmacokinetic model to characterize exposure of Americans to di-2-ethylhexyl phthalate. Journal of Exposure Science & Environmental Epidemiology 20(1): 38-53.

Lorber M, Koch HM, Angerer J. 2011. A critical evaluation of the creatinine correction approach: can it underestimate intakes of phthalates? A case study with di-2-ethylhexyl phthalate. Journal of Exposure Science & Environmental Epidemiology 21(6): 576-586.

Maruyama K, Ikeda H, Koizumi T, Tsuchida Y. 1999. Prenatal and postnatal histories of very low birthweight infants who developed hepatoblastoma. Pediatrics international : official journal of the Japan Pediatric Society 41(1): 82-89.

Melnick RL, Morrissey RE, Tomaszewski KE. 1987. Studies by the National Toxicology Program on di(2-ethylhexyl)phthalate. Toxicology and industrial health 3(2): 99-118.

Montori VM, Guyatt GH. 2008. Progress in evidence-based medicine. JAMA : the journal of the American Medical Association 300(15): 1814-1816.

National Research Council (US). Board on Environmental Studies and Toxicology. Review of the Environmental Protection Agency's Draft IRIS Assessment of Tetrachloroethylene. Washington: National Academy Press, 2010.

National Research Council (US). Committee to Review EPA's Draft IRIS Assessment of Formaldehyde. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Washington: National Academy Press, 2011.

National Research Council (US). Commission on Life Sciences. Monitoring Human Tissues for Toxic Substances. Washington: National Academy Press, 1991.

Oue T, Kubota A, Okuyama H, Kawahara H, Nara K, Kawa K, et al. 2003. Hepatoblastoma in children of extremely low birth weight: a report from a single perinatal center. Journal of pediatric surgery 38(1): 134-137; discussion 134-137.

Peters JM, Cheung C, Gonzalez FJ. 2005. Peroxisome proliferator-activated receptor-alpha and liver cancer: where do we stand? J Mol Med (Berl) 83(10): 774-785.

Ramdhan DH, Kamijima M, Wang D, Ito Y, Naito H, Yanagiba Y, et al. 2010. Differential response to trichloroethylene-induced hepatosteatosis in wild-type and PPARalpha-humanized mice. Environmental health perspectives 118(11): 1557-1563.

Reynolds P, Urayama KY, Von Behren J, Feusner J. 2004. Birth characteristics and hepatoblastoma risk in young children. Cancer 100(5): 1070-1076.

Roberts RA, Ganey PE, Ju C, Kamendulis LM, Rusyn I, Klaunig JE. 2007. Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. Toxicological Sciences 96(1): 2-15.

Rusyn I, Corton JC. 2012. Mechanistic considerations for human relevance of cancer hazard of di(2-ethylhexyl) phthalate. Mutation Research 750(2): 141-158.

Rusyn I, Peters JM, Cunningham ML. 2006. Modes of action and species-specific effects of di-(2-ethylhexyl)phthalate in the liver. Critical reviews in Toxicology 36(5): 459-479.

Sackett DL, Rosenberg WM. 1995. On the need for evidence-based medicine. Journal of public health medicine 17(3): 330-334.

Smith V, Devane D, Begley CM, Clarke M. 2011. Methodology in conducting a systematic review of systematic reviews of healthcare interventions. BMC medical research methodology 11(1): 15.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, et al. 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regulatory toxicology and pharmacology : RTP 34(2): 146-152.

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. 2000. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA : the journal of the American Medical Association 283(15): 2008-2012.

Svensson K, Hernandez-Ramirez RU, Burguete-Garcia A, Cebrian ME, Calafat AM, Needham LL, et al. 2011. Phthalate exposure associated with self-reported diabetes among Mexican women. Environmental Research 111(6): 792-796.

Teitelbaum SL, Mervish N, Moshier EL, Vangeepuram N, Galvez MP, Calafat AM, et al. 2012. Associations between phthalate metabolite urinary concentrations and body size measures in New York City children. Environmental Research 112: 186-193.

Voss C, Zerban H, Bannasch P, Berger MR. 2005. Lifelong exposure to di-(2-ethylhexyl)-phthalate induces tumors in liver and testes of Sprague-Dawley rats. Toxicology 206(3): 359-371.

Wilson VS, Blystone CR, Hotchkiss AK, Rider CV, Gray LE, Jr. 2008. Diverse mechanisms of anti-androgen action: impact on male rat reproductive tract development. International journal of andrology 31(2): 178-187.

Wittassek M, Wiesmuller GA, Koch HM, Eckard R, Dobler L, Muller J, et al. 2007. Internal phthalate exposure over the last two decades--a retrospective human biomonitoring study. International journal of hygiene and environmental health 210(3-4): 319-333.

Woodruff TJ, Sutton P. 2011. An evidence-based medicine methodology to bridge the gap between clinical and environmental health sciences. Health Aff (Millwood) 30(5): 931-937.