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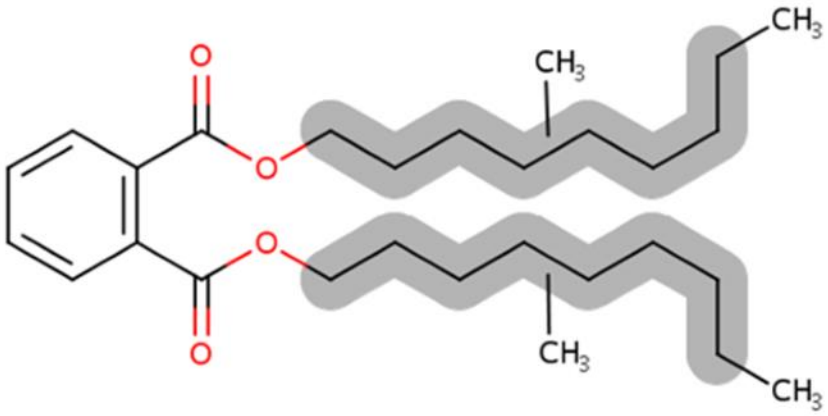
Pollution Prevention

Human Health Hazard Assessment for Diisodecyl Phthalate (DIDP)

Technical Support Document for the Risk Evaluation

CASRNs: 26761-40-0 and 68515-49-1



(Representative Structure)

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KEY ABBREVIATIONS AND ACRONYMS

α2u-globulin	Alpha 2u-globulin
ADME	Absorption, distribution, metabolism, and excretion
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMD	Benchmark dose
BMDL	Benchmark dose lower bound
BMR	Benchmark response
CASRN	Chemical Abstracts Service Registry Number
CPSC	(U.S.) Consumer Product Safety Commission

DEHP	Di-ethylhexyl phthalate
DIDP	Diisodecyl phthalate
DINP	Diisononyl phthalate
DNEL	Derived no effect level
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EPA	(U.S.) Environmental Protection Agency (or the Agency)
F344	Fischer 344 rat
FITC	Fluorescein isothiocyanate
GD	Gestational day
GGT	Gamma glutamyltransferase
GLP	Good Laboratory Practice
HEC	Human equivalent concentration
HED	Human equivalent dose
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
MCNP	Mono-(carboxynonyl) phthalate
MIDP	Mono-isodecyl phthalate
MMAD	Mass median aerodynamic diameter
MNCL	Mononuclear cell leukemia
MOA	Mode of action
MOE	Margin of exposure
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NTP-CERHR	National Toxicology Program Center for the Evaluation of Risks to Human Reproduction
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organisation for Economic Co-operation and Development
OPPT	Office of Pollution Prevention and Toxics
PBPK	Physiologically based pharmacokinetic
PECO	Population, exposure, comparator, and outcome
PESS	Potentially exposed or susceptible subpopulations
PND	Postnatal day
POD	Point of departure
PPARα	Peroxisome proliferator activated receptor alpha
ROS	Reactive oxygen species
SACC	Science Advisory Committee on Chemicals
SD	Sprague-Dawley rat
TSCA	Toxic Substances Control Act
TSD	Technical support document
UF	Uncertainty factor

SUMMARY

This technical support document (TSD) is for the TSCA *Risk Evaluation for Diisodecyl Phthalate (DIDP)* (U.S. EPA, 2024g). DIDP is a common chemical name for the category of chemical substances that includes the following substances: 1,2-benzenedicarboxylic acid, 1,2-diisodecyl ester (CASRN 26761-40-0) and 1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich (CASRN 68515-49-1). Both CASRNs contain mainly C10 dialkyl phthalate esters. This TSD describes the use of reasonably available information for both CASRNs to identify the non-cancer and cancer hazards associated with exposure to DIDP and identifies the points of departure (PODs) to be used to estimate risks from DIDP exposures in the risk evaluation of DIDP. See the completed risk evaluation for a complete list of all the TSDs for DIDP.

Non-cancer Human Health Hazards

An adequate toxicological database is available for DIDP. Available studies include one intermediate duration (>1–30 days) inhalation study of rats (General Motors, 1983b); seven intermediate duration oral exposure studies (5 of rats, 2 of mice) (Chen et al., 2019; Kwack et al., 2010; Kwack et al., 2009; Smith et al., 2000; Lake et al., 1991; BIBRA, 1990, 1986); three subchronic (>30–90 days) dietary studies (2 of rats, 1 of beagles) (BASF, 1969; Hazelton Labs, 1968a, b); two chronic (>90 days) dietary studies (1 of each of rats and mice) (Cho et al., 2011; Cho et al., 2010; Cho et al., 2008); two prenatal developmental studies of rats (Waterman et al., 1999; Hellwig et al., 1997); one developmental/reproductive toxicity screening study of mice (Hazleton Labs, 1983); and a pair of two-generation dietary studies of rats (Hushka et al., 2001; Exxon Biomedical, 2000, 1998). No repeated dose studies investigating the systemic toxicity of DIDP are available for the dermal route of exposure. Additionally, although the anti-androgenicity of DIDP is not discussed in detail in this document (see U.S. EPA (2023b) for further discussion), several mechanistic studies have demonstrated that gestational exposure during the critical window of development to DIDP does not induce antiandrogenic effects on the developing male reproductive system (Furr et al., 2014; Hannas et al., 2012). This conclusion was supported by the Science Advisory Committee on Chemicals (SACC) (U.S. EPA, 2023d).

EPA identified liver and developmental toxicity as the most sensitive and robust non-cancer hazards associated with oral exposure to DIDP in experimental animal models (Sections 3.1.1 and 3.1.2). Liver and developmental toxicity were also identified as the most sensitive and robust non-cancer effects following oral exposure to DIDP by the U.S. Consumer Product Safety Commission (U.S. CPSC, 2014), Health Canada (ECCC/HC, 2020), European Chemicals Agency (ECHA, 2013b), European Food Safety Authority (EFSA, 2019), and the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2015). Consistent, dose-related effects on development were observed across available experimental studies of rodent models. In two prenatal studies, increased incidences of skeletal and visceral variations were observed in Sprague-Dawley (SD) and Wistar rats at non-maternally toxic doses (Waterman et al., 1999; Hellwig et al., 1997). No-observed-adverse-effect levels (NOAELs)/lowest-observed-adverse-effect level (LOAELs) for developmental and maternal toxicity were 40/200 and 200/1,000 mg/kg-day, respectively, in the study by Hellwig et al. (1997), and 200/500 and 500/1,000 mg/kg-day, respectively, in the study by Waterman et al. (1999). The biological significance of the observed increases in skeletal and visceral variations are difficult to assess. However, EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991b) states that, "if variations are significantly increased in a dose-related manner, these should also be evaluated as a possible indication of developmental toxicity" and "Agents that produce developmental toxicity at a dose that is not toxic to the maternal animal are especially of concern." Therefore, EPA considered the increase in skeletal and visceral variations following gestational exposure to DIDP to be treatment-related adverse effects. Effects on developing offspring have also been observed consistently in a pair of two-generation studies of reproduction of SD rats (Hushka et al., 2001; Exxon Biomedical, 2000, 1998).

In the first two-generation study (Hushka et al., 2001; Exxon Biomedical, 1998), DIDP exposure (1) reduced F1 offspring survival on postnatal day (PND) PND4, (2) reduced F1 and F2 offspring body weight on PND0, and (3) reduced F1 and F2 offspring body weight gain through PND21 at doses equal to 524 to 637 mg/kg-day DIDP. DIDP also reduced F2 offspring survival on PND1 and PND4 at doses of 135 mg/kg-day and above. In the second two-generation study (Hushka et al., 2001; Exxon Biomedical, 2000), which tested lower doses than the first study (high-dose group received 254 to 356 mg/kg-day DIDP), DIDP reduced F2 offspring survival on PND1 and PND4 was observed at doses of 134 mg/kg-day and above.

To calculate non-cancer risks from oral exposure to DIDP for acute, intermediate, and chronic durations of exposure in the risk evaluation of DIDP, EPA selected a NOAEL of 38 mg/kg-day from a two-generation study of reproduction of rats based on reduced F2 offspring survival on PND1 and PND4 (Hushka et al., 2001; Exxon Biomedical, 2000). The NOAEL of 38 was converted to a human equivalent dose (HED) of 9.0 mg/kg-day based on allometric body weight scaling to the three-quarter power (U.S. EPA, 2011b). A total uncertainty factor (UF) of 30 was selected for use as the benchmark margin of exposure (based on a interspecies uncertainty factor [UF_A] of 3 and a intraspecies uncertainty factor [UF_H] of 10). The critical effect, reduced F2 offspring survival on PND1 and PND4, is clearly adverse and is assumed to be human-relevant. It is unclear whether decreased pup survival was due to a single, acute exposure or from repeated exposures. It is plausible that reduced offspring survival could result from a single exposure during gestation. However, it is also plausible that reduced offspring survival could result from repeated exposure during gestation or the postnatal period. Because repeated dose studies were used to investigate these hazard endpoints and the mode of action (MOA) for DIDP is uncertain, and because other studies did not provide a more sensitive or reliable endpoint, EPA considered reduced F2 offspring survival relevant for all exposure durations (U.S. EPA, 1996, 1991b). As discussed in Sections 6.1.1 through 6.1.3, several additional acute, intermediate, and chronic duration studies of DIDP provide similar, albeit slightly less-sensitive, candidate PODs, which further support EPA's decision to use the selected POD of 9.0 mg/kg-day to assess non-cancer risks for acute, intermediate, and chronic durations of exposure.

EPA reviewed the weight of scientific evidence and has robust overall confidence in the selected POD based on developmental outcomes for use in characterizing risk from exposure to DIDP for acute, intermediate, and chronic exposure scenarios. This conclusion was based on several weight of scientific evidence considerations (discussed in Section 6.1.4). First, exposure to DIDP resulted in consistent, dose-related, developmental toxicity in two prenatal developmental studies and in a pair of two-generation studies that adhered to relevant EPA guidelines (*i.e.*, OPPTS 870.3700 and OPPTS 870.3800). Further, developmental toxicity occurred at doses lower that those that caused overt maternal and/or parental toxicity. Second, across available studies, developmental toxicity was observed consistently at LOAELs ranging from 134 to 200 mg/kg-day. Third, the selected POD (NOAEL of 38.0 mg/kg-day) for developmental toxicity was the most sensitive and robust POD considered for acute, intermediate, and chronic exposures. Five additional acute, intermediate, and chronic duration studies of DIDP provide similar, although slightly less-sensitive, candidate PODs (*i.e.*, HEDs ranging from 9.3–13 mg/kg-day based on developmental or liver toxicity), which further supports EPA's decision to use the selected POD to assess non-cancer risks for acute, intermediate, and chronic durations of exposure. Finally, other regulatory and authoritative bodies have also concluded that DIDP is a developmental toxicant and that developmental effects are relevant for estimating human risk (EFSA, 2019; EC/HC, 2015; NICNAS, 2015; ECHA, 2013b; U.S. CPSC, 2010; EFSA, 2005; ECB, 2003; NTP-CERHR, 2003).

No data were available for the dermal or inhalation routes that were suitable for deriving route-specific PODs. Therefore, EPA used the oral POD to evaluate risks from dermal exposure to DIDP. Differences in absorption are accounted for in dermal exposure estimates in the risk evaluation for DIDP. For the inhalation route, EPA extrapolated the oral HED to an inhalation human equivalent concentration (HEC) using a human body weight and breathing rate relevant to a continuous exposure of an individual at rest (U.S. EPA, 1994). The oral HED and inhalation HEC values selected by EPA to estimate non-cancer risk from acute, intermediate, and chronic exposure to DIDP in the risk evaluation are summarized the table below and Section 8.

Cancer Human Health Hazards

Available data indicate that DIDP is not genotoxic or mutagenic (Section 4). In a 2-year dietary study of F344 rats (Cho et al., 2010; Cho et al., 2008), increased incidence of mononuclear cell leukemia (MNCL) was observed in high-dose male and female rats dosed with up to 479 to 620 mg/kg-day DIDP (Section 5.2.1). No other carcinogenic activity of DIDP was observed in this study. MNCL is a spontaneously occurring neoplasm of the hematopoietic system that reduces lifespan and is one of the most common tumor types occurring at a high background rate in the F344 strain of rat (also referred to as Fisher rat leukemia because it is so common) (Thomas et al., 2007). The MOA for induction of MNCL in F344 rats is unknown and there is uncertainty related to the human correlate to MNCL in F344 rats (Maronpot et al., 2016). The F344 strain of rat was used in the National Toxicology Program (NTP) 2-year chronic and carcinogenicity bioassays for nearly 30 years. However, in the early 2000s NTP stopped using the F344 strain of rat in large part because of high background incidence of MNCL and testicular Leydig cell tumors that confounded bioassay interpretation (King-Herbert et al., 2010; King-Herbert and Thayer, 2006). Given these considerations, EPA is not further considering MNCL as a factor in the determination of the cancer classification for DIDP, which is consistent with the recommendations of the Science Advisory Committee on Chemicals (SACC) (U.S. EPA, 2024h).

In a 26-week study of male and female wild-type and rasH2 transgenic mice (Cho et al., 2011), increased incidence of hepatocellular adenomas were observed in high-dose rasH2 males treated with 1,500 mg/kg-day DIDP. No other tumors were observed in any tissues in male or female wild-type mice or female rasH2 mice treated with up to 1,500 mg/kg-day (Section 5.2.2). However, hepatocellular adenomas were only observed in high-dose male rasH2 transgenic mice at a dose that exceeded the limit dose, causing a 31 percent decrease in terminal body weight. Per EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) "overt toxicity or qualitatively altered toxicokinetics due to excessively high doses may result in tumor effects that are secondary to the toxicity rather than directly attributable to the agent." No carcinogenic activity was observed in mid-dose male rasH2 mice treated with 495 mg/kg-day DIDP (a dose that caused no overt toxicity).

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), **EPA reviewed the weight of scientific evidence for the carcinogenicity of DIDP and determined that DIDP is not likely to be carcinogenic to humans** (Section 5.3). Consistent with this classification, the Agency is not conducting a dose-response assessment for DIDP or evaluating DIDP for carcinogenic risk to humans.

This human health hazard assessment for DIDP was released for public comment and peer-reviewed by the SACC during the July 30 to August 1, 2024, SACC meeting (U.S. EPA, 2024h).

Table ES-1. Non-cancer HECs and HEDs Used to Estimate Risks

Exposure Scenario	Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HEC (mg/m^3) [ppm]	HED (mg/ kg-day)	Benchmark MOE	References
Acute, intermediate, chronic	Developmental toxicity	SD rat	~35 weeks	NOAEL = 38	Reduced F2 offspring survival on PND1 and PND4	49 [2.7]	9.0	UF_A= 3[a] UF_H=10 *Total UF=30*	(Hushka et al., 2001; Exxon Biomedical, 2000)

HEC = human equivalent concentration; HED = human equivalent dose; MOE = margin of exposure; NOAEL = no-observed-adverse-effect level; POD = point of departure; SD = Sprague-Dawley; UF = uncertainty factor

[a] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011b), the UF_A was reduced from 10 to 3.

1 INTRODUCTION

On May 24, 2019, EPA received a request, pursuant to 40 CFR 702.37, from ExxonMobil Chemical Company, through the American Chemistry Council's High Phthalates Panel (ACC HPP, 2019), to conduct a risk evaluation for diisodecyl phthalate (DIDP) (chemical abstracts service registry numbers (CASRNs) 26761-40-0 and 68515-49-1) (Docket ID: EPA-HQ-OPPT-2018-0435). EPA determined that these two CASRNs should be treated as a category of chemical substances as defined in 15 U.S.C. section 2625(c). On August 19, 2019, EPA opened a 45-day public comment period to gather information relevant to the requested risk evaluation. EPA reviewed the request (along with additional information received during the public comment period) and assessed whether the circumstances identified in the request constitute conditions of use under 40 CFR 702.33, and whether those conditions of use warrant inclusion within the scope of a risk evaluation for DIDP. EPA determined that the request meets the applicable regulatory criteria and requirements, as prescribed under 40 CFR 702.37. The Agency granted the request on December 2, 2019, and published the draft and final scope documents for DIDP in 2020 and 2021, respectively (U.S. EPA, 2021b, 2020).

Following publication of the final scope document, one of the next steps in the Toxic Substances Control Act (TSCA) risk evaluation process is to identify and characterize the human health hazards of DIDP and conduct a dose-response assessment to determine the points of departure (PODs) to be used to estimate risks from DIDP exposures. This TSD for DIDP summarizes the non-cancer and cancer hazards associated with exposure to DIDP and identifies the PODs to be used to estimate risks from DIDP exposures.

1.1 Approach and Methodology

Over the past several decades the human health effects of DIDP have been reviewed by several regulatory and authoritative agencies, including the U.S. Consumer Product Safety Commission (U.S. CPSC); Health Canada; U.S. National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR); European Chemicals Bureau (ECB); European Chemicals Agency (ECHA); European Food Safety Authority (EFSA); and the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS). EPA relied on information published in existing assessments by these regulatory and authoritative agencies as a starting point for its human health hazard assessment of DIDP. Additionally, the Agency considered new literature published since the most recent existing assessments of DIDP to determine if this newer information might support the identification of new human health hazards or lower PODs for use in estimating human risk. EPA's process for considering and incorporating new DIDP literature is described in the *Systematic Review Protocol for Diisodecyl Phthalate (DIDP)* (U.S. EPA, 2024i) (also referred to as the DIDP Systematic Review Protocol). EPA's approach and methodology for identifying and using human epidemiologic data and experimental laboratory animal data are described in Sections 1.1.1 and 1.1.2, respectively.

1.1.1 Human Epidemiologic Data

To identify and integrate human epidemiologic data into the risk evaluation of DIDP, EPA first reviewed existing assessments of DIDP conducted by regulatory and authoritative agencies. Existing assessments reviewed by EPA are listed below. As described further in 0, most of these assessments have been subjected to external peer-review and/or public comment periods but have not employed formal systematic review protocols.

- *Toxicity Review of Di(isodecyl) Phthalate* (U.S. CPSC, 2010);
- *Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives* (U.S. CPSC, 2014);
- *State of the Science Report: Phthalates Substance Grouping: Long-chain Phthalate Esters. 1,2-Benzenedicarboxylic acid, diisodecyl ester (diisodecyl phthalate; DIDP) and 1,2-*

Benzenedicarboxylic acid, diundecyl ester (diundecyl phthalate; DUP). Chemical Abstracts Service Registry Numbers: 26761-40-0, 68515-49-1; 3648-20-2 (EC/HC, 2015);

- *Supporting Documentation: Carcinogenicity of Phthalates - Mode of Action and Human Relevance* (Health Canada, 2015);
- *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for hormonal effects, growth and development and reproductive parameters* (Health Canada, 2018b);
- *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for effects on behaviour and neurodevelopment, allergies, cardiovascular function, oxidative stress, breast cancer, obesity, and metabolic disorders* (Health Canada, 2018a);
- *Screening Assessment - Phthalate Substance Grouping* (ECCC/HC, 2020);
- *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-isodecyl Phthalate (DIDP)* (NTP-CERHR, 2003);
- *European Union Risk Assessment Report, vol 36: 1,2-Benzenedicarboxylic acid, Di-C9-11-Branched alkyl esters, C10-Rich and Di-"isodecyl"phthalate (DIDP)* (ECB, 2003);
- *Evaluation of New Scientific Evidence Concerning DINP and DIDP in Relation to Entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006* (ECHA, 2013b);
- *Committee for Risk Assessment (RAC) Opinion on the ECHA's Draft Review Report on "Evaluation of New Scientific Evidence Concerning DINP and DIDP in Relation to Entry 52 of Annex XVII to Regulation (EC) No 1907/2006 (REACH)" ECHA/RAC/A77-O-0000001412-86-10/F* (ECHA, 2013a);
- *Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) Related to Di-isodecylphthalate (DIDP) for Use in Food Contact Materials* (EFSA, 2005);
- *Update of the Risk Assessment of Di-butylphthalate (DBP), Butyl-benzyl-phthalate (BBP), Bis(2-ethylhexyl)phthalate (DEHP), Di-isononylphthalate (DINP) and Di-isodecylphthalate (DIDP) for Use in Food Contact Materials* (EFSA, 2019); and
- *Priority Existing Chemical Draft Assessment Report: Diisodecyl Phthalate & Di-n-octyl Phthalate* (NICNAS, 2015).

Next, EPA sought to identify new population, exposure, comparator, and outcome (PECO)-relevant literature published since the most recent existing assessment of DIDP. PECO-relevant literature published since the most recent existing assessment(s) of DIDP was identified by applying a literature inclusion cutoff date from existing assessments of DIDP. For DIDP, EPA used the applied cutoff date based on existing assessments of epidemiologic studies of phthalates by Health Canada (2018a, b), which included literature up to January 2018. The Health Canada (2018a, b) epidemiologic evaluations were considered the most appropriate existing assessments for setting a literature inclusion cutoff date because the assessments provided the most robust and recent evaluation of human epidemiologic data for DIDP. Health Canada evaluated epidemiologic study quality using the Downs and Black method (Downs and Black, 1998) and reviewed the database of epidemiologic studies for consistency, temporality, exposure-response, strength of association, and database quality to determine the level of evidence for association between urinary DIDP metabolites and health outcomes. New PECO-relevant literature published between 2018 to 2019 that was identified through the literature search conducted by EPA in 2019, as well as references published between 2018 to 2023 that were submitted with public comments to the DIDP Docket (EPA-HQ-OPPT-2018-0435) were evaluated for data quality and extracted consistent with EPA's *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (U.S. EPA, 2021a) and the DIDP Systematic Review Protocol (U.S. EPA, 2024i). Data quality evaluations for new studies reviewed by EPA are provided in the *Data Quality*

Evaluation Information for Human Health Hazard Epidemiology for Diisodecyl Phthalate (DIDP) (U.S. EPA, 2024d).

As described further in the DIDP Systematic Review Protocol (U.S. EPA, 2024i), EPA considers phthalate metabolite concentrations in urine to be the best proxy of exposure from all sources, including exposure through ingestion, dermal absorption, and inhalation. As described in the *Application of US EPA IRIS systematic review methods to the health effects of phthalates: Lessons learned and path forward* (Radke et al., 2020), from the U.S. EPA Integrated Risk Information System (IRIS) program, the "problem with measuring phthalate metabolites in blood and other tissues is the potential for contamination from outside sources (Calafat et al., 2015). Phthalate diesters present from exogenous contamination can be metabolized to the monoester metabolites by enzymes present in blood and other tissues, but not urine." Therefore, new epidemiologic studies that examined DIDP metabolites in matrices other than urine were considered supplemental and not evaluated for data quality.

EPA considered conclusions from Health Canada (2018a, b) regarding the level of evidence for association between urinary DIDP metabolites and each health outcome, as well as new epidemiologic studies identified by the Agency qualitatively during evidence integration to inform hazard identification and the weight of scientific evidence. EPA did not use epidemiology studies quantitatively for dose-response assessment, primarily due to uncertainty associated with exposure characterization.

Primary sources of uncertainty include the source(s) of exposure; timing of exposure assessment that may not be reflective of exposure during outcome measurements; measured urinary metabolites may represent exposure to more than one parent phthalate; and use of spot-urine samples, which due to rapid elimination kinetics may not be representative of average urinary concentrations that are collected over a longer term or calculated using pooled samples. Additional uncertainty results from co-exposure to mixtures of multiple phthalates that may confound results for the majority of epidemiologic studies, which examine one phthalate and one exposure period at a time such that they are treated as if they occur in isolation (Shin et al., 2019; Aylward et al., 2016). EPA's decision to use epidemiologic studies of DIDP qualitatively is consistent with existing assessments of DIDP by Health Canada, U.S. CPSC, ECHA, EFSA, and Australia NICNAS, which also only considered epidemiological studies qualitatively. As discussed further in Section 1.1.2, PODs for DIDP are derived from laboratory animal data.

1.1.2 Laboratory Animal Data

Figure 1-1 provides an overview of EPA's approach to identifying and integrating laboratory animal data into the risk evaluation of DIDP. The Agency first reviewed existing assessments of DIDP conducted by various regulatory and authoritative agencies. Existing assessments reviewed by EPA are listed above in Section 1.1.1. The purpose of this review was to identify sensitive and human relevant hazard outcomes associated with exposure to DIDP as well as key studies used to establish PODs for extrapolating human risk. As described further in 0, most of these assessments have been subjected to external peer-review and/or public comment periods but have not employed formal systematic review protocols.

EPA identified primary literature published since the most recent existing assessment of DIDP (as discussed further below, (EC/HC, 2015) and (NICNAS, 2015) were used to set a cutoff date). To do this, EPA systematically reviewed data sources identified in the literature search conducted by EPA in 2019. EPA first screened titles and abstracts and then full texts for relevancy using PECO screening criteria described in the DIDP Systematic Review Protocol (U.S. EPA, 2024i). EPA then identified PECO-relevant literature published since two recent and comprehensive existing assessments of DIDP by

applying a literature inclusion cutoff date from these assessments. For DIDP, assessments by Health Canada (EC/HC, 2015) and Australia NICNAS (NICNAS, 2015) included literature up to August 2014 and July 2014, respectively, and considered a full range of human health hazards (*i.e.*, acute toxicity, irritation, sensitization, developmental and reproductive toxicity, systemic toxicity to major organ systems, genotoxicity, carcinogenicity) across all durations of exposure (*i.e.*, acute [≤1 day], intermediate [>1–30 days], subchronic [>30–90 days], chronic [>90 days]) and routes of exposure (*i.e.*, oral, dermal, inhalation). Furthermore, assessments by both Health Canada and NICNAS were subject to public comment periods and the assessment by Health Canada was subject to external peer review (0). EPA preferred these assessments for setting a literature cutoff date instead of more recent assessments by EFSA (2019) and Health Canada (ECCC/HC, 2020) because the EFSA assessment was limited in scope (*i.e.*, considered a limited range of human health hazards) and was not subject to external peer review, whereas the Health Canada (ECCC/HC, 2020) assessment did not provide a specific literature inclusion cutoff date. Therefore, EPA considered literature published between 2014 to 2019 further as shown below in Figure 1-1.

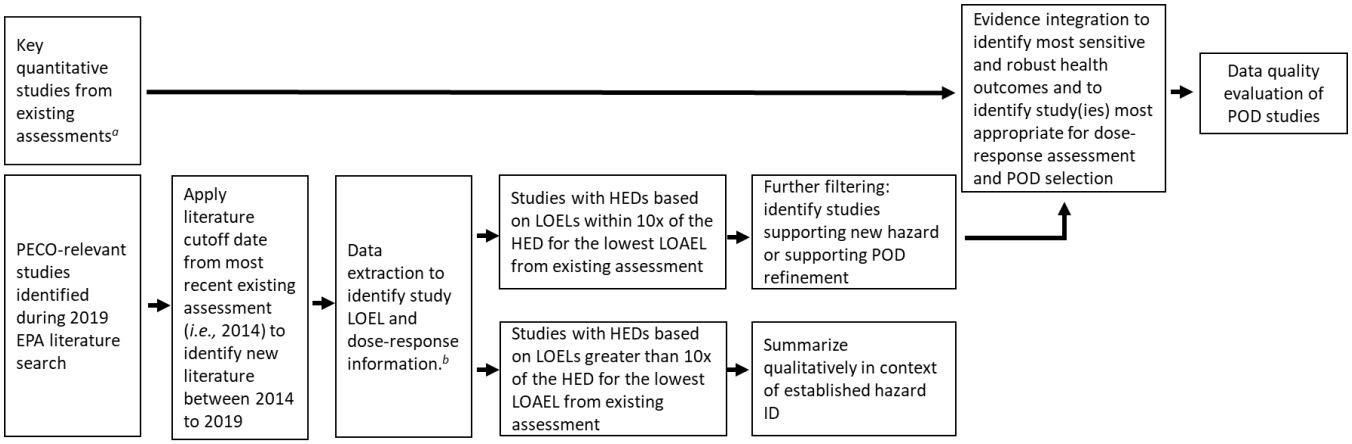

Figure 1-1. Overview of DIDP Human Health Hazard Assessment Approach

[a] Any study that was considered for dose-response assessment, not necessarily limited to the study used for POD selection.

[b] Extracted information includes PECO relevance, species, exposure route and type, study duration, number of dose groups, target organ/systems evaluated, study-wide lowest-observed-effect level (LOEL), and potentially exposed or susceptible subpopulations (PESS) categories.

Next, EPA reviewed new studies published between 2014 and 2019 and extracted key study information as described in the DIDP Systematic Review Protocol (U.S. EPA, 2024i). Extracted information included: PECO relevance; species tested; exposure route, method, and duration of exposure; number of dose groups; target organ/systems evaluated; information related to potentially exposed or susceptible subpopulations (PESS); and the study-wide LOEL (Figure 1-1).

New information for DIDP was limited to oral exposure studies and study LOELs were converted to HEDs based on LOELs by scaling allometrically across species using the three-quarter power of body weight ($BW^{3/4}$) for oral data, which is the approach recommended by EPA when physiologically based pharmacokinetic (PBPK) models or other information to support a chemical-specific quantitative extrapolation is absent (U.S. EPA, 2011b). EPA's use of allometric body weight scaling is described further in Appendix D. EPA did not conduct data quality evaluations for studies with HEDs based on LOELs that were greater than an order of magnitude of the lowest HED based on the LOAEL across existing assessments because they were not considered sensitive for subsequent POD selection. However, these studies were still reviewed and integrated into the hazard identification process. Studies

with HEDs for LOELs within an order of magnitude of the lowest LOAEL-based HED identified across existing assessments were considered sensitive and potentially relevant for POD selection. These studies were further reviewed by EPA to determine if they provide information that supports a new human health hazard not identified in existing assessments or to determine if they contain sufficient dose-response information to support a lower POD than identified in existing assessments of DIDP. New studies supporting dose-response assessment and POD selection for DIDP were evaluated for data quality consistent with EPA's *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances, Version 1.0: A Generic TSCA Systematic Review Protocol with Chemical-Specific Methodologies* (also called the Draft Systematic Review Protocol) (U.S. EPA, 2021a).

2024 TSCA Literature Search Update

Following release of the draft risk evaluation of DIDP in May 2024, EPA updated its literature searches for DIDP. The DIDP Systematic Review Protocol (U.S. EPA, 2024i) provides details regarding the updated DIDP literature search. No new PECO-relevant animal toxicology studies were identified for DIDP that met PECO screening criteria for full text data quality evaluation.

Data quality evaluations for DIDP animal toxicity studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Diisodecyl Phthalate (DIDP)* (U.S. EPA, 2024c).

1.2 Scope of DIDP Human Health Hazard Assessment

Existing Assessments

As described in Section 1.1, the human health hazards of DIDP have been evaluated in existing assessments by U.S. CPSC (2014, 2010), Health Canada (ECCC/HC, 2020; EC/HC, 2015), NTP-CERHR (2003), ECB (2003), ECHA (2013a, b), EFSA (2019, 2005), and Australia NICNAS (2015). These assessments have consistently identified effects on development and the liver to be the most sensitive for use in extrapolating human risk from exposure to DIDP, and the PODs selected for use in each existing risk assessment of DIDP are based on developmental and liver effects (Table 1-1).

Table 1-1. Summary of PODs Selected for Use in Existing Assessments of DIDP

Brief Study Description (Reference)	TSCA Data Quality [a]	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	U.S. CPSC (2014)	ECCC/HC (2020)	EFSA (2019)	NICNAS (2015)	ECHA (2013b)
Male and female beagles (3/sex/dose) fed dietary concentrations of 0, 0.05, 0.3, and 1% DIDP for 13 weeks (equivalent to 15, 75, 300 mg/kg-day) (Hazelton Labs, 1968a)	Medium	15/ 75	↑ liver weight, swelling and vacuolation of hepatocytes	✓[b]		✓[c]		✓[d]
Male and female rats (10–20/sex/dose) fed diet containing 0, 800, 1,600, 3,200, 6,400 ppm DIDP for 90 days (equivalent to 55, 100, 200, 400 mg/kg-day for males; 60, 120, 250, 500 mg/kg-day for females) (BASF, 1969) [d]	Not evaluated[e]	60/ 120	↑ relative liver weight [f]				✓[g]	✓[d]
Male and female F344 rats (52/sex/dose) fed diets of 0, 400, 2,000, 8,000 ppm DIDP for 2 years (equivalent to 22, 110, 479	Medium	None/ 22	↑ incidence of spongiosis hepatis and other		✓[b]			✓[d]

Brief Study Description (Reference)	TSCA Data Quality [a]	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	U.S. CPSC (2014)	ECCC/HC (2020)	EFSA (2019)	NICNAS (2015)	ECHA (2013b)
mg/kg-day for males); 23, 128, 620 mg/kg-day for females) (Cho et al., 2010; Cho et al., 2008)			signs of hepatotoxicity (males only)					
Male and female SD rats fed diets of 0, 0.02, 0.06, 0.2, 0.4% (Received doses in units of mg/kg-day shown in Table_Apx C-7) DIDP 10 weeks prior to mating, and throughout mating, gestation and lactation continuously for two-generations (adhered to OPPTS 870.3800) (Hushka et al., 2001)	Medium	33/ 115 [g]	↑ mortality of neonatal F2 pups					✓[d]
		52/ 166 [g]	↓ offspring bodyweight					✓[d]
Pregnant Wistar rats (7–10/dose) gavaged with 0 (corn oil), 40, 200, 1,000 mg/kg-day DIDP on GDs 6–15 (adhered to OPPTS 870.3700) (Hellwig et al., 1997)	Medium	100/ 200	↑ skeletal variations at non-maternally toxic doses				✓[g]	
Pregnant SD rats (22–25/dose) gavaged with 0 (corn oil), 100, 500, 1,000 mg/kg-day DIDP on GDs 6–15 (adhered to OPPTS 870.3700) (Waterman et al., 1999)	Medium							

[a] Studies evaluated for data quality consistent with the DIDP Systematic Review Protocol (U.S. EPA, 2024i) and EPA's Draft Systematic Review Protocol (U.S. EPA, 2021a).
[b] POD used for MOE calculations in risk assessments by U.S. CPSC and Health Canada.
[c] POD used to derive a tolerable daily intake by EFSA.
[d] ECHA calculated DNELs (derived no effect levels) for liver effects and developmental effects. The liver DNEL was based on the average of three DNELs derived from the 90-day studies of dogs and rats, and the 2-year study of rats. Two reproductive DNELs were derived, one for assessing risk based on exposure to adults (mortality) and for assessing risk based on exposure to children (bodyweight). The NOAEL/LOAEL values selected by ECHA for increased mortality are based on the received doses during the premating phase of the study for males of the second parental generation, while the NOAEL/LOAEL values for decreased offspring body weight are based on received doses during the postpartum phase of the study for F2 offspring.
[e] Reference available to EPA as a German language study. Study details provided in table are as reported in assessments by ECHA (2013b) and NICNAS (2015). Study was not evaluated for data quality.
[f] ECHA (2013b) and NICNAS (2015) identified a NOAEL of 60 mg/kg-day based on increased relative liver weight at a LOAEL of 120 mg/kg-day (BASF, 1969). As described further in Section 3.1.2, EPA generally does not consider liver weight changes to be adverse, unless accompanied by corroborating histopathology and/or biologically relevant changes in serum chemistry parameters indicative of liver toxicity.
[g] NICNAS identified PODs for liver and developmental (skeletal variations) effects that were used for MOE calculations. For the developmental NOAEL, results from two prenatal studies were integrated to support a NOAEL of 100 mg/kg-day.

New Literature (2014–2024)

As described in Section 1.1, EPA reviewed literature published between 2014 to 2024 for new information on sensitive human health hazards not previously identified in existing assessments, including information that may indicate a more sensitive POD. EPA identified three new PECO-relevant studies that provided information pertaining to four health outcomes: liver, kidney, neurotoxicity, and immune system. Further details regarding EPA's handling of this new information are provided below.

Chen et al. (2019) evaluated liver and kidney effects in a 2-week study of male mice at doses similar to those shown to cause liver and kidney toxicity in previous studies of rats, mice, and beagles. Results from Chen et al. are discussed in Sections 3.1.2 and 3.2.1. Ge et al. (2019) indicates that intermediate

duration oral exposure to DIDP in male mice can result in learning and memory impairment at doses similar to those that cause liver and developmental toxicity. Effects on learning and memory represent a new finding not previously seen in studies of DIDP. Neurotoxicity of DIDP is discussed further in Section 3.2.2. EPA identified one new study evaluating immune system effects (Shen et al., 2017). Results from Shen et al. indicate that intermediate duration oral exposure to DIDP in male mice pre-sensitized by exposure to fluorescein isothiocyanate can exacerbate allergic dermatitis. The immune adjuvant effects of DIDP are discussed further in Section 3.2.3.

Non-cancer Hazards Evaluated by EPA

Based on information provided in existing assessments of DIDP for liver and developmental effects and new information identified by EPA that encompasses additional hazard outcomes, the Agency focused its non-cancer human health hazard assessment on developmental toxicity (Section 3.1.1), liver toxicity (Section 3.1.2), kidney toxicity (Section 3.2.1), neurotoxicity (Section 3.2.2), and immune system toxicity (Section 3.2.3).

Genotoxicity and Cancer Hazards Evaluated by EPA

The genotoxicity and carcinogenicity of DIDP has been evaluated in several existing assessments (EC/HC, 2015; NICNAS, 2015; U.S. CPSC, 2014; ECHA, 2013b; U.S. CPSC, 2010; NICNAS, 2008a, b; ECB, 2003), which have consistently concluded that DIDP is not genotoxic or is not likely to be genotoxic. DIDP has not been classified for carcinogenicity by any international agencies. Genotoxicity and carcinogenicity data for DIDP are reviewed by EPA in Sections 4 and 5, respectively.

2 TOXICOKINETICS

2.1 Oral Route

No controlled human exposure studies are available that evaluate the absorption, distribution, metabolism, and excretion (ADME) of DIDP for the oral route. Four experimental animal studies are available that provide data useful in evaluating ADME of DIDP for the oral route. The ADME properties of DIDP have been evaluated in two *in vivo* studies of male rats (Jeong et al., 2021; General Motors, 1983a), whereas the metabolism of DIDP has been evaluated in two *in vivo* studies of female rats (Kato et al., 2007; Calafat et al., 2006).

In the first study, canulated adult male Sprague-Dawley (SD) rats were gavaged with a single dose of 0.1, 11.2, or 1,000 mg/kg carbon-14 (^{14}C) labelled DIDP (^{14}C-DIDP) and then sacrificed 72 hours post-exposure (General Motors, 1983a). Radioactivity in urine and feces was determined at 12- and 24-hour time intervals, respectively, and evaluated out to 72 hours to estimate urinary and fecal elimination, while the bile duct was canulated prior to dosing with DIDP to estimate biliary elimination. After 72 hours, low levels of radioactivity were detectable in the carcass (0.5, 0.8, and 0.2% of administered dose in low-, mid-, and high-dose groups), gastrointestinal tract (0.5, 0.8, and 0.2% of administered dose), liver (0.06, 0.08, and 0.03% of administered dose), and kidney (0.01, 0.01, and 0.00% of administered dose). Over 99 percent of the administered radioactivity associated with ^{14}C-DIDP was recovered in urine and feces, regardless of dose, indicating almost complete excretion within 3 days. The percent of radioactivity associated with ^{14}C-DIDP recovered in urine (41.3, 32.1, and 12.6% across doses) and bile (14.3, 13.8, and 4.7% across doses) decreased with dose, while the percent of radioactivity in feces increased with dose (58, 66, and 82% across doses), indicating percent absorption is inversely proportional to dose. Based on combined urinary and biliary excretion, absorption across the gastrointestinal tract was estimated by study authors to be 55.6, 45.9, and 17.3 percent at the low-, mid-, and high-dose, respectively. Given the minimal distribution to tissues and the carcass, these percentages were not considered in estimating absorption. These results suggest that absorption of DIDP across the gastrointestinal tract is incomplete and/or may become saturated following single high doses of DIDP (General Motors, 1983a).

EPA applied linear regression analysis to further evaluate the oral absorption data for DIDP from the available rat ADME study (General Motors, 1983a). This analysis is presented in Appendix B. The linear regression model provided a good fit (R^2 = 0.8093) and provided reasonable predictions of the observed oral absorption values. Further, linear regression analysis predicted close to 100 percent oral absorption at human relevant exposure levels (*i.e.,* 1–5 µg/kg).

Available data indicate that DIDP is rapidly metabolized to monoisodecyl phthalate (MIDP) and undergoes further oxidative metabolism before being excreted in urine and/or feces. In the study by General Motors (1983a), metabolites of DIDP detected in urine included phthalic acid and oxidative derivatives of the monoester. No DIDP or MIDP were detected in urine. Urinary radioactivity associated with phthalic acid decreased with increasing dose (38, 40, and 18% across doses), whereas radioactivity associated with oxidative derivatives of the monoester (specific derivatives not identified) increased with dose (52, 49, and 72% across doses) potentially indicating saturation of metabolism to phthalic acid. In feces, metabolites included oxidative derivatives of the monoester, MIDP, and DIDP. No phthalic acid was detected in feces. In feces, radioactivity associated with oxidative derivatives of the monoester and with MIDP decreased with increasing dose (25, 14, and 13% and 30, 26, and 13% across doses for oxidative derivatives and MIDP, respectively), whereas radioactivity associated with DIDP increased with dose (30, 55, and 60% across doses).

Metabolism of DIDP has been evaluated in two additional oral exposure studies of female rats (Kato et al., 2007; Calafat et al., 2006). Table 2-1 provides a summary of urinary metabolites of DIDP detected in studies by General Motors (1983a), Calafat et al. (2006) and Kato et al. (2007). Not all of the urinary metabolites listed in Table 2-1 are unique to DIDP (*e.g.*, phthalic acid is a metabolite common to all phthalate diesters). However, metabolites such as MIDP and mono-(carboxynonyl) phthalate (MCNP) are unique to DIDP and are regularly used as biomarkers of exposure to DIDP in human urinary biomonitoring studies, such as those conducted by the National Health and Nutrition Examination Survey (NHANES). Calafat et al. (2006) administered a single gavage dose of 300 mg/kg DIDP to four female SD rats and then measured metabolites in 24-hour composite urine samples. Mono-(3-carboxypropyl) phthalate was the major urinary metabolite (24-hour urinary concentration = 3.1 µg/mg creatinine), while MIDP (0.05 µg/mg creatinine), mono-n-octyl phthalate (0.06 µg/mg creatinine), and mono-(3-methyl-5-dimethylhexyl) phthalate (0.008 µg/mg creatinine) were minor urinary metabolites. Kato et al. (2007) administered 300 mg/kg DIDP to four female SD rats in a separate study and used full scan negative ion electrospray ionization mass spectroscopy to identify urinary metabolites of DIDP at 24-hour intervals for four days. The major urinary metabolites of DIDP included mono(carboxy-isononyl) phthalate (MCiNP) and mono(hydroxy-isodecyl) phthalate (MHiDP) with urinary elimination half-lives of 13.3 to 13.5 hours, respectively. Other minor oxidative metabolites of DIDP identified by Kato et al. are shown in Table 2-1—most of which also had urinary elimination half-lives of approximately 14 hours—except mono(carboxy-isodecyl) phthalate, which had a urinary elimination half-life of 22 hours. Based on these results, Kato et al. (2007) proposed a metabolic pathway in which DIDP is first hydrolyzed to MINP, and then monoester metabolites undergo further omega (ω) or ω-1 oxidation (Figure 2-1).

In the study by Jeong et al. (2021), male SD rats were administered a single oral (via Zonde needle) (n = 5 rats) or intravenous (via tail vein injection; n = 10 rats) dose of 100 mg/kg DIDP. Blood samples were collected before administration (0 hours) and 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24, 36, and 48 hours after DIDP administration. Urine and feces were collected at intervals (0–6 [urine], 6–12 [urine], 0–12 [feces] 12–24 [both urine and feces], 24–36 [both], and 36–48 [both] hours) to determine urinary and fecal excretion of DIDP and its metabolites. For the intravenous study, five rats were sacrificed 24 hours after exposure to DIDP and tissues were collected to investigate distribution of DIDP and its metabolites. In the oral exposure study, metabolites of DIDP, including MIDP, MHiDP, MCiNP, and mono(oxo-isodecyl) phthalate (MOiDP), were detectable in plasma starting 0.25 hours after exposure indicating rapid metabolism, and remained detectable in plasma until 36 to 48 hours after exposure. Toxicokinetic parameters were estimated for DIDP, MIDP, MHiDP, MCiNP, and MOiDP alone and as total forms (*i.e.*, as conjugated forms of each metabolite). For total forms of each metabolite, peak plasma concentrations (C_{max}) ranged from 22.8 µg/ml for MOiDP to 1,581 µg/ml for MIDP. Time to reach C_{max} (*i.e.*, T_{max}) for each metabolite ranged from 6 to 8.5 hours with plasma half-lives of 4.73 to 8.65 hours. MIDP, MHiDP, MCiNP, and MOiDP were detectable in urine and feces at all time points, with MCiNP being identified as a major urinary metabolite of DIDP, which is consistent with the results of Kato et al. (2007). DIDP was detectable in feces—but not urine or plasma—suggesting some DIDP passed through the gastrointestinal tract unchanged, which is consistent with the results of (General Motors, 1983a).

In the intravenous exposure study by Jeong et al. (2021), MIDP, MHiDP, MCiNP, and MOiDP were detectable in plasma starting 0.25 hours after exposure indicating rapid metabolism, and remained detectable in plasma until 36 to 48 hours after exposure. For total forms of each metabolite, peak plasma concentrations (C_{max}) ranged from 0.77 µg/ml for MOiDP to 11.25 for MCiNP. The time to reach C_{max} (*i.e.*, T_{max}) for each metabolite ranged from 5.3 to 8 hours with plasma half-lives of 9.69 to 21.25 hours. Twenty-four hours after intravenous exposure, DIDP and its metabolites were found to be systemically

distributed. Tissue concentrations of DIDP were the highest in lung (162,054 ng/g), followed by the liver (65,347 ng/g), spleen (42,827 ng/g), kidney (4,437 ng/g)—and to a lesser extent the heart, brain, gastrointestinal tract, adipose tissue, testis, and thymus. Similarly high concentrations of MIDP were also detected in the lung (14,431 ng/g), liver (101,967 ng/g), and spleen (44, 663 ng/g), and to a lesser extent other tissues. Tissue concentrations of MHiDP, MCiNP, and MOiDP were generally lower than that of DIDP and MIDP, with tissue concentrations of each metabolite being less than 85 ng/ng in each tissue in all cases, except for MCiNP in the kidney (165 ng/g). MIDP, MHiDP, MCiNP, and MOiDP were detectable in urine and feces at all time points. DIDP was detectable in feces, but not urine or plasma, suggesting some DIDP passed through the gastrointestinal tract unchanged, which is consistent with the results of (General Motors, 1983a).

Collectively, available data from oral exposure studies of rats indicate that absorption of DIDP across the gastrointestinal tract ranges from 17.3 to 55.6 percent at high doses ranging from 0.1 to 1,000 mg/kg. However, linear regression analysis indicates that absorption across the gastrointestinal tract is close to 100 percent at human relevant exposure levels (*i.e.*, 1–5 µg/kg). Therefore, for input into the risk evaluation, EPA will assume that absorption is 100 percent in rats and humans following exposure to DIDP via the oral route. This assumption is consistent with assessments by Australia NICNAS (2015) and Health Canada (EC/HC, 2015).

Table 2-1. Summary of Urinary Metabolites of DIDP Detected in Rats and Humans

Urinary Metabolite	Abbreviation	Rat	Human[a]	Reference(s)
Monoisodecyl phthalate	MIDP	✓		(Jeong et al., 2021; Calafat et al., 2006)
Mono(hydroxy-isodecyl) phthalate	MHiDP	✓	✓	(Jeong et al., 2021; Koch et al., 2012; Kato et al., 2007; Silva et al., 2007)
Mono(oxo-isodecyl) phthalate	MOiDP	✓	✓	(Jeong et al., 2021; Koch et al., 2012; Kato et al., 2007; Silva et al., 2007)
Mono(carboxy-isodecyl) phthalate	MCiNP	✓		(Jeong et al., 2021; Kato et al., 2007)
Mono(carboxynonyl) phthalate	MCNP	✓	✓	(Koch et al., 2012; Kato et al., 2007; Silva et al., 2007)
Mono(carboxy-isononyl) phthalate	MCiNP	✓		(Jeong et al., 2021; Kato et al., 2007)
Mono(oxo-isononyl) phthalate	MOiNP	✓		(Kato et al., 2007)
Mono(hydroxy-isononyl) phthalate	MHiNP	✓		(Kato et al., 2007)
Mono(carboxy-isooctyl) phthalate	MCiOP	✓		(Kato et al., 2007)
Mono-n-octyl phthalate	MnOP	✓	✓	(Calafat et al., 2006)
Mono(carboxy-isoheptyl) phthalate	MCiHpP	✓		(Kato et al., 2007)
Mono(carboxy-isohexyl) phthalate	MCiHxP	✓		(Kato et al., 2007)
Mono(carboxy-isopentyl) phthalate	MCiPeP	✓		(Kato et al., 2007)
Mono(carboxy-isobutyl) phthalate	MCiBP	✓		(Kato et al., 2007)
Mono-(3-carboxy-propyl) phthalate	MCPP	✓	✓	(Calafat et al., 2006)
Mono(carboxy-ethyl) phthalate	MCEP	✓		(Kato et al., 2007)
Mono-(3-methyl-5-dimethylhexyl) phthalate	MINP	✓		(Calafat et al., 2006)

Urinary Metabolite	Abbreviation	Rat	Human[a]	Reference(s)
Phthalic acid	PA	✓		(General Motors, 1983a)
[a] Metabolites detected as part of human urinary biomonitoring studies (Koch et al., 2012; Silva et al., 2007; Calafat et al., 2006), not controlled exposure studies. Although biomonitoring studies do not distinguish between routes or pathways of exposure, urinary metabolites are shown for comparison to urinary metabolites detected in rodent models.				

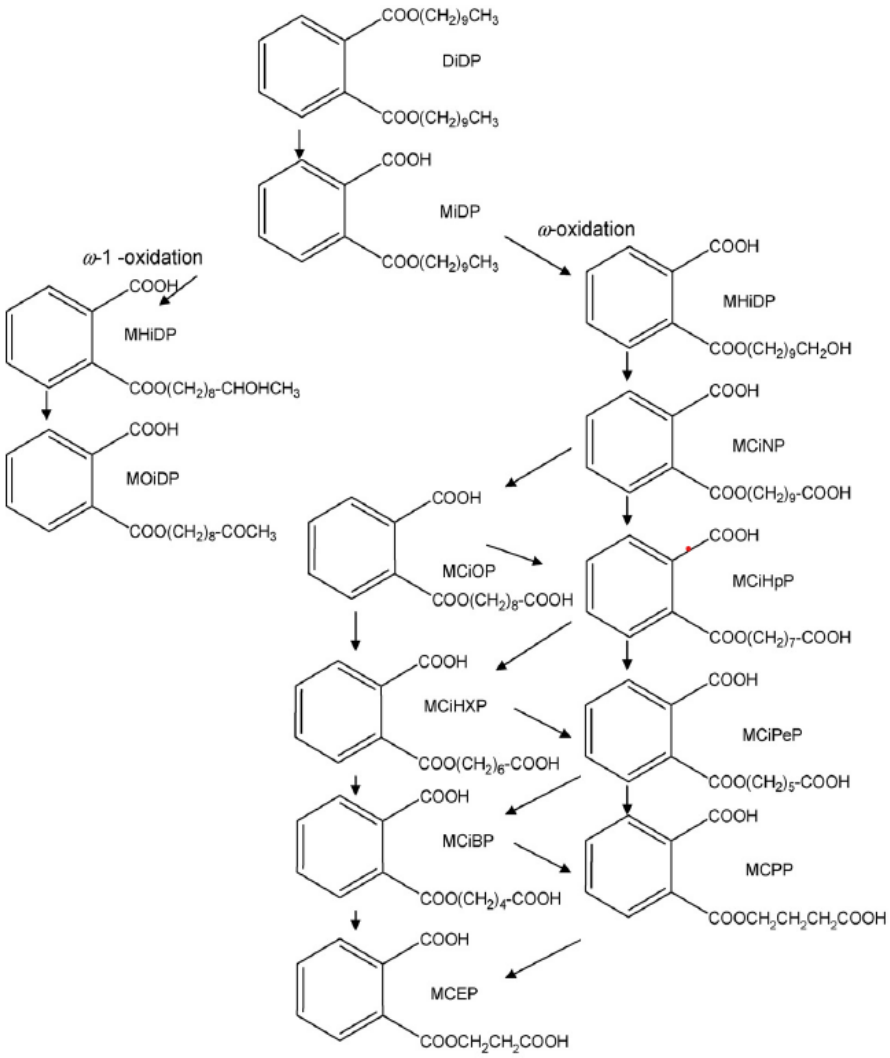

Figure 2-1. Proposed Metabolic Pathway of DIDP Following Oral Exposure (Kato et al., 2007)

2.2 Inhalation Route

No human studies are available that evaluate ADME of DIDP for the inhalation route.

EPA identified one *in vivo* study investigating the ADME properties of DIDP following inhalation exposure (General Motors, 1983b). Six adult male SD rats were exposed (head-only) to 91 mg/m^3 ^{14}C-DIDP aerosol (MMAD: 0.98 µm) for 6 hours. Immediately following exposure, three rats were sacrificed, and tissues were collected to determine radioactivity, while the remaining three rats were maintained in metabolic cages for 72 hours. ^{14}C-DIDP was absorbed and systemically distributed following inhalation exposure (Table 2-2). Immediately following exposure, the highest amounts of radioactivity were detected in the lung, followed by the gastrointestinal tract, liver, and kidney, and to a lesser extent in other tissues (Table 2-2). Seventy-two hours after exposure, radioactivity declined 60 to 92 percent in the gastrointestinal tract, liver, kidney, and thymus, and was no longer detectable in the

brain, spleen, and testes. Trace amounts of radioactivity were detectable in fat at both timepoints and did not appear to decline after 72 hours. In the lung, 27 percent of the radioactivity remained after 72 hours, indicating that absorption through the lung was approximately 73 percent over 72 hours. Over 72 hours, urinary and fecal excretion of radioactivity was approximately equal and accounted for 45.3 and 41.3 percent, of the total body burden, respectively. Metabolism and biliary excretion were not evaluated as part of this study.

As discussed further in Sections 3 and 6, no data from experimental animal models are available for the inhalation route that are suitable for deriving a route-specific PODs. Therefore, EPA extrapolated the inhalation POD from the oral POD. For this risk evaluation, EPA assumed similar absorption for the oral and inhalation routes, and no adjustment was made when extrapolating to the inhalation route.

Table 2-2. Distribution of Radioactivity in Rat Tissue Following Inhalation Exposure to DIDP[a]

Tissue	0 hours[b]	72 hours[b]	% Decline in Radioactivity over 72 hours
Lung	0.6630 ± 0.2556	0.1822 ± 0.0619	73%
Gastrointestinal tract	0.0948 ± 0.0080	0.0078 ± 0.0006	92%
Liver	0.0148 ± 0.0012	0.0013 ± 0.0004	91%
Kidney	0.0064 ± 0.0006	0.0006 ± 0.000	91%
Brain	0.0012 ± 0.0006	< LOD[c]	–
Thymus	0.0010 ± 0.0003	0.0004 ± 0.0002	60%
Heart	0.0009 ± 0.0001	Trace	–
Spleen	0.0007 ± 0.000	< LOD[c]	–
Fat	0.0003 ± 0.000	0.0004 ± 0.0001	–
Testes	0.0004 ± 0.000	< LOD[c]	–

[a] Adapted from General Motors (1983b).
[b] Data reported as mean ± SEM from three rats in units of µmole DIDP equivalents per gram of tissue.
[c] Limit of detection (LOD) reported to be 0.0001 µmole equivalents.

2.3 Dermal Route

No human studies are available that evaluate ADME of DIDP for the dermal route. No *in vitro* dermal absorption studies of DIDP are available. One *in vivo* study of male rats is available that investigated the ADME properties of DIDP following dermal exposure (Elsisi et al., 1989).

Elsisi et al. (1989) investigated the dermal absorption of eight phthalate diesters including DIDP by estimating the percentage of dose excreted in the urine and feces across several timepoints. Briefly, skin on the backs of male Fischer 344 (F344) rats was shaved one hour before test substance administration (rats with visual signs of abrasions were eliminated from the study). Then 5 to 8 mg/cm^2 neat ^{14}C-DIDP in an ethanol vehicle was applied to a circular area 1.3 cm in diameter. Ethanol was allowed to evaporate and then the application site was covered with a perforated circular plastic cup. Rats were then housed in metabolic cages for 7 days during which time urine and feces were collected every 24 hours. On the seventh day, rats were sacrificed, and organs were collected for determination of radioactivity. Low levels (less than one percent for combined tissues) of radioactivity associated with ^{14}C-DIDP were measured in adipose tissue, muscle, skin, and other tissues (*i.e.*, brain, lung, liver, spleen, small intestine,

kidney, testis, spinal cord, and blood) indicating dermally absorbed ^{14}C-DIDP was systemically distributed. The majority (75%) of the applied dose was recovered from skin at the application site. No radioactivity associated with ^{14}C-DIDP was detected in urine over the seven-day period, whereas only 0.04 and 0.5 percent of the applied dose was recovered in feces after 1 and 7 days of exposure, respectively. Based on the amount of radioactivity recovered from feces (0.5%) and other tissues (~1%), study authors estimated that approximately one to two percent of the applied dose of ^{14}C-DIDP was absorbed over 7 days.

Although the recovery of the applied dose of ^{14}C-DIDP in the study by Elsisi et al. (1989) (82%) is lower than recommended by the guideline (≥90%, OECD Test No. 428 (OECD, 2004)), this limitation has minimal impact on the usability of the absorption value for the following reasons. It is unlikely that the material unaccounted for was in any unanalyzed tissues (*e.g.*, carcass), given that the percent dose in the adipose tissue, muscle, and skin accounted for 0.57 percent dose, and the "other tissues" were less than 0.5 percent and represented the sum of the percent dose found in brain, lungs, liver, spleen, small intestine, kidneys, testes, spinal cord, and blood. It is more likely that the unaccounted for material was lost to evaporation, because, even though not highly volatile, the dermal exposure was 7 days, and the covering was only partially occluded (perforated plastic cap). The dermal absorption guideline (OECD Guidance Document No. 156 (OECD, 2022)) presents approaches for addressing recovery that is lower than recommended by the guideline and states that "losses from non-absorbed material will have no impact on the results." If it can be assumed that the chemical unaccounted for was lost to evaporation over 7 days, then it is reasonable that this material should not be included among what was absorbed, which would indicate that 1.5 percent was absorbed. If the material not accounted for was equally likely to have been absorbed as it is that it was not absorbed, then the recommended approach is to normalize the fraction absorbed by the percent recovery, which would indicate that 1.8 percent was absorbed (1.5/0.82). Although similar in magnitude, EPA opted to adjust the absorption based on the recovery and therefore considered dermal absorption to be 1.8 percent.

As described further in the *Environmental Release and Occupational Exposure Assessment for Diisodecyl Phthalate (DIDP)* (U.S. EPA, 2024f) and *Consumer and Indoor Dust Exposure Assessment for Diisodecyl Phthalate (DIDP)* (U.S. EPA, 2024b), for the risk evaluation of DIDP, EPA used data from the *in vivo* dermal absorption study of DIDP with rats (Elsisi et al., 1989) to estimate dermal absorptive flux, which is used to calculate occupational and consumer dermal exposure estimates.

3 NON-CANCER HAZARD IDENTIFICATION

3.1 Key Human Health Hazard Outcomes

The sections below focus on hazard identification, characterization, and evidence integration of developmental toxicity (Section 3.1.1) and liver toxicity (Section 3.1.2). These are the most sensitive human health hazard outcomes associated with oral exposure to DIDP in laboratory animals. In the risk evaluation of DIDP, developmental toxicity forms the basis of the POD used for acute (≤1 day), intermediate (>1–30 days), and chronic (>90 days) exposure scenarios.

An adequate toxicological database is available for DIDP. Available studies include the following: one intermediate duration inhalation study of rats (General Motors, 1983b); seven intermediate duration oral exposure studies (5 of rats, 2 of mice) (Chen et al., 2019; Kwack et al., 2010; Kwack et al., 2009; Smith et al., 2000; Lake et al., 1991; BIBRA, 1990, 1986); three subchronic dietary studies (2 in rats, 1 in beagles) (BASF, 1969; Hazelton Labs, 1968a, b); two chronic dietary studies (1 of each of rats and mice) (Cho et al., 2011; Cho et al., 2010; Cho et al., 2008); two prenatal developmental studies of rats (Waterman et al., 1999; Hellwig et al., 1997); one developmental/reproductive toxicity screening study of mice (Hazleton Labs, 1983); and a pair of two-generation dietary studies of rats (Hushka et al., 2001; Exxon Biomedical, 2000, 1998). No repeated dose studies investigating the systemic toxicity of DIDP are available for the dermal route of exposure.

3.1.1 Developmental Toxicity

Humans

Several epidemiologic studies investigating associations between urinary metabolites of DIDP and several developmental outcomes have been identified by EPA and other organizations. Health Canada (2018b) evaluated multiple studies that investigated the association between urinary metabolites of DIDP and several developmental outcomes, including birth measures (*i.e.*, birth weight, birth length, head circumference), preterm birth (births occuring before 37 weeks of gestation) and gestational age, and postnatal growth in infants and children (*i.e.*, body mass index, height, weight, head circumference, bone age, and bone age to chronological age ratio). Across available studies of DIDP, Health Canada found no evidence of association for urinary mono-(carboxynonyl) phthalate (MCNP), a metabolite of DIDP, and birth measures, preterm birth, or gestational age. The level of evidence of association for postnatal growth could not be established due to limitations in the database.

EPA identified three new medium quality studies that evaluated the association between urinary DIDP levels of one metabolite (MCNP) and developmental outcomes (Heggeseth et al., 2019; Mustieles et al., 2019; Philippat et al., 2019). All three identified studies were prospective cohort studies. Philippat et al. (2019) followed 457 mother-son pairs of the EDEN (Etude des Déterminants pré et postnatals du développement et de la santé de l'Enfant) cohort from France and evaluated the relationship between gestational exposure to MCNP (based on maternal urine spot samples collected during weeks 23 to 29 of gestation) and placental weight, birth weight, and placental-to-birth weight ratio. No association between maternal urinary MCNP levels and birth weight was found based on adjusted elastic net (ENET) penalized regression models. MCNP was negatively associated with both placental weight (β= −10.9 g [95% CI: −21.8, 0.09]) and the placental-to-birth weight ratio (−0.20 [95% CI: −0:54, 0.13]) by the ENET model.

In another cohort study, Mustieles et al. (2019) followed 68 fathers and 132 mothers, and their corresponding 132 singletons enrolled in the Environment and Reproductive Health (EARTH) study in Massachusetts. No association was observed between placental weight, birth weight, or the placental-to-

birth weight ratio and urinary MCNP levels collected during three different exposure window, including prior to conception for men and women (paternal preconception and maternal preconception) and during each trimester during pregnancy (median: 6, 21, 35 weeks of gestation).

Finally, Heggeseth at al. (2019) evaluated the relationship between prenatal DIDP exposure (based on maternal urinary MCNP levels) and body mass index trajectories in 335 children between ages 2 to 14 years from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort in California. No significant association between prenatal urinary MCNP and body mass index trajectories was identified for boys or girls.

Laboratory Animals

DIDP has been evaluated for developmental toxicity in several oral exposure studies, including two prenatal developmental studies of rats (Waterman et al., 1999; Hellwig et al., 1997), one developmental/reproductive toxicity screening study of mice (Hazleton Labs, 1983), and a pair of two-generation studies of reproduction of rats (Hushka et al., 2001). No studies of development are available for the dermal or inhalation exposure routes. Available studies are summarized in Appendix C.1, and discussed further below.

Additionally, several studies have evaluated the antiandrogenic effects of DIDP on the developing male reproductive system following gestational exposure during the critical window of development (*e.g.*, (Furr et al., 2014; Hannas et al., 2012)). Unlike other phthalate diesters (*e.g.*, DEHP), the available evidence indicates that DIDP does not induce effects on the developing male reproductive system consistent with a disruption of androgen action. Experimental evidence supporting this conclusion is discussed in EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (U.S. EPA, 2023b). EPA's conclusion was supported by the Science Advisory Committee on Chemicals (SACC) (U.S. EPA, 2023d) and the anti-androgenicity of DIDP is not discussed in detail in this document.

Dose-related increases in skeletal and visceral variations have consistently been observed in prenatal developmental studies of SD and Wistar rats at doses lower than those that caused maternal toxicity (Waterman et al., 1999; Hellwig et al., 1997). In the first study, which adhered to EPA 798.4900 (40 CFR Part 798, 1985), Waterman et al. (1999) gavaged pregnant SD rats (22–25 per dose) with 0, 100, 500, and 1,000 mg/kg-day DIDP on GDs 6 through 15. Statistically significant and dose-related increases in incidence of skeletal variations, including rudimentary lumbar ribs and supernumerary cervical ribs, were observed at 500 and 1,000 mg/kg-day (Table_Apx C-2), supporting a developmental NOAEL of 100 mg/kg-day. In the second study, Hellwig et al. (1997) gavaged pregnant Wistar rats with 0, 40, 200, and 1,000 mg/kg-day DIDP on GDs 6 through 15. The study was Good Laboratory Practice (GLP)-compliant and generally adhered to EPA 798.4900 (40 CFR pat 798, 1992), with the exception that 10 dams, instead of 20 were employed per dose group. Statistically significant and dose-related increases in the number of fetuses per litter with total variations (combined visceral [*i.e.*, dilated renal pelvis, hydroureter]) and skeletal variations (*i.e.*, rudimentary lumbar ribs and accessory 14th rib[s]) were observed at 200 and 1,000 mg/kg-day (Table_Apx C-3), supporting a developmental NOAEL of 40 mg/kg-day.

One study provided no evidence of developmental toxicity in mice (Hazleton Labs, 1983). As part of a screening study, pregnant CD-1 mice (50 per dose) were gavaged with 0 and 9,670 mg/kg-day DIDP on GDs 7 through 14, allowed to deliver pups naturally, and then sacrificed on PND3. No effects on the number of live pups per litter, mean litter weight, or mean pup weight per litter on PND1 or PND3 were

observed; however, no other developmental outcomes were evaluated potentially limiting the sensitivity of the study.

Dose-related, effects on offspring bodyweight gain, live births, and offspring survival have also been observed in a preliminary dose-range finding one-generation study and a pair of two-generation studies of reproduction with SD rats (termed Studies A and B), which were GLP-compliant and adhered to EPA draft Guideline 870.3800 (1994) (Hushka et al., 2001; Exxon Biomedical, 2000, 1998). Across available studies of reproduction, no treatment-related effects on any reproductive or fertility indices were observed. Further, across available studies of reproduction, developmental toxicity occurred at doses lower than those than those that caused overt parental toxicity, with the exception of increased liver and kidney weight (discussed further in Sections 3.1.2 and 3.2.1). In the first two-generation study (Study A), SD rats were continuously administered dietary concentrations of 0, 0.2, 0.4, and 0.8 percent DIDP (mean received doses in units of mg/kg-day reported in Table_Apx C-4) starting 10 weeks prior to mating, throughout mating, gestation, and lactation, until terminal sacrifice for two generations (Hushka et al., 2001; Exxon Biomedical, 1998). For F1 offspring, developmental effects were limited to the high-dose group and included decreased live births and survival on PND4 (Table_Apx C-5), and decreased male (6–23%) and female (4–20%) offspring body weight on PND0 through PND21 (Table_Apx C-6). For F2 offspring, effects included a dose-related decrease in offspring survival on PND1 and PND4 in all treatment groups, decreased survival on PND7, and viability at weaning in the high-dose group (Table_Apx C-5). High-dose F2 offspring also exhibited decreased body weight (9–22% in males and 6–21% in females) from PND0 through PND21 (Table_Apx C-6). EPA identified a developmental LOAEL (no NOAEL identified) of 0.2 percent DIDP (equivalent to 135 mg/kg-day) based on reduced F2 offspring survival on PND1 and PND4.

In the second two-generation study (Study B), male and female SD rats were continuously administered dietary concentrations of 0, 0.02, 0.06, 0.2, and 0.4 percent DIDP starting 10 weeks prior to mating, throughout mating, gestation, and lactation, until terminal sacrifice for two generations (Hushka et al., 2001; Exxon Biomedical, 2000). Mean received doses in units of mg/kg-day are shown in Table_Apx C-7. No developmental effects were observed in F1 offspring at any dose. However, consistent with the first two-generation study (Study A), a significant, dose-related, reduction in F2 survival on PND1 and PND4 in the 0.2 and 0.4 percent DIDP treatment groups was observed (Table_Apx C-8). EPA identified a developmental NOAEL of 0.06 percent (equivalent to 38 mg/kg-day) based on reduced F2 offspring survival on PND1 and PND4 at the LOAEL of 0.2 percent DIDP (equivalent to 134 mg/kg-day).

Mechanistic Information

Mechanisms underlying the developmental toxicity of DIDP have not been established. As discussed in EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act February* (U.S. EPA, 2023b) and endorsed in the SACC's Final Report (U.S. EPA, 2023d), DIDP is not antiandrogenic (Furr et al., 2014; Hannas et al., 2012; Hushka et al., 2001).

Available studies also indicate that DIDP is not an estrogen agonist or antagonist. DIDP showed no estrogenic activity in *in vivo* uterotrophic and vaginal cornification assays with SD rats (Zacharewski et al., 1998). *In vitro*, DIDP showed no estrogen receptor agonist or antagonist activity in Chinese hamster ovary cells transfected with either human estrogen receptor alpha or beta gene reporters (Takeuchi et al., 2005). Additionally, DIDP showed no competitive binding to the rat uterine estrogen receptor in a competitive ligand-binding assay in SD rat uterine homogenates; failed to induce estrogen receptor mediated gene expression in MCF-7 cells; did not induce estrogen receptor-mediated growth in yeast transformed with human estrogen receptor (Zacharewski et al., 1998); and was negative for estrogenic

activity in a recombinant yeast assay (Harris et al., 1997) and in a yeast two-hybrid assay (Nishihara et al., 2000).

Although available studies indicate that DIDP has no effect on estrogen- or androgen-related responses, studies investigating effects on the thyroid and adrenal endocrine systems, including effects on thyroid hormones or corticosterone levels, were not identified by EPA.

Conclusions on Developmental Toxicity

Consistent, dose-related effects on development were observed across available experimental studies of rodent models. In two prenatal studies, increased incidences of skeletal and visceral variations were observed in SD and Wistar rats (Waterman et al., 1999; Hellwig et al., 1997). In both studies, there was a dose-dependent increase in the incidence of variations, which occurred starting at doses that elicited no maternal toxicity. The biological significance of the observed increases in skeletal and visceral variations are difficult to assess. However, EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991b) states that, "if variations are significantly increased in a dose-related manner, these should also be evaluated as a possible indication of developmental toxicity" and "Agents that produce developmental toxicity at a dose that is not toxic to the maternal animal are especially of concern." Although rudimentary ribs may be transient and disappear during postnatal development, supernumerary cervical ribs are likely permanent and may ultimately become distinct ribs (Makris et al., 2009). Supernumerary ribs may be the result of abnormal gene expression and may interfere with blood flow and nerve function (Chernoff and Rogers, 2004). Therefore, EPA considered the increase in skeletal and visceral variations following gestational exposure to DIDP to be treatment-related adverse effects.

Effects on developing offspring have also been observed consistently in a pair of two-generation studies of reproduction of SD rats conducted by Exxon Biomedical (2000, 1998) and reported in Hushka et al. (2001). Observed effects include dose-related decreases in F1 and F2 offspring bodyweight and weight gain (Study A only), reduced live births (Study A only) and reduced F1 (Study A only) and F2 offspring survival on PND1 and PND4 (Study A and B). Notably, across the two studies, F2 offspring survival on PND1 and PND4 was consistently reduced at doses lower than those that reduced F1 offspring survival. Effects on F2 offspring survival occurred at doses at which no effects were observed on parental survival, body weight, or food consumption for either sex—indicating the effects were not secondary to parental toxicity.

There are several areas of uncertainty related to the developmental toxicity of DIDP. First, the mechanisms underlying the observed developmental effects have not been established, which makes it difficult to determine their human relevancy. Second, it is difficult to determine consistency across species, because evidence of developmental toxicity has only been observed in rat models. In the one available study of mice (Hazleton Labs, 1983), which tested one high-dose (9,670 mg/kg-day) of DIDP, no effects on F1 offspring survival or weight were observed on PND1 or PND3. However, that study is limited by the small number of evaluated outcomes and the timing of DIDP administration, which could further affect study sensitivity (*i.e.*, mice were exposed on GD 7–14; current OECD TG 414 recommend dosing from implantation to the day prior to scheduled caesarean section (OECD, 2018)). Third, there is uncertainty about the effect on humans, because human epidemiological studies generally did not identify effects in offspring (other than an association with placental weight). However, current DIDP exposure levels for the U.S. population based on NHANES urinary biomonitoring data are approximately four orders of magnitude below the exposure levels that cause developmental toxicity in rats, which may also explain the lack of observed developmental effects in human epidemiologic studies. For example, EPA estimated median and 95th percentile daily intake values for DIDP to be 1.17

and 3.5 µg/kg-day, respectively, for women of reproduction age in the 2017 to 2018 NHANES cycle (see EPA's *Environmental Media and General Population Exposure for Diisodecyl Phthalate (DIDP)* (U.S. EPA, 2024e)), compared to a human equivalent dose of 9,000 µg/kg-day (discussed further in Section 6.1.1) based on a NOAEL of 38,000 µg/kg-day for reduced F2 offspring survival on PND1 and PND4 in the study by Hushka et al. (2001). Further limitations associated with the available epidemiological studies related to exposure misclassification due to use of a single spot urine sample in several studies, periods of heightened susceptibility and timing of exposure assessment, and phthalate mixture effects. Until these limitations are addressed, results from the available epidemiological studies of DIDP should be interpreted with caution.

Although uncertainty exists, EPA considers the evidence of developmental effects observed across two prenatal studies of rats and a pair of two-generation studies of rats to provide strong evidence to support the conclusion that DIDP is a developmental toxicant in experimental animal models. The observed developmental effects are assumed to be relevant for extrapolating human risk. Developmental toxicity is considered further for dose-response assessment in Section 6. Notably, EPA's conclusion is consistent with that of other regulatory and authoritative bodies. NTP-CERHR (2003), European Chemicals Bureau (ECB, 2003), ECHA (2013b), EFSA (2019, 2005), Australia NICNAS (2015, 2008a, b), Health Canada (EC/HC, 2015) and U.S. CPSC (2014, 2010) have all consistently concluded that oral exposure to DIDP causes developmental toxicity in experimental animal models and is relevant for estimating human risk.

3.1.2 Liver Toxicity

Humans

No epidemiologic studies have been identified by EPA or other organizations for liver injury for DIDP and/or its metabolites.

Laboratory Animals

Liver effects of DIDP have been consistently reported in intermediate (>1–30 days), subchronic (>30–90 days) and chronic (>90 days) exposure studies. Available studies include: one intermediate duration inhalation study of rats (General Motors, 1983b); seven intermediate duration oral exposure studies (5 of rats, 2 of mice) (Chen et al., 2019; Kwack et al., 2010; Kwack et al., 2009; Smith et al., 2000; Lake et al., 1991; BIBRA, 1990, 1986); three subchronic oral exposure studies (2 of rats, 1 of beagles) (BASF, 1969; Hazelton Labs, 1968a, b); two chronic oral exposure studies (1 of each of rats and mice) (Cho et al., 2011; Cho et al., 2010; Cho et al., 2008); one prenatal developmental study of rats (Hellwig et al., 1997); and a pair of two-generation studies of rats (Hushka et al., 2001; Exxon Biomedical, 2000, 1998). No studies for the dermal route of exposure are available. Available studies are summarized in Appendix C.2, and discussed further below.

Considerations for Interpretation of Hepatic Effects: Consistent with previous guidance (Hall et al., 2012; U.S. EPA, 2002a), EPA considered hepatocellular hypertrophy and corresponding increases in liver size and weight to be adaptive non-adverse responses, unless accompanied by treatment-related, biologically significant changes (*i.e.*, two- to three-fold) in clinical markers of liver toxicity; that is, decreased albumin; or increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), bilirubin, cholesterol) and/or histopathology indicative of an adverse response (*e.g.*, hyperplasia, degeneration, necrosis, inflammation). Further, it is well documented that phthalates, including DIDP, can induce peroxisome proliferation in the livers of mice and rats, and there is evidence supporting a role for peroxisome-proliferator-activated receptor alpha (PPARα) activation in peroxisome-induced hepatic effects of DIDP. For purposes of identifying study no-observed-adverse-effect level (NOAEL) and LOAEL values,

effects consistent with peroxisome proliferation and PPARα activation were also considered relevant for setting the NOAEL and LOAEL.

Intermediate Duration Studies (>1–30 Days): Across available intermediate duration studies, consistent, treatment-related, effects on the liver are observed, including increased relative and absolute liver weight and increased biomarkers of peroxisome proliferation. Biologically significant changes in serum chemistry and histopathologic lesions are less commonly reported following intermediate duration oral exposure to DIDP.

Kwack et al. conducted two studies in which male SD rats were gavaged with 0 or 500 mg/kg-day DIDP for 2 (2010) or 4 weeks (2009). Both studies observed a 30 to 39 percent increase in relative liver weight (absolute weight not reported) and a 67 percent increase in serum ALP; however, other serum markers of liver toxicity (*e.g.,* ALT, AST, GGT, bilirubin) were unaffected and histopathology was not evaluated. Similarly, in a prenatal study of Wistar rats, relative and absolute liver weight were increased 9 to 13 percent in dams dosed with 1,000 mg/kg-day DIDP on GDs 6 through 15 (Hellwig et al., 1997).

Three additional studies in mice and rats provide additional evidence of liver effects, including increased liver weight and increased hepatic expression of biomarkers of PPARα activation. Smith et al. (2000) reported 25 to 50 percent increases in relative liver weight and 3- to 8-fold increases in peroxisomal beta-oxidation (biomarker of PPARα activation) in male B6C3F1 mice and male F344 rats administered approximately 600 and 900 mg/kg-day DIDP, respectively, in the diet for 2 and 4 weeks. In another study in which male F344 rats were fed diets containing 0, 0.02, 0.05, 0.1, 0.3 or 1.0 percent DIDP (equivalent to 25, 57, 116, 353, 1,287 mg/kg-day) for 28-days, a 9 to 120 percent increase in relative liver weight and increased palmitoyl-CoA oxidase activity was increased at 0.1 percent DIDP and above. Histologic findings were limited to the high-dose group and included increased incidence of cytoplasmic eosinophilia and hepatocellular hypertrophy (BIBRA, 1990). In the third study (BIBRA, 1986), male and female F344 rats were fed diets containing 0, 0.3, 1.2, or 2.5 percent DIDP (equivalent to 304, 1,134, and 2,100 mg/kg-day in males; 264, 1,042, and 1,972 mg/kg-day in females) for 21 days. Observed hepatic effects included: increased (21–154%) absolute and relative liver weight in both sexes in the mid- and high-dose groups; decreased hepatic cytoplasmic basophilia in both sexes in the mid- and high-dose groups and increased eosinophilia in both sexes at in the high-dose group; increased hepatic cyanide-insensitive palmitoyl-CoA oxidase activity in both sexes at 1.2 percent DIDP and above and increased hepatic lauric acid 11- and 12-hydroxylase activity in males at all doses and 12-hydroxylase activity in high-dose females. Additionally, electron microscopy demonstrated marked to very marked increases in peroxisome number and size in both sexes in the high-dose group.

EPA identified one new intermediate duration study that evaluated liver toxicity. Chen et al. (2019) gavaged male Balb/c mice (8/dose) with 0, 0.15, 1.5, 15, or 150 mg/kg-day DIDP for 14 days. Histopathologic findings in the liver were described qualitatively only (incidence data were not reported; no statistical analyses were performed). At 15 mg/kg-day, histological observations included, "broadened liver cords, expanded cells, and contracted liver sinuses," and liver sections were described as "fuzzy and edematous with extremely loose cytoplasm" at 150 mg/kg-day. Serum AST levels were significantly increased at 15 mg/kg-day and above while serum ALT was increased at 150 mg/kg-day and serum albumin was reduced at 150 mg/kg-day. The magnitude of changes in serum chemistry parameters was uncertain as data were presented graphically only and appeared variable. Liver weight and other serum markers of liver toxicity (ALP, GGT, bilirubin, and cholesterol) were not evaluated.

No histopathological findings were noted in the livers of male SD rats exposed to 505 mg/m^3 DIDP aerosol (mass median aerodynamic diameter [MMAD] = 0.98 µm) via whole-body inhalation for 6

hours/day, 5 days/week for 2 weeks (General Motors, 1983b). However, this study is limited by the timing of the histopathologic examination (*i.e.*, 3-weeks post-exposure) and lack of examination of organ weights and clinical chemistry.

Subchronic Studies (>30–90 Days): Increased absolute and/or relative liver weight has been consistently reported in two subchronic dietary studies of rats without any accompanying changes in clinical chemistry, urinalysis parameters, or histopathology (BASF, 1969; Hazelton Labs, 1968b). In the first study, male and female albino rats were fed diets contain 0, 500, 3,000, and 10,000 ppm DIDP (equivalent to 28, 170, 586 mg/kg-day for males; 35, 211, 686 mg/kg-day for females) for 90 days and a 35 to 67 percent increases in absolute and relative liver weight was observed in high-dose rats of both sexes (Hazelton Labs, 1968b). Similarly, in the second 90-day dietary study of Wistar rats, which was only available to EPA as a foreign language study in German ((BASF, 1969) as reported in (EC/HC, 2015; ECHA, 2013b; ECB, 2003)), male and female Wistar rats were fed diets containing 0, 800, 1,600, 3,200, or 6,400 ppm DIDP (equivalent to 55, 100, 200, 400 mg/kg-day for males; 60, 120, 250, 500 mg/kg-day for females). Absolute liver weight was increased 31 percent in high-dose males and 16 to 33 percent in females at 250 mg/kg-day and above, while treatment-related increases in relative liver weight were observed in females (but not males) at 120 mg/kg-day DIDP and above.

Consistent with findings from studies of mice and rats, liver effects have also been observed in a 13-week study of beagles (Hazelton Labs, 1968a). Male and female beagles (three per sex per dose) were fed diets containing 0, 500, 3,000, or 10,000 ppm DIDP (equivalent to 15, 75, 300 mg/kg-day) for 13 weeks. Mean absolute and relative liver weight appeared increased in high-dose males (25–37%) and females (44–51%); however, a statistical analysis was not conducted due to the small sample size. Slight to moderate swelling and vacuolation of hepatocytes was observed in mid- and high-dose males (incidence: 0/3, 0/2, 2/3, 1/3) and females (incidence: 0/3, 0/3, 2/3, 3/3). Clinical markers of hepatotoxicity were similar to control values (*i.e.*, AST, ALT, ALP, and bromsulphthalein clearance). Although this study is limited by its small sample size and lack of statistical analysis, there is consensus across existing assessments of DIDP by U.S. CPSC (2014), ECHA (2013b), EFSA (2019), Health Canada (ECCC/HC, 2020), and NICNAS (2015) that the study supports a NOAEL of 15 mg/kg-day, based on increased liver weight and histopathological findings (swelling and vacuolation of hepatocytes).

Chronic (>90 Days) Exposure: Similar to what has been reported in intermediate duration and subchronic studies of DIDP, dose-related increases in relative and/or absolute liver weight have also been consistently reported in chronic duration studies of DIDP. However, unlike intermediate duration and subchronic studies, histopathologic findings consistent with liver toxicity (*e.g.*, necrosis, spongiosis hepatis, parenchymal inflammation) have also been consistently been observed across available chronic studies of DIDP, including a 26-week study of wild-type and rasH2 transgenic mice (Cho et al., 2011), a 2-year study of F344 rats (Cho et al., 2010; Cho et al., 2008), and a two-generation study of SD rats (Hushka et al., 2001; Exxon Biomedical, 2000, 1998).

Cho et al. (2010; 2008) administered 0, 400, 2,000, 8,000 ppm DIDP to male and female F344 rats in the diet for 2 years (equivalent to 22, 110, and 479 mg/kg-day for males; 23, 128, and 620 mg/kg-day for females) and observed a 40 to 49 percent increase in relative liver weight in high-dose males and females (Cho et al., 2010; Cho et al., 2008). Evidence of peroxisome proliferation was apparent in the livers of high-dose males after 12 weeks, as demonstrated by increased expression of catalase protein and catalase activity. However, evidence of peroxisome proliferation was no longer apparent after 32 and 104 weeks of exposure. Non-neoplastic lesions that were statistically significantly increased in the liver included necrosis in high-dose males and females; oval cell hyperplasia, hypertrophy, and peliosis

in high-dose males; and microgranuloma and spongiosis hepatis in males at all dose levels (Table_Apx C-11)—supporting a LOAEL of 22 mg/kg-day. Similar results were obtained in a 26-week study of mice by Cho et al. (2011). Male and female wild-type mice were fed diets containing 0 or 1.0 percent DIDP (equivalent to approximately 1,500 mg/kg-day), while male and female transgenic rasH2 mice were fed diets containing 0, 0.1, 0.33, and 1.0 percent DIDP (equivalent to approximately 150, 495, 1,500 mg/kg-day) for 26 weeks. Relative (absolute not reported) liver weight increased 59 to 72 percent in high-dose wild-type mice of both sexes, 15 to 52 percent for mid- and high-dose rasH2 males, and 35 percent for high-dose rasH2 females. As shown in Table_Apx C-10, treatment-related histopathologic findings were observed in male and female wild-type and rasH2 mice—including parenchymal inflammation, focal necrosis, diffuse hepatocyte hypertrophy with eosinophilic granules, pigmented hepatocytes, pigmented Kupffer cells, and prominent Kupffer cells.

In a pair of two-generation studies of reproduction of SD rats (described in Section 3.1.1 and Appendix C.1), dose-related increases relative and absolute liver were observed in males and females of both parental generations (Hushka et al., 2001; Exxon Biomedical, 2000, 1998). In the first study (Study A), absolute and/or relative liver weight was significantly increased 11 to 29 percent in P1 and P2 males at 0.4 percent DIDP and above and 9 to 28 percent in P1 and P2 females at 0.2 percent DIDP and above (Hushka et al., 2001; Exxon Biomedical, 1998). Similarly, in the second study (Study B), absolute and/or relative liver weight was significantly increased 12 to 14 percent in P1 and P2 males and P1 females at 0.4 percent DIDP (highest dose tested) and 9 to 23 percent in P2 females at 0.2 percent DIDP and above. Histopathology examinations were only included in Study A. Notably, liver weight changes in Study A were accompanied by increased centrilobular or diffuse hepatocellular hypertrophy in P1 and P2 males and females at all doses, and the incidence and severity of the lesion increased with dose (Table_Apx C-12). Additionally, minimal to mild focal necrosis was observed in P1 males at 0.8 percent DIDP and P2 males at 0.4 percent DIDP and above but was not observed in P1 or P2 females. The liver effects observed in P1 and P2 females are consistent with an adaptive, non-adverse response. However, the increased incidence of focal necrosis in the livers of high-dose P1, and mid- and high-dose P2 males is adverse, supporting a NOAEL of 0.2 percent DIDP (equivalent to 117 mg/kg-day) based on increased incidence of necrosis at the LOAEL of 0.4 percent DIDP (equivalent to 229 mg/kg-day).

Mechanistic Information

DIDP is widely considered to be a PPARα activator. In an *in vitro* study, Bility et al. (2004) investigated the ability of MIDP to activate mouse and human PPARα, PPARγ, and PPARβ receptors using a trans-activation assay. MIDP activated both mouse and human PPARα and PPARγ, and mouse (but not human) PPARβ. Mouse PPARα was activated at lower concentrations of MIDP than human PPARα (lowest PPARα activation concentration 3 µM vs. 30 µM for human PPARα), and MIDP was a more potent inducer of mouse PPARα than human PPARα (maximal fold-induction of mouse and human PPARα were 26.9 and 3.9, respectively).

Consistent with activation of PPARα, intermediate duration *in vivo* studies with rats and mice have consistently demonstrated that oral exposure to DIDP can increase peroxisome number and size in hepatocytes, increase hepatic catalase, carnitine acetyl transferase, cyanide-insensitive palmitoyl-CoA oxidase and 11- and 12-hydroxylase activities, and increase hepatocyte peroxisomal beta-oxidation (Smith et al., 2000; BIBRA, 1990, 1985). Notably, biomarkers of PPARα activation increase at doses equivalent to or lower than those that cause increases in liver weight and hepatocellular hypertrophy *in vivo*. Peroxisome proliferation has also been examined after subchronic and chronic oral exposure to DIDP. Cho et al. (2008) fed male F344 rats diets containing 0, 400, 2,000, and 8,000 ppm DIDP and 12,000 ppm DEHP for 12 and 32 weeks and then evaluated hepatic catalase protein levels and catalase activity. After 12 weeks of exposure, hepatic catalase protein levels and activity were increased in rats

fed 8,000 ppm DIDP and 12,000 ppm DEHP. However, the effect on catalase levels and activity were no longer significant after 32 weeks of exposure to DIDP but remained elevated in rats exposed to DEHP. These results indicate peroxisome proliferation was not sustained with chronic exposure to DIDP.

One intermediate duration (14-day) *in vivo* study is available that provides evidence to suggest oral exposure to DIDP can induce oxidative stress, inflammation, and apoptosis in the liver of male Balb/c mice (Chen et al., 2019). Evidence of oxidative stress was limited to the livers of mice treated with 150 mg/kg-day DIDP and included increased ROS, malondialdehyde, and 8-hydroxy-2-deoxyguanosine levels, and decreased glutathione. Markers of inflammation and apoptosis included increased interleukin-1β and tumor necrosis factor-α content at 15 and 150 mg/kg-day, increased nuclear factor-κB levels in the liver at doses as low as 0.15 mg/kg-day, and increased caspase-3 levels in the liver at 150 mg/kg-day. Co-administration of vitamin E and DIDP attenuated markers of oxidative damage, inflammation, and apoptosis, further implicating a role for oxidative stress in the liver.

Conclusions on Liver Toxicity

No epidemiological studies evaluating DIDP exposure and liver effects were identified.

Across available intermediate, subchronic, and chronic oral exposure studies of rats, mice, and dogs, consistent, dose-related liver effects are observed. The most sensitive liver effect observed following oral exposure to DIDP was spongiosis hepatis. Cho et al. (2010; 2008) observed a statistically significant increase in the incidence of spongiosis hepatis in male F344 rats chronically exposed to DIDP through the diet for 2 years at 22 mg/kg-day DIDP (lowest dose tested), supporting a LOAEL of 22 mg/kg-day. However, several sources of uncertainty are associated with that study. First, although the incidence of spongiosis hepatis in male rats is statistically significantly increased in all dose groups, the dose-response for this lesion is flat, particularly in the low- and mid-dose groups. Second, spongiosis hepatis was not observed in female F344 rats in the chronic study by Cho et al. and has not been reported in any other studies of DIDP—including intermediate duration studies of rats that tested up to 2,100 mg/kg-day DIDP, subchronic studies of rats that tested up to 586 to 686 mg/kg-day DIDP, and a 26-week study of mice that tested 1,500 mg/kg-day DIDP (Table_Apx C-9). Finally, the MOA underlying spongiosis hepatis is unknown but is not believed to be related to peroxisome proliferation. Further, as discussed by ECHA (2013b), spongiosis hepatis has been observed in the livers of some strains of rats and certain species of fish (*e.g.*, medaka), but is (1) less common in mice; (2) has not been observed in non-human primates or dogs; and (3), with the exception of two case reports, has not been described in humans. These findings raise some uncertainty to the human relevance of spongiosis hepatis (Karbe and Kerlin, 2002).

EPA considers evidence of liver toxicity observed across intermediate, subchronic, and chronic duration studies of rats, mice, and beagles to provide strong evidence to support the conclusion that DIDP is a liver toxicant in experimental animal models. Liver toxicity is considered further for dose-response assessment in Section 6. Notably, EPA's conclusion is consistent with other existing assessments of DIDP, which have also identified the liver as a sensitive target organ and that liver toxicity is relevant for extrapolating human risk (EFSA, 2019; EC/HC, 2015; NICNAS, 2015; ECHA, 2013b; U.S. CPSC, 2010; EFSA, 2005; ECB, 2003; NTP-CERHR, 2003).

3.2 Other Human Health Hazard Outcomes

EPA identified new literature published between 2014 to 2024 that indicated potentially sensitive effects in laboratory animals orally exposed to DIDP related to kidney toxicity, neurotoxicity, and immune system toxicity. Sections 3.2.1, 3.2.2, and 3.2.3 describes hazard identification and evidence integration

for kidney toxicity, neurotoxicity, and immune system toxicity, respectively. Based on the results of evidence integration, none of these other human health hazard outcomes were considered to be critical to the risk evaluation of DIDP.

3.2.1 Kidney Toxicity

Humans

EPA identified one new high quality cross-sectional study (Malits et al., 2018). The study investigated both DIDP and its urinary metabolite (MIDP) and their association with estimated glomerular filtration rate, urinary protein-to-creatinine ratio, and systolic blood pressure in a total of 538 male and female children aged 1 to 17 years. The participants were part of the Chronic Kidney Disease in Children (CKiD) study, which is a multi-center prospective cohort study of children with mild to moderate impaired kidney function in the United States. Urinary MIDP levels corrected for creatinine were associated with a −0.74 unit change in estimated glomerular filtration rate (95% confidence interval: −1.26, −0.21; p = 0.007) by univariate (adjusted for urinary creatinine), but not multivariate (adjusted for body mass index (BMI), demographic characteristics, birthweight, prematurity, presence of glomerular disease, use of relevant medications (*e.g.*, ACE-inhibitors), urinary creatinine and cotinine), analyses. Urinary MIDP was not associated with any other outcomes.

Laboratory Animals

Intermediate (>1–30 Days) Exposure Studies: Effects on the kidney following intermediate duration oral exposure to DIDP are inconsistent and of uncertain toxicological significance. No effects were observed on relative or absolute kidney weight in pregnant Wistar rats gavaged with 0, 40, 200 or 1,000 mg/kg-day DIDP from GD6 to 15 and sacrificed on GD 20 (Hellwig et al., 1997). Similarly, no effects were observed on relative kidney weight (absolute weight not reported), serum chemistry (*i.e.*, blood urea nitrogen [BUN], creatinine), or urinalysis parameters in male SD rats (6 per dose) gavaged with 0 and 500 mg/kg-day DIDP for 28 days (Kwack et al., 2009). In a 14-day study in which male SD rats (6 per dose) were gavaged with 0 and 500 mg/kg-day DIDP, increased red blood cells in urine was reported (Kwack et al., 2010). However, no effects were observed on relative kidney weight (absolute weight not reported), clinical chemistry (*i.e.*, BUN, creatinine) or other urinalysis parameters. In another intermediate duration study, male and female F344 rats were fed diets containing 0, 0.3, 1.2, or 2 percent DIDP (equivalent to 304, 1,134, 2,100 for males and 264, 1,042, 1,972 mg/kg-day for females) for 21 days. Relative (but not absolute) kidney weight increased 10 to 19 percent in males of all dose groups, whereas relative kidney weight in females increased 9.5 percent in the mid- and high-dose groups. Changes in terminal body weight were limited to the high dose (32% decrease in males and 18% decrease in females), suggesting that the observed increase in relative kidney weight in the low- and mid-dose groups are not related to decreased body weight. No histopathological findings were reported in the kidneys of either sex at any dose (BIBRA, 1986).

EPA identified one new medium quality intermediate duration study of DIDP. Chen et al. (2019) gavaged male Balb/c mice (8 per dose) with 0, 0.15, 1.5, 15, or 150 mg/kg-day DIDP for 14 days and then evaluated several biomarkers of kidney toxicity (*i.e.*, serum creatinine and urinary nitrogen), kidney histology, and mechanistic endpoints. Histopathologic findings in the kidney were described qualitatively as follows: “a large reduction of tubular space and extreme edema of epithelial cells in the glomeruli were observed, with increasing damage observed from the DIDP15 and DIDP150 group.” No incidence data was reported, and no statistical analysis was conducted. Serum levels of creatinine were significantly increased at doses of 15 mg/kg-day and higher, whereas urinary nitrogen was increased in the high-dose group. The magnitude of change in these parameters could not be assessed because data was presented graphically only and appeared variable. Kidney weight was not evaluated, and no urinalysis was conducted. Sub-apical biomarkers of oxidative stress and inflammation were elevated in

kidney homogenates. Observed effects included increased ROS and malondialdehyde levels at 1.5 mg/kg-day and above; increased 8-hydroxy-2-deoxyguanosine and decreased glutathione levels at 150 mg/kg-day; and increased interleukin-1β at 150 mg/kg-day and tumor necrosis factor-alpha at 15 mg/kg-day and above. Immunohistochemistry showed increased nuclear factor-κβ at 1.5 mg/kg-day DIDP and above, while Hoechst staining showed increased caspase-3 levels in the kidney at 1.5 mg/kg-day DIDP and above. Collectively, this study provides some indication of effects on apical outcomes at 15 mg/kg-day DIDP and above and sub-apical mechanistic outcomes related to oxidative stress at 1.5 mg/kg-day DIDP and above. However, significant uncertainty remains due to limitations in the study (*i.e.*, histopathology reported qualitatively, uncertainty in the magnitude of changes in serum creatinine and urinary nitrogen, kidney weight not reported).

Subchronic (>30–90 Days) Exposure Studies: Similar to what was observed following intermediate oral exposure, effects on the kidney following subchronic oral exposure to DIDP were inconsistent and of uncertain toxicological significance. No effects were observed on absolute or relative kidney weight, histopathology, serum chemistry (*e.g.*, BUN), or urinalysis parameters in a 13-week study of male and female beagles (3 per sex per dose) at doses as high as 300 mg/kg-day DIDP (Hazelton Labs, 1968a) or in a 90-day study of male and female SD rats at doses as high as 400 (males) to 500 (females) mg/kg-day DIDP [(BASF, 1969) as reported in (EC/HC, 2015; ECB, 2003)]. In a 90-day study in which male and female albino rats were fed diets containing 0, 500, 3,000, or 10,000 ppm DIDP (equivalent to 28, 170, 586 mg/kg-day for males; 35, 211, 686 mg/kg-day for females), relative (but not absolute) kidney weight was increased 24 to 33 percent in mid- and high-dose males (but not females) (Hazelton Labs, 1968b). No effects on terminal body weight were observed for either sex at any dose. No histological findings, changes in serum chemistry (*e.g.*, BUN), or urinalysis parameters were observed for either sex.

Chronic (>90 Days) Exposure Studies: Kidney toxicity has been observed in two chronic oral exposure studies of rats and mice. Cho et al. (2011) administered 0, 0.1, 0.33, or 1.0 percent DIDP in feed (equivalent to 150, 495, 1,500 mg/kg-day) to male and female transgenic rasH2 mice and 0 or 1.0 percent DIDP (equivalent to 1,500 mg/kg-day) to male and female wild-type mice (15 per sex per strain per group) for 26 weeks. In transgenic mice, terminal body weight was reduced in high-dose males (31%) and females (15%). Relative kidney weight (absolute weight not reported) was increased in high-dose males (14%) and females (21%), and a significant increase was observed in tubular basophilia (incidence: 2/15, 0/15, 1/15, 11/15* [* indicates $p < 0.05$]) and hyperplasia (0/15, 0/15, 0/15, 13/15* [* indicates $p < 0.05$]) for high-dose males (but not females). The increased relative (to body weight) kidney weight may reflect decreased body weight. However, absolute organ weight is not reported, raising uncertainty. Similarly, in wild-type mice, a 36 percent increase in relative kidney weight (absolute weight not reported) was observed for females (but not males), and a significant increase in tubular basophilia (incidence: 1/15, 10/15* [* indicates $p < 0.05$]) and hyperplasia (incidence: 0/15, 5/15* [* indicates $p < 0.05$]) was observed for males (but not females). Terminal body weight was reduced 12 (females) to 27 (males) percent, and the increase in relative (to body weight) kidney weight may be related to decreased body weight. However, uncertainties remain because absolute organ weight was not reported.

Cho et al. (2010; 2008) administered 0, 400, 2,000, or 8,000 ppm DIDP in the diet (equivalent to 22, 110, 479 for males and 23, 128, 620 mg/kg-day for females) to male and female F344 rats for 2 years. In the high-dose group, a 26 to 32 percent increase in relative kidney weight (absolute weight not reported) was observed for both sexes, and terminal body weight was reduced 14 (males) to 18 (females) percent in the high-dose group. Histopathological findings were limited to high-dose males and included increased mineralization (incidence: 0/49, 1/48, 1/49, 13/39** [** indicates $p < 0.01$]) and interstitial nephritis (incidence: 2/49, 2/48, 5/49, 7/39** [** indicates $p < 0.01$]).

Kidney toxicity has also been observed in a pair of two-generation studies of reproduction. In the first study (study A), SD rats were continuously administered dietary concentrations of 0, 0.2, 0.4, or 0.8 percent DIDP starting 10 weeks prior to mating, continuing throughout mating, gestation, and lactation, and until terminal sacrifice for two generations (Hushka et al., 2001; Exxon Biomedical, 1998). Received doses in units of mg/kg-day are shown in Table_Apx C-4. As described in Section 3.1.1, effects on body weight and weight gain were generally limited to high-dose females of the first parental generation (P1) and high-dose males and females of the second parental generation (P2). Relative and absolute kidney weight increased 8.8 to 37 percent in P1 and P2 males in all treatment groups, while relative (but not absolute) kidney weight increased 8.8 to 14 percent for P1 and P2 females in the mid- and high-dose groups. Histopathology findings were limited to P1 and P2 males in all dose groups and included increased incidence of granular casts, focal degeneration of cortical tubules, and pigment in tubular epithelial cells (Table_Apx C-12). The study authors speculated that the observed histopathological changes in the kidneys of male rats were consistent with alpha 2u-globulin (α2u-globulin) nephropathy, which is a male rat specific phenomenon (U.S. EPA, 1991a), however, this MOA was not specifically evaluated in that study or by EPA.

In a second two-generation study (Study B), SD rats were continuously administered dietary concentrations of 0, 0.02, 0.06, 0.2, or 0.4 percent DIDP starting 10 weeks prior to mating, continuing throughout mating, gestation, and lactation, and until terminal sacrifice for two generations (Hushka et al., 2001; Exxon Biomedical, 2000). Received doses in units of mg/kg-day are shown in Table_Apx C-7. No effects on P1 or P2 body weight or body weight gain were observed at any dose for either sex. Histopathology was not evaluated in this study. Relative and absolute kidney weight increased 15 to 18 percent for P1 males at 0.4 percent DIDP and 6.9 to 20 percent for P2 males at 0.2 percent DIDP and above. Relative (but not absolute) kidney weight increased 6.0 percent for high-dose P1 females. Treatment-related effects on relative and absolute kidney weight were not observed for P2 females.

Conclusions on Kidney Toxicity

One epidemiological cross-sectional study is available that provides limited evidence of an association between urinary MCNP levels and decreased estimated glomerular filtration rate in children with mild to moderate impaired kidney function in the United States (Malits et al., 2018).

Available intermediate (five studies) and subchronic (three studies) duration oral exposure studies of DIDP in rats, mice, and beagles provide limited and somewhat inconsistent evidence of kidney effects that are not well-reported or are of uncertain toxicological relevance. Observed effects were mostly limited to sporadic increases in relative kidney weight (that appear to be unrelated to changes in body weight) and not accompanied by histopathological findings, changes in serum chemistry indicative of impaired kidney function, or significant urinalysis findings. One exception is an intermediate duration study by Chen et al. (2019) that identified histopathological effects and some changes in serum chemistry, but the results were poorly reported (*e.g.*, histopathological changes identified only qualitatively, organ weight not reported).

Available chronic studies of DIDP in rats and mice provide consistent evidence of kidney toxicity. Observed effects include increased incidence of tubular basophilia and hyperplasia in wild-type male (but not female) mice exposed to 1,500 mg/kg-day DIDP for 26-weeks (Cho et al., 2011); increased relative kidney weight and incidence of mineralization and interstitial nephritis in male F344 rats exposed to 479 mg/kg-day DIDP for 2 years (Cho et al., 2010; Cho et al., 2008); and increased relative and absolute kidney weight and incidence of granular casts, pigment in tubular epithelia cells, and focal degeneration of cortical tubules in P1 and P2 male SD rats fed diets containing 0.2 percent DIDP

(equivalent to approximately 117–216 mg/kg-day DIDP) for two generations (Hushka et al., 2001; Exxon Biomedical, 1998).

There are several areas of uncertainty associated with the observed kidney effects. First, the MOA associated with kidney toxicity has not been established. Hushka et al. (2001) speculated that the renal effects observed in male rats exposed to DIDP over two generations were consistent with α2u-globulin nephropathy, which is a male rat specific phenomenon (U.S. EPA, 1991a). However, this MOA has not been specifically evaluated for DIDP in that study or by EPA. Health Canada (EC/HC, 2015) concluded that the histopathology observed in rats chronically exposed to DIDP "could be related to alpha 2 u-globulin nephropathy (rat specific effect in male) and be of limited relevance to human health risk assessment." However, other existing assessments of DIDP by U.S. CPSC (2010), ECHA (2013b), and NICNAS (2015) have not drawn any conclusions regarding α2u-globulin nephropathy. Accumulation of α2u-globulin has been observed in the kidney of male rats following chronic oral exposure to diisononyl phthalate (DINP) (Caldwell et al., 1999), a phthalate structurally similar to DIDP, providing some evidence to support the plausibility of α2u-globulin nephropathy for DIDP. However, α2u-globulin nephropathy cannot explain the observed kidney toxicity at high doses (*i.e.*, 1,500 mg/kg-day) of DIDP in male mice (Cho et al., 2011).

Chronic studies of rats and mice provide consistent evidence of kidney toxicity. However, uncertainty related to the MOA remains. Further, kidney toxicity was only observed following exposure to high-doses of DIDP in mice (*i.e.*, 1,500 mg/kg-day), while kidney toxicity was observed at doses equivalent to or higher than those that caused liver toxicity in chronic studies of rats (Cho et al., 2010; Cho et al., 2008) and developmental toxicity in two-generation studies of rats (Hushka et al., 2001), demonstrating that liver and developmental toxicity were more sensitive outcomes across available studies. Therefore, EPA is not further considering kidney toxicity for dose-response analysis or for use in estimating risk to human health. Consistently, other existing assessments of DIDP by U.S. CPSC (2010), ECHA (2013b), NICNAS (2015), Health Canada (EC/HC, 2015) have also not used kidney effects to extrapolate risk from exposure to DIDP to human health, as liver and developmental toxicity are considered more sensitive and supportable endpoints.

3.2.2 Neurotoxicity

Humans

Several epidemiologic studies investigating associations between urinary metabolites of DIDP and neurological outcomes have been identified by EPA and other organizations. Health Canada (2018a) evaluated multiple studies that investigated the association between DIDP exposure and several behavioral and neurodevelopmental outcomes, including mental and psychomotor neurodevelopment, behavioral and cognitive functioning (*i.e.*, autism spectrum disorders, learning disabilities, attention-deficit disorder, and attention-deficit hyperactivity disorder), neurological function, and gender-related play behaviors. No studies evaluating DIDP and neurodevelopmental outcomes, neurological function, or gender-related play behaviors were identified by Health Canada, and the level of evidence of association for behavioral and cognitive functioning could not be established due to limitations in the database related to the quantity and quality of available studies (*i.e.*, only one cohort study was available, which found no association with levels of the DIDP metabolite, MCNP (Philippat et al., 2017)).

EPA identified four new prospective cohort studies (one high and three of medium quality) that evaluated the association between exposure to DIDP and behavioral and neurodevelopmental outcomes. In a high quality study, Shin et al., (2018) evaluated whether prenatal MCNP exposures may be associated with increased risk of autism spectrum disorder and non-typical development (defined by

study authors as participants with scores within three points of the Autism Diagnostic Observation Schedules cutoff and/or Mullen Scales of Early Leaning scores 1.5 to 2 standard deviations below average) in 201 mother-child pairs from the MARBLES cohort (Markers of Autism Risk in Babies Learning Early Signs) in California, which follows women at high risk for delivering a child with autism spectrum disorder. Maternal urinary MCNP levels were not significantly associated with risk of autism spectrum disorder for children of either sex. When stratified by sex, urinary MCNP levels were positively associated with non-typical development among boys (relative risk ratio = 1.85; 95% confidence interval: 1.09, 3.13; $p < 0.05$), but not girls. Further, urinary MCNP levels were significantly associated with increased risk of non-typical development in mothers that did not take prenatal vitamins during the first month of pregnancy (relative risk ratio = 3.67; 95% confidence interval: 1.80, 7.48; $p < 0.05$).

In a medium quality prospective cohort study, Li et al., (2019) evaluated the relationship between urinary MCNP levels at 1, 2, 3, 4, 5, and 8 years of age and children's cognitive abilities in 253 mother-child pairs as part of the Health Outcomes and Measures of the Environment (HOME) Study (Cincinnati, Ohio), a longitudinal pregnancy and birth cohort. No significant associations were found between urinary MCNP levels and full-scale intelligence quotient for children of either sex at any age. In a second medium quality study, Tanner et al. (2020) assessed the association between prenatal urinary DIDP metabolites (MhiDP, MCNP) measured during the first trimester and child full-scale intelligence quotient at 7 years of age in 718 mother-child pairs from the Swedish Environmental Longitudinal Mother and Child Asthma and Allergy study (SELMA). No significant associations for DIDP metabolites were observed. Jankowska et al. (2019) (medium quality) evaluated the relationship between the sum of three urinary DIDP metabolites (OH-MIDP, oxo-MIDP, cx-MIDP) and several outcomes in 250 early school-age children from the Polish Mother and Child Cohort. Child behavioral and emotional problems were assessed at seven years of age by the Strengths and Difficulties Questionnaire; cognitive and psychomotor development was assessed by the Intelligence and Development Scales. No significant associations were observed between summed urinary DIDP metabolites and any measures of behavior, emotional problems, or cognitive and psychomotor development.

Laboratory Animals

The database evaluating neurotoxicity following oral exposure to DIDP is limited to seven studies. Only one study, which EPA identified in the updated literature search, was specifically designed to evaluate neurotoxicity and multiple measurements including neurobehavioral and mechanistic evaluation of male mice (Ge et al., 2019). Remaining studies evaluated changes in brain weight and/or histopathology; these studies include one subchronic study of beagles (Hazelton Labs, 1968a), two chronic studies (one each of mice and rats) (Cho et al., 2011; Cho et al., 2010; Cho et al., 2008), and one two-generation study of reproduction (Hushka et al., 2001; Exxon Biomedical, 1998).

In the one subchronic study that evaluated neurological outcomes, male and female beagles (three per sex per dose) were fed 0, 15, 75, and 300 mg/kg-day DIDP in the diet for 13-weeks (Hazelton Labs, 1968a). No treatment-related effects were observed on absolute brain weight. Study methods state that histopathologic examination of the brain was conducted for control and high-dose dogs; however, no results are reported.

In a chronic study, Cho et al. (2011) administered male and female wild-type and rasH2 transgenic mice (15 per sex per dose per strain) with up to 1,500 mg/kg-day DIDP in the diet for 26 weeks. Relative brain weight increased 13 (female) to 36 (male) percent in wild-type mice and 45 percent in male rasH2 mice at 1,500 mg/kg-day. Absolute brain weight was not reported, and terminal body weight was reduced 12 (female) to 27 (male) percent in wild-type mice and 31 percent in male rasH2 mice at 1,500

mg/kg-day. Because brain weight is conserved in the presence of body weight changes, relative organ weight measures are less useful for studying brain weight changes (U.S. EPA, 2016, 1998). Changes in relative brain weight likely reflect decreases in body weight. A second chronic study, Cho et al. (2010; 2008) did not observe an effect on relative brain weight (absolute weight not reported) in male or female F344 rats (52/sex/dose) administered 0, 22, 110, or 479 mg/kg-day (males) and 0, 23, 128, or 620 mg/kg-day (females) in the diet for two years. In both chronic studies of mice and rats (Cho et al., 2011; Cho et al., 2010; Cho et al., 2008), the study authors state that the brain was examined microscopically; however, results were not reported.

In a two-generation study (Study A) (Hushka et al., 2001; Exxon Biomedical, 1998), SD rats were continuously administered dietary concentrations of 0, 0.2, 0.4, and 0.8 percent DIDP starting 10 weeks prior to mating, throughout mating, gestation, and lactation, until terminal sacrifice for two generations. Received doses in units of mg/kg-day are shown in Table_Apx C-4. No effect was observed on absolute brain weight at any dose for P1 males, P1 and P2 females, and F1 male and female offspring at weaning. Absolute brain weight was significantly reduced by 4.0 percent in high-dose P2 males, 7.2 percent in F2 high-dose male weanlings and 4.8 to 7.5 percent in F2 mid- and high-dose female weanlings. Histopathologic examination of the brain was limited to control and high-dose F1 and F2 weanlings (both sexes), and no findings were observed. Histopathological examination of the brain was not conducted for P1 or P2 adult animals. Notably, effects on brain weight occurred at doses higher than those that reduced F2 offspring survival on PND1 and PND4, which was reduced at 0.2 percent DIDP and above (Section 3.1.1), indicating that effects on development were a more sensitive outcome.

In another study, young (four-week old) male Kunming mice (10/group) were gavaged with 0 (saline control), 0.15, 1.5, 15, and 150 mg/kg-day DIDP for 21 days (Ge et al., 2019). Mice were evaluated for learning and memory impairment in the Morris Water Maze. Spatial learning in the Morris Water Maze was assessed over seven consecutive days starting on study day 13, and escape latency times were determined daily. The number of trials per day during the acquisition phase of the study was not stated. On the eighth day (study day 20), mice were kept away from the maze. On the ninth day (study day 21), mice were subjected to a probe trial to assess memory. Mice were sacrificed on study day 22, and brains were collected for histologic examination and evaluation of mechanistic endpoints. Escape latency times decreased over the seven-day acquisition phase of the study for mice in control and all DIDP exposure groups indicating learning. Study authors state that control mice showed the largest decrease in escape latency times, while high-dose mice showed the least decrease in escape latency times, indicating exposure to DIDP negatively impacted learning. However, it is unclear if statistical analysis was performed to determine if the difference in escape latency times between control and high-dose mice was significantly different, and sufficient information is not provided to enable an independent statistical analysis.

Other limitations of this study include additional reporting deficiencies. The path length to find the hidden platform for each trial during the acquisition phase was not reported. During the probe trial, study authors stated that control mice exhibited swimming trajectories that were more concentrated in the quadrant where the escape platform was located, whereas mice in the 15 and 150 mg/kg-day DIDP groups exhibited scattered and disorderly swimming trajectories indicating different spatial memory abilities. However, only single representative images of swimming trajectories were provided. Mice in the 15 and 150 mg/kg-day DIDP groups spent significantly less time in the target quadrant compared to control mice during the probe trial, indicating DIDP had an impact on memory. The magnitude of the effect was difficult to assess (data presented graphically only) but appeared relatively minor and did not exhibit a strong dose-response (*i.e.,* target quadrant retention times were similar in the 15 and 150 mg/kg-day groups). The number of platform crossings per exposure group during the probe trial was not

reported. In addition to not reporting path length per trial during the acquisition phase and number of platform crossings during the probe trial, the study did not evaluate swim speed or include cued-trials, which are important performance controls that can be used to dissociate cognitive deficits from sensorimotor performance impairments (U.S. EPA, 2016).

Brain histopathology was described qualitatively only (no incidence data presented, and no statistical analysis performed) and study authors do not state how many animals in each dose group were examined histologically. Study authors report that with increasing doses of DIDP, "damage to the pyramidal neurons in the hippocampal CA1 region was gradually made worse, showing loose and disordered arrangements, and swelling deformations" and "pyramidal neurons in the hippocampal CA1 region showed loss of Nissl substance and swelling deformations. Partial pyramidal neurons in the DIDP15 and DIDP150 groups were deeply stained and shrunken" (Ge et al., 2019).

Mechanistic Information

EPA identified one *in vivo* mouse study that provided mechanistic evidence. Ge et al. (2019) gavaged young male Kunming mice with 0, 0.15, 1.5, 15, or 150 mg/kg-day DIDP for 21 days and then examined markers of oxidative stress, inflammation, and apoptosis in brain homogenate. Study authors do not state what tissues were used to generate the homogenate (*i.e.*, whole brain or tissue from more specific regions of the brain). Levels of ROS, malondialdehyde, 8-hydroxy-2-deoxyguanosine increased, while glutathione decreased in a dose-dependent manner at 15 mg/kg-day DIDP and above. Similarly, levels of nuclear factor-κβ (NF- κβ) and caspase-3 increased, while brain derived neurotrophic factor (BDNF) and phosphorylated cAMP response-element (p-CREB) levels decreased in a dose-dependent manner at doses of 15 mg/kg-day DIDP and above (except for p-CREB, which increased only at 150 mg/kg-day).

Conclusions on Neurotoxicity

Available human epidemiologic studies show no consistent association between exposure to DIDP and neurological outcomes. However, there are limitations associated with the available epidemiological studies related to exposure misclassification due to use of a single spot urine sample in several studies, periods of heightened susceptibility and timing of exposure assessment, and phthalate mixture effects. Until these limitations are addressed, results from the available epidemiological studies of DIDP should be interpreted with caution.

The database of studies evaluating neurotoxicity following oral exposure to DIDP in laboratory animals is limited. No effects on absolute brain weight were observed in one 13-week study of beagles treated with up to 300 mg/kg-day DIDP. In a two-generation study of reproduction, slight reductions (4.0–7.5%) in absolute brain weight were observed in P2 males and F2 male and female offspring but were not accompanied by histopathology findings in male or female F2 weanlings. Further, high-dose F2 offspring exhibited decreased body weight gain throughout the postnatal period and weaning, and therefore the observed decrease in F2 weanling absolute brain weight may be related to reduced body weight gain and development during the postnatal period. Further, effects on absolute brain weight in the two-generation study occurred at doses higher than those that reduced F2 offspring survival on PND1 and PND4 (*i.e.*, brain weight changes occurred at 0.4 percent DIDP and above, while reduced F2 survival occurred at 0.2 percent DIDP and above), indicating effects on development are a more sensitive outcome.

One intermediate duration study of young male mice provides some evidence of cognitive deficits and neurotoxicity following oral exposure to DIDP (Ge et al., 2019). However, the study is limited by several reporting deficiencies (*e.g.*, histopathology reported qualitatively only, path length in acquisition phase and number of platform crossings during probe trial were not reported); statistical analysis (no

statistical analysis of histopathological findings, unclear statistical analysis of escape latency data set); and lack of inclusion of performance controls (*e.g.*, swim speed, cued-trials) that would help distinguish between cognitive deficits and sensorimotor performance impairments. These limitations reduced EPA's confidence in the study findings.

Overall, available laboratory animal studies provide some limited evidence that DIDP can cause neurotoxicity in experimental laboratory animals. However, given the limited database of studies evaluating neurological outcomes and the limitations and uncertainties associated with the available studies, EPA is not further considering neurotoxicity for dose-response analysis or for use in estimating risk to human health.

3.2.3 Immune System (Skin Sensitization and Adjuvant Properties)

The skin sensitizing properties of DIDP have been evaluated in several existing assessments. U.S. CPSC (2010) concluded that DIDP is "not a strong sensitizer," while Australia's NICNAS (2015) concluded that that, "there is insufficient information to indicate that DIDP causes skin sensitization." ECB (2003) concluded that "the weight of evidence is deemed insufficient to justify a classification [for sensitization]," while ECHA (2013b) concluded that DIDP (and other phthalates) "lack intrinsic sensitizing potential." These conclusions are based on results from experimental animal models and human patch testing that indicate DIDP is not sensitizing. Available studies of DIDP include: two Buehler tests (one positive and one negative for sensitization) (Huntingdon Research Center, 1994; Exxon Biomedical, 1992); one guinea pig maximization test (result: non-sensitizer) (Inveresk Research International, 1981); irritant and allergic patch test studies of 310 participants in which no allergic reactions were observed (Kanerva et al., 1999); and repeated insult patch testing of 104 participants in which no positive skin reactions were observed (Medeiros et al., 1999).

EPA identified no new studies evaluating skin sensitization. However, one new study was identified that indicated that DIDP can have adjuvant effects on dermatitis-like reactions in mice (Shen et al., 2017). The adjuvant properties of DIDP and other phthalates have been reviewed in existing assessments by ECHA (2013b) and EFSA (2019). ECHA (2013b) concluded "both DINP and DIDP share at least some of the adjuvant properties demonstrated for phthalates and an effect on atopic responses in humans cannot be excluded." Because the new study identified by EPA provided a potentially sensitive endpoint, EPA evaluated the weight of evidence for immune adjuvant effects.

Humans

EPA identified three medium quality studies evaluating the association between DIDP and its metabolites and immune/allergy outcomes. In a prospective cohort study, Shu et al. (2018) examined the association between phthalate metabolites (including two metabolites of DIDP [MCNP and MhiDP]) measured in prenatal urine samples among pregnant women in the Swedish Environmental Longitudinal Mother and Child Asthma and Allergy study (SELMA) cohort and immune outcomes (*i.e.*, croup, wheeze, and otitis media) in infants up to 12 months of age. No associations with croup, wheeze, or otitis media up to 12 months of age were found for either DIDP metabolite. In a second cohort study, Soomro et al., (2018) examined the association between maternal urinary phthalate metabolite levels (including 1 metabolite of DIDP, MCNP) in a subset of the mother-son French EDEN prospective birth cohort and eczema and total serum IgE status in their sons up to 5 years of age. The adjusted odds ratios for the relationship between maternal MCNP concentrations and eczema at year 3 (odds ratio: 1.61; 95% confidence interval: 1.00, 2.59; $p < 0.05$), year 5 (odds ratio: 1.37; 95% confidence interval: 1.04, 1.80; $p < 0.05$), and late-onset eczema (odds ratio: 1.29; 95% confidence interval: 1.02, 1.64; $p < 0.05$) were statistically significant, suggesting prenatal exposure to DIDP in boys may influence the occurrence of eczema in early childhood.

In a cross-sectional study, Strassle et. al. (2018) evaluated whether house dust endotoxin levels may modify the association between urinary phthalate metabolites (including 1 metabolite of DIDP, MCNP) and asthma, wheeze, hay fever, and rhinitis in 1,091 adults aged 18 years or older in the 2005 to 2006 NHANES data set. Multivariable logistic regression of MCNP exposure on wheeze symptoms, hay fever, and rhinitis found no significant associations when adjusted or unadjusted for endotoxins. Multivariable logistic regression of MCNP exposure on current asthma found no significant associations when adjusted or unadjusted for endotoxins.

Laboratory Animals

The database of studies evaluating immune adjuvant effects of DIDP is limited to two studies investigating IgG1 and IgE antibody responses in sensitized mice (Larsen et al., 2002; Larsen et al., 2001) and a study investigating dermatitis-like reactions in sensitized mice (Shen et al., 2017).

Larsen et al. (2001) investigated the adjuvant effects of MIDP in female BALB/cj mice (10–12 per group). Exposure groups included (1) an ovalbumin control (model antigen) in which mice received subcutaneous injections in the neck with 1 µg ovalbumin in 100 µL solvent (0.9% saline, PEG 400, 99.9% ethanol in ratio 89:10:1); (2) a positive control in which mice were injected with 1 µg ovalbumin in combination with the adjuvant aluminium hydroxide at concentrations of 0.27 or 2.7 mg/mL (vehicle: sterile water); and (3) test groups in which mice were subcutaneously injected with 1 µg ovalbumin with 100 µL of 1, 10, 100, or 1,000 µg/mL MIDP. Mice in all treatment groups were given two booster immunizations with 0.1 µg ovalbumin in 100 µL 0.9 percent saline 10 and 15 days after the first ovalbumin injection. Blood was collected 4 days after each booster injection and analyzed for ovalbumin-specific antibodies (*i.e.*, IgE, IgG1, IgG2a) by enzyme-linked immunosorbent assay. Serum IgG2a antibody levels were below detectable limits in all treatment groups at all timepoints. The aluminium hydroxide positive control gave equivocal results. After one booster, both doses of aluminum hydroxide significantly increased serum IgE levels over the ovalbumin control, whereas after two boosters, serum IgE levels were significantly higher in the ovalbumin control group compared to the positive control. Similarly, after one booster the high-dose positive control had significantly higher IgG1 levels over the ovalbumin control, while after two boosters no significant effect was observed. Mice treated with 100 or 1,000 µg/mL MIDP had significantly lower serum IgE and IgG1 levels compared to the ovalbumin control after two boosters. Study authors concluded that MIDP may have an immuno-suppressive effect in sensitized animals. Limitations of the study include the lack of a vehicle control group, an equivocal positive control response, and the relevance of the route of test substance administration (subcutaneous injection).

Larsen et al. (2002) investigated the adjuvant effects of DIDP in female BALB/cj mice (9–11 per group). Treatment groups included (1) an ovalbumin control in which mice were subcutaneously injected in the neck with 1 µg ovalbumin in 50 µL solvent (PEG 400, ethanol 99.9% and sterile 0.9% saline in a ratio of 494:5:1); (2) a positive control in which mice were injected with 1 µg ovalbumin in combination with 100 µL the adjuvant aluminium hydroxide at concentrations of 0.27 or 2.7 mg/mL (vehicle: sterile water); and (3) test groups in which mice were subcutaneously injected with 1 µg ovalbumin in combination with 50 µL of 2, 20, 200, or 2,000 µg/mL DIDP. Mice in all treatment groups were given two booster immunizations with 0.1 µg ovalbumin in 100 µL 0.9 percent saline 10 and 15 days after the first injection. Blood was collected 4 days after each booster injection and blood was analyzed for ovalbumin-specific antibodies (*i.e.*, IgE, IgG1, IgG2a) by enzyme-linked immunosorbent assay. Consistent with the first study, serum IgG2a antibody levels were low in all treatment groups. Study authors state that it was not possible to compare the positive control group to other treatment groups because PEG 400 (solvent used for ovalbumin and DIDP groups) can have immunosuppressive properties, and it was unclear how this may have affected the positive control response.

Use of PEG 400 as a solvent may explain the equivocal results obtained for the positive control in the first study of MIDP (Larsen et al., 2001). Treatment with DIDP had no effect on serum IgG1 levels after one booster. After two boosters, serum IgG1 levels were significantly increased above the cumulative ovalbumin control group (n = 30) in mice administered 2 and 2,000 µg/mL DIDP (no significant effect at 20 and 200 µg/mL), however, when compared to the corresponding ovalbumin control (n=10) the effect on serum IgG1 levels was not significant at any dose. Serum IgE levels were significantly increased in mice administered 2,000 µg/mL DIDP after one booster compared to both the cumulative and corresponding ovalbumin controls; however, serum IgE levels were unaffected by treatment with DIDP after two boosters. Study authors concluded that DIDP may have weak adjuvant properties and increase serum IgE and IgG1 levels in sensitized animals. Limitations of the study included the choice of selected vehicle (*i.e.*, PEG 400, which may have slight immunosuppressive properties), uncertainties related to the positive control response, relevancy of the route of test substance administration (subcutaneous injection), and inconsistency across endpoints (*e.g.*, effects on serum IgE and IgG1 responses).

In the one new study identified by EPA, Shen et al. (2017) investigated the adjuvant effects of DIDP on allergic contact dermatitis in male Balb/c mice (7 per group). Treatment groups included (1) saline control; (2) 200 mg/kg-day DIDP; (3) 0.5 percent fluorescein isothiocyanate (FITC) sensitized group; (4) 0.5 percent FITC in combination with 2, 20, or 200 mg/kg-day DIDP; and (5) 200 mg/kg-day DIDP in combination with 10 mg/kg-day melatonin and 0.5 percent FITC. For all treatment groups, mice were administered the saline vehicle, DIDP, and melatonin via daily oral gavage for 21 days. On study days 22 and 23, 120 µL of saline (treatment groups 1 and 2) or 0.5 percent FITC (vehicle: 1:1 acetone/DBP) (groups 3 through 5) was topically applied to the shaven backs of mice. On study day 28, mice were challenged with 20 µL of saline (groups 1 and 2) or 0.5 percent FITC (groups 3–5) to the right ear and 20 µL of saline (groups 1–2) or vehicle (groups 3–5) to the left ear. Twenty-four hours after the challenge, ear swelling and bilateral ear weight were determined. Compared to the saline control, treatment with FITC and FITC in combination with all dose levels of DIDP led to increases in: the number of inflammatory cells infiltrating skin lesions, ear swelling, bilateral ear weight, serum total IgE, and levels of IL-4 and tryptase (a marker for mast cell degranulation), but not IFN-γ, in ear homogenate.

Treatment with FITC in combination with 200 mg/kg-day DIDP significantly increased these outcomes over the FITC alone group, indicating exacerbation of the allergic dermatitis-like effects induced by FITC, while co-treatment with melatonin attenuated these effects. There were no significant differences in response between the saline control and 200 mg/kg-day DIDP groups, indicating that DIDP alone did not induce an allergic response. Treatment with FITC and FITC in combination with all dose levels of DIDP significantly increased ROS levels and reduced glutathione levels in ear tissue compared to controls. Co-exposure to FITC and 200 mg/kg-day DIDP increased ROS levels above the FITC alone group, while co-exposure to melatonin attenuated this the observed effects on ROS and glutathione. Similarly, treatment with FITC and FITC in combination with 200 mg/kg-day DIDP increased expression of thymic stromal lymphopoietin, pSTAT5, pSTAT6, and STAT3 protein, with the increase being significantly greater in the FITC in combination with 200 mg/kg-day DIDP co-exposure group compared to FITC alone. Study authors concluded that DIDP does not directly cause allergic dermatitis but can exacerbate FITC-induced allergic dermatitis with potential roles for oxidative stress and enhanced thymic stromal lymphopoietin production.

Conclusions on Immune System Toxicity

Studies of DIDP and MIDP on serum IgE and IgG1 responses in albumin sensitized mice provide inconsistent evidence for an immune adjuvant effect. Larsen et al. (2001) found that mice treated with MIDP had lower serum IgE and IgG1 levels compared to albumin controls, suggesting an

immunosuppressive effect. In contrast, Larsen et al. (2002) report results indicating DIDP may have immune adjuvant properties on serum IgE and IgG1. However, both studies are limited by somewhat inconsistent serum IgE and IgG1 responses after one and two boosters, lack of inclusion of a vehicle control group, relevancy of selected vehicle (*i.e.*, PEG 400, which may have slight immunosuppressive properties), uncertainties related to the positive control response, and route of test substance administration (subcutaneous injection). In a more recent study, Shen et al. (2017) found that DIDP alone does not induce a allergic dermatitis-like response in mice, but can exacerbate allergic dermatitis-like effects in mice sensitized with FITC. Treatment with 200, but not 2 or 20 mg/kg-day, in combination with FITC caused an elevated immune response compared to animals sensitized with FITC alone indicating that the adjuvant effects of DIDP were limited to the high-dose group (200 mg/kg-day).

Although available studies of laboratory animals provide some evidence for immune adjuvant effects of DIDP in sensitized animals, EPA is not further considering these effects for dose-response assessment or for use in extrapolating human risk. Several sources of uncertainty reduce the Agency's confidence in this outcome. First, the database of experimental animal studies is limited to three studies with inconsistent results. Second, available studies evaluate the adjuvant properties of DIDP in experimental rodent models pre-sensitized by exposure to other compounds (*i.e.*, FITC, ovalbumin). Co-exposure to DIDP and other compounds is another source of uncertainty that further reduced EPA's confidence in this outcome.

4 GENOTOXICITY HAZARD IDENTIFICATION

The mutagenic and genotoxic potential of DIDP has been evaluated in five studies (Table 4-1). Available studies include two bacterial reverse mutation assays (Zeiger et al., 1985; Seed, 1982), two *in vitro* mouse lymphoma assays (Barber et al., 2000; Hazleton Biotechnologies Company, 1986), and one *in vivo* mouse micronucleus test (McKee et al., 2000). No evidence of mutagenic activity was observed in the two *in vitro* bacterial reverse mutation assays of DIDP with or without metabolic activation using S9 mix (Zeiger et al., 1985; Seed, 1982). DIDP was inactive in two mouse lymphoma forward mutation assays with or without metabolic activation (Barber et al., 2000; Hazleton Biotechnologies Company, 1986). In an *in vivo* mouse micronucleus test, DIDP gave negative results when CD-1 mice were gavaged with a single dose of up to 5,000 mg/kg DIDP (McKee et al., 2000).

Although the database of genotoxicity studies of DIDP is limited to a few studies, other phthalate diesters have also been demonstrated to be non-genotoxic. For example, as described in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* (U.S. EPA, 2024a), available studies indicate that DINP is not mutagenic in bacterial reverse mutation assays or *in vitro* mouse lymphoma assays (with or without metabolic activation); does not induce chromosomal aberrations in Chinese hamster ovary cells; does not cause unscheduled DNA repair in primary rat hepatocytes; and does not induce clastogenic effects or micronuclei formation *in vivo*. Notably, findings for DINP are consistent with results for DIDP, providing further evidence that DIDP is unlikely to be genotoxic.

Available studies that evaluated the mutagenic and genotoxic potential of DIDP are consistently negative. Therefore, EPA considers DIDP not likely to be genotoxic or mutagenic. Consistently, existing assessments of DIDP by ECB (2003), ECHA (2013b), Australia NICNAS (2015, 2008a, b), Health Canada (EC/HC, 2015), and U.S. CPSC (2014, 2010) have also concluded that DIDP is not genotoxic or is not likely to be genotoxic.

Table 4-1. Summary of Genotoxicity Studies of DIDP

Test Type	Test System (Species/ Strain/Sex)	Dose/Duration	Metabolic Activation	Result	Reference(s)
In vivo studies					
Micronucleus (bone marrow) (adhered to OECD 474)	Male and female CD-1 mice	Single oral (gavage) dose of 0, 1.25, 2.5, 5 g/kg DIDP; sacrificed 24, 48, and 72 hours post-dosing	Not applicable	Negative	(McKee et al., 2000)
In vitro studies					
Reverse mutation[a]	*S. typhimurium* strain TA 100	Not reported	Unclear[a]	Negative	(Seed, 1982)
Reverse mutation	*S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537	0, 100, 333, 1,000, 3,333, 10,000 µg/plate	± Rat and hamster liver S9	Negative	(NTP, 2018; Zeiger et al., 1985)
Mouse lymphoma mutation assay	L5178Y TK$^{+/-}$ mouse lymphoma cells	0, 2, 4, 5, 6, 8, 10 µL/mL (− S9); 0, 0.25, 0.5, 1, 2 µL/mL (+ S9)	± Rat liver S9	Negative	(Hazleton Biotechnologies Company, 1986)
Mouse lymphoma mutation assay	L5178Y TK$^{+/-}$ mouse lymphoma cells	0, 2, 4, 6, 8, 10 µL/mL (− S9); 0, 0.25, 0.5, 1, 2 µL/mL (+ S9)	± Rat liver S9	Negative	(Barber et al., 2000)

[a] Seed (1982) tested bacteria for mutations to azaguanine resistance and reversion to histidine prototrophy. Tested concentrations of DIDP were not reported. The maximal concentration tested was determined by either the solubility limit or cytotoxicity exceeding more than 90% of control values. Study authors report that experiments were conducted with S9 mix, however, assay results for DIDP are reported as negative and it is unclear if this negative result was for studies with or without S9 mix.

5 CANCER HAZARD IDENTIFICATION AND CHARACTERIZATION

This section summarizes the available human (Section 5.1) and animal (Section 5.2) evidence for the carcinogenicity of DIDP.

5.1 Human Evidence

EPA identified one new medium quality case-control study. Parada et al. (2018) evaluated the association between exposure to urinary phthalate metabolites (including one metabolite of DIDP, MCNP) and incidence of breast cancer in females recruited from a rapid reporting system created for the Long Island Breast Cancer Study Project. Compared to the lowest quintile, the highest quintile of urinary MCNP was inversely associated with breast cancer (odds ratio: 0.72 [95% CI: 0.40, 1.03]). However, age-adjusted odd ratios, multivariable adjusted odd ratios, all-cause mortality hazard ratios with multivariate adjustment, and breast cancer-specific mortality hazard ratios with multivariate adjustment were not statistically significant.

5.2 Animal Evidence

Two studies have evaluated the carcinogenicity of DIDP in rodent models. Available studies include one 2-year dietary study of male and female F344 rats (Cho et al., 2010; Cho et al., 2008) and one 26-week dietary study of male and female CB6F1-rasH2 transgenic mice and wild-type mice (Cho et al., 2011). Across the two available studies of DIDP, increased incidence of mononuclear cell leukemia (MNCL) was observed in male and female F344 rats (Cho et al., 2010; Cho et al., 2008), whereas hepatocellular adenomas were observed in male CB6F1-rasH2 transgenic mice (Cho et al., 2011). No other neoplastic findings have been reported following chronic exposure to DIDP. Evidence for MNCL and hepatocellular adenomas are discussed further in Sections 5.2.1 and 5.2.2, respectively.

5.2.1 Mononuclear Cell Leukemia

Increased incidence of MNCL has been observed in one study in which male and female F344 rats were fed diets containing 0, 400, 2,000, or 8,000 ppm DIDP for 2 years (equivalent to 22, 110, and 479 mg/kg-day for males and 23, 128, and 620 mg/kg-day for females) (Cho et al., 2010; Cho et al., 2008). The incidence of MNCL was statistically significantly increased in high-dose males (23/50 vs. 10/50 in controls) and females (22/49 vs. 11/48 in controls) (Table 5-1). In contrast, MNCL was not observed in male or female CB6F1-rasH2 transgenic or wild-type mice exposed to up to 1,500 mg/kg-day DIDP through the diet for 26 weeks (Cho et al., 2011).

Table 5-1. Summary of Incidence of MNCL in Chronic Studies of DIDP

Brief Study Description	Incidence of MNCL	Remark
Male and female (52/sex/dose) F344 rats fed 0, 400, 2,000, or 8,000 ppm DIDP for 2 years (equivalent to 22, 110, 479 mg/kg-day for males; 23, 128, 620 mg/kg-day for females) (Cho et al., 2010; Cho et al., 2008)[a]	Males: 10/50 (20%), 16/50 (32%), 14/50 (28%), 23/50** (46%) Females: 11/48 (23%), 7/50 (14%), 11/49 (22%), 22/49* (45%)	Laboratory historical control data for MNCL not reported. Time to first occurrence of MNCL not reported.
Male and female (15/sex/dose) CB6F1-rasH2 transgenic mice fed 0, 0.1, 0.33, or 1.0% DIDP for 26 weeks (equivalent to 130, 429, 1500 mg/kg-day)[b] (Cho et al., 2011)	–	MNCL not observed in either sex at any dose.
Male and female (15/sex/dose) wild-type mice fed 0 or 1.0% DIDP for 26 weeks (equivalent to 1500 mg/kg-day)[b] (Cho et al., 2011)	–	MNCL not observed in either sex at any dose.

[a] Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$ by the poly-3 test as reported by Cho et al. (2008).
[b] Mean received doses in mg/kg-day and food consumption were not reported by (Cho et al., 2011). To estimate the mean received doses of DIDP in mg/kg-day, a food factor of 0.15 was used (% DIDP in diet × food factor × 10,000 = mean dose in mg/kg-day) (WHO, 1987).

MNCL is a spontaneously occurring neoplasm of the hematopoietic system that reduces lifespan and is one of the most common tumor types occurring at a high background rate in the F344 strain of rat (also referred to as Fisher rat leukemia because it is so common) (Thomas et al., 2007). Historical control data from NTP have demonstrated an increase in the spontaneous background incidence of MNCL in untreated male and female F344 rats from 7.9 and 2.1 percent in males and females, respectively, in 1971 to 52.5 and 24.2 percent in males and females, respectively, from 1995 through 1998 (Thomas et al., 2007). Spontaneous incidence of MNCL in other strains of rat appear to be rare. Brix et al. (2005) report the incidence of MNCL in female Harlan SD rats to be 0.5 percent in NTP 2-year studies. Further, MNCL does not appear to occur naturally in mice (Thomas et al., 2007). The F344 strain of rat was used in NTP 2-year chronic and carcinogenicity bioassays for nearly 30 years (King-Herbert et al., 2010; King-Herbert and Thayer, 2006). However, in the early 2000s NTP stopped using the F344 strain of rat in large part because of high background incidence of MNCL and testicular Leydig cell tumors that confounded bioassay interpretation. NTP subsequently replaced the F344 strain of rats with the Harlan Sprague Dawley strain (King-Herbert et al., 2010; King-Herbert and Thayer, 2006).

Given the high and variable background rate of MNCL in F344 rats, it is important to consider historical control data, concurrent control data, and time to onset of MNCL to assist in determining whether observed increases in MNCL are exposure-related. Cho et al. (2008) reported that survival was significantly reduced in high-dose male rats (survival: 85, 73, 83, and 37% in control, low-, mid-, and high-dose groups, respectively) and female rats (survival: 85, 75, 75, 56%). However, study authors do not report the cause of unscheduled deaths, and no information regarding the time to onset for MNCL was reported. Additionally, historical control data for MNCL in the laboratory conducting the study was not provided. Cho et al. stated that the incidence of MNCL following exposure to DIDP was within the range of historical control data for feed studies using F344 rats from NTP dietary carcinogenicity studies over a seven-year period (from approximately 1990 to 1997) for male (32–74%) and female (14–52%)

F344 rats (Haseman et al., 1998). EPA's *Guidelines for Carcinogen Risk Assessment* (2005) state that the most relevant historical control data comes from the same laboratory and supplier and are within 2 to 3 years of the study under review, and that other historical control data should be used with extreme caution. Given the high and variable background rate of MNCL in F344 rats, EPA does not consider use of NTP historical control data by Cho et al. (2008) to be an appropriate comparator for their study. Lack of relevant laboratory historical control data and data pertaining to the time to onset of MNCL make it challenging to determine if the increase in MNCL observed in high-dose F344 rats treated with DIDP, which was statistically significant compared to concurrent controls, is treatment-related and is a source of uncertainty.

Another source of uncertainty is lack of MOA information for induction of MNCL in F344 rats. The MOA for induction of MNCL in F344 rats is unknown. Lack of MOA information makes it difficult to determine human relevancy. There is additional uncertainty related to the human correlate to MNCL in F344 rats. Some researchers have suggested that based on the biological and functional features in the F344 rat, MNCL is analogous to large granular lymphocyte (LGL) in humans (Caldwell et al., 1999; Caldwell, 1999; Reynolds and Foon, 1984). There are two major human LGL leukemias, including CD3+ LGL leukemia and CD3- LGL leukemia with natural killer cell activity (reviewed in (Maronpot et al., 2016; Thomas et al., 2007)). Thomas et al. (2007) contend that MNCL in F344 rats shares some characteristics in common with aggressive natural killer cell leukemia (ANKCL) in humans, and that ANKCL may be a human correlate. However, Maronpot et al. (2016) point out that ANKCL is extremely rare with less than 98 cases reported worldwide, and its etiology is related to infection with Epstein-Barr virus, not chemical exposure. This is in contrast to MNCL in F344 rats, which is a more common form of leukemia and is not associated with a viral etiology. However, under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), site concordance is not always assumed between animals and humans.

Overall, the SACC recommended that "*the observation of an increased incidence of MNCL in a chronic bioassay employing the Fisher 344 rat should not be considered a factor in the determination of the cancer classification for DIDP*" and "*Most Committee members agreed that given the material presented in a retrospective review, MNCL and Leydig Cell Tumors, among other tumor responses in F344 rat carcinogenicity studies lack relevance in predicting human carcinogenicity (Maronpot et al., 2016)*" (U.S. EPA, 2024h). Consistent with the recommendations of the SACC, and based on the above discussion, EPA is not further considering MNCL as a factor in the determination of the cancer classification for DIDP.

5.2.2 Hepatocellular Adenomas

Increased incidence of hepatocellular adenomas has been observed in one study in which male and female CB6F1-rasH2 transgenic mice were fed diets containing 0, 0.1, 0.33, or 1.0 percent DIDP for 26 weeks (equivalent to approximately 150, 495, or 1,500 mg/kg-day) (Cho et al., 2011). The rasH2 mouse model was developed as an alternative to the traditional 2-year bioassay for use in pharmaceutical drug development (Bogdanffy et al., 2020). The rasH2 mouse carries multiple copies of the human H-ras protooncogene making it more susceptible to tumorigenesis. Incidence of adenomas was statistically significantly increased in high-dose males (5/15 vs. 0/15 in controls), but not females (Table 5-2). Carcinomas were not observed in either sex at any dose. In contrast to the study of male rasH2 mice, no significant increases were observed in liver tumors in male or female wild-type mice administered 1,500 mg/kg-day DIDP in the diet for 26 weeks (Cho et al., 2011) or in male and female F344 rats administered up to 479 (males) to 630 (females) mg/kg-day DIDP in the diet for 2 years (Cho et al., 2010; Cho et al., 2008).

However, there is evidence that the high-dose (1,500 mg/kg-day DIDP) used in the 26-week study of wild-type and CB6F1-rasH2 transgenic mice that coincided with an increase in hepatocellular adenomas is excessively high. This is demonstrated by the fact that terminal body weight was reduced 27 and 12 percent in male and female wild-type mice, respectively, and 31 and 15 percent in male and female transgenic mice, respectively, at 1,500 mg/kg-day. Per EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) "Other signs of treatment-related toxicity associated with an excessive high dose may include (a) significant reduction of body weight gain (e.g., greater than 10%)." Further, EPA's *Guidelines for Carcinogen Risk Assessment* state that "overt toxicity or qualitatively altered toxicokinetics due to excessively high doses may result in tumor effects that are secondary to the toxicity rather than directly attributable to the agent."

As discussed Section 3.1.2 (Liver Toxicity), DIDP is a peroxisome proliferator that can activate PPARα. Health Canada (EC/HC, 2015; Health Canada, 2015) and ECHA (2013b) have hypothesized that liver tumors in male rasH2 mice occur through a PPARα MOA (described in (Corton et al., 2018)). However, a complete analysis of the MOA for liver tumors consistent with U.S. EPA (2005) and International Programme on Chemical Safety (2007) guidance has not been completed.

Table 5-2. Summary of Liver Tumors Observed in Chronic Studies of DIDP

Brief Study Description	Incidence of Hepatocellular Adenomas[a]	Remark
Male and female (52/sex/dose) F344 rats fed 0, 400, 2,000, and 8,000 ppm DIDP for 2 years (equivalent to 22, 110, 479 mg/kg-day for males; 23, 128, 620 mg/kg-day for females) (Cho et al., 2010; Cho et al., 2008)	–	No liver tumors observed in either sex at any dose.
Male and female (15/sex/dose) CB6F1-rasH2 transgenic mice fed 0, 0.1, 0.33, 1.0% DIDP for 26 weeks (equivalent to 0, 150, 495, 1500 mg/kg-day)[b] (Cho et al., 2011)	Males: 0/15, 1/15 (7%), 1/15 (7%), 5/15* (33%)	Carcinomas not observed in either sex at any dose. Adenomas not observed in females at any dose.
Male and female (15/sex/dose) wild-type mice fed 0 or 1.0% DIDP for 26 weeks (equivalent to 0 or 1,500 mg/kg-day)[b] (Cho et al., 2011)	Males: 0/15, 1/15 (7%)	Incidence of adenomas in males not statistically significant. No liver tumors observed in females at any dose.

[a] Asterisk indicates a statistically significant ($p < 0.05$) difference compared to the concurrent control group by the Chi-square test as reported by Cho et al. (2011).
[b] Mean received doses in mg/kg-day and food consumption were not reported by (Cho et al., 2011). To estimate the mean received doses of DIDP in mg/kg-day, a food factor of 0.15 was used (% DIDP in diet * food factor * 10,000 = mean dose in mg/kg-day) (WHO, 1987).

5.3 Weight of Scientific Evidence: Conclusions on Carcinogenicity

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), **EPA reviewed the weight of evidence for the carcinogenicity of DIDP and concluded that DIDP is not likely to be carcinogenic to humans.** According to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), a descriptor of *Not Likely to Be Carcinogenic to Humans* is appropriate when:

> "the available data are considered robust for deciding that there is no basis for human hazard concern. In some instances, there can be positive results in experimental animals when there is strong, consistent evidence that each mode of action in experimental animals does not operate in humans. In other cases, there can be convincing evidence in both humans and animals that the agent is not carcinogenic. The judgment may be based on data such as: animal evidence that demonstrates lack of carcinogenic effect in both sexes in well-designed and well-conducted studies in at least two appropriate animal species (in the absence of other animal or human data suggesting a potential for cancer effects), convincing and extensive experimental evidence showing that the only carcinogenic effects observed in animals are not relevant to humans, convincing evidence that carcinogenic effects are not likely by a particular exposure route (see Section 2.3), or convincing evidence that carcinogenic effects are not likely below a defined dose range."

Weight of scientific evidence considerations supporting EPA's determination are listed below. Consistent with this cancer classification, EPA is not conducting a dose-response assessment for DIDP or evaluating DIDP for carcinogenic risk to humans.

- Hepatocellular adenomas were observed only in high-dose male CB6F1-rasH2 transgenic mice at 1,500 mg/kg-day but not in female transgenic mice or in wild-type male or female mice, which are more appropriate for use in human health risk assessment (Cho et al., 2011). However, in the studies of wild-type and transgenic mice the highest dose tested, 1,500 mg/kg-day, was above the limit dose. This is demonstrated by the fact that terminal body weight was reduced 27 and 12 percent in male and female wild-type mice, respectively, and 31 and 15 percent in male and female transgenic mice, respectively, at 1,500 mg/kg-day. Per EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) "signs of treatment-related toxicity associated with an excessive high dose may include (a) significant reduction of body weight gain (*e.g.*, greater than 10%)." Further, EPA's *Guidelines for Carcinogen Risk Assessment* state that "overt toxicity or qualitatively altered toxicokinetics due to excessively high doses may result in tumor effects that are secondary to the toxicity rather than directly attributable to the agent."
- No evidence of carcinogenic activity was observed in male or female CB6F1-rasH2 transgenic mice dosed with 150 or 495 mg/kg-day DIDP (Cho et al., 2011). Evidence of overt treatment-related toxicity associated with exceedance of the limit dose was not apparent at these dose levels.
- EPA acknowledges that increased MNCL was observed in male and female F344 rats treated with DIDP for 2 years (Cho et al., 2010; Cho et al., 2008). However, MNCL was only observed at in the high-dose group and coincided with high mortality. No other preneoplastic or neoplastic findings were observed in any tissue for either sex at any dose.
- MNCL has a high rate of spontaneous occurrence in F344 rats. Although the historical control data are not available for the laboratory that conducted this study, historical control data from NTP (1995–1998) show 52.5 percent in males and 24.2 percent in females (Thomas et al., 2007). The F344 strain of rat was used in NTP 2-year chronic and carcinogenicity bioassays for nearly 30 years (King-Herbert et al., 2010; King-Herbert and Thayer, 2006). However, in the early 2000s NTP stopped using the F344 strain of rat in part because of high background incidence of MNCL and testicular Leydig cell tumors and replaced the F344 strain of rats with the Harlan Sprague Dawley strain (King-Herbert et al., 2010; King-Herbert and Thayer, 2006). Consistent with recommendations of the SACC (U.S. EPA, 2024h), EPA is not further considering MNCL

as a factor in the determination of the cancer classification for DIDP because this is likely a strain-specific effect.

- EPA's weight of scientific evidence conclusion is consistent with Health Canada (EC/HC, 2015), U.S. CPSC (2014, 2010), NICNAS (2015), and ECHA (2013b). None of these regulatory agencies have evaluated DIDP for carcinogenic risk to human health.

6 DOSE-RESPONSE ASSESSMENT

EPA considered two non-cancer hazard endpoints—liver and developmental toxicity—for dose-response analysis. These two hazard endpoints were selected for dose-response analysis because (1) the Agency has the highest confidence in these hazard endpoints for estimating risk to human health, and (2) the effects were consistently observed across species and durations of exposure and occurred in a dose-related manner. EPA considered liver and developmental effects observed in experimental animal models to be relevant for estimating risk to human health. Other non-cancer hazard endpoints considered by the Agency (*i.e.*, kidney toxicity, neurotoxicity, and immune system toxicity) were not considered for dose-response analysis due to limitations and uncertainties that reduce EPA's confidence in using these endpoints for estimating risk to human health.

EPA is not considering cancer hazard endpoints for dose-response analysis (discussed in Section 5).

For the risk evaluation of DIDP, EPA considered NOAEL and LOAEL values from oral toxicity studies in experimental animal models. The use of a NOAEL/LOAEL approach is supported by consistency across several studies that have evaluated liver and developmental toxicity are similar and cluster around a single human equivalent dose (HED) NOAEL value, which supports identification of a consensus NOAEL. Acute, intermediate, and chronic non-cancer NOAEL and LOAEL values identified by EPA are discussed further in Sections 6.1.1, 6.1.2 and 6.1.3, respectively. Benchmark dose (BMD) modeling on select liver endpoints from one chronic dietary study (Cho et al., 2010; Cho et al., 2008) was conducted to refine the dose-response, since the study supported a potentially sensitive LOAEL and did not allow for the identification of a NOAEL (discussed in Section 6.1.3).

Data for the dose-response assessment were selected from oral toxicity studies in animals. No toxicological data were available by the dermal or inhalation route that could be used for dose-response assessment, and no PBPK models are available to extrapolate between animal and human doses or between routes of exposure using DIDP-specific information.

The PODs estimated based on effects in animals were converted to HEDs for the oral and dermal routes and HECs for the inhalation route. For this conversion, EPA used guidance from U.S. EPA (2011b) to allometrically scale oral data between animals and humans. Although the guidance is specific for the oral route, the Agency used the same HEDs for the dermal route of exposure as the oral route because the extrapolation from oral to dermal routes is done using the human oral doses, which do not need to be scaled across species. EPA accounts for dermal absorption in the dermal exposure estimates, which can then be directly compared to the dermal HEDs. Appendix D provides further details on the Agency's approach to calculating HEDs and use of oral HEDs.

For the inhalation route, EPA extrapolated the daily oral HEDs to HECs using human body weight and breathing rate relevant to a continuous exposure of an individual at rest (U.S. EPA, 1994). The Agency assumed similar absorption for the oral and inhalation routes (*i.e.*, 100% absorption) and no adjustment was made when extrapolating to the inhalation route. For consistency, all HEDs are expressed as daily doses and all HECs are based on daily, continuous concentrations (24 hours per day) using a breathing rate for individuals at rest. Adjustments to exposure durations, exposure frequencies, and breathing rates are made in the exposure estimates used to calculate risks for individual exposure scenarios. Appendix D provides further information on extrapolation of inhalation HECs from oral HEDs.

6.1 Selection of Studies and Endpoints for Non-cancer Toxicity

EPA considered the suite of oral animal toxicity studies for adverse liver and developmental effects when considering non-cancer PODs for estimating risks for acute, intermediate, and chronic exposure scenarios, as described in Sections 6.1.1, 6.1.2, and 6.1.3, respectively. The Agency selected studies and relevant health effects based on the following considerations:

- exposure duration;
- dose range;
- relevance (*e.g.*, what species was the effect in, was the study directly assessing the effect, is the endpoint the best marker for the toxicological outcome?);
- uncertainties not captured by the overall quality determination;
- endpoint/POD sensitivity; and
- total uncertainty factors (UFs).

The following sections provide comparisons of the above attributes for studies and hazard outcomes for each of these exposure durations and details related to the studies considered for each exposure duration scenario.

6.1.1 Non-cancer Oral Points of Departure for Acute Exposures

EPA considered developmental effects from two prenatal studies and a pair of two-generation studies of reproduction with rats relevant to acute exposure durations (U.S. EPA, 1996, 1991b). The endpoints considered relevant to acute exposure durations include skeletal and visceral variations as well as reduced F2 offspring survival on PND1 and PND4. Available studies are summarized in Table 6-1 and the dose-response array for these studies is depicted graphically in Figure 6-1.

In the first prenatal developmental toxicity study of SD rats that adhered to EPA guidelines (OPPTS 870.3700) (Waterman et al., 1999), increased incidence of skeletal variations (*i.e.*, rudimentary lumbar and supernumerary cervical ribs) were observed at doses that caused no maternal toxicity and no effects on fetal weight. This study supports a LOAEL of 500 mg/kg-day and a NOAEL of 100 mg/kg-day (HED of 24 mg/kg-day) for developmental toxicity. Similarly, in a second prenatal study of Wistar rats increased incidence of combined skeletal and visceral variations were observed at doses that did not cause maternal toxicity or effects on fetal body weight (Hellwig et al., 1997). That study supports a LOAEL of 200 mg/kg-day and a NOAEL of 40 mg/kg-day (HED of 9.5 mg/kg-day) for developmental toxicity. Although the prenatal study by Hellwig et al. is GLP-compliant and generally adhered to EPA guidelines (OPPTS 870.3700) available when the study was conducted, it has some limitations including a small sample size (7–10 dams included per dose group) and a non-standard statistical analysis of combined skeletal and visceral variations. Overall, the two prenatal studies of SD and Wistar rats provide consistent evidence of effects on the developing fetus (*i.e.*, increased skeletal and visceral variations at doses that did not cause maternal toxicity); however, as discussed further below, these studies are less sensitive than the two-generation studies of reproduction, which provide a lower POD.

Hushka et al. (2001) report the results of the pair of two-generation studies of reproduction of SD rats conducted by Exxon Biomedical (2000, 1998). Both studies are GLP-compliant and adhered to EPA testing guidelines available at the time of when the study was conducted (*i.e.*, OPPTS 870.3800, 1994 Draft Test Guidelines for Reproduction and Fertility Effects). In the first study, dose-related decreases in F2 offspring survival on PND1 and PND4 were observed at all doses, supporting a LOAEL of 135 mg/kg-day (HED of 32 mg/kg-day) for developmental effects. No NOAEL for developmental toxicity was established. Additional effects were noted at higher doses in this study, including decreased F1 and F2 male and female offspring body weight from PND0 through PND21 in the high-dose group, and

decreases in P1 and P2 male and female food consumption and body weight in the high-dose group (see Appendix C.1 for further details. In the second two-generation study, which tested lower doses than the first study, dose-related decreases in F2 offspring survival on PND1 and PND4 were observed, supporting a LOAEL of 134 mg/kg-day and a NOAEL of 38 mg/kg-day (HED of 9.0 mg/kg-day) for developmental toxicity. Overall, the (1998) two-generation study reported by Exxon Biomedical provides the most sensitive POD.

To calculate risks for the acute exposure duration in the risk evaluation of DIDP, EPA selected the daily HED of 9.0 mg/kg (NOAEL of 38 mg/kg-day) from the two-generation study of reproduction of SD rats based on reduced F2 offspring survival on PND1 and PND4 (Hushka et al., 2001; Exxon Biomedical, 2000). A total uncertainty factor of 30 was selected for use as the benchmark margin of exposure (based on an interspecies uncertainty factor [UF_A] of 3 and an intraspecies uncertainty factor [UF_H] of 10). Consistent with EPA guidance (2022, 2002b, 1993), the Agency reduced the UF_A from a value of 10 to 3 because allometric body weight scaling to the three-quarter power was used to adjust the POD to obtain a HED (see Appendix D). The selected HED is the most sensitive acute HED identified by EPA; however, the prenatal study by Hellwig et al. supports a similar HED of 9.5 mg/kg-day (Table 6-1). The critical effect, reduced F2 offspring survival on PND1 and PND4, is clearly adverse and is assumed to be human relevant. It is unclear whether decreased pup survival was due to a single, acute exposure or from repeated exposures. It is plausible that reduced offspring survival could result from a single exposure during gestation. However, it is also plausible that reduced offspring survival could result from repeated exposure during gestation or the postnatal period. Because repeated dose studies were used to investigate these hazard endpoints and the MOA for DIDP is uncertain, and because other studies did not provide a more sensitive or reliable endpoint, EPA considered reduced F2 offspring survival relevant for all exposure durations (U.S. EPA, 1996, 1991b).

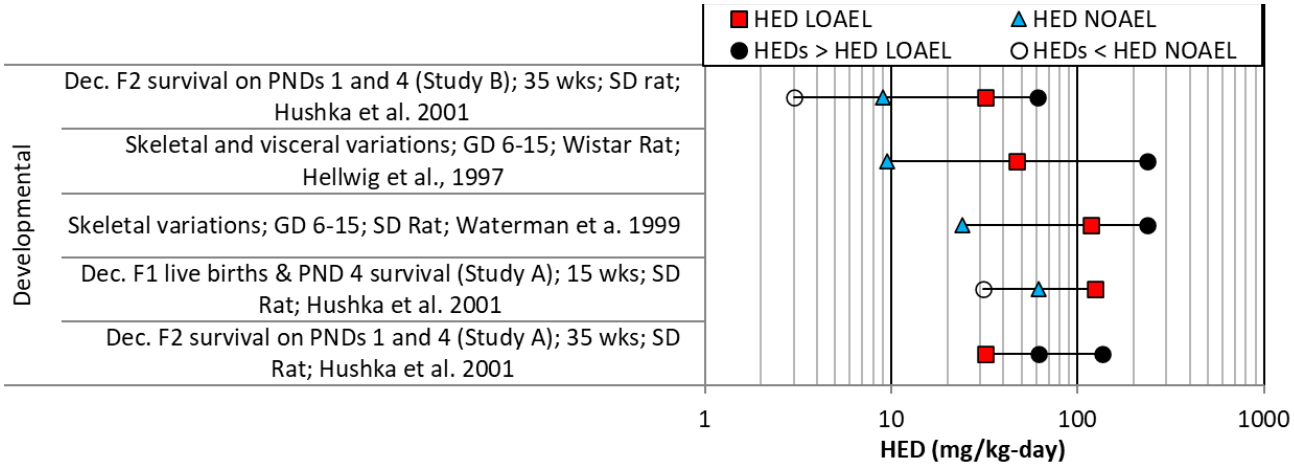

Figure 6-1. Exposure Response Array of Selected Studies Considered for Acute Exposure Scenarios

Table 6-1. Dose-Response Analysis of Selected Studies Considered for Acute Exposure Scenarios

Target Organ/ System	Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HEC (mg/m^3) [ppm]	HED (mg/kg)	Uncertainty Factors[a] [b]	Reference(s)
Developmental Toxicity	Sprague-Dawley rats; approximately 35 weeks; oral/dietary; 0, 13, 38, 134, 256 (Study B)	NOAEL = 38	Decreased F2 survival on PND1 and PND4	49 [2.7]	9.0	UF_A = 3 UF_H = 10 *Total UF = 30*	(Hushka et al., 2001; Exxon Biomedical, 2000)
Developmental Toxicity	Wistar rats; GDs 6–15; oral/gavage; 0, 40, 200, 1000	NOAEL = 40	Skeletal and visceral variations	51 [2.8]	9.5	UF_A = 3 UF_H = 10 *Total UF = 30*	(Hellwig et al., 1997)
Developmental Toxicity	Sprague-Dawley rats; GDs 6–15; oral/gavage; 0, 100, 500, 1000	NOAEL = 100	Skeletal variations	129 [7.0]	24	UF_A = 3 UF_H = 10 *Total UF = 30*	(Waterman et al., 1999)
Developmental Toxicity	Sprague-Dawley rats; approximately 35 weeks; oral/dietary; 0, 135, 262, 574 (Study A)	LOAEL = 135	Decreased F2 survival on PND1 and PND4	174 [9.5]	32	UF_A = 3 UF_H = 10 $UF_L = 10^c$ *Total UF = 300*	(Hushka et al., 2001; Exxon Biomedical, 1998)
Developmental Toxicity	Sprague-Dawley rats; approximately 15 weeks; oral/dietary; 0, 131, 262, 524 (Study A)	NOAEL = 262	Decreased F1 live births and PND4 survival	337 [18]	62	UF_A = 3 UF_H = 10 *Total UF = 30*	(Hushka et al., 2001; Exxon Biomedical, 1998)

[a] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011b), the interspecies uncertainty factor (UF_A), was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics.
[b] EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations due to limited information regarding the degree to which human variability may impact the disposition of or response to DIDP.
[c] EPA used a LOAEL-to-NOAEL uncertainty factor (UF_L) of 10 to account for the uncertainty inherent in extrapolating from the LOAEL to the NOAEL.

6.1.2 Non-cancer Oral Points of Departure for Intermediate Exposures

EPA used the acute HED (9.0 mg/kg-day) and benchmark MOE (30) identified in Section 6.1.1 to evaluate risk from intermediate exposures (*i.e.*, ranging from >1–30 days) to DIDP. The acute HED is more sensitive than the four candidate intermediate HEDs based on liver toxicity and is therefore protective of intermediate duration exposures to DIDP. In addition, it is based on a repeated exposure study and EPA considers it to be relevant for intermediate exposures.

As can be seen from Figure 6-2 and Table 6-2, three of the intermediate HED NOAELs based on liver toxicity are extremely similar to the acute HED NOAEL. These intermediate HED NOAELs based on liver toxicity range from 10 to 13 mg/kg-day compared to the acute HED NOAEL of 9.0 mg/kg-day. For all of these studies, a total uncertainty factor of 30 was selected (UF_A of 3; UF_H of 10); therefore, EPA's selected acute HED is more sensitive and protective. One study supports an HED LOAEL of 72 mg/kg-day (BIBRA, 1986) and a total uncertainty factor of 300 was selected (UF_A of 3; UF_H of 10; UF_L of 10). EPA considered whether this intermediate HED LOAEL and total uncertainty factor may provide a more protective endpoint to use in the risk evaluation of DIDP than the acute HED. However, the study by BIBRA (1986) did not allow for the identification of a NOAEL and is limited by dose selection. Further, the remaining three intermediate studies of mice and rats all tested lower doses allowing for the identification of NOAELs—all of which were slightly less sensitive than the acute HED NOAEL. This further supports EPA's decision to use the acute HED to evaluate risk from intermediate exposures to DIDP.

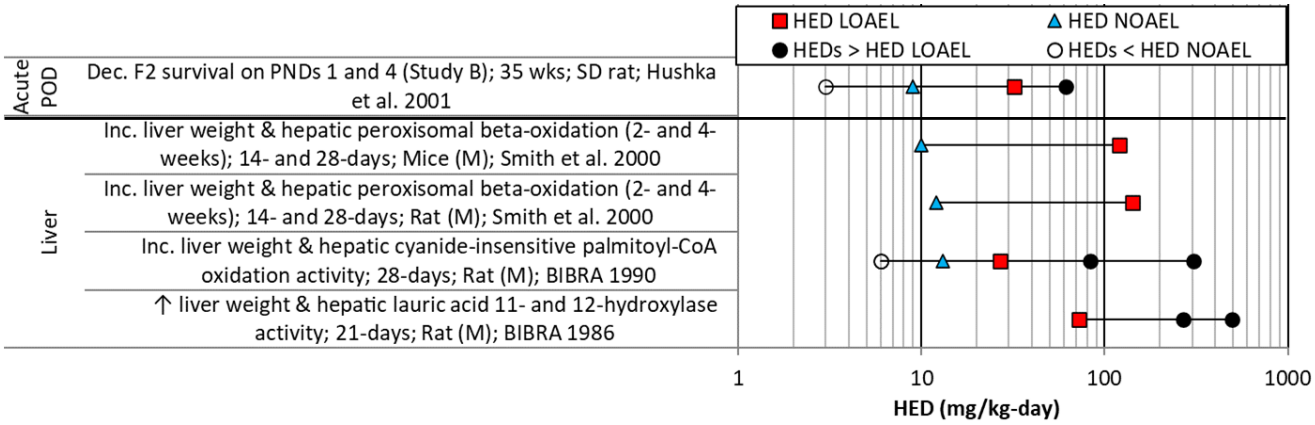

Figure 6-2. Exposure Response Array of Selected Studies Considered for intermediate Exposure Scenarios

Table 6-2. Dose-Response Analysis of Selected Studies Considered for Intermediate Exposure Scenarios

Target Organ/ System	Study Details (Species, Duration, Exposure Route/Method, Doses (mg/kg-day)	Study POD/ Type (mg/kg-day)	Effect	HEC (mg/m^3) [ppm]	HED (mg/kg-day)	Uncertainty Factors [a] [b]	Reference
Liver Toxicity	B6C3F1 mice; 14 and 28 days; oral/dietary; 0, 75, 900	NOAEL = 75	↑ relative liver weight & hepatic peroxisomal beta-oxidation (at 2 and 4weeks)	54 [3.0]	10	UF_A = 3 UF_H = 10 *Total UF = 30*	(Smith et al., 2000)
Liver Toxicity	F344 rats; 14 and 28 days; oral/dietary; 0, 50, 600	NOAEL = 50	↑ relative liver weight & hepatic peroxisomal beta-oxidation (at 2 and 4 weeks)	65 [3.5]	12	UF_A = 3 UF_H = 10 *Total UF = 30*	(Smith et al., 2000)
Liver Toxicity	F344 rats; 28 days; oral/ dietary; 0, 25, 57, 116, 353, 1287	NOAEL = 57	↑ relative liver weight & hepatic cyanide-insensitive palmitoyl-CoA oxidation activity	73 [4.0]	13	UF_A = 3 UF_H = 10 *Total UF = 30*	(Lake et al., 1991; BIBRA, 1990)
Liver Toxicity	F344 rats; 21 days; oral/dietary; 0, 304, 1134, 2100	LOAEL = 304	↑ liver weight & hepatic lauric acid 11- and 12-hydroxylase activity	391 [21]	72	UF_A = 3 UF_H = 10 $UF_L = 10^c$ *Total UF = 300*	(BIBRA, 1986)

[a] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011b), the interspecies uncertainty factor (UF_A), was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics.

[b] EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations due to limited information regarding the degree to which human variability may impact the disposition of or response to DIDP.

[c] EPA used a LOAEL-to-NOAEL uncertainty factor (UF_L) of 10 to account for the uncertainty inherent in extrapolating from the LOAEL to the NOAEL.

6.1.3 Non-cancer Oral Points of Departure for Chronic Exposures

Table 6-3 and Figure 6-3 presents EPA's dose-response analysis of selected experimental animal studies considered for deriving chronic HEDs.

EPA used the acute HED (9.0 mg/kg-day) based on reduced F2 offspring survival on PND1 and PND4 and benchmark MOE (30) identified in Section 6.1.1 to evaluate risk from chronic exposures to DIDP. As discussed in Section 6.1.1, it is unclear whether decreased pup survival was due to a single, acute exposure or from repeated exposures. It is plausible that reduced offspring survival could result from a single exposure during gestation. However, it is also plausible that reduced offspring survival could result from repeated exposure during gestation or the postnatal period. Because repeated dose studies were used to investigate these hazard endpoints and the MOA for DIDP is uncertain, and because other studies did not provide a more sensitive or reliable endpoint, EPA considered reduced F2 offspring survival relevant for all exposure durations (U.S. EPA, 1996, 1991b). Notably, this HED is more sensitive than all but one of the candidate chronic HEDs (*i.e.*, the HED LOAEL of 5.2 mg/kg-day) based on liver toxicity (Table 6-3). However, as discussed further below, there is significant uncertainty associated with the spongiosis hepatis and microgranuloma HED LOAEL, which reduced EPA's confidence in using the HED for assessing risks from chronic exposures to DIDP.

In a 2-year dietary study of F344 rats (Cho et al., 2010; Cho et al., 2008), statistically significant increases in spongiosis hepatis and microgranuloma were observed in the livers of male rats at all tested doses supporting a LOAEL of 22 mg/kg-day and an HED LOAEL of 5.2 mg/kg-day (benchmark MOE of 300). This HED is more sensitive than the selected HED NOAEL of 9.0 mg/kg-day (benchmark MOE of 30) based on reduced F2 offspring survival on PND1 and PND4. However, there are several sources of uncertainty associated with the study that reduced EPA's confidence in using it for risk characterization. First, the dose-response curve for incidence of microgranuloma is flat across the range of tested doses (2, 10, 12, and 10% across doses), while the dose-response curve for spongiosis hepatis is flat—particularly in the low- and mid-dose groups (0, 6.3, 6.1, and 13% across doses). Beyond increased incidence of spongiosis hepatis and microgranuloma, hepatotoxic effects were limited to male and female rats of the high-dose group (*e.g.*, increased relative liver weight [both sexes], necrosis [both sexes], oval cell hyperplasia [males only], hypertrophy [males only], peliosis [males only]), for which a significant reduction in survival was also observed. Another source of uncertainty stems from the fact that spongiosis hepatis and microgranuloma have not been reported in any other studies of DIDP, including intermediate duration studies of rats that tested up to 2,100 mg/kg-day DIDP, subchronic studies of rats that tested up to 586 to 686 mg/kg-day DIDP, and a 26-week study of mice that tested 1.500 mg/kg-day DIDP (Section 3.1.2).

Because the study by Cho et al. (2010; 2008) did not allow for the identification of a NOAEL, EPA conducted BMD modeling of the incidence data for histopathologic lesions in the liver of male and female F344 rats to refine the dose-response (Appendix E). The 95 percent lower confidence limit on the BMD (BMDL) associated with a benchmark response (BMR) of 10 percent (BMDL10) for spongiosis hepatis and microgranuloma in male rats were 172 and 314 mg/kg-day, respectively (Table_Apx E-1). Further, BMDL10 values for other histopathologic lesions in the liver ranged from 94 mg/kg-day for oval cell hyperplasia in the liver of male rats to 253 mg/kg-day for peliosis in the liver of male rats (Table_Apx E-1).

Collectively, the sources of uncertainty discussed above (*i.e.*, occurrence of spongiosis hepatis and microgranuloma in only one study; spongiosis hepatis only observed in male, but not female rats; both lesions displayed low incidence and flat dose-responses; low survival of high-dose male and female rats

in the key study; unknown MOA; uncertain human-relevance) reduced EPA's confidence in using the LOAEL of 22 mg/kg-day (HED LOAEL of 5.2 mg/kg-day) as a POD for assessing risks from chronic exposures to DIDP. Furthermore, BMD modeling of liver histopathology incidence data indicate that the observed liver effects in the 2-year dietary study by Cho et al. (*i.e.*, BMDL10 values ranged from 94 to 314 mg/kg-day) actually occur at higher doses (approximating mid- to high-dose) than indicated by the LOAEL of 22 mg/kg-day at the low-dose group and are less sensitive than the selected chronic POD based on a NOAEL of 38 mg/kg-day (HED of 9.0 mg/kg-day) for decreased F2 offspring survival on PND1 and PND4.

In contrast, numerous factors increase EPA's confidence in using the HED NOAEL of 9.0 mg/kg-day based on reduced F2 offspring survival on PND1 and PND4 to assess risks from chronic exposure to DIDP. First, the key study was GLP-compliant and adhered to EPA testing guidelines (OPPTS 870.3800). Further, decreased F2 survival on PND1 and PND4 was observed consistently in a pair of two-generations studies, and in both studies F2 offspring survival was reduced in a clear dose-dependent manner. Additionally, two prenatal developmental toxicity studies have also reported increased incidence of skeletal and visceral variations in rats. Collectively, there is a robust database of studies supporting the conclusion that DIDP can cause developmental toxicity in experimental animal models. Given these factors, EPA selected the HED NOAEL of 9.0 mg/kg-day based on reduced F2 offspring survival to evaluate risk from chronic exposures to DIDP.

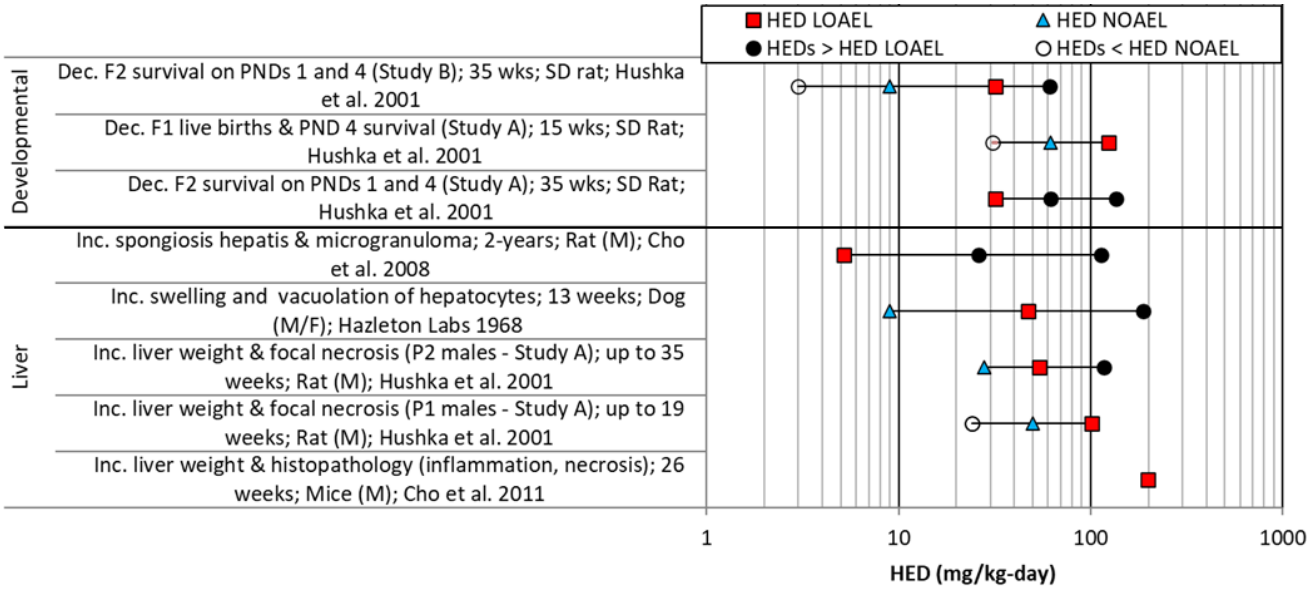

Figure 6-3. Exposure Response Array of Selected Studies Considered for Chronic Exposure Scenarios

Table 6-3. Dose-Response Analysis of Selected Studies Considered for Chronic Exposure Scenarios

Target Organ/ System	Study Details (Species, Duration, Exposure Route/ Method, Doses (mg/kg-day)	Study POD/ Type (mg/kg-day)	Effect	HEC (mg/m^3) [ppm]	HED (mg/kg)	Uncertainty Factors[a] [b]	Reference
Liver toxicity	F344 rats; 2 years; oral/dietary; 0, 22, 110, 479	LOAEL = 22	↑ incidence of spongiosis hepatis and microgranuloma	28 [1.5]	5.2	UF_A = 3 UF_H = 10 UF_L = 10[c] *Total UF = 300*	(Cho et al., 2010; Cho et al., 2008)
Developmental toxicity	Sprague-Dawley rats; up to approximately 35 weeks (F2 offspring – Study B); oral/dietary; 0, 13, 38, 134, 256	NOAEL = 38	Decreased F2 survival on PND1 and PND4	49 [2.7]	9.0	UF_A = 3 UF_H = 10 *Total UF = 30*	(Hushka et al., 2001; Exxon Biomedical, 2000)
Liver toxicity	Beagles; 13 weeks; oral/dietary; 0, 15, 75, 300	NOAEL = 15	↑ swelling and vacuolation of hepatocytes	51 [2.8]	9.3	UF_A = 3 UF_H = 10 *Total UF = 30*[d]	(Hazelton Labs, 1968a)
Liver toxicity	Sprague-Dawley rats; up to approximately 35 weeks (P2 males); oral/dietary; 0, 117, 229, 494	NOAEL = 117	↑ liver weight, histopathology (focal necrosis)	151 [8.2]	28	UF_A = 3 UF_H = 10 *Total UF = 30*	(Hushka et al., 2001; Exxon Biomedical, 1998)
Developmental toxicity	Sprague-Dawley rats; up to approximately 35 weeks (F2 offspring – Study A); oral/dietary; 0, 135, 262, 574	LOAEL = 135	Decreased F2 survival on PND1 and PND4	174 [9.5]	32	UF_A = 3 UF_H = 10 UF_L = 10[c] *Total UF = 300*	(Hushka et al., 2001; Exxon Biomedical, 1998)
Liver toxicity	Sprague-Dawley rats; up to approximately 19 weeks (F1 offspring – Study A); oral/dietary; 0, 103, 211, 427	NOAEL = 211	↑ liver weight, histopathology (focal necrosis)	271 [15]	50	UF_A = 3 UF_H = 10 *Total UF = 30*	(Hushka et al., 2001; Exxon Biomedical, 1998)
Developmental toxicity	Sprague-Dawley rats; approximately 15 weeks (F1 offspring – Study A); oral/dietary; 0, 131, 262, 524	NOAEL = 262	Decreased F1 live births and PND4 survival	337 [18]	62	UF_A = 3 UF_H = 10 *Total UF = 30*	(Hushka et al., 2001; Exxon Biomedical, 1998)
Liver toxicity	Wild-type mice; 26-weeks; oral/dietary; 0, 1500	LOAEL = 1500	↑ liver weight, histopathology (inflammation, necrosis)	1085 [59]	199	UF_A = 3 UF_H = 10 UF_L = 10[c] *Total UF = 300*	(Cho et al., 2011)

[a] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011b), the interspecies uncertainty factor (UF_A), was reduced from 10 to 3 to account remaining uncertainty associated with interspecies differences in toxicodynamics.

[b] EPA used a default intraspecies (UF_H) of 10 to account for variation in sensitivity within human populations due to limited information regarding the degree to which human variability may impact the disposition of or response to DIDP.

[c] EPA used a LOAEL-to-NOAEL uncertainty factor (UF_L) of 10 to account for the uncertainty inherent in extrapolating from the LOAEL to the NOAEL.

[d] EPA considered applying a subchronic-to-chronic uncertainty factor (UF_s) of 10 for the 13-week study of beagles. However, retrospective analyses of 13-week and 1-year dog studies have shown that dog studies beyond 13-weeks do not have a significant impact on the derivation of chronic PODs (Bishop et al., 2023; Dellarco et al., 2010; Box and Spielmann, 2005). Therefore, EPA did not consider a UF_s of 10 necessary.

6.1.4 Weight of Scientific Evidence Conclusion: POD for Acute, Intermediate, and Chronic Durations

EPA has concluded that the HED of 9.0 mg/kg (NOAEL of 38 mg/kg-day) from the two-generation study of reproduction of SD rats based on reduced F2 offspring survival on PND1 and PND4 (Hushka et al., 2001; Exxon Biomedical, 2000) is appropriate for calculation of risk from acute, intermediate and chronic durations. A total uncertainty factor of 30 was selected for use as the benchmark margin of exposure (based on an interspecies uncertainty factor (UF_A) of 3 and an intraspecies uncertainty factor (UF_H) of 10). Consistent with EPA guidance (2022, 2002b, 1993), the Agency reduced the UF_A from a value of 10 to 3 because allometric body weight scaling to the three-quarter power was used to adjust the POD to obtain a HED (Appendix D**). EPA has robust overall confidence in the selected POD based on the following weight of scientific evidence:**

- DIDP exposure resulted in treatment related developmental toxicity in two prenatal studies of Wistar and SD rats (Waterman et al., 1999; Hellwig et al., 1997) and a pair of two-generation studies of SD rats (Hushka et al., 2001; Exxon Biomedical, 2000, 1998) (Section 3.1.1). Available studies adhered to relevant EPA guidelines (*i.e.,* OPPTS 870.3700 and OPPTS 870.3800).
- DIDP exposure consistently resulted in increased incidence of skeletal and visceral variations in prenatal studies of SD and Wistar rats at doses that did not cause maternal toxicity. NOAELs/LOAELs for developmental and maternal toxicity were 40/200 and 200/1,000 mg/kg-day, respectively, in the study by Hellwig et al. (1997), and 200/500 and 500/1,000 mg/kg-day, respectively, in the study by Waterman et al. (1999).
- In the first two-generation study (Study A) by Exxon Biomedical (1998), DIDP exposure reduced F1 offspring survival on PND4, reduced F1 and F2 offspring body weight on PND0, and reduced F1 and F2 offspring body weight gain through PND21 at doses equal to 524 to 637 mg/kg-day DIDP. Effects on F1 offspring survival, and offspring body weight and weight gain were not observed in the second two-generation study (Study B) by Exxon Biomedical (2000), which tested lower doses of DIDP (high-dose group received approximately 254 to 356 mg/kg-day).
- DIDP exposure reduced F2 offspring survival on PND1 and PND4 at doses that did not cause overt toxicity to either parental generation. Reduced F2 offspring survival on PND1 and PND4 was observed at doses greater than or equal to 134 to 135 mg/kg-day in both two-generation studies of reproduction (Hushka et al., 2001; Exxon Biomedical, 2000, 1998).
- As discussed in Sections 6.1.1 through 6.1.3, the prenatal developmental study by Hellwig et al. (1997) supports an HED of 9.5 mg/kg based on increased incidence of skeletal variations, while three additional intermediate duration studies of rats and mice support HEDs ranging from 10 to 13 mg/kg-day based on liver effects (Smith et al., 2000; Lake et al., 1991; BIBRA, 1990), and a subchronic study of beagles supports an HED of 9.3 mg/kg-day based on liver effects (Hazelton Labs, 1968a). Overall, these 5 studies support HEDs ranging from 9.3 to 13 mg/kg-day, which are similar to the selected HED of 9.0 mg/kg based on reduced F2 offspring survival on PND1 and PND4 (Hushka et al., 2001; Exxon Biomedical, 2000), and further support EPA's selected HED.
- As discussed in Section 6.1.3, the 2-year dietary study of F344 rats by Cho et al. (2010; 2008) provided a slightly lower POD (HED of 5.2 mg/kg-day) based on a LOAEL for increased incidence of spongiosis hepatis and microgranuloma. However, several sources of uncertainty reduced EPA's confidence in this POD, including (1) the dose-response for incidence of

spongiosis hepatis was flat; (2) spongiosis hepatis was not observed in female rats from the same study or in any other study of DIDP; (3) the MOA for spongiosis hepatis is unknown; and (4) there is uncertainty related to the human relevance of the spongiosis hepatis. Further, BMD modeling indicate that the selected POD based on reduced F2 offspring survival is more sensitive than the observed liver effects in the two year dietary study by Cho et al. (*i.e.*, BMDL10 values ranged from 94 to 314 mg/kg-day).

- Other regulatory and authoritative bodies have also concluded that DIDP is a developmental toxicant and that developmental effects are relevant for estimating human risk (EFSA, 2019; EC/HC, 2015; NICNAS, 2015; ECHA, 2013b; U.S. CPSC, 2010; EFSA, 2005; ECB, 2003; NTP-CERHR, 2003).

There are no studies conducted via the dermal and inhalation route relevant for extrapolating human health risk. Therefore, EPA is using the oral HED of 9.0 mg/kg to extrapolate to the dermal route. EPA's approach to dermal absorption for workers, consumers, and the general population is described in EPA's *Environmental Release and Occupational Exposure Assessment for Diisodecyl Phthalate (DIDP)* (U.S. EPA, 2024f) and *Consumer and Indoor Dust Exposure Assessment for Diisodecyl Phthalate (DIDP)* (U.S. EPA, 2024b).

EPA is also using the oral HED of 9.0 mg/kg to extrapolate to the inhalation route. The Agency assumes similar absorption for the oral and inhalation routes based on toxicokinetic information provided in Section 2, and no adjustment was made when extrapolating to the inhalation route. For the inhalation route, EPA extrapolated the daily oral HEDs to inhalation HECs using a human body weight and breathing rate relevant to a continuous exposure of an individual at rest. Appendix D provides further information on extrapolation of inhalation HECs from oral HEDs.

Route-to-route extrapolation of the oral HED to an inhalation HEC and dermal HED results in additional uncertainty. EPA cannot predict whether the assumptions regarding route extrapolation for the chosen POD would lead to over- or underprediction of risk for the dermal and inhalation routes.

7 CONSIDERATION OF PESS AND AGGEGRATE EXPOSURE

7.1 Hazard Considerations for Aggregate Exposure

For use in the risk evaluation and assessing risks from other exposure routes, EPA conducted route-to-route extrapolation of the toxicity values from the oral studies for use in the dermal and inhalation exposure routes and scenarios. Because the health outcomes are systemic and are based on the oral studies, EPA considers it is possible to aggregate risks across exposure routes for all exposure durations and endpoints for the selected PODs in Section 8.

7.2 PESS Based on Greater Susceptibility

In this section, EPA addresses subpopulations expected to be more susceptible to DIDP exposure than other populations. Table 7-1 presents the data sources that were used in the PESS analysis evaluating susceptible subpopulations and identifies whether and how the subpopulation was addressed quantitatively in the risk evaluation of DIDP.

Several conclusions can be made regarding factors that may increase susceptibility to the effects of DIDP. Limited human data are available on health effects of DIDP and EPA did not identify differences in susceptibility among human populations. Animal studies identified developmental effects (Hushka et al., 2001; Exxon Biomedical, 2000; Waterman et al., 1999; Exxon Biomedical, 1998; Hellwig et al., 1997) and EPA is quantifying risks based on developmental toxicity in the risk evaluation of DIDP. The critical effect that is the basis of the POD is reduced F2 offspring survival on PND1 and PND4. Based on the selected POD, pregnant women, women of reproductive age, and infants may be more susceptible to DIDP exposure than other populations.

As identified in Table 7-1, there are many other susceptibility factors that are generally considered to increase susceptibility of individuals to chemical hazards. These factors include pre-existing diseases, alcohol use, smoking, physical activity, diet, stress, among others. The effect of these factors on susceptibility to health effects of DIDP is not known; therefore, EPA is uncertain about the magnitude of any possible increased risk from effects associated with DIDP exposure for relevant subpopulations.

For non-cancer endpoints, EPA used a default value of 10 for human variability (UF_H) to account for increased susceptibility when quantifying risks from exposure to DIDP. The Risk Assessment Forum, in *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), discusses some of the evidence for choosing the default factor of 10 when data are lacking and describe the types of populations that may be more susceptible, including different lifestages (*e.g.*, of children and elderly). U.S. EPA (2002b), however, did not discuss all the factors presented in Table 7-1. Thus, uncertainty remains whether additional susceptibility factors would be covered by the default UF_H value of 10 chosen for use in the risk evaluation of DIDP.

Table 7-1. PESS Evidence Crosswalk for Biological Susceptibility Considerations

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DIDP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DIDP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Embryos/ fetuses/infants	Direct quantitative animal evidence for developmental toxicity (*e.g.*, increased skeletal and visceral variations, decreased live births, decreased offspring body weight gain, and decreased offspring survival with increased severity in the second generation). Lack of effects on the developing male reproductive system consistent with a disruption of androgen action.	Hellwig et al. (1997) Waterman et al. (1999) Hushka et al. (2001) U.S. EPA (2023b, d)	–	–	POD for developmental endpoints protective of effects in offspring
	Females of reproductive age/pregnancy/ lactating status	Rodent dams not particularly susceptible during pregnancy and lactation, except for effects related to reduced maternal weight gain, which occurred at doses higher than those that caused developmental toxicity.	Waterman et al. (1999) Hushka et al. (2001)	–	–	POD for developmental endpoints protective of effects in dams (*i.e.*, developmental effects occurred at lower doses than effects in dams)
	Males of reproductive age	No direct evidence identified	–	–	–	Use of default UF_H
	Children	Reduced rodent offspring bodyweight gain between PND1 to PND21 was observed in one two-generation study of reproduction.	Hushka et al. (2001)	–	–	POD for developmental endpoints protective of effects of offspring bodyweight gain Use of default UF_H
	Elderly	No direct evidence identified	–	–	–	Use of default UF_H

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DIDP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DIDP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Pre-existing disease or disorder	Health outcome/ target organs	No direct evidence identified	–	Several preexisting conditions may contribute to adverse developmental outcomes (*e.g.*, diabetes, high blood pressure, certain viruses). Viruses such as viral hepatitis can cause liver damage.	CDC (2023e) CDC (2023g)	Use of default UF_H
	Toxicokinetics	No direct evidence identified	–	–	–	Use of default UF_H
Lifestyle activities	Smoking	No direct evidence identified	–	Smoking during pregnancy may increase susceptibility for developmental outcomes (*e.g.*, early delivery and stillbirths).	CDC (2023f)	Qualitative discussion in Section 7.2 and this table
	Alcohol consumption	No direct evidence identified	–	Alcohol use during pregnancy can cause developmental outcomes (*e.g.*, fetal alcohol spectrum disorders). Heavy alcohol use may affect susceptibility to liver disease.	CDC (2023d) CDC (2023a)	Qualitative discussion in Section 7.2 and this table
	Physical activity	No direct evidence identified	–	Insufficient activity may increase susceptibility to multiple health outcomes. Overly strenuous activity may also increase susceptibility.	CDC (2022)	Qualitative discussion in Section 7.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DIDP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DIDP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Sociodemo-graphic status	Race/ethnicity	No direct evidence identified (*e.g.*, no information on polymorphisms in DIDP metabolic pathways or diseases associated race/ethnicity that would lead to increased susceptibility to effects of DIDP by any individual group).	–	–	–	Qualitative discussion in Section 7.2 and this table
	Socioeconomic status	No direct evidence identified	–	Individuals with lower incomes may have worse health outcomes due to social needs that are not met, environmental concerns, and barriers to health care access.	ODPHP (2023b)	
	Sex/gender	No direct evidence identified	–	–	–	Use of default UF_H

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DIDP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DIDP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Nutrition	Diet	No direct evidence identified	–	Poor diets can lead to chronic illnesses such as heart disease, type 2 diabetes, and obesity, which may contribute to adverse developmental outcomes.	CDC (2023e) CDC (2023b)	Qualitative discussion in Section 7.2 and this table
	Malnutrition	No direct evidence identified	–	Micronutrient malnutrition can lead to multiple conditions that include birth defects, maternal and infant deaths, preterm birth, low birth weight, poor fetal growth, childhood blindness, undeveloped cognitive ability. Thus, malnutrition may increase susceptibility to some developmental outcomes associated with DIDP.	CDC (2021) CDC (2023b)	Qualitative discussion in Section 7.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DIDP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DIDP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Genetics/ epigenetics	Target organs	Increased incidence of hepatocellular adenomas in male rasH2 mice, but not wild-type mice.	(Cho et al., 2011)	–	–	Qualitative discussion in Section 7.2 and this table
	Toxicokinetics	No direct evidence identified	–	Polymorphisms in genes encoding enzymes (*e.g.*, esterases) involved in metabolism of DIDP may influence metabolism and excretion of DIDP.		Use of default UF_H to assess variability among humans
Other chemical and nonchemical stressors	Built environment	No direct evidence identified	–	Poor-quality housing is associated with a variety of negative health outcomes.	ODPHP (2023a)	Qualitative discussion in Section 7.2 and this table
	Social environment	No direct evidence identified	–	Social isolation and other social determinants (*e.g.*, decreased social capital, stress) can lead to negative health outcomes.	CDC (2023c) ODPHP (2023c)	Qualitative discussion in Section 7.2 and this table
	Chemical co-exposures	No direct evidence identified	–	Co-exposure to toxicologically similar chemicals may increase susceptibility to the developmental and hepatic effects associated with exposure to DIDP.	U.S. EPA (2023a, c)	Qualitative discussion in Section 7.2 and this table

8 POINTS OF DEPARTURE USED TO ESTIMATE RISK FROM DIDP EXPOSURE

After considering hazard identification and evidence integration, dose-response evaluation, and weight of scientific evidence of POD candidates, EPA chose one non-cancer endpoint for evaluating acute, intermediate, and chronic exposure scenarios in the risk evaluation of DIDP (Table 8-1). HECs are based on daily continuous (24-hour) exposure whereas HEDs are daily values.

As described in Section 5, EPA is not evaluating DIDP for cancer risk. No inhalation unit risk or cancer slope factors were derived for DIDP.

Table 8-1. Non-cancer HECs and HEDs Used to Estimate Risks

Exposure Scenario	Target Organ System	Species	Duration	POD (mg/kg-day)	Effect	HED (mg/kg-day)	HEC (mg/m^3) [ppm]	Benchmark MOE	References
Acute, intermediate, chronic	Developmental toxicity	SD rat	Approx. 35 weeks	NOAEL = 38	Reduced F2 offspring survival on PND1 and PND4	9.0	49 [2.7]	UF_A= 3[a] UF_H=10 *Total UF=30*	(Hushka et al., 2001; Exxon Biomedical, 2000)

HEC = human equivalent concentration; HED = human equivalent dose; MOE = margin of exposure; NOAEL = no-observed-adverse-effect level; POD = point of departure; UF = uncertainty factor

[a] EPA used allometric body weight scaling to the three-quarters power to derive the HED. Consistent with EPA Guidance (U.S. EPA, 2011b), the UF_A was reduced from 10 to 3.

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APPENDICES

Appendix A EXISTING ASSESSMENTS FOR OTHER REGULATORY AGENCIES OF DIDP

Table_Apx A-1 summarizes the available existing assessments of DIDP, including details regarding external peer-review, public consultation, and systematic review protocols employed.

Table_Apx A-1. Summary of Peer Review, Public Comments, and Systematic Review for Existing Assessments of DIDP

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
U.S. CPSC	*Toxicity Review of Di(isodecyl) Phthalate* (U.S. CPSC, 2010) *Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives* (U.S. CPSC, 2014)	Yes	Yes	No	- Peer-reviewed by panel of four experts. Peer-review report available at: https://www.cpsc.gov/s3fs-public/Peer-Review-Report-Comments.pdf - Public comments available at: https://www.cpsc.gov/chap - No formal systematic review protocol employed. - Details regarding CPSC's strategy for identifying new information and literature are provided on p. 12 of (U.S. CPSC, 2014)
Health Canada	*State of the Science Report: Phthalates Substance Grouping: Long-chain Phthalate Esters. 1,2-Benzenedicarboxylic acid, diisodecyl ester (diisodecyl phthalate; DIDP) and 1,2-Benzenedicarboxylic acid, diundecyl ester (diundecyl phthalate; DUP). Chemical Abstracts Service Registry Numbers: 26761-40-0,*	Yes	Yes	No (Animal studies) Yes (Epidemiologic studies)	- Ecological and human health portions of the screening assessment report (ECCC/HC, 2020) were subject to external review and/or consultation. See p. 2 of (ECCC/HC, 2020) for additional details. - State of the science report (EC/HC, 2015) and draft screening assessment report for the phthalate substance group subjected to 60-day public comment periods. Summaries of received public comments available at:

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	68515-49-1; 3648-20-2 (EC/HC, 2015) *Supporting Documentation: Carcinogenicity of Phthalates - Mode of Action and Human Relevance* (Health Canada, 2015) *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for hormonal effects, growth and development and reproductive parameters* (Health Canada, 2018b) *Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for effects on behaviour and neurodevelopment, allergies, cardiovascular function, oxidative stress, breast cancer, obesity, and metabolic disorders* (Health Canada, 2018a) *Screening Assessment - Phthalate Substance Grouping* (ECCC/HC, 2020)				https://www.canada.ca/en/health-canada/services/chemical-substances/substance-groupings-initiative/phthalate.html#a1 - No formal systematic review protocol employed to identify or evaluate experimental animal toxicology studies. - Details regarding Health Canada's strategy for identifying new information and literature are provided in Section 1 of (EC/HC, 2015) and (ECCC/HC, 2020) - Human epidemiologic studies evaluated using Downs and Black Method (Health Canada, 2018a, b)
NICNAS	*Priority Existing Chemical Draft Assessment Report: Diisodecyl Phthalate & Di-n-octyl Phthalate* (NICNAS, 2015)	No	Yes	No	- NICNAS (2015) states "The report has been subjected to internal peer review by NICNAS during all stages of preparation." However, a formal external peer-review was not conducted.

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
					- NICNAS (2015) states "In accordance with the Act, NICNAS makes a draft report of the assessment available to the applicants for comment during the correction and variation stages of the PEC consultation process." See Section 1.5 of (NICNAS, 2015) for more details. - No formal systematic review protocol employed. - Details regarding NICNAS's strategy for identifying new information and literature are provided in Section 1.3 of (NICNAS, 2015)
ECHA	*Evaluation of New Scientific Evidence Concerning DINP and DIDP in Relation to Entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006* (ECHA, 2013b)	Yes	Yes	No	- Peer-reviewed by ECHA's Committee for Risk Assessment (ECHA, 2013a) - Subject to 12-week public consultation - No formal systematic review protocol employed.. - Details regarding ECHA's strategy for identifying new information and literature are provided on pages 14-15 of (ECHA, 2013b)
EFSA	*Update of the Risk Assessment of Di-butylphthalate (DBP), Butyl-benzyl-phthalate (BBP), Bis(2-ethylhexyl)phthalate (DEHP), Di-isononylphthalate (DINP) and Di-isodecylphthalate (DIDP) for Use in Food Contact Materials* (EFSA, 2019)	No	Yes	No	- Draft report subject to public consultation. Public comments and EFSA's response to comments are available at: https://doi.org/10.2903/sp.efsa.2019.EN-1747 - No formal systematic review protocol employed.

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
					- Details regarding EFSA's strategy for identifying new information and literature are provided on page 18 and Appendix B of (EFSA, 2019)
NTP-CERHR	*NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-isodecyl Phthalate (DIDP)* (NTP-CERHR, 2003)	No	Yes	No	- Report prepared by NTP-CERHHR Phthalates Expert Panel and was reviewed by CERHR Core Committee (made up of representatives of NTP-participating agencies, CERHR staff scientists, member of phthalates expert panel) - Public comments summarized in Appendix III of (NTP-CERHR, 2003) - No formal systematic review protocol employed.

Appendix B ANALYSIS OF ORAL ABSORPTION DATA FOR DIDP AND DEHP

No information on the oral absorption of DIDP in humans is available; data are limited to rat studies, which indicate that oral absorption of DIDP is approximately 50 percent. ECHA (2013b) concluded that oral absorption of DIDP is 50 percent in rats and 100 percent in humans based on read-across from di-ethylhexyl phthalate (DEHP) and applied a correction factor of two to account for species difference in absorption (*i.e.*, PODs derived from experimental animal models were divided by two). ECHA assumed 100 percent oral absorption of DEHP in humans based on results from several controlled human exposure studies that estimated urinary excretion of DEHP to be up to 70 percent over 24 hours based on recovery of four urinary metabolites (Kessler et al., 2012; Anderson et al., 2011; Koch et al., 2005; Koch et al., 2004). ECHA assumed that measuring all urinary metabolites of DEHP would most likely result in close to 100 percent recovery of administered DEHP and that an unknown amount of biliary excretion would contribute further to the absorption estimate. Based on these considerations, ECHA concluded that it was reasonable to assume 100 percent oral absorption of DEHP in humans. In contrast to the conclusions of the assessment by ECHA, other existing assessments of DIDP by Health Canada (ECCC/HC, 2020; EC/HC, 2015), Australia NICNAS (2015), and U.S. CPSC (2014, 2010) did not apply a correction factor because they assumed that oral absorption is similar in rats and humans.

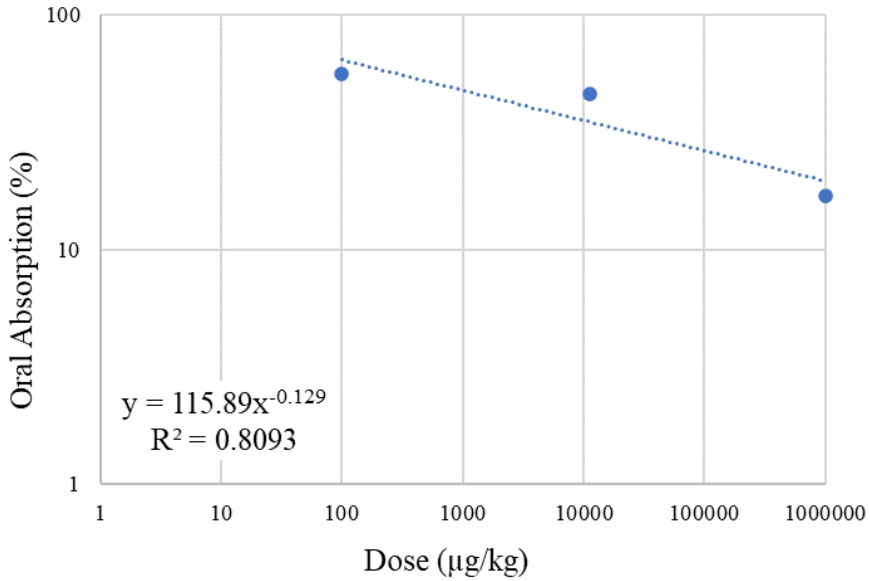
EPA reviewed available controlled human exposure studies of DEHP and considered whether they support the application of a correction factor that accounts for differences in oral absorption of DIDP between humans and rats (Table_Apx B-1). As noted by ECHA (2013b), the controlled human exposure studies of DEHP were designed to estimate fractional urinary excretion of DEHP metabolites, not to evaluate oral absorption. Available studies report total fractional urinary excretion estimates ranging from 0.291 to 0.705 (Table_Apx B-1). Koch et al. (2005) evaluated urinary DEHP elimination in a single participant, which provided a high-end estimate of approximately 70 percent excretion over 24 hours. In contrast, Anderson et al. (2011) evaluated urinary DEHP excretion in 10 male and 10 female volunteers, which provided an estimate of approximately 45 percent excretion over 24 hours. Notably, Anderson et al. provides an excretion estimate similar to that observed in the ADME study of DIDP with rats (oral absorption ranged from 46 to 56 percent (General Motors, 1983a)).

Variability in the total fractional urinary excretion estimates of DEHP reported in the available human studies is partially due to differences in measured metabolites, sample size, and study population. For example, Anderson et al. (2011) included 20 participants, whereas the studies by Koch et al. (2005; 2004) included only a single participant, the senior study author. EPA concluded that the DEHP human exposures studies, when accounting for the strengths and uncertainties, do not provide adequate evidence that absorption of DEHP differs between humans and rats. Therefore, the Agency will not apply a correction factor to account for potential differences in oral absorption of DIDP between humans and rats.

Table_Apx B-1. Summary of Controlled Human Exposure Studies of DEHP

Study Population (Reference)	Metabolites	24 Hour Fue	24 Hour Fue Sum
10 men (20–42 years of age) and 10 women (18–77 years of age) administered (gavage) single doses of 4.7 and 47 µg/kg-bw deuterated-DEHP (Anderson et al., 2011).	MEHP	0.062	0.453
	MEHHP	0.149	
	MEOHP	0.109	
	MECPP	0.132	
1 man (61 years of age) (senior study author) self-administered (dietary, spiked into butter and administered on bread) single dose of 640 µg/kg-bw deuterated-DEHP (Koch et al., 2004).	MEHP	0.07318	0.460
	MEHHP	0.2409	
	MEOHP	0.1461	
1 man (61 years of age) (senior study author) administered (dietary, spiked into butter and administered on bread) single doses of 4.7, 28.7, and 650 µg/kg-bw deuterated-DEHP (Koch et al., 2005).	MEHP	0.062–0.073	0.658 (low-dose) 0.64.6 (mid-dose) 0.705 (high-dose)
	MEHHP	0.227–0.241	
	MEOHP	0.130–0.173	
	MECPP	0.155–0.207	
4 men (28–61 years of age) administered (via oral syringe) single dose of 618–665 µg/kg-bw deuterated-DEHP (Kessler et al., 2012).	MEHP	0.025	0.291 (22 hour Fue)
	MEHHP	0.125	
	MEOHP	0.141	
Fue = urinary excretion fraction; MEHP = mono-2-ethylhexyl phthalate; MEHHP = mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = mono-(2-ethyl-5-oxyhexyl) phthalate; MECPP = mono-(2-ethyl-5-carboxypentyl) phthalate			

EPA applied linear regression analysis to further evaluate the oral absorption data for DIDP from the available rat ADME study (General Motors, 1983a). The model provided a good fit ($rR^2 = 0.8093$) and provided reasonable predictions of the observed oral absorption values (Figure_Apx B-1 and Table_Apx B-2). Next, EPA used the model to predict oral absorption at exposure levels commonly encountered by humans (*e.g.*, Health Canada (EC/HC, 2015) calculated median and 95th percentile exposure estimates of up to 1.4 and 4.9 µg/kg-day DIDP for various exposure scenarios). The model predicted 116 and 94 percent oral absorption at doses of 1 and 5 µg/kg, respectively. Although the regression is based on a limited data set (*i.e.*, three datapoints), it provides evidence to indicate that oral absorption can be expected to be close to 100 percent in rats at exposure levels similar to those encountered by humans. Based on this result, EPA did not apply a correction factor for differences in oral absorption across species.



Figure_Apx B-1. Linear Regression of Rat Oral Absorption Data for DIDP

Table_Apx B-2. Summary of Observed and Predicted Oral Absorption Values for DIDP

Dose (µg/kg)	Observed Oral Absorption (%)	Predicted Oral Absorption (%)	% Difference between Observed and Predicted Values
1	–	115.9	–
5	–	94.2	–
100	56	64.0	14
11,200	46	34.8	–24
1,000,000	17	19.5	15

Appendix C SUMMARY OF ANIMAL TOXICOLOGY STUDIES

Appendices C.1 and C.2 provide summaries of available animal toxicology studies evaluating developmental and liver toxicity, respectively.

C.1 Developmental Toxicity Studies

DIDP has been evaluated for developmental toxicity in several oral exposure studies, including two prenatal developmental studies of rats (Waterman et al., 1999; Hellwig et al., 1997), one developmental/reproductive toxicity screening study of mice (Hazleton Labs, 1983), and a pair of two-generation studies of reproduction of rats (Hushka et al., 2001). No studies of development are available for the dermal or inhalation exposure routes. Available studies are summarized in Table_Apx C-1 and discussed further below.

Table_Apx C-1. Summary of DIDP Studies Evaluating Effects on Development

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Pregnant SD rats (22–25/dose) gavaged with 0 (corn oil vehicle), 100, 500, 1,000 mg/kg-day DIDP (CASRN 68515-49-1) on GDs 6–15. Dams sacrificed on GD 21 (Waterman et al., 1999) Adhered to EPA 798.4900 (40 CFR Part 798, 1985)	100/ 500[a]	↑ Skeletal variations	Maternal Effects - ↓ food consumption (9–15%) on GDs 6–9, 9–12, 6–15 (1,000) - ↓ body weight gain on GDs 6–9, 9–12, 6–15 (1,000) Developmental Effects - ↑ incidence of rudimentary lumbar and supernumerary cervical ribs (≥500 mg/kg-day) Unaffected Outcomes - Maternal survival, clinical signs, resorptions, post-implantation loss, fetal viability, fetal body weight (both sexes), sex ratio, incidence of fetal malformations
Pregnant Wistar rats (7–10/dose) gavaged with 0 (corn oil vehicle), 40, 200, 1,000 mg/kg-day DIDP (CASRN 26761-40-0) on GDs 6–15. Dams sacrificed on GD 20 (Hellwig et al., 1997; BASF, 1993) Adhered to EPA 798.4900 (40 CFR Part 798, 1992), GLP compliant	40/ 200	↑ number of fetuses per litter with variations	Maternal Effects - ↓ food consumption on GDs 8–10 (1000) - Clinical signs (vaginal hemorrhage [3/10], urine-smeared fur [2/10]) (1,000) - ↑ relative and absolute (10–13%) liver weight (1,000) Developmental Effects - ↑ fetal variations at ≥200 mg/kg-day based on combined visceral and skeletal variations (↑ incidence of rudimentary cervical and accessory 14th ribs at 1,000 mg/kg-day; ↑ incidence of dilated renal pelvis and hydroureter at ≥40 mg/kg-day) Unaffected Outcomes - Maternal survival; maternal body weight gain; maternal kidney and uterus weight; post-implantation loss; resorptions; live fetuses/dam; fetal weight
Pregnant CD-1 mice (50/dose) gavaged with 0 (corn oil vehicle) or 9,650 mg/kg-day DIDP on GDs 7–14. Dams allowed to deliver pups naturally. Dams and litters sacrificed on PND3. (Hazleton Labs, 1983)	9,650/ None[b]	None	Maternal Effects - Clinical signs [rough hair coat (1/50 dams on GDs 7–14); oily coat (16/50 dams on GDs 7–14 and 48/50 on GDs 15–18); wet stains (3/50 dams on GDs 7–14); dry stains (5/50 dams on GDs 7–14 and 5/50 on GDs 15–18)] Developmental Effects - None Unaffected Outcomes - Maternal survival; maternal body weight; maternal body weight gain; reproductive index; # live pups per litter; mean litter or pup weight (PND1, PND3)
Male and female SD rats (30/sex/dose) fed diets containing 0, 0.2, 0.4, 0.8% DIDP starting 10 weeks prior to mating, through mating, gestation, and lactation	None/ 135[c]	↓ F2 offspring survival on PND1 and PND4	Parental (P1, P2) Effects - ↓ P1 body weight (both sexes) (0.8%); ↓ P2 male (≥0.4%) and female (0.8%) body weight - ↓ P1 (female only) food consumption (0.8%); ↓ P2 food consumption (both sexes) (0.8%)

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
continuously for two-generations (Study A). Received doses in units of mg/kg-day shown in Table_Apx C-4. (Hushka et al., 2001; Exxon Biomedical, 1998) Adhered to OPPTS 870.3800 (1994), GLP compliant			- ↑ relative and absolute liver weight in P1 males (≥0.4%), P1 females (≥0.2%), P2 males (0.8%), P2 females (≥0.2%) - Liver pathology: centrilobular or diffuse hepatocellular hypertrophy in P1/P2 males and females (≥0.2%); focal necrosis in P1 (0.8%) and P2 (≥0.4%) males (but not female) - ↑ relative and absolute kidney weight in P1 and P2 males (≥0.2%) and P1 and P2 females (≥0.4%) -Kidney pathology: pigment in tubular epithelial cells in P1/P2 males (≥0.2%); cortical tubular degeneration in P1/P2 males (≥0.2%); granular casts in renal tubules in P1 (0.8%) and P2 males (≥0.2%) Fertility Effects - None Offspring (F1, F2) Effects - ↓ F1 and F2 offspring body weight on PND0 (0.8%) and body weight gain through PND21 (0.8%) - ↓ F1 percent live births (0.8%) - ↓ F1 survival on PND4 (0.8%); ↓ F2 survival on PND1 and PND4 (≥0.2%), and PND7 (0.8%) - ↑ age (≤2 days) of vaginal patency for F1 (≥0.4%) Unaffected Outcomes - Survival (P1, P2); clinical signs (P1, P2); prostate, testis, epididymis, seminal vesicle weight (P1, P2); mating indices, fertility indices, gestational index, gestation length, litter size (P1, P2); P2 male sperm parameters (sperm count, quality indices, motility, morphology); P2 female estrous cycle length, percent normal cycles, oocyte count
Male and female SD rats (30/sex/dose) fed diets containing 0, 0.02, 0.06, 0.2, 0.4% DIDP starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for two-generations (Study B). Received doses in units of mg/kg-day shown in Table_Apx C-7. (Hushka et al., 2001; Exxon Biomedical, 2000)	38/ 134[c]	↓ PND1 and PND4 survival of F2 offspring	Parental (P1, P2) Effects - ↑ absolute and relative liver weight in P1 males and females (0.4%); P2 males (0.4%); P2 females (≥0.2%) - ↑ absolute and relative kidney weight in P1 males and females (0.4%); P2 males (≥0.2%) Fertility Effects - None Offspring (F1, F2) Effects - ↓ F2 offspring survival on PND1 and PND4 (≥0.2%) - ↑ age at preputial separation (1.2 day increase) (F2 only) Unaffected Outcomes - Survival (P1, P2); clinical signs (P1, P2); body weight (P1, P2); food consumption (P1, P2); mating indices, fertility indices, gestational index, mean gestation length,

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Adhered to OPPTS 870.3800 (1994), GLP compliant			mean litter size (P1, P2); percent live births (F1, F2); survival (F1); viability at weaning (F1, F2); body weight gain (F1, F2); anogenital distance (F1, F2); male nipple retention (F1, F2); preputial separation (F1); vaginal patency (F1, F2)

[a] Waterman et al. originally identified a developmental NOAEL of 500 mg/kg-day DIDP based on increased incidence of skeletal variations. However, a re-analysis of the data by study sponsors using the generalized estimating equation approach to the linearized model supported a NOAEL of 100 mg/kg-day DIDP. Results from the statistical re-analysis are reported in (NTP-CERHR, 2003).
[b] The observed clinical signs were considered to be of uncertain toxicological significance and may be related to oral and/or incidental dermal exposure (*e.g.*, regurgitation) from the corn oil vehicle.
[c] The LOAEL value of 135 mg/kg-day for decreased F2 offspring survival in Study A corresponds to the lowest dietary concentration of DIDP tested (0.2% DIDP). NOAEL/LOAEL values of 38/134 mg/kg-day for decreased F2 offspring survival in Study B correspond to the 0.06 and 0.2% DIDP treatment groups. Mean measured doses of DIDP for Study A and B are provided in Table_Apx C-4 and Table_Apx C-7, respectively.

Increased incidence of skeletal and visceral variations have been observed in the two available prenatal studies of rats. In the first study, which adhered to EPA 798.4900 (40 CFR Part 798, 1985), Waterman et al. (1999) gavaged pregnant SD rats (22–25 per dose) with 0, 100, 500, and 1,000 mg/kg-day DIDP on GDs 6 through 15. Maternal toxicity was limited to the high-dose group and included a reduction in maternal body weight gain (magnitude of effect not reported) and a 9 to 15 percent decrease in food consumption on GDs 6 through 9, 9 through 12, and 6 through 15. Food consumption and bodyweight gain significantly increased after cessation of exposure between GDs 18 through 21 and mean maternal body weight recovered to control levels by GD 21. No effects on maternal survival, clinical signs, resorptions, post-implantation loss, fetal viability, male and female fetal body weight, and fetal sex ratio were observed. No malformations were observed at any dose. Fetal effects were limited to treatment-related increases in skeletal variations, including increased incidence of rudimentary lumbar ribs and supernumerary cervical ribs at 500 and 1,000 mg/kg-day (Table_Apx C-2). EPA identified a developmental NOAEL of 100 mg/kg-day DIDP based on increased incidence of skeletal variations at 500 mg/kg-day and above and a maternal NOAEL of 500 mg/kg-day based on reduced maternal weight gain and food consumption at 1000 mg/kg-day DIDP.

Table_Apx C-2. Mean Percent of Fetuses in Litter with Skeletal Variations (Waterman et al., 1999) [a] [b]

	0 mg/kg-day	100 mg/kg-day	500 mg/kg-day	1,000 mg/kg-day
Skeletal variations	19.8	20.6	**31.9***	**64.1****
Rudimentary lumbar ribs	8.4	9.4	**21.9***	**51.9****
Supernumerary cervical ribs	1.1	3.1	**6.2***	**10.2****

[a] Adapted from Table 3 in (NTP-CERHR, 2003)
[b] * indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$. Data was re-analyzed by study sponsors using the generalized estimating equation approach to the linearized model to account for potential litter effects. The statistical re-analysis conducted by study sponsors is reported in (NTP-CERHR, 2003).

In a second prenatal study, Hellwig et al. (1997) gavaged pregnant Wistar rats with 0, 40, 200, and 1,000 mg/kg-day DIDP on GDs 6 through 15. The study was Good Laboratory Practice (GLP)-compliant and generally adhered to EPA 798.4900 (40 CFR pat 798, 1992), with the exception that 10 dams, instead of 20 were employed per dose group. Maternal toxicity was limited to the high-dose group and included increased clinical signs (*i.e.*, vaginal hemorrhage in 3/10 dams, urine-smeared fur in 2/10 dams), slight reductions (magnitude of effect not reported) in food consumption on GDs 8 through 10 and increased (9.7–13%) relative and absolute liver weight. No treatment-related malformations were observed. A significant increase in the number of fetuses per litter with total variations (combined visceral and skeletal variations) was observed at 200 and 1,000 mg/kg-day (percent of fetuses per litter with variations: 24.3, 37.2, 38.4*, 44.2* [* indicates $p < 0.05$]). At 1,000 mg/kg-day there was a clear increase in the incidence of fetuses and litters with rudimentary cervical ribs and accessory 14th ribs (Table_Apx C-3). The number of fetuses and litters with dilated renal pelves also appeared increased at all doses compared to the control; however, the effect was not clearly dose-related (Table_Apx C-3). Additionally, the number of fetuses and litters with hydroureter was slightly increased at all dose levels compared to the control, and the effect on fetuses, but not litters, appeared dose-related (Table_Apx C-3).

Across existing assessments of DIDP, there is some discrepancy in interpretation of the developmental NOAEL supported by Hellwig et al. (1997). NTP-CERHR (2003), U.S. CPSC (2010), ECHA (2013b), and Australia NICNAS (2015) consider Hellwig et al. to support a developmental NOAEL of 40 mg/kg-

day based on the increased incidence of total skeletal and visceral variations at 200 mg/kg-day, whereas Health Canada (EC/HC, 2015) set the developmental NOAEL at 200 mg/kg-day. Although the study by Hellwig et al. (1997) is limited, it includes fewer dams per dose group than recommend by EPA and Organisation for Economic Co-operation and Development Test Guideline (TG) 414 (OECD, 2018), EPA considers the study to support a developmental NOAEL of 40 mg/kg-day based on the increased incidence of total fetal variations at 200 mg/kg-day and above.

Table_Apx C-3. Incidence of Visceral and Skeletal Variations (Hellwig et al., 1997)[a]

Variation	0 mg/kg-day	40 mg/kg-day	200 mg/kg-day	1,000 mg/kg-day
Dilated renal pelvis	4 (4)	14 (8)	14 (5)	15 (8)
Hydroureter	0	3 (3)	5 (3)	8 (3)
Rudimentary lumbar ribs	1 (1)	0	0	15 (6)
Accessory 14th rib(s)	1 (1)	0	1 (1)	21 (8)

[a] Table adapted from Table 8 in Hellwig et al. (1997). Values indicate the number of fetuses and litters (in parentheses) in which variations were observed.

DIDP has also been evaluated in a developmental/reproductive toxicity screening study of mice. Pregnant CD-1 mice (50 per dose) were gavaged with 0 and 9,670 mg/kg-day DIDP on GDs 7 through 14, allowed to deliver pups naturally, and then sacrificed on PND3 (Hazleton Labs, 1983). No effects on maternal weight gain, the number of dams producing viable litters, the number of live pups per litter, mean litter weight, or mean pup weight per litter on PND1 or PND3 were observed. No other developmental or reproductive outcomes were evaluated. The dosing was shorter than some other prenatal studies and did not fully cover the entire period of gestation; OECD TG 414 recommends dosing from implantation (*e.g.*, day 5 post-mating) to the day prior to scheduled caesarean section (OECD, 2018). Observed effects were limited to increased clinical signs, including rough hair coat in 1 dam between GDs 7 to 14; oily coat in 16/50 dams between GDs 7 to 14 and 48/50 dams between GDs 15 to 18; wet stains in 3/50 dams between GDs 7 to 14; and dry stains in 5/50 dams between GDs 7 to 14 and 5/50 dams between GDs 15 to 18. The observed clinical signs were considered to be of uncertain toxicological significance and may be related to oral and/or incidental dermal exposure (*e.g.*, regurgitation) from the corn oil vehicle.

DIDP has also been evaluated in a preliminary one-generation study (dose-range finding study for two-generation study) and a pair of two-generation studies of reproduction (termed Studies A and B), which were GLP-compliant and adhered to EPA draft Guideline 870.3800 (1994) (Hushka et al., 2001; Exxon Biomedical, 2000, 1998). In the one generation study, SD rats (10/sex/dose) were continuously administered dietary concentrations of 0, 0.25, 0.5, 0.75, and 1.0 percent DIDP starting 10 weeks prior to mating, throughout mating, gestation, and lactation. Males were sacrificed after mating, whereas females were sacrificed at weaning on PND21. Effects on the parental generation included decreased body weight, suppression of body weight gain, and/or decreased food consumption in both sexes at 0.75 percent DIDP and above (magnitude of effects not reported). Food consumption was also decreased in females of the 0.5 percent group during the postpartum period. No effects on any reproductive indices were observed. Offspring effects were limited to suppression of body weight gain in the 0.75 percent group on PND14 through PND28 and 1.0 percent group on PND0 through PND28, and possibly the 0.5 percent group on PND14 through PND21 (magnitude not reported). Based on reductions in offspring and adult body weight in the 0.75 and 1.0 percent dose groups, 0.8 percent DIDP was selected as the high dose for the subsequent two-generation study of reproduction (Study A).

In the first two-generation study (Study A), SD rats were continuously administered dietary concentrations of 0, 0.2, 0.4, and 0.8 percent DIDP starting 10 weeks prior to mating, throughout mating, gestation, and lactation, until terminal sacrifice for two generations. Mean received doses in units of mg/kg-day are shown in Table_Apx C-4. Multiple outcomes were measured in P1 and P2 male and female parents. For the first parental generation (P1), no treatment-related clinical signs or effects on survival were reported. Food consumption was decreased throughout gestation (5.5% between GDs 0–21) and the postpartum phase of the study (12 percent on postpartum days 0 through 21) in high-dose females (but not males). Changes in food consumption coincided with decreases in maternal weight during gestation (up to 6% on GDs 0–21) and the postpartum phase of the study (6–11% on postpartum days 0–21) in high-dose P1 females. For the second parental generation (P2), no treatment-related clinical signs or effects on survival were observed. Food consumption was decreased in high-dose P2 males during the premating phase (up to 11%) and in high-dose P2 females during the postpartum phase (17% on postpartum days 0–21). No effects on P2 female body weight were observed during premating or gestation. Small decreases (8–9%) in high-dose P2 female body weight were observed on postpartum days 10 and 14. However, no effects on overall body weight gain were observed over the entire postpartum period in P2 females. Body weight was reduced (7–14%) in high-dose P2 males throughout the premating period until sacrifice, and small (<6%), but significant, decreases in body weight were observed in mid-dose males starting on day 56 of the premating period until sacrifice. Hepatic and kidney effects are discussed in Sections 3.1.1 and 3.2.1, respectively.

Table_Apx C-4. Mean Measured Doses (mg/kg-day) from the Two-Generation Study of DIDP in SD Rats (Study A) (Hushka et al., 2001; Exxon Biomedical, 1998)[a]

P1 Generation					P2 Generation			
Dose (%)	Premating-Males	Premating-Females	Gestation	Postpartum	Premating-Males	Premating-Females	Gestation	Postpartum
0.2	103–198	127–203	131–149	172–361	117–216	135–218	135–152	162–379
0.4	211–405	253–416	262–287	359–734	229–437	273–433	262–297	334–761
0.8	427–787	508–775	524–551	641–1582	494–929	566–927	574–611	637–1424

[a] Adapted from Table 9 in Hushka et al. (2001).

No treatment-related effects on any reproductive indices were observed at any dose in either generation. Effects on F1 and F2 offspring survival and body weight throughout the postnatal period were observed. For F1 offspring, effects were limited to the high-dose group and included decreased live births and survival on PND4 (Table_Apx C-5), and decreased male (6–23%) and female (4–20%) offspring body weight on PND0 through PND21 (Table_Apx C-6). For F2 offspring, effects included a dose-related decrease in offspring survival on PND1 and PND4 in all treatment groups, decreased survival on PND7, and viability at weaning in the high-dose group. High-dose F2 offspring also exhibited decreased body weight (9–22% in males; 6–21% in females) from PND0 through PND21. As can be seen from Table_Apx C-5 and Table_Apx C-6, statistically significant effects on F1 and F2 offspring survival and body weight were generally outside of the range of historical control data from the laboratory conducting the study (historical control data from 14 dietary studies conducted between 10/27/1988 to 09/25/1994; in life test period for study A: 07/11/1995 to 04/07/1996). EPA identified a LOAEL (no NOAEL identified) of 0.2 percent DIDP (equivalent to 135 mg/kg-day) based on reduced F2 offspring survival on PND1 and PND4.

Table_Apx C-5. F1 and F2 Offspring Survival Indices from the Two-Generation Study of Reproduction in SD Rats (Study A) (Hushka et al., 2001; Exxon Biomedical, 1998) [a] [b]

F1 Offspring							
Group	**Live Birth %**	**PND1 Survival %**	**PND4 Survival %**	**PND7 Survival %**	**PND14 Survival %**	**PND21 Survival %**	**Viability at Weaning %**
0%	98.7	95.5 h	93.9	97.8	95.5	100.0	93.4
0.2%	97.6	95.8 h	93.0	100.0	100.0**	100.0	100.0**
0.4%	96.8	94.2 h	91.5 h	99.4	99.4*	100.0	98.9*
0.8%	94.2**h	92.2 h	88.8*h	98.0	98.4	100.0	96.4
Historical control	95.2–99.2	96.2–100	92.8–99.7	92.8–100	93.7–100	98.8–100	86.9–100
F2 offspring							
0%	98.5	96.6	94.0	99.3	99.3	100.0	98.7
0.2%	94.7*h	92.1*h	85.8**h	100.0	100.0	100.0	100.0
0.4%	98.2	89.6**h	86.7**h	99.3	98.5	100.0	97.8
0.8%	96.8	85.2**h	77.6**h	95.4*	98.4	98.9	92.9*
Historical control	95.2–99.2	96.2–100	92.8–99.7	92.8–100	93.7–100	98.8–100	86.9–100

[a] Data from Tables 21 and 49 in Exxon Biomedical (1998).
[b] * and ** indicate the mean is significantly different from the control mean by $p < 0.05$ and $p < 0.01$, respectively. "h" indicates the mean is outside of laboratory historical control range.

Table_Apx C-6. F1 and F2 Offspring Postnatal Body Weight (Grams) from the Two-Generation Study of Reproduction in SD Rats (Study A) (Hushka et al., 2001; Exxon Biomedical, 1998)[a] [b]

F1 Offspring												
Group	**Male**						**Female**					
	PND0	**PND1**	**PND4**	**PND7**	**PND14**	**PND21**	**PND0**	**PND1**	**PND4**	**PND7**	**PND14**	**PND21**
0%	6.66	7.21	10.20	16.99	36.64	60.54	6.28	6.80	9.54	15.99	34.97	56.19
0.2%	6.66	7.15	10.27	16.95	35.15	59.05	6.34	6.97	10.09	16.77	34.62	56.98
0.4%	6.62	7.21	10.24	16.38	33.90	58.33	6.29	6.76	9.63	15.93	33.29	56.09
0.8%	6.27**h	6.75	9.33*	14.50*	28.18**h	48.10**	6.01*	6.60	9.09	14.21*	28.06**	47.23**
Historical control	6.35-7.02	6.68-7.49	8.53-11.43	13.64-18.74	28.81-37.09	44.89-62.34	5.96-6.74	6.30-7.16	8.32-11.05	13.33-17.69	27.22-35.89	42.39-61.19
F2 offspring												
0%	6.72	7.05	9.96	16.19	34.25	56.74	6.30	6.63	9.40	15.17	32.31	53.45
0.2%	6.57	6.98	10.08	16.10	34.31	57.18	6.27	6.68	9.61	15.26	32.95	54.94
0.4%	6.41	6.82	9.64	15.29	32.79	54.38	6.05	6.32	8.82	14.03	30.35	49.89
0.8%	6.12**h	6.32**h	8.17**h	12.55**h	27.36**h	44.20**h	5.95h	6.14*h	7.79**h	12.08**h	25.69**h	42.02**h
Historical Control	6.35-7.02	6.68-7.49	8.53-11.43	13.64-18.84	28.81-37.09	44.89-62.34	5.96-6.74	6.30-7.16	8.32-11.05	13.33-17.69	27.22-35.89	42.39-61.19

[a] Data from Tables 23 and 51 in Exxon Biomedical (1998).
[b] * and ** indicate the mean is significantly different from the control mean by $p < 0.05$ and $p < 0.01$, respectively. "h" indicates the mean is outside of laboratory historical control range.

Study A also included two satellite experiments, including a cross-fostering study and a switched diet study. For the cross-fostering study, 10 high-dose litters from the F1 generation were switched with 10 F1 control litters; the high-dose offspring were fostered by control dams and control offspring were fostered by high-dose dams. No effects on offspring survival indices on PND1, PND4, PND7, PND14, or PND21 were observed for offspring cross-fostered with either control or high-dose dams. There were no significant differences between the mean body weight of high-dose offspring cross-fostered to control dams and main study control offspring of either sex throughout the postnatal period. Mean bodyweights on PND14 and PND21 of control offspring cross-fostered to high-dose dams were significantly lower (by up to 19%) than the main study control offspring of both sexes. These results indicate that offspring may be exposed to DIDP through lactational transfer and that this exposure may contribute to observed effects, particularly on postnatal body weight gain. It is difficult to determine the contribution of gestational and lactation exposure to DIDP on F1 offspring survival from the current study design. Significant effects on F1 offspring survival were only observed for high-dose offspring on PND4 in the main study, and the magnitude of the effect was small (PND4 survival 88.8% compared to 93.9% in controls); the cross-fostering study included fewer litters than the main study, reducing the sensitivity of the cross-fostering study to detect small effects on F1 survival.

For the switched diet study, F1 control and high-dose offspring of both sexes were switched to high-dose and control diet, respectively, starting on PND21 through the duration of the P2 premating period. No effect on food consumption was observed in either switched diet groups. High-dose offspring of both sexes switched to control diet had lower (11–14%) body weights compared to control offspring of the main study after one week on the control diet. Although body weight recovered to control levels for both sexes after two weeks, it was reduced (7–10%) in high-dose males (but not females) on control diet compared to main study control males from study day 42 until sacrifice. Control male and female offspring switched to the high-dose diet generally had lower (6–10%) body weights compared to main study control offspring throughout the entirety of the switched dose study until sacrifice.

Study A did not allow for the identification of a developmental NOAEL. Therefore, Hushka et al. (2001) conducted a second two-generation study (Study B) at lower doses than Study A, to identify a NOAEL and to determine the reproducibility of the observed effects on offspring survival. In Study B, SD rats were continuously administered dietary concentrations of 0, 0.02, 0.06, 0.2, and 0.4 percent DIDP starting 10 weeks prior to mating, throughout mating, gestation, and lactation, until terminal sacrifice for two generations. Mean received doses in units of mg/kg-day are shown in Table_Apx C-7. No treatment-related effects on survival, food consumption, or body weight were observed for males or females of the P1 or P2 generations at any dose, nor were any treatment-related clinical signs observed for P1 and P2 males and females. No effects on any mating or fertility indices were observed at any dose in either generation, which is consistent with the first two-generation study. For F1 offspring, no significant effects on development were observed (*i.e.*, no effect on body weight gain, percent live births, postnatal survival, viability at weaning, age at preputial separation). For F2 offspring, there was a significant reduction in F2 survival on PND1 and PND4 in the 0.2 and 0.4 percent DIDP treatment groups (Table_Apx C-8) and a delay (1.2 day) in preputial separation in high-dose F2 males. Effects on offspring survival were generally outside of historical control ranges from the laboratory conducting the study (historical control data from 19 dietary studies conducted between 10/27/1988 to 03/02/1998; in life test period for study B: 12/07/1998 to 10/08/1999). EPA identified a developmental NOAEL of 0.06 percent (equivalent to 38 mg/kg-day) based on reduced F2 offspring survival on PND1 and PND4.

Table_Apx C-7. Mean Measured Received Doses (mg/kg-day) from the Two-Generation Study of DIDP (Study B) (Hushka et al., 2001; Exxon Biomedical, 2000) [a]

P1 Generation					P2 Generation			
Dose (%)	Premating-Males	Premating-Females	Gestation	Postpartum	Premating-Males	Premating-Females	Gestation	Postpartum
0.02	12–23	14–20	13–15	19–37	11–26	14–25	13–15	19–40
0.06	33–68	40–58	39–43	57–112	33–76	41–77	38–44	52–114
0.2	114–225	139–191	127–147	178–377	144–254	137–266	134–150	166–352
0.4	233–453	274–380	254–295	356–744	144–254	271–524	256–284	356–747

[a] Adapted from Table 9 in Hushka et al. (2001)

Table_Apx C-8. F2 Offspring Survival Indices from the Two-Generation Study of Reproduction in SD Rats (Study B) (Hushka et al., 2001; Exxon Biomedical, 2000) [a] [b]

Group	Live Birth %	PND1 Survival %	PND4 Survival %	PND7 Survival %	PND14 Survival %	PND21 Survival %	Viability at Weaning %
0%	97.7	99.0	97.7	98.5	95.4	100.0	94.0
0.02%	98.7	98.4	96.8	99.0	99.5*	100.0	98.5*
0.06%	97.4	97.4	96.6	99.0	100.0*	99.5	98.5*
0.2%	99.4 h	95.2**h	92.3**	98.8	98.8	98.7 h	96.3
0.4%	95.5	89.1**h	84.8**h	99.0	98.5	98.5 h	96.0
Historical control	95.2–99.2	95.5–100	88.9–99.5	92.8–100	93.7–100	98.8–100	86.9–100

[a] Data from Table 49 in Exxon Biomedical (2000).
[b] * and ** indicate the mean is significantly different from the control mean by $p<0.05$ and $p<0.01$, respectively. "h" indicates the mean is outside of laboratory historical control range.

C.2 Liver Toxicity Studies

Liver effects of DIDP have been reported in intermediate (>1–30 days), subchronic (>30–90 days) and chronic (>90 days) exposure studies. Available studies include: one intermediate duration inhalation study of rats (General Motors, 1983b); seven intermediate oral exposure studies (5 of rats, 2 of mice) (Chen et al., 2019; Kwack et al., 2010; Kwack et al., 2009; Smith et al., 2000; Lake et al., 1991; BIBRA, 1990, 1986); three subchronic oral exposure studies (2 of rats, 1 of beagles) (BASF, 1969; Hazelton Labs, 1968a, b); two chronic oral exposure studies (1 of each of rats and mice) (Cho et al., 2011; Cho et al., 2010; Cho et al., 2008); one prenatal developmental study of rats (Hellwig et al., 1997); and a pair of two-generation studies of rats (Hushka et al., 2001; Exxon Biomedical, 2000, 1998). No studies for the dermal route of exposure are available. Available studies are summarized in Table_Apx C-9 and discussed further below.

Considerations for Interpretation of Hepatic Effects

Consistent with previous guidances (Hall et al., 2012; U.S. EPA, 2002a), EPA considered hepatocellular hypertrophy and corresponding increases in liver size and weight to be adaptive non-adverse responses, unless accompanied by treatment-related, biologically significant changes in clinical markers of liver

toxicity (*i.e.*, decreased albumin; or increased alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], gamma glutamyltransferase [GGT], bilirubin, cholesterol) and/or histopathology indicative of an adverse response (*e.g.*, hyperplasia, degeneration, necrosis, inflammation). Further, it is well documented that phthalates, including DIDP, can induce peroxisome proliferation in the livers of mice and rats, and there is evidence supporting a role for peroxisome-proliferator-activated receptor alpha (PPARα) activation in peroxisome-induced hepatic effects of DIDP. For purposes of identifying study NOAEL and LOAEL values, effects consistent with peroxisome proliferation and PPARα activation were also considered relevant for setting the LOAEL.

Intermediate (>1–30 Days) Exposure Studies

EPA identified seven intermediate duration animal studies that evaluated liver effects following DIDP exposure. One intermediate duration inhalation study exposed adult male SD rats to 0 or 505 mg/m^3 DIDP aerosol (mass median aerodynamic diameter [MMAD] = 0.98 µm) via whole-body inhalation for 6 hours/day, 5 days/week for 2 weeks (General Motors, 1983b). Animals were sacrificed and necropsy was performed 3 weeks after the end of exposure. No histopathological findings were observed in the liver, and no signs of systemic effects were observed (*i.e.*, no effect on body weight gain, clinical signs, or survival). Evidence of local lung effects were observed, including moderate increases in the width of alveolar septa with slight interstitial mixed inflammatory reactions and increases in the numbers of alveolar macrophages and type II pneumocytes. Limitations of this study include the timing of the histopathologic examination (*i.e.*, 3 weeks post-exposure) and lack of examination of organ weights, clinical chemistry, and hematology.

Two studies by Kwack et al. gavaged male SD rats with 0 or 500 mg/kg-day DIDP for 2 (2010) or 4 weeks (2009). Both studies observed a 30 to 39 percent increase in relative liver weight (absolute weight not reported) and a 67 percent increase in serum ALP. There were no effects on body weight and no changes in other serum markers of liver toxicity, including ALT, AST, GGT, albumin, total bilirubin, and triglycerides. Histopathology was not evaluated in either study. Because liver weight changes were only accompanied by a slight (less than 2-fold) increase in ALP and other serum markers of hepatoxicity were unaffected, and histopathology was not evaluated, EPA determined that there was not sufficient evidence to conclude the liver findings from either study were adverse.

Table_Apx C-9. Summary of DIDP Studies Evaluating Liver Effects

Brief Study Description	NOAEL/ LOAEL (mg/kg-day, unless otherwise noted)	Effect at LOAEL	Remarks
Intermediate exposure studies (>1–30 days)			
Male SD rats (6–8/dose) exposed (whole-body) to DIDP aerosol (MMAD: 0.98 µm) nominally at 0 and 500 mg/m^3 (analytical: 505 ± 7 mg/m^3) for 6 hours/day, 5 days/week for 2 weeks. Rats sacrificed after 3-week observation period (General Motors, 1983b)	505 (mg/m^3)/ None	No systemic effects reported	Liver Effects - None (no histopathologic findings in liver) - Not examined: organ weight, clinical chemistry Other Toxicity - None (no effect on body weight gain, clinical signs, survival, spleen, and kidney histopathology)
Male Balb/c mice (8/dose) gavaged with 0 (saline vehicle), 0.15, 1.5, 15, and 150 mg/kg-day DIDP for 14 days (Chen et al., 2019)	NOEL/LOEL: 1.5/ 15	LOEL: ↑ serum AST, histopathology, ↑ IL-1β, ↑ TNF-α , and ↑ NF-κB	Liver Effects - ↑ serum ALT (150 mg/kg-day) and AST (≥15); ↓ albumin (150) - Histology (qualitative only) (broadened liver cords, expanded cells, contracted liver sinuses at 15 mg/kg-day; fuzzy and edematous with extremely loose cytoplasm at 150) - ↑ ROS (150 mg/kg-day), ↓ GSH (150), ↑ MDA (150), ↑ 8-OHdG (150), ↑ IL-1β (≥15), ↑ TNF-α (≥15), ↑ Casp-3 (150) in liver homogenate - ↑ NF-κB in the liver (≥0.15 mg/kg-day) - Not examined: organ weight Other Toxicity - Survival, body weight, clinical signs not evaluated
Young (5 weeks old) male SD rats (6/dose) were gavaged with 0 (corn oil vehicle) or 500 mg/kg-day DIDP for 14 days (Kwack et al., 2010)	NOEL/LOEL: None/ 500	LOEL: ↑ relative liver weight, ↑ ALP	Liver Effects - ↑ (30%) relative liver weight - ↑ serum ALP (67%), ↓ total cholesterol (14%) - Unaffected: serum AST, ALT, GGT, total bilirubin, albumin, triglycerides - Not examined: histopathology Other Toxicity - None (no effect on survival, body weight, food consumption)
Male and female F344 rats (5/sex/dose) were fed diets containing 0, 0.3, 1.2, or 2.5% DIDP for 21 days (equivalent to 0, 304, 1,134, 2,100 mg/kg-day for	None/ 304 (males)	↑ liver weight and hepatic lauric acid 11- and 12-hydroxylase activity	Liver Effects - ↑ absolute and relative liver weight for males (≥304 mg/kg-day) and females (≥1,042) - ↓ serum triglycerides (males only) (≥1,134 mg/kg-day)

Brief Study Description	NOAEL/ LOAEL (mg/kg-day, unless otherwise noted)	Effect at LOAEL	Remarks
males; 0, 264, 1,042, 1,972 mg/kg-day for females) (BIBRA, 1986)			- ↑ hepatic palmitoyl CoA oxidation activity (both sexes) (≥1134/1042 mg/kg-day) - ↑ hepatic lauric acid 11- and 12-hydroxylase activity (males at ≥304 mg/kg-day) and 12-hydroxylase (females at 1972) - Histopathology: ↓ hepatocyte basophilia (both sexes) (≥1,134/1,042 mg/kg-day); ↑ hepatocyte eosinophilia (both sexes) (2,100/1,972) - Marked to very marked increase in peroxisomes in hepatocytes (both sexes) (2,100/1,972 mg/kg-day) Other Toxicity - Clinical signs (piloerection in 2/5 males [2,100 mg/kg-day]) - ↓ (20–32%) body weight gain and terminal body weight (both sexes) (2100/1972 mg/kg-day) - ↓ food consumption for males (≥1,134 mg/kg-day) & females (1,972) - Unaffected: survival
Male F344 rats (5/dose) fed diets containing 0, 1,000, 12,000 ppm DIDP (CASRN 68515-49-1) (equivalent to 50, 600 mg/kg-day) for 2 and 4 weeks (Smith et al., 2000)	50/ 600	↑ relative liver weight and peroxisomal beta-oxidation	Liver Effects - ↑ relative liver weight at 2 and 4 weeks (600 mg/kg-day) - ↑ peroxisomal beta-oxidation activity at 2 and 4 weeks (600 mg/kg-day) - ↑ Hepatocellular replicative DNA synthesis at 2 weeks (600 mg/kg-day) and 4 weeks (50 mg/kg-day) - Not examined: histopathology, serum chemistry - Unaffected: GJIC
Male B6C3F1 mice (5/dose) fed diets containing 0, 500, 6,000 ppm DIDP (CASRN 68515-49-1) (equivalent to 75, 900 mg/kg-day) for 14 and 28 days (Smith et al., 2000)	75/ 900	↑ relative liver weight and/or peroxisomal beta-oxidation	Liver Effects - ↑ relative liver weight at 2 weeks (900 mg/kg-day) - ↑ peroxisomal beta-oxidation activity at 2 and 4 weeks (900 mg/kg-day) - ↑ Hepatocellular replicative DNA synthesis at 2 and 4 weeks (75 mg/kg-day) - Not examined: histopathology, serum chemistry - Unaffected: GJIC, relative liver weight at 4 weeks
Young (5 weeks old) male SD rats (6/dose) were gavaged with 0 (corn oil vehicle) or 500 mg/kg-day DIDP for 28 days (Kwack et al., 2009)	NOEL/ LOEL: None/ 500	LOEL: ↑ relative liver weight, ↑ ALP	Liver Effects - ↑ (39%) relative liver weight (500) - ↑ (67%) serum ALP (500)

Brief Study Description	NOAEL/ LOAEL (mg/kg-day, unless otherwise noted)	Effect at LOAEL	Remarks
			- Unaffected: serum AST, ALT, GGT, total bilirubin, albumin, triglycerides, total cholesterol - Not examined: histopathology Other Toxicity - None (no effect on survival, body weight, food consumption, clinical signs)
Young (6 weeks old) male F344 rats (5/dose) fed diets containing 0, 0.02, 0.05, 0.1, 0.3, 1.0% DIDP (equivalent to 25, 57, 116, 353, 1287 mg/kg-day) for 28 days (Lake et al., 1991; BIBRA, 1990)	57/ 116	↑ relative liver weight and cyanide-insensitive palmitoyl-CoA oxidation activity	Liver Effects - ↑ absolute (≥353 mg/kg-day) and relative liver weight (≥116 mg/kg-day) - ↑ incidence of hepatocellular hypertrophy and cytoplasmic eosinophilia (1,287 mg/kg-day) - ↑ cyanide-insensitive palmitoyl-CoA oxidation activity (≥116 mg/kg-day) Other Toxicity - None (no effect on body weight, food consumption, clinical signs)
Subchronic exposure studies (>30–90 days)			
Male and female SD rats (20/sex/dose) administered 0, 800, 1,600, 3,200, or 6400 ppm DIDP in feed for 90 days (equivalent to 55, 100, 200, 400 and 60, 120, 250, 500 mg/kg-day for males and females, respectively) [(BASF, 1969); available to EPA only as a German language study. Reported information based on study summaries provided in (EC/HC, 2015; ECB, 2003)	NOEL/ LOEL: 200/ 400 (males); 60/ 120 (females)	LOEL: ↑ absolute liver weight (males); ↑ relative liver weight (females)	Liver Effects - ↑ absolute (400 mg/kg-day) and relative liver weight in males (≥55 mg/kg-day) (relative weight changes not dose-related) - ↑ absolute (≥250 mg/kg-day) and relative (≥120 mg/kg-day) liver weight in females - Unaffected: clinical chemistry, histopathology, urinalysis Other Toxicity - ↓ body weight gain in males from day 77 onward (≥100 mg/kg-day) - Unaffected: survival, clinical signs, food consumption, body weight gain (females)
Male and female albino rats (10/sex/dose) fed 0, 500, 3,000, 10,000 ppm DIDP for 90 days (equivalent to 28, 170, 586 mg/kg-day for males; 35, 211, 686 for females) (Hazelton Labs, 1968b)	NOEL/LOEL: 170/586 (males); 211/686 (females)	LOEL: ↑ absolute and relative liver weight	Liver Effects - ↑ absolute/ relative liver weight (both sexes) (586/686 mg/kg-day) - Unaffected: histopathology, clinical chemistry, urinalysis Other Toxicity - None (no effect on survival, clinical signs, body weight gain, food consumption)

Brief Study Description	NOAEL/ LOAEL (mg/kg-day, unless otherwise noted)	Effect at LOAEL	Remarks
Male and female beagles (3/sex/dose) fed diets containing 0, 500, 3,000, and 10,000 ppm DIDP for 13 weeks (equivalent to 15, 75, 300 mg/kg-day DIDP) (Hazelton Labs, 1968a)	15/ 75	↑ swelling and vacuolation of hepatocytes	Liver Effects - ↑ absolute/relative liver weight (both sexes) (300 mg/kg-day) - Slight to moderate swelling and vacuolation of hepatocytes (both sexes) (75 mg/kg-day) - Unaffected: clinical chemistry (*e.g.*, ALT, AST, ALP, bromsulphthalein clearance), urinalysis parameters
Chronic exposure studies (>90 days)			
Male and female wild-type mice (15/sex/dose) were fed diets containing 0 and 1% DIDP for 182 days (equivalent to approximately 1,500 mg/kg-day) (Cho et al., 2011)	None/ 1,500	↑ relative liver weight, histopathology	Liver Effects - ↑ relative liver weight (both sexes) - Hepatocyte hypertrophy with eosinophilic granules (both sexes), parenchymal inflammation (males), pigmented hepatocytes and Kupffer cells (males), prominent Kupffer cells (males) - Not measured: clinical chemistry Other Toxicity - ↓ terminal body weight in males and females - Unaffected: survival, clinical signs
Male and female F344 rats (52/sex/dose) were fed diets 0, 400, 2,000, 8,000 ppm DIDP for 2 years (equivalent to 22, 110, 479 mg/kg-day for males; 23, 128, 620 mg/kg-day for females) (Cho et al., 2010; Cho et al., 2008)	None/ 22	↑ incidence of spongiosis hepatis	Liver Effects - ↑ relative liver weight (both sexes) (479/620 mg/kg-day) - Necrosis (both sexes) (479/620), oval cell hyperplasia (males) (479), hypertrophy (males) (479), peliosis (males) (479), microgranuloma (males) (≥22), spongiosis hepatis (males) (≥22) - Not examined: clinical chemistry Other Toxicity - ↓ survival (both sexes) (479/620) - ↓ body weight gain and terminal body weight (both sexes) (479/620) - Unaffected: clinical findings
Prenatal and two-generation studies			

Brief Study Description	NOAEL/ LOAEL (mg/kg-day, unless otherwise noted)	Effect at LOAEL	Remarks
Pregnant Wistar rats (7-10/dose) gavaged with 0, 40, 200, or 1,000 mg/kg-day DIDP on GDs 6–15. Dams sacrificed on GD 20 (Hellwig et al., 1997). *See* Table_Apx C-1 *for additional study details.*	NOEL/LOEL: 200/ 1,000	LOEL: ↑ Relative liver weight	Liver Effects - ↑ relative and absolute liver weight in dams (1,000 mg/kg-day) - Not examined: histopathology, clinical chemistry
Male and female SD rats (30/sex/dose) fed diets containing 0, 0.2, 0.4, 0.8% DIDP starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for two generations (Study A) (Hushka et al., 2001; Exxon Biomedical, 1998). *See* Table_Apx C-1 *for additional study details.*	117/ 229[a]	↑ liver weight, histopathology (focal necrosis) in P2 males	Liver Effects - ↑ relative and absolute liver weight in P1 males (≥0.4%), P1 females (≥0.2%), P2 males (≥0.4%), P2 females (≥0.2%) - Centrilobular or diffuse hepatocellular hypertrophy in P1 and P2 males and females (≥0.2%); ↑ incidence of focal necrosis in P1 (0.8%) and P2 (≥0.4%) males - Not examined: clinical chemistry
Male and female SD rats (30/sex/dose) fed diets containing 0, 0.02, 0.06, 0.2, 0.4% DIDP starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for two generations (Study B) (Hushka et al., 2001; Exxon Biomedical, 2000). *See* Table_Apx C-1 *for additional study details.*	NOEL/LOEL: 52/ 166[a]	LOEL: ↑ relative and absolute liver weight in P2 females	Liver Effects - ↑ absolute and relative liver weight in P1 males and females (0.4%), P2 males (0.4%), P2 females (≥0.2%) - Not examined: clinical chemistry, histopathology

[a] NOAEL/LOAEL values of 117/ 229 mg/kg-day for increased liver weight and focal necrosis in P2 males during the premating phase of Study A correspond to the 0.2 and 0.4% DIDP treatment groups. NOEL/LOEL values of 52/166 mg/kg-day for increased relative and absolute liver weight in P2 females during the postpartum phase of Study B correspond to the 0.06 and 0.2% treatment groups. Mean measured doses of DIDP for Study A and B are provided in Table_Apx C-4 and Table_Apx C-7, respectively.

Three additional studies in mice and rats provide evidence of peroxisome proliferation following intermediate duration oral exposure to DIDP. Smith et al. (2000) fed male B6C3F1 mice diets containing 0, 500, and 6,000 ppm DIDP (equivalent to 0, 75, 900 mg/kg-day) and male F344 rats diets containing 0, 1,000, and 12,000 ppm DIDP (equivalent to 0, 50, 600 mg/kg-day) for 2 and 4 weeks. In rats, relative liver weight (absolute weight not reported) increased approximately 50 percent in the high-dose group after 2 and 4 weeks, and relative liver weight increased approximately 25 percent in high-dose mice afer 2-, but not 4 weeks of exposure. Serum chemistry and histopathology were not evaluated. However, consistent with an induction of peroxisome proliferation, peroxisomal beta-oxidation was increased at the high dose by approximately 6- to 7-fold in rats and 3- to 8-fold in mice at both 2 and 4 weeks.

In BIBRA (1986), male and female F344 rats were fed diets containing 0, 0.3, 1.2, or 2.5 percent DIDP (equivalent to 304, 1,134, 2,100 mg/kg-day in males; 264, 1,042, 1,972 mg/kg-day in females) for 21 days. Body weight gain and terminal body weight were reduced (20–32%) in high-dose males and females, while food consumption was reduced for high-dose females and males at 1.2 percent DIDP and above. Absolute and relative liver weights were significantly increased in a dose-dependent manner for males (21–154% increase) and females (60–138% increase) at 1.2 percent DIDP and above. Histopathologic examinations revealed decreased hepatic cytoplasmic basophilia in both sexes at 1.2 percent DIDP and above, and increased eosinophilia in both sexes at 2.5 percent DIDP. Serum triglycerides were reduced (34%) in males (but not females) at 1.2 percent DIDP and above. No other serum chemistry parameters were evaluated. Consistent with an induction of peroxisome proliferation, hepatic cyanide-insensitive palmitoyl-CoA oxidase activity was significantly increased (approximately 6.5- to 14.5-fold) in both sexes at 1.2 percent DIDP and above, while hepatic lauric acid 11- and 12-hydroxylase activity was increased in males at all doses and 12-hydroxylase activity was increased in high-dose females. Electron microscopy demonstrated marked to very marked increases in peroxisome number and size in both sexes at 2.5 percent DIDP.

In a third study, male F344 rats (5/dose) were fed diets containing 0, 0.02, 0.05, 0.1, 0.3 or 1.0 percent DIDP (equivalent to 25, 57, 116, 353, 1287 mg/kg-day) for 28-days (BIBRA, 1990). Absolute liver weight increased 20 to 98 percent at 0.3 percent DIDP and above, while relative liver weight increased 9 to 120 percent at 0.1 percent DIDP and above. Histologic findings were limited to the high-dose group and included increased incidence of cytoplasmic eosinophilia and hepatocellular hypertrophy in males (incidence of both lesions: 5/5). Consistent with an induction of peroxisome proliferation, cynanide-insensitive palmitoyl-CoA oxidation activity was significantly increased (22–2,100%) at 0.1 percent DIDP and above.

New Literature: EPA identified one new medium quality intermediate duration study published between 2014 and 2019 that evaluated liver toxicity. Chen et al. (2019) gavaged male Balb/c mice (8/dose) with 0, 0.15, 1.5, 15, or 150 mg/kg-day DIDP for 14 days and then evaluated several serum chemistry markers of liver toxicity (*i.e.*, AST, ALT, albumin), liver histology, and several sub-apical mechanistic endpoints. Histopathologic findings in the liver were described qualitatively only (incidence data were not reported; no statistical analyses were performed). At 15 mg/kg-day, histological observations included, “broadened liver cords, expanded cells, and contracted liver sinuses,” and liver sections were described as “fuzzy and edematous with extremely loose cytoplasm” at 150 mg/kg-day. Serum AST levels were significantly increased at 15 mg/kg-day and above, while serum ALT was increased at 150 mg/kg-day and serum albumin was reduced at 150 mg/kg-day. The magnitude of changes in serum chemistry parameters could not be determined, as data were presented graphically only and appeared variable. Liver weight and other serum markers of liver toxicity (ALP, GGT, bilirubin, cholesterol) were not evaluated. Sub-apical mechanistic outcomes were also evaluated. Evidence of oxidative stress was

limited to the livers of mice treated with 150 mg/kg-day DIDP and included increased reactive oxygen species (ROS), malondialdehyde, and 8-hydroxy-2-deoxyguanosine levels, and decreased glutathione. Markers of inflammation and apoptosis included increased interleukin-1β and tumor necrosis factor-α content at 15 mg/kg-day DIDP and above, increased nuclear factor-κB levels in the liver at 0.15 mg/kg-day DIDP and above, and increased caspase-3 levels in the liver at 150 mg/kg-day. Co-administration of vitamin E attenuated markers of oxidative damage, inflammation, and apoptosis, further implicating a role for oxidative stress in the liver. Collectively, results from this study indicate effects on apical outcomes at 15 mg/kg-day DIDP and above (liver histopathology, increased serum AST) and sub-apical mechanistic outcomes at 0.15 mg/kg-day DIDP and above. However, the biological significance and adversity of the observed effects is uncertain due to limitations in the study (*i.e.*, histopathology reported qualitatively; uncertainty in the magnitude of changes in serum chemistry; liver weight not reported).

Subchronic (>30–90 Days) Exposure Studies: DIDP has been evaluated in two subchronic dietary studies of rats and one dietary study of beagles. In the first study, which was only available to EPA as a foreign language study in German [(BASF, 1969) as reported in (EC/HC, 2015; ECHA, 2013b; ECB, 2003)], male and female SD rats were fed diets containing 0, 800, 1,600, 3,200, or 6,400 ppm DIDP in feed for 90 days (equivalent to 55, 100, 200, 400 mg/kg-day for males; 60, 120, 250, 500 mg/kg-day for females). In the males, absolute liver weight increased (31%) in the high-dose group, while relative liver weight was significantly increased at all doses but without dose-concordance. In females, absolute liver weight increased (16–33%) in rats at 3,200 ppm and above, while relative liver weight increased at 1,600 ppm and above in a dose-dependent manner. Clinical chemistry and urinalysis parameters were reported to be within the normal range, and no histopathologic findings were reported in the liver of either sex. Based upon study summaries provided in existing assessments (EC/HC, 2015; ECHA, 2013b; ECB, 2003), this study supports a NOEL of 60 mg/kg-day based on a dose-related increase in relative liver weight in female rats at 120 mg/kg-day and above. However, because the study by BASF (1969) was not reasonably available to EPA in English, it is not further considered in the risk evaluation of DIDP.

In a second study by Hazelton Labs (1968b), male and female albino rats were fed diets containing 0, 500, 3,000, or 10,000 ppm DIDP for 90 days (equivalent to 28, 170, 586 mg/kg-day for males; 35, 211, 686 mg/kg-day for females). Hepatic effects were limited to increased absolute (35–42%) and relative (37–62%) liver weight in high-dose male and female rats. Clinical chemistry and urinalysis parameters were unaffected by exposure to DIDP, and no treatment-related histopathologic findings were noted in the liver of either sex.

In a third subchronic study, male and female beagles (three per sex per dose) were fed diets containing 0, 500, 3,000, or 10,000 ppm DIDP (equivalent to 15, 75, 300 mg/kg-day) for 13 weeks (Hazelton Labs, 1968a). Mean absolute and relative liver weight appeared increased in high-dose males (25–37%) and females (44–51%); however, a statistical analysis was not conducted due to the small sample size. Slight to moderate swelling and vacuolation of hepatocytes was observed in mid- and high-dose males (incidence: 0/3, 0/2, 2/3, 1/3) and females (incidence: 0/3, 0/3, 2/3, 3/3). Clinical markers of hepatotoxicity were similar to control values (*i.e.*, AST, ALT, ALP, bromsulphthalein clearance). Although this study is limited by its small sample size and lack of statistical analysis, existing assessments of DIDP by U.S. CPSC (2014), ECHA (2013b), EFSA (2019), Health Canada (ECCC/HC, 2020), and NICNAS (2015) have all identified a NOAEL of 15 mg/kg-day, based on increased liver weight and histopathological findings (swelling and vacuolation of hepatocytes).

Chronic (>90 Days) Exposure: Liver effects following DIDP exposure have been evaluated in two chronic studies, including one 26-week dietary study of mice (Cho et al., 2011) and a 2-year dietary

study of rats (Cho et al., 2010; Cho et al., 2008). Cho et al. (2011) fed male and female wild-type mice diets containing 0 or 1.0 percent DIDP (equivalent to approximately 1,500 mg/kg-day) and male and female transgenic rasH2 mice 0, 0.1, 0.33, and 1.0 percent DIDP (equivalent to approximately 150, 495, 1,500 mg/kg-day) for 26 weeks. No significant effects on survival were reported at any dose for wild-type or rasH2 mice of either sex. In wild-type mice, terminal body weight was reduced by 27 and 12 percent in males and females, respectively. Liver effects included an increase in relative liver weight in male and female mice (59–72%). Lesions with increased incidence included hepatocyte hypertrophy with eosinophilic granules in both sexes, and parenchymal inflammation, pigmented hepatocytes, pigmented Kupffer cells, and prominent Kupffer cells in males (Table_Apx C-10). A non-statistically significant increase in the incidence of focal necrosis was observed in males (5/15 vs. 1/15 in controls). Similarly, in rasH2 mice, terminal body weight was reduced by 31 and 15 percent in males and females, respectively. Relative liver weight was increase 15 to 52 percent for mid- and high-dose males and 35 percent for high-dose females. Lesions with increased incidence included parenchymal inflammation in females, hepatocyte hypertrophy with eosinophilic granules in both sexes, and focal necrosis, pigmented hepatocytes, pigmented Kupffer cells, and prominent Kupffer cells in males (Table_Apx C-10).

Table_Apx C-10. Incidence of Non-neoplastic Lesions in the Liver of Wild-Type and RasH2 Mice Exposed to DIDP in the Diet for 26 Weeks (Cho et al., 2011)[a]

Sex	Lesion	RasH2 Mice				Wild-type Mice	
		0	0.1% DIDP	0.33% DIDP	1.0% DIDP	0	1.0% DIDP
# of males examined		15	15	15	15	15	15
Male	Parenchymal inflammation	6	12*	11	11	7	13*
	Diffuse hepatocyte hypertrophy with eosinophilic granules	0	4*	15*	13*	0	11*
	Necrosis, focal	0	0	0	4*	1	5
	Pigmented hepatocytes	0	0	4*	6*	0	7*
	Pigmented Kupffer cells	0	0	4*	7*	0	7*
	Prominent Kupffer cells	0	4*	11*	13*	0	13*
Number of females examined		15	15	15	15	15	15
Female	Parenchymal inflammation	1	12*	13*	12*	6	3
	Diffuse hepatocyte hypertrophy with eosinophilic granules	0	0	1	12*	0	11*

[a] Data from Tables 4 in Cho et al. (2011). * ($p < 0.05$) indicate a significant difference from the control group by Chi-square test.

In a second chronic study, male and female F344 rats were administered 0, 400, 2,000, 8,000 ppm DIDP in the diet for 2 years (equivalent to 22, 110, 479 mg/kg-day for males; 23, 128, 620 mg/kg-day for females) (Cho et al., 2010; Cho et al., 2008). Overt toxicity was observed in the high-dose group, and included reduced survival of male (37 vs. 85% in control) and female (56 vs. 85% in control) rats, reduced bodyweight gain (both sexes), and a 14 to 18 percent decrease in terminal body weight for both sexes. Liver effects included a 40 to 49 percent increase in relative liver weight in high-dose males and females (absolute weight not reported). Non-neoplastic lesions were observed in the livers of high-dose females (necrosis) and males (*i.e.*, necrosis, hypertrophy, peliosis, microgranuloma, spongiosis hepatis, and oval cell hyperplasia) at doses of 400 ppm and higher (Table_Apx C-11). Evidence of peroxisome proliferation was apparent in the livers of high-dose males after 12 weeks of exposure to DIDP, as demonstrated by increased expression of catalase protein by western blot analysis and increased catalase

activity. However, evidence of peroxisome proliferation was no longer apparent after 32 or 104 weeks of exposure to DIDP indicating that peroxisome proliferation was not maintained. Collectively, this study supports a LOAEL of 400 ppm DIDP (equivalent to 22 mg/kg-day) (no NOAEL identified) based on increased incidence of spongiosis hepatis and microgranuloma in male rats. Consistently, Health Canada (EC/HC, 2015) and ECHA (2013b) have also concluded that the study by Cho et al. supports a LOAEL of 22 mg/kg-day. In contrast, Australia NICNAS (2015, 2012) did not consider spongiosis hepatis relevant as a critical endpoint for human health risk assessment and concluded that Cho et al. (2008) supports a NOAEL of 2,000 ppm DIDP (equivalent to 110 mg/kg-day) based on an increased liver weight and other non-neoplastic lesions.

Table_Apx C-11. Incidence of Non-neoplastic Lesions in the Liver of F344 Rats Exposed to DIDP in the Diet for 2 Years (Cho et al., 2008) [a]

Sex	Lesion	0	400 ppm	2,000 ppm	8,000 ppm
Female	Necrosis	2/49 (4.1%)	4/47 (8.5%)	6/47 (13%)	9/40** (21%)
	Altered cell foci	31/49 (63%)	26/47 (55%)	27/47 (57%)	17/40* (43%)
	Inflammation	2/49 (4.1%)	8/47* (17%)	11/47** (23%)	3/40 (7.5%)
	Microgranuloma	10/49 (20%)	6/47 (13%)	12/47 (26%)	3/40*(7.5%)
Male	Oval cell hyperplasia	1/49 (2.0%)	3/48 (6.3%)	2/49 (4.1%)	6/39* (15%)
	Hypertrophy	0/49	0/48	1/49 (2.0%)	4/39* (10%)
	Microgranuloma	1/49 (2.0%)	5/48* (10%)	6/49* (12%)	4/39* (10%)
	Necrosis	3/49 (6.1%)	7/48 (15%)	5/49 (10%)	8/39* (21%)
	Peliosis	1/49 (2.0%)	0/48	2/49 (4.1%)	4/39* (10%)
	Spongiosis hepatis	0/49	3/48* (6.3%)	3/49* (6.1%)	5/39** (13%)
	Fatty change	4/49 (8.2%)	6/48 (12.5%)	1/49 (2.0%)	0/39 (0%)
	Altered cell foci	27/49 (55%)	19/48 (40%)	18/49* (37%)	3/39** (7.7%)

[a] Data from Tables 3 and 4 in Cho et al. (2008). * ($p < 0.05$) and ** ($p < 0.01$) indicate a significant difference from the control group by the poly-3 test.

Studies of Development and Reproduction: Liver effects have also been observed in one prenatal developmental study (Hellwig et al., 1997) and in a pair of two-generation studies of reproduction (Hushka et al., 2001; Exxon Biomedical, 2000, 1998). In the prenatal study, pregnant Wistar rats were gavaged with 0, 40, 200, and 1,000 mg/kg-day on GDs 6 through 15 and then sacrificed on GD 20 (Hellwig et al., 1997). In high-dose dams a 9.3 to 13 percent increase in relative and absolute liver weight was observed. Clinical chemistry and histopathology were not evaluated.

In the first two-generation study (Study A), SD rats were continuously administered dietary concentrations of 0, 0.2, 0.4, and 0.8 percent DIDP starting 10 weeks prior to mating, continuing throughout mating, gestation, and lactation, and lasting until terminal sacrifice for two generations (Hushka et al., 2001; Exxon Biomedical, 1998). Received doses in units of mg/kg-day are shown in Table_Apx C-4. Hepatic effects were observed in male and female rats of both the P1 and P2 generations at all dose levels. Absolute and/or relative liver weight was significantly increased 11 to 29 percent in P1 and P2 males at 0.4 percent DIDP and above and 9 to 28 percent in P1 and P2 females at 0.2 percent DIDP and above. Liver weight changes were accompanied by increased centrilobular or diffuse hepatocellular hypertrophy in P1 and P2 males and females at all doses, and the incidence and severity of the lesion increased with dose (Table_Apx C-12). Minimal to mild focal necrosis was observed in P1 males at 0.8 percent DIDP and P2 males at 0.4 percent DIDP and above but was not

observed in P1 or P2 females (Table_Apx C-12). Diffuse hepatocellular hypertrophy was observed in the livers of F1 and F2 offspring sacrificed after weaning at 0.4 percent DIDP and above. However, necrosis was not observed in the livers of F1 or F2 offspring at any dose. Clinical chemistry was not evaluated. The liver effects observed in P1 and P2 females and F1 and F2 offspring are consistent with an adaptive, non-adverse response. However, the increased incidence of focal necrosis in the livers of high-dose P1, and mid- and high-dose P2 males is adverse, supporting a NOAEL of 0.2 percent DIDP in P2 males (equivalent to 117 mg/kg-day).

In the second two-generation study (Study B), SD rats were continuously administered dietary concentrations of 0, 0.02, 0.06, 0.2, and 0.4% DIDP starting 10 weeks prior to mating, throughout mating, gestation, and lactation, until terminal sacrifice for two generations (Hushka et al., 2001; Exxon Biomedical, 2000). Received doses in units of mg/kg-day are shown in Table_Apx C-7. Clinical chemistry and histopathology were not evaluated. Absolute and/or relative liver weight was significantly increased 12 to 14 percent in high-dose P1 males and females, 13 to 14 percent in high-dose P2 males, and 9 to 23 percent in P2 females at 0.2 percent DIDP and above. Liver weight changes were not observed for F1 or F2 offspring of either sex at weaning.

Table_Apx C-12. Incidence of Non-neoplastic Lesions in the Liver and Kidney of Rats Exposed to DIDP over Two Generations (Study A) (Hushka et al., 2001; Exxon Biomedical, 1998) [a] [b]

Group	Organ: Lesion (Severity)	Males				Females			
		0	0.2%	0.4%	0.8%	0	0.2%	0.4%	0.8%
P1	Liver: Hypertrophy, hepatocellular, centrilobular (minimal/mild)	0/45	6/30 (2/4)	21/30 (1/20)	2/45 (0/2)	0/50	0/30	0/30	1/50 (0/1)
	Liver: Hypertrophy, hepatocellular, diffuse (minimal/mild/moderate)	0/45	0/30	9/30 (0/9/0)	42/45 (0/2/40)	0/50	22/30 (1/18/3)	24/30 (1/9/14)	43/50 (4/5/34)
	Liver: Necrosis, focal (minimal/mild)	1/45 (1/0)	2/30 (2/0)	0/30	6/45 (2/4)	2/50 (1/1)	0/30	0/30	2/50 (1/1)
	Kidney: Granular cast(s)	2/45 (4.4%)	1/30 (3.2%)	4/30 (13%)	14/45 (31%)	1/50 (2.0%)	0/30	0/30	0/50
	Kidney: Focal degeneration, cortical tubules (minimal/mild/ moderate)	33/45 (73%) (15/18/0)	26/30 (87%) (18/7/1)	27/30 (90%) (14/11/2)	44/45 (98%) (8/32/4)	5/50 (10%) (5/0/0)	0/30	0/30	4/50 (8%) (2/2/0)
	Kidney: Pigment in tubular epithelia cells (minimal/mild/ moderate/marked)	34/45 (76%) (11/16/6/1)	28/30 (93%) (9/18/1/0)	30/30 (100%) (6/22/2/0)	45/45 (100%) (0/9/27/9)	0/50	0/30	0/30	0/50
P2	Liver: Hypertrophy, hepatocellular, centrilobular (minimal/ mild/moderate)	0/30	15/30 (1/11/3)	8/30 (0/0/8)	0/30	0/30	0/30	0/30	0/30
	Liver: Hypertrophy, hepatocellular, diffuse (minimal/mild/moderate/marked)	0/30	15/30 (0/11/4/0)	22/30 (0/9/13/0)	30/30 (2/13/14/1)	0/30	23/30 (12/11/0/0)	26/30 (9/17/0/0)	30/30 (2/5/23/0)
	Liver: Necrosis, focal (minimal/mild)	1/30 (0/1)	2/30 (2/0)	4/30 (0/4)	9/30 (4/5)	3/30 (1/2)	0/30	1/30 (0/1)	0/30
	Kidney: Granular cast(s)	0/30	2/30 (6.7%)	4/30 (13%)	5/30 (17%)	0/30	–	–	0/30
	Kidney: Focal degeneration, cortical tubules (minimal/mild/moderate)	24/30 (80%) (11/12/1)	25/30 (83%) (8/15/2)	28/30 (93%) (11/15/2)	27/30 (90%) (4/20/3)	3/30 (10%) (3/0/0)	–	–	6/30 (20%) (4/2/0)
	Kidney: Pigment in tubular epithelia cells (minimal/mild/moderate/marked)	23/30 (77%) (12/8/3/0)	27/30 (90%) (5/18/4/0)	26/30 (87%) (7/13/6/0)	30/30 (100%) (0/7/15/8)	0/30	–	–	0/30
F1	Hypertrophy, hepatocellular, diffuse (minimal/mild)	0/21	0/22	12/21 (12/0)	21/30 (15/6)	0/20	0/19	8/23 (8/0)	18/29 (13/5)

F2	Hypertrophy, hepatocellular, diffuse (minimal/mild)	0/19	0/21	10/17 (10/0)	19/26 (15/4)	0/18	0/21	11/19 (11/0)	19/26 (17/2)

[a] P1 and P2 refer to the 1st and 2nd parental generations, respectively. P1and P2 males were sacrificed after the last dam gave birth, while females were sacrificed after weaning on PND21. F1 and F2 refer to the offspring sired by the P1 and P2 generations, respectively. F1 offspring not selected for mating, necropsy, or switched diet groups, and all surviving F2 offspring were sacrificed after weaning.
[b] Incidence data from Appendix BA of Exxon Biomedical (1998).

Appendix D CALCULATING DAILY ORAL HUMAN EQUIVALENT DOSES AND HUMAN EQUIVALENT CONCENTRATIONS

For DIDP, all data considered for PODs are obtained from oral animal toxicity studies in rats, mice, or beagles. Because toxicity values for DIDP are from oral animal studies, EPA must use an extrapolation method to estimate human equivalent doses (HEDs). The preferred method would be to use chemical-specific information for such an extrapolation. However, there are no DIDP-specific PBPK models, and EPA did not locate other DIDP information to conduct a chemical-specific quantitative extrapolation. In the absence of such data, the Agency relied on the guidance from U.S. EPA (2011b), which recommends scaling allometrically across species using the three-quarter power of body weight ($BW^{3/4}$) for oral data. Allometric scaling accounts for differences in physiological and biochemical processes, mostly related to kinetics.

For application of allometric scaling in risk evaluations, EPA uses dosimetric adjustment factors (DAFs), which can be calculated using Equation_Apx D-1.

Equation_Apx D-1. Dosimetric Adjustment Factor

$$DAF = \left(\frac{BW_A}{BW_H}\right)^{1/4}$$

Where:

DAF	=	Dosimetric adjustment factor (unitless)
BW_A	=	Body weight of species used in toxicity study (kg)
BW_H	=	Body weight of adult human (kg)

U.S. EPA (2011b), presents DAFs for extrapolation to humans from several species. However, because those DAFs used a human body weight of 70 kg, EPA has updated the DAFs using a human body weight of 80 kg for the risk evaluation of DIDP (U.S. EPA, 2011a). The Agency used the body weights of 0.025, 0.25, and 12 kg for mice, rats and dogs, respectively, as presented in U.S. EPA (2011b). The resulting DAFs for mice, rats, and dogs are 0.133, 0.236, and 0.622, respectively.

Use of allometric scaling for oral animal toxicity data to account for differences among species allows EPA to decrease the default intraspecies uncertainty factor (UF_A) used to set the benchmark MOE; the default value of 10 can be decreased to 3, which accounts for any toxicodynamic differences that are not covered by use of $BW^{3/4}$. Using the appropriate DAF from Equation_Apx D-1, EPA adjusts the POD to obtain the HED using Equation_Apx D-2:

Equation_Apx D-2. Daily Oral Human Equivalent Dose

$$HED_{Daily} = POD_{Daily} \times DAF$$

Where:

HED_{Daily}	=	Human equivalent dose assuming daily doses (mg/kg-day)
POD_{Daily}	=	Oral POD assuming daily doses (mg/kg-day)
DAF	=	Dosimetric adjustment factor (unitless)

For this risk evaluation, EPA assumes similar absorption for the oral and inhalation routes, and no adjustment was made when extrapolating to the inhalation route. For the inhalation route, the Agency

extrapolated the daily oral HEDs to inhalation HECs using a human body weight and breathing rate relevant to a continuous exposure of an individual at rest, as follows:

Equation_Apx D-3. Extrapolating from Oral HED to Inhalation HEC

$$HEC_{Daily,\ continuous} = HED_{Daily} \times (\frac{BW_H}{IR_R * ED_C})$$

Where:

$HEC_{Daily,continuous}$	=	Inhalation HEC based on continuous daily exposure (mg/m^3)
HED_{Daily}	=	Oral HED based on daily exposure (mg/kg-day)
BW_H	=	Body weight of adult humans (kg) = 80
IR_R	=	Inhalation rate for an individual at rest (m^3/h) = 0.6125
ED_C	=	Exposure duration for a continuous exposure (h/day) = 24

Based on information from U.S. EPA (2011a), EPA assumes an at rest breathing rate of 0.6125 m^3/h. Adjustments for different breathing rates required for individual exposure scenarios are made in the exposure calculations, as needed.

It is often necessary to convert between ppm and mg/m^3 due to variation in concentration reporting in studies and the default units for different OPPT models. Therefore, EPA presents all PODs in equivalents of both units to avoid confusion and errors. Equation_Apx D-4 presents the conversion of the HEC from mg/m^3 to ppm.

Equation_Apx D-4. Converting Units for HECs (mg/m3 to ppm)

$$X\ ppm = Y\ \frac{mg}{m^3} \times \frac{24.45}{MW}$$

Where:

24.45	=	Molar volume of a gas at standard temperature and pressure (L/mol), default
MW	=	Molecular weight of the chemical (MW of DIDP = 446.7 g/mol)

D.1 DIDP Non-cancer HED and HEC Calculations for Acute, Intermediate, and Chronic Exposures

The acute non-cancer POD is based on a NOAEL of 38 mg/kg-day, and the critical effect is decreased F2 offspring survival on PND1 and PND4 in a two-generation study of reproduction (Hushka et al., 2001; Exxon Biomedical, 2000). This non-cancer POD is considered protective of effects observed following intermediate and chronic exposures to DIDP. EPA used Equation_Apx D-1 to determine a DAF specific to rats (0.236), which was in turn used in the following calculation of the daily HED using Equation_Apx D-2:

$$8.98\ \frac{mg}{kg-day} = 38 \frac{mg}{kg-day} \times 0.236$$

EPA then calculated the continuous HEC for an individual at rest using Equation_Apx D-3:

$$48.9\ \frac{mg}{m^3} =\ 8.98\ \frac{mg}{kg-day} \times (\frac{80\ kg}{0.6125 \frac{m^3}{hr} * 24\ hr})$$

Equation_Apx D-4 was used to convert the HEC from mg/m^3 to ppm:

$$2.68\ ppm = 48.9\ \frac{mg}{m^3} \times \frac{24.45}{446.7}$$

Appendix E BENCHMARK DOSE ANALYSIS OF CHO ET AL. (2008, 2010)

E.1 Summary of Benchmark Dose Modeling Approach

EPA performed benchmark dose (BMD) modeling using EPA's BMD modeling software version 3.3.2 (BMDS 3.3.2) for select dichotomous endpoints (listed below) from a 2-year chronic dietary exposure study of DIDP with male and female F344 rats (Cho et al., 2010; Cho et al., 2008). All standard BMDS 3.3.2 dichotomous models that use maximum likelihood (MLE) optimization and profile likelihood-based confidence intervals were used in this analysis. Standard forms of these models (defined below) were run so that auto-generated model selection recommendations accurately reflect current EPA model selection procedures in EPA's Benchmark Dose Technical Guidance (U.S. EPA, 2012). BMDS 3.3.2 models that use Bayesian fitting procedures and Bayesian model averaging were not applied in this work.

Dichotomous Endpoints Modeled

- Incidence of spongiosis hepatis in the liver (male F344 rats only)
- Incidence of necrosis in the liver (male and female F344 rats)
- Incidence of hypertrophy in the liver (male F344 rats only)
- Incidence of oval cell hyperplasia in the liver (male F344 rats only)
- Incidence of peliosis in the liver (male F344 rats only)
- Incidence of microgranuloma in the liver (male F344 rats only)

Standard BMDS 3.3.2 Models Applied to Dichotomous Endpoints

- Gamma-restricted
- Log-Logistic-restricted
- Weibull-restricted
- Dichotomous Hill-restricted
- Multistage 1, 2, 3-restricted
- Logistic (log)-unrestricted
- Log-Probit-unrestricted
- Probit (pro)-unrestricted
- Quantal Linear-unrestricted

General Model Options Used for Individual Endpoint Analyses

- Risk Type: Extra Risk
- Preferred Dichotomous Endpoint BMR: 0.1 (10%)
- Confidence Level: 0.95
- Background response: Estimated
- Model Restrictions: Restrictions for BMDS 3.3.2 models are defined in the BMDS 3.3.2 User Guide and are applied in accordance with EPA BMD Technical Guidance (U.S. EPA, 2012).

Model Selection

The preferred model for the BMD derivations was chosen from the standard set of dichotomous models listed above. The modeling restrictions and the model selection criteria facilitated in BMDS 3.3.2, and defined in the BMDS User Guide, were applied in accordance with EPA BMD Technical Guidance (U.S. EPA, 2012) for non-cancer endpoints.

E.2 Summary of Benchmark Dose Modeling Results

A summary of EPA's BMD modeling results is provided in Table_Apx E-1.

Table_Apx E-1. Summary of Benchmark Dose Modeling Results from Selected Endpoints in Male and Female F344 Rats Following 2-Year Exposure to DIDP (Cho et al. 2008, 2010)

Section	Endpoint	Sex	Selected Model	BMD10 (mg/kg-day)	BMDL10 (mg/kg-day)
E.3	Spongiosis hepatis in the liver	Male	Log-Logistic	391	172
E.4.1	Necrosis in the liver	Male	Multistage 3	427	172
E.4.2	Necrosis in the liver	Female	Log-Logistic	290	144
E.5	Hypertrophy in the liver	Male	Dichotomous Hill	161	120
E.6	Oval cell hyperplasia in the liver	Male	Log-Probit	471	94
E.7	Peliosis in the liver	Male	Multistage 2/3	518	253
E.8	Microgranuloma in the liver	Male	Log-Logistic	2,856	314

E.3 Spongiosis Hepatis in the Liver of Male F344 Rats

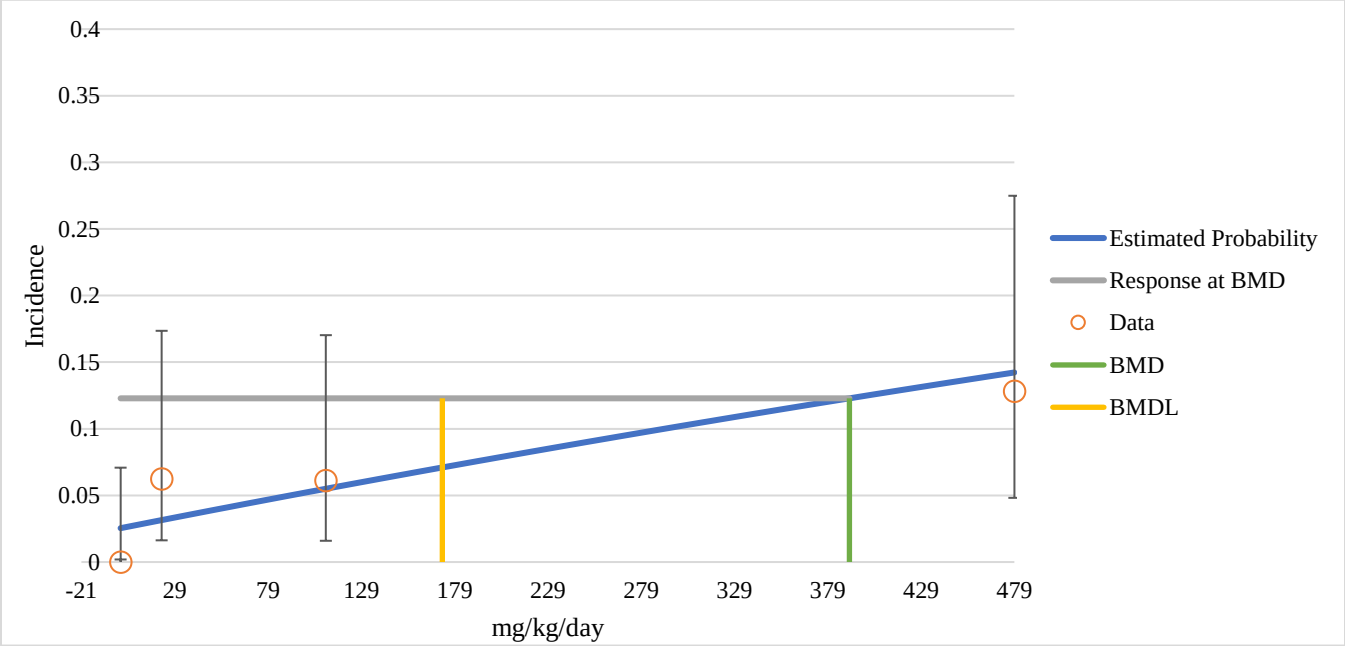
Table_Apx E-2. Incidence of Spongiosis Hepatis in the Livers of Male F344 Rats Dosed with DIDP for 2 Years (Cho et al., 2010; Cho et al., 2008)

Dose (mg/kg-day)	Number of Animals	Incidence
0	49	0
22	48	3
110	49	3
479	39	5

Table_Apx E-3. Summary of Benchmark Dose Modeling Results for Spongiosis Hepatis in the Liver of Male F344 Rats Following 2-Year Exposure to DIDP (Cho et al., 2010; Cho et al., 2008) [a]

Model	Restrictions	Goodness of Fit (Means)		BMD 10%ER (mg/kg-day)	BMDL 10%ER (mg/kg-day)	BMDS Recommendation Notes
		p-value	AIC			
Dichotomous Hill	Restricted	0.661	79.7	307.7507	0	Unusable (BMD computation failed; lower limit includes zero BMDL not estimated)
Gamma	Restricted	0.236	82.7	401.3942	189.701	Viable – Alternate
Log-Logistic	**Restricted**	**0.235**	**82.7**	**390.6084**	**172.4344**	**Viable – Recommended (Lowest AIC)**
Multistage 3	Restricted	0.236	82.7	401.3942	189.697	Viable – Alternate
Multistage 2	Restricted	0.236	82.7	401.3943	189.6967	Viable – Alternate
Multistage 1	Restricted	0.236	82.7	401.3942	189.6965	Viable – Alternate
Weibull	Restricted	0.236	82.7	401.3942	189.701	Viable – Alternate
Logistic	Unrestricted	0.240	83.3	471.7638	318.4489	Viable – Alternate
Log-Probit	Unrestricted	0.816	79.3	265.0933	0	Unusable (BMD computation failed; lower limit includes zero BMDL not estimated)
Probit	Unrestricted	0.241	83.2	466.4551	301.8694	Viable – Alternate
Quantal Linear	Unrestricted	0.236	82.7	401.3942	189.701	Viable – Alternate

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit
[a] Selected model is bolded and shaded gray; scaled residuals for doses 0, 22, 110, and 479 mg/kg-day were −1.13, 1.23, 0.19, and −0.25, respectively.



Figure_Apx E-1. Frequentist Log-Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

User Input

Info	
Model	Log-Logistic
Model Restriction	Restricted
Dataset Name	Male - Liver Spongiosis Hepatis
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g+(1-g)/[1+exp(-a-b*Log(dose))]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	mg/kg/day
Independent Variable	Incidence
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	390.6084258
BMDL	172.4343542
BMDU	1996.021182
AIC	82.69569071
P-value	0.235388531
D.O.F.	2
Chi^2	2.89303561

Model Parameters				
# of Parameters	3			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.025417951	0.04666981	-0.0660532	0.1168891
a	-8.164930167	0.608465746	-9.3575011	-6.9723592
b	Bounded	NA	NA	NA

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.025417951	1.245479618	0	49	-1.13047
22	0.031478998	1.510991892	3	48	1.230868
110	0.054987594	2.694392115	3	49	0.1915206
479	0.142285551	5.54913648	5	39	-0.251708

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-37.44330992	4	-	-	NA
Fitted Model	-39.34784535	2	3.80907087	2	0.1488918
Reduced Model	-41.71335758	1	8.54009531	3	0.0360738

Figure_Apx E-2. Results for Selected Model – Log-logistic (Restricted) – Extra Risk, BMR = 0.1

E.4 Necrosis in the Liver

E.4.1 Male F344 Rats

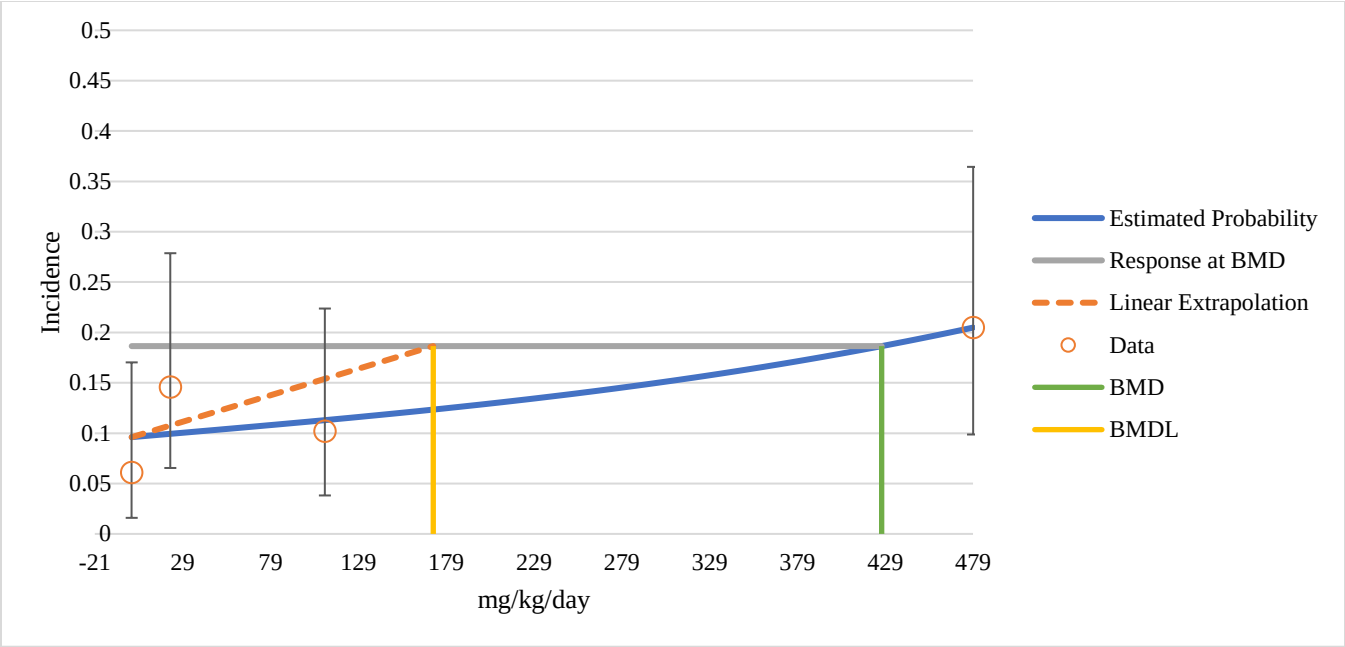
Table_Apx E-4. Incidence of Necrosis in the Livers of Male F344 Rats Dosed with DIDP for 2 Years (Cho et al., 2010; Cho et al., 2008)

Dose (mg/kg-day)	Number of Animals	Incidence
0	49	3
22	48	7
110	49	5
479	39	8

Table_Apx E-5. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver of Male F344 Rats Following 2-Year Exposure to DIDP (Cho et al., 2010; Cho et al., 2008) [a]

Model	Restrictions	Goodness of Fit (Means)		BMD 10%ER (mg/kg-day)	BMDL 10%ER (mg/kg-day)	BMDS Recommendation Notes
		p-value	AIC			
Dichotomous Hill	Restricted	NA	144.2	440.2657	103.038	Questionable (BMD/BMDL ratio > 3; d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Gamma	Restricted	0.377	140.2	393.0433	171.2347	Viable – Alternate
Log-Logistic	Restricted	0.376	140.2	389.0818	156.5007	Viable – Alternate
Multistage 3	**Restricted**	**0.386**	**140.2**	**426.9737**	**171.7**	**Viable – Recommended (Lowest AIC)**
Multistage 2	Restricted	0.382	140.2	411.1486	171.4042	Viable – Alternate
Multistage 1	Restricted	0.377	140.2	393.0434	171.2223	Viable – Alternate
Weibull	Restricted	0.377	140.2	393.0433	171.2347	Viable – Alternate
Logistic	Unrestricted	0.383	140.2	416.0951	246.3016	Viable – Alternate
Log-Probit	Unrestricted	0.259	141.7	175.1345	0	Unusable (BMD computation failed; lower limit includes zero BMDL not estimated)
Probit	Unrestricted	0.382	140.2	412.6344	235.1003	Viable – Alternate
Quantal Linear	Unrestricted	0.377	140.2	393.0435	171.2348	Viable –- Alternate

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit
[a] Selected model is bolded and shaded gray; scaled residuals for doses 0, 22, 110, and 479 mg/kg-day were −0.83, 1.08, −0.24, and 0.0045, respectively.



Figure_Apx E-3. Frequentist Multistage Degree 3 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

User Input

Info	
Model	Multistage degree 3
Model Restriction	Restricted
Dataset Name	Male - Liver Necrosis
User notes	[Add user notes here]
Dose-Response Model	[dose] = g + (1-g)*[1-exp(-b1*dose^1-b2*dose^2 - ...)]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	mg/kg/day
Independent Variable	Incidence
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	426.9737381
BMDL	171.6999586
BMDU	Infinity
AIC	140.1900563
P-value	0.386491773
D.O.F.	2
Chi^2	1.901289395
Slope Factor	0.000582411

Model Parameters				
# of Parameters	4			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.096068384	2.47E-02	0.04764193	0.14449484
b1	0.000165931	0.314449295	-0.6161434	0.61647523
b2	Bounded	NA	NA	NA
b3	Bounded	NA	NA	NA

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.096068384	4.70735083	3	49	-0.827688
22	0.099366401	4.769587249	7	48	1.0761449
110	0.112941264	5.534121933	5	49	-0.241068
479	0.204838813	7.988713704	8	39	0.004478

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-67.16310593	4	-	-	NA
Fitted Model	-68.09502815	2	1.86384444	2	0.393796
Reduced Model	-69.45885437	1	4.59149688	3	0.2042727

Figure_Apx E-4. Results for Selected Model – Multistage Degree 3 – Extra Risk, BMR = 0.1

E.4.2 Female F344 Rats

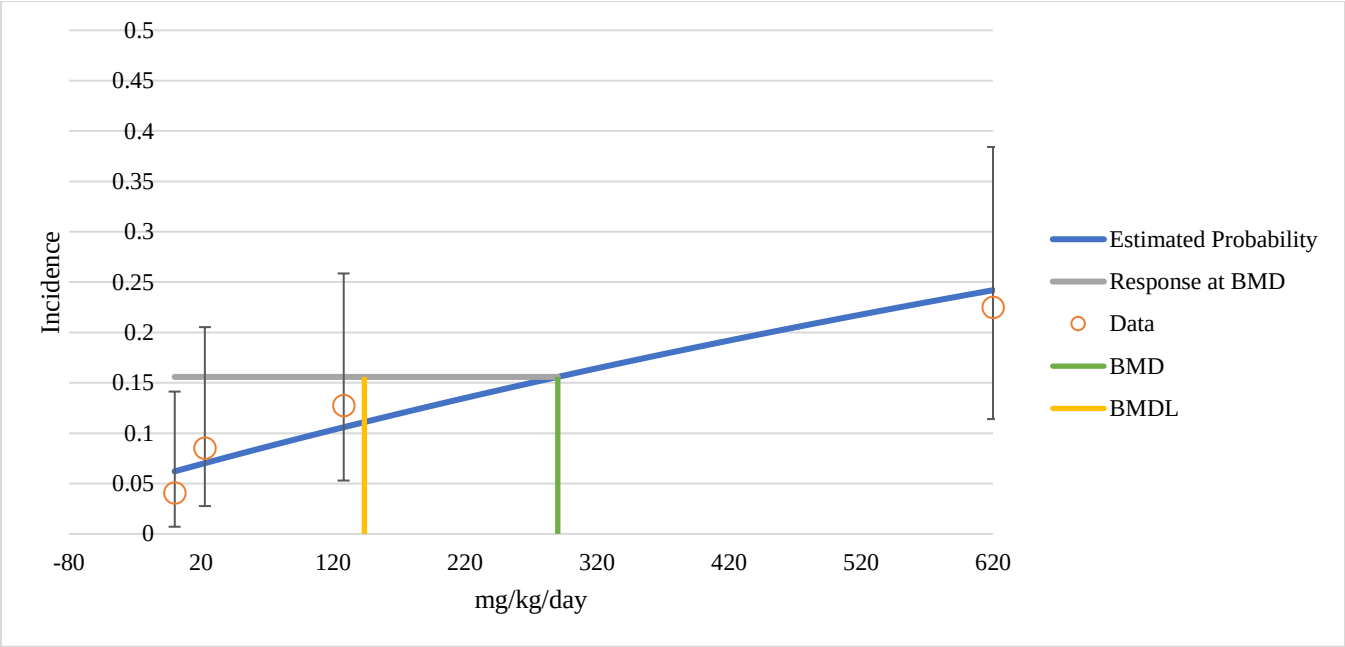
Table_Apx E-6. Incidence of Necrosis in the Livers of Female F344 Rats Dosed with DIDP for 2 Years (Cho et al., 2010; Cho et al., 2008)

Dose (mg/kg-day)	Number of Animals	Incidence
0	49	2
23	47	4
128	47	6
620	40	9

Table_Apx E-7. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver of Female F344 Rats Following 2-Year Exposure to DIDP (Cho et al., 2010; Cho et al., 2008) [a]

Model	Restrictions	Goodness of Fit (Means)		BMD 10%ER (mg/kg-day)	BMDL 10%ER (mg/kg-day)	BMDS Recommendation Notes
		p-Value	AIC			
Dichotomous Hill	Restricted	0.639	128.8	131.6645	0.10489	Questionable (BMD/BMDL ratio > 20; BMD/BMDL ratio > 3; BMDL 3× lower than lowest non-zero dose; BMDL 10× lower than lowest non-zero dose)
Gamma	Restricted	0.629	127.6	311.8711	167.5532	Viable – Alternate
Log-Logistic	**Restricted**	**0.658**	**127.5**	**290.2961**	**143.7633**	**Viable – Recommended (Lowest AIC)**
Multistage 3	Restricted	0.629	127.6	311.8712	167.5534	Viable – Alternate
Multistage 2	Restricted	0.629	127.6	311.8711	167.5534	Viable – Alternate
Multistage 1	Restricted	0.629	127.6	311.8711	167.5555	Viable – Alternate
Weibull	Restricted	0.629	127.6	311.8711	167.5532	Viable – Alternate
Logistic	Unrestricted	0.491	128.1	424.6112	296.5777	Viable – Alternate
Log-Probit	Unrestricted	0.846	128.7	135.3035	2.578588	Questionable (BMD/BMDL ratio > 20; BMD/BMDL ratio > 3; BMDL 3× lower than lowest non-zero dose)
Probit	Unrestricted	0.507	128.1	409.5236	278.0353	Viable – Alternate
Quantal Linear	Unrestricted	0.629	127.6	311.8711	167.5532	Viable – Alternate

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit
[a] Selected model is bolded and shaded gray; scaled residuals for doses 0, 23, 128, and 620 mg/kg-day were −0.62, 0.40, 0.49, and −0.25, respectively.



Figure_Apx E-5. Frequentist Log-Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

User Input

Info	
Model	Log-Logistic
Model Restriction	Restricted
Dataset Name	Female - Liver Necrosis
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g+(1-g)/[1+exp(-a-b*Log(dose))]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	mg/kg/day
Independent Variable	Incidence
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	290.29606
BMDL	143.7633306
BMDU	941.9942586
AIC	127.4905506
P-value	0.657995606
D.O.F.	2
Chi^2	0.837114051

Model Parameters				
# of Parameters	3			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.062082489	2.75E-02	0.00827604	0.11588894
a	-7.868125876	0.508074217	-8.863933	-6.8723187
b	Bounded	NA	NA	NA

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.062082489	3.042041943	2	49	-0.616908
23	0.070267181	3.302557495	4	47	0.3980193
128	0.105886989	4.976688494	6	47	0.4851113
620	0.241967797	9.678711873	9	40	-0.250572

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-61.31259719	4	-	-	NA
Fitted Model	-61.74527529	2	0.86535621	2	0.6487693
Reduced Model	-65.21038847	1	7.79558257	3	0.0504308

Figure_Apx E-6. Results for Selected Model – Log Logistic – Extra Risk, BMR = 0.1

E.5 Hypertrophy in the Liver of Male F344 Rats

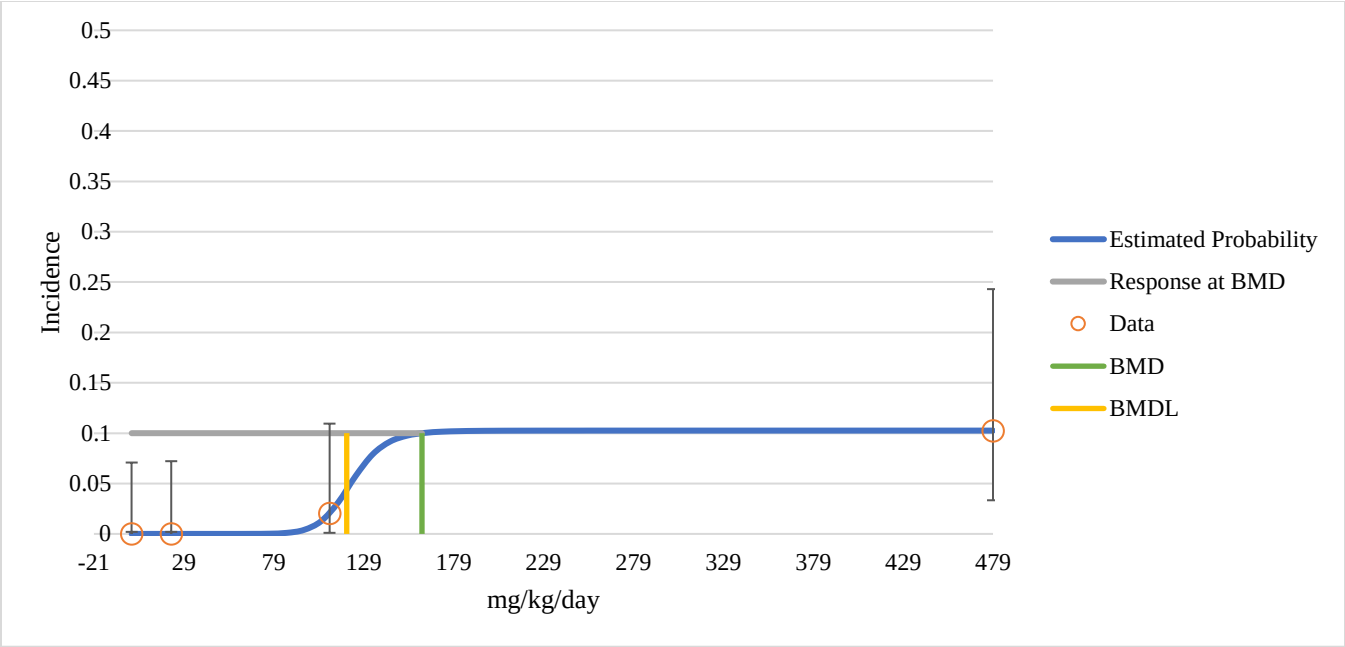
Table_Apx E-8. Incidence of Hypertrophy in the Livers of Male F344 Rats Dosed with DIDP for 2 Years (Cho et al., 2010; Cho et al., 2008)

Dose (mg/kg-day)	Number of Animals	Incidence
0	49	0
22	48	0
110	49	1
479	39	4

Table_Apx E-9. Summary of Benchmark Dose Modeling Results for Hypertrophy in the Liver of Male F344 Rats Following 2-Year Exposure to DIDP (Cho et al., 2010; Cho et al., 2008) [a]

Model	Restrictions	Goodness of Fit (Means)		BMD 10%ER (mg/kg-day)	BMDL 10%ER (mg/kg-day)	BMDS Recommendation Notes
		p-value	AIC			
Dichotomous Hill	**Restricted**	**0.999**	**41.6**	**161.4316**	**119.5791**	**Viable – Recommended (Lowest BMDL)**
Gamma	Restricted	0.928	39.8	458.7922	269.4713	Viable – Alternate
Log-Logistic	Restricted	0.929	39.8	459.041	265.2225	Viable – Alternate
Multistage 3	Restricted	0.979	37.9	465.5527	268.033	Viable – Alternate
Multistage 2	Restricted	0.979	37.9	465.5527	268.0221	Viable – Alternate
Multistage 1	Restricted	0.968	38.0	507.7941	263.5718	Viable – Alternate (BMD higher than maximum dose)
Weibull	Restricted	0.927	39.8	459.8554	269.8823	Viable – Alternate
Logistic	Unrestricted	0.559	41.0	476.2509	381.0337	Viable – Alternate
Log-Probit	Unrestricted	0.959	39.7	455.7691	249.4561	Viable – Alternate
Probit	Unrestricted	0.591	40.8	472.7884	362.5556	Viable – Alternate
Quantal Linear	Unrestricted	0.968	38.0	507.7941	263.5695	Viable – Alternate (BMD higher than maximum dose)

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit
[a] Selected model is bolded and shaded gray; scaled residuals for doses 0, 22, 110, and 479 mg/kg-day were −0.00086, −0.00086, −5.3E−09 and 2.6E−08, respectively.



Figure_Apx E-7. Frequentist Dichotomous Hill Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

User Input

Info	
Model	Dichotomous Hill
Model Restriction	Restricted
Dataset Name	Male - Hypertrophy
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g +(v-v*g)/[1+exp(-a-b*Log(dose))]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	mg/kg/day
Independent Variable	Incidence
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	161.4316178
BMDL	119.5791075
BMDU	283.0086458
AIC	41.55618433
P-value	0.999029969
D.O.F.	1
Chi^2	1.47806E-06

Model Parameters	
# of Parameters	4

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	Bounded	NA	NA	NA
v	0.102564089	3.28E-02	0.03825026	0.16687792
a	-63.34975877	2246.214021	-4465.8484	4339.14886
b	13.18100897	477.8684619	-923.42397	949.785991

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	7.46269E-07	0	49	-0.000864
22	1.52456E-08	7.31788E-07	0	48	-0.000855
110	0.020408163	1.000000005	1	49	-5.31E-09
479	0.102564101	3.999999951	4	39	2.568E-08

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-17.77809069	4	-	-	NA
Fitted Model	-17.77809216	3	2.9561E-06	1	0.9986282
Reduced Model	-22.98640492	1	10.4166285	3	0.0153373

Figure_Apx E-8. Results for Selected Model – Dichotomous Hill Model – Extra Risk, BMR = 0.1

E.6 Oval Cell Hyperplasia in the Liver of Male F344 Rats

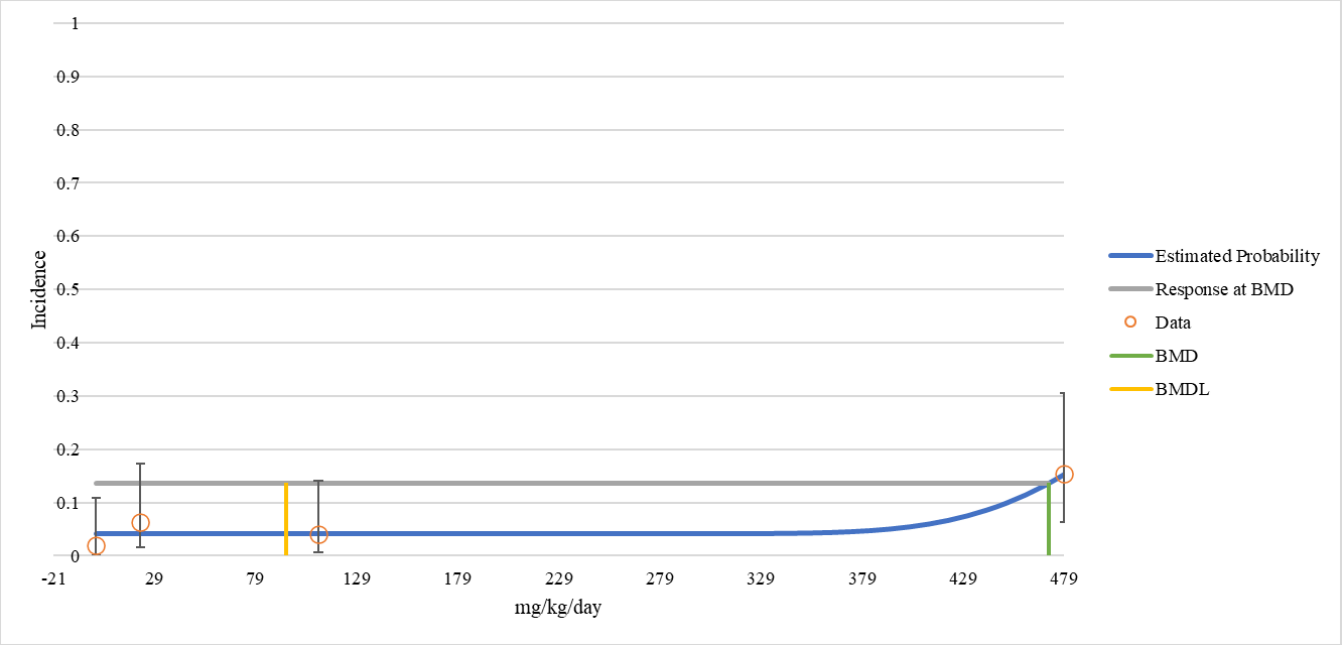
Table_Apx E-10. Incidence of Oval Cell Hyperplasia in the Livers of Male F344 Rats Dosed with DIDP for 2 Years (Cho et al., 2010; Cho et al., 2008)

Dose (mg/kg-day)	Number of Animals	Incidence
0	49	1
22	48	3
110	49	2
479	39	6

Table_Apx E-11. Summary of Benchmark Dose Modeling Results for Oval Cell Hyperplasia in the Liver of Male F344 Rats Following 2-Year Exposure to DIDP (Cho et al., 2010; Cho et al., 2008) [a]

Model	Restrictions	Goodness of Fit (Means)		BMD 10%ER (mg/kg-day)	BMDL 10%ER (mg/kg-day)	BMDS Recommendation Notes
		p-value	AIC			
Dichotomous Hill	**Restricted**	NA	91.5	472.6549	117.7685	Questionable (BMD/BMDL ratio > 3; d.f. = 0, saturated model (Goodness of fit test cannot be calculated)
Gamma	Restricted	0.296	89.5	463.4325	200.4703	Viable – Alternate
Log-Logistic	Restricted	0.296	89.5	474.1878	189.5827	Viable – Alternate
Multistage 3	Restricted	0.569	87.5	441.755	201.0995	Viable – Alternate
Multistage 2	Restricted	0.555	87.6	431.494	200.1476	Viable – Alternate
Multistage 1	Restricted	0.508	87.7	403.8577	196.8671	Viable – Alternate
Weibull	Restricted	0.296	89.5	474.4541	200.4693	Viable – Alternate
Logistic	Unrestricted	0.550	87.5	428.4377	300.3815	Viable – Alternate
Log-Probit	**Unrestricted**	**0.296**	**89.5**	**471.3352**	**93.87571**	**Viable – Recommended (Lowest BMDL; BMD/BMDL ratio > 3)**
Probit	Unrestricted	0.545	87.6	423.6878	284.5076	Viable – Alternate
Quantal Linear	Unrestricted	0.508	87.7	403.8577	196.8785	Viable – Alternate

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit
[a] Selected model is bolded and shaded gray; scaled residuals for doses 0, 22, 110, and 479 mg/kg-day were −0.73, −0.75, −0.0099 and 9.6E−09, respectively.



Figure_Apx E-9. Frequentist Log-Probit Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

User Input

Info	
Model	Log-Probit
Model Restriction	Unrestricted
Dataset Name	Male - Oval cell hyperplasia
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g+(1-g) * CumNorm(a+b*Log(Dose))

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	mg/kg/day
Independent Variable	Incidence
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	471.3351744
BMDL	93.87570544
BMDU	Infinity
AIC	89.53933729
P-value	0.29640474
D.O.F.	1
Chi^2	1.090300039

Model Parameters				
# of Parameters	3			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.041095891	2.60E-02	-0.0099305	0.09212231
a	-37.30126478	NA	NA	NA
b	5.851564737	NA	NA	NA

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.041095891	2.013698649	1	49	-0.729498
22	0.041095891	1.972602758	3	48	0.7470179
110	0.041095891	2.013698649	2	49	-0.009858
479	0.153846153	5.999999978	6	39	9.572E-09

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-41.20310652	4	-	-	NA
Fitted Model	-41.76966864	3	1.13312424	1	0.2871099
Reduced Model	-44.42750199	1	6.44879094	3	0.0917041

Figure_Apx E-10. Results for Selected Model – Log Probit – Extra Risk, BMR = 0.1

E.7 Peliosis in the Liver of Male F344 Rats

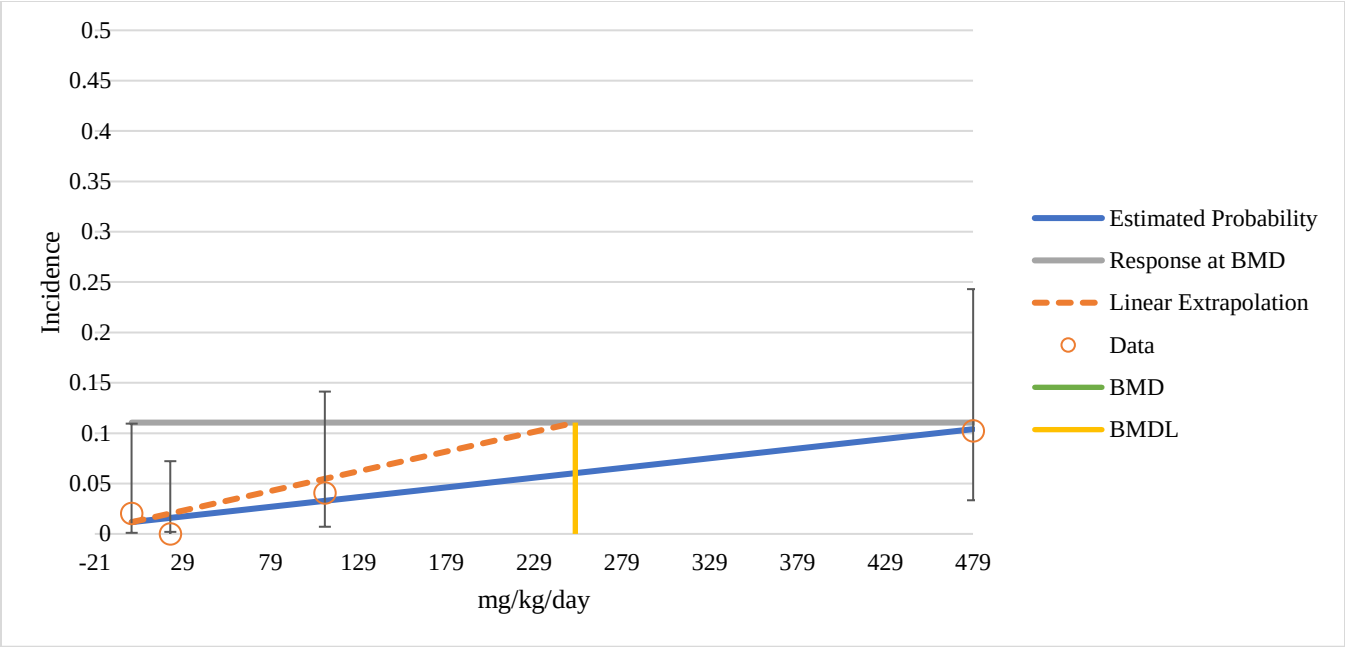
Table_Apx E-12. Incidence of Peliosis in the Livers of Male F344 Rats Dosed with DIDP for 2 Years (Cho et al., 2010; Cho et al., 2008)

Dose (mg/kg-day)	Number of Animals	Incidence
0	49	1
22	48	0
110	49	2
479	39	4

Table_Apx E-13. Summary of Benchmark Dose Modeling Results for Peliosis in the Liver of Male F344 Rats Following 2-Year Exposure to DIDP (Cho et al., 2010; Cho et al., 2008) [a]

Model	Restrictions	Goodness of Fit (Means)		BMD 10%ER (mg/kg-day)	BMDL 10%ER (mg/kg-day)	BMDS Recommendation Notes
		p-value	AIC			
Dichotomous Hill	Restricted	0.275	60.1	496.7284	253.7998	Unusable (BMD computation failed)
Gamma	Restricted	0.276	60.1	497.4194	247.2017	Viable – Alternate (BMD higher than maximum dose)
Log-Logistic	Restricted	0.549	58.2	513.0092	252.5906	Viable – Alternate
Multistage 3	**Restricted**	**0.549**	**58.2**	**513.0092**	**252.5742**	**Viable – Recommended (Lowest AIC; BMD higher than maximum dose)**
Multistage 2	**Restricted**	**0.550**	**58.2**	**518.0987**	**252.5382**	**Viable – Recommended (Lowest AIC; BMD higher than maximum dose)**
Multistage 1	Restricted	0.274	60.1	497.73	253.6747	Viable – Alternate (BMD higher than maximum dose)
Weibull	Restricted	0.493	58.4	499.6025	359.4279	Viable – Alternate (BMD higher than maximum dose)
Logistic	Unrestricted	0.289	60.0	494.7802	230.8129	Viable – Alternate (BMD higher than maximum dose)
Log-Probit	Unrestricted	0.504	58.4	499.1052	342.5722	Viable – Alternate (BMD higher than maximum dose)
Probit	Unrestricted	0.550	58.2	518.0987	252.566	Viable – Alternate (BMD higher than maximum dose)
Quantal Linear	Unrestricted	0.275	60.1	496.7284	253.7998	Viable – Alternate (BMD higher than maximum dose)

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit
[a] Selected model (Multistage 2) is bolded and shaded gray; scaled residuals for doses 0, 22, 110, and 479 mg/kg-day were 0.57, -0.88, 0.31, and −0.029 respectively.



Figure_Apx E-11. Frequentist Multistage Degree 2 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

User Input

Info	
Model	Multistage degree 2
Model Restriction	Restricted
Dataset Name	Male - Peliosis
User notes	[Add user notes here]
Dose-Response Model	[dose] = g + (1-g)*[1-exp(-b1*dose^1-b2*dose^2 - ...)]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	mg/kg/day
Independent Variable	Incidence
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	513.0091617
BMDL	252.5741919
BMDU	Infinity
AIC	58.16420507
P-value	0.549265541
D.O.F.	2
Chi^2	1.198346547
Slope Factor	0.000395923

Model Parameters				
# of Parameters	3			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.011677288	6.17E-02	-0.1091832	0.13253773
b1	0.000194505	0.121715862	-0.2383642	0.23875321
b2	Bounded	NA	NA	NA

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.011677288	0.572187121	1	49	0.5688993
22	0.015907485	0.76355926	0	48	-0.880853
110	0.032846422	1.609474666	2	49	0.3130109
479	0.10396566	4.054660739	4	39	-0.028677

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-26.13405365	4	-	-	NA
Fitted Model	-27.08210254	2	1.89609777	2	0.3874963
Reduced Model	-29.78698464	1	7.30586198	3	0.0627622

Figure_Apx E-12. Results for Selected Model – Multistage 2 – Extra Risk, BMR = 0.1

E.8 Microgranuloma in the Liver of Male F344 Rats

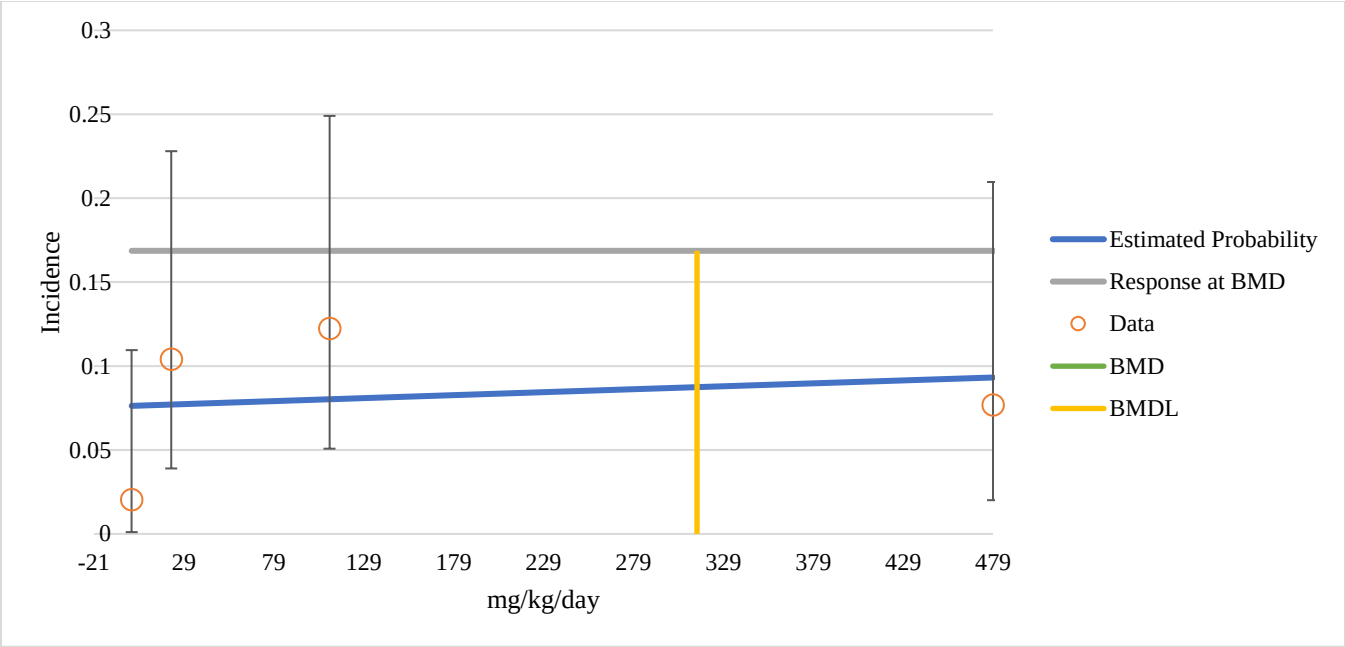
Table_Apx E-14. Incidence of Microgranuloma in the Livers of Male F344 Rats Dosed with DIDP for 2 Years (Cho et al., 2010; Cho et al., 2008)

Dose (mg/kg-day)	Number of Animals	Incidence
0	49	1
22	48	5
110	49	6
479	39	4

Table_Apx E-15. Summary of Benchmark Dose Modeling Results for Microgranuloma in the Liver of Male F344 Rats Following 2-Year Exposure to DIDP (Cho et al., 2010; Cho et al., 2008) [a]

Model	Restrictions	Goodness of Fit (Means)		BMD 10%ER (mg/kg-day)	BMDL 10%ER (mg/kg-day)	BMDS Recommendation Notes
		p-value	AIC			
Dichotomous Hill	Restricted	–	–	–	–	Unusable (BMD Computation failed)
Gamma	Restricted	0.048	110.1	18812.99	481.0798	Questionable (Goodness of fit p-value < 0.1; BMD/BMDL ratio > 20; BMD/BMDL ratio > 3; BMD higher than maximum dose; BMDL higher than maximum dose)
Log-Logistic	**Restricted**	**0.137**	**108.0**	**2856.478**	**314.3809**	**Viable – Recommended (Lowest AIC; BMD/BMDL ratio > 3; BMD higher than maximum dose)**
Multistage 3	Restricted	0.137	108.0	2803.398	330.2234	Viable – Alternate (BMD/BMDL ratio > 3; BMD higher than maximum dose)
Multistage 2	Restricted	0.137	108.0	2803.398	330.222	Viable – Alternate (BMD/BMDL ratio > 3; BMD higher than maximum dose)
Multistage 1	Restricted	0.137	108.0	2803.4	330.2357	Viable – Alternate (BMD/BMDL ratio > 3; BMD higher than maximum dose)
Weibull	Restricted	0.137	108.0	2803.398	330.2348	Viable – Alternate (BMD/BMDL ratio > 3; BMD higher than maximum dose)
Logistic	Unrestricted	0.138	108.1	2484.523	413.7805	Viable – Alternate (BMD/BMDL ratio > 3; BMD higher than maximum dose)
Log-Probit	Unrestricted	–	–	–	–	Unusable (BMD Computation failed)
Probit	Unrestricted	0.138	108.1	2540.797	404.5589	Viable – Alternate (BMD/BMDL ratio > 3; BMD higher than maximum dose)
Quantal Linear	Unrestricted	0.137	108.0	2803.4	330.2351	Viable – Alternate (BMD/BMDL ratio > 3; BMD higher than maximum dose)

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit
[a] Selected model is bolded; scaled residuals for doses 0, 22, 110, and 479 mg/kg-day were −1.47, 0.70, 1.09, and −0.35, respectively.



Figure_Apx E-13. Frequentist Log-Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL

User Input

Info	
Model	Log-Logistic
Model Restriction	Restricted
Dataset Name	Male - Microgranuloma
User notes	[Add user notes here]
Dose-Response Model	P[dose] = g+(1-g)/[1+exp(-a-b*Log(dose))]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	mg/kg/day
Independent Variable	Incidence
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	2856.477591
BMDL	314.3808975
BMDU	Infinity
AIC	108.0408258
P-value	0.137159475
D.O.F.	2
Chi^2	3.97322196

Model Parameters				
# of Parameters	3			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.076271859	2.30E-02	0.03115239	0.12139132
a	-10.15456911	3.745521559	-17.495657	-2.8134817
b	Bounded	NA	NA	NA

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.076271859	3.737321092	1	49	-1.47324
22	0.077061668	3.698960061	5	48	0.7041488
110	0.080207444	3.930164734	6	49	1.0886429
479	0.093168057	3.633554227	3	39	-0.349023

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-49.71381844	4	-	-	NA
Fitted Model	-52.02041289	2	4.6131889	2	0.0995999
Reduced Model	-52.05934032	1	4.69104378	3	0.1958696

Figure_Apx E-14. Results for Selected Model – Log Logistic – Extra Risk, BMR = 0.1