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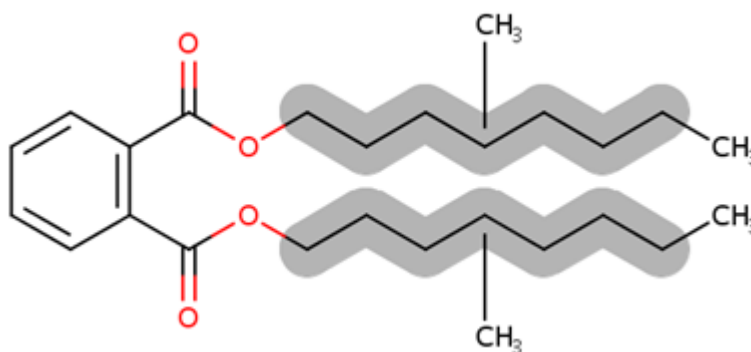
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Office of Chemical Safety and
Pollution Prevention

Non-cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)

Technical Support Document for the Risk Evaluation

CASRN: 28553-12-0 and 68515-48-0



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KEY ABBREVIATIONS AND ACRONYMS

α 2u-globulin	Alpha 2u-globulin
ACE	Angiotensin converting enzyme
ADME	Absorption, distribution, metabolism, and excretion
AGD	Anogenital distance
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AT1R	Angiotensin-II type 1 receptor
BMD	Benchmark dose
BMDL	Benchmark dose (lower confidence limit)
CASRN	Chemical Abstracts Service registry number
CPSC	Consumer Product Safety Commission (U.S.)

DINP	Diisononyl phthalate
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
eNOS	Endothelial nitric oxide synthase
EPA	Environmental Protection Agency (U.S.) (or the Agency)
F344	Fischer 344 (rat)
GD	Gestation day(s)
GI	Gastrointestinal tract
GLP	Good Laboratory Practice
GSH	Glutathione
HEC	Human equivalent concentration
HED	Human equivalent dose
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LABC	Levator ani-bulbocavernosus muscle
LD50	Lethal dose (or median lethal dose), is the amount of a substance that is lethal to 50 percent of a group of test animals
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
MNG	Multinucleated gonocytes
MOA	Mode of action
MOE	Margin of exposure
MWM	Morris Water Maze
NF κ B	Nuclear factor kappa B
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
Nrf2	Nuclear factor erythroid 2–related factor 2
NTP-CERHR	National Toxicology Program Center for the Evaluation of Risks to Human Reproduction
OCSPP	Office of Chemical Safety and Pollution Prevention (EPA)
OECD	Organisation for Economic Co-operation and Development
8-OH-dG	8-Hydroxydeoxyguanosine
OPPT	Office of Pollution Prevention and Toxics (EPA)
PECO	Population, exposure, comparator, and outcome
PESS	Potentially exposed or susceptible subpopulations
PND	Postnatal day(s)
POD	Point of departure
PPAR α	Peroxisome proliferator activated receptor alpha
ROS	Reactive oxygen species
SACC	Science Advisory Committee on Chemicals
SD	Sprague-Dawley (rat)
TNF α	Tumor necrosis factor alpha
TSCA	Toxic Substances Control Act
TSD	Technical support document
UF	Uncertainty factor
U.S.	United States

SUMMARY

This technical support document (TSD) is for diisononyl phthalate (DINP) summarizes the non-cancer hazards associated with exposure to DINP and identifies the points of departure (PODs) to be used to estimate risks from DINP exposures in the risk evaluation of DINP. EPA summarizes the cancer hazards associated with exposure to DINP in a separate assessment/TSD, the *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025a](#)).

EPA identified developmental, liver, and kidney toxicity as the most sensitive and robust non-cancer hazards associated with oral exposure to DINP in experimental animal models (Sections 3.1 through 3.3). Liver, kidney, and developmental toxicity were also identified as the most sensitive and robust non-cancer effects following oral exposure to DINP by existing assessments of DINP—including those 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](#)). EPA is using a point of departure (POD) of 49 mg/kg-day (human equivalent dose [HED] of 12 mg/kg-day) to estimate non-cancer risks from oral exposure to DINP for acute and intermediate durations of exposure in the risk evaluation of DINP. The POD was derived through meta-regression analysis and benchmark dose (BMD) modeling of fetal testicular testosterone data from two prenatal exposure studies of rats by the National Academies of Sciences, Engineering, and Medicine ([NASEM, 2017](#)). The POD of 49 mg/kg-day is the 95 percent lower confidence limit of the BMD associated with a benchmark response (BMR) of 5 percent.

As discussed further in Sections 4.1.1 and 4.1.2, several additional acute and intermediate duration studies of DINP provide similar, although less-sensitive, candidate PODs, which further support EPA's use of the selected POD of 12 mg/kg-day for decreased fetal testicular testosterone production. The Agency has performed $\frac{3}{4}$ body weight scaling to yield the HED and is applying the animal to human extrapolation factor (*i.e.*, interspecies extrapolation; UF_A) of $3\times$ and a within human variability extrapolation factor (*i.e.*, intraspecies extrapolation; UF_H) of $10\times$. Thus, a total uncertainty factor (UF) of $30\times$ is applied for use as the benchmark margin of exposure (MOE). Based on the strengths, limitations, and uncertainties discussed in Section 4.2.1, *EPA has robust overall confidence in the selected POD based on fetal testicular testosterone for use in characterizing risk from exposure to DINP for acute and intermediate exposure scenarios*. For purposes of assessing non-cancer risks, the selected POD is considered most applicable to women of reproductive age, pregnant women, male infants, and male children. Use of this POD to assess risk for other age groups (*e.g.*, adult males) is conservative.

EPA selected a no-observed-adverse-effect level (NOAEL) of 15 mg/kg-day (HED of 3.5 mg/kg-day) from a high quality 2-year study of rats based on liver toxicity to estimate non-cancer risks from oral exposure to DINP for chronic durations of exposure in the risk evaluation of DINP. More specifically, liver toxicity in the key study ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)) was characterized by increased liver weight, increased serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and histopathological findings (*e.g.*, focal necrosis, spongiosis hepatis). EPA considers the observed liver effects to be adverse and relevant for extrapolating human risk from chronic exposures ([U.S. EPA, 2002a](#)). As discussed further in Sections 4.1.1 through 4.1.3, several additional studies of DINP provide similar, albeit less-sensitive, candidate PODs, which further support EPA's decision to use the selected POD of 3.5 mg/kg-day for chronic exposures. The Agency has performed $\frac{3}{4}$ body weight scaling to yield the HED and is applying the animal to human extrapolation factor (*i.e.*, interspecies extrapolation; UF_A) of $3\times$ and a within human variability extrapolation factor (*i.e.*, intraspecies extrapolation; UF_H) of $10\times$. Thus, a total UF of $30\times$ is applied for use as the benchmark MOE. Overall, based on the strengths, limitations, and uncertainties discussed in Section

4.2.2, EPA has robust overall confidence in the selected POD based on hepatic outcomes for use in characterizing risk from exposure to DINP for chronic exposure scenarios. For purposes of assessing non-cancer risks, the selected POD is considered most applicable to adults (16+ years). Use of this POD for characterization of risk to infants and children from chronic exposure to DINP may be conservative and may not be relevant (discussed further in Appendix I).

No data were available for the dermal or inhalation routes that were suitable for deriving route-specific PODs. Therefore, EPA used the acute/intermediate and chronic oral PODs to evaluate risks from dermal exposure to DINP. Differences in absorption are accounted for in dermal exposure estimates in the risk evaluation for DINP. 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 DINP in the risk evaluation of DINP are summarized below in Table ES-1 and Section 6.

This non-cancer human health hazard assessment for DINP was released for public comment and was peer-reviewed by the Science Advisory Committee on Chemicals (SACC) during the July 30 to August 1, 2024, SACC meeting ([U.S. EPA, 2024g](#)).

Table ES-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	Development	Rat	5 to 14 days throughout gestation	BMDL ₅ = 49 ^a NOAEL = 50 ^b	↓ fetal testicular testosterone, ↑ incidence of MNGs	12 ^c	63 [3.7]	UF _A = 3 UF _H = 10 Total UF = 30	(NASEM, 2017 ; Clewett et al., 2013a)
Chronic	Liver	Rat	2 years	NOAEL = 15	↑ liver weight, ↑ serum chemistry, histopathology ^{d,e}	3.5	19 [1.1]	UF _A = 3 UF _H = 10 Total UF = 30	(Lington et al., 1997 ; Bio/dynamics, 1986)

BMDL = benchmark dose lower limit; 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 The BMDL₅ was derived by NASEM (2017) through meta-regression and BMD modeling of fetal testicular testosterone data from two studies of DINP with rats ([Boberg et al., 2011](#); [Hannas et al., 2011](#)). R code supporting NASEM's meta-regression and BMD analysis of DINP is publicly available through [GitHub](#).

^b The NOAEL was derived from the gestational exposure study conducted by Clewett et al. (2013a), which supports a NOAEL of 50 mg/kg-day based decreased fetal testicular testosterone and increased incidence of multinucleated gonocytes (MNGs).

^c The BMDL₅ of 49 mg/kg-day and NOAEL of 50 mg/kg-day both support an HED of 12 mg/kg-day.

^d Liver toxicity included increased relative liver weight, increased serum chemistry (*i.e.*, AST, ALT, and ALP) and histopathologic findings (*e.g.*, focal necrosis, spongiosis hepatitis) in F344 rats following 2 years of dietary exposure to DINP ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)).

^e The Lington et al. study presents a portion of the data from a larger good laboratory practice (GLP)-certified study by Bio/dynamics (1986).

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 diisononyl phthalate (DINP) (CASRN 28553-12-0 and 68515-48-0) (Docket ID: [EPA-HQ-OPPT-2018-0436](#)). EPA determined that these two CASRN 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. The Agency 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 DINP. 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 DINP in August 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 DINP and conduct a dose-response assessment to determine the toxicity values to be used to estimate risks from DINP exposures. This technical support document for DINP summarizes the non-cancer hazards associated with exposure to DINP and identifies toxicity values to be used to estimate non-cancer risks from DINP exposures. EPA summarizes the cancer hazards associated with exposure to DINP in a separate TSD, the *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025a](#)).

Over the past several decades the human health effects of DINP 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); the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS); NASEM; and EPA. The Agency relied on information published in existing assessments by these regulatory and authoritative agencies as a starting point for its human health hazard assessment of DINP. Additionally, EPA considered new literature published since the most recent existing assessments of DINP to determine if 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 DINP literature is described in the *Systematic Review Protocol for Diisononyl Phthalate (DINP)* (also referred to as the DINP Systematic Review Protocol) ([U.S. EPA, 2025h](#)). EPA's approach and methodology for identifying and using human epidemiologic data and experimental laboratory animal data is described in Section 1.1.

1.1 Human Epidemiologic Data: Approach and Conclusions

To identify and integrate human epidemiologic data into the risk evaluation of DINP, EPA first reviewed existing assessments of DINP conducted by regulatory and authoritative agencies, as well as several systematic reviews of epidemiologic studies of DINP published by Radke and colleagues in the open literature. Although the authors (*i.e.*, Radke et al.) are affiliated with EPA's Center for Public Health and Environmental Assessment, the reviews do not reflect Agency policy. Existing assessments reviewed by EPA are listed below. As described further in Appendix A, most of these assessments have been subjected to peer review and/or public comment periods and have employed formal systematic review protocols.

- *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](#));
- *Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence* ([Radke et al., 2018](#));
- *Phthalate exposure and female reproductive and developmental outcomes: A systematic review of the human epidemiological evidence* ([Radke et al., 2019b](#));
- *Phthalate exposure and metabolic effects: A systematic review of the human epidemiological evidence* ([Radke et al., 2019a](#)); and
- *Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence* ([Radke et al., 2020a](#)).

Next, EPA sought to identify new population, exposure, comparator, and outcome (PECO)-relevant literature published since the most recent existing assessment(s) of DINP by applying a literature inclusion cutoff date. For DINP, the applied cutoff date was 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 those assessments provided the most robust and recent evaluation of human epidemiologic data for DINP. 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 DINP metabolites and health outcomes. New PECO-relevant literature published between 2018 to 2019 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 DINP Docket ([EPA-HQ-OPPT-2018-0436](#)), were evaluated for data quality and extracted 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 referred to as the "Draft Systematic Review Protocol") ([U.S. EPA, 2021a](#)). Data quality evaluations for new studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Epidemiology for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025d](#)).

As described further in the DINP Systematic Review Protocol ([U.S. EPA, 2025h](#)), EPA considers phthalate metabolite concentrations in urine to be an appropriate 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., 2020b](#)), 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, EPA has focused its epidemiologic evaluation on urinary biomonitoring data; new epidemiologic studies that examined DINP metabolites in matrices other than urine were considered supplemental and not evaluated for data quality.

The Agency is using epidemiologic studies of DINP qualitatively; this is consistent with Health Canada, U.S. CPSC, ECHA, EFSA, and Australia NICNAS. 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](#)). Conclusions from Health Canada ([2018a, b](#)) and systematic review articles by Radke and colleagues. ([Radke et al., 2020a](#); [Radke et al., 2019b](#); [Radke et al., 2019a](#); [Radke et al., 2018](#)) regarding the level of evidence for association between urinary DINP metabolites and each health outcome were reviewed by EPA and used as a starting point for its human health hazard assessment. The Agency also evaluated and summarized new epidemiologic studies identified by EPA's systematic review process to use qualitatively during evidence integration to inform hazard identification and the weight of scientific evidence.

1.2 Laboratory Animal Findings: Summary of Existing Assessments from Other Regulatory Organizations

The human health hazards of DINP 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 ([2013b](#)), EFSA ([2019, 2005](#)), and Australia NICNAS ([2012](#)). These assessments have consistently identified developmental, liver, and kidney toxicity as the most sensitive outcomes for use in estimating human risk from exposure to DINP. The PODs from these assessments are shown in Table 1-1.

U.S. CPSC ([2010](#)), Health Canada ([EC/HC, 2015](#)), ECB ([2003](#)), ECHA ([2013b](#)), and Australia NICNAS ([2012](#)) have consistently concluded that DINP is not acutely toxic via the oral ($LD_{50} > 10$ g/kg), dermal ($LD_{50} > 3$ g/kg), or inhalation ($LC_{50} > 4.4$ mg/L) routes of exposure. DINP only resulted in slight irritation in primary skin and eye irritation studies in rabbits. Dermal sensitization studies with rodent models (*e.g.*, Buehler tests) indicate that DINP is not a dermal sensitizer. EPA identified no new information that would change these conclusions; therefore, these hazards are not discussed further in this hazard assessment.

Table 1-1. Summary of DINP Non-cancer PODs Selected for Use by Other Regulatory Organizations

Brief Study Description	TSCA Data Quality ^f	NOAEL/ LOAEL (mg/kg-day)	Critical Effect	U.S. CPSC (2014)	ECCC/HC (2020)	EFSA (2019)	NICNAS (2012)	ECHA (2013b)
Male and female F344 rats (110/sex/dose) fed diets containing 0, 300, 3,000, 6,000 ppm DINP (CASRN 68515-48-0) for 2 years (equivalent to 15, 152, 307 mg/kg-day for males; 18, 184, 375 mg/kg-day for females) (GLP-compliant, non-guideline study) (Lington et al., 1997 ; Bio/dynamics, 1986)	High	15/ 152	↑ in absolute and relative liver and kidney weight with increase in histopathological changes (e.g., spongiosis hepatis) and other signs of hepatotoxicity	✓ ^a	✓ ^b	✓ ^c	✓ ^d	✓ ^e
Male and female F344 rats (70–85/sex/dose) administered 0, 500, 1,500, 6000, 12,000 ppm in the diet for 104 weeks (equivalent to 29, 88, 358, 733 mg/kg-day in males; 36, 108, 4422, 885 mg/kg-day in females) (GLP-compliant, adhered to 40 CFR part 798 (section 798.330)) (Covance Labs, 1998c)	High	88/ 358	↑ Liver and kidney weight, biochemical changes (↑ serum ALT, AST), and histopathological findings				✓ ^d	
Pregnant female SD rats (6/dose) gavaged with 0, 10, 100, 500, 1,000 mg/kg-day DINP on GD12–21. Dams were allowed to give birth naturally, and then dams and pups were sacrificed (non-guideline study) (Li et al., 2015)	Medium	10 (LOEL)/ 100 (LOAEL)	↑ MNGs and Leydig cell clusters/ aggregation		✓ ^b			
Hershberger assay: young (6-week old) castrated male SD rats treated with testosterone propionate (0.4 mg/kg-day) were gavaged with 0, 20, 100, 500 mg/kg-day DINP for 10 days and then sacrificed (non-guideline study) (Lee and Koo, 2007)	Medium	100/500	↓ absolute seminal vesicle and LABC weights		✓ ^b			
Pregnant SD rats (8/dose) gavaged with 0, 50, 250, 500 mg/kg-day DIINP on GD12–19 (non-guideline study) (Clewett et al., 2013a)	High	50/ 250	Transient reduced fetal testosterone level and histopathological changes (MNGs)	✓ ^a		✓ ^c	✓ ^d	✓ ^e
Pregnant Wistar rats (16/dose) gavaged with 0, 300, 600, 750, 900 mg/kg-day DINP from GD7 to PND17 (non-guideline study) (Boberg et al., 2011)	Medium	300/600	↑ Nipple retention	✓ ^a			✓ ^d	
Pregnant Harlan SD rats (5–9/group) gavaged with 0, 500, 750, 1,000, 1,500 mg/kg-day DINP from GD14 to 18 (non-guideline study) (Hannas et al., 2011)	Medium	–/500	↓ fetal testicular testosterone production	✓ ^a			✓ ^d	
	Medium	250/750	↓ male pup AGDon PND14	✓ ^a				

Brief Study Description	TSCA Data Quality ^f	NOAEL/LOAEL (mg/kg-day)	Critical Effect	U.S. CPSC (2014)	ECCC/HC (2020)	EFSA (2019)	NICNAS (2012)	ECHA (2013b)
Pregnant SD rats (20–24/group) fed diets containing 0, 760, 3800, 11,400 ppm DINP from GD12 to PND14 (target doses: 0, 50, 250, 750 mg/kg-day; received doses: 56, 288, 720, mg/kg-day on GD13–20) (non-guideline study) (Clewell et al., 2013b)		50/250	↓ male pup body weight on PND14				✓ ^e	
Male and female SD rats fed diets containing 0, 0.2, 0.4, 0.8% (Received doses in units of mg/kg-day shown in Table 3-7) DINP 10 weeks prior to mating, and throughout mating, gestation and lactation continuously for two generations (GLP-compliant, adhered to 40 CFR part 798 (section 798.4700)) (Waterman et al., 2000 ; Exxon Biomedical, 1996b)	High	–/114-395	↓ F1 and F2 pup body weight on PND7 and 21				✓ ^e	

CPSC = Consumer Product Safety Commission (U.S.); ECCC/HC = Environment and Climate Change Canada/Health Canada; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; NICNAS = Australia National Industrial Chemicals Notification and Assessment Scheme; ALT = Alanine aminotransferase; AGD= Anogenital distance; AST = Aspartate aminotransferase; LABC = Levator ani/bulbocavernosus; MNG = Multinucleated gonocytes; GD= Gestational day; PND = Postnatal day; GLP = Good Laboratory Practice

^a NOAELs from antiandrogenic endpoints (*i.e.*, nipple retention, fetal testosterone production, MNGs) across several studies ([Clewell et al., 2013a](#); [Clewell et al., 2013b](#); [Boberg et al., 2011](#); [Hannas et al., 2011](#)) were used by U.S. CPSC to assign a NOAEL for developmental toxicity of 50 mg/kg-day based on antiandrogenic endpoints (see p. 98 of ([U.S. CPSC, 2014](#))).

^b NOAELs from [Lington et al. \(1997\)](#) and [Li et al. \(2015\)](#) were used by Health Canada to calculate MOEs for individual DINP exposure scenarios (see Table 9-58 of ([ECCC/HC, 2020](#))). NOAELs from [Li et al.](#) and [Lee and Koo \(2007\)](#) were used to estimate hazard quotients for DINP as part of the cumulative risk assessment (see Tables F-5 through F-9 in ([ECCC/HC, 2020](#))).

^c NOAEL from [Lington et al. \(1997\)](#) was used by EFSA to derive a stand-alone tolerable daily intake (TDI) for DINP based on liver and kidney effects, while the NOAEL from [Clewell et al. \(2013a\)](#) was used to establish a group-TDI for several phthalates (*e.g.*, DEHP, DBP, BBP, and DINP) based on developmental effects related to a plausible common mechanism (*i.e.*, reduced fetal testosterone).

^d NICAS derived a NOAEL for systemic effects (liver and kidney toxicity) based on the results from two 2-year dietary studies of F344 rats ([Covance Labs, 1998c](#); [Lington et al., 1997](#)), which were similar in design and collectively supported a NOAEL of 88 mg/kg-day. Similarly, NICNAS derived a NOAEL of 50 mg/kg-day for fertility-related effects (*i.e.*, reduced fetal testosterone) based on results from three studies ([Clewell et al., 2013a](#); [Boberg et al., 2011](#); [Hannas et al., 2011](#)) and a NOAEL of 50 mg/kg-day for developmental effects (*i.e.*, reduced pup weight) based on results from two studies ([Clewell et al., 2013b](#); [Waterman et al., 2000](#)) (see Table 7.1 in ([NICNAS, 2012](#))).

^e NOAELs used by ECHA to calculate derived no effect levels (DNELs) (see Section 4.4.11.2 of ([ECHA, 2013b](#))).

^f Studies evaluated for data quality consistent with the DINP Systematic Review Protocol ([U.S. EPA, 2025h](#)) and EPA’s Draft Systematic Review Protocol ([U.S. EPA, 2021a](#)).

1.3 Laboratory Animal Data: Approach and Methodology

1.3.1 Approach to Identifying and Integrating 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 DINP. The Agency first reviewed existing assessments of DINP conducted by various regulatory and authoritative agencies. Existing assessments reviewed by EPA are listed below. The purpose of this review was to identify sensitive and human relevant hazard outcomes associated with exposure to DINP, and identify key studies used to establish PODs for estimating human risk. As described further in Appendix A, 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 Diisononyl Phthalate (DINP)* ([U.S. CPSC, 2010](#));
- *Chronic Hazard Advisory Panel on phthalates and phthalate alternatives* ([U.S. CPSC, 2014](#));
- *State of the science report: Phthalate substance grouping 1,2-Benzenedicarboxylic acid, diisononyl ester; 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich (Diisononyl Phthalate; DINP). Chemical Abstracts Service Registry Numbers: 28553-12-0 and 68515-48-0* ([EC/HC, 2015](#));
- *Supporting documentation: Carcinogenicity of phthalates - mode of action and human relevance* ([Health Canada, 2015](#));
- *Screening assessment - Phthalate substance grouping* ([ECCC/HC, 2020](#));
- *NTP-CERHR monograph on the potential human reproductive and developmental effects of diisononyl phthalate (DINP)* ([NTP-CERHR, 2003](#));
- *European union risk assessment report: DINP* ([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](#));
- *Committee for Risk Assessment (RAC) Opinion proposing harmonised classification and labelling at EU level of 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkylesters, C9- rich; [1] di-"isononyl" phthalate; [2] [DINP]* ([ECHA, 2018](#));
- *Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to di-isononylphthalate (DINP) 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](#));
- *Priority existing chemical assessment report no. 35: Diisononyl phthalate* ([NICNAS, 2012](#));
- *Application of Systematic Review Methods in an Overall Strategy for Evaluating Low-Dose Toxicity from Endocrine Active Chemicals* ([NASEM, 2017](#));
- *Revised technical review of diisononyl phthalate* ([U.S. EPA, 2005b](#)); and
- *Technical review of diisononyl phthalate (Final assessment)* ([U.S. EPA, 2023c](#)).

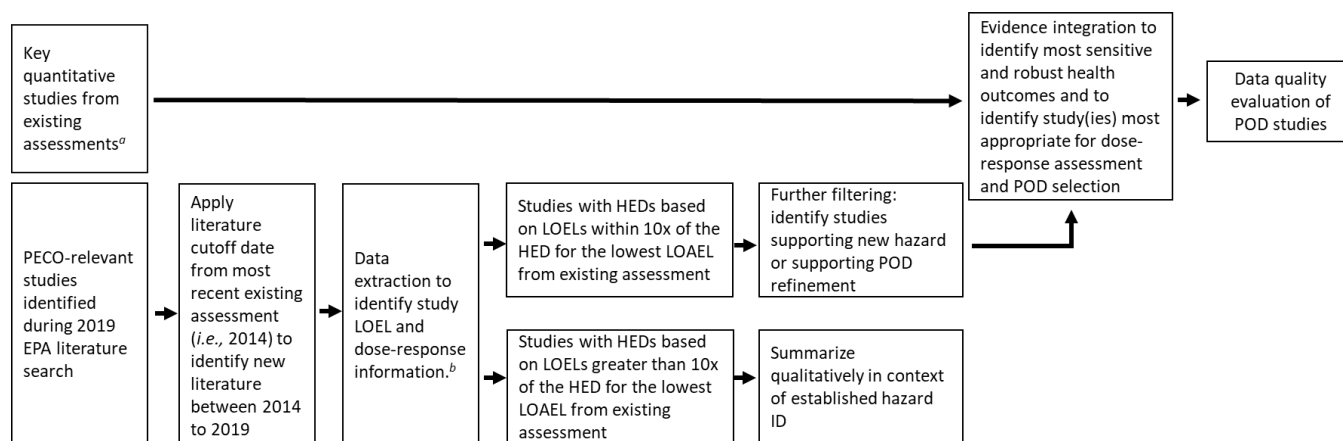


Figure 1-1. Overview of DINP Human Health Hazard Assessment Approach

^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 LOEL, and PESS categories.

EPA used the 2015 Health Canada assessment ([EC/HC, 2015](#)) as the key starting point for this document. The Health Canada assessment included scientific literature up to August 2014 and considered a range of human health hazards (*e.g.*, developmental and reproductive toxicity, systemic toxicity to major organ systems, genotoxicity, carcinogenicity) across all durations (*i.e.*, acute, intermediate, subchronic, chronic) and routes of exposure (*i.e.*, oral, dermal, inhalation). The EFSA ([2019](#)) 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 and the EPA ([2023c](#)) assessment did not describe its approach to identifying literature. Therefore, the Agency considered literature published between 2014 to 2019 further as shown in Figure 1-1. EPA first screened titles and abstracts and then full texts for relevancy using PECO screening criteria described in the DINP Systematic Review Protocol ([U.S. EPA, 2025h](#)). EPA then identified PECO-relevant literature published since the most recent and comprehensive existing assessment of DINP by applying a literature inclusion cutoff date from this assessment.

Next, EPA reviewed new studies published between 2014 and 2019 and extracted key study information as described in the DINP Systematic Review Protocol ([U.S. EPA, 2025h](#)). 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 lowest-observed-effect level (LOEL) (Figure 1-1).

New information for DINP was primarily limited to oral exposure studies, and study LOELs were converted to HEDs by scaling allometrically across species using the $\frac{3}{4}$ power of body weight ($BW^{3/4}$) for oral data, which is the approach recommended by U.S. EPA when physiologically based pharmacokinetic 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 F. 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 lowest-observed-adverse-effect level (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 human health hazard not identified in previous assessments or to determine if they contain sufficient dose-response information to support a potentially lower POD than identified in existing assessments of DINP.

2024 TSCA Literature Search Update

Following release of the draft human health hazard assessments of DINP in May 2024, EPA updated its literature searches for DINP. The DINP Systematic Review Protocol ([U.S. EPA, 2025h](#)) provides details regarding the updated DINP literature search. Ten new PECO-relevant animal toxicology studies were identified for DINP that met PECO screening criteria and were evaluated for data quality as described in the DINP Systematic Review Protocol ([U.S. EPA, 2025h](#)).

Data quality evaluations for DINP animal toxicity studies reviewed by EPA are provided in the *Data Quality Evaluation Information for Human Health Hazard Animal Toxicology for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025c](#)).

1.3.2 New Literature Identified and Hazards of Focus for DINP

As described in Section 1.3.1, 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. As described further in the DINP Systematic Review Protocol ([U.S. EPA, 2025h](#)), EPA identified 23 new PECO-relevant studies that provided information pertaining to 8 primary hazard outcomes, including reproduction/development, neurological, cardiovascular, immune system, musculoskeletal system, and gastrointestinal system. Further details regarding EPA's handling of this new information are provided below.

- **Reproductive/Developmental.** EPA identified 11 new studies evaluating reproductive/developmental outcome ([Santacruz-Márquez et al., 2024](#); [Bhurke et al., 2023](#); [Laws et al., 2023](#); [Chen et al., 2022](#); [Chiang et al., 2020a, b](#); [Chiang and Flaws, 2019](#); [Neier et al., 2019](#); [Neier et al., 2018](#); [Li et al., 2015](#); [Sedha et al., 2015](#)). These new studies of DINP are discussed further in Section 3.1.
- **Liver.** EPA identified one new study evaluating liver toxicity ([Liang and Yan, 2020](#)). This new study of DINP is discussed further in Section 3.2 and Appendix B.
- **Kidney.** EPA identified two new studies evaluating kidney toxicity ([Gu et al., 2021](#); [Liang and Yan, 2020](#)). These new studies of DINP are discussed further in Section 3.3.
- **Neurotoxicity.** EPA identified four new studies evaluating neurological outcomes, including two that evaluate neurobehavioral outcomes ([Ma et al., 2015](#); [Peng, 2015](#)) and two that evaluate brain weight ([Neier et al., 2018](#); [Setti Ahmed et al., 2018](#)). Neurotoxicity is a new health outcome that has not been seen in previous studies of DINP or been the focus of existing assessments of DINP. The neurologic effects of DINP are discussed further in Section 3.4.
- **Cardiovascular.** EPA identified one new study evaluating cardiovascular outcomes ([Deng et al., 2019](#)). Results from Deng et al. provide evidence of a new health hazard associated with exposure to DINP that has not been previously seen in studies of DINP. The cardiovascular effects of DINP are discussed further in Section 3.5.
- **Immune System.** EPA identified three new studies evaluating immune system effects ([Kang et al., 2016](#); [Wu et al., 2015](#); [Sadakane et al., 2014](#)). Results from these studies indicate that DINP

can have adjuvant-like effects on immune responses. The immune adjuvant effects of DINP are discussed further in Section 3.6.

- ***Musculoskeletal.*** EPA identified one new study evaluating effects on the musculoskeletal system ([Hwang et al., 2017](#)). Results from Hwang et al. provide evidence of a new health hazard associated with exposure to DINP that has not been previously seen in studies of DINP. Musculoskeletal effects of DINP are discussed further in Section 3.7.
- ***Gastrointestinal.*** EPA identified three new studies evaluating effects on the gastrointestinal system ([Chiu et al., 2021](#); [Chiu et al., 2020](#); [Setti Ahmed et al., 2018](#)). Gastrointestinal system effects of DINP are discussed in Section 3.8.

Based on information provided in existing assessments of DINP for liver, kidney, and developmental effects in combination with 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); liver toxicity (Section 3.2); kidney toxicity (Section 3.3); neurotoxicity (Section 3.4); cardiovascular health effects (Section 3.5); immune system toxicity (Section 3.6); musculoskeletal toxicity (Section 3.7); and gastrointestinal toxicity (Section 3.8).

Genotoxicity and carcinogenicity data for DINP are summarized in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025a](#)).

2 TOXICOKINETICS

2.1 Oral Route

Three experimental animal studies are available that provide useful data in evaluating absorption, distribution, metabolism, and excretion (ADME) of DINP for the oral route. DINP is shown to be predominantly metabolized in the liver in rodents, and urinary excretion is the primary route of elimination for metabolites. In one of the few studies designed to investigate the metabolism of phthalates in humans, a male volunteer (aged 63) was given a single oral dose of 1.27 mg of deuterium-labeled DINP/kg bodyweight. DINP was found to be rapidly eliminated in a manner similar to rats ([Koch and Angerer, 2007](#)). The postulated metabolic pathway of DINP in humans is shown in Figure 2-1. Results indicated that approximately 44 percent of the administered dose was recovered in urine over 48 hours in the form of the following metabolites: (1) 20.2 percent as OH-MINP (MHINP; based on measured standard of 7OH-MMeOP); (2) 10.7 percent as carboxy-MINP (MCiOP; based on measured standard of 7-carboxy-MMeHP); (3) 10.6 percent as oxo-MINP (MOINP; based on measured standard of 7oxo-MMeOP); and (4) 2.2 percent as MINP ([Koch and Angerer, 2007](#)).

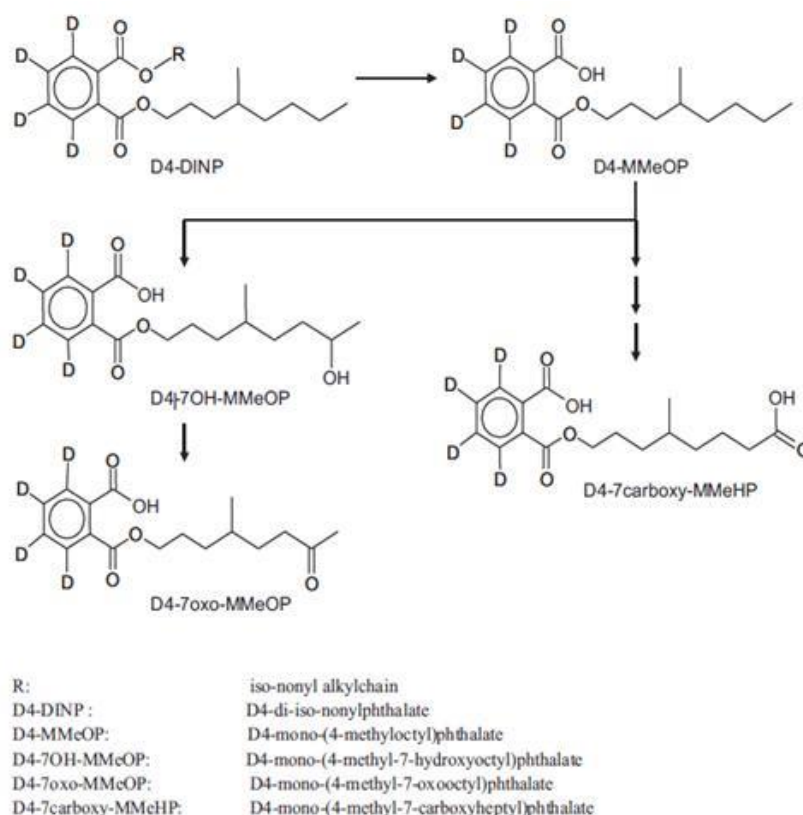


Figure 2-1. Postulated DINP Metabolism in Humans ([Koch and Angerer, 2007](#))

In Anderson et al., 20 volunteers were given two doses of DINP (0.78 mg and 7.3 mg) to examine its metabolism and excretion. More than 33 percent of the labelled DINP was found as metabolites in urine after 48 hours ([Anderson et al., 2011](#)).

Several studies investigated the toxicokinetics of DINP in animals. McKee et al. ([2002](#)) examined the ADME of DINP in male and female F344 rats. Rats were administered single oral doses of 50 or 500 mg/kg [^{14}C]DINP, and data on tissue distribution indicated that 2 to 4 hours following administration,

the highest levels of radioactivity were found to be in the blood, liver, and kidneys. The distribution of radiolabeled DINP to other tissues after 7 days of exposure, was gastrointestinal (GI) tract (0.097%), fat (0.053%), muscle (0.024%), and other organs ($\leq 0.009\%$). No differences in excretion were apparent in either sex at either dose. In the single dose studies, 50 percent of the radioactivity was recovered in the urine and the remainder in the feces at the low dose; whereas at the high dose, 35 to 40 percent of the radioactivity was excreted in the urine and the remainder in the feces, suggesting an inverse relationship between dose level and absorption. In repeated dose studies, rats were administered 50, 150, and 500 mg/kg-day [^{14}C]DINP for 5 days, and excretion was evaluated ([McKee et al., 2002](#)). In the repeated dose studies, about 60 percent of the administered dose was excreted at all doses, suggesting an elevation of esterase activity and more rapid conversion to monoester following repeated treatment (Table 2-1). The elimination (half-life) of absorbed [^{14}C]DINP was about 7 hours.

In another study by Clewell et al. ([2013a](#)), pregnant Sprague-Dawley (SD) rats received 50, 250, and 750 mg/kg-day of DINP from gestation day (GD) 12 to 19 via oral gavage. The percentage of DINP absorbed following oral exposure was lower at the higher doses of 750 mg/kg-day compared to the 250 mg/kg-day group. Additionally, Clewell et al. ([2013a](#)) characterized the metabolite disposition of DINP in the fetus and demonstrated that MINP and its oxidative metabolites along with its glucuronidated form (MINP-Gluc) were all present in the fetal plasma, testes, and amniotic fluid. MINP-Gluc was present at higher concentrations in the fetal plasma than the maternal plasma (in contradiction with what was observed with the other metabolites), indicating potential placental transfer of MINP-Gluc, or, more likely, that conjugation could occur in the fetus by phase II detoxification enzyme systems. Because these metabolites were localized in maternal plasma and MINP was present at similar concentrations as MCiOP, it was suggested that (1) urinary clearance of both MINP and MINP-Gluc is limited, and (2) these metabolites were poor predictors of plasma and tissue disposition for DINP.

A summary of different metabolites found in human and rat urine after oral administration of DINP is presented in Table 2-2.

Table 2-1. Absorption and Excretion Summary of DINP

Species	Dose	Source	Absorption	Reference
Human	1.28 mg/kg	Urine	44% over 48 hours	(Koch and Angerer, 2007)
Human	0.78 and 7.3 mg/kg	Urine	33 \pm 6.4% over 48 hours	(Anderson et al., 2011)
Rat	50 mg/kg 500 mg/kg 50–500 mg/kg	Urine Urine Estimated urine + bile	49% over 72 hours 39% over 72 hours 75% over 72 hours	(McKee et al., 2002)
	50, 150, or 500 mg/kg-day for 5 days	Urine Estimated urine + bile	56–62% over 24 hours, 62–64% over 72 hours 90% over 72 hours	
Rat (non-pregnant)	Single dose of 300 mg/kg	Urine	Mono(carboxy-isooctyl)phthalate (MciOP) 82% Other metabolites 18%	(Silva et al., 2006)

Silva et al. (2006) administered a single oral gavage dose of 300 mg/kg DINP to non-pregnant SD rats and quantified the metabolites in urine daily for 4 days. MciOP accounted for 82 percent of the identified metabolites, and the other metabolites constituted 18 percent. This study characterized the different ω - and ω -1-oxidation metabolites found in urine and found that MciOP was the major urinary metabolite recovered, while MINP and DINP were not found in significant amounts in the urine.

Based on the available data, EPA assumes an oral absorption of 100 percent for the risk evaluation of DINP.

Table 2-2. Metabolites of DINP Identified in Urine from Rats and Humans after Oral Administration

Metabolite(s)	Abbreviation(s)	Reference(s) (Species)
Monoisobutyl phthalate	MINP	(Anderson et al., 2011) (human) (Suzuki et al., 2012) (human) (Koch and Angerer, 2007) (human) (Calafat et al., 2006a) (rat)
Glucuronidated MINP	MINP-Gluc	(Clewell et al., 2013a) (rat)
[mono-(4-methyl-7-carboxyheptyl) phthalate] representing: Mono(carboxyisooctyl) phthalate	[D4-7carboxy-MmeHP] CO2-MINP; MCIOP	(Anderson et al., 2011) (human) (Koch and Angerer, 2007) (human)
[D4-mono-(4-methyl-7-hydroxyoctyl) phthalate] representing: Mono(hydroxyisononyl) phthalate	[7OH-MmeOP] for OH-MINP; MHINP	(Anderson et al., 2011) (human) (Koch et al., 2012) (human) (Koch and Angerer, 2007) (human) (Silva et al., 2006) (rat)
[D4-mono-(4-methyl-7-oxooctyl)phthalate] representing: Mono(oxoisononyl) phthalate	[7oxo-MmeOP] for Oxo-MINP; MOINP	(Anderson et al., 2011) (human) (Koch et al., 2012) (human) (Koch and Angerer, 2007) (human) (Silva et al., 2006) (rat)
Monocarboxylisononyl phthalate	cx-MINP	(Koch et al., 2012) (human)
Mono-carboxy-isooctyl phthalate	MCIOP (MCOP is sometimes used to represent MCIOP)	(Silva et al., 2006) (rat)
Mono(carboxy-isoheptyl) phthalate	MciHpP	(Silva et al., 2006) (rat)
Mono-(3-carboxypropyl) phthalate	MCPP	(Calafat et al., 2006b; Calafat et al., 2006a) (rat)
Mono-n-octyl phthalate	MnOP	(Calafat et al., 2006b) (rat)
Phthalic acid	PA	(McKee et al., 2002) (rat)

2.2 Inhalation Route

No controlled human exposure studies or *in vivo* animal studies are available that evaluate the ADME properties of DINP for the inhalation route. Therefore, EPA is assuming 100 percent absorption via inhalation. Similarly, ECHA concluded 75 percent absorption via inhalation for adults and 100 percent for newborns and infants as a vulnerable subpopulation (ECHA, 2013b; ECB, 2003).

2.3 Dermal Route

In vivo and *in vitro* studies have shown that absorption of phthalates through rat and human skin decreases as the length of the alkyl chain increases ([Mint et al., 1994](#); [Elsisi et al., 1989](#); [Scott et al., 1987](#)). Dermal absorption data specific to DINP are limited. EPA only identified one study directly related to the dermal absorption of DINP ([McKee et al., 2002](#); [Midwest Research Institute, 1983](#)). In this study, neat [¹⁴C]DINP at 50 mg/kg-day was applied to the freshly shaven backs (3 cm × 4 cm) of three groups of male F344 rats as “conditioned skin,” “non-conditioned skin,” and “occluded” (styrofoam cup lined with aluminum foil) ([McKee et al., 2002](#); [Midwest Research Institute, 1983](#)). Dermal absorption was estimated to be 2 to 4 percent over 7 days, with an absorption rate of approximately 0.3 to 0.6 percent per day based on amount of applied dose recovered in urine, feces, and other tissues. Additionally, radioactivity increased with time on skin: 0.12, 0.26, and 0.27 percent of the applied dose following exposure of 1, 3, and 7 days, respectively. For all dermal absorption experiments with DINP, material recovery fell within the Organisation for Economic Co-operation and Development (OECD) 156 (2022) Guidelines of 90 to 110 percent for non-volatile chemicals. The metabolic profile of dermally absorbed DINP was similar to DINP metabolic profile from oral administration.

Although specific data on DINP dermal absorption in humans is lacking, several regulatory agencies (*e.g.*, Danish EPA, ECHA, NICNAS) recognize that absorption of phthalates would likely be lower in human skin than through rat skin. This observation is based on data from *in vitro* migration studies conducted with DEHP and other phthalates. Notably, other regulatory agencies (*e.g.*, Australia NICNAS, ECHA) have reached similar conclusions regarding the low dermal absorption of DINP ([ECHA, 2013b](#); [NICNAS, 2012](#)).

As described further in the *Environmental Release and Occupational Exposure Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025f](#)) and the *Consumer and Indoor Exposure Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025b](#)), for the risk evaluation of DINP, EPA used data from the *in vivo* dermal absorption study of DINP with rats ([McKee et al., 2002](#); [Midwest Research Institute, 1983](#)) to estimate dermal absorptive flux, which is used to calculate occupational and consumer dermal exposure estimates.

2.4 Summary

Toxicokinetic data indicates that orally administered DINP is rapidly metabolized in the gut to MINP and distributed via blood to major tissues, particularly the liver and kidneys. DINP metabolites were excreted in urine and to a lesser extent in feces. Repeated dosing did not result in accumulation of DINP and/or its metabolites in blood and tissues but did result in increased formation and elimination of the monoester oxidation products.

Tissue distribution patterns of DINP revealed that absorption from the GI tract was rapid after both single and repeated oral dosing. DINP is then primarily hydrolyzed in the GI tract after oral administration. DINP translocated from the GI tract via the blood rapidly to liver and kidney. The metabolic profile suggests that DINP is recovered primarily as oxidized products and phthalic acid and very little as the parent or the metabolite MINP, suggesting that DINP is rapidly metabolized in the GI tract to the corresponding monoester with a second hydrolysis step in liver to phthalic acid.

DINP is primarily eliminated in urine following oral exposures. Available studies have reported that more than 90 percent of [¹⁴C] DINP was eliminated over 72 hours, with the majority through urine and to a minor extent through feces ([Anderson et al., 2011](#); [Koch and Angerer, 2007](#); [Silva et al., 2006](#); [McKee et al., 2002](#)). The total radioactivity recovered from the previously identified metabolites

combined was 33 ± 6.4 percent of the labeled DINP in urine over 48 hours. Metabolite half-lives were estimated to be 4 to 8 hours with over 90 percent excreted in the first 24 hours of urine collection.

In contrast to absorption following oral exposure, dermal absorption of DINP in adult male F344 rats is low, ranging from 2 to 4 percent of the applied dose when measured 7 days after application ([McKee et al., 2002](#)). This finding agrees with data from other *in vivo* and *in vitro* studies that show absorption of phthalates through rat and human skin decreases as the length of the alkyl chain increases. The dermally absorbed fraction is distributed to multiple tissues, including skin, GI tract, muscle, fat, and liver. The recovery of radioactivity in feces and the GI tract suggests excretion of DINP or its metabolites in the bile, which in turn suggests that after dermal absorption, DINP undergoes a similar metabolic fate as orally administered DINP.

3 HAZARD IDENTIFICATION

EPA has developed detailed hazard characterization and mode of action (MOA) analysis for the effects on fetal testicular testosterone and liver cancer, with an emphasis on liver effects leading to liver tumors. Effects on fetal testicular testosterone are presented in Section 3.1.2.1. Non-cancer liver effects are presented in Section 3.2, while liver cancer and EPA's MOA analysis of liver tumors is presented in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025a](#)). The scientific MOA analysis is presented in accordance with EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)) and the *IPCS Mode of Action Framework* ([IPCS, 2007](#)) and includes a description of the state of the science with regards to key events, pathways of toxicity and weight of evidence following the modified Bradford Hill criteria. Other hazards considered by EPA—such as kidney, neurotoxicity, cardiovascular health effects, immune system toxicity, musculoskeletal toxicity, and gastrointestinal toxicity—are presented in Sections 3.3 through 3.8.

3.1 Developmental and Reproductive Toxicity

3.1.1 Summary of Available Epidemiological Studies

EPA reviewed and summarized conclusions from previous assessment conducted by Health Canada ([2018b](#)) and systematic review articles by Radke et al. ([2019b](#); [2018](#)) that investigated the association between DINP exposure and male and female development and reproductive outcomes. In the Health Canada ([2018b](#)) assessment, there were no studies that evaluated the association between DINP and its metabolites and reproductive outcomes such as altered male puberty, pregnancy complication and loss, uterine leiomyoma, sexual dysfunction in females, and age at menopause. There was inadequate evidence for the association between DINP and its metabolites and reproductive outcomes such as altered female puberty, changes in semen parameters, sexual dysfunction in males, polycystic ovary syndromes, and sex ratios. There was also no evidence for the association between DINP and its metabolites and reproductive outcomes such as gynecomastia, endometriosis and adenomyosis. Overall, Health Canada found that the evidence could not be established for the association between DINP and its metabolites and any reproductive outcomes, such as altered fertility.

In the conclusions from the systematic review articles by Radke et al. ([2018](#)), examining the association between DINP male reproductive outcomes the authors found moderate evidence linking DINP metabolites to lower testosterone levels. However, they could not find clear evidence linking DINP and male reproductive outcomes such as AGD, time until pregnancy in males, and sperm parameters due to a combination of low exposure levels (*i.e.*, poor sensitivity) and data availability (*i.e.*, fewer accessible studies). In terms of the association between female reproductive and developmental outcomes and DINP, Radke et al. ([2019b](#)) found that the evidence was indeterminate.

EPA identified 11 new epidemiological studies published between 2018 and 2019 that were not evaluated by Health Canada or Radke et al. ([2019b](#); [2018](#)). Eight of the available studies were of medium quality and three were of low quality. Overall, conclusions of the 11 new studies were consistent with that of Health Canada and the systematic review articles by Radke et al. EPA concluded that the existing epidemiological studies do not support quantitative dose-response assessment, but rather provide qualitative support as part of weight of scientific evidence. Further information on the 11 new studies identified by EPA can be found in Appendix D.

3.1.2 Summary of Laboratory Animals Studies

The developmental effects of exposure to DINP in experimental animal models have been evaluated as part of several existing assessments. NTP-CERHR ([2003](#)), ECHA ([2013b](#)), EFSA ([2019](#)), Australia

NICNAS ([2012](#)), Health Canada ([EC/HC, 2015](#)) and U.S. CPSC ([2014, 2010](#)) have all consistently concluded that oral exposure to DINP can cause developmental toxicity in experimental animal models. Oral exposure to DINP has been shown to cause skeletal and visceral variations, reduced pup body weight gain, and effects on the developing male reproductive system consistent with a disruption of androgen action. Effects on the developing male reproductive system and other developmental and reproductive toxicity are discussed in Sections 3.1.2.1 and 3.1.2.2, respectively.

3.1.2.1 Developing Male Reproductive System

EPA has previously considered the weight of scientific evidence and concluded that oral exposure to DINP can induce effects on the developing male reproductive system consistent with a disruption of androgen action (see EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* (also called "Draft Proposed Approach for CRA for Phthalates") ([U.S. EPA, 2023a](#))). Notably, EPA's conclusion was supported by the Science Advisory Committee on Chemicals (SACC) ([U.S. EPA, 2023b](#)). A summary of available studies evaluating effects on the developing male reproductive system are provided in Section 3.1.2.1.1, while a brief MOA summary is provided in 3.1.2.1.2. Readers are directed to see EPA's Draft Proposed Approach for CRA for Phthalates ([U.S. EPA, 2023a](#)) for a more thorough discussion of DINP's effects on the developing male reproductive system and EPA's MOA analysis. Effects on the developing male reproductive system are considered further for dose-response assessment in Section 4.

3.1.2.1.1 Summary of Studies Evaluating Effects on the Developing Male Reproductive System

Available studies (including 16 studies of rats) evaluating the antiandrogenic effects of DINP on the male reproductive system are summarized below in Table 3-1.

Table 3-1. Summary of DINP Studies Evaluating Effects on the Developing Male Reproductive System

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Pregnant SD rats (8/dose/ timepoint evaluated) gavaged with 0 (corn oil vehicle), 50, 250, 750 mg/kg-day DINP (CASRN 68515-48-0) on GD12–19. Dams sacrificed on GD19 (2 hours post-dosing) or GD20 (24 hours post-dosing) (Clewett et al., 2013a)	50/ 250	↓ fetal testicular testosterone and testicular pathology (MNGs)	<u>Maternal Effects</u> - ↑ (12%) absolute and relative maternal liver weight (≥250 mg/kg-day) <u>Developmental Effects</u> - ↓ (50–65%) testicular testosterone on GD19 (≥250 mg/kg-day) - Testicular pathology on GD20 (↑ MNGs [≥250 mg/kg-day], Leydig cell aggregates [750 mg/kg-day]) <u>Unaffected outcomes</u> - Maternal body weight gain; terminal maternal body weight; fetal body weight; male AGD(GD20); testicular testosterone on GD20; seminiferous tubule diameter on GD20
Pregnant SD rats (20–24 litters/dose) fed diets containing 0, 760, 3,800, or 11,400 ppm DINP (CASRN 68515-48-0) on GD12 through PND14 (equivalent to: 56, 288, 720 mg/kg-day on GD13–20 and 109, 555, 1,513 mg/kg-day on PND2–14). Dams allowed to deliver pups naturally, and pups sacrificed on PND49 or 50 (Clewett et al., 2013b)	56/ 288	↓ male pup body weight on PND14 and ↑ incidence of MNGs on PND2	<u>Maternal Effects</u> - ↓ body weight on GD20, PND2 and 14 (11,400 ppm) - ↓ (30%) body weight gain on GD10–20 (11,400 ppm) - ↓ food consumption on GD10–20 (11,400 ppm) and PND2–14 (≥3,800 ppm) <u>Developmental Effects</u> - ↓ (10–27%) male pup weight on PND2 (720 mg/kg-day) and 14 (≥288 mg/kg-day) - Testicular pathology on PND2 (↑ Leydig cell aggregates (720 mg/kg-day), MNGs (≥288 mg/kg-day)) - ↓ AGDon PND14 (720 mg/kg-day) - ↓ (10%) absolute LABC weight on PND49–50 (720 mg/kg-day) <u>Unaffected outcomes</u> - Live pups/litter; testicular testosterone (PND49); PPS; AGD(PND2, 49); NR (PND14, 49); absolute testis and epididymis weight (PND2, 49); gubernacular cord length (PND49); male offspring body weight (PND49); absolute testes, epididymis, SV, ventral prostate, glans penis, Cowper's Glands weight (PND49); reproductive tract malformations (PND49) (<i>e.g.</i> , hypospadias, exposed os penis, undescended testes, epididymal agenesis); testicular pathology (PND49)
Pregnant Wistar rats (# of litters per dose not stated) fed soy-free diets containing 0, 40, 400, 4,000, or 20,000 ppm DINP (CASRN 28553-	None/ 2	↓ male pup AGD, ↓ pup body weight, ↓	<u>Maternal Effects</u> - Not examined or reported <u>Developmental Effects</u> - ↓ male/female body weight on PND1 (≥2 mg/kg-day)

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
12-0) from GD15 through PND21 and allowed to deliver pups naturally [received doses, as estimated by (EC/HC, 2015): 2, 20, 200, 1,000 mg/kg-day] (Lee et al., 2006a)		female lordosis quotient	<ul style="list-style-type: none"> - ↓ male AGDon PND1 (≥ 2 mg/kg-day) - ↓ frequency of mounts, intromissions, ejaculations in male rats (PNW 20) (only at 2 mg/kg-day, no dose-response) - ↓ Lordosis quotient of females in PNW 20 (≥ 2 mg/kg-day) <u>Unaffected outcomes</u> <ul style="list-style-type: none"> - Serum testosterone and estradiol (PND7); serum testosterone, luteinizing hormone, follicle stimulating hormone, estradiol (PNW 20)
Pregnant SD rats (5/dose) fed soy-free diets containing 0, 400, 4,000, 20,000 ppm DINP (CASRN 28553-12-0) on GD15 through PND10 (equivalent to: 31, 307, 1,165 mg/kg-day on GD15–20 and 66, 657, 2,657 mg/kg-day on PND2–10) (Masutomi et al., 2003)	66/ 657	↓ male body weight on PND27	<u>Maternal Effects</u> <ul style="list-style-type: none"> - ↓ body weight gain and food consumption between GD15–20 & PND2–10 (20,000 ppm) <u>Developmental Effects</u> <ul style="list-style-type: none"> - ↓ body weight gain between PND2-10 (both sexes) (20,000 ppm) - ↓ (18–43%) body weight on PND27 for males ($\geq 4,000$ ppm) and females (20,000 ppm) - ↓ Absolute testes weight on PND27 (20,000 ppm) - Testicular pathology on PND77 (20,000 ppm) (<i>i.e.</i>, vacuolar degeneration of Sertoli cells, degeneration of meiotic spermatocytes at stage XIV, scattered cell debris in ducts of epididymis) <u>Unaffected outcomes</u> <ul style="list-style-type: none"> - Number of live offspring; pup body weight (PND2); AGD(PND2); pup body weight gain (PND10–21); PPS; vaginal opening; absolute testes weight (PND77)
Pregnant Wistar rats gavaged with 0 (corn oil vehicle), 300, 600, 750, 900 mg/kg-day DINP (CASRN 28553-12-0) on GD7 through PND17. Dams sacrificed on GD21 (subgroup 1) or allowed to give birth naturally and offspring sacrificed on PND90 (subgroup 2) (Boberg et al., 2016, 2011)	300/ 600	↑ MNGs in fetal testis and ↓ sperm motility on PND90	<u>Maternal Effects</u> <ul style="list-style-type: none"> - None <u>Developmental Effects</u> <ul style="list-style-type: none"> - Testis pathology on GD21 (↑ incidence of MNGs (≥ 600 mg/kg-day); enlarged diameter of seminiferous cords (≥ 750 mg/kg-day); gonocytes with central location in chords (≥ 750 mg/kg-day)) - ↓ Testicular testosterone on GD21 (600 mg/kg-day, no dose-response) - ↓ male pup body weight on PND13 (900 mg/kg-day) - ↓ male pup AGDon PND1 (900 mg/kg-day) and ↑ male pup NR on PND13 (≥ 750 mg/kg-day) - ↓ sperm motility on PND90 (≥ 600 mg/kg-day) <u>Unaffected Outcomes</u>

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			- Maternal body weight and weight gain; gestation length, post-implantation loss, litter size, sex ratio, perinatal loss; testicular testosterone production (GD21); plasma testosterone and luteinizing hormone (GD21); fetal birth weight; male and female body weight (PND90); absolute reproductive organ weight (PND90) (<i>e.g.</i> , testis, prostate LABC, SV, ovary, uterus); AGDor NR (PND90); testis testosterone (PND90); SV, prostate, testis pathology
Pregnant Harlan SD rats (5–9/dose) gavaged with 0, 500, 750, 1,000, or 1,500 mg/kg-day DINP (CASRNs 28553-12-0 and 68515-48-0 tested) on GD14–18. Dams sacrificed on GD18, approximately 2 hours post-dosing (Hannas et al., 2011)	None/ 500	↓ fetal testicular testosterone production	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ (30–69%) <i>ex vivo</i> fetal testicular testosterone production (≥500 mg/kg-day, both CASRNs) - ↓ expression of <i>StAR</i> and <i>Cyp11a</i> mRNA in fetal testes (≥1,000 mg/kg-day, both CASRNs) <u>Unaffected Outcomes</u> - Dam mortality; dam body weight gain; litter size
Pregnant Harlan SD rats gavaged with 0, 500, 750, 1,000, or 1,500 mg/kg-day DINP (CASRNs 28553-12-0 and 68515-48-0 tested) on GD14–18. Dams sacrificed on GD18, approximately 2 hours post-dosing (Hannas et al., 2012)	NOEL/ LOEL: None/ 500	↓ steroidogenic gene expression in the fetal testes	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ mRNA expression of <i>StAR</i> , <i>Cyp11a</i> , <i>Cyp11b1</i> , <i>Cyp11b2</i> , <i>Hsd3b</i> , <i>Cyp17a1</i> , <i>Scarb1</i> , <i>Insl3</i> , <i>Dhcr7</i> in the fetal testes (≥500 mg/kg-day, both CASRNs) <u>Unaffected Outcomes</u> - Dam mortality; dam body weight gain; litter size
Pregnant SD rats (5–8/dose) gavaged with 0 (corn oil vehicle), 250, or 750 mg/kg-day DINP (CASRN not reported) on embryonic days 13.5–17.5. Dams sacrificed on embryonic day 19.5 (Adamsson et al., 2009)	NOEL/ LOEL: 250/ 750	↑ <i>GATA-4</i> , <i>Insl3</i> , <i>P450scc</i> mRNA in the fetal testes	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↑ Testicular mRNA expression of <i>GATA-4</i> , <i>Insl3</i> , <i>P450scc</i> (750 mg/kg-day) <u>Unaffected Outcomes</u> - Plasma corticosterone; litter size; sex ratio; fetal body weight; testicular testosterone; testicular mRNA expression of <i>Star</i> , <i>3β-HSD</i> , <i>SF-1</i> ; testicular protein expression of <i>StAR</i> , <i>P450scc</i> , <i>3β-HSD</i> , androgen receptor; testicular pathology

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Pregnant SD rats (14–19/dose) gavaged with 0 (corn oil vehicle) or 750 mg/kg-day DINP (CASRN 68515-48-0) from GD14 through PND3. Dams were allowed to give birth naturally and mall offspring were sacrificed between 3–7 months of age (Gray et al., 2000)	None/ 750	↑ male pup NR, reproductive malformations	<u>Maternal Effects</u> - ↓ (10%) maternal weight gain to GD21 <u>Developmental Effects</u> - ↑ percent of males with areolas (22.4%) on PND13 - Reproductive malformations at 3–7 months: permanent nipples in 2/52 males from 2 litters, small and atrophic testes in 1/52 males; flaccid, fluid-filled in 1/52 males; unilateral epididymal agenesis with hypospermatogenesis in 1/52 males <u>Unaffected outcomes</u> - Maternal mortality; maternal weight gain to PND3; male pup weight at birth; PPS; absolute reproductive organ weight at 3–7 months (<i>i.e.</i> , testes, LABC, SV, glans penis, ventral prostate, epididymis, cauda epididymis, caput-corpus epididymis); serum testosterone (3–7 months); male AGD(PND2); reproductive malformations at 3–7 months (hypospadias, cleft phallus, vaginal pouch, SV agenesis, undescended testes, testis absent, abnormal gubernacular cord)
Pregnant Harlan SD rats (3–5/dose) gavaged with 0 (corn oil vehicle) or 750 mg/kg-day DINP on GD14–18. Dams sacrificed on GD18, approximately 2 hours post-dosing. Study completed over several blocks. Block 1 and 5 tested CASRN 68515-48-0, Block 7 tested CASRN 28553-12-0 (Furr et al., 2014)	None/ 750	↓ fetal testicular testosterone production	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ (24–50% across Blocks 1, 5, and 7) <i>ex vivo</i> fetal testicular testosterone production <u>Unaffected Outcomes</u> - Maternal weight gain, fetal viability (all blocks)
Pregnant Wistar rats (8/dose) gavaged with 0 or 750 mg/kg-day DINP (CASRN 28553-12-0) on GD7–21. Dams sacrificed on GD21 (Borch et al., 2004)	None/ 750	↓ fetal testicular testosterone content and production	<u>Maternal Effects</u> - Not examined or reported <u>Developmental Effects</u> - ↓ <i>ex vivo</i> fetal testicular testosterone production and testicular testosterone content (magnitude of effect not reported, only presented graphically) <u>Unaffected Outcomes</u> - Plasma testosterone and luteinizing hormone
Hershberger assay: Testosterone propionate-treated (0.4 mg/kg-day) castrated immature (7 week old) male	NA	NA	- ↓ absolute SV (≥ 20 mg/kg-day) (lacked dose-response) and LABC (500) weight <u>Unaffected Outcomes</u>

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
SD rats were administered DINP via gavage at 0, 20, 100, or 500 mg/kg-day for 10 days (Lee and Koo, 2007)			- Terminal body weight; absolute liver, kidney, adrenal; ventral prostate, Cowper's gland; Glans penis weight
Pregnant SD rats (6/dose) gavaged with 0 (corn oil vehicle) , 10, 100, 500, 1,000 mg/kg-day DINP (CASRN not provided) on GD12–21. Dams were allowed to give birth naturally and then pups were sacrificed (Li et al., 2015)	None/ 10	↓ male pup body weight and fetal Leydig cell aggregation	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ male pup body weight (≥ 10 mg/kg-day) (lacked dose-response) - ↓ testicular testosterone (1,000 mg/kg-day) - ↑ testis dysgenesis (≥ 100 mg/kg-day) - ↑ incidence of MNGs (≥ 100 mg/kg-day) - Fetal Leydig cell aggregation (≥ 10 mg/kg-day) - ↓ testicular gene expression (<i>Insl3</i> (≥ 10), <i>Lhcgr</i> (≥ 500), <i>Star</i> (≥ 500), <i>Cyp11a1</i> (≥ 100), <i>Hsd3b1</i> (≥ 100), <i>Cyp17a1</i> (≥ 100), <i>Hsd17b3</i> (1,000)) <u>Unaffected outcomes</u> - Gestation length; number of dams delivering litters; pups per litter; sex ratio; dam body weight; male AGD
Pregnant Harlan SD rats gavaged with 0, 500, 750, 1,000, or 1,500 mg/kg-day DINP on GD14–18. Dams sacrificed on GD18, approximately 2 hours post-dosing (Gray et al., 2021)	None/ 500	↓ <i>ex vivo</i> fetal testicular testosterone production & ↓ steroidogenic gene expression in the fetal testes	<u>Developmental Effects</u> - ↓ (29–68%) <i>ex vivo</i> fetal testicular testosterone production (≥ 500 mg/kg-day) - ↓ mRNA expression of <i>Nr0b1</i> , <i>Star</i> , <i>Cyp11a1</i> , <i>Hsd3b</i> , <i>Cyp17a1</i> , <i>Scarb1</i> , <i>Insl3</i> , <i>Dhcr7</i> , <i>Cyp11b1</i> and <i>Inha</i> in the fetal testis (≥ 500 mg/kg-day) and <i>Cyp11b2</i> ($\geq 1,000$), <i>Lhcgr</i> ($\geq 1,000$), and <i>Rox10</i> ($\geq 1,000$) <u>Additional Comments</u> - Gray et al. (2021) summarizes combined testosterone data originally reported in (Furr et al., 2014) and (Hannas et al., 2011)
Pregnant SD rats (6–7 per dose) gavaged with 0, 1,000, or 1,500 mg/kg-day DINP (CASRN 68515-48-0) on GD14 through PND3. F1 males were euthanized on PND220–240 (Gray, 2023)	None/ 1,000	↑ F1 male offspring nipple retention	<u>Maternal Effects</u> - ↓ body weight gain ($\leq 5\%$) throughout DINP administration ($\geq 1,000$ mg/kg-day) <u>Developmental Effects</u> - ↓ absolute AGD for F1 males on PND2 (1,500 mg/kg-day) - ↓ F1 male and female body weight on PND2 (body weight effect no longer apparent by PND13) (1,500 mg/kg-day) - ↑ F1 male offspring nipple retention on PND13 and F1 adult males (~210 days of age) ($\geq 1,000$ mg/kg-day)

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			<ul style="list-style-type: none"> - ↓ absolute glans penis, LABC, seminal vesicle weight in F1 adult males (~210 days of age) (1,500 mg/kg-day) - Increased incidence of total reproductive tract malformations ($\geq 1,000$ mg/kg-day) (observed malformations include seminiferous tubule hypospermatogenesis/atrophy; bilateral fluid filled testis in 3 males of each dose group, hypoplasia and epididymal atrophy in 1 male of high-dose group; unilateral testis and gubernacular testis agenesis and undescended atrophic epididymis in 1 male of high-dose group) <p><u>Unaffected outcomes</u></p> <ul style="list-style-type: none"> - No overt signs of maternal toxicity observed; survival at birth; litter sizes at PND2; female AGD on PND2; timing of preputial separation; absolute weight of Cowper's gland, epididymis, ventral prostate, testes, liver, adrenal, kidney <p><u>Additional Comments</u></p> <ul style="list-style-type: none"> - Gray (2023) also reports a statistical analysis of combined data from Gray (2023) and Gray et al. (2000), which was a study of similar design and tested a lower dose of DINP (<i>i.e.</i>, 750 mg/kg-day). Results from this statistical analysis of combined data are not reported in this table.
Pregnant SD rats (7–8 dams per dose) gavaged with 0, 750 mg/kg-day DINP, 250 mg/kg-day DBP, or binary mixture of 750 mg/kg-day DINP and 250 mg/kg-day DBP on GD14–18. Dams were euthanized 2–4 hours after the final dose on GD18 (Gray et al., 2024)	None/ 750	↓ <i>ex vivo</i> fetal testicular testosterone production and ↓ steroidogenic gene expression in the fetal testes	<p><u>Developmental Effects</u></p> <ul style="list-style-type: none"> - <i>Ex vivo</i> fetal testis testosterone production was reduced 45% - ↓ mRNA expression of <i>Cyp11b1</i>, <i>Cyp11a1</i>, <i>Cyp11b2</i>, <i>Cyp17a1</i>, <i>Cyp51</i>, <i>Ebp</i>, <i>Hmgcr</i>, <i>Hmgcs1</i>, <i>Hsd3b3</i>, <i>Iidi1</i>, <i>Inha</i>, <i>Insl3</i>, <i>Lhcgr</i>, <i>Mvd</i>, <i>Scarb1</i>, <i>Star</i>, <i>TM7SF2</i>, <i>SUPERGENE</i>, <i>Rhox10</i>, <i>TESTIN</i> in fetal testis <p><u>Unaffected outcomes</u></p> <ul style="list-style-type: none"> - Maternal body weight or weight gain; litter size; fetal viability <p><u>Additional Comments</u></p> <ul style="list-style-type: none"> - Only results for the DINP component of the study are reported in this table.
<p>Abbreviations: ↓ = statistically significant decrease; ↑ = statistically significant increase; ND = NOAEL or LOAEL not established; NOAEL = no observed-adverse-effect-level; LOAEL = lowest-observed-adverse-effect level; GD = gestation day; PND = postnatal day; PNW = postnatal week; AGD= anogenital distance; MNGs = multinucleated gonocytes</p>			

3.1.2.1.2 Mode of Action for Phthalate Syndrome

The proposed MOA for phthalate syndrome is shown in Figure 3-1, which explains the link between gestational and/or perinatal exposure to DINP and effects on the male reproductive system in rats. The MOA has been described in greater detail in EPA's Draft Proposed Approach for CRA for Phthalates ([U.S. EPA, 2023a](#)) and is described briefly below.

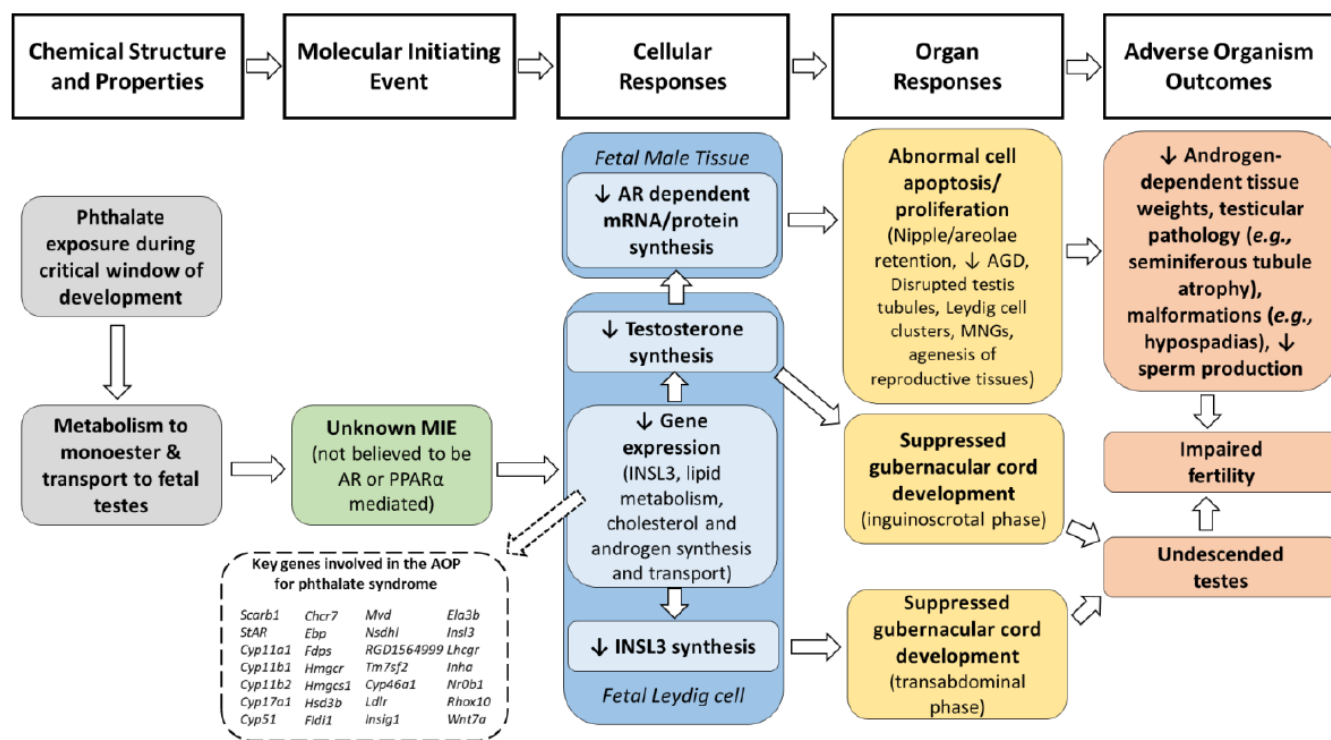


Figure 3-1. Hypothesized Phthalate Syndrome Mode of Action Following Gestational Exposure

Source: Figure taken directly from ([U.S. EPA, 2023a](#)) and adapted from ([Conley et al., 2021](#); [Gray et al., 2021](#); [Schwartz et al., 2021](#); [Howdeshell et al., 2017](#)).

AR = androgen receptor; INSL3 = insulin-like growth factor 3; MNG = multinucleated gonocyte; PPARα = peroxisome proliferator-activated receptor alpha.

The MOA underlying phthalate syndrome has not been fully established; however, key cellular-, organ-, and organism-level effects are generally understood (Figure 3-1). The molecular events preceding cellular changes remain unknown. Although androgen receptor antagonism and peroxisome proliferator-activated receptor alpha activation have been hypothesized to play a role, studies have generally ruled out the involvement of these receptors ([Foster, 2005](#); [Foster et al., 2001](#); [Parks et al., 2000](#)).

Exposure to DINP during the masculinization programming window (*i.e.*, GD15.5–18.5 for rats; GD14–16 for mice; gestational weeks 8–14 for humans) in which androgen action drives development of the male reproductive system can lead to antiandrogenic effects on the male reproductive system ([MacLeod et al., 2010](#); [Welsh et al., 2008](#); [Carruthers and Foster, 2005](#)). *In vivo* pharmacokinetic studies with rats have demonstrated that monoester metabolites of DINP can cross the placenta and be delivered to the target tissue, the fetal testes ([Clewett et al., 2013a](#); [Clewett et al., 2010](#)). Consistent with the MOA outlined in Figure 3-1, studies of DINP have demonstrated that exposure to DINP during the masculinization programming window in rats can reduce mRNA levels of insulin-like growth factor 3 (INSL3), as well as genes involved in steroidogenesis in the fetal testes ([Gray et al., 2024](#); [Gray et al.,](#)

[2021](#); [Li et al., 2015](#); [Hannas et al., 2011](#); [Adamsson et al., 2009](#)). Consistently, studies have also demonstrated that exposure to DINP during the masculinization programming window can reduce fetal testicular testosterone content and/or testosterone production ([Gray et al., 2024](#); [Gray et al., 2021](#); [Li et al., 2015](#); [Furr et al., 2014](#); [Clewell et al., 2013a](#); [Boberg et al., 2011](#); [Hannas et al., 2011](#); [Borch et al., 2004](#)). Exposure to DINP during the masculinization programming window can also reduce male pup anogenital distance (AGD) and cause male pup nipple retention (NR) ([Gray, 2023](#); [Boberg et al., 2016](#); [Clewell et al., 2013b](#); [Gray et al., 2000](#)), which are two hallmarks of antiandrogenic substance; however effects on AGD and NR are less consistently observed following oral exposure to DINP in rats (see Sections 3.1.3.3 and 3.1.3.4 of ([U.S. EPA, 2023a](#)) for additional discussion). In contrast, exposure to DINP generally does not induce severe reproductive tract malformations such as hypospadias and/or cryptorchidism, but has been shown to cause epididymal agenesis, seminiferous tubule hypospermatogenesis/atrophy, fluid filled testis, and other reproductive tract malformations at high doses ranging from 750 to 1,500 mg/kg-day ([Gray, 2023](#); [Gray et al., 2000](#)). Further, a spectrum of other effects consistent with phthalate syndrome, including increased numbers of multinucleated gonocytes (MNGs) ([Li et al., 2015](#); [Clewell et al., 2013a](#); [Clewell et al., 2013b](#); [Boberg et al., 2011](#)), fetal Leydig cell aggregation ([Li et al., 2015](#); [Clewell et al., 2013a](#); [Clewell et al., 2013b](#)), and decrease sperm motility ([Boberg et al., 2011](#)) have also been observed following gestational exposure to DINP during the critical window of development.

Based on available data, EPA previously concluded that the weight of scientific evidence demonstrates that oral exposure to DINP can induce effects on the developing male reproductive system consistent with a disruption of androgen action and the MOA outlined in Figure 3-1 (see EPA's Draft Proposed Approach for CRA for Phthalates ([U.S. EPA, 2023a](#))). Notably, EPA's conclusion was supported by the SACC ([U.S. EPA, 2023b](#)).

3.1.2.2 Other Developmental and Reproductive Outcomes

EPA has evaluated several oral exposure studies, including two prenatal developmental studies of rats ([Waterman et al., 1999](#); [Hellwig et al., 1997](#)), a one-generation study of reproduction of rats ([Waterman et al., 2000](#); [Exxon Biomedical, 1996a](#)), and a two-generation study of reproduction of rats ([Waterman et al., 2000](#); [Exxon Biomedical, 1996b](#)). The Agency identified several studies published from 2015 to 2024 evaluating estrogenic potential ([Sedha et al., 2015](#)), reproductive effects ([Santacruz-Márquez et al., 2024](#); [Chen et al., 2022](#); [Chiang et al., 2020a, b](#); [Chiang and Flaws, 2019](#)), developmental effects ([Bhurke et al., 2023](#); [Laws et al., 2023](#); [Neier et al., 2018](#)), and metabolic effects ([Neier et al., 2019](#)) of DINP in mice and rats treated in the perinatal period. No studies of development are available for the dermal or inhalation exposure routes. Available studies are summarized in Table 3-2 and discussed further below.

Table 3-2. Summary of DINP Studies Evaluating Effects on Reproduction and Development

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Pregnant SD rats (23–25/dose) gavaged with 0 (corn oil vehicle), 100, 500, 1,000 mg/kg-day DINP (CASRN 68515-48-0) on GD6–15. Dams sacrificed on GD21 (Waterman et al., 1999)	100/ 500 ^a	↑ Skeletal variations	<u>Maternal Effects</u> - ↓ (13%) food consumption on GD6–9 (1,000 mg/kg-day) - ↓ body weight gain on GD6–9, 6–15 (1,000 mg/kg-day) <u>Developmental Effects</u> - ↑ incidence of rudimentary lumbar (≥ 500 mg/kg-day), supernumerary cervical ribs (1,000 mg/kg-day), renal pelves (1,000 mg/kg-day) <u>Unaffected Outcomes</u> - Maternal survival, clinical signs, resorptions, post-implantation loss, fetal viability, fetal body weight, sex ratio, incidence of fetal malformations
Pregnant Wistar rats (10/dose) gavaged with 0 (corn oil vehicle), 40, 200, 1,000 mg/kg-day DINP-1 (CASRN 68515-48-0) on GD6–15. Dams sacrificed on GD20 (Hellwig et al., 1997)	200/ 1,000	↑ Skeletal variations	<u>Maternal Effects</u> - ↓ food consumption (1,000 mg/kg-day) - Clinical signs (vaginal hemorrhage and urine-smear fur in one dam) (1,000 mg/kg-day) - ↑ (13%) relative kidney weight (1,000 mg/kg-day) <u>Developmental Effects</u> - ↑ skeletal variations (rudimentary cervical and accessory 14th ribs) (1,000 mg/kg-day) <u>Unaffected Outcomes</u> - Survival; maternal body weight; uterus weight; relative liver weight; resorptions; post-implantation loss; number of live fetuses per dam; fetal weight
Pregnant Wistar rats (10/dose) gavaged with 0 (corn oil vehicle), 40, 200, 1,000 mg/kg-day DINP-2 (CASRN 28553-12-0) on GD6–15. Dams sacrificed on GD20 (Hellwig et al., 1997)	200/ 1,000	↑ Skeletal variations	<u>Maternal Effects</u> - Clinical signs (vaginal hemorrhage in one dam) (1,000 mg/kg-day) <u>Developmental Effects</u> - ↑ skeletal variations (rudimentary cervical and accessory 14th ribs) (1,000 mg/kg-day) <u>Unaffected Outcomes</u> - Survival; food consumption; maternal body weight; uterus weight; relative liver and kidney weight; resorptions; post-implantation loss; number of live fetuses per dam; fetal weight

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Pregnant Wistar rats (10/dose) gavaged with 0 (corn oil vehicle), 40, 200, 1,000 mg/kg-day DINP-3 (CASRN 28553-12-0, resulting from a different production line than DINP-2) on GD6–15. Dams sacrificed on GD20 (Hellwig et al., 1997)	200/ 1,000	↑ incidence of skeletal, visceral, and soft tissue variations	<u>Maternal Effects</u> <ul style="list-style-type: none"> - ↓ food consumption (1,000 mg/kg-day) - ↓ body weight gain from GD6–15 (1,000 mg/kg-day) - ↑ (11%) relative liver weight (1,000 mg/kg-day) <u>Developmental Effects</u> <ul style="list-style-type: none"> - ↑ skeletal retardations (unossified or incompletely ossified sternebrae) (1,000 mg/kg-day) - ↑ soft tissue variations (hydroureter) (1,000 mg/kg-day) - ↑ skeletal variations (rudimentary cervical and accessory 14th ribs) (1,000 mg/kg-day) <u>Unaffected Outcomes</u> <ul style="list-style-type: none"> - Survival; clinical signs; uterus weight; resorptions; post-implantation loss; number of live fetuses per dam; fetal weight
Male and female SD rats (30/sex/dose) fed diets containing 0, 0.5, 1.0, 1.5% DINP (CASRN 68515-48-0) starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for one generation. Received doses in units of mg/kg-day shown in Table 3-5. (Waterman et al., 2000 ; Exxon Biomedical, 1996a)	None/ 377	↓ F1 male and female body weight on PND0, 14, 21	<u>Parental (P1) Effects</u> <ul style="list-style-type: none"> - ↓ P1 body weight (both sexes) ($\geq 1.0\%$) - ↓ P1 food consumption (both sexes) ($\geq 1.0\%$) - ↑ absolute and relative liver weight (both sexes) ($\geq 0.5\%$) - ↑ absolute and/or relative kidney weight (both sexes) ($\geq 0.5\%$) - ↑ absolute testes, right epididymis, and ovary weight (1.5%) <u>Fertility Effects</u> <ul style="list-style-type: none"> - None <u>Offspring (F1) Effects</u> <ul style="list-style-type: none"> - ↓ live births, ↓ PND4 survival, ↓ PND14 survival, ↓ viability at weaning (all at 1.5%) - ↓ male and female body weights on PND0, 1, 14, 21 ($\geq 0.5\%$) <u>Unaffected Outcomes</u> <ul style="list-style-type: none"> - Clinical signs (P1); survival (P1); reproductive indices (male mating, male/female fertility, female fecundity, gestational indices); litter size; number of live/dead offspring at birth; sex ratio
Male and female SD rats (30/sex/dose) fed diets containing 0, 0.2, 0.4, 0.8% DINP (CASRN 68515-48-0) starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for two-generations. Received doses in units	None/133	↓ F1 and F2 male and female body weight on PND7 and 21	<u>Parental (P1, P2) Effects</u> <ul style="list-style-type: none"> - ↓ P1 female body weight on PND14 and 21 (0.8%) - ↓ P2 male and female body weight ($\geq 0.4\%$) - ↓ P1 female food consumption during lactational period (0.8%) - ↓ P2 male and female food consumption during premating, gestation, and lactational periods (0.8%)

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
of mg/kg-day shown in Table 3-7. (Waterman et al., 2000 ; Exxon Biomedical, 1996b)			<ul style="list-style-type: none"> - ↑ relative and/or absolute liver weight for P1 males and females ($\geq 0.4\%$) & P2 males and females (0.8%) - ↑ absolute kidney weight for P1 males ($\geq 0.4\%$) and females ($\geq 0.2\%$) & P2 males (0.8%) - ↑ incidence of minimal to moderate cytoplasmic eosinophilia (both sexes in P1 and P2) ($\geq 0.2\%$) - ↑ incidence of minimal to moderate dilation of the renal pelves for P2 males ($\geq 0.4\%$) <u>Fertility Effects</u> <ul style="list-style-type: none"> - None <u>Offspring (F1, F2) Effects</u> <ul style="list-style-type: none"> - ↓ F1 male and female offspring body weight on PND21 ($\geq 0.2\%$) - ↓ F2 female offspring body weight on PND7 ($\geq 0.2\%$) <u>Unaffected Outcomes</u> <ul style="list-style-type: none"> - Clinical signs (P1, P2); survival (P1, P2); reproductive indices (male mating, male/female fertility, female fecundity, gestational indices) (P1, P2); litter size (F1, F2); number of live/dead offspring at birth (F1, F2); sex ratio (F1, F2)
Uterotrophic Assay: 20 day old female Wistar rats (6/group) were gavaged with 0 (untreated), 0 (corn oil vehicle), 276, 1380 mg/kg-day DINP (CASRN 68515-48-0), or 40 µg/kg-day diethylstilbesterol for 3 days. Animals sacrificed 24 hours after dosing (Sedha et al., 2015)	None/ 276	↓ body weight gain	<ul style="list-style-type: none"> - ↓ body weight gain (≥ 276 mg/kg-day) - Positive control gave anticipated results <u>Unaffected Outcomes</u> <ul style="list-style-type: none"> - Uterine and pair ovary wet weight
Pubertal Assay: 20 day old female Wistar rats were gavaged with 0 (untreated), 0 (corn oil vehicle), 276, 1380 mg/kg-day DINP (CASRN 68515-48-0), or diethylstilbesterol 6 µg/kg-day diethylstilbesterol for 20 days starting on PND21. Animals were sacrificed on PND41 (Sedha et al., 2015)	None/ 276	↓ body weight gain	<ul style="list-style-type: none"> - ↓ body weight gain (≥ 276 mg/kg-day) - ↓ (10–28%) relative and absolute ovary weight (1,380 mg/kg-day) - Positive control gave anticipated results <u>Unaffected Outcomes</u> <ul style="list-style-type: none"> - Absolute and relative uterine wet weight and vaginal weight; vaginal opening
CD-1 female mice (4–12/dose) were gavaged with 0 (corn oil vehicle), 0.02,	200/ None	NA	<u>Maternal Effects</u> ^b <ul style="list-style-type: none"> - None definitively related to treatment

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
0.1, 20, or 200 mg/kg-day DINP (CASRN not provided) for 10 days and then mated with untreated males immediately after, as well as 3 and 9 months post-dosing (Chiang and Flaws, 2019)			<u>Developmental Effects</u> ^b - None definitively related to treatment <u>Unaffected Outcomes (all timepoints, unless otherwise noted)</u> - Body weight; absolute ovary, uterine, liver weight; time to mating; fertility index; gestational index; gestation length; litter size; pup weight; pup mortality; estrous cyclicity (0, 9 months)
CD-1 female mice (6–12/dose) were gavaged with 0 (corn oil vehicle), 0.02, 0.1, 20, or 200 mg/kg-day DINP (CASRN not provided) for 10 days and outcomes evaluated immediately after dosing, as well as 3-, 6-, and 9-months post-dosing (Chiang et al., 2020a)	200/ None	NA	<u>Maternal Effects</u> ^c - None definitively related to treatment <u>Developmental Effects</u> ^c - None definitively related to treatment <u>Unaffected Outcomes (all timepoints, unless otherwise noted)</u> - Hormones (serum progesterone, estradiol, FSH, and inhibin B) - Ovarian histopathology (total number of follicles)
CD-1 female mice (6–12/dose) were gavaged with 0 (corn oil vehicle), 0.02, 0.1, 20, or 200 mg/kg-day DINP (CASRN not provided) for 10 days and then mated with untreated males 12- or 15-months post-dosing. Outcomes were evaluated at 12-, 15-, and 18-months post-dosing (Chiang et al., 2020b)	200/ None	NA	<u>Maternal Effects</u> ^c - None definitively related to treatment <u>Developmental Effects</u> ^c - None definitively related to treatment <u>Unaffected Outcomes (12 and 15 months)</u> - Estrus cyclicity, time to mating, litter size, percent of females who gave birth, average live litter weight, serum levels of testosterone, progesterone, estradiol, FSH, or inhibin B, total number of ovarian follicles <u>Unaffected Outcomes (18 months)</u> - Total number of ovarian follicles, percent of follicle type (<i>i.e.</i> , primordial, primary, preantral, antral)
Female yellow agouti mice (resulting in 15–17 litters/dose) were fed diets of 0 or 75 mg/kg feed DINP (equivalent to 0 or 15 mg/kg-day) from 2 weeks prior to mating through weaning (PND21) with body and organ weights were collected on PND21 (Neier et al., 2018)	None/ 15	↑ maternal body weight gain; ↑ pup body weight; ↑ pup relative liver weight	<u>Maternal Effects</u> - ↑ body weight gains <u>Developmental Effects (PND21)</u> - ↑ pup body weight (both sexes) - ↑ pup relative liver weight (females) <u>Unaffected Outcomes (PND21)</u> - Number of live pups per litter; maternal body weight; pup hepatic triglycerides; pup gonadal fat, brain, spleen, and kidney weights; pup liver weight (male); pup A ^{vy} DNA methylation
Female yellow agouti mice (17–21/group) were fed diets of 0 or 75	None/ 15	↓ birth rates;	<u>Maternal Effects</u> - ↓ birth rates

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
mg/kg feed DINP (equivalent to 0 or 15 mg/kg-day) from 2 weeks prior to mating through weaning (PND21). 1 male and female pup/litter were allowed to recover for 10 months (Neier et al., 2019)		↑ pup liver masses; altered pup body composition; ↓ glucose tolerance	<u>Developmental Effects (PND21)</u> - ↑ liver masses (males) - ↑ body fat (females, longitudinal 2–8 months) - ↓ lean mass percentage (females, longitudinal 2–8 months) - ↓ glucose tolerance (females, longitudinal 2–8 months) <u>Unaffected Outcomes (at 2 and 8 months unless noted)</u> - Pup body weight across life course (PND21–10 months); pup physical activity; pup food intake; pup energy expenditure; resting metabolic rate, respiratory exchange rate, fat oxidation rate, glucose oxidation rate; pup plasma adipokines
Pregnant female CD-1 mice (25/group) were administered 0 or 20 µg/kg-day DINP by pipetting DINP directly into the mouth of the mice on GD1–7. Seven mice from each treatment group were allowed to deliver litters naturally, while the remaining mice were euthanized on GD7, 13, and 18 (6 mice/dose/time point) (Bhurke et al., 2023)	None/ 0.02	↓ litter size, ↓ gestation length, ↓ fetal and pup weight, and other placental defects (<i>i.e.</i> , perturbation in placental histopathology)	<u>Maternal Effects</u> - Study authors do not report examination of any maternal outcomes <u>Developmental Effects (PND21)</u> - ↓ average litter size (decreased from 16–11 in DINP group) - ↓ fetal and placental weight on GD13 and ↓ fetal weight on GD18 - ↓ gestation length (by 20–24 hours) and ↓ pup weight on PND1 - ↓ mRNA expression of genes involved in decidualization and angiogenesis in uterine tissue on GD7 - ↓ mRNA expression of genes involved in trophoblast differentiation and glucose transporters in placental tissue on GD13 <u>Unaffected Outcomes</u> - # of implantation sites on GD7; sex ratio; serum estrogen; serum progesterone
Adult female CD-1 mice (12–14 mice per dose) fed diets containing 0, 0.15, 1.5 and 1,500 ppm DINP continuously for 11 months (equivalent to 0.024, 0.24, 240 mg/kg-day) and then mated with untreated males after 11 months of exposure (Laws et al., 2023). Estrus cyclicity was evaluated after 1, 3, 5, 7, and 11 months of phthalate exposure.	0.24/ 240	↓ gestation index and birth rate	<u>Maternal Effects</u> - None <u>Developmental Effects</u> - ↓ gestation index and birth rate (240 mg/kg-day) <u>Unaffected Outcomes</u> - Body weight and weight gain; estrous cyclicity; mating index, fertility index, pregnancy rate, dystocia rate
Adult female CD-1 mice fed diets containing 0, 0.15, 1.5, and 1,500 ppm DINP for 1 and 6 months (equivalent to	0.24/ 240	Altered ovarian follicles	<u>Maternal Effects</u> - Maternal outcomes (body weight, food consumption, clinical signs) not evaluated

Brief Study Description	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
0, 0.024, 0.24, and 240 mg/kg-day) (Santacruz-Márquez et al., 2024)			<p>Reproductive Effects</p> <ul style="list-style-type: none"> - ↑ percentage of primordial follicles & ↓ percentage of preantral and antral follicles (1,500 ppm at 6 months) - ↓ serum follicle stimulating hormone after 1 month (1.5 ppm) and ↑ follicle stimulating hormone after 6 months (1.5 ppm) (not dose-related) - ↓ serum luteinizing hormone after 6 months (≥1.5 ppm) <p>Unaffected Outcomes</p> <ul style="list-style-type: none"> - Ovarian follicles (1 month); serum progesterone, testosterone, estradiol (1 and 6 months); mRNA expression of steroidogenic genes, follicle stimulating hormone receptor, luteinizing hormone receptor in ovary (1 and 6 months); serum luteinizing hormone levels (1 month)
<p>^a Waterman et al., (1999) originally identified a developmental NOAEL of 500 mg/kg-day DINP 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 DINP. Results from the statistical re-analysis are reported in (NTP-CERHR, 2003).</p> <p>^b The study authors in Chiang (2019) reported several statistically significant findings as related to treatment with DINP; however, EPA considered these differences to be spurious and incidental to treatment because they were unrelated to dose, transient, and/or not adverse. These significant differences included: differences in estrous cyclicity at 20 and 100 µg/kg-day and 200 mg/kg-day DINP and fewer pregnant females at 20 µg/kg-day at 3 months post-dosing; differences in estrous cyclicity at 100 µg/kg-day and reduced time to mating at 100 µg/kg-day to 200 mg/kg-day DINP and increased percent males in litters at 100 µg/kg-day and 20 and 200 mg/kg-day DINP at 9 months post-dosing.</p> <p>^c The study authors of Chiang et al. (2020a, b) reported statistically significant findings related to exposure to DINP; however, EPA considered these effects to be unrelated to dose, transient, and/or not adverse. These findings included: changes in the percent of type of follicle (<i>i.e.</i>, primordial, primary, preantral, antral) at doses as low as 0.02 mg/kg-day (decreased percent antral follicles at 6 months, but no other timepoint), changes in serum levels of estradiol, progesterone, and/or inhibin B, percent of female pups (12 months), and gestation length (12 months).</p>			

In the first study, which adhered to EPA section 798.4900 (40 CFR part 798, 1985), Waterman et al. (1999) gavaged pregnant SD rats (23–25 per dose) with 0, 100, 500, and 1,000 mg/kg-day DINP (CASRN 68515-48-0) on GD6 through 15. Maternal toxicity was limited to the high-dose group and included a reduction in maternal body weight gain on GD6 through 9 and 6 through 15 (magnitude of effect not reported), and a 13 percent decrease in food consumption on GD6 through 9. Food consumption and bodyweight gain significantly increased after cessation of exposure between GD18 through 21 and mean maternal body weight recovered to control levels by GD21. No treatment-related effects on maternal survival, clinical signs, resorptions, post-implantation loss, fetal viability, sex ratio, or fetal body weight were observed. No malformations were observed at any dose. Fetal effects were limited to treatment-related increases in skeletal and visceral variations, including increased incidence of renal pelves at 1,000 mg/kg-day, rudimentary lumbar ribs at 500 and 1,000 mg/kg-day, and supernumerary cervical ribs at 1,000 mg/kg-day (Table 3-3). EPA identified a developmental NOAEL of 100 mg/kg-day DINP 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 1,000 mg/kg-day DINP.

Table 3-3. Mean Percent of Fetuses in Litter with Skeletal Variations (Waterman et al., 1999) ^{a b}

Type of Fetal Variation	0 (mg/kg-day)	100 (mg/kg-day)	500 (mg/kg-day)	1,000 (mg/kg-day)
Skeletal variations	16.4	15.0	28.3*	43.4**
Visceral variations	0.5	3.3	3.7	5.8*
Renal pelves	0.0	3.3	3.7	5.3*
Rudimentary lumbar ribs	3.5	4.7	18.1*	34.2**
Supernumerary cervical ribs	1.6	1.5	1.0	5.5*

^a Adapted from Tables 5 and 6 in (NTP-CERHR, 2003).
^b * indicates $P \leq 0.05$ and ** indicates $p \leq 0.01$. Skeletal variation data was re-analyzed by study sponsors using the generalized estimating equation (GEE) 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). Renal pelves data could not be re-analyzed using the GEE methodology due to the zero incidence in the control. Renal pelves data was re-analyzed using two approaches, including a nested analysis that considered litter effects and by changing one control fetus to affected and using the GEE approach. Both approaches provided similar results (significant increase at 1,000 mg/kg-day).

In a second prenatal study, Hellwig et al. (1997) gavaged pregnant Wistar rats (10 per dose) with 0, 40, 200, and 1,000 mg/kg-day DINP on GD6 through 15. Three different formulations of DINP were evaluated, including: DINP-1 (CASRN 68515-48-0, purity $\geq 99\%$), commercially available with the alcohol moiety consisting of roughly equivalent amounts of 3,4-, 4,6-, 3,6-, 3,5-, 4,5-, and 5,6-dimethylheptanol-1; DINP-2 (28553-12-0), with at least 95% of the alcohol components as alkyl-substituted octanol or heptanol derived from *n*-butene; and DINP-3 (28553-12-0), resulting from a different production line from DINP-2, with main alcohol components synthesized from *n*-isobutene, resulting in $>60\%$ alkyl-substituted hexanols. The studies were Good Laboratory Practice (GLP)-compliant and generally adhered to EPA section 798.4900 (40 CFR part 798, 1992), with the exception that 10 dams, instead of 20 were employed per dose group. For DINP-1, maternal toxicity was limited to the high-dose group and included reduced food consumption (magnitude of effect not reported), clinical signs (*i.e.*, vaginal hemorrhage and urine smeared fur in one dam), and a 13 percent increase in relative kidney (but not liver) weight. No treatment-related effects on maternal body weight, maternal survival, resorptions, post-implantation loss, number of live fetuses per dam, or fetal weights were observed.

Developmental effects were limited to the high-dose group and included a statistically significant increase in the percent fetuses per litter with variations (35.3, 41.5, 29.5, and 58.4% across dose groups). Variations showing dose-related increases included rudimentary cervical and accessory 14th rib(s), and an apparent, non-statistically significant, increase in dilated renal pelvis (Table 3-4). For DINP-2, there was no statistically significant maternal toxicity that was treatment-related. One dam given 1,000 mg/kg-day DINP-2 had vaginal hemorrhage on GD14 and 15. No effects on food consumption, maternal body weight, maternal survival, relative liver or kidney weight, resorptions, post-implantation loss, number of live fetuses per dam, or fetal weights were observed. Study authors state that “the only substance-related fetal effect was an increased incidence of a skeletal variation [accessory 14th rib(s)]” in the high-dose group, although the incidence of rudimentary cervical rib(s) also appeared slightly increased (Table 3-4). Multiple malformations were observed in one high-dose fetus, including globular-shaped heart, unilobular lung, hydrocephaly, dilation of aortic arch, and anasarca, which were regarded as spontaneous and not treatment related by study authors.

For DINP-3, maternal toxicity was limited to the high dose group, and included reduced food consumption (magnitude of effect not reported), decreased body weight gain from GD6 to 15, increased (11%) relative liver weight, and a non-statistically significant increase (9%) in relative kidney weight. No effects on maternal survival, resorptions, post-implantation loss, number of live fetuses per dam, or fetal weights were observed. Developmental effects were limited to the high-dose group and included a statistically significant increase in the percent fetuses per litter with variations (35.3, 29.6, 39.5, and 60.7% across dose groups), including increased incidences of skeletal retardations (unossified or incompletely ossified sternebrae), skeletal variations (rudimentary cervical and/or accessory 14th rib[s]) and soft tissue variations (hydroureter, dilated renal pelvis) (Table 3-4). Additionally, study authors attributed some soft tissue malformations (predominately affecting the urogenital tract) and skeletal malformations (shortened and bent humerus and femur in a single fetus) in the high-dose group to be treatment-related. Overall, similar developmental findings were observed for all three tested formulations of DINP and support a developmental NOAEL of 200 mg/kg-day based on increased skeletal and visceral variations at 1,000 mg/kg-day.

Table 3-4. Incidence of Visceral, Skeletal, and Soft Tissue Variations ([Hellwig et al., 1997](#)) ^a

DINP Formulation	Number of Fetuses Evaluated and Type of Fetal Variation	Control	40 (mg/kg-day)	200 (mg/kg-day)	1,000 (mg/kg-day)
DINP-1	No. fetuses (litters) evaluated	135 (9)	116 (9)	111 (8)	131 (10)
	Rudimentary cervical rib(s)		2 (1)	1	11 (5)
	Accessory 14th rib			2 (2)	37 (10)
	Dilated renal pelvis	12 (7)	11 (4)	8 (4)	22 (9)
DINP-2	No. fetuses (litters) evaluated	135 (9)	116 (9)	135 (10)	141 (10)
	Rudimentary cervical rib(s)		1	4 (2)	10 (5)
	Accessory 14th rib			1	4 (4)
DINP-3	No. fetuses (litters) evaluated	135 (9)	138 (10)	135 (9)	120 (9)
	Rudimentary cervical rib(s)			2 (1)	12 (7)
	Accessory 14th rib			9 (5)	34 (8)
	Sternebrae not ossified	6 (3)	1	3 (2)	26 (7)
	Sternebrae incompletely ossified or reduced in size	20 (7)	11 (7)	16 (6)	36 (9)
	Dilated renal pelvis	12 (7)	15 (8)	13 (9)	20 (9)

DINP Formulation	Number of Fetuses Evaluated and Type of Fetal Variation	Control	40 (mg/kg-day)	200 (mg/kg-day)	1,000 (mg/kg-day)
	Hydroureter	4 (3)	5 (3)	1	12 (8)

^a Table adapted from Tables 10, 12, and 14 in Hellwig et al. (1997).

DINP has also been evaluated in both one- and two-generation studies of reproduction, which were GLP compliant and conducted in accordance with EPA Test Guidelines for Reproductive and Fertility Effects (40 CFR part 798, 1985) ([Waterman et al., 2000](#); [Exxon Biomedical, 1996a, b](#)). In the one generation study, SD rats (30/sex/dose) were continuously administered dietary concentrations of 0, 0.5, 1.0, and 1.5 percent DINP (CASRN 68515-48-0) starting 10 weeks prior to mating, throughout mating, gestation, and lactation. Mean received doses in units of mg/kg-day are shown in Table 3-5. P1 males were sacrificed following delivery of the last litter of F1 pups, while P1 females were sacrificed at F1 weaning on postnatal day (PND) 21. No treatment-related clinical signs or effects on survival were reported for P1 males or females. Body weight was statistically significantly reduced in mid- and high-dose males and females during the premating phase, and in mid- (5.3–15.3% decrease) and high-dose (10.8–23.3% decrease) P1 females during gestation and lactation. Similarly, food consumption was significantly reduced in mid- (5.3–8.7% decrease) and high-dose (5.8–10.5% decrease) males and females during the premating phase, and in mid- (16.7–27.4% decrease) and high-dose (11.6–42.2% decrease) P1 females during gestation and lactation.

Treatment with DINP had no significant effects on any reproductive indices, including male mating, male/female fertility, female fecundity, or gestational indices. Mean litter size, mean number of live and dead offspring, and sex ratio were unaffected by treatment with DINP. At the high dose, treatment with DINP significantly reduced percent live births (95.2 vs. 98.2% in controls), PND4 survival (85.6 vs. 93.1% in controls), PND14 survival (92.7 vs. 98.5% in controls), and viability at weaning (87.2 vs. 93.9% in controls). Male and female F1 offspring body weight was significantly reduced in all treatment groups on PND0 (7.9–11.5%) and continued to be reduced, although not always statistically significantly, in all treatment groups for both sexes through PND21 (Table 3-6). Overall, this study supports a developmental LOAEL of 377 mg/kg-day (no NOAEL identified), based on reduced F1 offspring body weight throughout the lactational period.

Table 3-5. Mean Measured Doses (mg/kg-day) from the One-Generation Study of DINP in SD Rats ([Waterman et al., 2000](#); [Exxon Biomedical, 1996a](#))^a

Dose (%)	Premating – Males	Premating – Females	Gestation	Postpartum
0.5	301–591	363–624	377–395	490–923
1.0	622–1,157	734–1,169	741–765	1,034–1,731
1.5	966–1,676	1114–1,694	1087–1,128	1,274–2,246

^a Adapted from Table 12 in Exxon Biomedical (1996a).

Table 3-6. F1 Offspring Postnatal Body Weight (Grams) from the One-Generation Study of Reproduction in SD Rats (Waterman et al., 2000; Exxon Biomedical, 1996a) ^{a b c}

F1 Offspring												
Group	Male						Female					
	PND0	PND1	PND4	PND7	PND14	PND21	PND0	PND1	PND4	PND7	PND14	PND21
0%	6.98	7.34	9.80	16.02	33.77	54.34	6.68	7.05	9.58	15.60	32.72	52.19
0.2%	6.49**	6.83	9.18	14.52	30.00**	48.94*	6.15**	6.52*	8.81	14.07	29.40**	47.77**
0.4%	6.42**	6.92*	9.12	14.00*	26.23**	39.93**	6.05**	6.49**	8.56*	13.24*	25.04**	38.13**
0.8%	6.27**	6.58**	8.19**	11.04**	20.18**	29.32**	5.91**	6.25**	7.84**	10.71**	19.31**	27.71**
Historical Control	6.35-7.02	6.68-7.46	8.53-11.43	13.64-18.74	28.81-36.73	44.89-60.77	5.96-6.74	6.30-7.16	8.32-11.05	13.33-17.69	27.22-35.74	42.39-61.19

^a Data from Table 4 in Waterman et al. (2000).
^b '*' and '**' indicate the mean is significantly different from the control mean by $p < 0.05$ and $p < 0.01$, respectively.
^c Historical control data reported to be from the laboratory conducting the study. Further details (e.g., number of studies data collected from, timespan of studies) regarding the source of historical control data were not provided in Exxon Biomedical (1996a).

In the two-generation study, SD rats (30/sex/dose) were continuously administered dietary concentrations of 0, 0.2, 0.4, and 0.8 percent DINP (CASRN 68515-48-0) starting 10 weeks prior to mating, throughout mating, gestation, and lactation continuously for two generations (Waterman et al., 2000; Exxon Biomedical, 1996b). Mean received doses in units of mg/kg-day are shown in Table 3-7. For the first parental generation (P1), no treatment-related clinical signs or effects on survival were reported for P1 animals. No significant effects on P1 body weight were observed, except for a 6.7 to 7.8 percent decrease in high-dose female body weight on PND14 and 21. Food consumption was significantly reduced (9%) for high-dose females during the postnatal phase of the study but was not reduced for males or females during other phases of the study. For the second parental generation (P2), no treatment-related clinical signs or effects on survival were reported. At the start of the premating period (six weeks after weaning), mean body weights for mid and high dose males and females were lower than control. Females in the high-dose group had consistently lower body weight gain compared to the control group during the premating (statistically significant for first 2 weeks), gestation (not significant), and lactational (significant for PND4–21) phases. Small (<8%), but statistically significant, decreases in food consumption were observed in high-dose males and females during the premating period and in high-dose females during gestation (13–16% decrease) and lactation (9% decrease). No treatment-related effects on any reproductive indices were observed for either generations, including male mating, male/female fertility, female fecundity, or gestational indices.

Similarly, gestation length, mean litter size, mean number of live and dead offspring, sex ratio, percent live births, survival on PND1, 4, 7, 14, and 21, and viability at weaning were unaffected by treatment with DINP for both the F1 and F2 generations. F1 and F2 offspring body weight was significantly reduced throughout PND0 to 21 (Table 3-8). For F1 offspring, bodyweight was significantly reduced 6.8 percent for high-dose males on PND0; 10 to 15 percent for mid- and high-dose males and females on PND7 and 14; and 8.9 to 19 percent for males and females on PND21 in all dose groups. For F2 offspring, bodyweight was significantly reduced 14 to 17 percent for mid- and high-dose females on PND4; 14 to 19 percent for mid- and high-dose males and 10 to 21 percent for females in all dose groups on PND7; 12 to 21 percent for mid- and high-dose males and females on PND14; and 12 to 22 percent for mid- and high-dose males and females on PND21. Study authors state that the observed body weight changes were within historical control ranges from the laboratory conducting the study and that effects on body weight at 0.2 and 0.4 percent DINP “seem unrelated to treatment.” However, no information regarding the source of the historical control data is provided (e.g., number of studies, years study conducted, strain/species tested, and diet animals were maintained on were not reported), so it is

unclear if an appropriate historical control data set was used. Overall, this study suggests a developmental LOAEL of 0.2 percent DINP (equivalent to approximately 133 mg/kg-day during gestation) for decrements in F1 and F2 body weight during lactation.

Table 3-7. Mean Measured Doses (mg/kg-day) from the Two-Generation Study of DINP in SD Rats (Waterman et al., 2000; Exxon Biomedical, 1996b) ^a

Dose (%)	P1 Generation				P2 Generation			
	Premating – Males	Premating – Females	Gestation	Postpartum	Premating – Males	Premating – Females	Gestation	Postpartum
0.2	118–212	145–215	139–153	159–350	114–264	140–254	133–153	174–395
0.4	236–426	278–425	274–301	347–731	235–523	271–522	271–307	348–758
0.8	477–852	562–830	543–571	673–1,379	467–1,090	544–1,060	544–577	718–1,541

^a Adapted from Tables 12 and 32 in Exxon Biomedical (1996b).

Table 3-8. F1 and F2 Offspring Postnatal Body Weight (Grams) from the Two-Generation Study of Reproduction in SD Rats (Waterman et al., 2000; Exxon Biomedical, 1996b) ^{a b c}

F1 Offspring												
Group	Male						Female					
	PND0	PND1	PND4	PND7	PND14	PND21	PND0	PND1	PND4	PND7	PND14	PND21
0%	6.90	7.49	10.63	17.62	35.01	57.25	6.47	7.11	10.26	16.70	33.52	53.99
0.2%	6.78	7.39	10.26	16.44	33.28	51.40*	6.36	6.96	9.61	15.54	31.89	49.19*
0.4%	6.48	7.03	9.54	15.28**	30.43**	47.95**	6.16	6.67	9.24	14.21**	29.14**	45.63**
0.8%	6.43*	7.05	9.74	15.67*	29.66**	46.52**	6.08	6.70	9.36	15.03*	28.41**	44.68**
Historical Control	6.35–7.02	6.68–7.46	8.53–11.43	13.64–18.74	28.81–36.73	44.89–60.77	5.96–6.74	6.30–7.16	8.32–11.05	13.33–17.69	27.22–35.74	42.39–61.19
F2 offspring												
0%	6.67	7.30	10.63	18.08	37.09	62.34	6.44	7.10	10.48	17.47	35.89	59.37
0.2%	6.49	7.12	10.05	16.43	34.80	57.89	6.13	6.75	9.60	15.72*	33.64	55.50
0.4%	6.55	7.08	9.73	15.48**	32.51**	54.82**	6.11	6.59	9.05**	14.56**	31.22**	51.98**
0.8%	6.18	6.64	9.05	14.70**	29.88**	49.12**	5.92	6.41	8.68**	13.76**	28.20**	46.20**
Historical Control	6.35–7.02	6.68–7.46	8.53–11.43	13.64–18.74	28.81–36.73	44.89–60.77	5.96–6.74	6.30–7.16	8.32–11.05	13.33–17.69	27.22–35.74	42.39–61.19

^a Data from Tables 8 and 11 in Waterman et al. (2000).

^b * and ** indicate the mean is significantly different from the control mean by $p < 0.05$ and $p < 0.01$, respectively.

^c Historical control data reported to be from the laboratory conducting the study. Further details (e.g., number of studies data collected from, timespan of studies) regarding the source of historical control data were not provided in (Exxon Biomedical 1996b).

Chiang and Flaws (2019) gavaged adult CD-1 female mice (4–12 per group) with 0 (corn oil vehicle), 0.02, 0.1, 20, or 200 mg/kg-day DINP (CASRN not provided) for 10 days and then evaluated effects on organ weight, estrous cyclicity, and mating behavior with untreated male mice immediately after dosing, as well as 3 and 9 months post-dosing. Treatment with DINP had no effect on body weight, absolute ovary, uterine or liver weight at any timepoint. Three months post-dosing, females treated with 0.02 and 200 mg/kg-day spent significantly less time in proestrus and more time in metestrus and diestrus. However, no dose-related effects on estrous cyclicity were observed immediately following dosing or nine months post-dosing and the effects observed at three months appeared slight (magnitude of effect not reported) and of uncertain toxicological significance. No adverse, dose-related, effects on time to

mating, fertility index, gestational index, gestation length, the number of females able to produce pups, litter size, pup weight on PND20, pup mortality, sex ratio were observed at any timepoint. Several parameters were statistically significantly altered (*e.g.*, fertility index decreased at 0.02 mg/kg-day at 3 months [but not at higher doses or other timepoints], number of females able to produce pups decreased at 0.02 mg/kg-day at 3 months and 20 mg/kg-day at 9 months [but not at higher doses]); however, these findings were of uncertain toxicological significance, given the non-monotonic dose relationship and the lack of mechanistic data from other studies supporting an effect of DINP on these endpoints.

Two follow-up studies by Chiang et al. ([2020a, b](#)) further evaluated the effects of DINP on follicle populations and hormone levels at timepoints where fertility was disrupted in the prior study ([Chiang et al., 2020a](#)) or at additional timepoints several months after exposure has ended ([Chiang et al., 2020b](#)). The exposure paradigm for both was similar to the prior Chiang and Flaws study ([2019](#)), where adult female CD-1 mice (at least 6/group; 12 in controls) were dosed orally via pipette beginning at PND39 or 40 with 0, 0.02, 0.1, 20, or 200 mg/kg-day DINP for 10 consecutive days. In Chiang et al. ([2020a](#)), outcomes were evaluated in non-mated females immediately after dosing, as well as at 3, 6, and 9-months and included ovarian histopathology (*i.e.*, % follicle types and total number of follicles) and serum hormone levels (testosterone, progesterone, estradiol, FSH, and inhibin B). Similar to the results of Chiang and Flaws ([2019](#)), there were no effects that exhibited a linear dose-response. There were no changes in total follicle number at any timepoint. Although there were significant changes in the percent of follicles of a given type, there was no dose-response, and the directionality and/or dose at which a significant effect was observed differed across timepoints (*e.g.*, the percent of primary follicles in the 200 mg/kg-day group was increased at 6 months, but there was no change at this dose for other timepoints, and the percent was decreased at 0.02 mg/kg-day at 9-months). Changes in serum hormone levels were observed including significant decreases in testosterone immediately after dosing at doses as low as 0.02 mg/kg-day, as well as the three, 6-month timepoints at doses as low as 0.1 mg/kg-day, but there was no dose-response in any data set. Changes in estradiol and progesterone were also observed, but the directionality was not consistent across timepoints.

In Chiang et al. ([2020b](#)), two groups of mice were used. The first group was used for cyclicity monitoring (14 days) followed by a breeding trial at 12 and 15 months and ovary and sera collection at 18 months post-dosing. The second group was used for ovary and sera collection at 12 and 15 months post-dosing. Altogether, outcomes were evaluated 12, 15, or 18 months after dosing and included estrus cyclicity (*i.e.*, % time spent in proestrus estrus, metestrus, diestrus at 12 or 15 months); pregnancy loss, fertility, and sex ratio of pups at 12 months; ovarian histopathology (*i.e.*, ovarian follicle populations) at 12, 15, and 18 months; and hormone levels (serum testosterone, progesterone, and estradiol at 12, 15, and 18 months). Similar to Chiang and Flaws ([2019](#)) and Chiang et al. ([2020a](#)), there were no effects that exhibited a linear dose-response. There were no significant changes in the percent of time in any particular phase of estrus at 12 or 15 months, aside from a non-significant increase in estrus at the 12-month timepoint in the 0.02 mg/kg-day group. There were no changes in total follicle numbers at 12, 15, or 18 months. Although there were significant changes in the number and percentages of various follicle types at 15 and 18 months, there was no consistent dose-related trend. Changes in hormone levels were only noted at 18 months and included decreases in testosterone and estradiol in the 0.1 mg/kg-day group, and an increase in inhibin B in the 20 mg/kg-day group.

Overall, the studies by Chiang et al. ([2020a, b](#); [2019](#)) provide some evidence of effects of DINP on the female reproductive system; however, the lack of a linear dose-response relationship and inconsistent effects across evaluated timepoints makes it difficult to interpret whether or not the observed effects are directly related to DINP exposure.

Two perinatal exposure studies of DINP have also been conducted using the viable yellow agouti mouse model ([Neier et al., 2019](#); [Neier et al., 2018](#)). In the first study, *a/a* dams were fed phytoestrogen-free diets containing 0 or 75 mg/kg DINP (CASRN not reported) (equivalent to approximately 15 mg/kg-day DINP) starting 2 weeks prior to mating with *A^{vy}/a* males and continuing throughout gestation and lactation until weaning on PND21 ([Neier et al., 2018](#)). The exact number of mating pairs per treatment group is not provided in the 2018 study; however, 15 to 17 litters were produced for the control and DINP treatment groups. Treatment with DINP had no effect on maternal body weight at PND21, offspring sex ratio, mean pups per litter, pup mortality through PND21, or pup genotype. Body weight was significantly increased 10 to 20 percent for females (of genotypes *a/a* and *Avy/a*) and 15 percent for males (of genotype *Avy/a*) on PND21 in the DINP treatment group. Treatment with DINP correlated to increased relative liver weight for female pups. There was no change in absolute or relative liver weight (males only), gonadal fat, brain, spleen, or kidney weights in pups. Additionally, no change in pup DNA methylation was observed. In summary, treatment with DINP showed modest decreases in pup body weights and increased relative liver weight (females only).

The Neier et al. ([2019](#)) study followed the same dosing scheme with viable yellow agouti mouse dams exposed from 2 weeks prior to mating through PND21 in diet at dosages of 0 and 75 mg/kg feed DINP (equivalent to 0 and 15 mg/kg-day). A total of 17 control pairs and 21 DINP pairs were dosed to produce a minimum of 15 litters per treatment group. The largest male and female from each litter (10 per sex per dose) were fed a phthalate-free diet until 10 months old; one male in the 15 mg/kg-day group died during glucose gavage at 2 months. The DINP treatment group showed a decreased birth rate, and a non-significant increase in liver masses in males at 10 months (9.1% control vs. 33% treated). Effects were not reported in dams and pups body weights were not altered. The Neier et al. ([2019](#)) study also evaluated metabolic effects through adulthood in mice exposed to DINP perinatally with evaluations at 2, 8, and 10 months. The DINP treatment group showed altered body fat, lean mass percentage in females longitudinally; however, these effects were not significant when accounting for multiple comparisons. DINP treated females showed a moderate reduction in glucose tolerance longitudinally driven by decreased glucose tolerance at two months that improved slightly at eight months. There was no change in pup body weight across life, physical activity, or food intake. Additionally, there was no alteration in energy expenditure, resting metabolic rate, respiratory exchange rate, fat oxidation rate, glucose oxidation rate, or plasma adipokines. Overall, treatment with DINP resulted decreased birth rate, as well as modest alterations to female pup body composition and glucose tolerance, without corresponding alterations to diet, physical activity, or other markers for metabolic activity.

Bhurke et al. ([2023](#)) dosed pregnant CD-1 mice (25 per dose group) with 0 or 20 µg/kg-day DINP by pipetting DINP directly into the mouth of the mice from GD1 through GD7. Seven mice from each treatment group were allowed to deliver litters naturally, while the remaining mice were euthanized on GD7, 13, and 18 (6 mice/dose/time point). Study authors did not report evaluating maternal outcomes such as food consumption, body weight, weight gain, or clinical signs; however, given the low dose of DINP utilized in the study, overt maternal toxicity is not expected. Treatment with DINP reduced litter size from an average of 16 pups per litter for concurrent controls to 11 pups per litter for DINP treated mice. Treatment with DINP had no effect on sex ratio or number of implantation sites. Decreased mRNA expression of genes involved in decidualization (*i.e.*, *Hand2*, *Bmp2*, *Wnt4*, and *Cebpβ*) and angiogenesis (*i.e.*, *Epas1*, *Vegfa*, *Angpt1*, and *Angp2*) were observed in uterine tissue from mice treated with DINP on GD7; however, no effect on serum estrogen or progesterone was observed. On GD13, fetal and placental weight were significantly reduced, and histologic examination demonstrated alterations in placental architecture, reflected by a disorganized junctional zone, with cells from the junctional zone having infiltrated the labyrinth layer. The average area of the labyrinth was significantly reduced, while area of the junctional zone was increased by exposure to DINP. In the placenta, mRNA

expression of several genes involved in trophoblast differentiation (*i.e.*, *SynA*, *SynB*, and *Gem1*) and glucose transport (*i.e.*, *Slc2a1*, *Slc2a3*, *Slc6a1*, *Slc6a4*, and *Gjb2*) were downregulated following exposure to DINP. Overall, study authors concluded that the placental effects observed following exposure to DINP were the cause of fetal loss and reduced litter sizes. Although this study provides some evidence that DINP can reduced litter size and cause placental abnormalities, several limitations, particularly with regard to exposure methods, exist that reduce confidence in the study. Limitations include the fact that only a single dose level was evaluated and was administered via pipetting, which is a non-standard method of administration that contributes uncertainty related to the actual received doses. Additionally, effects in Bhurke et al. are difficult to reconcile with guideline studies that did not observe reductions in litter size or placental weight at doses greater than three orders of magnitude higher (*e.g.*, one- and two-generation studies by Waterman et al. ([2000](#))).

In Laws et al. ([2023](#)) adult female CD-1 mice (12–14 mice per dose) were fed diets containing 0, 0.15, 1.5 and 1,500 ppm DINP continuously for 11 months and then mated them with unexposed males after 11 months. Estrus cyclicity was examined after 1, 3, 5, 7, and 11 months of exposure (via vaginal lavage for 14 days at each timepoint). Study authors reported that these doses corresponded to mean received doses of 0.024, 0.24, and 240 mg/kg-day based on an assumption that a 25 g mouse eats a dose of approximately 5 grams of food per day. Treatment with DINP had no effect on body weight gain or food consumption throughout the 11-month study. Treatment with DINP had no significant effects on the amount of time mice spent in estrus or metestrus/diestrus after 1, 5, or 11 months of exposure. After 3 months of exposure, mice treated with 1.5 ppm DINP tended to spend more time in estrus and less time in metestrus/diestrus, while after 7 months of exposure, mice treated with 0.15 ppm DINP tended to spend less time in estrus and more time in metestrus/diestrus. However, the effect at both 3 and 7 months was not statistically significant, was not observed consistently across timepoints, and did not occur in a dose-dependent manner (no effect was observed at 1,500 ppm at either timepoint). Therefore, EPA did not consider the changes in estrous cyclicity to be related to treatment. After 11 months of exposure, DINP treated females were mated with untreated males. Treatment with DINP had no effect on mating index, pregnancy rate, fertility index, or dystocia rate. However, treatment with 1,500 ppm DINP led to a dose-related decrease in gestation index and birth rate, supporting a LOAEL of 240 mg/kg-day and a NOAEL of 0.24 mg/kg-day. Notably, this study is limited by large dose spacing between the mid- and high-dose groups (*i.e.*, 4 orders of magnitude).

In another study by the same research group, Santacruz-Márquez et al. ([2024](#)) fed adult (33 days old) female CD-1 mice diets containing 0, 0.15, 1.5, and 1,500 ppm DINP for 1 and 6 months. Mean achieved doses were reported to be 0, 0.024, 0.24, and 240 mg/kg-day DINP based on the previous study from ([Laws et al., 2023](#)). Body weight and food consumption were not evaluated as part of this study. Treatment with DINP had no effect on ovarian follicles after 1 month. After 6 months, treatment with 1,500 ppm DINP increased the percentages of primordial follicles and decreased the percentage of preantral and antral follicles. At 1 and 6 months, serum sex hormone levels were determined; however, treatment with DINP had no effect on serum levels of progesterone, testosterone, or estradiol. Treatment with 1.5 ppm DINP significantly decreased serum follicle stimulating hormone (FSH) levels after 1 month and increased FSH levels after 6 months; however, no effect on FSH levels were observed at 1,500 ppm. No effect on serum luteinizing hormone levels were observed after 1 month; however, luteinizing hormone levels were significantly decreased at 1.5 and 1,500 ppm after 6 months. Expression of genes that regulate FSH and luteinizing hormones in the pituitary were investigated. Treatment with DINP had no effect on mRNA expression of *Fshb* or *Lhb*, while *Nr5a1* and *Cga* mRNA was increased at 0.15 ppm after 6 months; however, these change in gene expression did not occur in a dose-related manner. Treatment with DINP similarly did not affect mRNA expression of steroidogenic genes (*i.e.*,

Star, *Hsd3b1*, *Hsd17b1*, *Cyp19a1*), follicle-stimulating hormone receptor (*Fshr*), or luteinizing hormone receptor (*Lhcgr*) in the ovary after 1 or 6 months.

Chen et al. (2022) gavaged (5 week old) female Kunming mice with 0, 2, 20, or 200 mg/kg-day DINP for 14 days and then evaluated ovarian histopathology and serum levels of estradiol at the end of study. Body weight and food consumption were not evaluated, nor were animals monitored for clinical signs. Study authors report that DINP caused follicular granulosa cells of the ovary to become disorganized; however, the doses at which this effect was observed is not stated. Serum estradiol levels were decreased by approximately 15 percent at 20 mg/kg-day DINP and above, but these data are difficult to interpret given that the time of outcome evaluation is likely to overlap with the onset of the first estrus in the mice. The authors also reported mechanistic evidence consistent with increased oxidative stress, increased apoptosis, and autophagy in ovarian tissue, as demonstrated by increased levels of apoptosis-related proteins (caspase-8, caspase-3, Bax), decreased levels of the anti-apoptosis protein Bcl-2, and increased levels of autophagy-related proteins (LC3-II/LC3-I, Beclin 1, Atg 5) at all doses of DINP. Levels of malondialdehyde were increased at 200 mg/kg-day, while levels of glutathione (at 20 mg/kg-day and above) and activities of glutathione peroxidase (at 2 mg/kg-day and above) and superoxide dismutase (at 20 mg/kg-day and above) were decreased, suggesting increased oxidative stress in ovary tissue. *In vitro* experiments with primary mouse ovarian granulosa cells were further conducted to investigate the mechanisms of DINP in the ovary. Similar to the *in vivo* study, DINP was found to induce apoptosis, autophagy, and oxidative stress in primary mouse ovarian granulosa cells. While these data may suggest perturbation of ovarian tissue following exposure to DINP, there were several limitations. For instance, no quantification of the histopathology data was provided (*i.e.*, only single representative images were provided, no incidence data, etc.), and insufficient detail is provided on the methods used to evaluate histopathology (*e.g.*, information on the number of animals examined per group). Other reporting deficiencies mentioned above contribute additional uncertainty. Altogether, these limitations impact the ability to interpret the results of the study.

Sedha et al. (2015) investigated the estrogenic potential of DINP in a three-day uterotrophic assay and a 20-day pubertal assay. For the uterotrophic assay, 20-day old female Wistar rats (6/dose/group) were gavaged with 0 (corn oil vehicle), 276, or 1380 mg/kg-day DINP (CASRN 68515-48-0) for three consecutive days, while an additional group was treated with diethylstilbesterol (40 µg/kg-day), which served as the positive control. Body weight gain was reduced in both DINP treatment groups compared to the control; however, treatment with DINP had no significant effect on uterine or paired ovary wet weight, while the positive control increased ovary and uterus wet weight. For the pubertal assay, 20-day old female Wistar rats were gavaged with 0 (corn oil vehicle), 276, or 1380 mg/kg-day DINP and diethylstilbesterol (6 µg/kg-day) from PND21 to sacrifice on PND41. Body weight gain was significantly reduced in all DINP treatment groups compared to the control. Absolute and relative uterine wet weight and vaginal weight were unaffected by treatment with DINP, while relative and absolute ovary weight was significantly reduced 10 to 28 percent by treatment with 1380 mg/kg-day DINP. Timing of vaginal opening was unaffected by treatment with DINP. Collectively, results from these assays indicate that DINP lacks estrogenic potential *in vivo*.

3.1.2.3 Conclusions on Reproductive and Developmental Toxicity

EPA previously proposed a MOA for male reproductive effects in rodents due to antiandrogenic activity of DINP as part of a proposed approach for cumulative risk assessment of phthalates (U.S. EPA, 2023a), which was supported by the SACC (U.S. EPA, 2023b). As outlined in Table 3-1, male reproductive effects were observed in 16 rat studies with gestational or perinatal exposures. Collectively, these data support EPA's conclusion that exposure of pregnant female rodents to DINP during gestation results in effects on male offspring consistent with androgen insufficiency.

Additional developmental studies in mice and rats were included in the data set covering a wide developmental window. Available studies included a one-generation study of reproduction in rats ([Waterman et al., 2000](#); [Exxon Biomedical, 1996a](#)) and two-generation study of reproduction in rats ([Waterman et al., 2000](#); [Exxon Biomedical, 1996b](#)), and a uterotrophic assay in rats ([Sedha et al., 2015](#)), along with multiple studies covering the pre-mating, gestation, and lactation periods. All studies were limited to oral exposures in rodents.

The evidence for effects on the female endocrine system and reproduction is less clear than the evidence supporting androgen insufficiency. The uterotrophic assay in rats showed decreased body weight gains, but no change to uterine or paired ovary wet weight ([Sedha et al., 2015](#)). In the pubertal assay, absolute and relative uterine wet weight and vaginal weight were unaffected by treatment with DINP, while relative and absolute ovary weight was significantly reduced at the high dose (1,380 mg/kg-day DINP). Sexual maturation (time to vaginal opening) was unaffected by treatment with DINP. In the study by Chiang and Flaws ([2019](#)) in which adult CD-1 female mice were administered DINP via oral gavage and mated with untreated male mice, there were no adverse effects of treatment on body weight, weights of the uterus or ovaries, time to mating, fertility index, gestational index, gestation length, the number of females able to produce pups, litter size, pup weight on PND20, pup mortality, or sex ratio. Several parameters were significantly different from controls (*e.g.*, decreases in fertility index and number of females able to produce pups and differences in estrous cycle; however, these findings were of uncertain toxicological significance, given the findings were often transient, and the non-monotonic dose relationship and the lack of mechanistic data from other studies supporting an effect of DINP on these endpoints. These limitations were also apparent in the two follow-up studies by Chiang et al. ([2020a, b](#)), which evaluated the effects of DINP on follicle populations and hormone levels at timepoints where fertility was disrupted in the prior study or at additional timepoints several months after exposure has ended. In contrast, Bhurke et al. ([2023](#)) report decreased litter size and gestation length in female CD-1 mice dosed with an extremely low dose of DINP (0.02 mg/kg-day). Santacruz-Márquez et al. ([2024](#)) report evidence of altered ovarian follicles in female CD-1 mice after 6 months of dietary exposure to DINP, while Chen et al. ([2022](#)) reports that DINP can induce apoptosis, autophagy, and oxidative stress in the ovaries of female Kunming mice. Laws et al. ([2023](#)) report that treatment with DINP does not alter estrous cyclicity in female CD-1 mice. Similarly, Santacruz-Márquez et al. ([2024](#)) found that treatment with DINP had no effect on serum estradiol in female CD-1 mice, while in contrast Chen et al. ([2022](#)) reported that DINP slightly reduced serum estradiol levels in female Kunming mice. Collectively, results from these assays generally indicate that DINP lacks estrogenic potential *in vivo*, and the results of *in vitro* receptor-binding assays ([Kruger et al., 2008](#); [Takeuchi et al., 2005](#); [Roy et al., 2004](#)) are consistent with the lack of effects in the uterotrophic and female pubertal assays in Sedha et al. ([2015](#)).

Skeletal variations ([Waterman et al., 1999](#); [Hellwig et al., 1997](#)) and reduced body weights were observed in rat pups across multiple studies ([Setti Ahmed et al., 2018](#); [Sedha et al., 2015](#); [Waterman et al., 2000](#); [Exxon Biomedical, 1996a](#)). Maternal body weights and food consumption were decreased in several studies on rats ([Setti Ahmed et al., 2018](#); [Waterman et al., 1999](#); [Hellwig et al., 1997](#)). The one generation reproduction study showed decreased live births and postnatal survival ([Waterman et al., 2000](#); [Exxon Biomedical, 1996a](#)). Two studies of yellow agouti mice dosed with 15 mg/kg-day DINP from 2 weeks prior to mating through lactation found increased pup body weights, altered body compositions, and decreased glucose tolerances ([Neier et al., 2019](#); [Neier et al., 2018](#)), as well as decreased birth rates ([Neier et al., 2019](#)). Although these data show different effects in mice and rats, the low number of studies in mice make it difficult to confidently determine species sensitivity.

Oral exposure to DINP has consistently been shown to cause developmental effects in animal models as illustrated by the studies described above and concluded by previous assessments by NTP-CERHR (2003), ECHA (2013b), EFSA (2019), Australia NICNAS (2012), Health Canada (EC/HC, 2015) and U.S. CPSC (2014, 2010). Therefore, EPA is considering developmental toxicity for dose-response analysis in Section 4.

3.2 Liver Toxicity

The non-cancer health effects and carcinogenicity of DINP have been evaluated primarily in animal toxicological studies; no human epidemiologic studies evaluating hepatic effects were identified by EPA's review of existing assessments (primarily Health Canada (2018a)). Moreover, existing assessments have consistently identified the liver as one of the most sensitive target organs following oral exposure to DINP in experimental animal studies (ECCC/HC, 2020; EFSA, 2019; EC/HC, 2015; ECHA, 2013b; NICNAS, 2012; U.S. CPSC, 2010; EFSA, 2005; ECB, 2003; NTP-CERHR, 2003; U.S. CPSC, 2001).

EPA identified twenty-five animal toxicology studies that evaluated non-cancer effects on the liver following intermediate (>1 to 30 days), subchronic (>30 to 90 days), or chronic (>90 days) oral exposure to DINP, and two following intermediate dermal exposure to DINP. Available studies include: 12 intermediate oral studies (7 studies on rats, 4 studies on mice, 1 study on cynomolgus monkeys); 9 subchronic oral exposure studies (6 on rats, 1 on mice, 1 on beagle dogs, and 1 on marmosets); 4 chronic 2-year oral exposure studies (3 on rats and 1 on mice); one-generation and two-generation studies of reproduction of rats that report non-cancer liver effects; and two intermediate dermal studies in mice. More detailed information on the available studies is provided in Appendix B, including information on individual study design.

Exposure to DINP resulted in adverse non-cancer effects on the liver across study designs. Adverse non-cancer effects such as increased absolute and/or relative liver weight consistently coincided with increased incidences of non-neoplastic lesions or changes in clinical chemistry parameters, indicative of liver toxicity. Adverse non-cancer effects on the liver were primarily observed in rats and mice of both sexes, although there was also evidence of hepatotoxicity from one study in beagles. Two studies in non-human primates with dose ranges comparable to those in the rodent and beagle studies did not provide evidence of non-cancer or pre-neoplastic effects on the liver following 14- (Pugh et al., 2000) and 90-day oral exposures to DINP (Hall et al., 1999). Changes in liver weights, histopathology, and clinical chemistry parameters in rodents coincided with mechanistic endpoints indicative of Peroxisome proliferator activated receptor alpha (PPAR α) activation, which is discussed further in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* (U.S. EPA, 2025a).

In general, intermediate (9 of the 12 studies) and subchronic duration studies (9 of 9) consistently reported increases in absolute and/or relative liver weight, sometimes in parallel with exposure-related histopathological effects on the liver (e.g., hepatocellular hypertrophy) or coinciding with increases in liver enzymes (e.g., ALT, AST, ALP), suggesting impaired liver function. These effects were generally dose-dependent, observed in both sexes, and in multiple species, including rats, mice, and beagle dogs. One 13-week study in marmoset monkeys reported non-statistically significant increases in liver weight, but there was no dose-response, and the authors attribute the lack of statistical significance to high variability and small sample size (Hall et al., 1999). More detailed study information for intermediate and subchronic studies is available in Appendix B within Table_Apx B-1, and Table_Apx B-2, respectively.

Three chronic 2-year studies in rats ([Covance Labs, 1998c](#); [Lington et al., 1997](#); [Bio/dynamics, 1987](#)) and one in mice ([Covance Labs, 1998b](#)) consistently reported non-cancer liver effects, while all except the Lington et al. (1997) study reported statistically significant increased incidences of liver tumors (*i.e.*, hepatocellular adenomas and/or carcinomas). Non-cancer liver effects that were observed across these four studies included consistent increases in liver weight that corresponded with histopathological alterations (*e.g.*, spongiosis hepatitis, necrosis) and/or increases in serum enzyme levels or activity in both sexes. An additional one- and two-generation study in rats by Waterman et al. (2000; [Exxon Biomedical, 1996a](#)) found increases in liver weight in the parental generation that coincided with minimal to moderate cytoplasmic eosinophilia in the liver. More detailed study information for intermediate and subchronic studies is available in Appendix B within Table_Apx B-1, and Table_Apx B-2.

The NOAEL and LOAEL for non-cancer hepatic effects in F344 rats in Lington et al. (1997) were 15 and 152 mg/kg-day, respectively; based on a statistically significant increase in the incidence of spongiosis hepatitis in mid-dose male rats that was accompanied by increased absolute and relative liver weights and changes in serum enzyme activities (*i.e.*, increased ALT and AST). These effects are also the basis for the LOAEL of 359 mg/kg-day (NOAEL of 88 mg/kg-day) in the Covance study (1998c) of F344 rats. The incidence of spongiosis hepatitis was dose-related and significantly increased in male rats exposed to DINP in both studies. Moreover, a Histopathology Peer Review and Pathology Working Group ([EPL, 1999](#)) independently evaluated the liver slides from rats chronically treated with DINP and confirmed that the incidence of spongiosis hepatitis was increased in male rats in each study. Bio/dynamics (1987) also reported a significant increase incidence of spongiosis hepatitis in male SD rats of the two highest dose groups, and dose-related trends in both males and females. Detailed information on lesion incidence is available in Appendix B within Table_Apx B-6.

Conclusions on Non-cancer Liver Toxicity

Collectively, intermediate, subchronic, and chronic studies of mice, rats, and beagles provide consistent evidence that oral exposure to DINP can cause liver toxicity. The lowest non-cancer NOAEL identified by EPA was 15 mg/kg-day based on increased liver weight, increase serum ALT and AST, and increased incidence of non-neoplastic lesions (*e.g.*, spongiosis hepatitis, enlargement, and granular and pitted rough changes in hepatocytes, central vein dilation, enlarged, discolored, congestion, oedema, and narrowing sinusoidal with loose cytoplasm) in 2-year study of F344 rats ([Lington et al., 1997](#)). EPA further considers liver toxicity for dose-response assessment in Section 4.

EPA summarizes the liver cancer associated with exposure to DINP in a separate technical support document, the *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025a](#)).

3.3 Kidney Toxicity

Kidney effects generally occur at higher doses than liver effects and occur inconsistently across study designs and species ([EFSA, 2019](#); [EC/HC, 2015](#); [ECHA, 2013b](#); [NICNAS, 2012](#); [U.S. CPSC, 2010](#); [EFSA, 2005](#); [ECB, 2003](#); [NTP-CERHR, 2003](#)).

Humans

Although the systematic review process used by Radke et al. identified five epidemiological studies that investigated the association between DINP and renal effects, the evidence was deemed inadequate.

Three of the five studies had critical deficiencies in exposure measurement, and the other two studies were of low confidence and had evidence of selection bias and reverse causality ([Radke et al., 2019a](#)).

EPA did not identify any new epidemiologic studies that examine the association between DINP and its metabolites and/or biomarkers of kidney injury.

Laboratory Animals

Many experimental animal studies have evaluated the kidney toxicity of DINP following oral exposure. Studies have evaluated the effects on kidney function (*i.e.*, urinalysis parameters, serum BUN levels), kidney weight, and histopathology. Seventeen studies are available that provide data on histopathological effects of the kidney, 16 of which also provide data on absolute and/or relative kidney weights. Six studies report changes in indices of kidney function such as serum BUN levels or urinalysis parameters. One study was available for the dermal exposure route ([Hazleton Laboratories, 1969](#)). No studies were available for the inhalation exposure route.

Intermediate (≥ 1 to 30 Days) Exposure Studies: EPA identified five intermediate studies in rodent models that provide data on the effects of DINP on the kidney ([Ma et al., 2014](#); [Kwack et al., 2010](#); [Kwack et al., 2009](#); [BIBRA, 1986](#); [Bio/dynamics, 1982a](#)). A study by Bio/dynamics ([1982a](#)) exposed male Fischer 344 (F344) rats to 0 or 2 percent (equivalent to 1,700 mg/kg-day) DINP for one week via feed and evaluated kidney weights and histopathology at study termination. Significant increases in absolute (7.5% increase) and relative (12.2% increase) kidney weights were observed in rats exposed to DINP. No abnormal histopathological findings were observed in the kidneys. Another study in F344 rats reported similar findings ([BIBRA, 1986](#)). BIBRA ([1986](#)) administered 0, 0.6, 1.2, 2.5 percent DINP for 21 days (equivalent to 0, 639, 1,192, 2,195 mg/kg-day [males]; 0, 607, 1,198, 2,289 mg/kg-day [females]) in the diet to male and female rats and evaluated kidney weights and histopathology at study termination. Dose-related increases in relative kidney weights were observed in males and females at all dose levels; the LOAEL was 639 and 607 mg/kg-day for males and females, respectively. No exposure-related histopathological findings were observed in the kidneys.

Not all intermediate studies reported dose-related changes in kidney weights that coincide with other effects of the kidney. Two studies in male SD rats reported no change in relative kidney weights and/or no change in BUN or other urinalysis parameters ([Kwack et al., 2010](#); [Kwack et al., 2009](#)), while another in B6C3F1 mice reported changes in weights without a consistent dose-related trend ([Hazleton Labs, 1991a](#)). The studies by Kwack et al. exposed male SD rats to 0 or 500 mg/kg-day DINP via gavage for 14 days ([Kwack et al., 2010](#); [Kwack et al., 2009](#)), while the Hazleton study ([1991a](#)) exposed mice to 0, 3,000, 6,000, or 12,500 ppm DINP for 4 weeks (equivalent to 0, 635, 1,377, 2,689, or 6,518 mg/kg-day [males]; 0, 780, 1,761, 3,287, or 6,920 mg/kg-day [females]). In the Hazleton study ([1991a](#)), significant increases in relative kidney weight were observed at the highest dose in males (6,518 mg/kg-day) and females (6,920 mg/kg-day), but significant decreases were observed at lower dose-levels in both sexes, which was also true for absolute kidney weights. Nevertheless, the increased relative kidney weights coincided with significant increased serum BUN levels in high-dose males and increased incidences of tubular nephrosis in all high-dose males and females, supporting an exposure-related effect on the kidney ([Hazleton Labs, 1991a](#)). In a study in which male Kunming mice were exposed to 0.2, 2, 20 or 200 mg/kg-day DINP for 14 days via gavage, Ma et al. ([2014](#)) reported significantly increased incidences in histopathologic lesions of the kidney, including large reduction in tubular space and extreme edema of epithelial cells in the glomeruli in animals exposed to the highest dose of DINP. However, this publication only described these findings qualitatively in text and did not include quantitative data on incidence or severity.

New Literature: EPA identified three new studies published from 2015 through 2024 that provided data on toxicological effects of the kidney following intermediate exposure to DINP ([Gu et al., 2021](#); [Liang and Yan, 2020](#); [Neier et al., 2018](#)). The developmental exposure study by Neier et al. ([2018](#)) reported no

change in absolute or relative kidney weights at PND21 in male and female yellow agouti (*Avy*) mice offspring. In that study, dams were administered 0 or 75 ppm DINP in the diet (equivalent to 15 mg/kg-day) beginning 2-weeks before mating and continuing through PND21.

In a dermal exposure study, Liang and Yan (2020) applied 0, 0.02, 0.2, 2, 20, or 200 mg/kg-day DINP to the shaved skin on the backs of male Balb/c mice (6/group) for 28 days and evaluated kidney weights, kidney histopathology, and markers of oxidative stress in kidney tissue at the end of the study (*i.e.*, ROS via DCF-DA assay, GSH content, MDA content, and DNA-protein crosslinks (DPCs)). Increased relative kidney weight was observed only in the high dose group (*i.e.*, 200 mg/kg-day), and there was no change in body weight or absolute kidney weight. Histopathological lesions were reported in the kidneys, including a “large reduction of tubular space and extreme edema of epithelial cells in the glomeruli.” Although representative images of the histopathological effects were provided, no quantitative data was provided, which impacts the interpretation of these results. Gu et al. (2021) evaluated the effects of lower doses of DINP on the kidney. Male ICR mice (8/group) were exposed to 0, 0.05, or 4.8 mg/kg-day DINP daily for 5 weeks and then the lipidomic profile of kidney tissues collected at the end of the study, and kidney weights and markers of oxidative stress were measured. No significant changes were observed on kidney weights, nor body weights of the mice exposed to DINP.

Subchronic (>30 to 90 days) Exposure Studies: EPA identified six dietary studies from existing assessments that provide data on the toxicological effects of DINP on the kidneys following subchronic oral exposure (Hazleton Labs, 1991b; Bio/dynamics, 1982b, c; Hazleton Labs, 1981; Hazleton Laboratories, 1971; Hazleton Labs, 1971) and one gavage study in marmoset monkeys (Hall et al., 1999). These studies provided data across a range of species and strains as well as both sexes. Increases in absolute and/or relative kidney weights and histopathological effects were reported in all of the studies, (Hazleton Labs, 1991b; Bio/dynamics, 1982b, c; Hazleton Labs, 1981; Hazleton Laboratories, 1971; Hazleton Labs, 1971), albeit the effects were sometimes attributable to decreased body weight. Dose-related increases in absolute and/or relative kidney weights sometimes corresponded with increased incidences of histopathological lesions or altered urine chemistry, but these trends were not consistent across all studies.

A study by Bio/dynamics labs (1982b) exposed F344 rats to 0, 0.1, 0.3, 0.6, 1.0, or 2.0 percent DINP for 13 weeks via feed (equivalent to 0, 77, 227, 460, 767, 1,554 mg/kg-day). Dose-dependent increases in kidney weight were noted in males, where doses as low as 227 mg/kg-day DINP resulted in increased absolute (9.7%) and relative (21.9%) weights. The increase in kidney weight was accompanied by a dose-dependent increase in dark brown discoloration in the kidney from 460 mg/kg-day. A similar study from Bio/dynamics labs (1982c) exposed Sprague Dawley rats to 0.3 or 1.0 percent DINP in the diet for 13 weeks (equivalent to 201 or 690 mg/kg-day [males]; 251 or 880 mg/kg-day [females]). The authors reported dose-related increases in absolute and relative kidney weights in males and females that corresponded with altered clinical chemistry parameters in males, most notably a dose-dependent decrease in triglycerides and increased calcium in high-dose males. The LOEL was 201 or 251 mg/kg-day for males or females, respectively.

These results were similar to three studies from Hazleton Labs (1991b, 1981, 1971), each using a different strain of rats. Hazleton Laboratories (1971) reported increases in absolute (9.3–17.6% increases) and relative (14.4–25.5% increases) kidney weight in male and female albino rats of the highest dose group (500 mg/kg-day). In that study, animals were exposed to 0, 50, 150, 500 mg/kg-day DINP for 13 weeks. Hazleton Labs (1991b) administered 0, 2,500, 5,000, 10,000, or 20,000 ppm DINP via diet to CDF (F344)/CrI/Br rats for 13 weeks (equivalent to 176, 354, 719, or 1,545 mg/kg-day [males]; 218, 438, 823, or 1,687 mg/kg-day [females]). Dose-dependent increases in absolute and

relative kidney weights were observed in both sexes, which coincided with a dose-related increase in granular casts and regenerative /basophilic tubules in the kidneys, beginning at 354 mg/kg-day in males. Hazleton Laboratories (1981) administered 0, 1,000, 3,000, or 10,000 ppm DINP to SD rats via feed for 13 weeks (equivalent to 0, 60, 180, and 600 mg/kg-day). A LOAEL of 60 mg/kg-day was identified based on an increased incidence of kidney lesions (focal mononuclear cell infiltration and mineralization) in exposed males. Absolute and relative kidney weights were also increased in males and females exposed to 600 mg/kg-day. Absolute weights increased 20 percent in males and 10.8 percent in females, while relative weight increased 17.7 percent in males and 13.7 percent in females.

Although there is ample evidence that the kidney is a target organ for DINP in rodents, the evidence is less consistent and less numerous across other species, including dogs, monkeys, and rabbits. Increased kidney weights were observed in high-dose animals in a study of beagle dogs by Hazleton Laboratories (1971), but were attributed to decreased body weight. In that study, animals were administered 0.125, 0.5, or 2 percent DINP in feed for 13 weeks (equivalent to 37, 160, or 2,000 mg/kg-day). The study also reported increased incidences of tubular epithelial cell hypertrophy in high-dose (2,000 mg/kg-day) males and females. Urinalysis parameters were comparable between control and test groups. In contrast, a study in marmoset monkeys by Hall et al. (1999) did not observe any kidney effects. In that study, male and female marmoset monkeys were exposed to 0, 100, 500, or 2,500 mg/kg-day DINP via gavage for 13 weeks. No histological findings were exposure related, and there were no changes in kidney weights. Similarly, no effects on the kidney were observed in a dermal study of New Zealand White rabbits exposed to up to 2,500 mg/kg-day DINP for 6 weeks (Hazleton Laboratories, 1969).

New Literature: EPA identified one new study published from 2015 through 2024 that provided data on toxicological effects of the kidney following subchronic exposure to DINP (Deng et al., 2019). Deng et al. (2019) exposed male C57BL/6 mice to 0, 0.15, 1.5 or 15 mg/kg-day DINP for 6 weeks via gavage. The authors reported vacuoles and hyaline degeneration in the glomerulus of the kidney, as well as smaller glomeruli and a thickened glomerular basement membrane. However, the authors do not specify at which doses the effects were observed and only single images are provided.

Chronic (>90 days) Exposure: EPA identified five rodent studies from existing assessments that provide information on the toxicological effects of DINP on the kidney, including four studies following chronic oral exposure to DINP (CASRN 68515-48-0) (Covance Labs, 1998b, c; Lington et al., 1997), or DINP (CASRN 71549-78-5) (Bio/dynamics, 1987), and one study following a one- or two-generation exposure in SD rats (Waterman et al., 2000). These studies provide data on absolute and/or relative kidney weights, histopathology, and urinalysis measures that reflect kidney function (i.e., BUN levels).

Lington et al. (1997) and Covance Labs (1998c) evaluated kidney weights, urinalysis parameters, and kidney histopathology in F344 rats following exposure to DINP for 2 years. Both studies observed increases in kidney weights in the mid- and high-dose animals but reported inconsistent results for urinalysis parameters and histopathology. Significant increases were observed in relative and absolute kidney weights in males and females of the mid- and high-dose groups (i.e., 152 and 307 mg/kg-day [males] or 184 and 375 mg/kg-day [females] at most time points (i.e., 6, 12, 18, and 24 months). Moreover, relative kidney weight at study termination was increased 10 to 20 percent and 7 to 10 percent in males and females, respectively. In the 2-year study by Covance Labs (1998c), increased relative kidney weights were observed in rats receiving dietary doses greater than 359 mg/kg-day for males (>25% increase) and 442 mg/kg-day for females (>14% increase) at study termination. Kidney weights in the recovery groups were comparable to the same-sex control values at the end of the 26-week recovery period.

In Lington et al. (1997), there were no exposure-related changes in serum chemistry parameters such as blood urea nitrogen (BUN). Some of the urine chemistry parameters were affected by DINP exposure in males. Increased urine volume, potassium, and glucose were observed in high-dose (307 mg/kg-day) males at most time intervals; potassium and glucose levels were also increased in mid dose males. Excretion of renal epithelial cells was increased in high-dose males at 6 months, but not at other timepoints. No urinalysis changes were observed in females. In contrast, Covance Labs (1998c) reported increases in serum urea (BUN) levels in males and females from the two highest dose groups at multiple timepoints during the study including study termination (*i.e.*, weeks 26, 52, 78, and 104). BUN was increased up to 32 percent over controls in the mid-dose (359 mg/kg-day [male] or 442 mg/kg-day [female]), and 50 percent over controls at the high dose (733 mg/kg-day [male] or 885 mg/kg-day [female]).

In Covance Labs (1998c), exposure-related increases in the severity of tubule cell pigment occurred in the kidneys of males exposed to 733 mg/kg-day DINP (Table 3-9). At study termination, a dose-related increase was observed in the incidence and severity of mineralization of the renal papilla in males at 359 and 733 mg/kg-day DINP as well as in the recovery group. Increased severity of tubule cell pigment was observed at the two highest dose groups in both sexes (Table 3-9).

Table 3-9. Incidence and Severity of Selected Non-neoplastic Lesions in the Kidneys of Male and Female F344 Rats Fed DINP for 2 Years (Covance Labs, 1998c)

	Dose Group mg/kg-day (ppm)					
	Control	29 M / 36 F (500)	88 M / 109 F (1,500)	359 M / 442 F (6,000)	733 M / 885 F (12,000)	Recovery ^a 637 M / 774 F (12,000)
Number M/F examined ^b	36/37	35/38	39/40	31/33	27/32	29/34
Mineralization of renal papilla (males)						
Minimal	6	11	9	6	2	0
Slight	0	0	0	24	1	2
Moderate	0	0	0	0	22	27
Total	6	11	9	30	25	29
Tubule cell pigment (males)						
Minimal	24	21	18	0	0	0
Slight	10	12	21	23	7	26
Moderate	0	1	0	6	17	3
Moderately severe	0	1	0	2	3	0
Total	34	35	39	31	27	29
Tubule cell pigment (Females)						
Minimal	22	27	34	4	0	1
Slight	14	10	5	27	21	33
Moderate	0	1	1	1	10	0
Moderately severe	0	0	0	1	1	0
Total	36	38	40	33	32	34

Source: Table 10D on page 350 of Covance Labs (1998c)

M = Male; F = female

^a The 12,000 ppm recovery group received 12,000 ppm DINP in the diet for 78 weeks, followed by a 26-week recovery period during which the test animals received basal diet alone.

^b Number examined at terminal sacrifice; does not include unscheduled deaths.

Bio/dynamics (1987) also conducted a 2-year chronic dietary study in rats, albeit of a different strain (SD), and noted significant increases in absolute and relative kidney weights in high-dose males at both the interim (19 and 25%, respectively) and terminal (13 and 12%) timepoints. Kidney weights of mid-dose group males (271 mg/kg-day) were increased by 11 percent, although this was not a statistically significant change. In high-dose females (672 mg/kg-day), increased relative kidney weights were observed (20% increase) at interim sacrifice as well as terminal sacrifice (14% increase). Increased incidence of medullary mineral deposits in the kidney were observed in high-dose males (25/70 treated vs. 3/70 controls). However, in females, incidences of renal medullary mineral deposits at the high dose (15/70) were comparable to controls (14/70). No histopathological evaluation was conducted on samples from the low- or mid-dose groups, which limits the assessment of dose-dependency and effect levels.

Waterman et al. (2000) assessed the potential kidney toxicity of DINP in one- and two-generation studies conducted in SD rats. In the one-generation study, absolute and relative kidney weights in both sexes were significantly increased at all doses, except in high-dose P1 females, and generally in a dose-related fashion. In the two-generation study, absolute kidney weights of P1 males and females were increased over controls at all DINP treatment levels. Although decreased mean body weights and body weight gains were also observed in P1 males and females for all doses, the changes in kidney weight are not solely attributable to changes in body weight. Increased incidence of minimal to moderate renal pelvis dilation was observed in F2 males of the two highest dose groups (0.4 and 0.8%, equivalent to 741–796, 1,087–1,186 mg/kg-day). No changes were observed in the females; therefore, the authors attributed the increased incidence of kidney lesions to induction of male rat-specific alpha 2u-globulin (α 2u-globulin).

In contrast to the studies in rats which consistently reported increases in relative and/or absolute kidney weight, a study in male B6C3F1 mice reported decreased kidney weights (Covance Labs, 1998b). In that study, male and female mice were exposed to 0, 1,500, 4,000, or 8,000 ppm DINP for 2 years via feed (equivalent to 0, 276, 742, or 1,560 mg/kg-day). No effects were observed in females. In addition to the weight changes in males, the authors reported significant increases in urine output, decreases in mean urine osmolality; and decreased sodium, potassium, and chloride levels in male and female mice from the 1,560 mg/kg-day dose group at 26, 52, 78, and 104 weeks. The study authors concluded that there was no DINP-related change in glomerular filtration rate; however, they suggested that this pattern of urinalysis findings may indicate a compromised ability to concentrate urine in the renal tubule epithelium, as an increased incidence of chronic progressive nephropathy was observed in high-dose females (1,888 mg/kg-day). The kidneys of 1,888 mg/kg-day females also had a granular pitted/rough appearance. The effects of DINP on the kidney, including decreased kidney weights in males, were partially attenuated in the recovery groups, which were evaluated 26 weeks after the end of exposure. The reversibility of the kidney effects in the recovery groups was not as pronounced as that for liver effects (Section 3.1). The incidences of chronic progressive nephropathy in female mice were comparable to those of the control group upon termination, suggesting that nephropathy is reversible or that exacerbation of this lesion halted when exposure to DINP was discontinued.

New Literature: EPA did not identify any new studies published from 2015 through 2024 that provided data on toxicological effects of the kidney following chronic exposure to DINP.

Mechanistic Information

EPA identified four *in vivo* studies that provide data that may inform mechanisms of action of the observed nephrotoxic effects of DINP. Mechanisms evaluated include oxidative stress in mice and male rat-specific α 2u-globulin.

Ma et al. (2014) evaluated the contribution of oxidative stress to the aforementioned tissue lesions observed in the kidneys of male Kunming mice, which were primarily observed at 200 mg/kg-day. In that study, mice were exposed to 0.2, 2, 20, or 200 mg/kg-day DINP for 14 days via gavage, and endpoints relevant to oxidative stress were evaluated in renal and hepatic tissue homogenates. Increases in reactive oxygen species (ROS) and MDA, in parallel with decreases in glutathione (GSH) content, were observed at 200 mg/kg-day DINP, indicative of oxidative stress. Some indices of oxidative stress were observed at lower doses than those that resulted in kidney lesions. Indeed, the authors also reported DNA-protein-crosslinks at 200 mg/kg-day and increases in 8-hydroxydeoxyguanosine (8-OH-dG) at 20 and 200 mg/kg-day, which indicate oxidative damage to DNA. Levels of interleukin (IL)-1 and tumor necrosis factor alpha (TNF α) were also increased at 20 and 200 mg/kg-day, which would be consistent with enhancement of an inflammatory response. The authors also evaluated the effect of combined exposure of 200 mg/kg-day DINP and melatonin (50 mg/kg-day). Mice exposed to 200 mg/kg-day DINP plus 50 mg/kg-day melatonin showed glomerular cell proliferation and mild renal tubule epithelial cell edema, and attenuated indices of oxidative stress (ROS, GSH, MDA, DNA-protein-crosslinks, and cytokine levels). These data indicate that melatonin can attenuate the oxidative stress that results from exposure to DINP in mice, but not fully attenuate damage to renal tissue, and support an MOA where oxidative stress may contribute to the toxicological effects of DINP on the kidney.

Both Liang and Yan (2020) and Gu et al. (2021) also evaluated the effect of DINP on oxidative stress. Liang and Yan (2020) applied 0, 0.02, 0.2, 2, 20, or 200 mg/kg-day DINP to the shaved skin on the backs of male Balb/c mice (6/group) for 28 days. In kidney homogenates, levels of ROS, MDA, and DPCs were significantly increased at 20 and 200 mg/kg-day, with concomitant decreases in GSH at the same doses. These data are further described in Appendix B. Gu et al. (2021) evaluated the effect of lower doses of DINP (*i.e.*, 0.05 and 4.8 mg/kg-day) in kidney tissues of male ICR mice exposed daily to DINP for 5 weeks. No changes in markers of oxidative stress were observed in the kidney, aside from a significant increase in GSH content in the 4.8 mg/kg-day dose group. However, a limitation of the data set is that it is difficult to interpret increases in GSH content without understanding changes in the ratio of reduced to oxidized glutathione. Collectively, studies by Ma et al. (2014) and Liang and Yan (2020) provide some evidence that DINP can induce ROS in the kidneys of male mice; however, ROS has not been evaluated in the kidneys of female mice or other species such as the rat.

Caldwell et al. (1999) followed up on observations from Lington et al. (1997), that kidney tumors were observed in male rats, but not female rats. The male-specific nature of the findings led them to evaluate a mechanism of action involving the male rat-specific α 2u-globulin. Tissue sections from male and female F344 rats at the 12-month interim sacrifice were evaluated. In male rats, a dose-dependent increase in α 2u-globulin accumulation was observed in regions of the kidney where increased cell proliferation was also observed. In parallel, tubular epithelial hypertrophy and tubular regeneration were observed. α 2u-globulin was not detected in the kidneys of female rats, and renal cell proliferation of DINP-exposed female rats was comparable to controls. These results are consistent a mechanism where α 2u-globulin accumulation leads to kidney tissue damage, cell proliferation, and subsequent neoplastic lesions of the kidney in male rats. The two-generation study by Waterman et al. (2000) also attributed their observations of renal pelvis dilation in the kidney of F2 male rats to induction of α 2u-globulin. However, these effects are not regarded as relevant to humans (Swenberg and Lehman-McKeeman,

1999; [U.S. EPA, 1991a](#)). Kidney tumors and evidence for an α 2u-globulin MOA are further discussed in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025a](#)).

Conclusions on Kidney Toxicity

Twenty studies in experimental animal models have evaluated toxicologic effects of DINP on the kidney following intermediate, subchronic, developmental, or chronic exposure to DINP. Findings were similar across study designs, including increased absolute and/or relative kidney weights, and observed in both sexes, but these data predominantly reflect rat studies, and the toxicological effects of DINP on the kidney is less certain in other species.

Increases in absolute and/or relative kidney weight have been observed primarily in rat studies across multiple study designs and often coincide with increased incidences of non-neoplastic lesions of the kidney or altered urinalysis parameters. Indeed, increased kidney weights were reported in two intermediate studies in F344 rats ([BIBRA, 1986](#); [Bio/dynamics, 1982a](#)), five subchronic studies in various strains of rats ([Hazleton Labs, 1991b](#); [Bio/dynamics, 1982b, c](#); [Hazleton Labs, 1981, 1971](#)), three chronic studies in rats ([Covance Labs, 1998c](#); [Lington et al., 1997](#); [Bio/dynamics, 1987](#)) and one developmental study in rats ([Waterman et al., 2000](#)).

In the 2-year study conducted by Lington et al. ([1997](#)), increased relative kidney weights of male and female rats were observed following exposure to dietary levels of 152 and 307 mg/kg-day (males) or 184 and 375 mg/kg-day (females). In the 2-year study reported by Covance Labs ([1998c](#)), increased relative kidney weights occurred in rats receiving dietary doses greater than 359 mg/kg-day for males and 442 mg/kg-day for females. Urinalysis findings from the chronic studies included significant increases in urine output and corresponding decreases in electrolyte levels in high-dose males, suggesting compromised ability to concentrate urine in the renal tubule epithelium. These effects occurred at the same dosages that produced changes in kidney weights. In the Covance Labs ([1998c](#)) study, serum urea levels (a marker of kidney toxicity) were significantly increased in rats exposed to 359 mg/kg-day and higher during the second half of the study. Increases in urine volume and kidney lesions were observed in the recovery group exposed to 733 mg/kg-day.

In many of the chronic studies, effects on the kidney generally occurred at doses equivalent to those where effects on the liver were observed in rats ([Covance Labs, 1998c](#); [Lington et al., 1997](#)) and mice ([Covance Labs, 1998b](#)). Moreover, the LOAELs ranged from 152 to 923 mg/kg-day which reflect effects on both the liver and kidneys, including increases in absolute and relative kidney weight as well as histopathologic findings in the kidney in two chronic studies of male rats ([Covance Labs, 1998c](#); [Lington et al., 1997](#)). The NOAEL in the Lington et al. study was 15 mg/kg-day (males) or 18 mg/kg-day (females). However, in a third chronic exposure study in rats ([Bio/dynamics, 1987](#)), effects on the kidney were observed, but not at the LOAEL, suggesting that the kidney may be less sensitive than the liver to the effects of DINP.

The findings of increased kidney weight in rats were inconsistent with one study of mice, which reported decreased absolute kidney weight in males (LOAEL = 276 mg/kg-day; NOAEL = 90 mg/kg-day in males) ([Covance Labs, 1998b](#)). That study also reported chronic progressive nephropathy in female mice of the high-dose group (1,888 mg/kg-day) but no effects in males ([Covance Labs, 1998b](#)). The lack of coherence of effects (*e.g.*, organ weight, histopathology data do not coincide in males or females) is a limitation of this study.

The MOA of kidney toxicity is not currently known, and effects on the kidney are primarily observed in one species (rats). Furthermore, kidney effects observed in the rat are less sensitive than effects on the

liver and on developmental outcomes. EPA is considering kidney toxicity for dose-response analysis in Section 4.

3.4 Neurotoxicity

Humans

Health Canada ([2018a](#)) evaluated multiple studies that investigated the association between DINP 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. Across available studies of DINP, Health Canada determined that the level of evidence for association between DINP and its metabolites and neurological effects could not be established.

Radke et al. ([2020a](#)) evaluated the association between DINP and neurodevelopment and found that there was no clear association between DINP and neurodevelopment. Three research studies examined the relationship between DINP and cognition; however, two of the studies found no relationship and one revealed an inverse relationship. As a result, the evidence supporting the relationship between DINP and cognition is deemed inconclusive. Because of the limited number of studies examining this relationship, the evidence linking DINP to motor ability is regarded as weak. The data supporting the link between boys' behavior and DINP found no increased odds of ADHD with DINP exposure, and the authors considered the evidence preliminary. Because of the inconsistent reports about the relationship between DINP and newborn neurobehavior, the evidence was considered indeterminate. The inconsistent nature of the currently available research renders the evidence for a connection between DINP and autism/social impairment as unclear.

New Literature: EPA identified eleven new studies (2 high quality and 9 medium quality) that evaluated the association between urinary DINP and neurological effects. The first high-quality study, by Shin et al. ([2018](#)), examined a subset of the of mother-child pairs from Markers of Autism Risk in Babies Learning Early Signs (MARBLES) cohort to evaluate the association between exposure to DINP metabolite (MCOP) and Autism spectrum disorder (ASD) and non-typical development (Non-TD). Among mothers who did not take prenatal vitamins, prenatal MCOP exposure during mid to late pregnancy was associated with higher risk of non-TD (vs. typical development) (MCOP RRR = 1.86 [95% CI: 1.01, 3.39]). Among mothers who did take prenatal vitamins, prenatal MCOP exposure during mid-to-late pregnancy was associated with lower risk of autism spectrum disorder (vs. typical development) (MCOP RRR = 0.49 [95% CI: 0.27, 0.88]). There was an association in multinomial logistic regression of MCOP during 2nd trimester and ASD (vs. TD) among mothers who took prenatal vitamins (RRR = 0.41 [95% CI: 0.21, 0.79]).

Another high-quality, cross-sectional study, by Jankowska et al. ([2019b](#)), conducted from a subset of the Polish Mother and Child Cohort (REPRO_PL), examined the association between Child behavioral and emotional problems at age 7 years as well as child cognitive and psychomotor development and DINP exposure. Negative associations in peer relationship problems were noted for sum DINP metabolites, and lower Intelligence and Development Scales (IDS) scores were generally positively associated with higher phthalate concentrations.

The first medium quality prospective analysis, by Balalian et al. ([2019](#)), of maternal prenatal and child age 3, 5, and 7 postnatal DINP metabolite (MCOP) exposures with motor skills at age 11 year as assessed by the short form of the BOT-2 were selected from participants in an ongoing longitudinal birth cohort study of mothers and newborns conducted by the Columbia Center for Children's Environmental

Health (CCCEH). MCOP measured at age 3 was inversely associated with BOT-2 total, fine motor, and gross motor composite scores among boys. In linear regression models, a 1 log-unit increase in age 3 MCOP was associated with lower total (beta: -3.08 [95% CI: $-5.35, -0.80$]), fine motor (beta: -1.64 [95% CI: $-3.16, -0.12$]), and gross motor (beta: -1.44 [95% CI: $-2.60, -0.28$]) composite scores in boys. Comparisons of the 4th vs. 1st quartiles of age 3 years MCOP was also associated with all three outcomes in boys (Q4 vs. Q1) total composite score (beta: -7.47 [95% CI: $-12.60, -2.34$]); fine motor composite score (beta: -4.18 [95% CI: $-7.51, -0.85$]); gross motor composite score (beta: -3.29 [95% CI: $-6.06, -0.52$]). No significant associations were found between MCOP at age 3 and outcomes in girls. There were no significant association for sex differences at age 3. There were also no significant associations between prenatal MCOP and outcomes in either girls or boys. There were no significant associations between MCOP measured at ages 5 or 7 and outcomes in either girls or boys.

A medium quality study, by Li et al. (2019), used data from children in the Cincinnati Health Outcomes and Measures of the Environment (HOME) cohort to analyze associations between DINP metabolites (MCOP, MCNP) and child cognition measured at ages 5 and 8 years. The pattern of associations for MCOP and MCNP measures was heterogeneous ($p < 0.20$ for MCNP), and no adjusted associations reached significance. Associations between child IQ scores and urinary MCOP measured at different ages were not statistically significant and were heterogeneous (positive and negative). For exposure at age 3 years, when associations with several other phthalate metabolites were significantly inverse, adjusted beta for MCOP was -1.2 (95% CI: $-3.2, 0.9$)

Another medium quality cohort study, by Tanner et al. (2020), examined mother-child pairs from the Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy (SELMA) study and the association between prenatal urinary DINP metabolite (MHiDP, MCNP, MHiNP, MOiNP, MCiOP) exposure and child IQ at age 7 years. Because this is a mixtures analysis, the DINP metabolites of interest were not directly analyzed as they were only above the threshold of concern in sensitivity analyses using positive weights.

A medium quality prospective cohort study, by Jankowska et al. (2019a), evaluated the association between prenatal and postnatal (age 2 years) OH-MINP and child behavior, cognition, and psychomotor development at age 7 years. The study included a subset of mother-child pairs from the Polish Mother and Child Cohort. There were no statistically significant associations between prenatal or postnatal OH-MINP and any of the study outcomes. There was also no clear pattern of associations with behavioral outcomes, and associations with cognitive and psychomotor scores were generally weakly negative. oxo-MINP was measured, but associations with outcomes were not analyzed, as detection rates were less than 70 percent (56 and 65% for pre- and postnatal measures, respectively).

A medium quality cohort study by Hyland et al. (2019) analyzed associations between prenatal DINP metabolites and neurodevelopment in live singletons in Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), a birth cohort of low-income Mexican American children in Salinas, California. Associations between IQ scores and MCOP were shown only for combined sexes, and not significant.

A medium quality longitudinal cohort study, by Jacobson et al. (2021), used data from the NYU Children's Health and Environment Study, to evaluate urinary DINP metabolites (MCiOP, MINP) levels in pregnant women and assessed the association with postnatal and postpartum depression following delivery. There were no significant associations for the Edinburgh Postnatal Depression Scale (EPDS) score or postpartum depression for sum DINP phthalates.

A medium quality study by Dzwilewski et al. ([2021](#)) used data from a subset of participants in the Illinois Kids Development Study (IKIDS) to evaluate associations between prenatal exposure to DINP metabolites (MINP, MCOP, MONP), and infant cognition assessed at 7 to 8 months of age. The authors presented results of analyses using the sum of 2 (DINP2) or 3 (DINP3) metabolites, and MONP individually. Associations varied by infant sex and by the set of images used in testing. DINP2 was associated with longer processing time for image set 2, and DINP3 with longer processing time among males viewing image set 2. DINP2 and DINP3 had weak negative associations with visual recognition memory (novelty preference). Urinary Σ DINP2 metabolites (MINP and MCOP) was associated with significant increases in average information processing speed (run duration) among infants viewing image set 2. DINP2 was also associated with a non-significant decrease in visual recognition memory (novelty preference). Urinary Σ DINP3 metabolites (MINP, MCOP, and MONP) were associated with significant increases in average information processing speed (run duration) among male infants viewing image set 2. DINP3 was also associated with a non-significant decrease in visual recognition memory (novelty preference) overall, while MONP was associated with a non-significant increase in novelty preference among infants viewing image set 2. DINP3 was associated with a non-significant decrease in visual attention (time to familiarization) for image set 2.

A medium quality case-cohort study by Kamai et al. ([2021](#)) nested in the Norwegian Mother and Child Cohort (MoBa) analyzed the association between prenatal DINP measured in spot urines at about 17 weeks' gestation and ADHD at age 3 years. DINP was non-linearly associated with increased odds of preschool ADHD. Results of multivariate logistic regression found an association between increasing DINP quintile 2 vs. quintile 1, OR = 2.04 (95% CI: 1.2 to 3.33; includes adjustment for DEHP).

The final medium quality study, a population-based nested case-control study by Engel et al. ([2018](#)), assessed the association of DINP metabolites and ADHD in children of at least 5 years of age of mothers within the Norwegian Mother and Child Cohort (MoBa). The authors reported no association of ADHD with sumDINP metabolites. In Bayesian logistic regression models, there was no association (OR= 0.85, [95% CI: 0.61, 1.15]) with log sum of DINP and ADHD. Associations with individual DINP metabolites were also not significant.

Laboratory Animals

A limited number of experimental animal studies have evaluated the neurotoxicity of DINP following oral exposure. Existing assessments of DINP have not drawn human health hazard conclusions on the neurotoxicity of DINP, but have evaluated effects on behavior, brain weight, and/or brain histopathology ([U.S. EPA, 2023c](#); [ECCC/HC, 2020](#); [U.S. CPSC, 2014](#); [ECHA, 2013b](#); [NICNAS, 2012](#); [ECB, 2003](#)). Only three rodent studies ([Boberg et al., 2016](#); [Ma et al., 2015](#); [Peng, 2015](#); [Boberg et al., 2011](#)) are available that are specifically designed to evaluate neurotoxicity. Remaining studies evaluated brain weight and/or brain histopathology. These included three subchronic exposure duration studies and three chronic studies, as well as six developmental exposure studies (*i.e.*, one- or two-generation studies of reproduction, perinatal, postnatal, or peri-and-postnatal exposure studies). No studies were available for the dermal or inhalation exposure routes.

One developmental study in Wistar rats ([Boberg et al., 2011](#)) reviewed in existing assessments ([U.S. CPSC, 2014](#); [NICNAS, 2012](#)) provides data on behavior, including an evaluation of learning and memory following DINP exposure. Boberg et al. ([2011](#)) exposed pregnant Wistar rats to 300, 600, 750, or 900 mg/kg-day DINP via oral gavage daily from GD7 through PND17 and evaluated several neurobehavioral endpoints on male and female offspring at later timepoints. Behavioral examinations included those of motor activity levels at PND27 through 28, Morris Water Maze (MWM) at 2 to 3 months of age, sweet preference at 4 months, and radial arm maze performance at 5 to 7 months of age.

The MWM test is used to evaluate learning and memory. In this test, animals are placed in a circular pool of water and required to escape from water onto a hidden platform using spatial memory. No changes were observed in motor activity levels and radial arm maze performances in male or female offspring exposed to DINP during development. An increase in saccharin intake in the sweet preference test was observed in female offspring of the 750 mg/kg-day group; however, this effect was not dose-dependent, and the study authors concluded that it may be a chance finding. In the MWM test, dose-dependent improvements in swim length and latency were observed on the first day of memory testing, with significantly shorter swim length and latency in the 900 mg/kg-day females. The study authors asserted that performance in the MWM test is sexually dimorphic, and concluded that DINP affected spatial learning, as female offspring performed better than controls and similarly to control males in the MWM, indicating masculinization of behavior in DINP exposed females. However, the effects were no longer apparent on the second day of memory testing or when the platform was moved to a new position in the maze. Performance was unaffected by exposure to DINP in males. Notably, the male reproductive parameters were affected at a lower dose than the apparent effects on learning in memory in females, with increased MNGs and decreased sperm motility at 600 mg/kg-day and above, increased nipple retention at 750 mg/kg-day and above, and decreased AGD at 900 mg/kg-day.

Several rodent studies were identified in existing assessments that provide data on absolute and/or relative brain weight following exposure to DINP. These include three chronic studies ([Covance Labs, 1998a, c](#); [Lington et al., 1997](#)) and two developmental studies ([Masutomi et al., 2003](#)). In general, changes in absolute and/or relative brain weight were not observed or were only observed at the highest doses tested in both males and females. No changes in brain index (*i.e.*, relative brain weight) were observed in male Kunming mice exposed to 1.5, 15, or 150 mg/kg-day DINP for 9 days via gavage ([Peng, 2015](#)). Similarly, no changes were observed in relative and/or absolute brain weight of: B6C3F1 mice exposed to up to 8,000 ppm DINP in feed for two years (equivalent to 1,600 mg/kg-day) ([Covance Labs, 1998b](#)); F344 rats exposed for 2 years to up to 12,000 ppm (equivalent to 733 mg/kg-day in males; 885 mg/kg-day in females) ([Covance Labs, 1998c](#)); or up to 0.6 percent (equivalent to 307 mg/kg-day in males; 375 mg/kg-day in females) ([Lington et al., 1997](#)). In contrast, changes in brain weight were observed in one perinatal exposure study ([Masutomi et al., 2003](#)). In Masutomi et al. (2003), maternal SD rats were fed test diets containing 0, 400, 4,000, or 20,000 ppm DINP from GD15 through PND10 (equivalent to 31, 307, or 1,164 mg/kg-day during gestation and 66, 657, or 2,657 mg/kg-day during lactation). Significant decreases in absolute brain weight were observed in male (12.9%) and female (11.1%) rat pups from the highest dose group at PND27, while significant increases in relative brain weight were observed in males (53.5%) and females (46%), which likely reflects decreased terminal body weight at PND27 in the highest dose group in both males and females. Body weight gain of male and female pups was decreased as well.

Data from existing assessments on the histopathological effects on the brain following DINP exposure have been reported. Identified literature includes one intermediate exposure duration study ([Midwest Research Institute, 1981](#)) and three chronic studies ([Covance Labs, 1998b, c](#); [Lington et al., 1997](#)). In general, there were no exposure-related histopathological findings in the 28-day exposure study by the Midwest Research Institute (1981) nor in the chronic exposure studies in mice ([Covance Labs, 1998b](#)) and rats ([Covance Labs, 1998c](#); [Lington et al., 1997](#)).

New Literature: Four new studies were identified by EPA that had not been reviewed in existing assessments ([Neier et al., 2018](#); [Setti Ahmed et al., 2018](#); [Ma et al., 2015](#); [Peng, 2015](#)), which provide data on neurobehavioral outcomes, brain weights, and brain histopathology following exposure to DINP. Results of Ma et al. (2015) and Peng et al. (2015) were not fully evaluated in the 2020 Health Canada Screening Assessment ([ECCC/HC, 2020](#)), and are therefore considered new literature.

Two intermediate exposure duration studies in male Kunming mice ([Ma et al., 2015](#); [Peng, 2015](#)) are available that provide data on behavior. Impaired learning and memory following DINP exposure was observed consistently across the two intermediate studies. Peng et al. ([2015](#)) and Ma et al. ([2015](#)) have similar study designs. Peng et al. ([2015](#)) exposed mice to 1.5, 15, or 150 mg/kg-day DINP daily via oral gavage for 9 days, while Ma et al. ([2015](#)) exposed mice to 0.2, 2, 20, or 200 mg/kg-day DINP daily via oral gavage for 14 days. In both studies, the authors evaluated the effect of DINP on learning and memory using the MWM test. In both studies, escape latency (*i.e.*, time it took mice to locate submerged escape platform) was evaluated throughout the exposure period (“training period”), and memory was evaluated on the last day of exposure (“probe trial”) following one day of no testing (a “forget” period). Each study also investigated the combined effect of DINP and an antioxidant; these endpoints are discussed in the mechanistic section. Mice were euthanized 24 h after the last DINP exposure, at which point brain tissue was harvested for histological examination as well as various non-apical measures of oxidative stress and inflammation (discussed in mechanistic information section below).

In both Ma et al. ([2015](#)) and Peng et al. ([2015](#)) escape latency in the MWM test was reduced in each exposure group at the end of the training periods compared to the first day. Escape latency was increased in all groups exposed to DINP compared to controls, indicating impaired learning in DINP groups. Peng et al. ([2015](#)) reported decreased retention time in the target quadrant in the MWM test during the probe trial, indicative of impaired memory. Similarly, Ma et al. ([2015](#)) reported decreased time and number of entries into the target quadrant in the MWM test during the probe trial, indicative of impaired memory. In addition to MWM, Ma et al. ([2015](#)) conducted an open field test to evaluate locomotor activity. Decreased time and number of entries into the central area were observed for mice exposed to 200 mg/kg-day DINP, which the authors attributed to anxiety-like behavior.

Four new rodent studies were identified that provide data on absolute and/or relative brain weight following exposure to DINP, three of which were oral exposure studies. These include one intermediate exposure duration study ([Peng, 2015](#)), and two developmental studies ([Neier et al., 2018](#); [Setti Ahmed et al., 2018](#)). In general, changes in absolute and/or relative brain weight were not observed, with the exception of one study weight in yellow agouti (A^{vy}) mice, where biologically significant (*i.e.*, >10% change) changes in brain weight were observed at the highest doses tested in male mice, which may be exposure-related. No changes in brain index (*i.e.*, relative brain weight) were observed in male Kunming mice exposed to 1.5, 15, or 150 mg/kg-day DINP for 9 days via gavage ([Peng, 2015](#)). Ahmed et al. ([2018](#)) observed similar results. In that study, pregnant Wistar rats (36 dams/group) were exposed to 0 or 380 mg/kg-day DINP via oral gavage beginning on GD8 and continuing up to PND30. Interim sacrifices were conducted on PND7, PND15, and PND21. Brain weight was determined at interim and terminal timepoints. No changes were observed in absolute brain weights (relative brain weights not reported) at PND7, PND15, or PND30. Body weight was significantly reduced in pups exposed to DINP at PND15 and PND30. In contrast to the findings of Ahmed et al. ([2018](#)), a developmental study by Neier et al. ([2018](#)) reported changes in relative brain weight in yellow agouti (A^{vy}) mice fed diets containing 5 ppm (equivalent to 15 mg/kg-day) DINP from 2 weeks prior to mating until weaning. The authors reported absolute and relative brain weights in PND21 offspring. Decreased relative brain weights were observed in PND21 males only, and no changes in absolute weights were observed. Increased terminal body weights were observed for females, but not males, at PND21, indicating that brain weight is decreased in males even when adjusted for body weight. Although it is likely this observation is exposure-related, uncertainty exists due to the use of the yellow agouti (A^{vy}) mouse model in the Neier study.

New data on the histopathological effects on the brain following DINP exposure have been reported. Identified literature includes two intermediate exposure duration studies ([Ma et al., 2015](#); [Peng, 2015](#)),

which both reported histopathological alterations in the pyramidal cells of the CA₁ region of the hippocampus following intermediate exposure to DINP via gavage. Ma et al. (2015) reported damaged pyramidal neurons in the 20 and 200 mg/kg-day dose groups. Peng et al. (2015) reported that with increasing DINP exposure, the arrangement of hippocampal cells became more disordered, cells swelled, and apical dendrites shortened or disappeared. Limitations of the histopathological data set from both studies include qualitative presentation of data that lacks incidence or severity information.

Mechanistic Information

EPA identified five *in vivo* studies and one *in vitro* study that provide data that may inform mechanisms of the observed neurological effects of DINP. Three of the *in vivo* studies investigated mechanisms involving oxidative stress in mouse models (Duan et al., 2018; Ma et al., 2015; Peng, 2015). The aforementioned studies by Peng et al. (2015) and Ma et al. (2015) exposed male Kunming mice to DINP via oral gavage daily for 9 or 14 days and evaluated several endpoints related to oxidative stress. Both studies observed increases in ROS, decreases in superoxide dismutase activity, decreases in GSH content, increases in inflammatory cytokines, and increases in caspase-3 levels, activity, or staining intensity at the highest dose (200 mg/kg-day) (Ma et al., 2015) or two highest doses (15 and 150 mg/kg-day) (Peng, 2015). Ma et al. also reported increases in DNA-protein-crosslinks at 200 mg/kg-day and increases in 8-OH-dG at 20 and 200 mg/kg-day, indicating oxidative damage to DNA. Although Ma et al. did not quantify histopathological changes observed in the hippocampus (Section 3.4), they quantified immunohistochemistry staining of glial fibrillary acidic protein, in addition to caspase-3 in the hippocampus CA₁ region and cerebral cortex. Staining intensity of caspase-3 and glial fibrillary acidic protein was increased at 200 mg/kg-day in both regions of the brain and increased in the cerebral cortex at the 20 mg/kg-day dose.

Both studies also evaluated the combined effects of the highest tested dose of DINP in addition to vitamin E or melatonin (*i.e.*, 150 mg/kg-day + 50 mg/kg-day vitamin E (Peng, 2015); or 200 mg/kg-day + 50 mg/kg-day melatonin (Ma et al., 2015)). Mice exposed to 200 mg/kg-day DINP plus 50 mg/kg-day melatonin had less caspase-3 and glial fibrillary acidic protein staining than DINP alone, indicating that melatonin can rescue the increase in caspase-3 and glial fibrillary acidic protein expression that follows DINP exposure. The addition of melatonin was also sufficient to attenuate the effects consistent with an oxidative stress response (*i.e.*, increases ROS, DNA-protein-crosslinks, 8-OH-dG, cytokines; decreases in superoxide dismutase activity and GSH content), implying that DINP induces oxidative stress in the cerebral cortex which contributes to neuronal damage (Ma et al., 2015). Similarly, Peng et al. (2015) observed that combined exposure of DINP + vitamin E, which has antioxidant properties, attenuated effects consistent with an oxidative stress response, implying that the observed effects were consequent to a pro-oxidant cellular environment in the brain.

In Duan et al. (2018), specific pathogen-free male Balb/c mice were divided into several groups designed to evaluate the impact of DINP on an allergic response to an ovalbumin (OVA) antigen. The authors also investigated the modulatory effect of melatonin, which they state has antioxidant properties, as well as the role of nuclear factor kappa B (NFκB) signaling and oxidative stress using an inhibitor of NFκB, dehydroxymethylepoxyquinomicin (DHMEQ). DINP exposure exacerbated effects consistent with an oxidative stress response in brain homogenates (*i.e.*, increase in ROS levels and decreases in superoxide dismutase activity). DINP also increased IL-1β and IL-17 levels in brain homogenates as well as nerve growth factor staining in the prefrontal cortex; all of which were attenuated by the combined exposure of DINP + melatonin or DINP + DHMEQ—suggesting that the inflammation is mediated by a pro-oxidant environment and activation of NFκB signaling. Other endpoints in this study included: brain histopathology of pyramidal cells in the prefrontal cortex, and immunohistochemistry

staining in the prefrontal cortex for eosinophil cationic proteins, nuclear factor erythroid 2–related factor 2 (Nrf2), NFκB. Limitations include lack on quantitative results for histopathology.

The other identified study provides a diverse set of data evaluating sexually dimorphic gene expression in relation to effects on sexual behavior in rodents ([Lee et al., 2006b](#)). Lee et al. investigated the effects of perinatal exposure to DINP on expression of sex-steroid-regulated genes in the hypothalamus of offspring and sexual behaviors as adults. Pregnant rats were administered 40, 400, 4,000, or 20,000 ppm DINP in the diet from GD15 through PND21. At PND7, male and female pups were sacrificed, and the hippocampus was dissected from brains to quantify expression of sexually dimorphic genes such as *granulin* (*grn*) and *p130*. After maturation, the authors evaluated and sexual behaviors (*e.g.*, lordosis, copulatory behavior), reproductive endpoints (*e.g.*, estrus cycles, serum levels of estradiol, LH, FSH); these data are discussed in detail in Section 3.1. In male PND7 pups, there was no change in hypothalamic *grn* expression, and a non-monotonic dose response was observed in *p130*, but expression was increased at all dose levels. In females, *grn* was increased in the 40 and 400 ppm, and 20,000 ppm exposure groups, and no change was observed in *p130*. While the increased *p130* expression in males coincided with impaired male sexual behavior (*i.e.*, decreased copulatory behavior), serum hormone levels (*i.e.*, testosterone, FSH, LH) were not changed. The authors suggest that DINP may act on regions of the hypothalamus that alter sexual behavior, but not gonadotropin secretion, to influence sex-specific adult behavior.

Conclusions on Neurotoxicity

Fifteen studies in experimental animal models have evaluated neurotoxicological endpoints (*i.e.*, behavior, brain weight, or histopathology) following exposure to DINP. However, only three of these were specifically designed to evaluate behavioral neurotoxicity, which typically may provide insight into more sensitive effects of DINP and supplement the neurobehavioral data from the epidemiological database.

Two intermediate duration exposure studies with similar designs in male Kunming mice ([Ma et al., 2015](#); [Peng, 2015](#)) provide consistent evidence for impaired learning and memory following DINP exposure for 9 or 14 days, with parallel perturbations in the pyramidal cells of the hippocampus at doses up to 200 mg/kg-day. The developmental exposure study by Boberg et al. ([2011](#)) exposed rats to doses up to 900 mg/kg-day from GD7 to PND17 and conducted behavioral examinations at later timepoints. No evidence of impairment was observed in males or females (2–3 months for MWM; radial arm maze performance at 5–7 months). One consideration regarding the study design in Boberg et al. ([2011](#)) is that a considerable amount of time had elapsed between the cessation of exposure and time of outcome evaluation, which could make it more difficult to detect an exposure-related effect (*i.e.*, bias towards the null), and this difference makes a direct comparison to the studies by Ma et al. ([2015](#)) and Peng ([2015](#)) challenging. However, this design also helps determine the extent to which perinatal exposures influence behavior later in life. Nevertheless, discordant results across these studies may reflect study design differences that influence the degree to which the received dose influences the test animals. Moreover, Ma et al. ([2015](#)) and Peng et al. ([2015](#)) exposed adult male Kunming mice and measured outcomes in adults, while Boberg et al. ([2011](#)) exposed pregnant rats and evaluated outcomes in the offspring. In addition to the inconsistent findings across study designs, a limitation of the behavioral data set is the relative lack of studies that consider outcomes in both sexes—especially given the fact that performance in the MWM test is sexually dimorphic.

Although histopathological alterations were observed in the pyramidal cells of the hippocampus in two independent intermediate exposure duration studies by Ma et al., ([2015](#)) and Peng et al., ([2015](#)), these studies were limited by the lack of quantitative data and were inconsistent with findings of the 28-day

exposure study by the Midwest Research Institute ([1981](#)) as well as all the chronic exposure studies in mice ([Covance Labs, 1998b](#)) and rats ([Covance Labs, 1998c](#); [Lington et al., 1997](#)). Strengths of the data set include coherence with the behavioral data sets from the Ma et al. ([2015](#)) and Peng ([2015](#)) studies; pyramidal cells of the hippocampus are involved in learning and memory, and the mechanistic data set from these studies provides evidence of biological plausibility via a mechanism involving ROS damage by DINP to the pyramidal neurons. Limitations of the data set include lack of quantitative results for incidence and severity of histopathology effects and lack of chronic exposure studies with histopathology of neural tissues.

Overall, available laboratory animal studies provide some evidence that DINP may cause behavioral effects in rodents. Although some uncertainty exists, EPA considered neurotoxicity further for dose-response analysis in Section 4. Specifically, neurobehavioral endpoints from Ma et al. ([2015](#)) and Peng ([2015](#)) were further considered.

3.5 Cardiovascular Health Effects

Humans

Health Canada ([2018a](#)) evaluated multiple studies that investigated the association between phthalate exposure and several cardiovascular outcomes and/or associated risk factors (*i.e.*, cholesterol, diastolic and systolic blood pressure, HDL-cholesterol, LDL-cholesterol, and blood glucose levels); however only two studies directly looked at evidence of an association between DINP and/or its metabolites and cardiovascular effects. A cross-sectional study of good quality by Trasande et al. ([2014](#)) looked at albumin/creatinine ratio (ACR), a biomarker of endothelial dysfunction and increased risk of CVD in children and adolescents found that there was inadequate evidence for an association between ACR and MCOP in children and adolescents ([Health Canada, 2018a](#)).

New Literature: EPA identified three new medium quality studies that evaluated the association between urinary DINP levels of metabolite and cardiovascular effects. The first medium quality study, a prospective birth cohort study, by Heggeseth et al. ([2019](#)), used data from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort to assess the association between prenatal urinary DINP measurements and BMI trajectories throughout childhood. The authors did not report any significant results; however, functional principal components analysis found that MCOP was an explanatory variable in variation of BMI trajectories among girls.

Another medium quality study, a cross-sectional and longitudinal analysis, by Diaz Santana et al. ([2019](#)), of participants from a nested case-control included using data from the Women's Health Initiative (WHI) evaluated the association between overweight and obesity as well as weight change and DINP exposure. The study found no significant results in cross-sectional analyses by quartile of exposure. However, there was significant association across quartiles with MCOP and overweight as well as obese women, with p-trend <0.001 and p-trend=0.001 respectively.

Finally a medium quality study, a longitudinal cohort study, by Zettergren et al. ([2021](#)), examined associations between DINP metabolites (MHiNP, MOiNP, MCiOP) and obesity measures through age 24y in a subset of participants in the Swedish Abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) cohort. The study found significant associations between increases in DINP metabolites at age 4y and obesity measures obtained at ages 8 years and above. Urinary MHiNP, MOiNP, MCiOP and DINP measures were significantly associated with an increased odds of overweight at ages 8, 16, and 24 years, and with higher BMI [$\beta = 1.60$ (95% CI: 0.37–2.84)], waist circumference ($\beta = 4.42$ [95% CI: 1.35–7.49]), body fat percent ($\beta = 2.65$ [95% CI: 0.52–4.77]),

and trunk fat percent ($\beta = 2.70$ [0.33–5.07]) at 24 years. The cross-sectional association between DINP metabolites and obesity at age 4 were not significant.

Laboratory Animals

A limited number of experimental animal studies have evaluated the cardiovascular effects of DINP following oral exposure. Existing assessments of DINP have not drawn human health hazard conclusions on the cardiotoxicity of DINP. Nevertheless, data are available on the effects of DINP on blood pressure, heart rate, other indicators of adverse cardiac events, heart weight and/or heart histopathology ([U.S. EPA, 2023c](#); [NICNAS, 2012](#); [U.S. CPSC, 2010](#); [ECB, 2003](#)). Only one study was available that was specifically designed to evaluate cardiotoxicity ([Deng et al., 2019](#)). Remaining studies evaluated heart weight and/or heart histopathology ([Kwack et al., 2009](#); [Lington et al., 1997](#); [Bio/dynamics, 1982b](#); [Midwest Research Institute, 1981](#)). No studies are available for the dermal or inhalation exposure routes.

Three studies of varying study designs were identified that provide data on the effect of DINP exposure on heart rate, blood pressure, or other indicators of adverse cardiac events, including levels of total cholesterol and triglycerides. A subchronic duration study by Deng et al. ([2019](#)) investigated the mechanisms associated with increased blood pressure following exposure to DINP. Groups of C57BL/6 mice were administered 0, 0.15, 1.5 or 15 mg/kg-day DINP via oral gavage daily for 6 weeks. At study termination, systolic blood pressure, diastolic blood pressure, mean blood pressure, and heart rate were measured. Additionally, blood samples were collected for measurements of serum nitric oxide levels and levels of angiotensin converting enzyme (ACE), angiotensin-II type 1 receptor (AT1R), and endothelial nitric oxide synthase (eNOS), were evaluated via immunohistochemistry staining. Increased systolic, diastolic, and mean blood pressure was observed in mice of the two highest dose groups (1.5 and 15 mg/kg-day). Immunohistochemistry of the aorta showed increased staining intensity of ACE and AT1R as well as decreased staining intensity of eNOS and nitric oxide. These latter endpoints are discussed more in detail below under *Mechanistic Information*.

One additional study is available that provides data on changes in triglycerides and cholesterol following intermediate duration exposure to DINP ([Kwack et al., 2009](#)). Kwack et al. exposed male SD rats to 0 or 500 mg/kg-day DINP daily for 4 weeks via oral gavage and evaluated several cardiovascular outcomes including serum levels of total cholesterol and triglycerides. Serum triglycerides were significantly increased (50% increase compared to controls), while no change was observed in serum total cholesterol.

Three studies were identified that provide data on the effect of DINP on heart weight, including one intermediate exposure duration study in male SD rats ([Kwack et al., 2009](#)), one intermediate study in male and female F344 ([Bio/dynamics, 1982b](#)), and one chronic study in male and female F344 rats ([Lington et al., 1997](#)). In general, no statistically or biologically significant (*i.e.*, >10% change) exposure-related changes in absolute or relative heart weight were observed across studies.

Two studies were identified that report histopathology of the heart and/or aorta following exposure to DINP. The subchronic study in male mice by Deng et al. ([2019](#)) also evaluated histopathology of the heart and aorta. Lesions were observed in the high-dose group (15 mg/kg-day), including ventricular wall thickening and cardiomyocyte hypertrophy. In contrast, the study by the Midwest Research Institute ([1981](#)) did not observe discernable lesions in the heart at study termination. In this study, male and female F344 rats were exposed to 0, 0.2, 0.67, or 2 percent DINP for 28-days via feed (estimated doses: 0, 150, 500, 1,500 mg/kg-day [males]; 0, 125, 420, 1,300 mg/kg-day [females]). A limitation of these studies is that histopathology was reported qualitatively.

Table 3-10. Summary of Study Evaluating Cardiovascular Outcomes

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Comments
C57BL/6 mice (males only); oral gavage; 0, 0.15, 1.5, 15 mg/kg-day; 6 weeks; with or without induction of hypertension (Deng et al., 2019)	0.15/ 15	↑ in systolic, diastolic, and mean blood pressure; ventricular wall thickening & cardiomyocyte hypertrophy; immunohistochemistry of aorta showed ↑ACE & AT1R & ↓eNOS & NO.	<u>15 mg/kg-day:</u> ↑ Heart Rate and diastolic blood pressure. Pathological changes in the heart, aorta, and kidney <u>Kidney histopathology (qualitative only):</u> Study authors also state that “DINP exposure and DEXA treatment could both induce vacuoles and hyaline degeneration in the glomerulus as compared to the saline group. EPA also found that DINP exposure resulted in smaller glomeruli and a thickened glomerular basement membrane, and that ACEI effectively inhibited these lesions.” Doses at which this occurred are not stated.

Mechanistic Information

EPA identified one *in vivo* study ([Deng et al., 2019](#)) that provides data that may inform mechanisms of the observed cardiovascular effects of DINP. The mouse study by Deng et al. (2019) investigated mechanisms associated with increased blood pressure following exposure to DINP. Groups of C57BL/6 mice were exposed to 0, 0.15, 1.5, or 15 mg/kg-day DINP daily for 6 weeks via gavage. Parallel groups of mice also received a subcutaneous injection of 1 mg/kg-day dexamethasone to induce hypertension and/or 5 mg/kg-day of an ACE inhibitor via gavage in addition to the highest dose of DINP. In addition to the evaluations of blood pressure described above, the authors measured serum nitric oxide (NO) levels and determined levels (*i.e.*, staining intensity) of ACE, AT1R, and eNOS in the aorta via immunohistochemistry staining. The authors observed increased staining intensity of ACE and AT1R as well as decreased staining intensity of eNOS in the aorta using immunohistochemistry following exposure to 1.5 or 15 mg/kg-day DINP (AT1R and eNOS) or all doses (ACE). Co-exposure of 15 mg/kg-day DINP and dexamethasone resulted in similar changes in expression of ACE, AT1R, and eNOS. Co-exposure of dexamethasone + 15 mg/kg-day DINP + the ACE-inhibitor did not fully attenuate the changes. Serum levels of NO were decreased following DINP exposure (all doses) as well as with co-exposure to dexamethasone and/or the ACE inhibitor. Given the aforementioned increases in systolic, diastolic, and mean blood pressure, in mice of the two highest dose groups (1.5 and 15 mg/kg-day), these results provide some evidence to support a mechanism whereby DINP acts through the ACE pathway to increase blood pressure.

Conclusions on Cardiovascular Health Effects

The database of studies in experimental animals that has evaluated cardiovascular toxicity and associated risk factors following exposure to DINP is limited and findings were generally inconsistent across study designs and species. Only one subchronic study was available that was specifically designed to evaluate cardiotoxicity ([Deng et al., 2019](#)). Limitations of the study included failure to consider both sexes and reporting deficiencies, including the qualitative reporting of histopathology data. Nevertheless, the consistency across endpoints within Deng et al. (2019), including increased blood pressure and histopathological effects in the aorta suggest that DINP may be toxic to the cardiovascular system. Mechanistic data from the same study suggest the underlying mechanism for these effects involves the ACE pathway.

Overall, there is limited evidence that DINP can elicit cardiotoxicity in experimental laboratory animals. Only one study in one species of one sex evaluates cardiovascular outcomes ([Deng et al., 2019](#)). Additionally, the clinical implications, or relevance to humans, is uncertain for cardiovascular effects of DINP. Due to these limitations and uncertainty, EPA is not further considering cardiotoxicity for dose-response analysis.

3.6 Immune System Toxicity

Humans

Health Canada ([2018a](#)) evaluated multiple studies that investigated the association between urinary metabolite and immunological outcomes. Across available studies of DINP, Health Canada found that there was limited or inadequate evidence for association between DINP and its metabolites and immunological outcomes.

New Literature: EPA identified three new studies (two medium quality studies and one low quality) that evaluated the association between DINP and its metabolites and immune/allergy outcomes. The first medium quality study, a prospective birth cohort, by Soomro et al. ([2018](#)), of the Etude des Déterminants pré et postnatals du développement de la santé de l'Enfant (EDEN) study measured one maternal urinary DINP metabolite (MCOP) and its association with eczema diagnosed at ages 1-5 in boys, and with elevated serum IgE at age 5 years. Results for the main effect association between MCOP and elevated IgE were described only as not significant for MCOP. There were no significant associations found with MCOP and elevated serum IgE (≥ 60 IU/mL). However, multivariate logistic regression of MCOP and odds of diagnosed eczema was only significant for age 5 (OR = 1.60 [95% CI: 1.16, 2.23]). There was a significant association found in multivariate logistic regression of MCOP and association with early onset eczema (first 2 years of life) (OR = 1.29 [95% CI: 1.04, 1.60], $p < 0.05$) and late-onset (age 3–5 years) eczema (OR = 1.63 [95% CI: 1.20, 2.21], $p < 0.05$). There was also a significant association in Cox proportional hazard model of MCOP and ever diagnosed with eczema (HR = 1.09 [95% CI: 0.95, 1.25], $p = 0.05$).

Another medium quality study, a cross-sectional study, by Ait Bamai et al. ([2018](#)), that used data from Hokkaido study on Environment and Children's Health examined the association between DINP and eczema within the past 12 months. Logistic regression of DINP ($\mu\text{g/g}$ dust) exposure on eczema found significant gene-environmental interaction with FLG mutation (OR total = 1.17 [95% CI: 0.91, 1.52]; $p = 0.039$). No other significant associations were found between eczema and DINP exposure.

Finally, a low quality cohort study by Wan et al. ([2021](#)), which used data from the Kingston Allergy Birth Cohort (KABC), examined the association between skin prick testing and DINP exposure. The authors did not find any statistically significant results in adjusted logistic regression models for DINP exposure relation to allergic sensitization.

Laboratory Animals

A limited number of studies are available that have been evaluated for the toxicological effects of DINP on the immune system. Available studies have provided data on the adjuvant properties of DINP; an adjuvant is a substance that can enhance immune responsiveness without itself being an antigen. ECB ([2003](#)) summarized the irritation and sensitization data and determined that DINP is a very slight skin and eye irritant, with effects reversible in short time. The U.S. CPSC ([2010](#)) concluded that “*in vivo* studies in guinea pigs suggest that DINP is not a skin sensitizer”; however, “*in vivo* studies in mice show that DINP or other o-DAP's may augment an antigen mediated IL-4, IgE, and/or IgG1 reaction.” These findings suggest that DINP may potentiate allergic and/or asthmatic responses.

The database of studies from existing assessments that evaluate the immune adjuvant effects of DINP is limited to two studies ([Koike et al., 2010](#); [Imai et al., 2006](#)), which investigate the effects of DINP on atopic dermatitis and skin sensitization.

Koike et al. ([2010](#)) investigated the effect of DINP on atopic dermatitis resulting from contact with a dust mite allergen. Male NC/NgaTndCrlj mice were injected intradermally on the ventral side of their right ears with saline or extract of the dust mite, *Dermatophagoides pteronyssinus* (Dp) on study days 0, 3, 5, 8, 10, 12, 15, and 17. On study days 2, 5, 9, and 16, DINP was administered via intraperitoneal (i.p.) injection dose levels: 0, 0.15, 1.5, 15, or 150 mg/kg-day. The authors evaluated several endpoints including histopathology of the ears, protein expression (from ear homogenates) of Th₁-type vs. Th₂-type cytokines, as well as chemokines such as eotaxin, eotaxin-2, and thymic stromal lymphopoietin (TSLP), via ELISA. DINP exposure significantly increased ear thickening and macroscopic features of the ears from 4 and 6 days after the first injection of Dp. However, no dose-dependent effects of DINP were observed. Animals exposed to 15 mg/kg-day DINP + Dp had more skin lesions when compared to animals exposed to Dp or saline (no Dp). Histopathological evaluation of the ears showed that while Dp had increased infiltration of eosinophils into the skin lesions when compared with saline controls, 15 mg/kg-day DINP + Dp potentiated the infiltration of eosinophils into the skin lesion (compared to Dp) in parallel with increased mast cell degranulation. Alterations in cytokine levels were observed in the ears of animals exposed to Dp (compared to saline), including increased IL-4, -5, and -13 and decreased interferon- γ (IFN- γ). There was a decrease in expression of IFN- γ , eotaxin and eotaxin-2, and increased expression of TSLP were also observed in the ears of mice exposed to DINP, compared to those exposed to Dp + vehicle. These data suggest that DINP aggravates allergic dermatitis-like skin lesions caused by the Dp antigen. To evaluate the adjuvant capacity of DINP for immunoglobulin (Ig) production, the authors also measured serum levels of anti-DP-IgG₁, IgE, as well as histamine release. Intradermal injection of Dp increased the levels of Dp-specific IgG₁, total IgE, and histamine levels in serum compared to saline alone. Exposure to DINP significantly increased histamine levels in serum compared to saline alone. However, no significant changes in serum levels of Dp-specific IgG₁, total IgE, or histamine were observed in groups exposed to DINP compared to Dp. Collectively, these data support the conclusion that DINP is not an adjuvant in an atopic dermatitis mouse model.

Imai et al. ([2006](#)) investigated whether different phthalate esters (including DINP) have adjuvant effects on skin sensitization using FITC as a sensitizer. Female CD-1 (ICR) and BALB/c mice were used for this skin sensitization study. Experimental groups include having multiple phthalates mixed with acetone at a 1:1 ratio and the control group with acetone alone. ICR mice were epicutaneously sensitized with FITC dissolved in an acetone solution containing one of various phthalate esters, including DINP. The applications on the forelimbs were repeated on day 7 and on day 14; ear thickness and ear swelling were measured. There were no significant differences in ear thickness/swelling between the DINP treated group compared to the acetone control group. Similar results with DINP were confirmed using BALB/c mice. Twenty-four hours following skin sensitization, draining lymph node cells were examined for FITC fluorescence by means of flow cytometry. Mice sensitized with FITC in acetone containing DINP did not show consistent ear-swelling response. DINP also showed no significant increase in the FITC-positive cell number in the draining lymph nodes. These data suggest that DINP does not act as an adjuvant in a FITC skin sensitization model in mice.

New Literature: EPA identified two new studies that investigated the effects of DINP exposure on atopic dermatitis ([Wu et al., 2015](#); [Sadakane et al., 2014](#)).

Wu et al. (2015) investigated the effects of DINP on allergic dermatitis in a FITC-induced allergic dermatitis model and the role of oxidative stress and inflammatory factors in skin lesions of the model mice and characterize the mechanism involved in the DINP. Additionally, uncovering the protective role of melatonin (MT) on allergic dermatitis and exploring its mechanism as an antioxidant. Forty-nine male Balb/c mice were divided randomly into seven groups: control, melatonin (30 mg/kg-day) 3 hours after saline skin exposure, 0.5 percent FITC-sensitized group (FITC), 1.4 mg/kg-day DINP skin exposure+0.5 percent FITC-sensitized group (FITC + DINP1.4), 14.0 mg/kg-day DINP skin exposure+0.5 percent FITC-sensitized group (FITC+DINP 14), 140.0 mg/kg-day DINP skin exposure+0.5 percent FITC sensitized group (FITC+DINP 140), and MT (30 mg/kg-day) 3 hours after 140.0 mg/kg-day DINP skin exposure combined with 0.5 percent FITC sensitized group (FITC+DINP 140.0+MT). The mice were exposed for 40 days, then given saline or FITC on days 41 and 42. Sensitization was terminated on day 47 to measure ear thickness. This experiment was terminated on day 48 and blood samples were collected to measure IgE levels and immunohistochemistry were conducted on the sections from the right ear for TSLP, p-STAT3, p-STAT5, p-STAT6, NF- κ B, and p65. Markers of oxidative stress—including ROS, MDA, GSH, along with cytokines, IL-4 and IFN- γ —were evaluated from the ear tissue.

The highest concentration of DINP (140 mg/kg-day) with FITC significantly increased the number of infiltrating inflammatory cells when compared with the FITC exposed only group. Moreover, the pathological alterations and the number of infiltrating inflammatory cells were alleviated in the FITC+DINP 140+MT group as compared with the FITC+DINP 140 group. Ear swelling and bilateral ear weight were significantly altered in all FITC-immunized groups. Dermal DINP exposure significantly increased ear swelling and bilateral ear weight when compared to the group exposed to FITC only, and this adverse effect was potentiated. Also, when MT was added, it diminished the DINP-induced ear swelling and the bilateral ear weight when compared to the same concentration of DINP without MT. FITC alone and all concentrations of FITC+DINP exposure significantly enhanced serum T IgE levels, at all concentrations. The highest dose of DINP (140 mg/kg) exposure drastically elevated serum T-IgE levels compared with the FITC-sensitization only group. Further, T-IgE levels in the FITC + DINP 140 group significantly decreased when compared to the FITC+DINP 140+MT group. Compared with the FITC only group, co-exposure with any concentration of DINP induced a significant increase in IL-4, IL-5, and a resulting skew in the ratio of IL-4 to IFN- γ . These adverse effects exacerbated by DINP were concentration-dependent. However, MT alleviated the DINP-induced effects, suggesting that DINP is associated with Th2 cytokine expression by FITC-mediated allergic inflammation. Their results of histopathological examinations and measurements of ear swelling as well as immunological and inflammatory biomarkers (total-immunoglobulin IgE and Th cytokines) supported their conclusion that high doses of DINP might aggravate atopic dermatitis.

Lastly, Sadakane et al. (2014) investigated the role of DEHP and DINP on atopic dermatitis at doses lower than the NOAEL for chronic liver toxicity (*i.e.*, 15 mg/kg-day). Herein, only results for DINP are discussed. The study included a control and 4 experimental treatment groups, each of which included 12 male NC/Nga mice. Animals in the experimental groups were exposed to the allergen, *Dermatophagoides pteronyssinus* (Dp), by subcutaneous injection of 5 mg of Dp dissolved in 10 mL of saline in the ventral side of the right ear for 2 to 3 days a week (a total of 8 times) under anesthesia. Animals in the experimental DINP groups were exposed to the allergen and treated with 0 (Dp + vehicle), 6.6 μ g DINP/animal (Dp + DINP 6.6), 131.3 μ g DINP/animal (Dp + DINP 131.3), or 2,625 μ g DINP/animal (Dp + DINP 2625). In the experimental groups, mice were orally administered DINP dissolved in 0.1 mL of olive oil 5 days before the first injection of the allergen. Control group animals (saline + vehicle and Dp + vehicle groups) were not exposed to DINP and were orally administered 0.1 mL of olive oil.

Twenty-four hours following Dp injections, skin disease symptomatology and ear thickness were evaluated and scored for symptoms of skin dryness and eruption, edema, crusting and erosion. Also, the clinical scores of the Dp + DINP 6.6 and Dp + DINP 131.3 groups began increasing when compared with the Dp + vehicle group from day 16, the Dp + DINP 131.3. The Dp + DINP 131.3 group had a higher (not significant) wound score compared with the Dp + vehicle group while the Dp + DINP 2625 did not change. Statistical tests revealed no significant differences between DINP treated groups and the control at any doses to contribute to ASDLs. The dorsal skin of the Dp-treated groups with or without DINP exposure exhibited epidermal and dermal thickening, eosinophil accumulation and mast cell degranulation. The eosinophil counts of both DP+DINP treatments increased but not significantly. However, oral exposure to DINP did not increase the eotaxin levels. Exposure to DINP modestly increased mean total IgE levels. The rank of mean skin scores with specific DINP doses (Dp + DINP 131.34 > Dp + DINP 6.64 > Dp + DINP2625 > Dp + vehicle) was found to be strongly positively correlated with the number of eosinophils, the number of severely degranulated mast cells, and moderately positively correlated with the total number of mast cells. Overall, this study provides some evidence that DINP can aggravate the allergic response in animal allergic dermatitis models.

Mechanistic Information

EPA identified seven studies that describe the mechanism of action for the adverse immunological effects of DINP ([Yun-Ho et al., 2019](#); [Duan et al., 2018](#); [Kang et al., 2017](#); [Kang et al., 2016](#); [Chen et al., 2015](#); [Koike et al., 2010](#); [Lee et al., 2004](#)).

Koike et al. ([2010](#)) evaluated the adjuvant effects of DINP on bone-marrow-derived dendritic cells or splenocytes *in vitro*. Bone-marrow-derived dendritic cells and splenocytes were exposed to DINP for 24 hours at concentrations of 0 (control), 0.1, 1, 10, and 100 μ M. At 100 μ M, DINP exposure for 24 hours led to significantly increased the production of Th₂ chemokines, TARC/CCL17 and MDC/CCL22, in bone-marrow-derived dendritic cells when compared with vehicle control. However, Th₁ cytokine IL-12p40 was not detected in any bone-marrow-derived dendritic cell culture. Moreover, DINP also significantly increased the expression of the chemokine receptors CCR7, CXCR4, MHC class II, CD80, and CD86 on bone-marrow-derived dendritic cells compared with controls. DINP exposure for 24 hours significantly increased IL-4 production from splenocytes compared with controls. After 72-hours of exposure to DINP in the presence of Dp, there was a significant increase in proliferation of splenocytes at 0.001 to 1 μ M and decreased proliferation at 10 μ M compared with controls. These results show that DINP augmented IL-4 production and Dp-stimulated proliferation of splenocytes and suggest that DINP can aggravate allergic dermatitis-like skin lesions through TSLP-related activation of dendritic cells and by direct or indirect activation of other immune cells.

Kang et al. ([2016](#)) examined the effects of DINP exposure on the development of allergies and the underlying mechanisms. Male Balb/c mice were gavaged with 2, 20, or 200 mg/kg-day DINP for 21 days, then sensitized with either saline or 0.5 percent FITC (in 1:1 acetone/DBP) on days 22 and 23 via dermal application to shaved skin. On day 28, the mice received a 0.5 percent FITC challenge (or saline) to the right ear, and saline or vehicle (1:1 acetone/DBP) to the left ear and the baseline ear thickness was measured. On day 29, the study was terminated, and blood samples were collected to determine IgE levels. Immunohistochemistry staining was performed on the sections from the right ear to visualize the localization and staining intensity of TSLP, p-STAT3, p-STAT5, p-STAT6, NF- κ B, and p65. The authors also evaluated ROS, MDA, and GSH levels in the ear tissue as well as levels of the cytokines, IL-4 and IFN- γ . In mice administered DINP + FITC, there was an increase in the number of infiltrating inflammatory immune cells in their ear tissue. Dose-dependent, significant increases in IL-4 and IL-5 were observed in all groups exposed to FITC + DINP. In contrast, there was a dose-dependent decrease in IFN- γ , which increased the IL-4/IFN- γ ratio, showing DINP only increases Th₂-specific cytokines.

However, no significant pathological changes were observed in the ears of mice exposed to DINP alone, but the ears of mice from the FITC only group showed inflammatory cell infiltration into the skin. Additionally, to uncover the pathway of these adverse effects, treatment with FITC + 200 mg/kg-day DINP, and pyrrolidine dithiocarbamate, a well-known inhibitor of NF- κ B, markedly reduced the ear swelling when compared to the FITC + 200 mg/kg-day DINP group. Further, bilateral ear weight decreased significantly when the FITC + DINP-immunized groups were treated with pyrrolidine dithiocarbamate. There was an increase in ROS and MDA levels and a decrease in GSH levels observed in the FITC + 200 mg/kg-day DINP exposure group compared to FITC alone, but pyrrolidine dithiocarbamate reversed these effects. The adverse pathological effects observed in higher dose groups were attenuated with pyrrolidine dithiocarbamate treatment, which suggest that the effects are facilitated by the NF- κ B signaling pathway. Results support the conclusion that DINP aggravates FITC-induced allergic contact dermatitis through exacerbating increased MDA and ROS accumulation and IL-4 and IL-5 production, while also decreasing GSH and IFN- γ , which then activates the NF- κ B pathway. Following activation, TSLP expression and activation is increased, causing increased production of STATs 3, 5, and 6.

A subsequent study by Kang et al. (2017) expanded on the previously mentioned underlying mechanisms of DINP and the role of transient receptor potential (TRP) cation channel, subfamily A, member 1 (TRPA1) on the NF- κ B pathway. In this allergic dermatitis model, male BALB/c mice were gavaged with saline (control) or DINP (2, 20, 200 mg/kg-day) from days 1 to 21. On days 22 and 23, mice were smeared with saline or 0.5 percent FITC on their backs to sensitize them, then on day 28, mice are given saline or FITC on their right ear. Following sensitization, skin lesions showed enhanced levels of IgG1, IL-6, IL-13, and TRPA1 expression with DINP potentiating these levels. To determine the role of TRPA1 and NF- κ B for allergic dermatitis, on days 22, 23, and 28, mice were injected with HC-030031, a TRPA1 antagonist, and NF- κ B inhibitor, pyrrolidine dithiocarbamate. Blocking NF- κ B inhibited TRPA1 expression; however, TRPA1 antagonism did not have any effect on NF- κ B or TSLP expression. These findings suggest that TRPA1 is dependent on NF- κ B activation and TSLP expression for DINP aggravated allergic dermatitis.

Similarly, Lee et al. (2004) examined the effects of DINP on IL-4 production in CD4⁺ T-cells and the associated mechanisms. BALB/c mice were injected with Keyhole limpet hemocyanin in alum adjuvant twice at 7-day intervals while being intraperitoneally injected with 2 or 5 mg/kg of DINP every other day. Lymph node cells were harvested and cultured from these mice after 7 days of treatment and used to measure IL-4 and IFN- γ . DINP was shown to enhance IL-4 production in lymph node cells, which originated from CD4⁺ T-cells in a concentration dependent manner and increase IgE serum levels *in vivo*. Additionally, DINP exposure also increased IL-4 gene promotion activity in Phorbol-12-myristate-13-acetate stimulated EL4 T-cells. IL-4 gene promoter contains multiple binding sites to nuclear factor of activated T-cells (NF-AT), and DINP was shown to potentiate IL-4 production via enhancing P1 and P4 binding site activity on NF-AT. These results support the conclusion that DINP augments the allergic response of IL-4 production in CD4⁺T-cells via increased NF-AT binding activity.

Chen et al. (2015) investigated how DINP exposure during gestation and lactation affects the allergic response of pups and the role of the PI3K/Akt pathway. Female Wistar rats are treated with 0, 5, 50, and 500 mg/kg-day from GD7 to PND21. On PND22, 23, and 37, pups were sensitized with ovalbumin. Then, protein expression and production of cytokines associated with PI3K/Akt were measured. In the 50 mg/kg-day DINP group, pups displayed significantly increased lung resistance (RI) when compared to the controls. Moreover, all DINP-treated groups had significantly increased eosinophil infiltration into the airways when compared to the control group, as indicated by immunohistochemistry. Pups exposed to 50 mg/kg-day DINP had increased Akt phosphorylation, NF- κ B translocation, and increased Th₂

cytokine (IL-13) expression, while having decreased Th₁ cytokine (INF-r) expression, when compared to the vehicle control group. These results suggest DINP aggravates the ovalbumin-induced response and enhances expression of the PI3K/Akt pathway and NF-κB translocation.

Using a neuroinflammation mouse asthma model, Duan et al. (2018) exposed (via intraperitoneal injection) groups of Balb/c mice (8 mice/group) to: 1) saline only (control); 2) Ovalbumin (OVA) only; 3) OVA and formaldehyde (1 mg/m³, 5h/day) exposure (OVA + FA group); 4) OVA and 20 mg/kg-DINP (OVA + DINP); 5) OVA and formaldehyde (1mg/m³, 5h/day) and 20 mg/kg-day DINP (OVA + FA + DINP); 6-9) melatonin (10 mg/kg-day) blocking groups (OVA + MT group, OVA + FA + MT group, OVA + DINP + MT group, OVA + FA + DINP + MT group); 10-13) were dehydroxymethylepoxyquinomicin (DHMEQ; a NF-κB inhibitor) (10mg/kg-day) NF-κB blocking groups (OVA + DHMEQ, OVA + FA + DHMEQ, OVA + DINP + DHMEQ, OVA + FA + DINP + DHMEQ). Following 18 days of exposure and 7 days of sensitization, allergic asthma symptoms (eosinophilic catatonic protein) levels and mucus secretion), markers of oxidative stress (ROS fluorescence, superoxide dismutase, and Nrf2 levels), cytokines (IL-1β and IL-17), and NF-κB signaling were measured in the brain. Exposure to DINP increased eosinophilic catatonic protein levels and the number of mucus secreting cells in the airway of the mice with OVA sensitization. Additionally, DINP exposure increased levels of IL-1β, IL-17, and nerve growth factor levels in the brain and increased NF-κB activation in the pre-frontal cortex. Moreover, DINP exposure increased ROS fluorescence in the brain, Nrf2, and decreased superoxide dismutase. Results of this study indicate that DINP promotes neuroinflammation through potentiating oxidative stress and NF-κB signal pathway activation in this mouse asthma model.

Lastly, Yun-Ho et al. (2019) investigated the role of TLR4 and HMGB1 in DINP-induced asthma. In this study, female C57BL/6 mice were intraperitoneally injected with 50 mg/kg-day DINP for a week to sensitize them and then challenged with saline or DINP on days 19, 21, and 23. During the challenge, mice were injected in their tail vein with either 3 mg/kg TAK-242 (TLR4 inhibitor) or 10 mg/kg anti-HMGB1 antibody, respectively, on each day of the challenge. DINP significantly increased airway hyperresponsiveness, number of infiltrating cells in bronchoalveolar fluid, numbers of inflammatory cells in blood, pulmonary fibrosis, mucus production, Th2 cytokine production (IL-4, IL-5, IL-13), and lung cell apoptosis. In contrast, adding the TLR4 inhibitor or anti-HMGB1 antibody following DINP exposure reduces airway hyperresponsiveness, reduced production of IL-4, IL-5, and IL-13 cytokines, and number of inflammatory cells in the airway. Overall, this study provides evidence that HMGB1 and TLR4 signaling pathways can contribute to DINP-induced asthma.

Conclusions on Immune System Toxicity

There are multiple animal toxicity studies that support the adjuvant effects of DINP exposure on the immune response in dermatitis models and *in vitro* experiments (Koike et al., 2010; Imai et al., 2006). Koike et al. (2010) stated that DINP exposure did not aggravate serum levels of IgG1, IgE, and histamine levels *in vivo*. Further, Imai et al. (2006) concluded that DINP is not considered a skin sensitizer based on no significant increase in the FITC-positive cell number in the draining lymph nodes. Additionally, there were three new studies that all support that DINP aggravates atopic dermatitis via causing oxidative stress and NF-κB cellular pathway activation (Kang et al., 2016; Wu et al., 2015; Sadakane et al., 2014). Similarly, EPA identified six mechanistic studies that support DINP enhancing NF-κB signaling, TSLP transcription, NF-AT, PI3K/Akt, TLR4, and HMGB1 in allergic dermatitis, atopic dermatitis, and asthma mouse models (Yun-Ho et al., 2019; Duan et al., 2018; Kang et al., 2017; Kang et al., 2016; Chen et al., 2015; Lee et al., 2004). Overall, available studies provide evidence that DINP augments the inflammatory responses in several sensitization models and the underlying mechanisms. Specifically, there are several studies that demonstrate DINP's role in potentiating ROS

production, TSLP transcription, PI3K/Akt, TLR4, and NF-κB pathway activation, and Th2 cytokine production in allergic dermatitis, neuroinflammation, and asthma in animal models.

Although available studies of laboratory animals provide some evidence for immune adjuvant effects of DINP in sensitized animals, EPA is not further considering these effects for dose-response assessment or for use in extrapolating human risk. Available studies evaluate the adjuvant properties of DINP in experimental rodent models pre-sensitized by exposure to other compounds (*e.g.*, FITC, ovalbumin). While these studies may be useful for hazard identification for a specific population (pre-sensitized individuals), the fact that the outcome evaluated in these studies requires prior exposure to another chemical precludes its broader applicability.

3.7 Musculoskeletal Toxicity

Humans

Four epidemiologic studies, three cross-sectional and one cohort study examined the association between DINP urinary levels of metabolites and bone mineral density, Osteoporosis and Vitamin D in adults; however the evidence was considered inadequate due to inconsistent results ([Health Canada, 2018a](#)).

New Literature: EPA did not identify any new epidemiologic studies that examine the association between DINP and its metabolites and musculoskeletal toxicity.

Laboratory Animals

Hwang et al. ([2017](#)) was the only study that investigated the relationship between DINP and osteopenia, which is characterized by bone loss and deterioration of bone structure leading to fractures. DINP (2, 20, or 200 mg/kg-day) was administered via intraperitoneal injection to 8-week-old female C3H/HeN ovariectomized (OVX) mice (5 animals/group), including: a sham-operated control group injected with PBS; a vehicle treated OVX group injected with PBS; and three DINP groups of 2, 20, or 200 mg/kg-day. The vehicle and DINP were administered for 6 weeks, and the body weights were recorded weekly. There was significant increase in body weights of OVX mice compared to sham control mice 6 weeks after OVX surgery. DINP also significantly increased body weight compared to sham control mice. DINP-treated mice had significantly reduced uterus weight and decreased tibia and femur lengths. Tibia weights were decreased in OVX mice and in the DINP-treated mice. However, no differences were noted in femur weights among the groups. DINP treatment of the normal mice increased the inorganic phosphorus release. Lactate dehydrogenase was unaffected by OVX or DINP treatments.

Further, tartrate-resistant acid phosphatase activity (bone resorption marker) was significantly increased in both OVX mice and in the mice treated with 200 mg/kg-day DINP at a similar magnitude over controls. Bone ALP activity was lower than sham controls in the OVX mice and in the DINP mice treated with 2 and 20 mg/kg-day; however, bone ALP activity in mice treated with 200 mg/kg-day DINP was comparable to sham controls, indicating that these decreases were not dose-related. Further, the microarchitecture of the femur and tibia were affected by OVX and DINP. The bone volume, tissue volume, bone volume/tissue volume ratio, bone surface, bone surface/tissue volume ratio, trabecular thickness, and trabecular number were all reduced, while the trabecular pattern factor, structure model index, and trabecular separation were increased in the DINP-treated mice, although these differences were not as substantial as in the OVX mice compared to sham controls. Similarly, the bone mineral density of the femur and tibia was dose-dependently decreased in the DINP-treated mice, but not decreased to the extent noted in the OVX mice, compared to the sham controls. The authors concluded that these results indicate that DINP contributes to an increased risk of osteopenia via destruction of the

microarchitecture and enhancement of osteoclastic activity, although it is difficult to conclude as the mechanism of action is currently unknown.

Conclusions on Musculoskeletal Toxicity

Four epidemiological studies and one study in experimental animals have provided data on the associations between exposure to DINP and musculoskeletal outcomes such as osteoporosis or osteopenia. The human evidence was considered inadequate due to inconsistent results across study designs and not further evaluated by EPA. The animal evidence suggests that DINP can reduce bone mineral density in female mice. Overall, there is limited evidence that DINP can elicit musculoskeletal toxicity in experimental laboratory animals; only one study in one species of one sex evaluates musculoskeletal outcomes. Additionally, the clinical implications, or relevance to humans, is uncertain given the limitations of the epidemiologic database. Due to these limitations and uncertainty, EPA is not further considering musculoskeletal toxicity for dose-response analysis.

3.8 Gastrointestinal System Toxicity

Humans

EPA did not identify any epidemiologic studies of the gastrointestinal system for DINP and/or its metabolites.

Laboratory Animals

EPA identified three animal toxicology studies that provide data on the effects of DINP on the gastrointestinal system ([Chiu et al., 2021](#); [Chiu et al., 2020](#); [Setti Ahmed et al., 2018](#)). Setti Ahmed et al. (2018) investigated the effects of DINP on development of the small intestine. Pregnant Wistar rats (36 per dose) were gavaged with 0 (corn oil vehicle) or 380 mg/kg-day DINP (CASRN 68515-48-0) from GD8 through PND30. Treatment with DINP reduced maternal food consumption 14 to 39 percent during gestation and 48 to 62 percent during lactation (PND1–21); however, it is unclear if reduced food consumption led to reduced dam body weight, as this outcome was not reported. Pup body weight gain was significantly reduced (54–56%) from PND15 to 30. Study authors report that pup small intestine weight was significantly reduced 41 percent by treatment with DINP; however, there are apparent discrepancies between the text and tabular organ weight data (unclear if a statistical analysis as done on individual organs). Histologically, offspring small intestine (duodenal, jejunal and ileal samples) showed villous atrophy following exposure to DINP; however, no incidence data is reported (only representative photomicrographs are provided). Lactase, maltase, sucrase, and ALP activity in the duodenum, ileum, and jejunum were also reported to be impacted by treatment with DINP on PND7, PND15, and PND30. Although results from this study suggest that DINP has effects on the developing small intestine in offspring exposed via maternal exposure during gestation and lactation, these effects may be related to the substantial decreases in offspring body weight gain which may be secondary to decreased maternal food consumption during gestation and lactation.

Chiu et al. (2020) evaluated the effect of DINP on the intestinal endocrine system including levels of testosterone and estradiol in the colon as well as distal colon histopathology and gross measurements of colon length and weight. Female CD-1 mice (6 per group) were orally administered 0, 0.02, 0.2, 2, 20, and 200 mg/kg-day DINP (CASRN 28553-12-0) for 10 to 14 days. There was no effect of DINP on colon weight, length, or weight-to-length ratio. Increased damage to the colon was noted in animals exposed to 0.02, 0.2, 2, and 200 mg/kg-day, but there was no dose-response and there are limitations in the methods for histopathological quantification (noted below). The authors qualitatively attribute changes at 0.02 and 0.2 mg/kg-day DINP to cellular infiltration and aberrant colon walls, and attribute changes at 2 and 200 mg/kg-day to edema based on their scoring criteria, but quantitative information (*i.e.*, incidence data) are not reported. There were no significant or dose-related changes in testosterone

in colon tissues. Although significant decreases in estradiol were observed in mice exposed to 0.2, 20, and 200 mg/kg-day DINP, there was no dose-response. There were no other dose-related effects. Several additional factors exist in Chiu et al. (2020) which decrease confidence in the study. These include the method of test substance administration (*i.e.*, pipetting chemical into the mouth of the mice) and methodological deficiencies in the histopathologic quantification of the colon that impact the ability to interpret the results. Indeed, incidence data was not provided, and information of tissue sampling (*i.e.*, number of sites evaluated in a given sample, number of slices, thickness of slices, *etc.*) was not provided. Overall, EPA did not consider this study further for dose-response assessment given the limitations.

In a second study, adult (2 months of age) female CD-1 mice (6 per group) were dosed with 0, 0.02, 0.02, 2, 20, and 200 mg/kg-day DINP (CASRN 28553-12-0) for 10 to 14 days (Chiu et al., 2021). Mice were administered DINP via pipetting the chemical into the mouth of the mouse, which raises uncertainty around the received doses of DINP. Colon tissue samples from 6 animals in each treatment group were pooled and 40 different cytokines were evaluated in the pooled samples using a cytokine array. Treatment with DINP appeared to decrease protein levels of CXCL12 and depending on the dose increased or decreased levels of IL-1RA; however, because samples were pooled for the array, no statistical analysis could be performed. Further analysis of CXCL12 and IL-1RA protein levels in the colon were evaluated via ELISA; however, no statistically significant effects were observed. Finally, serum levels of estradiol were determined. Treatment with DINP significantly increased serum levels of estradiol at 20 mg/kg-day, but not at any other doses. Additionally, this finding is somewhat inconsistent with that of Chiu et al. (2020), where estradiol measured from colon samples was found to be decreased at several doses. The inconsistent directionality of these effects increases uncertainty in the data set.

Mechanistic Information

Mechanistic data are limited to two studies from the same authors (Chiu et al., 2021; Chiu et al., 2020). Chiu et al. (2020) also evaluated molecular mediators involved in the aforementioned effects of DINP on the intestinal endocrine system, including gene expression of cell adhesion molecules (*Zo-1*, *Zo-2*, *Zo-3*, *Ocln*, *Cldn-1*, *Cldn-4*), cytokines (*IL-4*, *IL-5*, *IL-13*, *IL-17a*, *IL-6*, *Ifn- γ* , and *Tnf*), cell-cycle regulators (*Ccna-2*, *Ccnb1*, *Ccnd2*, *Ccne1*, *Cdk4*, *Cdkn1a*), apoptotic factors (*Aifm1*, *Bcl2*, *Bcl2l10*), and cell proliferation (*Ki67*). Additionally, TUNEL staining was conducted to evaluate apoptosis, and protein levels of the cell-adhesion molecule, sICAM-1, were quantified. In parallel with histopathologic findings of the colon at 200 mg/kg-day (*i.e.*, those attributed to edema), decreases in sICAM-1 levels and gene expression of *Zo-3* were observed. These data, albeit limited to a single study, may suggest that DINP elicits effects on cell-adhesion molecules at the molecular level. Increased expression of *Tnf* was observed at 0.2 mg/kg-day DINP, but no other doses, and there were no corresponding changes in TNF- α protein levels, implying that the *Tnf* findings may be spurious. No other significant changes in gene expression of cytokines were observed. Gene expression was significantly increased for the apoptotic factors *Aifm1* at 0.2 mg/kg-day (approximately 1.3-fold) and *Bcl2l10* at 20 mg/kg-day (approximately 2-fold), but the fold-increases were small, no other changes were observed at other doses, and TUNEL staining did not reveal significant cell death. These data do not support involvement of apoptosis in the effects of DINP on the gastrointestinal system. No changes were observed in expression of *Ki67*.

Chiu et al. (2021) evaluated the effects of DINP on gene and/or protein expression of specialized epithelial cells of the colon and various immune factors. Treatment with DINP significantly increased the level of Ki67 protein (a marker of cell proliferation) in the colons of mice in all treatment groups. Notably, this differs from the findings of the prior publication from these authors, which reported no changes in gene expression of Ki67; the inconsistency across studies decreases confidence in the data. Treatment with DINP did not affect mRNA expression of leucine-rich repeat-containing G-protein

coupled receptor (*Lgr5*), cluster of differentiation 24 antigen (*Cd24a*), chromogranin A (*ChgA*), villin 1 (*Vill1*), which are markers for various cells in the colon; mRNA levels of several mucins (*Muc1*, *Muc2*, *Muc3a*, *Muc4*), which are secreted from goblet cells to trap microbes; or alter mRNA expression of Toll-like receptor 4 or 5 (*Tlr4*, *Tlr5*) in the colon. Treatment with DINP did increase colon mRNA levels of lysozyme 1 (*Lyz1*) (a marker of Paneth cells) at 200 mg/kg-day DINP.

Conclusions on Gastrointestinal System Toxicity

EPA did not identify any epidemiologic studies of the gastrointestinal system for DINP and/or its metabolites. Available animal evidence is limited to two studies. One study also identified gastrointestinal effects, including reduced small intestine weight and villous atrophy in duodenum, ileum, and jejunum, although these findings are likely related to decreased offspring body weight gain, and secondary to decreased maternal food consumption ([Setti Ahmed et al., 2018](#)). Chiu et al. ([2020](#)) reported histopathological alterations in the colon of mice exposed to DINP for 10 days, but there was no linear dose-response, and the histopathological evaluation had several methodological limitations that decreased confidence and impacted the ability to interpret the results. Chiu et al. ([2021](#)) found that exposure to DINP can increase Ki67, a marker for cell proliferation in the colon; however, the earlier study by Chiu et al. ([2020](#)) did not observe any change in *Ki67* expression. Additionally, other effects on mRNA and cytokines in the colon were generally unaffected in Chiu et al. ([2021](#)). Overall, there is limited evidence that DINP can elicit gastrointestinal toxicity in experimental laboratory animals. Therefore, EPA is not further considering gastrointestinal system toxicity for dose-response analysis.

4 DOSE-REPOSE ASSESSMENT

EPA is considering four non-cancer hazard endpoints related to liver, kidney, neurological and developmental toxicity for dose-response analysis as described in the following sections. These hazard endpoints were selected for dose-response analysis because EPA has the highest confidence in these hazard endpoints for estimating risk to human health in the non-cancer sections. The effects for liver, kidney, and developmental effects were consistently observed across multiple rodent 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 EPA (*i.e.*, cardiovascular toxicity (Section 3.5), immune system toxicity (Section 3.6), musculoskeletal toxicity (Section 3.7) and gastrointestinal (Section 3.8) were not considered for dose-response analysis due to limitations in the number of studies, unknown MOA and uncertainties that reduce EPA's confidence in using these endpoints for estimating risk to human health.

EPA considered two approaches, including a NOAEL/LOAEL approach, and benchmark dose modeling for liver effects and benchmark dose modeling of developmental effects performed by NASEM (2017). EPA considered NOAEL and LOAEL values from oral toxicity studies in experimental animal models. Acute, intermediate, and chronic non-cancer NOAEL/LOAEL and BMDL values identified by EPA are discussed further in Sections 4.1.1, 4.1.2, and 4.1.3, respectively. As described in Appendix F, EPA converted oral PODs derived from animal studies to human equivalent doses (HEDs) using allometric body weight scaling to the three-quarters power (U.S. EPA, 2011b). In the absence of dermal toxicology studies, EPA used the oral HED to assess risks from dermal exposures. Differences in dermal and oral absorption are corrected for as part of the dermal exposure assessment. In the absence of inhalation studies, EPA performed route-to-route extrapolation to convert oral HEDs to inhalation human equivalent concentrations (HECs) (Appendix F).

4.1 Selection of Studies and Endpoints for Non-cancer and Threshold Cancer Health Effects

EPA considered the suite of oral animal toxicity studies for adverse liver, kidney, neurological and developmental effects identified during hazard identification (Section 3) when determining non-cancer PODs for estimating risks for acute, intermediate, and chronic exposure scenarios, as described in Sections 4.1.1, 4.1.2, and 4.1.3, respectively. EPA assessed relevant non-cancer health effects in these studies 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 relevant to each of these exposure durations and details related to the studies considered for each exposure duration scenario.

4.1.1 Non-cancer Oral Points of Departure for Acute Exposures

EPA considered 15 developmental toxicity studies with endpoints relevant to acute exposure duration (U.S. EPA, 1991b), summarized in Table 4-2. The endpoints considered relevant to acute exposure

durations include skeletal and visceral variations, and effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of male reproductive development in rats. These studies were subjected to dose-response analysis to select the study and endpoint most appropriate to derive the POD for acute hazard. The dose-response array for these studies is depicted graphically in Figure 4-1. Although these studies entailed exposure durations that exceeded a single day, EPA considered endpoints from these developmental toxicity studies for which there is evidence that they can result from a single exposure day during a critical window of development during gestation. For example, several studies have demonstrated that a single dose of DBP, which is toxicologically similar to DINP ([U.S. EPA, 2023a, b](#)), during the critical window of development (*i.e.*, GD15.5–18.5) is sufficient to disrupt fetal testicular testosterone production and steroidogenic gene expression. Although analogous single dose studies are not available for DINP, studies of DBP support the conclusion that effects on the developing male reproductive system may occur following acute, single dose exposures in rodent models (see Appendix C for further justification). Notably, the SACC agreed with this scientific justification to use reduced fetal testicular testosterone and subsequent downstream apical outcomes linked with this MOA to determine the acute duration POD ([U.S. EPA, 2024g](#)).

In two prenatal developmental toxicity studies ([Waterman et al., 1999](#); [Hellwig et al., 1997](#)), an increased incidence of fetal skeletal variations (*e.g.*, rudimentary/supernumerary cervical or lumbar ribs) and urogenital variations ([Hellwig et al., 1997](#)) were observed following exposure during GD6 to 15. rudimentary/supernumerary cervical or lumbar ribs) and urogenital variations were observed following exposure during GD6 through 15. However, the doses at which fetal visceral and skeletal variations occurred (500 and 1,000 mg/kg-day) were higher than doses in other developmental toxicity studies in which more sensitive effects of androgen insufficiency were observed. Therefore, EPA did not select these studies and endpoints because they do not provide the most sensitive robust endpoint for an acute POD.

The remaining 13 developmental toxicity studies considered by EPA resulted in effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of development. EPA identified this hazard in the Draft Proposed Approach for CRA for Phthalates ([U.S. EPA, 2023a](#)) and concluded that the weight of scientific evidence indicates that DINP can induce effects on the developing male reproductive system consistent with a disruption of androgen action and rat phthalate syndrome. Notably, EPA's conclusion was supported by the SACC ([U.S. EPA, 2023b](#)). The exposure durations for these 13 studies ranged from initiation of dosing at implantation through the day prior to expected parturition (*i.e.*, GD7 to 21) as employed in most guideline studies, to more narrow windows of exposure during gestation in which the phthalate-specific effects on male rodent offspring are known to occur (*e.g.*, GD14 to 18) or extended to encompass the perinatal period (*e.g.*, GD14 to PND3). Observed effects included decreased steroidogenic gene expression in the fetal testes, decreased fetal testicular testosterone, decreased male offspring AGD, increased male offspring NR, effects on fetal Leydig cells, increased incidence of MNGs, and decreased sperm motility. LOAELs for these effects ranged from 100 to 1,165 mg/kg-day.

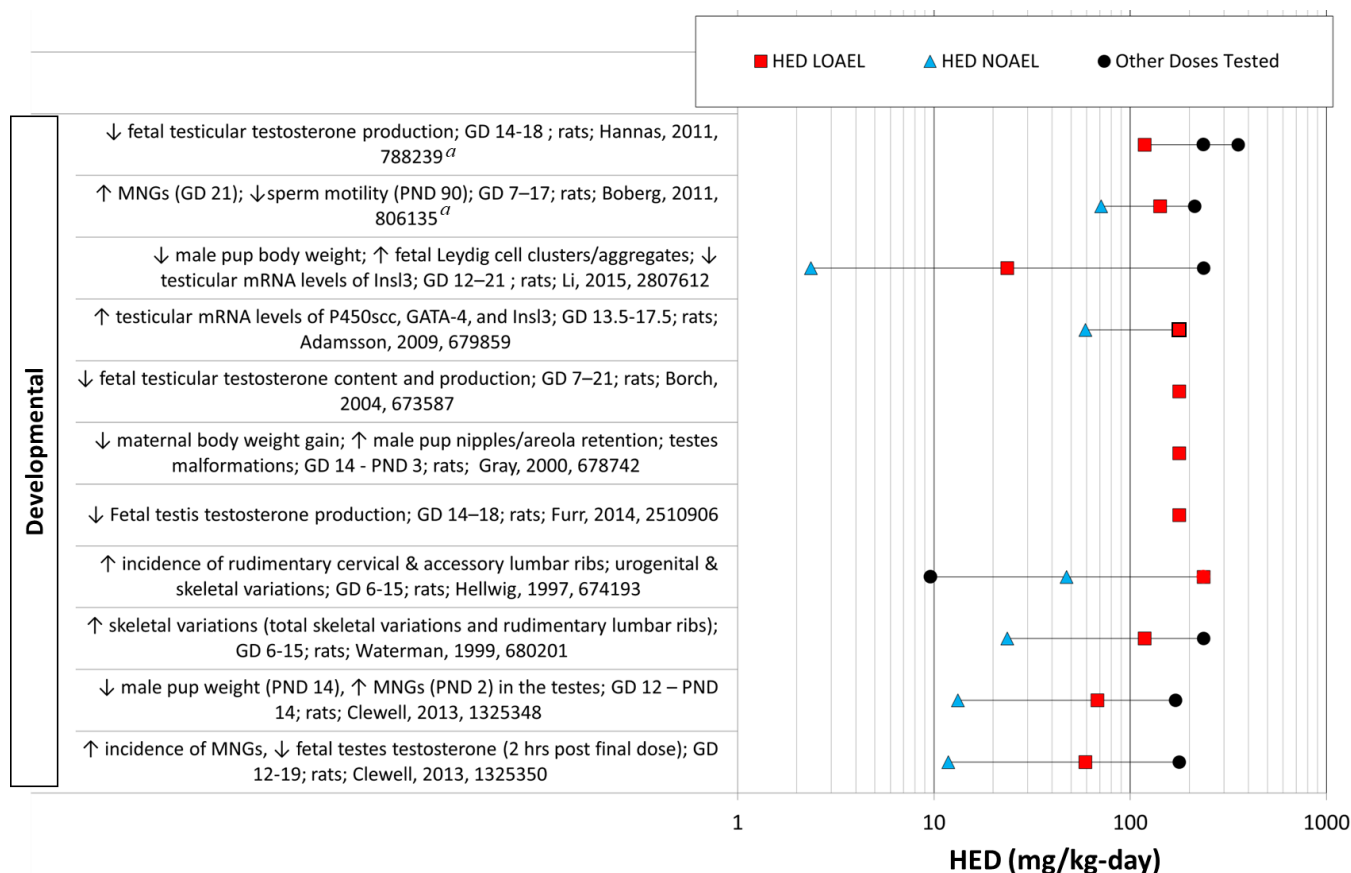


Figure 4-1. Dose-Response Array of Studies Considered for Deriving the Acute Duration Non-cancer POD

Notes: ↑ = statistically significant increase in response compared to controls; ↓ = statistically significant decrease in response compared to controls; M = males; F= females; GD= Gestational Day; PND = Postnatal Day; MNGs = multinucleated gonocytes; HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level.

^a Study included in NASEM (2017) meta-regression analysis and BMD modeling.

In 2017, NASEM (2017) assessed experimental animal evidence for effects on fetal testicular testosterone following *in utero* exposure to DINP using the systematic review methodology developed by the National Toxicology Program’s (NTP) Office of Health Assessment and Translation (OHAT). Based on results from four studies of rats (Li et al., 2015; Boberg et al., 2011; Hannas et al., 2011; Adamsson et al., 2009), NASEM found high confidence in the body of evidence and a high level of evidence that fetal exposure to DINP is associated with a reduction in fetal testosterone in rats. NASEM further conducted meta-regression analysis and benchmark dose (BMD) modeling on decreased fetal testicular testosterone production data from two medium-quality prenatal exposure studies of rats (Boberg et al., 2011; Hannas et al., 2011). Testosterone data from Li et al. (2015) was not included in the meta-regression and BMD modeling analysis because NASEM only included testosterone data measured during the fetal lifestage (Li et al. evaluated testosterone on PND1), while data from Adamson et al. (2009) was excluded because sufficient data were not reported to support its inclusion (*i.e.*, the exact number of litters per dose group was not report). NASEM found a statistically significant overall effect and linear trends in $\log_{10}(\text{dose})$ and dose, with an overall large magnitude of effect (>50%) in its meta-analysis for DINP (Table 4-1). Further BMD analysis determined BMDL₅ and BMDL₄₀ values of 49 and 552 mg/kg-day, the 95 percent lower confidence limit of the BMD associated with a benchmark

response (BMR) of 5 and 40 percent, respectively (Table 4-1). EPA has higher confidence in the NASEM meta-analysis since it takes into account data from multiples studies.

Table 4-1. Summary of NASEM (2017) Meta-Analysis and BMD Modeling for Effects of DINP in Fetal Testosterone (Using Metafor Version 2.0.0) ^{a b}

Database Supporting Outcome	Confidence in Evidence	Evidence of Outcome	Heterogeneity in Overall Effect	Model with Lowest AIC	BMD ₅ mg/kg-day (95% CI)	BMD ₄₀ mg/kg-day (95% CI)
4 rat studies	High	High	I ² = 83%	Linear quadratic	76 (49, 145)	701 (552, 847)
^a R code supporting NASEM’s meta-regression and BMD analysis of DINP is publicly available through GitHub . ^b NASEM (2017) calculated BMD40s for this endpoint because “previous studies have shown that reproductive-tract malformations were seen in male rats when fetal testosterone production was reduced by about 40%.”						

In response to recommendations from the SACC, EPA conducted an updated meta-analysis and BMD modeling analysis of decreased fetal rat testicular testosterone for DINP (U.S. EPA, 2024g). Using the publicly available R code provided by NASEM (<https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose>), EPA applied the same meta-analysis and BMD modeling approach used by NASEM, with the exception that the most recent Metafor package available at the time of EPA’s updated analysis was used (*i.e.*, NASEM used Metafor Version 2.0.0, while EPA conducted the updated meta-analysis with Metafor Version 4.6.0 and 2.0.0 so that results could be compared). EPA also evaluated an additional BMR of 10 percent. Appendix G provides justification for the evaluated BMRs of 5, 10, and 40 percent, while Appendix H provides a more detailed description of methods and results for the updated analysis. Fetal rat testicular testosterone data from four studies was included in the updated analysis, including new data from two studies (Gray et al., 2024; Furr et al., 2014), as well as data from the two studies included in the 2017 NASEM analysis (Boberg et al., 2011; Hannas et al., 2011). Overall, the meta-analysis conducted using Metafor Version 4.6.0 found a statistically significant overall effect and linear trends in log₁₀(dose) and dose, with an overall effect that is large in magnitude (>50% change) (Table_Apx H-3). There was substantial, statistically significant heterogeneity in the overall analysis (I²>80%). The statistical significance of these effects was robust to leaving out individual studies. The linear-quadratic model provided the best fit (based on lowest AIC) (Table_Apx H-4). BMD estimates from the linear-quadratic model were 74 mg/kg-day [95% confidence interval: 47, 158] for a 5 percent change (BMR = 5%), 152 mg/kg-day [97, 278] for a 10 percent change (BMR = 10%), and 699 mg/kg-day [539, 858] for a 40 percent change (BMR = 40%) (Table_Apx H-4). Notably, BMD₅ and BMD₄₀ estimates calculated by NASEM (2017) and as part of EPA’s updated analysis are nearly identical (*i.e.*, BMD₅ values of 49 and 47 mg/kg-day; BMD₄₀ values of 701 and 699 mg/kg-day).

One study (Li et al., 2015) appears to demonstrate similar effects on male offspring at lower doses than indicated in many of the other developmental toxicity studies. However, EPA did not consider this study further as the sole study on which to derive the POD because several areas of uncertainty reduced EPA’s confidence in the results when considered independently from the other studies in the analysis. While dose-dependent increases in testes dysgenesis and decreases in fetal testicular testosterone were noted, this study had limited statistical power (n = 6). It is also unclear what the study authors considered the broad description of “testes dysgenesis” to represent, although there is some indication that they are referring to seminiferous tubule atrophy. Further, effects on male pup body weight were not dose-related, with an essentially flat dose-response across doses spanning three orders of magnitude. A similar flat dose-response was noted in the frequency distribution of cluster sizes of fetal Leydig cells, and this endpoint is of uncertain adversity. Although this study supports EPA’s conclusions regarding

the endpoint for hazard identification, there is too much uncertainty in the dose-response in this study to use it quantitatively for determination of the acute POD.

Two additional developmental toxicity studies not included among the four studies considered in the meta-analysis by NASEM ([Clewell et al., 2013a](#); [Clewell et al., 2013b](#); [Hamner Institutes for Health Sciences, 2011](#)) resulted in decreased fetal testosterone production and other effects on the developing male reproductive system (*i.e.*, increased incidence of MNGs) at similar doses (LOAELs from 250 to 307 mg/kg-day and NOAELs from 50 to 56 mg/kg-day) to the BMDL₅ of 49 and 47 mg/kg-day derived from the NASEM meta-analysis and EPA's updated meta-analysis. Therefore, these studies support the selection of the BMDL₅ of 49 mg/kg-day for the acute POD.

Although several other additional studies were identified for effects on the developing male reproductive system and specifically for decreased fetal testicular testosterone, they were single doses studies ([Gray et al., 2024](#); [Furr et al., 2014](#); [Borch et al., 2004](#); [Gray et al., 2000](#)) with an identified LOAEL of 750 mg/kg-day. Similarly, several other studies identified effects on testosterone, steroidogenic gene expression, male offspring nipple retention, and reproductive tract malformations and support LOAELs of 500 to 1,000 mg/kg-day, but did not test sufficiently low doses to allow for the identification of a NOAEL ([Gray, 2023](#); [Gray et al., 2021](#)). These studies are not very sensitive and support LOAELs considerably higher than the LOAELs identified in the studies discussed above.

In a dietary study by Lee et al. ([2006a](#)), decreased male pup AGD was reported at the lowest dose tested, 40 ppm (estimated to be approximately 2 mg/kg-day). However, several factors reduce EPA's confidence in this study and its results. First, study authors did not report dam body weight, food intake, or calculate received doses in units of mg/kg-day, so there is uncertainty related to the achieved doses in the study. Further, the effect of DINP on male pup AGD normalized to the cube root of bodyweight was slight (overall magnitude of effect not reported), and treatment with DBP (a more potent antiandrogen compared to DINP) at equivalent or higher doses had no effect on male pup AGD normalized to the cube root of body weight. This calls into question the significances of the slight change in AGD observed for DINP. Given these uncertainties, EPA does not consider the study by Lee et al. ([2006a](#)) suitable for use as the acute POD.

Overall, the NASEM ([2017](#)) meta-analysis of fetal rat testicular testosterone supports a BMDL₅ of 49 mg/kg-day (HED of 11.6 mg/kg-day, which rounds to 12 mg/kg-day), while EPA's updated meta-analysis supports a BMDL₅ of 47 mg/kg-day (HED of 11.1 mg/kg-day, which rounds to 11 mg/kg-day), and the study by Clewell et al. ([2013a](#)) supports a NOAEL of 50 mg/kg-day (HED of 11.8 mg/kg-day, which rounds to 12 mg/kg-day) based on reduced fetal testicular testosterone and increased incidence of MNGs. HEDs were extrapolated from the BMDL₅ or NOAEL using allometric body weight scaling to the three-quarters power ([U.S. EPA, 2011b](#)). EPA selected an HED of 12 mg/kg-day to use as the acute exposure duration POD, which was extrapolated from the BMDL₅ from the NASEM ([2017](#)) meta-analysis and the NOAEL from the study by Clewell et al. ([2013a](#)). 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](#)), EPA 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 F). EPA considered reducing the UF_A further to a value of 1 based on apparent differences in toxicodynamics between rats and humans. As discussed in Section 3.1.4 of EPA's Draft Proposed Approach for CRA for Phthalates ([U.S. EPA, 2023a](#)), several explant ([Lambrot et al., 2009](#); [Hallmark et al., 2007](#)) and xenograft studies ([van Den Driesche et al., 2015](#); [Spade et al., 2014](#); [Heger et al., 2012](#); [Mitchell et al., 2012](#)) using human donor fetal testis tissue have been conducted to investigate the antiandrogenicity of mono-2-ethylhexyl

phthalate (MEHP; a monoester metabolite of DEHP), DBP, and monobutyl phthalate (MBP; a monoester metabolite of DBP) in a human model. Generally, results from human explant and xenograft studies suggest that human fetal testes are less sensitive than rat testes to the antiandrogenic effects of phthalates; however, effects on Sertoli cells and increased incidence of MNGs have been observed in two human xenograft studies of DBP ([van Den Driesche et al., 2015](#); [Spade et al., 2014](#); [Heger et al., 2012](#); [Mitchell et al., 2012](#)). As discussed in EPA's draft approach document ([U.S. EPA, 2023a](#)), the available human explant and xenograft studies have limitations and uncertainties, which preclude definitive conclusions related to species differences in sensitivity. For example, key limitations and uncertainties of the human explant and xenograft studies include: small sample size; human testis tissue was collected from donors of variable age and by variable non-standardized methods; and most of the testis tissue was taken from fetuses older than 14 weeks, which is outside of the critical window of development (*i.e.*, gestational weeks 8–14 in humans). Therefore, EPA did not reduce the UFA.

Table 4-2. Dose-Response Analysis of Selected Developmental Studies Considered for Deriving the Acute Non-cancer POD

Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	HEC (mg/m ³) [ppm]	Uncertainty Factors ^d	Reference
Wistar-Imamichi rats GD15 to PND21; estimated doses (as reported by (EC/HC, 2015)) 0, 2, 20, 200, 1,000 mg/kg-day; (28 days)	LOEL= 2	↓AGD& AGI, ↑ in hypothalamic granulin (grn, females) and p130 (males) mRNA levels; reduced lordosis quotient in females	0.473	2.57 [0.150]	UF _A = 3 UF _H = 10 UF _L =10 Total UF = 300	(Lee et al., 2006a) ^b
Pregnant SD rats; oral gavage (corn oil); 0, 10, 100, 500, 1,000 mg/kg-day; GD12–21	NOAEL = 10	↓ male pup body weight; ↑ fetal Leydig cell clusters/aggregates; ↓ testicular mRNA levels for <i>Ins13</i>	2.36	12.9 [0.75]	UF _A = 3 UF _H = 10 Total UF = 30	(Li et al., 2015) ^a
Meta-regression and BMD modeling of fetal testicular testosterone in rats	BMDL ₅ = 47	↓ Fetal testicular testosterone	11.1	60.5 [3.53]	UF _A = 3 UF _H = 10 Total UF = 30	EPA updated meta-analysis
Meta-regression and BMD modeling of fetal testicular testosterone in rats	BMDL ₅ = 49	↓ Fetal testicular testosterone	11.6	63.0 [3.68]	UF _A = 3 UF _H = 10 Total UF = 30	(NASEM, 2017) ^c
Pregnant SD rats; oral gavage; 0, 50, 250, 750 mg/kg-day; GD12–19	NOAEL = 50	↑ incidence of MNGs, ↓ fetal testes testosterone (2 hours post final dose)	11.8	64.3 [3.76]	UF _A = 3 UF _H = 10 Total UF = 30	(Clewell et al., 2013a)
Pregnant SD rats; dietary; 0, 760, 3,800, 11,400 ppm (estimated: 56, 288, 720 mg/kg-day on GD13–20; 109, 555, 1,513 mg/kg-day on PND2–14); GD12–PND14	NOAEL = 56	↓ male pup weight (PND14), ↑ MNGs (PND2) in the testes	13.2	72.1 [4.21]	UF _A = 3 UF _H = 10 Total UF = 30	(Clewell et al., 2013b)
Pregnant SD rats; oral gavage; 0, 100, 500, 1,000 mg/kg-day; GD6–15	NOAEL = 100	↑ skeletal variations (total skeletal variations and rudimentary lumbar ribs)	23.6	129 [7.52]	UF _A = 3 UF _H = 10 Total UF = 30	(Waterman et al., 1999)
Pregnant Wistar rats; oral gavage; 0, 40, 200, 1,000 mg/kg-day; GD6–15	NOAEL = 200	↑ incidences of rudimentary cervical and accessory lumbar ribs; urogenital and skeletal variations	47.3	257 [15.0]	UF _A = 3 UF _H = 10 Total UF = 30	(Hellwig et al., 1997)
Pregnant Wistar rats; oral gavage (corn oil); 0, 300, 600, 750, 900 mg/kg-day; GD7–17	NOAEL = 300	↑ MNGs (GD21); ↓sperm motility (PND90)	70.9	386 [22.6]	UF _A = 3 UF _H = 10 Total UF = 30	(Boberg et al., 2011) ^a

Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	HEC (mg/m ³) [ppm]	Uncertainty Factors ^d	Reference
Pregnant Harlan SD rats; Oral gavage (corn oil); 0, 500, 750, 1,000, 1,500 mg/kg-day; GD14–18	LOAEL = 500	↓ <i>ex vivo</i> fetal testicular testosterone production	118	643 [37.6]	UF _A = 3 UF _H =10 UF _L = 10 Total UF = 300	(Hannas et al., 2011) ^a
Pregnant Harlan SD rats; Oral gavage (corn oil); 0, 500, 750, 1,000, 1,500 mg/kg-day; GD14–18	LOAEL = 500	↓ <i>ex vivo</i> fetal testicular testosterone production and ↓ steroidogenic gene expression in the fetal testis	118	643 [37.6]	UF _A = 3 UF _H =10 UF _L = 10 Total UF = 300	(Gray et al., 2021)
Pregnant SD rats; oral gavage (corn oil); 0, 750 mg/kg-day; GD14–18	LOAEL = 750	↓ <i>ex vivo</i> fetal testis testosterone production	177	965 [56.4]	UF _A = 3 UF _H = 10 UF _L = 10 Total UF = 300	(Furr et al., 2014)
Pregnant Wistar rats; oral gavage (peanut oil); 0, 750 mg/kg-day; GD7–21	LOAEL = 750	↓ <i>ex vivo</i> fetal testicular testosterone content and production	177	965 [56.4]	UF _A = 3 UF _H =10 UF _L = 10 Total UF = 300	(Borch et al., 2004)
Pregnant SD rats; oral gavage (corn oil); 0, 750 mg/kg-day; GD14–PND3	LOAEL = 750	↓ maternal body weight gain; ↑ male pup nipples/areola retention; testes malformations (small, atrophic, flaccid, fluid-filled, azoospermia, epididymal agenesis)	177	965 [56.4]	UF _A = 3 UF _H = 10 UF _L =10 Total UF = 300	(Gray et al., 2000)
Pregnant SD rats; oral gavage (corn oil); 0, 750 mg/kg-day; GD14–18	LOAEL = 750	↓ <i>ex vivo</i> fetal testis testosterone production and ↓ steroidogenic gene expression in the fetal testis	177	965 [56.4]	UF _A = 3 UF _H = 10 UF _L = 10 Total UF = 300	(Gray et al., 2024)
Pregnant SD rats; oral gavaged (corn oil) 0, 1,000, 1,500 mg/kg-day; GD14– PND3	LOAEL = 1,000	↑ F1 male offspring nipple retention and ↑ incidence of total reproductive tract malformations in F1 males	236	1287 [75]	UF _A = 3 UF _H = 10 UF _L = 10 Total UF = 300	(Gray, 2023)
Pregnant SD rats; oral gavage (corn oil); 0, 250, 750 mg/kg-day; embryonic day 13.5–17.5	NOEL = 250	↑ testicular mRNA levels of <i>P450scc</i> , <i>GATA-4</i> , <i>Insl3</i>	–	–	–	(Adamsson et al., 2009) ^a

Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg-day)	HEC (mg/m ³) [ppm]	Uncertainty Factors ^d	Reference
<p>^a Study considered as part of NASEM analysis (NASEM, 2017). EPA did not consider this study (Li et al., 2015) further as the sole study on which to derive the POD because several areas of uncertainty (<i>e.g.</i>, low statistical power with n=6, questionable dose-response and uncertain adversity among several endpoints) reduced EPA's confidence in the results when considered independently from the other studies in a meta-analysis.</p> <p>^b Lee et al. (2006a) was not suitable for use to determine an acute POD due to uncertainties (<i>e.g.</i>, reporting deficiencies for dam body weight and food consumption for a dietary exposure study, and others described in the text).</p> <p>^c R code supporting NASEM's meta-regression and BMD analysis of DINP is publicly available through GitHub (https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose).</p> <p>^d 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. 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 DINP. 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.</p>						

4.1.2 Non-cancer Oral Points of Departure for Intermediate Exposures

EPA considered 12 intermediate (>1 to 30 days) oral exposure studies (6 of rats and 6 of mice) of DINP for establishing the intermediate duration POD (Table 4-3). Figure 4-2 depicts the dose-response array for available studies. Ultimately, EPA selected the acute POD (12 mg/kg-day) and benchmark MOE (total UF of 30) identified in Section 4.1.1 to evaluate risk from intermediate exposures (*i.e.*, ranging from 1 to 30 days) to DINP.

The acute POD is more sensitive than many of the intermediate HEDs based on liver, kidney, or developmental toxicity in rodents. As can be seen from Table 4-3 and Figure 4-2, of the 12 intermediate studies under consideration, 7 supported HEDs ranging from 15.6 to 401 ([Kwack et al., 2009](#); [Kaufmann et al., 2002](#); [Smith et al., 2000](#); [Hazleton Labs, 1991a](#); [BIBRA, 1986](#); [Bio/dynamics, 1982a](#); [Midwest Research Institute, 1981](#)). These studies are less sensitive than the acute POD (HED of 12 mg/kg-day). Further, several of these studies are limited by poor dose selection and did not test doses low enough to support NOAEL identification ([Hazleton Labs, 1991a](#); [BIBRA, 1986](#); [Midwest Research Institute, 1981](#)) or only tested a single high dose of DINP ([Kwack et al., 2009](#); [Bio/dynamics, 1982a](#)).

Five intermediate studies ([Ma et al., 2015](#); [Peng, 2015](#); [Ma et al., 2014](#); [Masutomi et al., 2003](#); [Smith et al., 2000](#)) report HEDs based on NOAELs ranging from 2.0 to 10 mg/kg-day, indicating that they are more sensitive than the HED that EPA selected for a POD. However, each of these studies had uncertainties that reduced EPA confidence in their use for deriving a POD for intermediate duration exposure.

Masutomi et al. (2003) supports a developmental NOAEL of 31 mg/kg-day (HED of 7.3 mg/kg-day) based on reduced F1 male offspring body weight on PND27. However, this study is limited by its small sample size (5 rats per dose group). Further, the biological significance of the effect on F1 male body weight is unclear, as F1 male bodyweight was unaffected on PND2, and no effect on F1 male bodyweight gain was observed from PND2 to PND10 or PND10 to PND21, and by PND77 F1 male body weight had recovered to control levels. These limitations and uncertainties reduce EPA's confidence in using the study by Masutomi et al. (2003) for the intermediate POD.

Three studies ([Ma et al., 2015](#); [Peng, 2015](#); [Ma et al., 2014](#)) reported treatment-related effects on endpoints indicating oxidative stress, but it is unclear if the apparent effects on neurotoxicity ([Ma et al., 2015](#); [Peng, 2015](#)) reported in Section 3.4, and the findings in the liver and kidney ([Ma et al., 2014](#)) reported in Section 3.2 and 3.3 can be directly attributed to the oxidative stress and inflammatory responses observed in the studies. Although there is some evidence showing protective effects of antioxidants in mitigating the effects of treatment with DINP, there is not enough data to determine the link to the apparent effects on neurotoxicity and on the liver and kidneys. This limitation is due, in large part, to the lack of quantitative data on the incidence or severity of the histopathology findings in the brain, liver, and kidney. These data were only described qualitatively, with representative micrographs of control and high dose groups presented as images, which precludes their usefulness to set a POD. Additional limitations in the two neurotoxicity studies are described below.

In the two neurotoxicity studies ([Ma et al., 2015](#); [Peng, 2015](#)), male Kunming mice were administered DINP by oral gavage at doses up to 200 mg/kg-day, followed by swim trials in the Morris Water Maze to determine effects on learning and memory, along with measurements of oxidative stress and histopathology evaluation of the brain. However, EPA identified several deficiencies in the study methods and reporting. First, both studies only report mean escape latency of each swimming trial over the 7-day acquisition phase but provide no measure of variability. Neither study conducted statistical

analysis on escape latency times *within* a given trial, but instead conducted statistical analysis on the average escape latency times *across* the 7 trials. Therefore, EPA is not able to determine whether there is a significant interaction between treatment and time to determine if the learning curve was steeper in the controls compared to the mice administered DINP. Second, path length provides another measure of learning, with path length decreasing over the acquisition phase if learning is occurring. The North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (TWG) - Developmental Neurotoxicity Study Guidance Document ([U.S. EPA, 2016](#)) indicates that the mean path length per trial should be reported, as this outcome is highly correlated with escape latency times. Both studies ([Ma et al., 2015](#); [Peng, 2015](#)) report use of camera tracking and computer software (ANY-Maze), which has the capabilities to determine path length. However, neither study reports the path length numerically for the swimming trials, but instead only depict an image of the swim path for a representative trial in the high dose and control groups. The lack of quantitative data on swim path length precludes EPA's ability to discern whether any increase in swim time is due to actual deficits in learning and memory, or if there is an increase in swim time due to general toxicity (*i.e.*, swimming more slowly). Neither study included performance controls.

Per the NAFTA guidance document, swim speed and cued-trials are two common performance controls that can be used to rule out treatment-related visual and motor impairments that can confound interpretation of cognitive deficits (*e.g.*, longer latency times may be due to slower swim speeds, not cognitive impairment). Third, for the probe trial, Ma et al. ([2015](#)) report both the target quarter retention time and the number of entries into the target quadrant, which is consistent with the NAFTA guidance document. There is a clear treatment related effect on target quadrant retention time; however, the controls spent only ~16 seconds in the target quadrant, which is only slightly above chance levels of 25 percent. NAFTA guidance states that controls must show an increase in percent time in the correct quadrant that exceeds chance levels of 25 percent. For the probe trial by Peng ([2015](#)), target quadrant retention time is reported, and controls spent approximately 25 seconds in the target quadrant, well above chance levels of 25 percent, but the number of entries into the target quadrant was not reported. Fourth, both of these studies ([Ma et al., 2015](#); [Peng, 2015](#)) reported alterations in pyramidal cells in hippocampus at the high dose (150 and 200 mg/kg-day); however, no quantitative data were provided on the incidence or severity of the histopathology findings; the data were only described qualitatively, with representative micrographs of control and high dose groups presented as images. Taken together, these uncertainties limit the utility of the neurological studies for use in determining an intermediate duration POD.

Finally, one study ([Smith et al., 2000](#)) provided a HED value for the NOAEL (10 mg/kg-day) in the same range as the acute HED value (12 mg/kg-day). Smith et al. ([2000](#)) report treatment-related increases in liver weights, hepatic peroxisomal beta oxidation (PBOX), and DNA synthesis, accompanied by inhibition of gap junctional intercellular communication (GJIC), in male B6C3F1 mice fed diets containing 6,000 ppm DINP (approximately 900 mg/kg-day) for up to 4 weeks. This study is limited in dose selection, with only two treated groups with doses spanning a wide range between the NOAEL in the low-dose group at 75 mg/kg-day and the LOAEL in the high dose at 900 mg/kg-day. Therefore, EPA did not consider the dose selection to be refined enough or endpoints examined to be comprehensive enough to establish a robust POD. However, the fact that the HED value from this study aligns with the HED from the acute POD adds further support to EPA's selection of the acute POD to be protective of intermediate exposure durations.

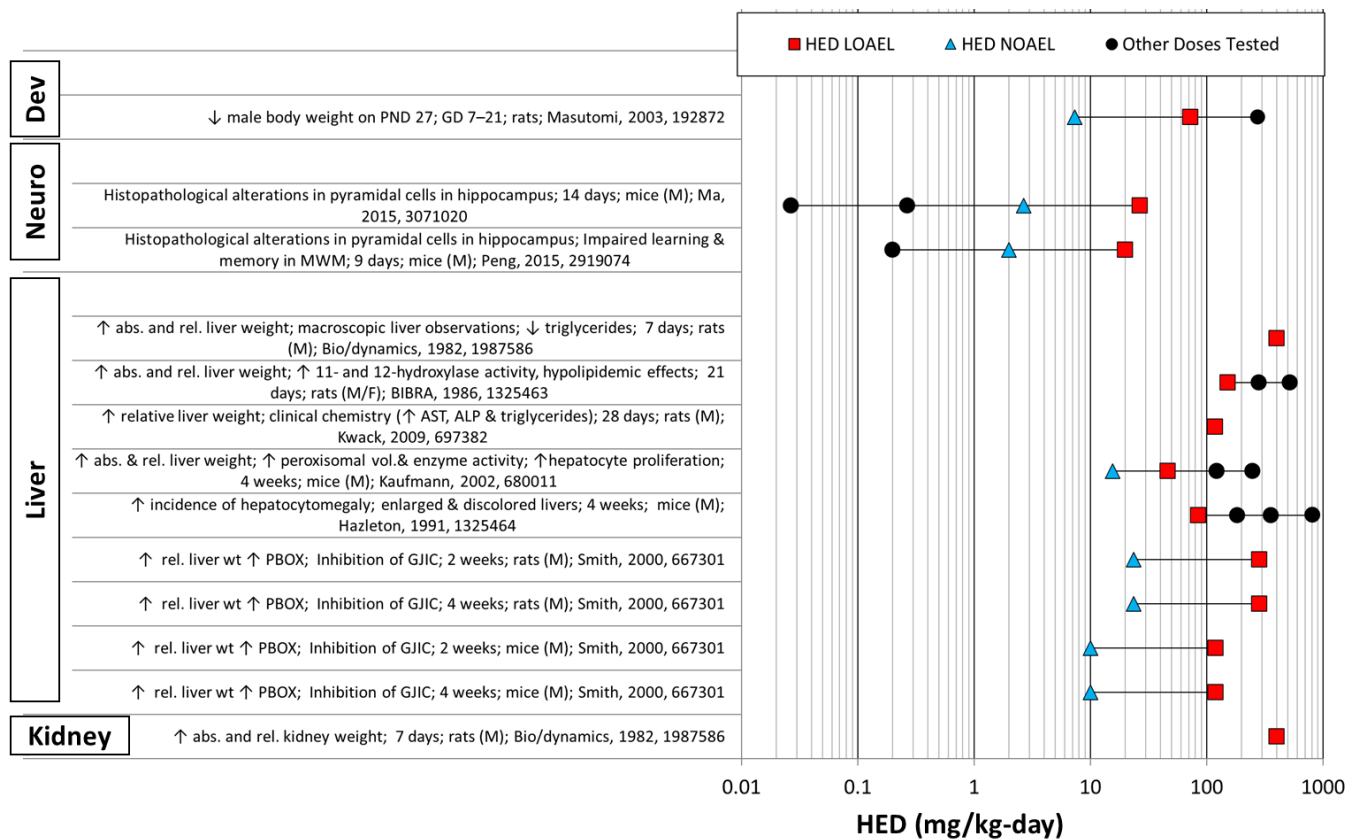


Figure 4-2. Dose-Response Array of Studies Considered for Deriving the Intermediate Duration Non-cancer POD

Notes: ↑ = statistically significant increase in response compared to controls; ↓ = statistically significant decrease in response compared to controls; Dev = Developmental; Neuro = Neurological; M = males; F= females; GD= Gestational Day; PND = Postnatal Day; ALT = alanine aminotransferase; AST= aspartate aminotransferase; ALP = alkaline phosphatase; HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level.

Table 4-3. Dose-Response Analysis of Selected Studies Considered for Deriving the Intermediate Non-cancer POD

Target Organ/System	Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	HEC (mg/m ³) [ppm]	Uncertainty Factors ^a	Reference
Neurotoxicity	Kunming mice (males only); oral gavage; 0, 1.5, 15, 150 mg/kg-day; 9 days	NOAEL = 15	↓ body weight gain; impaired learning & memory in Morris Water Maze; oxidative stress & inflammation; histopathological alterations in pyramidal cells in hippocampus	1.99	10.9 [0.634]	UF _A = 3 UF _H = 10 Total UF = 30	(Peng, 2015)
Neurotoxicity	Kunming mice (males only); oral gavage; 0, 0.2, 2, 20, 200 mg/kg-day; 14 days	NOAEL = 20	Histopathological alterations in pyramidal cells; oxidative stress & inflammation	2.66	14.5 [0.845]	UF _A = 3 UF _H = 10 Total UF = 30	(Ma et al., 2015)
Liver and Kidney	Kunming mice (males only); oral gavage; 0, 0.2, 2, 20, 200 mg/kg-day; 14 days	NOAEL = 20	Markers of oxidative stress (↑ ROS, ↓ GSH, ↑ MDA, ↑ 8-OH-dG) & inflammation (↑ IL-1, ↑ TNFα)	2.67	14.5 [0.845]	UF _A = 3 UF _H = 10 Total UF = 30	(Ma et al., 2014)
Developmental	Pregnant SD rats; dietary; 0, 400, 4,000, 20,000 ppm (estimated: 31–66, 307–657, 1,165–2,657 mg/kg-day); GD15 to PND10	NOAEL = 31 (males); 66 (females)	↓ male body weight on PND27	7.33	39.9 [2.33]	UF _A = 3 UF _H = 10 Total UF = 30	(Masutomi et al., 2003)
Liver	B6C3F1 mice (males only); dietary; 0, 500, 6,000 ppm (estimated: 75, 900 mg/kg-day); 2 and 4 weeks	NOEL = 75	Hepatic changes (↑ liver weight, ↑ PBOX, ↑ DNA synthesis; inhibition of GJIC)	9.97	54.3 [3.17]	UF _A = 3 UF _H = 10 Total UF = 30	(Smith et al., 2000)
Liver	B6C3F1 mice (both sexes); dietary; 0, 500, 1,500, 4,000, 8,000 ppm (estimated: 117, 350, 913, 1,860 mg/kg-day [males]; 167, 546, 1,272, 2,806 mg/kg-day [females]); 1 or 4 weeks	NOAEL = 117 (males)	↑ absolute and relative liver weight; ↑ peroxisomal volume, and peroxisomal enzyme activity; ↑ hepatocyte proliferation in males	15.6	84.7 [4.95]	UF _A = 3 UF _H = 10 Total UF = 30	(Kaufmann et al., 2002)
Liver	F344 rats (males only); dietary; 0, 1,000, 12,000 ppm (estimated: 100, 1,200 mg/kg-day); 2 and 4 weeks	NOAEL = 100	Hepatic changes (↑ liver weight, ↑ PBOX, ↑ DNA synthesis; inhibition of GJIC)	23.6	129 [7.52]	UF _A = 3 UF _H = 10 Total UF = 30	(Smith et al., 2000)
Liver & Kidney	F344 rats (both sexes); dietary; 0, 0.2, 0.67, 2% (estimated: 150, 500, 1,500 mg/kg-day [males]; 0, 125, 420, 1,300 mg/kg-day [females]); 28 days	LOEL = 125 (females)	↑ hepatic catalase and carnitine acetyltransferase activity	29.6	161 [9.39]	UF _A = 3 UF _H = 10 UF _L = 10 Total UF = 300	(Midwest Research Institute, 1981)

Target Organ/System	Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg-day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	HEC (mg/m ³) [ppm]	Uncertainty Factors ^a	Reference
Liver	B6C3F1 mice (both sexes); dietary; 0, 3,000, 6,000, 12,500 ppm (estimated: 635, 1,377, 2,689, 6,518 mg/kg-day [males]; 780, 1,761, 3,287, 6,920 mg/kg-day [females]); 4 weeks	LOAEL = 635 (males)	Enlarged and discolored livers; ↑ incidence of hepatocytomegaly	84.4	460 [26.8]	UF _A = 3 UF _H = 10 UF _L = 10 Total UF = 300	(Hazleton Labs, 1991a)
Liver	SD rats (males only); oral gavage; 0, 500 mg/kg-day; 28 days	LOAEL = 500	↓ body weight gain; ↑ relative liver weight; clinical chemistry (↑ AST, ALP, triglycerides)	118	643 [37.6]	UF _A = 3 UF _H = 10 UF _L = 10 Total UF = 300	(Kwack et al., 2009)
Liver	F344 rats (both sexes); diet; 0, 0.6, 1.2, 2.5% (estimated: 639, 1,192, 2,195 mg/kg-day [males]; 607, 1,198, 2,289 mg/kg-day [females]); 21 days	LOAEL= 607 (females)	↑ absolute and relative liver weight; ↑ 11- and 12-hydroxylase activity, hypolipidemic effects	144	781 [45.6]	UF _A = 3 UF _H = 10 UF _L = 10 Total UF = 300	(BIBRA, 1986)
Liver and Kidney	F344 rats (males only); dietary; 0, 2% (estimated: 1,700 mg/kg-day); 7 days	LOAEL = 1,700	↑ absolute and relative liver and kidney weight, macroscopic liver observations, changes in clinical chemistry	402	2,187 [128]	UF _A = 3 UF _H = 10 UF _L = 10 Total UF = 300	(Bio/dynamics, 1982a)
^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. 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 DINP. 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.							

4.1.3 Non-cancer Oral Points of Departure for Chronic Exposures

EPA considered four 2-year chronic dietary studies (3 of rats, 1 of mice), six 13-week subchronic dietary studies (4 of rats, and 1 each of mice and beagles), a one-generation study of reproduction of rats, and a two-generation study of reproduction of rats for establishing the chronic POD (Table 4-5). Across one- and two-generation studies of reproduction, reduced offspring bodyweight was the most sensitive effect, while liver and kidney toxicity were the most sensitive effects observed across chronic and subchronic studies, and these effects were considered for establishing the chronic POD. Figure 4-3 depicts the dose-response array for available studies.

Across the one- and two-generation studies of reproduction ([Waterman et al., 2000](#); [Exxon Biomedical, 1996a, b](#)), both of which were GLP-compliant and adhered to available guidelines (40 CFR part 798, section 798.4700), LOAELs for developmental effects were 377 mg/kg-day in the one-generation study based on reduced male and female F1 offspring body weight on PND0, 14, and 21; and 133 mg/kg-day in the two generation study based on reduced F1 and F2 offspring body weight on PND7 and 21. Neither study tested sufficiently low doses to establish a developmental NOAEL. Further, there is some uncertainty associated with the LOAEL from the two-generation study, as F1 offspring bodyweight (both sexes) was reduced on PND21, while F2 offspring body weight was reduced only on PND7 for females (Table 3-8). More consistent effects on F1 and F2 offspring body weight were observed in the mid-dose group. These sources of uncertainty reduce EPA's confidence in using the LOAEL of 133 mg/kg-day from the two-generation study as a chronic POD. Further, EPA identified more sensitive PODs based on liver toxicity from subchronic and chronic studies that tested lower doses of DINP and allowed for the identification of a NOAEL.

Across the six available subchronic studies, the lowest LOAELs for each of the tested species were 160 mg/kg-day in beagles (NOAEL = 37 mg/kg-day; HED = 23) based on increased absolute and relative liver weight and increase serum ALT ([Hazleton Laboratories, 1971](#)); 972 mg/kg-day in mice (NOAEL = 365; HED = 49 mg/kg-day) based on increased absolute and relative liver weight and histopathological findings (*e.g.*, necrosis) ([Hazleton Labs, 1992](#)); and 60 mg/kg-day in SD rats (no NOAEL identified; HED = 14 mg/kg-day) based on increased incidence of histopathological lesions in the kidney of male rats (*i.e.*, focal mononuclear cell infiltration and mineralization) ([Hazleton Labs, 1981](#)). LOAELs based on liver and kidney toxicity from the remaining three subchronic studies of rats were less sensitive and ranged from 176 to 227 mg/kg-day ([Hazleton Labs, 1991b](#); [Bio/dynamics, 1982b, c](#)). The study of beagles was conducted prior to the establishment of GLP principles and OECD test guidelines, and additionally only included four dogs per sex in each treatment group, so no statistical analysis was performed due to the small sample size ([Hazleton Laboratories, 1971](#)). These limitations reduced EPA's confidence in using the study to establish a chronic POD, and importantly, other subchronic and chronic studies of rats provide more sensitive and health protective candidate PODs. Similarly, the one subchronic study of mice ([Hazleton Labs, 1992](#)) provides a less sensitive candidate POD compared to studies of rats. The lowest subchronic LOAEL of 60 mg/kg-day in rats comes from a study conducted prior to the establishment of GLP principles and OECD test guidelines ([Hazleton Labs, 1981](#)), and did not test sufficiently low doses to establish a NOAEL. Furthermore, EPA did not consider this study sufficient for selection of a POD because it only reported effects on kidney in male rats which may be related to α_2 -globulin and not relevant for human health.

Across the four available 2-year dietary studies of rats and mice, the lowest LOAEL is 152 mg/kg-day (NOAEL = 15 mg/kg-day; HED = 3.5 mg/kg-day) from a 2-year dietary study of F344 rats ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)). The study by Lington et al. is GLP-compliant and received a high overall study quality determination. Although the study does not explicitly state compliance with any

testing guidelines, it generally follows the guidelines outlined by OECD Test Number 453 (Combined Chronic Toxicity/Carcinogenicity Studies). At the LOAEL, a spectrum of dose-related effects consistent with liver toxicity was observed in male and female rats, including treatment related increases in relative liver weight, serum ALT, AST, and ALP, and histopathological findings (*e.g.*, spongiosis hepatitis, sinusoid ectasia). One source of uncertainty associated with the findings of Lington et al. results from spongiosis hepatitis. The MOA underlying spongiosis hepatitis is unknown but is not believed to be related to peroxisome proliferation. Further, as discussed by ECHA ([2013b](#)), spongiosis hepatitis has been observed in the livers of some strains of rats and certain species of fish (*e.g.*, medaka), but is less common in mice, has not been observed in non-human primates or dogs, and with the exception of two case reports, has not been described in humans. These findings raise some uncertainty as to the human relevance of spongiosis hepatitis ([Karbe and Kerlin, 2002](#)). However, spongiosis hepatitis co-occurred with other hepatic effects that are more clearly adverse and relevant for use in human health risk assessment (*e.g.*, increase liver weight, serum ALT, AST, ALP, focal necrosis). Further supporting use of the LOAEL reported by Lington et al., similar hepatic effects (*e.g.*, increased relative liver weight, serum ALT, AST, and ALP, spongiosis hepatitis, necrosis) have consistently been reported in two other chronic dietary studies of DINP with F344 ([Covance Labs, 1998c](#)) and SD rats ([Bio/dynamics, 1987](#)), albeit at slightly higher doses of DINP (Table 4-5).

Given the broad dose spacing between the NOAEL of 15 mg/kg-day and LOAEL of 152 mg/kg-day identified in Lington et al. ([1997](#)), EPA attempted to refine the POD by conducting BMD modeling in accordance with EPA's *Benchmark Dose Technical Guidance* ([U.S. EPA, 2012](#)). Endpoints modeled included relative liver weight at terminal sacrifice (both sexes); serum ALT at 6-and 18-month sacrifices (males only); incidence of focal necrosis in the liver (both sexes); incidence of spongiosis hepatitis (males only); and incidence of sinusoid ectasia (males only). For each endpoint, multiple BMRs were modeled. BMD modeling results are presented in Appendix E, and results for representative BMRs are presented in Table 4-4. For dichotomous endpoints, BMDL₁₀ values ranged from 8.6 mg/kg-day for spongiosis hepatitis to 125 mg/kg-day for focal necrosis in male rats. BMDL₁₀ values for spongiosis (8.6 mg/kg-day) in the liver and sinusoid ectasia in the liver (14 mg/kg-day) were less than the study NOAEL of 15 mg/kg-day; however, BMD/BMDL ratios were greater than 3 (ranging from 3.7 to 8.9), indicating model uncertainty. For continuous endpoints, the BMDL₁₀ was 85 mg/kg-day for increased relative liver weights for males, while no models adequately fit relative liver weight data for female rats. For increase in serum ALT at 6 and 18 months, BMDL₁₀₀ values were 87 and 134 mg/kg-day, respectively. A BMR of 100 percent was selected for this endpoint since 2 to 3 fold changes in ALT are generally considered biologically significant and outside the range of normal variation ([Hall et al., 2012](#); [U.S. EPA, 2002a](#)). However, there is some uncertainty related to the BMR selection, so EPA also presents BMDL_{1SD} values in Table 4-4, which is consistent with EPA's *Benchmark Dose Technical Guidance* ([U.S. EPA, 2012](#)). BMDL_{1SD} values for increased serum ALT at 6 and 18 months were 16 and 33 mg/kg-day, respectively.

Overall, calculated BMDLs shown in Table 4-4 ranged from 8.6 to 134 mg/kg-day, which is similar to the study NOAEL and LOAEL values of 15 and 152 mg/kg-day. The wide variability in BMDLs and uncertainty in several modelled outcomes (*i.e.*, BMD/BMDL ratios greater than 3) reduce EPA's confidence in using the BMD modeling results for establishing a POD.

Table 4-4. Summary of BMD Model Results from Lington et al. (1997)

Endpoint	Sex	Selected Model	BMD _{1SD} / BMDL _{1SD} (mg/kg-day)	BMD ₁₀ / BMDL ₁₀ (mg/kg-day)	BMD ₁₀₀ / BMDL ₁₀₀ (mg/kg-day)
Dichotomous endpoints					
Focal necrosis in the liver	Male	Logistic	–	159/ 125	–
Focal necrosis in the liver	Female	Log-Probit	–	222/ 34	–
Spongiosis hepatitis in the liver	Male	Log-Probit	–	32/ 8.6	–
Sinusoid ectasia in the liver	Male	Log-Probit	–	125/ 14	–
Continuous endpoints					
Relative liver weight at terminal sacrifice	Male	Linear, CV	242/ 196	106/ 85	–
Relative liver weight at terminal sacrifice	Female	None selected; LOAEL (184 mg/kg-day) was used	–	–	–
Serum ALT at 6-month sacrifice	Male	Linear	23/ 16	–	125/ 87
Serum ALT at 18-month sacrifice	Male	Power	63/ 33	–	179/ 134

Overall, EPA selected the NOAEL of 15 mg/kg-day based on liver toxicity observed in a 2-year dietary study of F344 rats (Lington et al., 1997; Bio/dynamics, 1986) as the chronic POD for use in estimating non-cancer risk from exposure to DINP in the risk evaluation of DINP. This POD represents the most sensitive POD identified by EPA. Furthermore, the NOAEL of 15 mg/kg-day supports the suite of effects liver occurring at 152 mg/kg-day in Lington et al. (1997). Consistently, other regulatory bodies have selected the same chronic POD for use in quantifying risk from exposures to DINP (ECCC/HC, 2020; EFSA, 2019; U.S. CPSC, 2014; ECHA, 2013b). EPA used allometric body weight scaling to the three-quarters power to derive an HED of 3.5 mg/kg-day from the NOAEL of 15 mg/kg-day (U.S. EPA, 2011b). A total uncertainty factor of 30 was selected for use as the benchmark margin of exposure (based on an interspecies uncertainty factor (UFA) of 3 to account for intraspecies differences in toxicodynamics and an intraspecies uncertainty factor (UFH) of 10 to account for variability in the human population that might lead to increased susceptibility). Consistent with EPA guidance (2022, 2002b, 1993), EPA reduced the UFA 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, which accounts for interspecies differences in toxicokinetics (see Appendix F).

As discussed further in Appendix I, EPA considered reducing the toxicodynamics component of the UFA should be reduced from 3 to 1 based on differences in species sensitivity to the liver effects that form the basis of the chronic POD. As described in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* (U.S. EPA, 2025a), the weight of evidence indicates that humans are less sensitive than rodents to liver effects associated with PPAR α activation, which could support a reduction in the toxicodynamics component of the UFA from 3 to 1. However, the chronic POD of 3.5 mg/kg-day is based on a spectrum of liver effects, some of which are related to PPAR α activation (e.g., \uparrow liver weight, hypertrophy, necrosis) and some of which are PPAR α -independent (i.e., spongiosis hepatitis). Given that the chronic POD is based on liver effects that are both dependent and independent

of PPAR α and the uncertainty in mode of action associated with spongiosis hepatitis, EPA concluded that a reduction in the toxicodynamics component of the UF_A from 3 to 1 is not warranted.

Overall, EPA considers the selected chronic POD most applicable to adults (16 years and older). Use of this POD for characterization of risk to infants and children from chronic exposure to DINP may be conservative and may not be relevant. As described further in Appendix I, the chronic POD is based on liver effects dependent and independent of PPAR α activation. As discussed in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* (U.S. EPA, 2025a), there is evidence to suggest humans are less sensitive than rats to liver effects associated with PPAR α activation, while the PPAR α -independent effects (*i.e.*, spongiosis hepatitis) are most prevalent in the livers of aging rats.

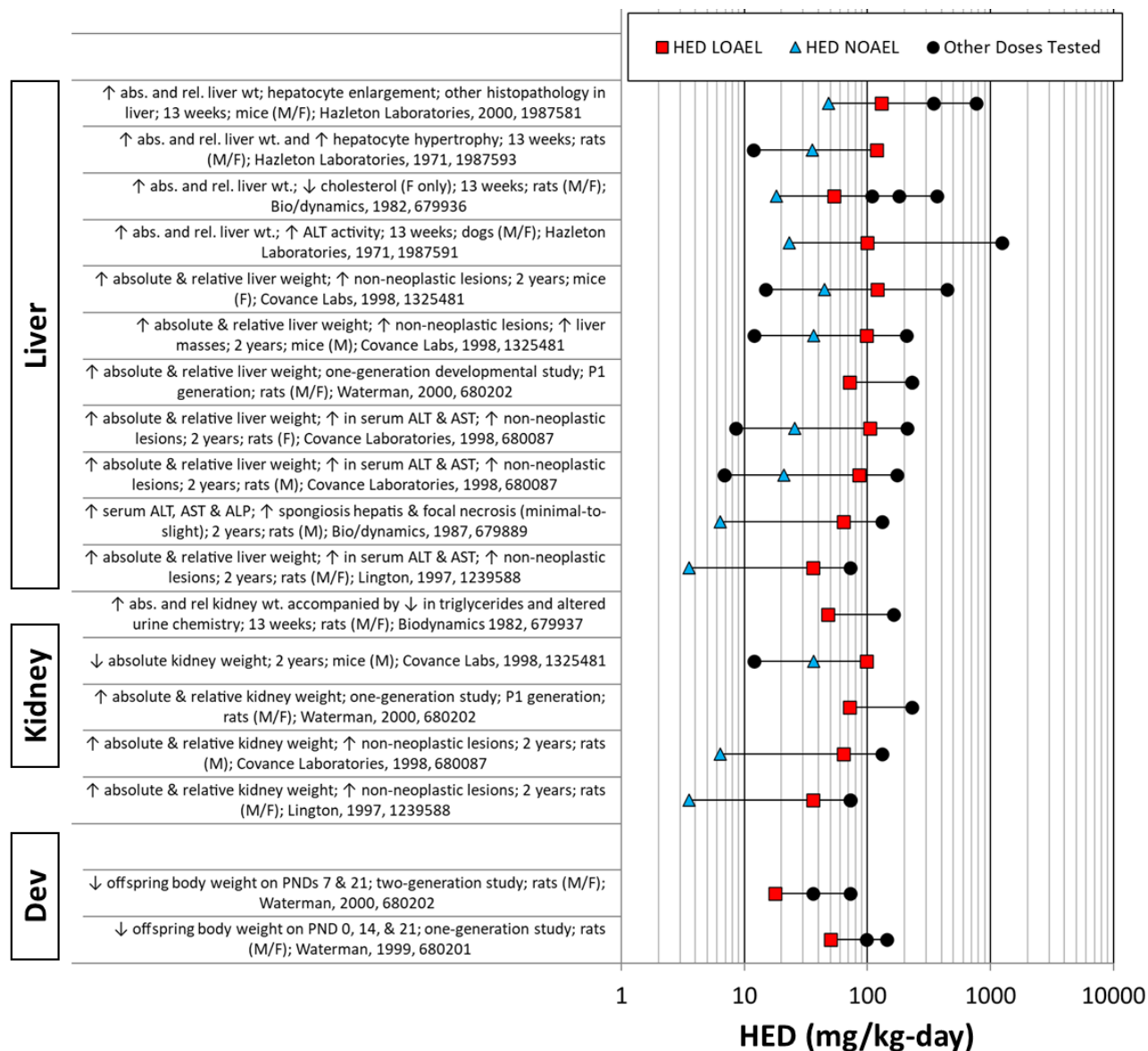


Figure 4-3. Dose-Response Array of Studies Considered for Deriving the Chronic Non-cancer POD

Notes: ↑ = statistically significant increase in response compared to controls; ↓ = statistically significant decrease in response compared to controls; M = males; F= females; P1 = parental generation; PND = Postnatal Day; ALT = alanine aminotransferase; AST= aspartate aminotransferase; ALP = alkaline phosphatase; HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level.

Table 4-5. Dose-Response Analysis of Selected Studies Considered for Deriving the Chronic Non-cancer POD

Target Organ/ System	Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg- day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	HEC (mg/m ³) [ppm]	Uncertainty Factors ^{a b}	Reference(s)
Liver and Kidney	F344 rats (both sexes); dietary; 0, 0.03, 0.3, 0.6% (estimated: 15, 152, 307 mg/kg-day [males]; 18, 184, 375 mg/kg-day [females]); 2 years	NOAEL= 15 (males) 18 (females)	↑ absolute and relative liver and kidney weight; ↑ in serum ALT and AST; histopathological alterations (<i>e.g.</i> , spongiosis hepatitis, focal necrosis)	3.55	19.3 [1.13]	UF _A = 3 UF _H = 10 <i>Total UF</i> = 30	(Lington et al., 1997 ; Bio/dynamics, 1986)
Liver	SD rats (both sexes); dietary; 0, 500, 5,000, 10,000 ppm (estimated: 27, 271, 553 mg/kg-day [males]; 33, 331, 672 mg/kg-day [females]); 2 years	NOAEL = 27	↑ serum ALT, AST, ALP (males); histopathological findings in the liver (<i>i.e.</i> , minimal-to-slight focal necrosis, spongiosis hepatitis)	6.38	34.7 [2.03]	UF _A = 3 UF _L = 10 <i>Total UF</i> = 30	(Bio/dynamics, 1987)
Liver and Kidney	F344 rats (both sexes); dietary; 0, 500, 1,500, 6,000, 12,000 ppm (estimated: 29, 88, 359, 733 mg/kg-day [males]; 36, 109, 442, 885 mg/kg-day [females]); 2 years	NOAEL = 88 (males) 109 (females)	↑ absolute and relative liver and kidney weight; ↑ in serum ALT, AST, BUN; histopathological findings in liver (<i>e.g.</i> , spongiosis hepatitis) and kidney (<i>e.g.</i> , mineralization of renal papilla, pigment in tubule cells)	20.8	113 [6.61]	UF _A = 3 UF _H = 10 <i>Total UF</i> = 30	(Covance Labs, 1998c)
Liver and Kidney	B6C3F1 mice (both sexes); dietary; 0, 500, 1,500, 4,000, 8,000 ppm (estimated: 90, 276, 742, 1,560 mg/kg-day [males]; 112, 336, 910, 1,888 mg/kg-day [females]); 2 years	NOAEL = 90 (males) 112 (females)	↑ absolute and relative liver weight, histopathological changes in the liver; ↓ body weight gain (females); ↑ incidence of liver masses and ↓ absolute kidney weight (males)	12.0	65.1 [3.80]	UF _A = 3 UF _H = 10 <i>Total UF</i> = 30	(Covance Labs, 1998b)
Developmental	<u>Two-generation study</u> : SD rats (30/group) administered 0, 0.2, 0.4, 0.8% DINP in the diet continuously starting 10 weeks prior to mating, throughout mating, gestation and lactation for two generations	LOAEL = 133	↓ F1 and F2 offspring body weight on PND7, 14, 21	31.4	171 [10.0]	UF _A = 3 UF _H = 10 UF _L = 10 <i>Total UF</i> = 300	(Waterman et al., 2000 ; Exxon Biomedical, 1996b)
Developmental	<u>One generation study</u> : SD rats (30/group); administered 0, 0.5, 1.0, 1.5% DINP in diet continuously starting 10 weeks prior to mating	LOAEL = 377	↓ male and female offspring body weight on PND0, 14, and 21	89.1	485 [28.3]	UF _A = 3 UF _H = 10 UF _L =10	(Waterman et al., 2000 ; Exxon Biomedical, 1996a)

Target Organ/ System	Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg- day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	HEC (mg/m ³) [ppm]	Uncertainty Factors ^{a b}	Reference(s)
	and throughout mating, gestation and lactation for one generation.					Total UF = 300	
Liver	Beagle dogs (both sexes); dietary; 0, 0.125, 0.5, 2% (estimated: 37, 160, 2,000 mg/kg-day); 13 weeks	NOAEL = 37	↑ absolute and relative liver weight; ↑ serum ALT	23.0	125 [7.32]	UF _A = 3 UF _H = 10 Total UF = 30 ^b	(Hazleton Laboratories, 1971)
Liver	B6C3F1 mice (both sexes); dietary; 0, 1,500, 4,000, 10,000, 20,000 ppm (estimated: 365, 972, 2,600, 5,770 mg/kg-day); 13 weeks	NOAEL = 365	↑ absolute and relative liver weight; liver histopathology (<i>e.g.</i> , necrosis, degeneration, hepatocyte enlargement)	48.5	264 [15.4]	UF _A = 3 UF _H = 10 UF _S = 10 Total UF = 300	(Hazleton Labs, 1992)
Liver and Kidney	F344 rats (both sexes); dietary; 0, 0.1, 0.3, 0.6, 1.0, 2.0% (estimated: 77, 227, 460, 767, 1,554 mg/kg- day); 13 weeks	NOAEL = 77	↑ absolute and relative liver and kidney weight; ↓ cholesterol level (females)	18.2	99.1 [5.79]	UF _A = 3 UF _H = 10 UF _S = 10 Total UF = 300	(Bio/dynamics, 1982b)
Liver and Kidney	F344 rats (both sexes); dietary; 0, 2,500, 5,000, 10,000, 20,000 ppm (estimated: 176, 354, 719, 1,545 mg/kg-day [males]; 218, 438, 823, 1,687 mg/kg-day [females]); 13 weeks	LOAEL = 176 (males) 218 (females)	↑ kidney and liver weights	41.6	226 [13.2]	UF _A = 3 UF _H = 10 UF _S = 10 UF _L = 10 Total UF = 3000	(Hazleton Labs, 1991b)
Liver and Kidney	SD rats (both sexes); dietary; 0, 0.3, 1.0% (estimated: 201, 690 mg/kg- day [males]; 251, 880 mg/kg-day [females]); 13 weeks	LOAEL = 201 (males) 251 (females)	↑ absolute and relative liver and kidney weight accompanied by ↓ in triglycerides and altered urine chemistry	47.5	259 [15.1]	UF _A = 3 UF _H = 10 UF _S = 10 UF _L = 10 Total UF = 3000	(Bio/dynamics, 1982c)
Kidney	SD rats (both sexes); dietary; 0, 1,000, 3,000, 10,000 ppm (estimated: 60, 180, 600 mg/kg-	LOAEL = 60 (males)	↑ incidence of histopathology lesions in the kidney [<i>i.e.</i> , focal	14.2	77.2 [4.51]	UF _A = 3 UF _H = 10	(Hazleton Labs, 1981)

Target Organ/ System	Study Details (Species, Duration, Exposure Route/ Method, Doses [mg/kg- day])	Study POD/ Type (mg/kg-day)	Effect	HED (mg/kg)	HEC (mg/m ³) [ppm]	Uncertainty Factors ^{a b}	Reference(s)
	day); 13 weeks		mononuclear cell infiltration and mineralization]; males only			UF _S = 10 UF _L = 10 <i>Total UF = 3000</i>	
<p>^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. 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 DINP. 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.</p> <p>^b EPA considered applying a subchronic-to-chronic UF (UF_S) of 10 for the intermediate (13-week) dog study under consideration for deriving a chronic POD. 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, a UF_S was not used.</p>							

4.2 Weight of Scientific Evidence

4.2.1 POD for Acute and Intermediate Durations

EPA has concluded that the HED of 12 mg/kg-day (based on BMDL₅ of 49 mg/kg-day and NOAEL of 50 mg/kg-day) from the NASEM (2017) meta-regression of reduced fetal testicular testosterone in rats and the study by Clewell et al. (2013a) is appropriate for calculation of risks for acute and intermediate exposure durations. A total UF of 30 was selected for use as the benchmark MOE (based on an interspecies UF (UF_A) of 3 and an intraspecies UF (UF_H) of 10). Consistent with EPA guidance (2022, 2002b, 1993), EPA 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 F). *EPA has robust overall confidence in the selected POD based on the following weight of scientific evidence:*

- EPA has previously considered the weight of scientific evidence and concluded that oral exposure to DINP can induce effects on the developing male reproductive system consistent with a disruption of androgen action (see EPA's Draft Proposed Approach for CRA for Phthalates (U.S. EPA, 2023a)). Notably, EPA's conclusion was supported by the SACC (U.S. EPA, 2023b).
- DINP exposure resulted in treatment-related effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of development in 16 studies of rats (Section 3.1.2.1). Observed effects included: reduced mRNA expression of INSL3 and genes involved in steroidogenesis in the fetal testes; reduced fetal testes testosterone content and/or production; reduced male pup anogenital distance; increased male offspring nipple retention; increased incidence of MNGs and fetal Leydig cell aggregation; and decreased sperm motility in adult rats exposed perinatally to DINP.
- The selected POD is based on meta-regression analysis of fetal testicular testosterone data from two studies of rats that supports a BMDL₅ of 49 mg/kg-day (Boberg et al., 2011; Hannas et al., 2011), and a gestational study of rats that supports a NOAEL of 50 mg/kg-day based on decreased fetal testicular testosterone and increased incidence of MNGs (Clewell et al., 2013a).
- EPA's updated meta-regression and BMD modeling analysis of fetal testicular testosterone data from four studies of rats supports a BMDL₅ of 47 mg/kg-day (Gray et al., 2024; Furr et al., 2014; Boberg et al., 2011; Hannas et al., 2011). EPA's updated analysis, which integrates more fetal testicular testosterone data, further support the selected POD.
- One additional developmental toxicity study (Clewell et al., 2013b) resulted in increased MNGs in the testis on PND2 and decreased male pup body weight on PND14 at similar doses (LOAEL of 307 mg/kg-day and NOAEL of 56 mg/kg-day) to the BMDL₅ of 49 mg/kg-day derived from the NASEM meta-analysis. This study supports the selection of the BMDL₅ of 49 mg/kg-day for the acute and intermediate duration PODs.
- Other regulatory and authoritative bodies have also concluded that DINP is a developmental toxicant and can induce effects on the developing male reproductive system consistent with a disruption of androgen action and phthalate syndrome and that these developmental effects are relevant for estimating human risk (Table 1-1) (ECCC/HC, 2020; EFSA, 2019; U.S. CPSC, 2014; ECHA, 2013b; NICNAS, 2012).

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 12 mg/kg-day to extrapolate to the dermal route. Differences in absorption will be accounted for in the dermal exposure estimates in the risk evaluation for DINP.

EPA is also using the oral HED of 12 mg/kg-day to extrapolate to the inhalation route. 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, 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 F 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.

4.2.2 POD for Chronic Durations

EPA has concluded that the HED of 3.5 mg/kg-day (NOAEL of 15 mg/kg-day) from the 2-year dietary study of F344 rats based on liver toxicity ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)) is appropriate for calculation of risk for chronic exposure durations. A total UF of 30 was selected for use as the benchmark MOE (based on an interspecies UF (UF_A) of 3 and an intraspecies UF (UF_H) of 10). Consistent with EPA guidance ([2022](#), [2002b](#), [1993](#)), EPA 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 F). *EPA has robust overall confidence in the selected POD based on the following weight of scientific evidence:*

- The NOAEL of 15 mg/kg-day (HED = 3.5 mg/kg-day) from the 2-year dietary study of F344 rats ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)) represents the most sensitive POD identified by EPA across the 12 relevant studies subjected to dose-response analysis, including four 2-year chronic dietary studies (3 of rats, 1 of mice), six 13-week subchronic dietary studies (4 of rats, and 1 each of mice and beagles), a one-generation study of reproduction of rats, and a two-generation study of reproduction of rats.
- This study received a high overall study quality determination and is GLP-compliant.
- At the LOAEL, a spectrum of dose-related effects consistent with liver toxicity was observed in male and female rats, including treatment related increases in relative liver weight, serum ALT, AST, and ALP, and histopathological findings (*i.e.*, spongiosis hepatitis, focal necrosis, sinusoid ectasia).
- Given the relatively broad dose-spacing between the NOAEL (15 mg/kg-day) and the LOAEL (152 mg/kg-day) in the principal study ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)), EPA attempted to refine the POD by conducting BMD modeling of relevant dose-related findings showing a substantial increase in magnitude over controls, including: relative liver weight at terminal sacrifice (both sexes); serum ALT at 6- and 18-month sacrifices (males only); incidence of focal necrosis in the liver (both sexes); incidence of spongiosis hepatitis (males only); and incidence of sinusoid ectasia (males only). Calculated BMDLs ranged from 8.6 to 125 mg/kg-day, which is similar to the study NOAEL and LOAEL values of 15 and 152 mg/kg-day. The wide variability in BMDLs and uncertainty in several modelled outcomes (*i.e.*, BMD/BMDL ratios greater than 3) reduce EPA's confidence in using the BMD modeling results for establishing a POD, and further affirm the use of the NOAEL for establishing the POD.
- The NOAEL of 15 mg/kg-day in Lington et al. ([1997](#)) also aligns with the BMD05 of 12 mg/kg-day for one of the more sensitive endpoints in this study, spongiosis hepatitis, determined by CPSC ([2010](#)). However, EPA considers it more appropriate to use the NOAEL of 15 mg/kg-day instead of the BMD05 of 12 mg/kg-day because the NOAEL supports the suite of effects on the liver occurring at 152 mg/kg-day instead of being based on the single effect of spongiosis hepatitis with its associated uncertainty regarding human relevance.

- The endpoints indicative of liver toxicity on which the POD is based were robust in that they were observed across species and durations.
 - The remaining three chronic studies in rodents ([Covance Labs, 1998b, c](#); [Bio/dynamics, 1987](#)) reported similar findings of liver toxicity (*e.g.*, increased liver weights; clinical chemistry changes such as increased ALT, AST, ALP; and histopathology findings such as liver necrosis and spongiosis hepatis), with similar but less sensitive NOAELs ranging from 27 to 112 mg/kg-day.
 - Similar findings indicative of liver toxicity were observed in the subchronic studies, although at higher doses than observed in the chronic study by Lington et al. ([1997](#)). In these subchronic studies, the lowest LOAELs for each of the tested species were: 160 mg/kg-day in beagles (NOAEL = 37 mg/kg-day; HED = 23) based on increased absolute and relative liver weight and increase serum ALT ([Hazleton Laboratories, 1971](#)) and 972 mg/kg-day in mice (NOAEL = 365; HED = 49 mg/kg-day) based on increased absolute and relative liver weight and histopathological findings (*e.g.*, necrosis) ([Hazleton Labs, 1992](#)). LOAELs based on liver toxicity from the remaining three subchronic studies of rats were less sensitive and ranged from 176 to 227 mg/kg-day ([Hazleton Labs, 1991b](#); [Bio/dynamics, 1982b, c](#)).
- Consistently, other regulatory bodies have selected the same chronic POD (NOAEL 15 mg/kg-day) for use in quantify risk from exposures to DINP (Table 1-1) ([ECCC/HC, 2020](#); [EFSA, 2019](#); [U.S. CPSC, 2014](#); [ECHA, 2013b](#)).

EPA considers this chronic POD most applicable for adults (16+ years). Use of this POD for characterization of risk to infants and children from chronic exposure to DINP may be conservative and may not be relevant. As described further in Appendix I, the chronic POD is based on liver effects dependent and independent of PPAR α activation. As discussed in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025a](#)), there is evidence to suggest humans are less sensitive than rats to liver effects associated with PPAR α activation, while the PPAR α -independent effects (*i.e.*, spongiosis hepatis) are most prevalent in the livers of aging rats.

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 3.5 mg/kg-day to extrapolate to the dermal route. Differences in absorption will be accounted for in the dermal exposure estimates in the risk evaluation of DINP.

EPA is also using the oral HED of 3.5 mg/kg-day to extrapolate to the inhalation route. 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, 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 F 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.

5 CONSIDERATION OF PESS AND AGGEGRATE EXPOSURE

5.1 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. Health outcomes that serve as the basis for acute, intermediate, and chronic hazard values are systemic and assumed to be consistent across routes of exposure. EPA therefore concludes that for consideration of aggregate exposures, it is reasonable to assume that exposures and risks across oral, dermal, and inhalation routes may be additive for the selected PODs in Section 6.

5.2 PESS Based on Greater Susceptibility

In this section, EPA addresses subpopulations expected to be more susceptible to DINP exposure than other populations. Table 5-1 presents the data sources that were used in the potentially exposed or susceptible subpopulations (PESS) analysis evaluating susceptible subpopulations and identifies whether and how the subpopulation was addressed quantitatively in the risk evaluation of DINP. EPA identified a range of factors that may have the potential to increase biological susceptibility to DINP, including lifestage, chronic liver or kidney disease, pre-existing diseases, physical activity, diet, stress, and co-exposures to other environmental stressors that contribute to related health outcomes.

Regarding lifestage, exposure to DINP during the masculinization programming window (*i.e.*, GD15.5–18.5 for rats; GD14–16 for mice; gestational weeks 8–14 for humans) can lead to antiandrogenic effects on the male reproductive system ([MacLeod et al., 2010](#); [Welsh et al., 2008](#); [Carruthers and Foster, 2005](#)). Animal studies demonstrating effects of DINP on male reproductive development and other developmental outcomes provide direct evidence that gestation is a particularly sensitive lifestage. EPA considered the sensitivity of this lifestage in its derivation of the POD for acute and intermediate exposure duration based on reduced fetal testicular testosterone in rats after evaluation of the weight of scientific evidence that DINP resulted in treatment-related effects on the developing male reproductive system consistent with a disruption of androgen action during the critical window of development in 13 studies of rats. In humans, there is moderate evidence for the association between DINP and testosterone and semen parameters, based on studies that found decreasing testosterone levels with increasing DINP exposure ([Radke et al., 2018](#)). Based on this evidence from animal and human studies, EPA has identified two groups that may be more susceptible to DINP exposure due to lifestages:

- pregnant women/women of reproductive age, and
- male infants, male toddlers, and male children.

Animal evidence also demonstrates that the liver, kidneys, nervous system, cardiovascular system, immune system, may be sensitive target organs. EPA is quantifying risks based on liver and developmental toxicity in the risk evaluation of DINP, and determining risk based these endpoints is protective of the other hazards which occur at higher doses.

Regarding the factor of co-exposure, studies have demonstrated that co-exposure to DINP and other toxicologically similar phthalates (*e.g.*, DEHP, DBP, BBP) and other classes of antiandrogenic chemicals (*e.g.*, certain pesticides and pharmaceuticals that are discussed more in ([U.S. EPA, 2023a](#))) can induce effects on the developing male reproductive system in a dose-additive manner. EPA details how it intends to evaluate risk to above-identified PESS from co-exposure to DINP and several other toxicologically similar phthalates in its Draft Proposed Approach for CRA for Phthalates ([U.S. EPA, 2023a](#)).

The effect of other factors on susceptibility to health effects of DINP is not known; therefore, EPA is uncertain about the magnitude of any possible increased risk from effects associated with DINP exposure for subpopulations that may be relevant to other factors.

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 DINP. 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 5-1. 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 DINP.

Table 5-1. PESS Evidence Crosswalk for Biological Susceptibility Considerations

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DINP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DINP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Embryos/ fetuses/infants	<p>Direct quantitative animal evidence for developmental toxicity (<i>e.g.</i>, increased skeletal and visceral variations, decreased live births, decreased offspring body weight gain, and decreased offspring survival with increased severity in the second generation).</p> <p>There is direct quantitative animal evidence for effects on the developing male reproductive system consistent with a disruption of androgen action.</p>	<p>(Hellwig et al., 1997)</p> <p>(Waterman et al., 1999)</p> <p>(Waterman et al., 2000)</p> <p>(U.S. EPA, 2023a)</p> <p>(U.S. EPA, 2023b)</p>			Acute and intermediate duration PODs for developmental endpoints protective of effects in offspring
	Pregnancy/ lactating status	Rodent dams not particularly susceptible during pregnancy and lactation, except for effects related to reduced maternal weight gain, food consumption, and increased organ weight (liver and kidney), which occurred at doses higher than those that caused developmental toxicity.	<p>(Hellwig et al., 1997)</p> <p>(Waterman et al., 1999)</p>			Acute and intermediate duration PODs for developmental endpoints protective of effects in dams

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DINP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DINP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Lifestage	Males of reproductive age	Increased testes, right epididymis, liver, and kidney weights. There was also decreased food consumption.	(Waterman et al., 2000 ; Exxon Biomedical, 1996a)			Use of default 10× UF _H
	Children	Reduced rodent offspring bodyweight gain between PND1 to 21 was observed in one and two-generation studies of reproduction.	(Waterman et al., 2000 ; Exxon Biomedical, 1996a, b)			Acute and intermediate duration PODs for developmental endpoints protective of effects of offspring bodyweight gain Use of default 10× UF _H
	Elderly	No direct evidence identified				Use of default 10× UF _H
Pre-existing disease or disorder	Health outcome/ target organs	No direct evidence identified		<p>Several preexisting conditions may contribute to adverse developmental outcomes (<i>e.g.</i>, diabetes, high blood pressure, certain viruses).</p> <p>Individuals with chronic liver and kidney disease may be more susceptible to effects on these target organs</p> <p>Viruses such as viral hepatitis can cause liver damage.</p>	<p>CDC (2023e)</p> <p>CDC (2023g)</p>	Use of default 10× UF _H

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DINP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DINP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Pre-existing disease or disorder	Toxicokinetics	No direct evidence identified		Chronic liver and kidney disease are associated with impaired metabolism and clearance (altered expression of phase 1 and phase 2 enzymes, impaired clearance), which may enhance exposure duration and concentration of DINP.		Use of default 10× UF _H
Lifestyle activities	Smoking	No direct evidence identified		Smoking during pregnancy may increase susceptibility for developmental outcomes (<i>e.g.</i> , early delivery and stillbirths).	CDC (2023f)	Qualitative discussion in Section 5.2 and this table
	Alcohol consumption	No direct evidence identified		Alcohol use during pregnancy can cause developmental outcomes (<i>e.g.</i> , fetal alcohol spectrum disorders). Heavy alcohol use may affect susceptibility to liver disease.	CDC (2023d) CDC (2023a)	Qualitative discussion in Section 5.2 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 5.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DINP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DINP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Sociodemographic status	Race/ethnicity	No direct evidence identified (<i>e.g.</i> , no information on polymorphisms in DINP metabolic pathways or diseases associated race/ethnicity that would lead to increased susceptibility to effects of DINP by any individual group).				Qualitative discussion in Section 5.2 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 10× UF _H
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. Additionally, diet can be a risk factor for fatty liver, which could be a pre-existing condition to enhance susceptibility to DINP-induced liver toxicity.	CDC (2023e) CDC (2023b)	Qualitative discussion in Section 5.2 and this table

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DINP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DINP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
Nutrition	Malnutrition	No direct evidence identified		<p>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.</p> <p>Thus, malnutrition may increase susceptibility to some developmental outcomes associated with DINP.</p>	<p>CDC (2021)</p> <p>CDC (2023b)</p>	Qualitative discussion in Section 5.2 and this table
	Target organs	No direct evidence identified		Polymorphisms in genes may increase susceptibility to liver, kidney, or developmental toxicity.		Use of default 10× UF _H
Genetics/ epigenetics	Toxicokinetics	No direct evidence identified		Polymorphisms in genes encoding enzymes (e.g., esterases) involved in metabolism of DINP may influence metabolism and excretion of DINP.		Use of default 10× UF _H

Susceptibility Category	Examples of Specific Factors	Direct Evidence this Factor Modifies Susceptibility to DINP		Indirect Evidence of Interaction with Target Organs or Biological Pathways Relevant to DINP		Susceptibility Addressed in Risk Evaluation?
		Description of Interaction	Key Citations	Description of Interaction	Key Citation(s)	
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 5.2 and this table
	Social environment	No direct evidence identified		Social isolation and other social determinants (<i>e.g.</i> , decreased social capital, stress) can lead to negative health outcomes.	CDC (2023c) ODPHP (2023c)	Qualitative discussion in Section 5.2 and this table
	Chemical co-exposures	Studies have demonstrated that co-exposure to DINP and other toxicologically similar phthalates (<i>e.g.</i> , DEHP, DBP, BBP) and other classes of antiandrogenic chemicals (<i>e.g.</i> , certain pesticides and pharmaceuticals – discussed more in (U.S. EPA, 2023a)) can induce effects on the developing male reproductive system in a dose-additive manner.	See (U.S. EPA, 2023a) and (U.S. EPA, 2023b)			Qualitative discussion in Section 5.2 and this table and will be quantitatively addressed as part of the phthalate cumulative risk assessment.

6 POINTS OF DEPARTURE USED TO ESTIMATE RISKS FROM DINP EXPOSURE

After considering hazard identification and evidence integration, dose-response evaluation, and weight of scientific evidence of POD candidates, EPA chose two non-cancer endpoints for the risk evaluation—one for acute and intermediate exposure scenarios and a second one for chronic scenarios (Table 6-1). HECs are based on daily continuous (24-hour) exposure, and HEDs are daily values.

For purposes of assessing acute/intermediate non-cancer risks, the selected acute/intermediate POD is considered most applicable to women of reproductive age, pregnant women, male infants, and male children. Use of this POD to assess risk for other age groups (*e.g.*, adult males) is conservative. For purposes of assessing chronic non-cancer risks, the selected POD is considered most applicable to adults (16+ years). Use of this POD for characterization of risk to infants and children from chronic exposure to DINP may be conservative and may not be relevant (discussed further in Appendix I).

Table 6-1. 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	Reference
Acute, Intermediate	Development	Rat	5–14 days throughout gestation	BMDL ₅ = 49 ^a NOAEL = 50 ^b	↓ fetal testicular testosterone, ↑ incidence of MNGs	12 ^c	63 [3.7]	UF _A = 3 UF _H = 10 Total UF = 30	(NASEM, 2017 ; Clewell et al., 2013a)
Chronic	Liver	Rat	2 years	NOAEL = 15	↑ liver weight, ↑ serum chemistry, histopathology ^{d e}	3.5	19 [1.1]	UF _A = 3 UF _H = 10 Total UF = 30	(Lington et al., 1997 ; Bio/dynamics, 1986)

BMDL = benchmark dose lower limit; HEC = human equivalent concentration; HED = human equivalent dose; MOE = margin of exposure; NOAEL = no observable adverse effect level; POD = point of departure; UF = uncertainty factor;

^a The BMDL₅ was derived by NASEM ([2017](#)) through meta-regression and BMD modeling of fetal testicular testosterone data from two studies of DINP with rats ([Boberg et al., 2011](#); [Hannas et al., 2011](#)). R code supporting NASEM's meta-regression and BMD analysis of DINP is publicly available through [GitHub](#).

^b The NOAEL was derived from the gestational exposure study conducted by Clewell et al. ([2013a](#)), which supports a NOAEL of 50 mg/kg-day based decreased fetal testicular testosterone and increased incidence of MNGs.

^c The BMDL₅ of 49 mg/kg-day and NOAEL of 50 mg/kg-day both support an HED of 12 mg/kg-day.

^d Liver toxicity included increased relative liver weight, increased serum chemistry (*i.e.*, AST, ALT, ALP), and histopathologic findings (*e.g.*, focal necrosis, spongiosis hepatis) in F344 rats following 2 years of dietary exposure to DINP ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)).

^e The Lington study presents a portion of the data from a larger good laboratory practice (GLP)-certified study by Bio/dynamics ([1986](#)).

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APPENDICES

Appendix A EXISTING ASSESSMENTS FROM OTHER REGULATORY AGENCIES OF DINP

The available existing assessments of DINP are summarized in Table_Apx A-1, which includes details regarding external peer review, public consultation, and systematic review protocols that were used.

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

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
U.S. EPA (authors are affiliated with the U.S. EPA's Center for Public Health and Environmental Assessment)	<i>Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence</i> (Radke et al., 2018) <i>Phthalate exposure and female reproductive and developmental outcomes: A systematic review of the human epidemiological evidence</i> (Radke et al., 2019b) <i>Phthalate exposure and metabolic effects: A systematic review of the human epidemiological evidence</i> (Radke et al., 2019a) <i>Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence</i> (Radke et al., 2020a).	No	No	Yes	- Publications were subjected to peer-review prior to being published in a special issue of <i>Environment International</i> - Publications employed a systematic review process that included literature search and screening, study evaluation, data extraction, and evidence synthesis. The full systematic review protocol is available as a supplemental file associated with each publication.
U.S. EPA	<i>Technical review of diisononyl phthalate (Final assessment)</i> (U.S. EPA, 2023c)	No	Yes	No	- Technical review of DINP was reviewed by two internal EPA reviewers, but was not subjected to external peer-review - Draft technical review of DINP was subjected to a public review period. Public comments available here:

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
					https://www.regulations.gov/docket/EPA-HQ-TRI-2022-0262/comments
U.S. CPSC	<i>Toxicity review of Diisononyl Phthalate (DINP)</i> (U.S. CPSC, 2010) <i>Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives</i> (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 page 12 of (U.S. CPSC, 2014)
NASEM	<i>Application of systematic review methods in an overall strategy for evaluating low-dose toxicity from endocrine active chemicals</i> (NASEM, 2017)	Yes	No	Yes	- Draft report was reviewed by individuals chosen for their diverse perspectives and technical expertise in accordance with the National Academies peer review process. See Acknowledgements Section of (NASEM, 2017) for more details. - Employed NTP's Office of Health Assessment and Translation (OHAT) systematic review method
Health Canada	<i>State of the science report: Phthalate substance grouping 1,2-Benzenedicarboxylic acid, diisononyl ester; 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich (Diisononyl Phthalate; DINP). Chemical Abstracts Service Registry Numbers: 28553-12-0 and 68515-48-0</i> (EC/HC, 2015) <i>Supporting Documentation: Carcinogenicity of Phthalates - Mode</i>	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 page 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: https://www.canada.ca/en/health-canada/services/chemical-

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
	<p><i>of Action and Human Relevance</i> (Health Canada, 2015)</p> <p><i>Supporting documentation: Evaluation of epidemiologic studies on phthalate compounds and their metabolites for hormonal effects, growth and development and reproductive parameters</i> (Health Canada, 2018b)</p> <p><i>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</i> (Health Canada, 2018a)</p> <p><i>Screening Assessment - Phthalate Substance Grouping</i> (ECCC/HC, 2020)</p>				<p>substances/substance-groupings-initiative/phthalate.html#a1</p> <ul style="list-style-type: none"> - 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	<p><i>Priority existing chemical assessment report no. 35: Diisononyl phthalate</i> (NICNAS, 2012)</p>	No	Yes	No	<ul style="list-style-type: none"> - NICNAS (2012) 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. - NICNAS (2012) states "Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
					during which requests for variation of the report may be made.” See Preface of (NICNAS, 2012) 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, 2012)
ECHA	<i>Evaluation of New Scientific Evidence Concerning DINP and DIDP in Relation to Entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006</i> (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	<i>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</i> (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. - Details regarding EFSA’s strategy for identifying new information and literature are provided on page 18 and Appendix B of (EFSA, 2019)
NTP-CERHR	<i>NTP-CERHR monograph on the potential human reproductive and developmental effects of di-isononyl phthalate (DINP)</i> (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)

Agency	Assessment(s) (Reference)	External Peer-Review?	Public Consultation?	Systematic Review Protocol Employed?	Remarks
					- No formal systematic review protocol employed.

Appendix B SUMMARY OF LIVER TOXICITY STUDIES

This Appendix contains more detailed information on the available studies described in the liver toxicity hazard identification (Section 3.2), including information on individual study design.

Humans

No epidemiologic studies were identified by Health Canada (2018a) or by Radke et al. that examined the association between DINP and/or its metabolites and biomarkers of liver injury.

New Literature: EPA considered new studies published since Health Canada's assessment (Health Canada, 2018a); however, no studies were identified that fall within this date range and evaluated liver injury for DINP and/or its metabolites.

Laboratory Animals

Existing assessments have consistently identified the liver as one of the most sensitive target organs following oral exposure to DINP in experimental animal studies (ECCC/HC, 2020; EFSA, 2019; EC/HC, 2015; ECHA, 2013b; NICNAS, 2012; U.S. CPSC, 2010; EFSA, 2005; ECB, 2003; NTP-CERHR, 2003; U.S. CPSC, 2001). Intermediate (>1 to 30 days), subchronic (>30 to 90 days) and chronic (>90 days) exposure studies have reported significant liver effects. Available studies include: 11 intermediate oral studies (six studies on rats, four studies on mice, 1 study on cynomolgus monkeys); nine subchronic oral exposure studies (six on rats, one on mice, one on beagle dogs, and one on marmosets) and five chronic oral exposure studies (four on rats and one on mice) Available studies are summarized in Table_Apx B-1, Table_Apx B-2, and Table_Apx B-6, and are discussed further below.

Considerations for Interpretation of Hepatic Effects: Consistent with previous guidance documents (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 exposure-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, bilirubin, cholesterol) and/or histopathology indicative of an adverse response (*e.g.*, hyperplasia, degeneration, necrosis, inflammation). Further, phthalates, including DINP, can induce peroxisome proliferation in the livers of mice and rats (Corton et al., 2018; Lapinskas et al., 2005; Valles et al., 2003), and EPA considered evidence supporting a role for PPAR α activation in peroxisome-induced hepatic effects of DINP. For purposes of identifying study NOAEL and LOAEL values, effects consistent with peroxisome proliferation and PPAR α activation were also considered relevant for setting the LOAEL.

Intermediate (≥ 1 to 30 Days) Exposure Studies: EPA evaluated 12 intermediate exposure animal studies from existing assessments that evaluated liver effects following oral exposure to DINP (Ma et al., 2014; Kwack et al., 2010; Kwack et al., 2009; Valles et al., 2003; Kaufmann et al., 2002; Pugh et al., 2000; Smith et al., 2000; Hüls AG, 1992; Hazleton Labs, 1991a; BIBRA, 1986; Bio/dynamics, 1982a; Midwest Research Institute, 1981). The database includes seven studies in various strains of rat, four studies in mice, and one study in monkeys. One intermediate dermal exposure study in female B6C3F1 mice was identified (Butala et al., 2004). These studies provide data on relative/and/or absolute liver weights, histopathology, hepatic enzyme levels and/or activity (*e.g.*, AST, ALT, ALP), and other parameters useful to determining the effects of DINP on the liver. These studies are summarized in Table_Apx B-1.

Eight of the available intermediate oral studies and one of the dermal exposure studies reported increases in absolute and/or relative liver weights or incidences of hepatocyte proliferation or other

nonneoplastic lesions following oral exposure to DINP ([Ma et al., 2014](#); [Kwack et al., 2009](#); [Valles et al., 2003](#); [Kaufmann et al., 2002](#); [Smith et al., 2000](#); [Hüls AG, 1992](#); [Hazleton Labs, 1991a](#); [BIBRA, 1986](#); [Bio/dynamics, 1982a](#)). These observations sometimes coincided with increases in peroxisomal volume, peroxisomal beta oxidation, and activity of enzymes such as palmitoyl-CoA oxidase, indicative of PPAR α activation, which is discussed in further detail in the mechanistic section.

The BIBRA ([1986](#)) study evaluated the ability of DINP to induce peroxisome proliferation in male and female F344 rats fed 0, 0.6, 1.2, or 2.5 percent DINP in the diet for 21 days (equivalent to 0, 639, 1,192, or 2,195 mg/kg-day [males] and 0, 607, 1,193, or 2,289 mg/kg-day [females]). Body weights were significantly reduced in males (6–12% decrease) and in females (6–14% decrease) in a time- and dose-dependent manner. Food intake was also significantly reduced (19–49%) in males and females. Significant dose-dependent increases in absolute and relative liver weight were observed in males and females beginning in animals from the low dose group (639 mg/kg-day in males; 607 mg/kg-day females). The effects observed on liver weight were considered exposure-related even though terminal body weights were significantly reduced in males and in females in a dose-dependent manner, and body weight gain was reduced in animals at the highest dose level. In parallel with the increases in liver weights, the authors reported dose-dependent increases in cyanide-insensitive palmitoyl-CoA oxidation levels in males and females of the mid- and high-dose groups, dose-dependent increases in microsomal protein levels of males and females (all dose levels) and increases in lauric acid 11- and 12-hydroxylase activities in males of the low-dose group (639 mg/kg-day in males). Hydroxylase activities were increased in high-dose females. The authors also reported decreases in total cholesterol in males (9–24%) and females (14–24%), as well as dose-dependent decreases in serum triglycerides in males (24–48%). However, dose-dependent increases in serum triglycerides (24–26%) were observed in females. The inconsistency of effects between sexes is source of uncertainty in the data set. The authors also examined liver tissue via electron microscopy and observed increases in peroxisome proliferation in males and females from the highest exposure groups. However, these effects were not further quantitatively described, which is another limitation of the data set.

Data from BIBRA ([1986](#)) were consistent with Kwack et al. ([2009](#)). In the Kwack study, male SD rats were administered 0 or 500 mg/kg-day DINP daily via gavage for 4 weeks. Increased relative liver weight (45%) was observed, which coincided with perturbations in several clinical chemistry parameters. Increases were observed in the serum levels of AST (32%), ALP (260%), and triglycerides (53%). The observed effects were considered adverse because the liver weight changes were accompanied by clinical chemistry markers of hepatotoxicity. Interestingly, these results were not wholly consistent with a study by the same authors with a shorter exposure duration ([Kwack et al., 2010](#)). In that study, male SD rats were again administered to 0 or 500 mg/kg-day DINP daily via gavage for 2 weeks. Increases in AST levels (31%) and ALP (159%) were observed as well as increases in serum triglycerides. There was no change in ALT levels and no significant change in relative liver weight.

Several other studies reported increases in relative and/or absolute liver weight with concomitant changes in other hepatic endpoints in B6C3F1 mice ([Valles et al., 2003](#); [Kaufmann et al., 2002](#); [Smith et al., 2000](#); [Hazleton Labs, 1991a](#)) and/or F344 rats ([Smith et al., 2000](#); [Hüls AG, 1992](#); [Bio/dynamics, 1982a](#); [Midwest Research Institute, 1981](#)), following oral exposure to DINP.

Smith et al. ([2000](#)) evaluated liver weights in mice and rats following 2- or 4-week dietary exposure to DINP. In rats, increased relative liver weights were observed after 4 weeks of exposure to 12,000 ppm DINP (equivalent to 1200 mg/kg-day). In mice, increased liver weights were observed after 2- or 4-weeks exposure to 6,000 ppm DINP (equivalent to 900 mg/kg-day). The LOEL in each species was the high-dose of DINP (1,200 mg/kg-day for rats, 900 mg/kg-day in mice). Valles et al. ([2003](#)) reported

similar findings in male and female B6C3F1 mice fed diets containing 0, 150, 1,500, 4,000, or 8,000 ppm of DINP (CASRN 68515-48-0) for 2 weeks. Relative liver weight was significantly increased in both sexes at the two highest dose groups and in females at the mid dose-group. The percent change in relative liver weight for the high dose group was 37 percent in males and over 50 percent in females. The other statistically significant increases in females were less than 10 percent over controls, while relative liver weight in males of the 4,000 ppm increased by almost 17 percent.

Two other studies ([Kaufmann et al., 2002](#); [Hazleton Labs, 1991a](#)) reported similar findings at lower doses after similar exposure durations (*i.e.*, 4 weeks). In Kaufmann et al. (2002), male and female B6C3F1 mice were exposed to 0, 500, 1,500, 4,000, or 8,000 ppm DINP in the diet for 4 weeks (equivalent to 0, 117, 350, 913, 1860 mg/kg-day [males]; or 0, 167, 546, 1272, or 2806 mg/kg-day [females]). Significant increases in absolute and relative liver weight were observed in males and females, which corresponded with increased peroxisomal volume and peroxisomal enzyme activity (cyanide-insensitive palmitoyl-CoA) at doses as low as 350 mg/kg-day in males or 546 mg/kg-day in females. The LOEL/NOEL was 350/117 mg/kg-day in males and 546/167 mg/kg-day in females. Hazleton Labs (1991a) reported similar LOEL values for liver effects in males (635 mg/kg-day) and females (780 mg/kg-day). That study exposed male and female B6C3F1 mice to 0, 3000, 6000, or 12,500 ppm DINP in the diet for 4 weeks (equivalent to 0, 635, 1,377, 2,689, or 6,518 mg/kg-day [males]; 0, 780, 1,761, 3,287, or 6,920 mg/kg-day [females]) and evaluated liver weights, histopathology, and serum liver enzymes at study termination.

Increases in absolute and relative liver weights were observed in all male and female exposure groups except the low dose, and increased ALT activity was observed in males and females from the high dose only. Additional findings included enlarged and discolored livers, increased incidence of hepatocytomegaly (all male dose groups; all female dose groups except low dose), and increased incidence of coagulative necrosis and/or separate chronic inflammatory foci in high-dose males (6,518 mg/kg-day) and females (6,920 mg/kg-day) as well as females of the 3,287 mg/kg-day group. Similar findings were reported in a study by Ma et al. (2014), which administered 0.2, 2, 20 or 200 mg/kg-day DINP to male Kunming mice via oral gavage daily for 14 days. This study established a NOAEL at 20 mg/kg-day and a LOAEL at 200 mg/kg-day based on increased histopathologic lesions (reported qualitatively only) of the liver, including central vein dilation, congestion, and narrowing of the sinusoid with loose cytoplasm in animals exposed to the highest dose of DINP.

The findings that support liver toxicity in mice and the rat study by Smith et al. (2000) were consistent with two additional rat studies. A study by the Midwest Research Institute (1981) fed male and female F344 rats 0, 0.2, 0.67, or 2 percent DINP in the diet for 28 days (estimated doses: 0, 150, 500, 1,500 mg/kg-day [males]; 0, 125, 420, 1,300 mg/kg-day [females]). Increases in hepatic catalase and carnitine acetyltransferase activity were observed in low dose males (150 mg/kg-day) and females (125 mg/kg-day). Increases in absolute and relative liver weight were also observed in the mid dose males (500 mg/kg-day) and females (420 mg/kg-day) with no corresponding change in body weight. Additionally, Bio/dynamics (1982a) administered 0 or 1,700 mg/kg-day DINP in the diet to male rats for 1 week and then evaluated liver weight, general appearance (*i.e.*, macroscopic observation), and clinical chemistry parameters, including serum ALP at study termination. At study termination, the treated animals had increased absolute and relative liver weight, as well as increased body weight, and the authors noted slight congestion in all lobes of the liver in animals exposed to DINP. No statistically or biologically significant changes were observed for serum ALP levels. A 14-day study by Hüls AG (1992) exposed female F344 rats to 0, 25, 75, 150, or 1,500 mg/kg-day and then evaluated liver weights, clinical chemistry parameters, and histopathology at study termination, as well as activities of several microsomal enzymes. In general, effects were observed at the highest dose, including increases in

absolute and relative liver weight, and increases in EROD. A dose-dependent increase was observed in lauric acid hydroxylase, beginning at 25 mg/kg-day. Of note, this study was not reasonably available to EPA, and data reported on this study reflect those reported by Health Canada's Hazard Assessment ([EC/HC, 2015](#)).

Not all studies identified in existing assessments reported hepatic effects consistent with peroxisomal beta-oxidation and/or PPAR α activation. Indeed, one study in cynomolgus monkeys ([Pugh et al., 2000](#)) reported no effect on relative liver weights, histopathology, or serum chemistry parameters in monkeys administered 0 or 500 mg/kg-day DINP daily via oral gavage for 14 days.

New Literature: EPA identified two new studies published between 2015 and 2024 that provided data on toxicological effects of the liver following intermediate exposure to DINP via the oral route ([Neier et al., 2018](#)) or dermal route ([Liang and Yan, 2020](#)). The developmental exposure study by Neier et al. (2018) evaluated absolute and relative liver weights as well as hepatic triglyceride levels in PND21 male and female yellow agouti (Avy) mice. Dams were administered 0 or 75 ppm DINP in the diet (equivalent to 15 mg/kg-day) beginning 2-weeks before mating and lasting through PND21. Increased absolute (27.6%) and relative (15.5%) liver weights were observed in exposed female offspring at PND21. No significant changes were observed in males. No significant changes were observed in hepatic triglyceride levels, suggesting that differences in liver weight were not attributed to increases in lipid accumulation in the liver in this study.

Liang and Yan (2020) applied 0, 0.02, 0.2, 2, 20, or 200 mg/kg-day DINP to the shaved skin on the backs of male Balb/c mice (6/group) for 28 days and evaluated liver weights, liver histopathology, and markers of oxidative stress in liver tissue at the end of the study. Significant increases in relative liver weight were observed in the 20 (7% increase) and 200 mg/kg-day groups (11% increase) and no significant changes in absolute liver weight were observed, nor changes in body weight. The increased liver weight corresponded with increases in histopathological findings in the 20 and 200 mg/kg-day group including enlarged hepatocytes, broadened liver cords, and expanded central veins. However, the histopathological data was not reported quantitatively, only single representative histological images were provided, which limits the ability to interpret these results. Changes were observed in various markers of oxidative stress, including ROS via the DCF-DA assay, DNA-protein-crosslinks (DPC), glutathione content (but not ratio of GSH:GSSH), and MDA. In general, a dose-dependent increase in ROS and MDA and corresponding dose-dependent decrease in GSH content was observed in liver samples, with statistically significant effects at 20 and 200 mg/kg-day. These data suggest a pro-oxidant environment in the liver. Additionally, DPCs were increased at 200 mg/kg-day in liver. While these data support that DINP negatively impacts the liver at doses as low as 20 mg/kg-day, the study has several limitations that impact the ability to interpret the results, namely those of reporting deficiencies (*e.g.*, qualitative reporting of histopathological data), and exposure methods characterization for the dermal application of DINP.

As discussed in Section 2.3, dermal absorption of DINP is low (*i.e.*, 2–4% over 7 days), which indicates that the dose of absorbed DINP that caused oxidative stress in the study by Liang and Yan (2020) is much lower than the dermally applied dose of 20 mg/kg-day. However, there are several uncertainties associated with the study by Liang and Yan (2020) that raise uncertainty with the actual received doses in the study, as only nominal doses are provided. Liang and Yan state that 20 μ L of test solution (concentration of applied test solution not provided) was applied evenly to a 2 cm² area of exposed skin on the center of the back of the mouse; however, additionally methodological details pertaining to how DINP was dermally administered were not provided. For example, study authors do not provide information relating to how hair was removed from the backs of mice and whether or not care was taken

to avoid applying solutions of DINP to abraded skin, which would be expected to increase dermal absorption; how frequently DINP solutions were applied and whether DINP was washed from the skin at the application site between dermal applications; and whether skin was covered with a bandage to help limit evaporation, as well as oral ingestion of DINP through grooming.

Table_Apx B-1. Summary of Liver Effects Reported in Animal Toxicological Studies Following Intermediate Duration Exposure to DINP

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
Kunming mice (males only); gavage; 0, 0.2, 2, 20, 200 mg/kg-day; 14 days (Ma et al., 2014)	20/ 200	Markers of oxidative stress (↑ ROS, ↓ GSH, ↑MDA, ↑ 8-OH-dG) and inflammation (↑IL-1, ↑ TNFα) at ≥ 20 mg/kg-day	Other liver effects: Liver histopathology: ↑ incidences of edema (20 mg/kg-day); central vein dilation, congestion, edema, & narrowing sinusoidal with extremely loose cytoplasm (200 mg/kg-day) <u>Considerations:</u> Bodyweight not reported <u>Limitations:</u> Histopathology qualitative only (no incidence data or statistical analysis); organ weight and clinical chemistry not evaluated
F344 rats (females only); gavage; 0, 25, 75, 150, 1,500 mg/kg-day; 14 days (Hüls AG, 1992)	25 (LOEL)	↑ lauric acid hydroxylase (dose-dependent beginning at 25 mg/kg-day)	Other liver effects: ↑ absolute and relative liver weight at 1,500 mg/kg-day; ↑ liver microsomal enzyme activities (pentoxyresorufin O-desalkylase (PROD) and lauryl-CoA oxidase) at 1,500 mg/kg-day
F344 rats (both sexes); dietary; 0, 0.2, 0.67, 2% (estimated: 150, 500, 1,500 mg/kg-day [males]; 0, 125, 420, 1,300 mg/kg-day [females]); 28 days (Midwest Research Institute, 1981)	ND/ 125 (females; LOEL) ND/ 150 (males; LOEL)	↑ in hepatic catalase and carnitine acetyltransferase activity	Other liver effects: ↑ absolute and relative liver weight (500 mg/kg-day [males]; 420 mg/kg-day [females])
B6C3F1 mice (both sexes); dietary; 0, 500, 1,500, 4,000, 8,000 ppm (estimated: 117, 350, 913, 1,860 mg/kg-day [males]; 0, 167, 546, 1,272, 2,806 mg/kg-day [females]); 1 or	117/ 350 (males) 167/ 546 (female)	↑ absolute and relative liver weight; ↑ peroxisomal volume, and peroxisomal enzyme activity; ↑ hepatocyte proliferation in males	Other liver effects: Liver histopathology: ↑ hepatocyte proliferation in females at ≥1,272 mg/kg-day. <u>Considerations:</u> Multiple zones of the liver examined for

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
4 weeks (Kaufmann et al., 2002)			quantitative measurement of hepatocyte proliferation; bodyweight not reported
SD rats (males only); oral gavage; 0, 500 mg/kg-day; 28 days (Kwack et al., 2009)	ND/ 500	↓ body weight gain; ↑ relative liver weight; clinical chemistry (↑ AST, ALP, triglycerides)	<u>Considerations</u> : ↓ body weight gain (~10%) in DINP exposed mice
F344 rats (both sexes); diet; 0, 0.6, 1.2, 2.5% (estimated: 639, 1,192, 2,195 mg/kg-day [males]; 607, 1,198, 2,289 mg/kg-day [females]); 21 days (BIBRA, 1986)	ND/ 639 (males) ND/ 607 (females)	↑ absolute and relative liver weight (absolute increase in males: 136, 150, and 165%; relative increase in males: 136, 173, 232%; absolute increase in females: 124, 164, and 198%; relative liver weights in females: 131, 175, 231%); ↑ 11- and 12-hydroxylase activity, hypolipidemic effects	<u>Considerations</u> : Body weights and food intake were significantly reduced in males (6–12%) and in females (6–14% decrease). Food intake was also significantly reduced (19–49%) in males and females.
B6C3F1 mice (both sexes); dietary; 0, 3,000, 6,000, 12,500 ppm (estimated: 635, 1,377, 2,689, 6,518 mg/kg-day [males]; 780, 1,761, 3,287, 6,920 mg/kg-day [females]); 4 weeks (Hazleton Labs, 1991a)	ND/ 635 (males) ND/ 780 (females, LOEL)	Enlarged and discolored livers; ↑ incidence of hepatocytomegaly	<u>Other liver effects</u> : ↑ incidence of coagulative necrosis and/or separate chronic inflammatory foci
B6C3F1 mice (males only); dietary; 0, 500, 6,000 ppm (estimated: 75, 900 mg/kg-day); 2 or 4 weeks (Smith et al., 2000) ^c	75 (NOEL)/ 900 (LOEL)	↑ in relative liver weight at 4 weeks	<u>Other liver effects</u> : ↑ PBOX, ↑ DNA synthesis; inhibition of GJIC <u>Limitations</u> : Bodyweight not reported
F344 rats (males only); dietary; 0, 1,000, 12,000 ppm (estimated: 100, 1,200 mg/kg-day); 2 or 4 weeks (Smith et al., 2000) ^c	100 (NOEL)/ 1,200 (LOEL)	↑ in relative liver weight at 4 weeks	<u>Other liver effects</u> : ↑ PBOX, ↑ DNA synthesis; inhibition of GJIC <u>Considerations</u> : Significant increases in relative liver weight

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
			observed at 4-week but not 2-week timepoint. <u>Limitations:</u> Only male rats were evaluated.
F344 rats (males only); dietary; 0, 2% (estimated: 1,700 mg/kg-day); 7 days (Bio/dynamics, 1982a)	ND/ 1,700	↑ absolute and relative liver weight; macroscopic liver observations; changes in clinical chemistry (↓ triglycerides)	
Cynomolgus monkeys (males only); 0, 500 mg/kg-day; oral gavage; 14 days (Pugh et al., 2000)	500/ ND		No statistically or biologically significant effects were observed
SD rats (male and female); 0 or 500 mg/kg-day; gavage; 14 days (Kwack et al., 2010)	ND/ 500	↑ AST activity (31%), ↑ ALP (159%); ↑ serum triglycerides	<u>Other liver effects:</u> ↑ liver weights, altered serum biochemistry, and altered urinalysis <u>Considerations:</u> No change in serum ALT
<p>^a Dose equivalent calculated from 75 mg DINP/kg chow/day based on the assumption that pregnant and nursing female mice weigh approximately 25g and eat approximately 5 g chow/day.</p> <p>^b Data for the Huls AG study (1992) were not reasonably available to EPA; data in this table reflect those reported by Health Canada's Hazard Assessment (EC/HC, 2015).</p> <p>^c Smith et al. (2000) evaluated two isomers of DINP: DINP-1 (CAS 68515-48-0) and DINP-A (CAS 71549-78-5). The DINP-A isomer is outside the scope of the hazard evaluation; all results herein refer to the DINP-1 isomer.</p>			

Subchronic (>30 to 90 Days) Exposure Studies: EPA identified nine studies from existing assessments that provide data on the toxicological effects of DINP on the liver following subchronic duration oral exposure, including six studies in rats ([Hazleton Labs, 1991b](#); [BASF, 1987](#); [Bio/dynamics, 1982b, c](#); [Hazleton Labs, 1981, 1971](#)), one in mice ([Hazleton Labs, 1992](#)), one study in dogs ([Hazleton Laboratories, 1971](#)), and one study in marmoset monkeys ([Hall et al., 1999](#)). The available studies are summarized in Table_Apx B-2 and discussed further below. One dermal exposure study in New Zealand white rabbits was also available ([Hazleton Laboratories, 1969](#)).

The lowest achieved dose across these rodent studies was 50 mg/kg-day and the highest was 5,770 mg/kg-day (Table_Apx B-2). All studies reported increases in absolute and/or relative liver weight, sometimes in parallel with exposure-related histopathological effects on the liver (*e.g.*, hepatocytic hypertrophy), and sometimes coinciding with increases in liver enzymes (*i.e.*, ALT, ALP), suggesting impaired liver function. These data suggest that the liver is a target organ for DINP, which is consistent with conclusions from previous assessments by regulatory agencies.

Hazleton Laboratories ([1971](#)) reported increased absolute and relative liver weights in both sexes at 500 mg/kg-day as well as exposure-related changes in liver histopathology in males (hepatocytic hypertrophy throughout the panlobular section). In that study, albino rats were exposed to 0, 50, 150, or 500 mg/kg-day DINP for 13 weeks via diet. Two additional dietary exposure studies in rats by Hazleton Labs ([1991b](#), [1981](#)) reported increased liver weights, and increased incidences of histopathological lesions or altered clinical chemistry parameters that suggest liver toxicity. Consistent with the earlier Hazleton study ([1971](#)), Hazleton Labs ([1991b](#)) found evidence to suggest liver toxicity in F344 rats exposed to 0, 2500, 5,000, 10,000 or 20,000 ppm DINP for 13 weeks via feed (equivalent to 0, 176, 354, 719, or 1,545 mg/kg-day [males]; 0, 218, 438, 823, or 1,687 mg/kg-day [females]). Increases in absolute and relative liver weight were accompanied by hepatocellular enlargement in the highest treatment group. The LOEL was 176 mg/kg-day in males and 218 mg/kg-day in females based on increased liver weights.

Another study from Hazleton Labs ([1981](#)) administered 0, 1,000, 3,000, or 10,000 ppm DINP to male and female albino rats for 13 weeks in feed (equivalent to 0, 60, 180, or 600 mg/kg-day). Exposure related increases in absolute and relative liver weights were observed in males and females from the high dose groups (absolute weights: 33% increase in males, 23.3% increase in females; relative liver weights: 30.2% increase in males; 33.3% in females). Unlike the other Hazleton rat studies ([1991b](#), [1971](#)), exposure-related nonneoplastic lesions in the liver were not observed, although hepatocellular degeneration was noted in two individual high-dose (600 mg/kg-day) males. Moreover, the authors note that exposure-related changes in histopathology were limited to the kidneys of high dose males. Dose-related decreases in several clinical chemistry parameters were observed in both sexes, including total protein, globulin, and total bilirubin, apart from total bilirubin from males of the mid-dose group (180 mg/kg-day). The decrease in globulin levels reached statistical significance in mid- (180 mg/kg-day) and high-dose (600 mg/kg-day) females. Decreased bilirubin reached statistical significance in high-dose males.

Two similarly designed studies in rats from Bio/dynamics ([1982b](#), [c](#)) also reported increased absolute and/or relative liver weight at similar doses in parallel with changes in clinical chemistry parameters. In the first Bio/Dynamics study, male and female F344 rats were administered 0, 0.1, 0.3, 0.6, 1.0, or 2.0 percent DINP in diet for 13 weeks (equivalent to 0, 77, 227, 460, 767, or 1,554 mg/kg-day) ([Bio/dynamics, 1982b](#)). In the second study, male and female SD rats were administered 0.3 or 1.0 percent DINP in diet for 13 weeks (equivalent to 0, 201 or 690 mg/kg-day [males]; 0, 251 or 880 mg/kg-day [females]) ([Bio/dynamics, 1982c](#)). In the first study, increased absolute and relative liver weights and decreased cholesterol were observed in females exposed to 227 mg/kg-day (LOAEL) ([Bio/dynamics, 1982b](#)). Other effects included increases in ALT in the two highest doses in males (767 or 1,554 mg/kg-day) and highest dose in females. In the second study, increased relative liver weight and decreased serum triglyceride levels were observed in males exposed to doses as low as 201 mg/kg-day and females exposed to 251 mg/kg-day (LOEL), as well as at higher doses. These changes were accompanied by a 49 or 53 percent increase in ALP (in males or females, respectively) and 31 percent increase in ALT (males) in rats from the high dose groups. In both studies, terminal body weight was decreased by at least 10 percent in high-dose males and females. In the SD rat study, terminal body weight was also reduced in the low dose animals by 24 percent (males; 201 mg/kg-day) or over 15 percent (females; 251 mg/kg-day) ([Bio/dynamics, 1982c](#)).

An additional study from BASF ([1987](#)) reported effects on clinical chemistry and other hepatic changes related to hepatotoxicity with similar LOAELs to the Bio/dynamics studies. In that study, male and female Wistar rats were fed 0, 3,000, 10,000, or 30,000 ppm DINP in the diet for 13 weeks (equivalent to 0, 152, 512, 1,543 mg/kg-day [males]; 0, 200, 666, 2,049 mg/kg-day [females]). Decreased

triglyceride levels and peripheral fat deposits in hepatocytes were reported in low-dose male (152 mg/kg-day) and female (200 mg/kg-day) rats. Increased absolute and relative liver weights were observed at 1,101 mg/kg-day [males] and 1,214 mg/kg-day [females]), which are doses much higher than those in which increased liver weights were observed in the two Bio/dynamics studies ([1982b, c](#)). The BASF study ([1987](#)) was not reasonably available to EPA in English; it was identified from Health Canada's Hazard Assessment ([EC/HC, 2015](#)) and therefore is not further considered.

One subchronic duration study in mice provided evidence that the liver is a target of DINP ([Hazleton Labs, 1992](#)). In that study, male and female B6C3F1 mice were administered 1,500, 4,000, 10,000, or 20,000 ppm DINP (equivalent to 365, 972, 2,600, or 5,770 mg/kg-day) in the diet for 13 weeks. Increases in absolute and relative liver weight, as well as histopathologic effects such as hepatocyte enlargement, liver degeneration, necrosis, and pigment in Kupffer cells as well as in the bile canaliculi were observed in the 972 mg/kg-day group (LOAEL). One limitation of this study was the small sample size, which results in limited statistical power to detect differences between treated groups and controls.

Not all studies have consistently demonstrated the liver toxicity of DINP. Indeed, studies in non-rodent species, including one study in beagle dogs ([Hazleton Laboratories, 1971](#)) and one study in marmoset monkeys ([Hall et al., 1999](#)), have reported contrasting findings. In a study by Hazleton Laboratories ([1971](#)), 0, 0.125, 0.5, 2 percent DINP was administered to beagles in the diet for 13 weeks (equivalent to 0, 37, 160, or 2,000 mg/kg-day). Increases in absolute and relative liver weights were observed at 160 mg/kg-day in males and 2,000 mg/kg-day in both sexes. Histopathologic changes were also observed, including hepatocyte hypertrophy associated with decreased prominence of hepatic sinusoids at 2,000 mg/kg-day in both sexes. Serum ALT levels increased by 37 percent in males and 48 percent in females from week 4 at 160 and 2,000 mg/kg-day. Dose-responsive increases in ALT levels were observed in males (47, 32 and 60% increase) and females (48, 74, and 107% increase) at study termination. Limitations of this study include the small sample size and lack of statistical analysis, which increase uncertainty in the data from this study. Nevertheless, existing assessments of DINP have supported NOAEL and LOAEL values of 37 and 160 mg/kg-day based on increased absolute and relative liver weights accompanied with histopathological changes at the highest dose (2,000 mg/kg-day) tested ([EC/HC, 2015](#)), or a LOAEL of 37 mg/kg-day with no NOAEL based on increase liver weight and serum ALT ([ECHA, 2013b](#); [ECB, 2003](#)). Additional limitations of this study include reporting deficiencies, including the lack of statistical analyses and inconsistencies between text and tables. These limitations increase uncertainty in the data from this study.

In contrast, a study in marmoset monkeys by Hall et al. ([1999](#)) did not observe any statistically significant liver effects. In that study, male and female marmoset monkeys were administered 0, 100, 500, or 2,500 mg/kg-day DINP daily via oral gavage for 13 weeks. Exposure to DINP increased liver weight in males, but the effect was not dose-dependent nor statistically significant at any dose, which the authors attribute to low sample size and high variability.

New Literature: EPA did not identify any new studies published from 2015 through 2024 that provided data on toxicological effects of liver following chronic exposure to DINP.

Table_Apx B-2. Summary of Liver Effects Reported in Animal Toxicological Studies Following Subchronic Exposure to DINP

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Comments
Beagle dogs (both sexes); dietary; 0, 0.125, 0.5, 2% (estimated: 37, 160, 2,000 mg/kg-day); 13 weeks (Hazleton Laboratories, 1971)	37/ 160	↑ absolute and relative liver weight; ↑ serum ALT	<u>Other liver effects:</u> Hepatocytic hypertrophy associated with decreased prominence of hepatic sinusoids at 2,000 mg/kg-day. Hepatocytic cytoplasm varied from fine granular to vacuolated appearance. <u>Considerations:</u> No NOAEL established due to absence of statistical analysis and some inconsistencies in data reporting (<i>i.e.</i> , text and tables in the study)
F344 rats (both sexes); dietary; 0, 0.1, 0.3, 0.6, 1.0, 2.0% (estimated: 77, 227, 460, 767, 1,554 mg/kg-day); 13 weeks (Bio/dynamics, 1982b)	77/ 227	↑ absolute and relative liver weight; ↓ cholesterol (females)	<u>Other liver effects:</u> ↑ ALT (males at ≥767 mg/kg-day and females at 1,554 mg/kg-day); ↓ cholesterol (females at ≥227 1,554 mg/kg-day) <u>Considerations:</u> ↓ bodyweight gains at 767 mg/kg-day (males only). ↓ terminal bodyweight (≥10%) at 1,554 mg/kg-day (both sexes).
Wistar rats (both sexes); dietary; 0, 3,000, 10,000, 30,000 ppm (estimated: 152, 512, 1,543 mg/kg-day [males]; 200, 666, 2,049 mg/kg-day [females]); 13 weeks ((BASF, 1987) as cited by Health Canada (EC/HC, 2015)) ^a	ND/ 152 (males) ND/ 200 (females)	Clinical chemistry and liver changes related to hepatotoxicity (↓ triglyceride level and ↓ peripheral fat deposits in hepatocytes)	<u>Considerations:</u> ↓ bodyweight for males at 152 and 1,543 mg/kg-day. Insufficient information to discern if reported bodyweight was terminal or bodyweight change.
F344 rats (both sexes); dietary; 0, 2,500, 5,000, 10,000, 20,000 ppm (estimated: 176, 354, 719, 1,545 mg/kg-day [males]; 218, 438, 823, 1,687 mg/kg-day [females]); 13	ND/ 176 (males) ND/ 218 (females)	↑ liver weights	<u>Other liver effects:</u> Hepatocellular enlargement at the highest dose. <u>Considerations:</u> ↓ bodyweight gain at 1,545 mg/kg-day (both sexes). ↓ terminal bodyweight (≥10%). (Body weight gains were

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Comments
weeks (Hazleton Labs, 1991b)			decreased in both sexes at 1,545 mg/kg-day, along with decreases in terminal body weight >10% relative to controls).
SD rats (both sexes); dietary; 0, 1,000, 3,000, 10,000 ppm (estimated: 60, 180, 600 mg/kg-day); 13 weeks Hazleton Labs (1981)	LOEL = 180	↓ total protein and globulin levels (males)	<u>Other liver effects:</u> ↑ liver weights (high dose for both sexes); ↓ total protein, and total bilirubin <u>Considerations:</u> histopathological findings limited to the kidney
SD rats (both sexes); dietary; 0, 0.3, 1.0% (estimated: 201, 690 mg/kg-day [males]; 251, 880 mg/kg-day [females]); 13 weeks (Bio/dynamics, 1982c)	ND/ 201 (males; LOEL) ND/ 251 (females; LOEL)	↓ terminal body weights in both sexes; ↑ absolute and relative liver weight accompanied by ↓ in triglycerides.	<u>Other liver effects:</u> ↑ ALP (males & females) and ↑ ALT (males) from the high dose groups <u>Considerations:</u> ↓ terminal bodyweight by 24% and 28% in 201 mg/kg-day and 690 mg/kg-day males, respectively. ↓ terminal bodyweight by ≥15% and 31% in 251mg/kg-day and 880 mg/kg-day females, respectively.
Albino rats (both sexes); dietary; 0, 50, 150, 500 mg/kg-day; 3 months (Hazleton Labs, 1971)	150 (NOEL)/ 500 (LOEL)	↑ absolute and relative liver weight and ↑ hepatocyte hypertrophy	<u>Considerations:</u> Slight non-significant ↓ bodyweight gain in 500 mg/kg-day males. Bodyweight gain similar across all female groups. Terminal bodyweight within 10% of controls for all male and female dose groups.
B6C3F1 mice (both sexes); dietary; 0, 1,500, 4,000, 10,000, 20,000 ppm (estimated: 365, 972, 2,600, 5,770 mg/kg-day); 13 weeks (Hazleton Labs, 1992)	365/ 972	↑ absolute and relative liver weight; hepatocyte enlargement; other histopathology in liver [<i>i.e.</i> , pigments in Kupffer cells and bile canaliculi, liver degeneration/ necrosis]	<u>Considerations:</u> ↓ bodyweight gain and ↓ terminal bodyweight of males and females at 5,770 mg/kg-day.

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Comments
Marmoset (both sexes); 0, 100, 500, 2,500 mg/kg-day; oral gavage; 13 weeks (Hall et al., 1999)	500/ ND	↓ body weight and body weight gain	<u>Considerations:</u> ↓ relative liver weight (males) but not dose-dependent and did not reach statistical significance
^a The BASF (1987) study was only available in German; EPA reports its use based on Health Canada's human health hazard assessment (EC/HC, 2015).			

Chronic (>90 days) Exposure: EPA identified five studies from existing assessments that provide information on the toxicological effects of DINP on the liver, including two oral exposure studies conducted in F344 rats ([Covance Labs, 1998c](#); [Lington et al., 1997](#)), one oral study in SD rats ([Bio/dynamics, 1987](#)), one oral exposure study conducted in B6C3F1 mice ([Covance Labs, 1998b](#)), and a combined one and two generation study in SD rats ([Waterman et al., 2000](#); [Exxon Biomedical, 1996a, b](#)). No chronic exposure data on DINP are available for humans or other primates. Available studies are summarized in Table_Apx B-6.

Two studies in F344 rats reported similar findings, most notably of nonneoplastic lesions of the liver including spongiosis hepatitis ([Covance Labs, 1998c](#); [Lington et al., 1997](#)). Lington et al. ([1997](#)) administered 0, 300, 3,000, or 6,000 ppm DINP to F344 rats in the diet for up to 24 months, corresponding to mean daily intakes of 0, 15, 152, or 307 mg/kg-day in males and 0, 18, 184, or 375 mg/kg-day in females, respectively. Male and female rats in the mid- and high-dose groups had statistically significant increases in absolute and relative liver weights throughout the exposure period and study termination, where relative weight increased 19 to 31 percent in males and 16 to 29 percent in females. Increases in liver weight corresponded with increases in liver enzyme levels. In males, dose-related increases of 1.5- to 3-fold were observed in ALP, AST, and ALT activities of mid- and high-dose groups throughout the study. No significant differences were observed in females. Increased incidences of several non-neoplastic histopathological lesions were observed in the liver at 18 months, including minimal to slight centrilobular to midzonal hepatocellular enlargement in high-dose males (incidence: 9/10 vs. 0/10 in controls) and females (10/10 vs 0/10 in controls). At study termination (*i.e.*, 24 months), dose-related increases were observed in the incidence of focal necrosis, spongiosis hepatitis, sinusoid ectasia, hepatocellular enlargement, and hepatopathy associated with leukemia (Table_Apx B-3).

The study authors did not report statistical significance for any of the observed lesions. EPA conducted an independent review of the incidences of spongiosis hepatitis and hepatopathy associated with leukemia and determined that these histopathology findings were significantly increased in mid- (152 mg/kg-day) and high-dose (307 mg/kg-day) male rats (Table_Apx B-3). Additionally at the high dose in the males, the incidences of sinusoid ectasia, hepatocellular enlargement, and focal necrosis were significantly increased over controls. In females, dose-related increases in the incidence of focal necrosis, hepatopathy associated with leukemia, and hepatocellular enlargement were noted at study termination. The independent statistical analysis determined that the incidences of hepatocellular enlargement and hepatopathy associated with leukemia were significantly increased in high-dose females. The NOAEL and LOAEL for non-cancer hepatic effects in this study were 15 and 152 mg/kg-day, respectively; both are based on a statistically significant increase in the incidence of spongiosis hepatitis in mid-dose male rats that was accompanied by increased absolute and relative liver weights and changes in serum enzyme activities.

Table_Apx B-3. Incidence of Selected Non-neoplastic Hepatic Lesions in F344 Rats Exposed to DINP for 24 Months ([Lington et al., 1997](#))

Lesion	Dose Group mg/kg-day (ppm)			
	Control	15 M/18 F (300)	152 M/184 (3,000)	307 M/375 (6,000)
Males ^a				
Spongiosis hepatitis	24/81 (29.6%)	24/80 (30%)	51/80* (63.8%)	62/80* (77.5%)
Hepatopathy associated with leukemia	22/81 (27.2%)	17/80 (21.3%)	34/80* (42.5%)	33/80* (41.3%)
Sinusoid ectasia	16/81 (19.8%)	16/80 (20.0%)	24/80 (30.0%)	33/80* (41.3%)
Hepatocellular enlargement	1/81 (1.2%)	1/80 (1.3%)	1/80 (1.3%)	9/80* (11.3%)
Focal necrosis	10/81 (12.3%)	9/80 (11.2%)	16/80 (20.0%)	26/80* (32.5%)
Females ^a				
Focal necrosis	13/81 (16.0%)	11/81 (13.6%)	19/80 (23.8%)	21/80 (26.3%)
Spongiosis hepatitis	4/81 (4.9%)	1/81 (1.2%)	3/80 (3.8%)	4/80 (5.0%)
Sinusoid ectasia	9/81 (11.1%)	4/81 (4.9%)	6/80 (7.5%)	10/80 (12.5%)
Hepatocellular enlargement	1/81 (1.2%)	0/81 (0%)	0/80 (0%)	11/80* (13.8%)
Source: Table 7 in Lington et al. (1997) M = male; F = female ^a Number of animals with lesion/total number of animals examined. Percent lesion incidence in parentheses. * Statistically significant at p < 0.05 when compared to the control incidence using Fischer's Exact test; statistical analysis performed by EPA.				

Another 2-year study in F344 rats with comparable dose levels to Lington et al. ([1997](#)) provided data to support the liver toxicity of DINP ([Covance Labs, 1998c](#)). In that study, DINP was administered to rats at dietary concentrations of 500, 1,500, 6,000, or 12,000 ppm (equivalent to average daily doses of 29, 88, 359, or 733 mg/kg-day in males, and 36, 109, 442, or 885 mg/kg-day in females for 104 weeks. Additional groups of male and female rats were given 12,000 ppm (637 and 774 mg/kg-day, respectively) for 78 weeks and received basal diet only for the remainder of the study (26 weeks) to evaluate the reversibility of DINP toxicity (recovery group). Increased absolute and relative liver weights were observed in the two highest dose groups in males and females at multiple timepoints throughout the study as well study termination. Relative liver weights were increased 35 to 61 percent in males and 26 to 71 percent in females. There were no significant changes in absolute liver weights in the recovery group at the end of the 26-week recovery period, suggesting a reversibility of liver enlargement. Significant increases in activities of serum enzymes (AST and ALT) were also observed in both sexes at the two highest doses at weeks 52, 78, and study termination. Serum liver enzyme activities were also increased in the recovery group. Increases in palmitoyl-CoA oxidase activity were observed in high dose male and female rats, which is further discussed in the mechanistic section below.

Histological evidence of liver toxicity was observed in parallel with increases in liver weight and alterations in serum enzyme activity. Incidences of select non-neoplastic lesions from the Covance study are summarized in Table_Apx B-4. A dose-responsive increase in the incidence of spongiosis hepatitis was observed at doses as low as 359 mg/kg-day in males. Other lesions observed in males, such as cytoplasmic eosinophilia, diffuse hepatocellular enlargement, pigment, and individual cell degeneration or necrosis were generally observed at higher doses, suggesting spongiosis hepatitis was the most sensitive histopathological response to DINP. EPA's independent review determined that diffuse hepatocellular enlargement was significantly increased in high-dose males and females at study termination.

Table_Apx B-4. Incidence of Selected Hepatic Lesions in F344 Rats Exposed to DINP in the Diet for 2 Years (Covance Labs, 1998c)

Lesion	Dose Group mg/kg-day (ppm)					
	Control	29 M/ 36 F (500)	88 M/ 109 F (1,500)	359 M/ 442 F (6,000)	733 M/ 885 F (12,000)	Recovery ^a 637 M/ 774 F (12,000)
Males						
Spongiosis hepatitis	5/55 ^b (9.1%)	5/50 (10.0%)	2/50 (4.0%)	13/55* (23.6%)	21/55* (38.2%)	9/50 (18.0%)
Cytoplasmic eosinophilia	0/55 (0%)	0/50 (0%)	0/50 (0%)	0/55 (0%)	31/55* (56.4%)	0/50 (0%)
Diffuse hepatocellular enlargement	0/55 (0%)	0/50 (0%)	0/50 (0%)	0/55 (0%)	17/55* (30.9%)	0/50 (0%)
Increased pigment	1/55 (1.8%)	0/50 (0%)	1/50 (2.0%)	0/55 (0%)	7/55* (12.7%)	9/50 (18.0%)
Individual cell degeneration/ necrosis	0/55 (0%)	0/50 (0%)	0/50 (0%)	1/55 (1.8%)	5/55* (9.1%)	0/50 (0%)
Females						
Spongiosis hepatitis	0/55 (0%)	0/50 (0%)	0/50 (0%)	1/55 (1.8%)	2/55 (3.6%)	0/50 (0%)
Cytoplasmic eosinophilia	0/55 (0%)	0/50 (0%)	0/50 (0%)	0/55 (0%)	35/55* (63.6%)	0/50 (0%)
Diffuse hepatocellular enlargement	0/55 (0%)	0/50 (0%)	0/50 (0%)	0/55 (0%)	33/55* (60.0%)	0/50 (0%)
Increased pigment	7/55 (12.7%)	8/50 (16.0%)	9/50 (18.0%)	5/55 (9.1%)	16/55* (29.1%)	10/50 (20.0%)
Individual cell degeneration/ necrosis	0/55 (0%)	0/50 (0%)	0/50 (0%)	0/55 (0%)	0/55 (0%)	0/50 (0%)

Lesion	Dose Group mg/kg-day (ppm)					
	Control	29 M/ 36 F (500)	88 M/ 109 F (1,500)	359 M/ 442 F (6,000)	733 M/ 885 F (12,000)	Recovery ^a 637 M/ 774 F (12,000)

Source: Tables 10A and 10C in Covance Labs (1998c)
M = male; F = female
* = significantly different from control ($p < 0.05$) by Fisher's Exact test as performed by EPA.
^a The 12,000 ppm recovery group received 12,000 ppm DINP in the diet for 78 weeks, followed by a 26-week recovery period during which the test animals received basal diet alone.
^b Number of animals with lesion/number of animals with livers examined; percentage is given in parentheses.
Incidence is sum of lesions observed in unscheduled deaths and at terminal sacrifice.

A third study in rats by Bio/dynamics (1987) provided data on liver weights, histopathology, and effects on clinical chemistry parameters following chronic exposure to DINP. In that study, male and female SD rats were administered 0, 500, 5,000, or 10,000 ppm DINP in the diet for up to 2-years (equivalent to 0, 27, 271, or 553 mg/kg-day in males and 0, 33, 331, or 672 mg/kg-day in females). Increased absolute and relative liver weights were observed in high-dose males and females at the 12-month interim sacrifice and study termination; all increases were between 14 and 34 percent. In the mid-dose females, there were non-significant increases in absolute (14%) and relative (11%) liver weight at interim sacrifice and absolute liver weight (15%) at terminal sacrifice, and a significant increase in relative liver weight (16%) at terminal sacrifice. In mid-dose males, a nonsignificant increase of 11 percent was seen in the mid-dose group at interim sacrifice. Histopathological findings were observed at lower doses than changes in liver weights. Increased incidences of spongiosis hepatitis and minimal-to-slight hepatic focal necrosis were observed in males from the mid-dose group (271 mg/kg-day). The increases in liver weights and incidences of nonneoplastic lesions were attributed to the administration of DINP.

In parallel with increases in liver weight and histopathological findings, changes in clinical chemistry parameters were observed. Serum ALT was significantly increased in high-dose males at interim sacrifices on months 6, 12, and 18 by 292, 203, and 232 percent, respectively. A non-statistically significant increase of 218 percent was observed in males at study termination (24 months). Serum ALP was significantly increased at months 6 and 12 in high-dose males by 88 and 76 percent, respectively. Non-significant increases in AST were observed in males from the mid and high dose groups. In females, non-significant increases in AST (63%) and ALT (89%) were observed at 6 months. Serum ALP was significantly increased in females of the high-dose group by 81 percent at 18 months, while a non-significant increase of 38 percent was observed at study termination. No exposure-related changes in serum ALP were observed at earlier timepoints in this group or in females of the low- or mid-dose groups. The increased serum AST, ALT, and ALP in treated males were for the most part not statistically significant; however, these findings were considered treatment-related due to the consistency with which they were noted in the treated males at most timepoints. The increased ALP in females of the high-dose group at month 18 and month 24 is considered treatment-related and adverse. However, the increased AST and ALT values in females of the high-dose group at month 6 were not considered treatment-related due to their isolated occurrence in only one animal at only one timepoint. Moreover, data from this animal were considered to be statistical outliers via the Grubb's outlier test.

Overall, the Bio/dynamics study (1987) supports a NOAEL of 27 mg/kg-day in male rats based on treatment related increases in histopathologic lesions (*i.e.*, spongiosis hepatitis, focal necrosis) and increases in serum ALT, AST, and ALP at the LOAEL of 271 mg/kg-day.

One chronic study in mice by Covance Labs ([1998b](#)) was identified from existing assessments. Covance Labs exposed male and female B6C3F1 mice to 500, 1,500, 4,000, or 8,000 ppm DINP for at least 104 weeks. These concentrations corresponded to average daily doses of 0, 90, 276, 742, and 1,560 mg/kg-day in males and 0, 112, 336, 910, and 1,888 mg/kg-day in females. Evidence of liver toxicity was observed in treated animals of both sexes. At interim sacrifice, significant increases were observed in relative liver weights in mid-dose males (742 mg/kg-day) and females (910 mg/kg-day) and in high-dose males (1,560 mg/kg-day). At study termination, significant increases were observed in absolute (13–33% increase) and relative (25–60% increase) liver weights in males exposed to 742 or 1,560 mg/kg-day DINP. Relative liver weight was also significantly increased 32 percent in the recovery group. In females, increases in absolute liver weight (18–34% increase) and relative liver weight (24–39%) were observed in females exposed to 910 or 1,888 mg/kg-day DINP, as well as in the recovery groups. However, the responses were not statistically significant.

Exposure-related changes in serum chemistry profiles were also observed and supported the liver as a target organ. AST and ALT activities were increased in high-dose males (1,560 mg/kg-day) and recovery group males and females. Exposure-related increases in the serum levels of total protein, albumin, and globulin were also observed in high-dose males. Increases in albumin and globulin were also observed in recovery males.

Gross findings, including liver masses, occurred with greatest frequency at the 910 and 1,560 mg/kg-day dose groups, as well as the recovery group. These masses corresponded to hepatocellular neoplasms or involvement by lymphoma or histiocytic sarcoma and are discussed further in ([U.S. EPA, 2025a](#)). Increased incidences of several nonneoplastic lesions were observed in the livers of high-dose males and females, including cytoplasmic eosinophilia, diffuse slight to moderate hepatocellular enlargement, and slight to moderate pigment (Table_Apx B-5). These changes were also observed in the recovery group, but generally at lower incidences than in the high-dose groups. No other statistically significant or dose-related nonneoplastic lesions of the liver were observed in the Covance study ([1998b](#)). Liver weights in recovery group animals were comparable to those of controls, and histological evidence of liver enlargement was not observed in the male or female recovery groups. The incidences of non-neoplastic lesions in the recovery groups were decreased at study termination relative to the high-dose groups, but in most cases were significantly greater than the control values. These data suggest that DINP-induced liver toxicity was partially reversed in the recovery groups.

EPA identified a LOAEL value from the Covance ([1998b](#)) study of 742 mg/kg-day in males and 910 mg/kg-day in females based on increased incidence of liver masses in males, and increased absolute and relative liver weights, and decreased absolute and relative kidney weights (Section 3.3). A NOAEL of 276 mg/kg-day in males or 336 mg/kg-day in females was identified based on non-cancer and cancer effects.

Table_Apx B-5. Incidence of Selected Non-neoplastic Lesions in B6C3F1 Mice Exposed to DINP in the Diet for 2 Years (Covance Labs, 1998b)

Lesion	Dose Group mg/kg-day (ppm)					
	Control	90 M 112 F (500)	276 M 336 F (1,500)	742 M 910 F (4,000)	1,560 M 1,888 F (8,000)	Recovery ^b 1,560 M 1,888 F (8,000)
Males						
Diffuse hepatocellular enlargement	0/55 ^a (0%)	1/50 (2.0%)	1/50 (2.0%)	2/50 (4.0%)	45/55* (81.8%)	10/50* (20.0%)
Increased cytoplasmic eosinophilia	0/55 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	52/55* (94.5%)	10/50* (20.0%)
Pigment	0/55 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	49/55* (89.1%)	6/50* (12.0%)
Females						
Diffuse hepatocellular enlargement	0/55 (0%)	0/51 (0%)	0/50 (0%)	1/50 (2.0%)	52/55* (94.5%)	6/50* (12.0%)
Increased cytoplasmic eosinophilia	0/55 (0%)	0/51 (0%)	0/50 (0%)	0/50 (0%)	53/55* (81.8%)	6/50* (12.0%)
Pigment	1/55 (1.8%)	1/51 (2.0%)	2/50 (4.0%)	2/50 (4.0%)	41/55* (74.5%)	3/50 (6.0%)
<p>Source: Tables 11A and 11C in Covance Labs (1998b). M = male; F = female * = significantly different from control (p < 0.05) by Fisher's Exact test performed by Syracuse Research Corporation. ^a Number of animals with lesion/total number of animals examined; percent incidence of lesion in parentheses. Incidences are sum of unscheduled deaths and lesions observed at terminal sacrifice. ^b The 8,000 ppm recovery group received 8,000 ppm for 78 weeks, followed by a 26-week recovery period during which the test animals received basal diet alone.</p>						

Waterman et al. (2000) assessed the potential toxicity of DINP in one- and two-generation studies conducted in SD rats. In the one-generation study, male and female animals were administered 0.5, 1.0, or 1.5 percent DINP in the diet for 10 weeks prior to mating and lasting throughout the mating period. The females were subsequently exposed throughout gestation and lactation until PND21. Mean received doses in units of mg/kg-day are shown in Table 3-5. Parental body weight gain was significantly reduced at the 1.0 and 1.5 percent dose groups in both sexes during the premating phase and in females during gestation and lactation. Absolute liver weights in both sexes were significantly increased at all doses, except in P1 females at the 1.5 percent level.

For the two-generation study, male and female SD rats were fed DINP at dietary concentrations of 0.0, 0.2, 0.4, or 0.8 percent for 10 weeks before mating and for an additional 7 weeks, through mating, gestation, and lactation continuously for two-generations. Mean received doses in units of mg/kg-day are shown in Table 3-7. Absolute liver weights of P1 males and females were increased over controls at all DINP treatment levels. Minimal to moderate increases in cytoplasmic eosinophilia were observed in all males and females from all dose groups of parents in both generations.

Table_Apx B-6. Summary of Liver Effects Reported in Animal Toxicological Studies Following Chronic Exposure to DINP

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
F344 rats (both sexes); dietary; 0, 0.03, 0.3, 0.6% (estimated: 15, 152, 307 mg/kg-day [males]; 18, 184, 375 mg/kg-day [females]); 2 years (Lington et al., 1997)	15/ 152 (males) 18/ 184 (females)	↑ absolute and relative liver weight; ↑ in serum ALT, AST; ↑ non-neoplastic lesions (e.g., focal necrosis, spongiosis hepatis)	
SD rats (both sexes); dietary; 0, 500, 5,000, 10,000 ppm (estimated: 27, 271, 553 mg/kg-day [males]; 33, 331, 672 mg/kg-day [females]); 2 years (Bio/dynamics, 1987) GLP-compliant study, non-guideline	27/ 271 (males)	↑ serum ALT, AST, ALP (males); ↑ spongiosis hepatis; ↑ hepatic focal necrosis	<u>Other liver effects:</u> ↑ absolute and relative liver weight (both sexes); ↑ serum ALP (females); ↑ incidence of hepatocyte necrosis at low- and high-doses (males) <u>Considerations:</u> ↓ bodyweight gains in females (672 mg/kg-day); no change in terminal bodyweight in males; ↑ food consumption for females at multiple timepoints during study (672 mg/kg-day)
Male and female SD rats (30/sex/dose) fed diets containing 0, 0.5, 1.0, 1.5% DINP (CASRN 68515-48-0) starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for one generation (received doses in units of mg/kg-day shown in Table 3-5) (Waterman et al., 2000 ; Exxon Biomedical, 1996a).	ND/ 301 (LOEL)	↑ absolute and relative liver weight for P1 and P2 males and females; ↑ incidence of minimal to moderate cytoplasmic eosinophilia	
Male and female SD rats (30/sex/dose) fed diets containing 0, 0.2, 0.4, 0.8% DINP (CASRN 68515-48-0) starting 10 weeks prior to mating, through mating, gestation, and lactation continuously for two-			

Brief Study Description (Reference)	NOAEL/ LOAEL (mg/kg-day)	Effect at LOAEL	Remarks
generations. Received doses in units of mg/kg-day shown in Table 3-7. (Waterman et al., 2000 ; Exxon Biomedical, 1996b).			
<p>B6C3F1 mice (both sexes); dietary; 0, 500, 1,500, 4,000, 8,000 ppm (estimated: 90, 276, 742, 1,560 mg/kg-day [males]; 112, 336, 910, 1,888 mg/kg-day [females]); 2 years</p> <p>Recovery study; 0, 1,377 [males]; 0, 1,581 [females]; diet; 78 weeks, followed by 26 weeks recovery. (Covance Labs, 1998b)</p> <p>GLP-compliant and adhere to EPA guidelines (40 CFR 798.330)</p>	<p>276/ 742 (males)</p> <p>336/ 910 (females)</p>	<p>↑ absolute liver weight, histopathological changes in the liver and ↓ body weight gain) (females); (↑ incidence of liver masses (males)</p>	<p><u>Significant neoplastic findings</u>: ↑ hepatocellular carcinoma; ↑ incidence of total liver neoplasms (combined carcinomas and adenomas)</p> <p><u>Considerations</u>: ↓ mean bodyweights in males (≥742 mg/kg-day) and females (≥336 mg/kg-day)</p>
<p>F344 rats (both sexes); dietary; 0, 500, 1,500, 6,000, 12,000 ppm (estimated: 29, 88, 359, 733 mg/kg-day [males]; 36, 109, 442, 885 mg/kg-day [females]); 2 years</p> <p>Recovery study: 0, 637 mg/kg-day [males]; 0, 774 mg/kg-day [females]; diet; 78-week exposure, followed by 26 week recovery period (Covance Labs, 1998c)</p> <p>GLP-compliant and adhere to EPA guidelines (40 CFR 798.330)</p>	<p>88/ 359 (males)</p> <p>109/ 442 (females)</p>	<p>↑ absolute and relative liver weight; ↑ in serum ALT and AST; histopathological findings in liver</p>	<p><u>Significant neoplastic findings</u> ↑ incidence of mononuclear cell leukemia; ↑ in hepatocellular carcinoma; ↑ in combined hepatocellular carcinoma and adenoma (See (U.S. EPA, 2025a) for further discussion)</p> <p><u>Limitations</u>: Did not report results of statistical analyses of lesion incidence data</p>

Appendix C FETAL TESTICULAR TESTOSTERONE AS AN ACUTE EFFECT

No studies of experimental animal models are available that investigate the antiandrogenic effects of DINP following single dose, acute exposures. However, there are studies of dibutyl phthalate (DBP) available that indicate a single acute exposure during the critical window of development (*i.e.*, GD14-19) can reduce fetal testicular testosterone production and disrupt testicular steroidogenic gene expression. Two studies were identified that demonstrate single doses of 500 mg/kg DBP can reduce fetal testicular testosterone and steroidogenic gene expression. Johnson et al. ([2012](#); [2011](#)) gavaged pregnant SD rats with a single dose of 500 mg/kg DBP on GD19 and observed reductions in steroidogenic gene expression in the fetal testes three (*Cyp17a1*) to six (*Cyp11a1*, *StAR*) hours post-exposure, while fetal testicular testosterone was reduced starting 18 hours post-exposure. Similarly, Thompson et al. ([2005](#)) reported a 50 percent reduction in fetal testicular testosterone 1-hour after pregnant SD rats were gavaged with a single dose of 500 mg/kg DBP on GD19, while changes in steroidogenic gene expression occurred 3 (*StAR*) to 6 (*Cyp11a1*, *Cyp17a1*, *Scarb1*) hours post-exposure, and protein levels of these genes were reduced 6 to 12 hours post-exposure. Additionally, studies by Carruthers et al. ([2005](#)) further demonstrate that exposure to as few as two oral doses of 500 mg/kg DBP on successive days between GD15 to 20 can reduce male pup AGD, cause permanent nipple retention, and increase the frequency of reproductive tract malformations and testicular pathology in adult rats that received two doses of DBP during the critical window.

In summary, studies of DBP provide evidence to support use of effects on fetal testosterone as an acute effect. However, the database is limited to just a few studies of DBP that test relatively high (500 mg/kg) single doses of DBP. Although there are no single dose studies of DINP that evaluate antiandrogenic effects on the developing male reproductive system, there are four studies that have evaluated effects on fetal testicular testosterone production and steroidogenic gene expression following daily gavage doses of 500 to 1,500 mg/kg-day DINP on GD14 to 18 (5 total doses) ([Gray et al., 2021](#); [Furr et al., 2014](#); [Hannas et al., 2012](#); [Hannas et al., 2011](#))—all of which consistently report antiandrogenic effects at the lowest dose tested (500 mg/kg-day).

Appendix D SUMMARY OF EPIDEMIOLOGY STUDIES ON REPRODUCTIVE OUTCOMES

Radke et al. (2018) report the results of a systematic review that evaluated the association between DINP and male reproductive outcomes. In examining the relationship between DINP exposure and AGD, the authors found that there is little evidence linking DINP to AGD. The combination of low exposure levels (*i.e.*, poor sensitivity) and data availability (*i.e.*, fewer accessible studies) may account for the weaker evidence of an association between AGD and DINP. When evaluating the relationship between DINP exposure and sperm parameters, the author determined that the association was moderate due to the morphology's consistency across studies. In examining the association between DINP and the time until pregnancy in males, the authors did not report a relationship for DINP, and the evidence was deemed inconclusive due to the small number of studies and narrow range of exposure. Finally, when examining the relationship between DINP metabolite (MINP or MCiOP) exposure and testosterone, the authors found that there is moderate evidence linking DINP metabolites to lower testosterone levels.

Another systematic review by Radke et al. (2019b) evaluated the association between DINP and female reproductive and developmental outcomes and also found no clear evidence of association due to inadequate sensitivity in the available data. When examining the relationship between DINP exposure and pubertal development the authors found that there was no association linking DINP and pubertal development and the strength of the evidence was deemed indeterminate. Study evaluations of the relationship between DINP and a woman's time to pregnancy found that the evidence of an association between fecundity and exposure to DINP was deemed indeterminate due to lack of the evidence of relationship for the key fecundity outcomes. The authors also found that in studies that measured the relationship between DINP and spontaneous abortion, there was no association between early loss and total loss. Thus, the evidence for an association between DINP and spontaneous abortion was deemed indeterminate. Finally, when evaluating the association between DINP and gestational duration, the authors found slight evidence for the association between DINP exposure and preterm birth; however while there was modest increase in the odds of preterm birth with higher DINP exposure the association was not statistically significant. In summary there was indeterminate evidence linking DINP and female reproductive and developmental outcomes.

EPA identified 11 new studies (8 medium quality and 3 low quality) that evaluated the association between DINP metabolites and developmental and reproductive outcomes. The first medium quality study, a longitudinal cohort study, by Berger et al. (2018), using data from Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort examined prenatal urinary DINP levels and the association with timing of puberty milestones (thelarche, menarche, pubarche, gonadarche) in children. The authors found an association between pubarche and menarche age increased in "normal" weight girls per log₂ increase in MCOP. The authors also found gonadarche and pubarche age decreased in all obese boys. There was not significant a significant association between thelarche age increased in all girls per log₂ increase in MCOP.

A medium quality birth cohort study, by Philipat et al. (2019), Etude des Déterminants pré et postnatals du développement et de la santé de l'Enfant (EDEN) cohort, evaluated associations between DINP metabolites (MCOP, MCNP) and a set of outcomes measured at birth (birth weight, placental weight, placental-to-birth weight ratio). MCNP and MCOP were both associated with lower placental-to-birth weight ratio; MCNP was additionally associated with lower placental weight. MCOP was associated with lower placental-to-birth weight ratio (PFR) in multipollutant elastic net penalized regression models. MCOP was not associated with birth weight or placental weight based on elastic net regression models.

A medium quality cross-sectional pilot study, by Zota et al. (2019), included a racially diverse population of premenopausal women within the Fibroids Observational Research on Genes and the Environment (FORGE) study presenting to a university gynecology clinic and undergoing either hysterectomy or myomectomy for symptomatic uterine fibroids to examine the potential associations between urinary DINP biomarkers and two measures of fibroid burden (uterine volume and fibroid size). Higher urinary concentrations of MCOP and MCNP were significantly associated with odds of greater uterine volume. In multivariate logistic regression analyses, each log-unit increase in MCOP was significantly associated with 2.1 (95% CI: 1.2–3.5) times increased odds of greater uterine volume, and each log-unit increase in MCNP was associated with 2.8 (95% CI: 1.2–3.5) times increased odds of greater uterine volume, $p < 0.05$. Results from additional multivariate linear regression analyses of urinary phthalate exposure on percent increase in uterine volume were positive but not significant. Results from multivariate logistic regression analysis of urinary DINP exposure on odds of fibroid size increase for MCOP were non-significant. Results from additional multivariate linear regression analyses of urinary MCOP phthalate exposure on percent increase in fibroid size (cm) were also non-significant.

A medium quality cross-sectional study, by Chang et al. (2019), evaluated the association between sex hormone levels (luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone binding globulin (SHBG), inhibin B, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), androstenedione (AD), estrone (E1), estradiol (E2), total testosterone (TT), free testosterone (FT), dihydrotestosterone (DHT), dihydrotestosterone/total testosterone ratio, estradiol/total testosterone ratio, estradiol/estrone ratio), Oxidative stress/Inflammation [(malondialdehyde (MDA), inducible nitric oxide synthetase (iNOS), 8-hydroxy-2'-deoxyguanosine (8-OHdG)] and benign prostatic hyperplasia (prostate specific antigen (PSA), prostate volume) and DINP exposure. There were significant positive associations between the outcomes, FSH, Inhibin B, DHEA, iNOS and MINP with regression coefficients of 0.91 (95% CI: 0.85, 0.98), 0.90 (95% CI: 0.83, 0.97), 1.58 (95% CI: 1.40, 1.79) and 1.61 (95% CI: 1.29, 2.03) respectively, $p < 0.05$. Multivariate regression coefficients showed significant results for FHS, Inhibin B, iNOS and DHEA, but showed nonsignificant results for LH, SHBG, DHEA-s, AD, E1, E2, TT, FT, DHT, MDA, 8-OHdG, PSA, and prostate volume.

A medium quality study, by Mustieles et al. (2019), used data from a small cohort of subfertile couples in the Environment and Reproductive Health (EARTH) study to analyze the association between paternal and maternal preconception urinary DINP metabolites (MCOP), as well as maternal prenatal DINP metabolites, and measures of placental weight. The authors did not find any significant association between paternal and maternal preconception urinary phthalates, as well as maternal prenatal phthalates, and measures of placental weight and MCOP.

A medium quality cohort, by Machtinger et al. (2018), examined the association between urinary concentrations of DINP with intermediate and clinical *in vitro* fertilization (IVF) outcomes. There was an association (adjusted means) between urinary MCOP concentration and intermediate outcomes of assisted reproduction (total oocytes and mature oocytes) [total oocytes T2 = 10.2 (95% CI: 9.3, 11.2), T2 vs. T1 < 0.05 ; mature oocytes T2 = 8.4 (95% CI: 7.6, 9.3) T2 vs. T1 < 0.05]. However, there was no significant association (adjusted means) between urinary MCOP concentration and intermediate outcomes of assisted reproduction (fertilized oocytes, top quality embryos). While there was an association (adjusted means) between urinary MINP concentration and intermediate outcomes of assisted reproduction (total oocytes) [total oocytes T2 = 9.2 (95% CI: 8.2, 10.2), T2 vs. T1 < 0.05]; there was not an association (adjusted means) between urinary MINP concentration and intermediate outcomes of assisted reproduction (mature oocytes, fertilized oocytes, top quality embryos). Associations between MOiNP or MONP and intermediate outcomes of assisted reproduction (total oocytes, mature oocytes, fertilized oocytes, top quality embryos) and live birth following assisted

reproduction were all non-significant for T2, T3 vs. T1 intermediate outcomes and for p-trend of live birth.

A medium quality case-control study, by Lee et al. (2020), assessed the relationship between uterine fibroids and DINP metabolite concentrations. The authors did not find any statistically significant associations between uterine fibroids and DINP metabolite concentrations. The authors did find associations between cases and controls for OH-MINP concentrations (p-value: 0.042) as mono(4-methyl-7-hydroxyoctyl) phthalate (OH-MINP) concentrations were significantly higher in the cases than controls, but it was not statistically significant.

A medium quality occupational short longitudinal study, by Henrotin et al. (2020), observed the three-day changes in levels of total and free testosterone and oxidized MINP exposure in male factory workers. A significant inverse association was found between the decrease in serum total testosterone (TT) concentrations between T1 and T2 and an increase in urinary OXO-MINP. There were no significant associations observed for total testosterone and models for OH-MINP, or CX-MINP. No significant associations were noted for free testosterone and oxo-MINP, OH-MINP, or CX-MINP. Bivariate analyses of sexual health scales (IIEF-5 and ADAM) between DINP exposed and non-exposed groups: No association was observed between the level of urinary oxo-MINP concentrations and FSH, LH, index of aromatase activity (ratio of total testosterone to estradiol (TT/E2)). No association was observed between the level of urinary OXO-MINP concentrations and bone turnover biomarkers (P1NP, CTX).

The first low quality study, a case control study, by Durmaz et al. (2018), examined the association between DINP metabolites (MINP, MHiNP, MOiNP, MCiOP) and serum luteinizing hormone (LH), plasma follicle stimulating hormone (FSH) and serum estradiol in non-obese girls aged 4 to 8 years with premature thelarche. DINP metabolites (MINP, MHiNP, MOiNP, MCiOP and their sum) measured in spot urine samples were compared among cases and controls. Spearman correlations with uterine volumes, ovarian volume and pubic hair growth varied but were largely weak, negative and/or not significant, with some significant positive correlation for the association between MCiOP, MINP and pubic hair growth, $\rho = 0.440$, $p = 0.002$ and $\rho = 0.480$, $p = 0.000$, respectively. Thyroid hormone levels had largely negative Spearman correlations with DINP metabolites; however MCiOP had a significant negative correlation with fT4 ($\rho = -0.335$, $p = 0.041$). Spearman correlations between DINP metabolites (MCiOP, MiNP, MHiNP, MOiNP, SumDiNP) and BMI and weight were positive and significant.

A low quality case-control study, by Moreira Fernandez et al. (2019), of women in Brazil evaluated the association between one DINP metabolite (MINP) and endometriosis. The authors found that there was a positive but non-significant association for the relationship between MINP and endometriosis (OR=2.5 [95% CI: 0.46, 13.78]).

A final low quality study, a case-control study, by Liao et al. (2018), examined associations between exposure to one DINP metabolite (MINP) measured in urine samples and recurrent pregnancy loss among women in Taiwan. The MINP samples was below the limit of detection. The highest sample was 70.4 ng/mL in controls (detection rate 2.6%) and 1.43 ng/mL in cases (detection rate 2.9%).

Appendix E BENCHMARK DOSE ANALYSIS OF LINGTON ET AL. (1997)

E.1 Background

OCSPP requested that CPHEA run benchmark dose (BMD) models that are available in EPA's Benchmark Dose Software version 3.3.2 (BMDS 3.3.2), to estimate risk from DINP for select endpoints from a chronic exposure study ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)) using specified benchmark response (BMR) levels. The specific endpoints and BMRs provided by OCSPP for analysis are as follows:

1. Liver weight relative to bodyweight at terminal sacrifice (males and females)
 - BMR: 1 control SD, 5%, 10%, 25%
2. Serum ALT at 6- and 18-month sacrifices (males only)
 - BMR: 1 control SD, 10%, 20%, 100% (*i.e.*, 2x)
3. Incidence of focal necrosis in the liver (males and females)
 - BMR: 5%, 10%
4. Incidence of spongiosis hepatitis in the liver (males only)
 - BMR: 5%, 10%
5. Incidence of sinusoid ectasia in the liver (males only)
 - BMR: 5%, 10%

Although BMD and BMDL values are provided for all of the BMRs, this report provides detailed model run outputs for only the models that were run using the standard BMRs generally recommended by EPA for these endpoints, 10 percent relative deviation from the control mean (10% RD) for the dichotomous endpoints and organ weight change and 1 standard deviation change from the control mean (1 SD). Detailed modeling results for all standard non-cancer models are provided for all six endpoints using all of the BMRs requested by OCSPP in separately delivered BMDS Excel output files.

E.2 Summary of BMD Modeling Approach

All standard BMDS 3.3.2 dichotomous and continuous 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 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.

Standard BMDS 3.3.2 Models Applied to Continuous Endpoints:

- Exponential 3-restricted (exp3-r)
- Exponential 5-restricted (exp5-r)
- Hill-restricted (hil-r)
- Polynomial Degree 3-restricted (ply3-r)
- Polynomial Degree 2-restricted (ply2-r)
- Power-restricted (pow-r)
- Linear-unrestricted (lin-ur)

Standard BMDS 3.3.2 Models Applied to Dichotomous Endpoints:

- Gamma-restricted (gam-r)
- Log-Logistic-restricted (lnl-r)

- Weibull-restricted (wei-r)
- Dichotomous Hill-unrestricted (dhl-ur)
- Logistic (log)
- Log-Probit-unrestricted (lnp-ur)
- Probit (pro)

General Model Options Used for Individual Endpoint Analyses:

- Risk Type: Extra Risk
- Preferred Continuous Endpoint BMRs
 - Relative Liver Weight: 0.1 (10%)
 - Serum ALT: 1 Standard Deviation (1 SD)
- 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 and continuous 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.

With respect to the continuous endpoints, responses were first assumed to be normally distributed with constant variance across dose groups. If no model achieved adequate fit to response means (BMDS Test 4 $p > 0.1$) and response variances (BMDS Test 2 $p > 0.05$) under that assumption, models that assume normal distribution with non-constant variance, variance modeled as a power function of the dose group mean ([U.S. EPA, 2012](#)), were considered. If no model achieved adequate fit to response means and variances (BMDS Test 2 $p > 0.05$) under that assumption, a BMD/BMDL was not derived, and a LOAEL was selected as POD for the endpoint.

E.3 Summary of BMD Modeling Results

Table_Apx E-1. Summary of Benchmark Dose Modeling Results from Selected Endpoints in Male and Female F344 Rats Following a 2-Year Exposure to DINP ([Lington et al., 1997](#))

Section	Endpoint	Sex	Selected Model ^a	BMD ₁₀ (mg/kg-day)	BMDL ₁₀ (mg/kg-day)
E.4	Continuous endpoints				
E.4.1.1	Relative Liver weight at terminal sacrifice	Male	Linear, CV	106	85.0
E.4.1.2	Relative Liver weight at terminal sacrifice	Female	LOAEL (184 mg/kg-day)		
E.4.2.1	Serum ALT at 6-month sacrifice	Male	Linear, NCV	12.5	8.68
E.4.2.2	Serum ALT at 18-month sacrifice	Male	Power, NCV	37.2	17.4
E.5	Dichotomous endpoints				

Section	Endpoint	Sex	Selected Model ^a	BMD ₁₀ (mg/kg-day)	BMDL ₁₀ (mg/kg-day)
E.5.1.1	Focal necrosis in the liver	Male	Logistic	159	125
E.5.1.2	Focal necrosis in the liver	Female	Log-Probit	222	34.3
E.5.2	Spongiosis hepatitis in the liver	Male	Log-Probit	31.9	8.57
E.5.3	Sinusoid ectasia in the liver	Male	Log-Probit	125	14.4
CV = constant variance model; NCV = non-constant variance model ^a As described in Section 2, BMDs for non-cancer endpoints were derived from the standard set of models as defined in EPA BMD technical guidance and as identified in BMDS 3.3.2 as defaults. Since the standard approach gave adequate results for all endpoints, non-standard models were not considered for BMD derivations.					

E.4 Continuous Endpoints

E.4.1 Relative Liver Weight – Terminal Sacrifice

E.4.1.1 Male F344 Rats

Table_Apx E-2. Dose-Response Modeling Data for Relative Liver Weight at Terminal Sacrifice in Male F344 Rats Following a 2-Year Exposure to DINP ([Lington et al., 1997](#))

Dose (mg/kg-day)	Number per Group	Mean	Standard Deviation
0	61	0.032	0.006
15	54	0.034	0.008
152	50	0.038	0.008
307	51	0.042	0.008

Table_Apx E-3. Summary of Benchmark Dose Modeling Results for Relative Liver Weight at Terminal Sacrifice in Male F344 Rats Following a 2-Year Exposure to DINP (Constant Variance) ([Lington et al., 1997](#))

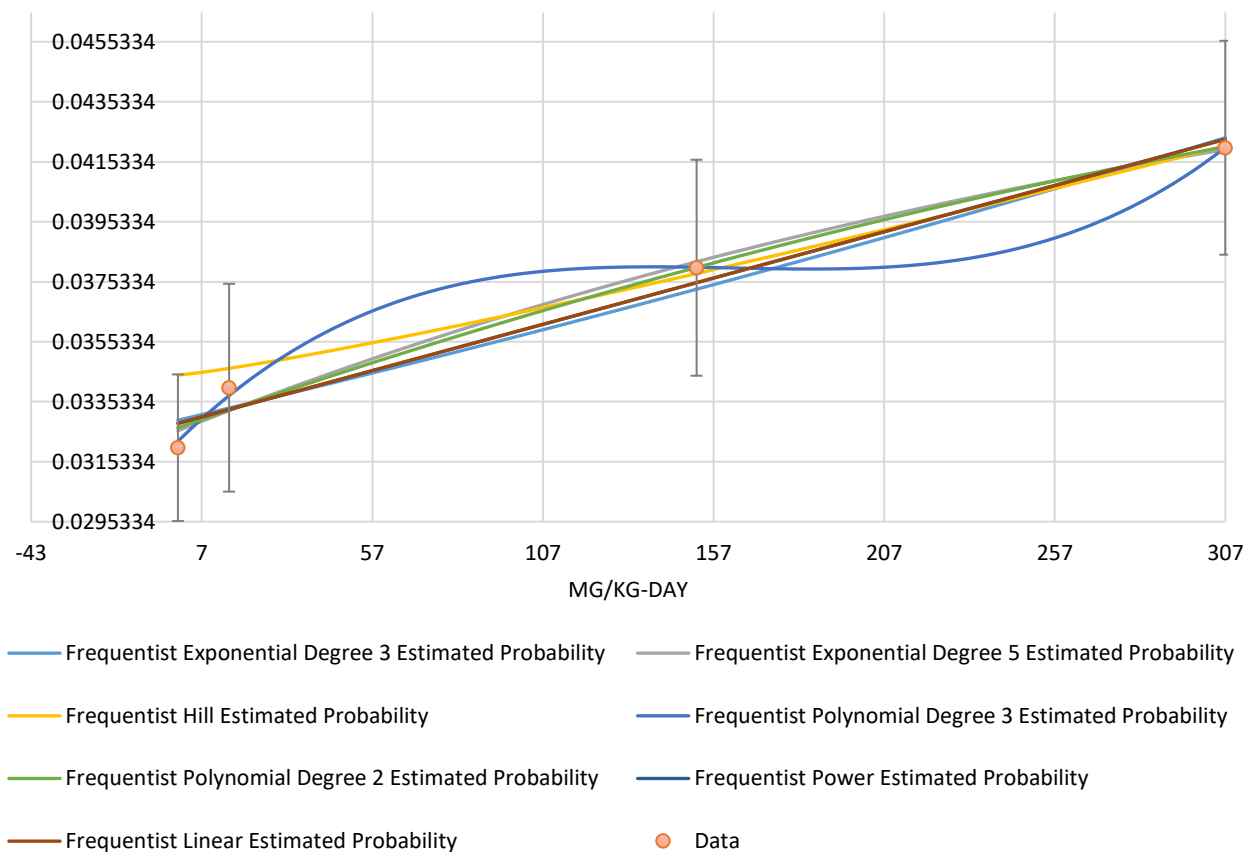
Models ^a	Restriction ^b	BMR = 10%		P Value	AIC	BMDS Recommends	BMDS Recommendation Notes	BMR = 5%		BMR = 1 SD		BMR = 25%	
		BMD	BMDL					BMD	BMDL	BMD	BMDL	BMD	BMDL
Exponential 3	Restricted	116.26	95.59	0.3786	-1497.4 98773	Viable – Alternate	Modeled control response std. dev. > 1.5 actual response std. dev.	59.51	48.93	248.94	206.95	272.19	223.80
Exponential 5	Restricted	79.84	36.41	0.3253	-1496.4 71899	Viable – Alternate		37.70	16.38	218.32	131.93	248.11	147.52
Hill	Restricted	154.16	151.00	NA	-1488.6 14597	Questionable	Residual at control > 2 d.f.=0, saturated model (Goodness of fit test cannot be calculated)	85.09	83.34	303.22	296.39	340.22	333.23
Polynomial Degree 3	Restricted	36.76	10.37	NA	-1495.3 18631	Questionable	BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)	16.01	4.92	272.09	29.48	283.55	31.16
Polynomial Degree 2	Restricted	88.20	49.76	0.3087	-1496.4 03289	Viable – Alternate		42.54	23.75	225.74	141.55	254.52	155.99
Power	Restricted	106.22	85.08	0.4626	-1497.8 97726	Viable – Alternate		53.11	42.54	241.06	195.89	265.55	212.69
Linear	Unrestricted	106.44	84.96	0.4627	-1497.8 97925	Viable – Recommended	Lowest AIC	50.59	42.54	241.50	195.75	266.10	211.11

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

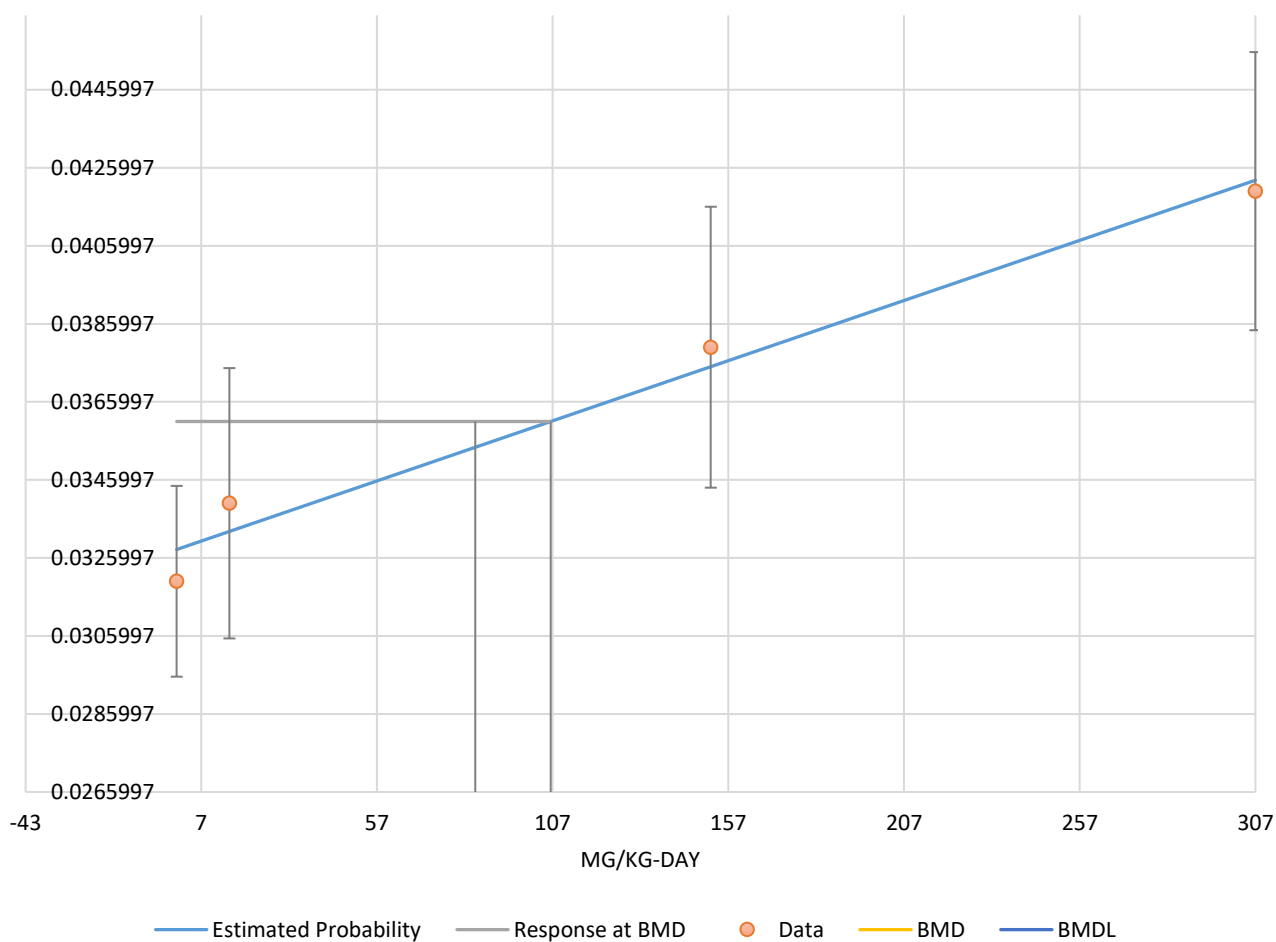
^a Selected Model (bolded and shaded gray); residuals for doses 0, 15, 152, and 307 mg/kg-day were -0.8549, 0.7132, 0.4739, and -0.2682, respectively.

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

Model Summary with BMR of 0.1 Relative Deviation for the BMD and 0.95 Lower Confidence Limit for the BMDL



Selected Frequentist Linear Model with BMR of 0.1 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



Results for Selected Model – Linear, CV (Unrestricted) – Rel. Dev., BMR = 0.1

User Input			
Info		Options	Model Data
Model	frequentist Linear, CV	Risk Type	Rel. Dev.
Dataset Name	"Male F344 Rats_RelLiverWt_2yr"	BMR	0.1
Formula	$M[\text{dose}] = g + b1 * \text{dose}$ $\text{Var}[j] = \alpha$	Confidence Level	.95
		Distribution	Normal
		Variance	Constant
		Dependent Variable	mg/kg-day
		Independent Variable	
		Total # of Observation	4

Model Results

Benchmark Dose	
BMD	106.440033
BMDL	84.96359659
BMDU	139.9032525
AIC	-1497.897925
Test 4 P-value	0.462657772
D.O.F.	2

Model Parameters	
# of Parameters	3
Variable	Estimate
g	0.032814937
beta	3.08295E-05
alpha	5.54312E-05

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	61	0.032814937	0.032	0.032	0.00744522	0.006	0.006	-0.854892965
15	54	0.03327738	0.034	0.034	0.00744522	0.008	0.008	0.713230418
152	50	0.037501021	0.038	0.038	0.00744522	0.008	0.008	0.47390353
307	51	0.042279594	0.042	0.042	0.00744522	0.008	0.008	-0.268185348

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	752.7197303	5	-1495.43946
A2	755.9925165	8	-1495.98503
A3	752.7197303	5	-1495.43946
fitted	751.9489626	3	-1497.89793
R	726.8720033	2	-1449.74401

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	58.24102629	6	<0.0001
2	6.545572396	3	0.08788246
3	6.545572396	3	0.08788246
4	1.541535303	2	0.46265777

E.4.1.2 Female F344 Rats

Table_Apx E-4. Dose-Response Modeling Data for Relative Liver Weight at Terminal Sacrifice in Female F344 Rats Following a 2-Year Exposure to DINP ([Lington et al., 1997](#))

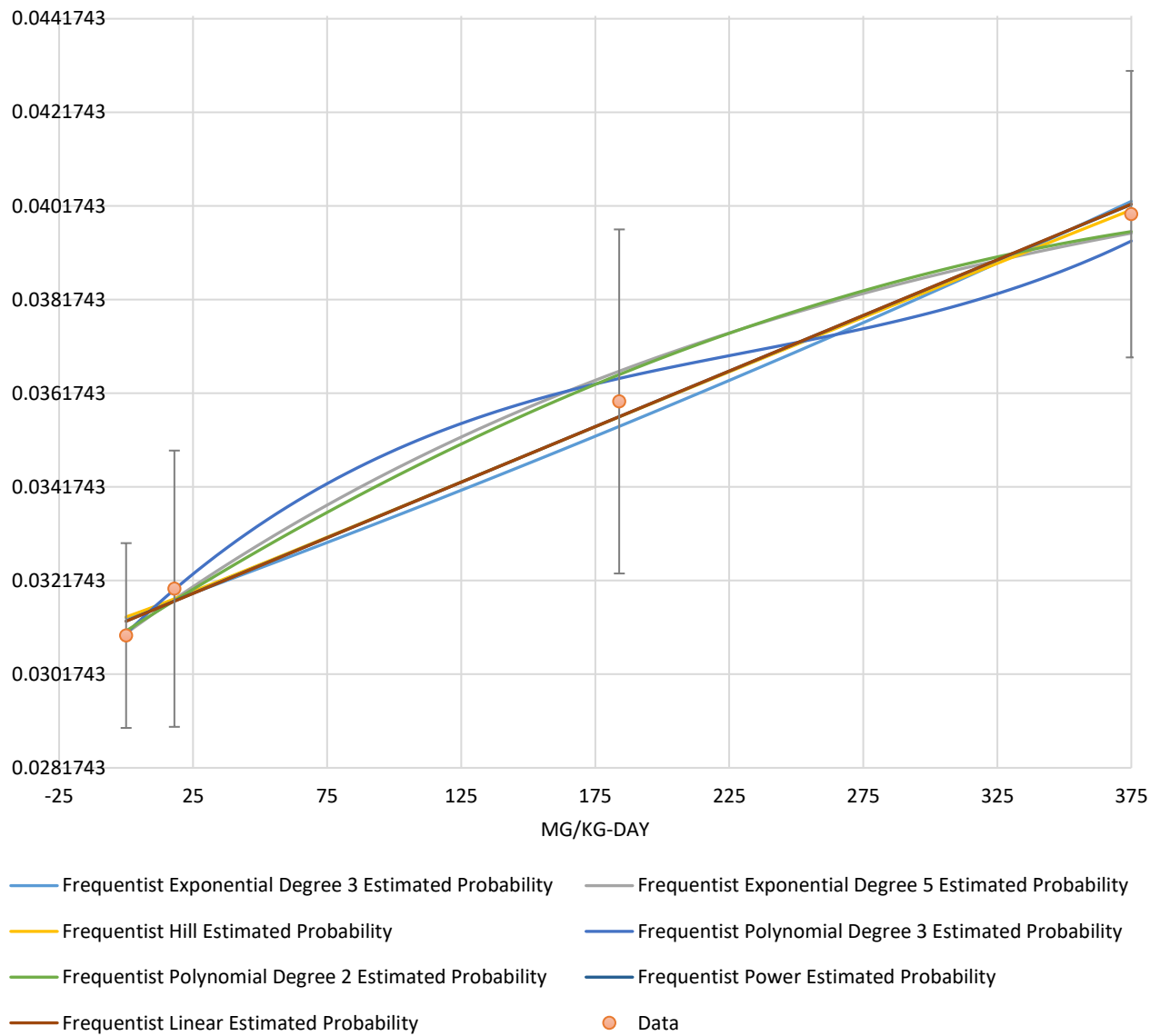
Dose (mg/kg-day)	Number per Group	Mean	Standard Deviation
0	65	0.031	0.005
18	57	0.032	0.007
184	48	0.036	0.008
375	53	0.04	0.007

Table_Apx E-5. Summary of Benchmark Dose Modeling Results for Relative Liver Weight at Terminal Sacrifice in Female F344 Rats Following a 2-Year Exposure to DINP (Non-constant Variance) ([Lington et al., 1997](#))

Standard Models ^a	Restriction ^b	BMR = 10%		P Value	AIC	BMDS Recommends	BMDS Recommendation Notes	BMR = 5%		BMR = 1 SD		BMR = 25%	
		BMD	BMDL					BMD	BMDL	BMD	BMDL	BMD	BMDL
Exponential 3	Restricted	143.27	118.57	0.2610	-1596.49	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Modeled control response std. dev. > 1.5 actual response std. dev.	73.34	60.66	268.59	219.51	335.42	277.61
Exponential 5	Restricted	86.77	35.03	0.3336	-1596.24	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)	39.99	15.51	199.97	114.18	309.91	167.83
Hill	Restricted	135.95	99.63	NA	-1592.96	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)	69.29	48.44	263.02	194.84	338.00	256.96
Polynomial Degree 3	Restricted	72.04	14.45	NA	-1594.31	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)	31.23	6.76	207.53	28.21	350.14	44.06
Polynomial Degree 2	Restricted	91.72	58.72	0.3068	-1596.13	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)	44.59	27.86	204.48	123.24	308.82	189.00
Power	Restricted	131.94	106.23	0.3428	-1597.04	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)	65.97	53.08	257.01	205.66	329.86	265.74
Linear	Unrestricted	128.47	105.83	0.3429	-1597.04	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)	62.63	53.11	256.89	204.62	329.42	264.54

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable
^a No selected model due to inadequate fit of constant or non-constant variance models.
^b Restrictions defined in the [BMDS 3.3 User Guide](#).

Model Summary with BMR of 0.1 Relative Deviation for the BMD and 0.95 Lower Confidence Limit for the BMDL



E.4.2 Serum ALT – Male F344 Rats

E.4.2.1 6-Month Sacrifice

Table_Apx E-6. Dose-Response Modeling Data for Serum ALT Levels in Male F344 Rats Following a 6-Month Exposure to DINP ([Lington et al., 1997](#))

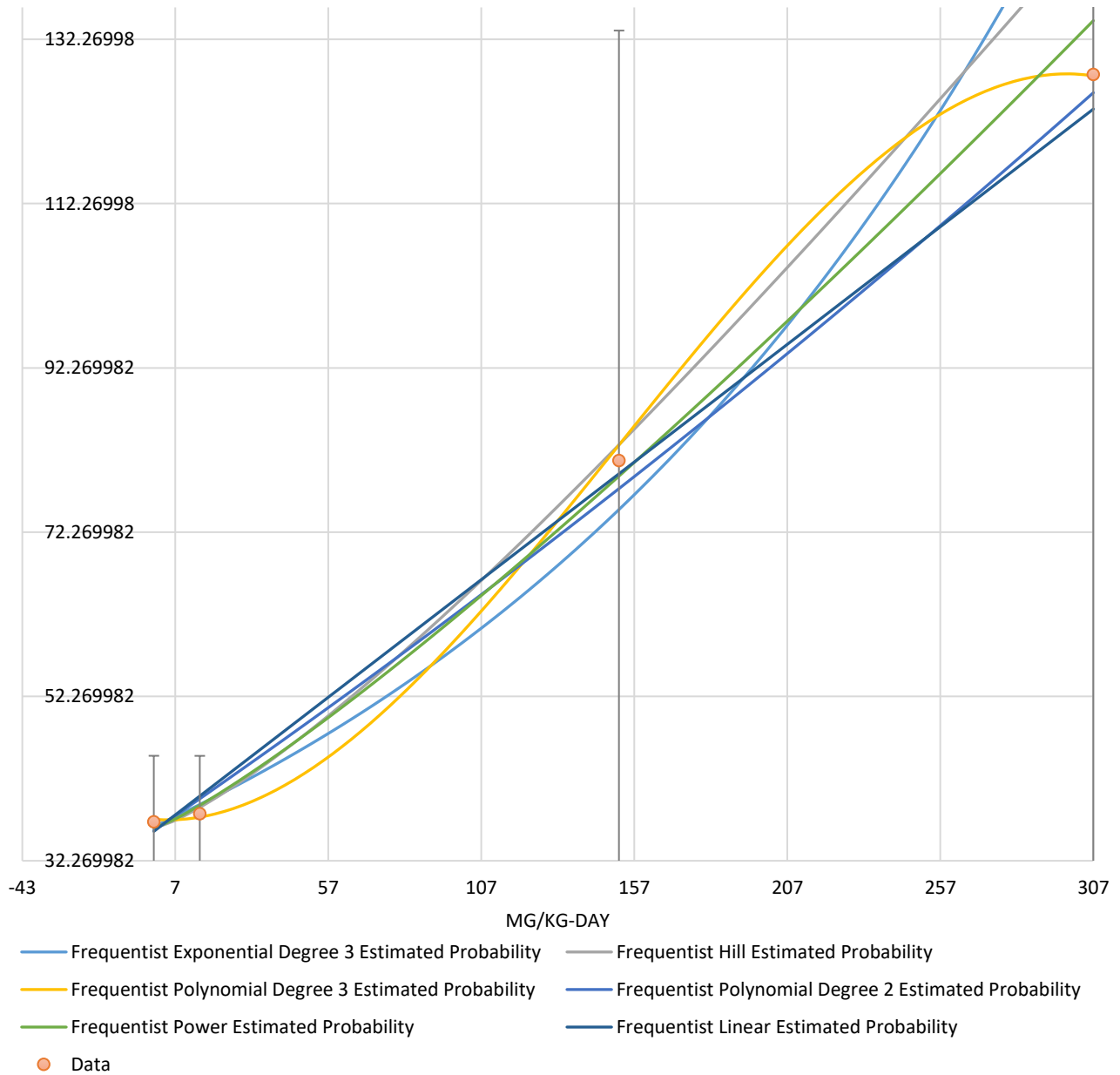
Dose (mg/kg-day)	Number per Group	Mean	Standard Deviation
0	10	37	8
15	10	38	7
152	10	81	52
307	10	128	145

Table_Apx E-7. Summary of Benchmark Dose Modeling Results for Serum ALT Levels in Male F344 Rats Following a 6-Month Exposure to DINP (Non-constant Variance) ([Lington et al., 1997](#))

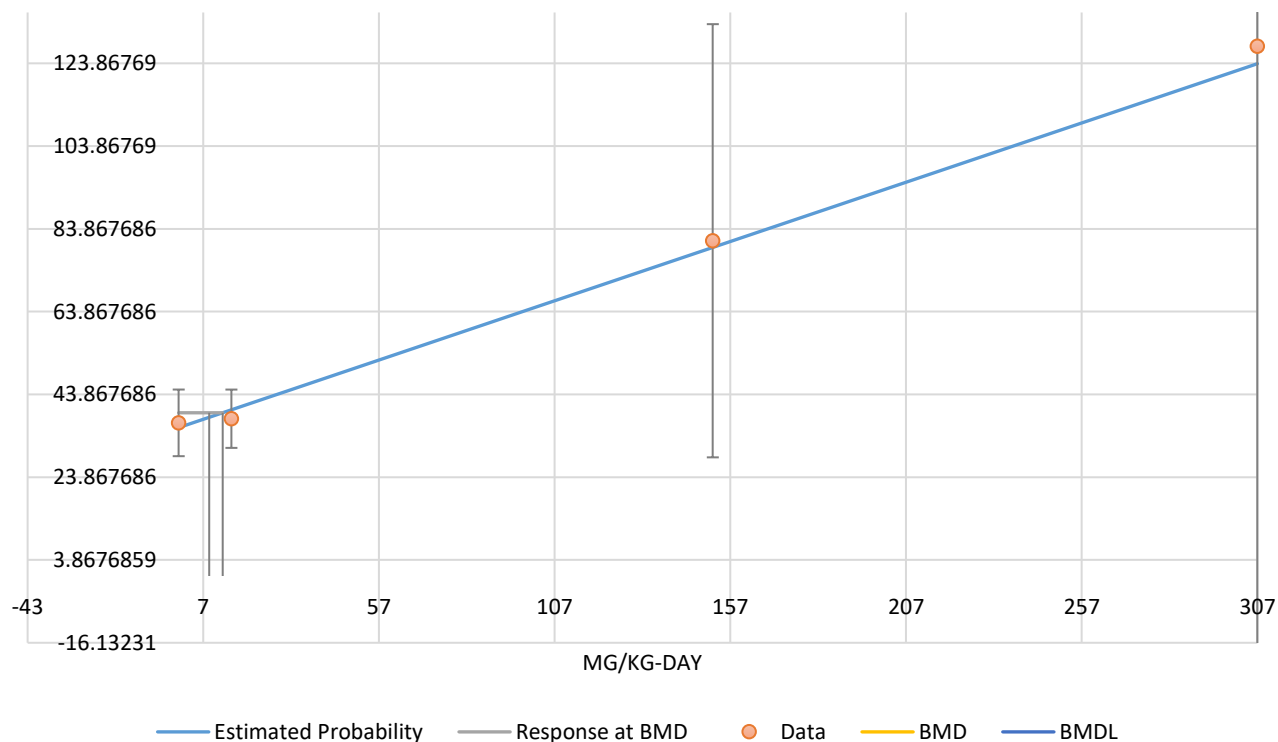
Models ^a	Restriction ^b	BMR = 10%		P Value	AIC	BMDS Recommends	BMDS Recommendation Notes	BMR = 1 SD		BMR = 20%		BMR = 100%	
		BMD	BMDL					BMD	BMDL	BMD	BMDL	BMD	BMDL
Exponential 3	Restricted	20.05	15.84	0.0692	382.00	Questionable	Goodness of fit p-value < 0.1 Modeled control response std. dev. > 1.5 actual response std. dev.	40.15	28.50	38.35	30.29	CF	CF
Exponential 5	Restricted	CF	CF	CF	CF	Unusable	BMD computation failed	124.58	27.19	CF	CF	CF	CF
Hill	Restricted	19.94	9.12	NA	382.16	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)	34.15	16.39	CF	CF	123.97	90.11
Polynomial Degree 3	Restricted	40.68	11.16	NA	380.67	Questionable	BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)	55.33	20.32	56.49	22.31	134.04	98.56
Polynomial Degree 2	Restricted	13.99	0	0.1351	380.89	Unusable	BMD computation failed; lower limit includes zero BMDL not estimated	26.33	14.94	27.79	16.84	132.49	87.19
Power	Restricted	18.76	9.26	0.2143	380.20	Viable – Alternate		32.59	16.63	33.74	18.51	131.87	91.22
Linear	Unrestricted	12.52	8.68	0.3050	379.03	Viable – Recommended	Lowest AIC	23.42	15.50	25.04	17.37	125.20	86.83

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable; CF = computation failed
^a Selected Model (bolded and shaded gray); residuals for doses 0, 15, 152, and 307 were 0.5396, -0.7686, 0.1084, 0.0955, respectively.
^b Restrictions defined in the BMDS 3.3 User Guide

Model Summary with BMR of 0.1 Relative Deviation for the BMD and 0.95 Lower Confidence Limit for the BMDL



Frequentist Linear Model with BMR of 0.1 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



Results for Selected Model – Linear, NCV (Unrestricted) – Rel. Dev., BMR = 0.1

User Input

Info		Options		Model Data	
Model	Frequentist Linear, NCV	Risk Type	Rel. Dev.	Dependent Variable	mg/kg-day
Dataset Name	Male F344 Rats Serum ALT_6mon	BMR	0.1	Independent Variable	
Formula	$M[\text{dose}] = g + b1 * \text{dose}$ $\text{Var}[i] = \alpha$ $* \text{mean}[i] ^ \rho$	Confidence Level	0.95	Total # of Observation	4
		Distribution	Normal		
		Variance	Non-Constant		

Model Results

Benchmark Dose	
BMD	12.51986155
BMDL	8.683091255
BMDU	12.77902268
AIC	379.0287425
Test 4 P-value	0.304955816
D.O.F.	2

Model Parameters	
# of Parameters	4
Variable	Estimate
g	35.85553524
beta	0.286389228
rho	4.902699939
alpha	1.07545E-06

Goodness of Fit		Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
Dose	Size							
0	10	35.85553524	37	37	6.7074289	8	8	0.539568203
15	10	40.15137365	38	38	8.85168002	7	7	-0.768581876
152	10	79.38669783	81	81	47.0696879	52	52	0.108386302
307	10	123.7770281	128	128	139.825984	145	145	0.095505923

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-228.508524	5	467.017048
A2	-184.1836225	8	384.367245
A3	-184.3267829	6	380.653566
fitted	-185.5143713	4	379.028743

E.4.2.2 18-Month Sacrifice

Table_Apx E-8. Dose-Response Modeling Data for Serum ALT Levels in Male F344 Rats Following an 18-Month Exposure to DINP ([Lington et al., 1997](#))

Dose (mg/kg-day)	Number per Group	Mean	Standard Deviation
0	9	42	10
15	10	39	7
152	10	69	39
307	10	128	126

Table_Apx E-9. Summary of Benchmark Dose Modeling Results for Serum ALT Levels in Male F344 Rats Following an 18-Month Exposure to DINP (Non-constant Variance) ([Lington et al., 1997](#))

Models ^a	Restriction ^b	BMR=10%		P Value	AIC	BMDS Recommends	BMDS Recommendation Notes	BMR=1 SD		BMR=20%		BMR=100%	
		BMD	BMDL					BMD	BMDL	BMD	BMDL	BMD	BMDL
Exponential 3	Restricted	28.31	19.66	0.0433	371.30	Questionable	Goodness of fit p-value < 0.1 Modeled control response std. dev. > 1.5 actual response std. dev.	56.70	37.76	52.87	37.61	191.28	143.00
Exponential 5	Restricted	103.76	21.91	NA	370.80	Questionable	BMD/BMDL ratio > 3; d.f.=0, saturated model (Goodness of fit test cannot be calculated)	113.99	40.10	113.67	39.87	154.96	134.70
Hill	Restricted	61.57	28.62	NA	371.00	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)	CF	CF	82.15	46.68	182.90	133.66
Polynomial Degree 3	Restricted	63.43	20.61	NA	370.94	Questionable	BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)	85.51	40.83	84.98	40.09	200.71	131.37
Polynomial Degree 2	Restricted	29.49	14.27	0.0428	371.32	Questionable	Goodness of fit p-value < 0.1	56.99	28.32	55.73	28.45	210.39	132.17
Power^c	Restricted	37.19	17.45	0.0925	370.04	Questionable	Goodness of fit p-value < 0.1	62.51	33.36	59.71	33.45	179.20	134.31
Linear	Unrestricted	20.06	12.52	0.0655	370.67	Questionable	Goodness of fit p-value < 0.1	40.61	24.79	40.11	25.04	200.56	125.22

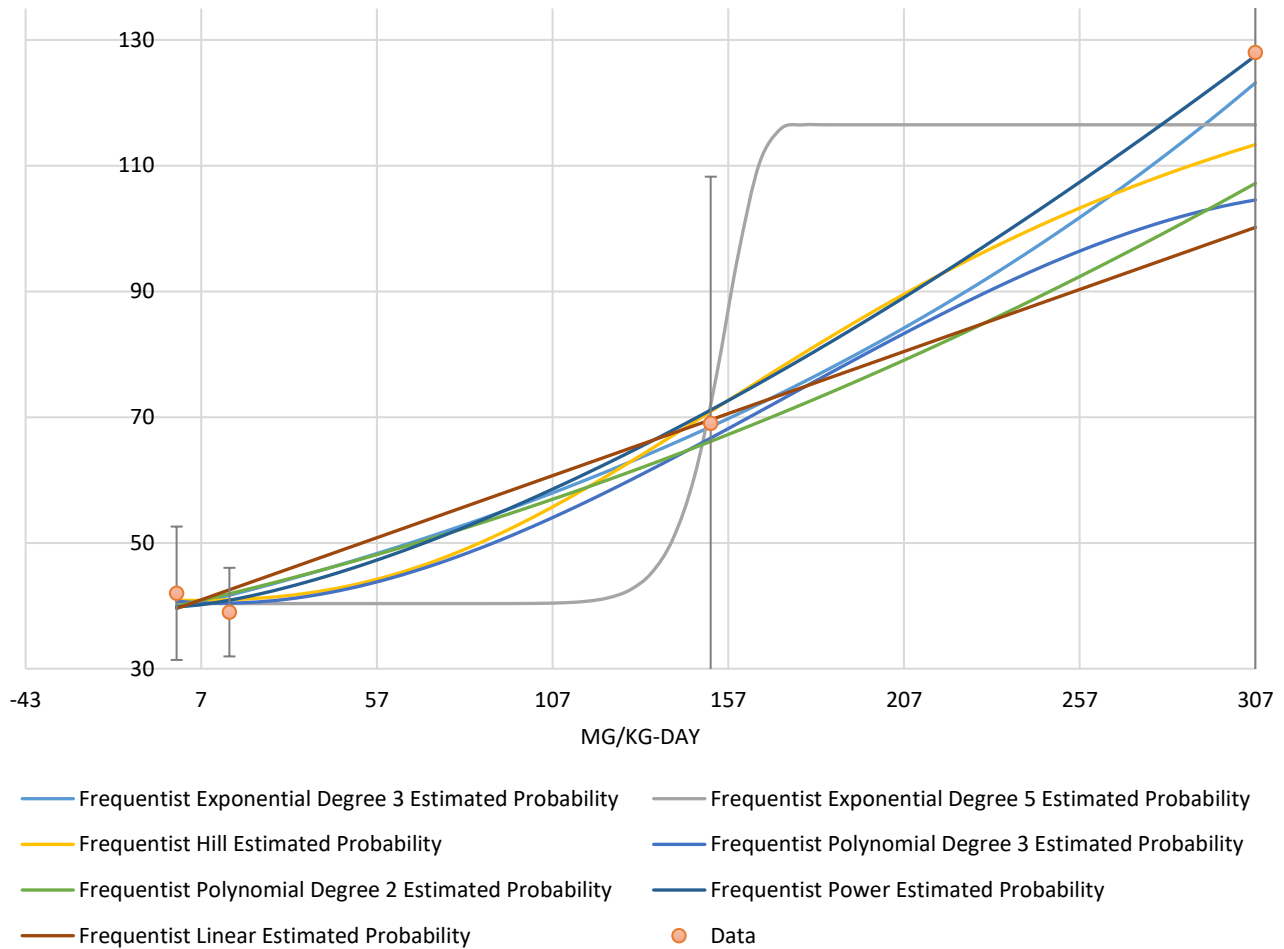
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model is bolded and shaded gray; residuals for doses 0, 15, 152, and 307 were 0.7610, -0.6609, -0.2070, and 0.0131, respectively.

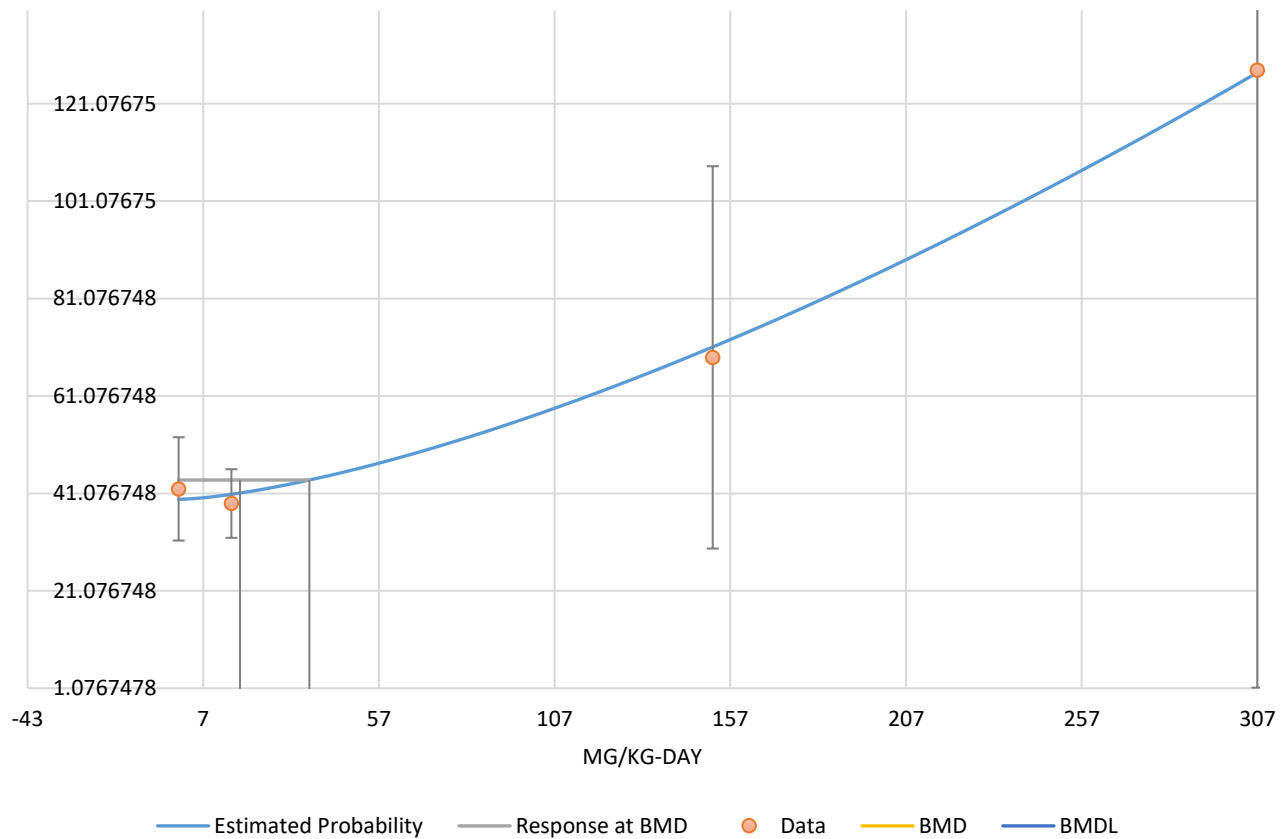
^b Restrictions defined in the BMDS 3.3 User Guide

^c Despite p < 0.1, the Power model fit would pass at p > 0.05, the variance model passed p>0.05, and visual fit of model to data is still adequate for BMD calculation.

Model Summary with BMR of 0.1 Relative Deviation for the BMD and 0.95 Lower Confidence Limit for the BMDL



Frequentist Power Model with BMR of 0.1 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



Results for Selected Model – Power, NCV (Restricted) – Rel. Dev., BMR = 0.1

User Input

Info		Options		Model Data	
Model	Frequentist Power, NCV	Risk Type	Rel. Dev.	Dependent Variable	mg/kg-day
Dataset Name	MaleF344Rats_Serum ALT_18mon	BMR	0.1	Independent Variable	
Formula	$M[\text{dose}] = g + v * \text{dose}^n$ $\text{Var}[i] = \alpha * \text{mean}[i]^{\rho}$	Confidence Level	0.95	Total # of Observations	4
		Distribution	Normal		
		Variance	Non-Constant		

Model Results

Benchmark Dose	
BMD	37.19126348
BMDL	17.45080887
BMDU	37.96112263
AIC	370.0444752
Test 4 P-value	0.092488008
D.O.F.	1

Model Parameters	
# of Parameters	5
Variable	Estimate
g	39.8382544
v	0.019980069
n	1.464367921
rho	4.643124981
alpha	2.69559E-06

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	9	39.8382544	42	42	8.5216504	10	10	0.761030608
15	10	40.89222207	39	39	9.05422294	7	7	-0.66087743
152	10	71.14361683	69	69	32.7473294	39	39	-0.207000441
307	10	127.4742711	128	128	126.82257	126	126	0.013108871

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-217.2980126	5	444.596025
A2	-178.4089743	8	372.817949
A3	-178.6069741	6	369.213948
fitted	-180.0222376	5	370.044475

E.5 Dichotomous Endpoints

E.5.1 Focal Necrosis in the Liver

E.5.1.1 Male F344 Rats

Table_Apx E-10. Dose-Response Modeling Data for Focal Necrosis of the Liver in Male F344 Rats Following a 2-Year Exposure to DINP ([Lington et al., 1997](#))

Dose (mg/kg-day)	Number per Group	Incidence
0	81	10
15	80	9
152	80	16
307	80	26

Table_Apx E-11. Summary of Benchmark Dose Modeling Results for Focal Necrosis of the Liver in Male F344 Rats Following a 2-Year Exposure to DINP (Lington et al., 1997)

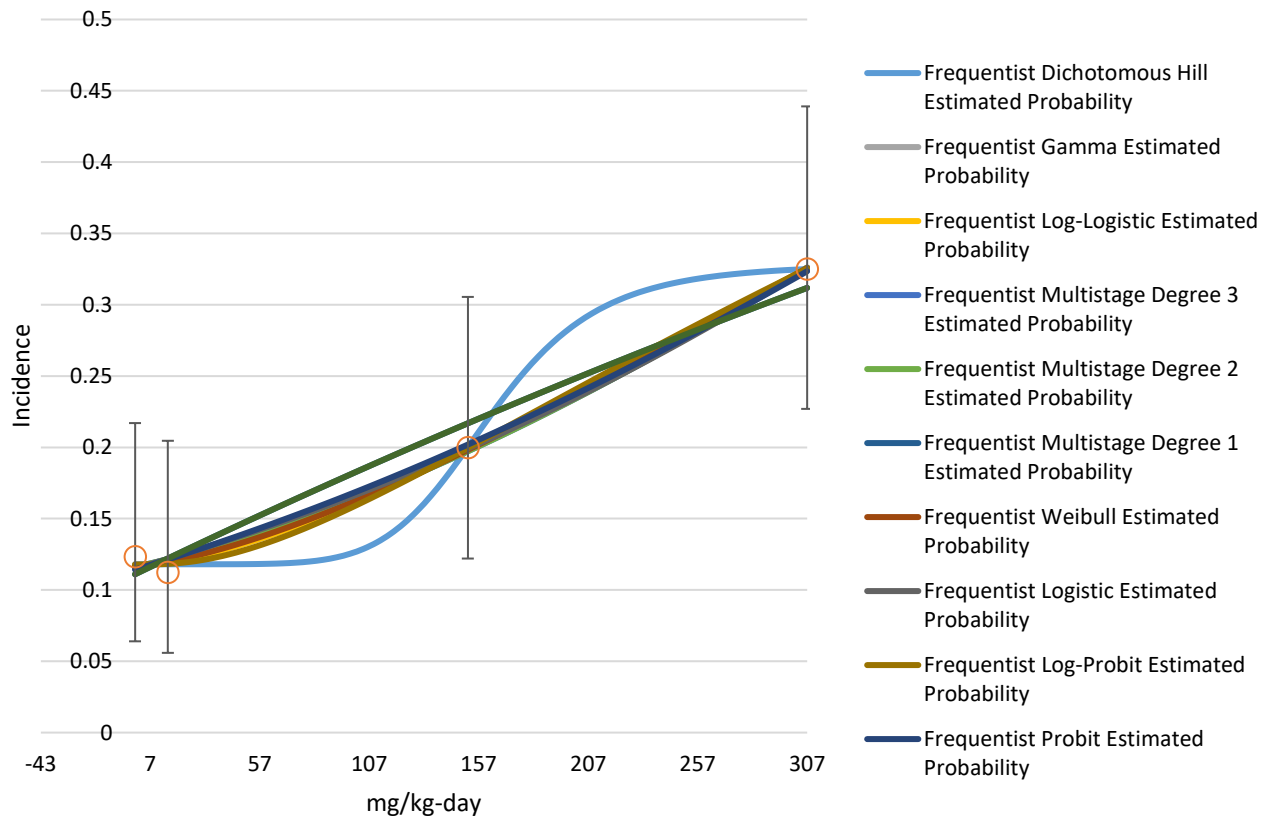
Models ^a	Restriction ^b	BMR = 10%		P Value	AIC	BMDS Recommends	BMDS Recommendation Notes	BMR = 5%	
		BMD	BMDL					BMD	BMDL
Dichotomous Hill	Restricted	154.87	48.90	NA	305.83	Questionable	BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)	132.94	18.97
Gamma	Restricted	161.40	85.98	0.7925	303.85	Viable – Alternate		100.26	41.86
Log-Logistic	Restricted	160.91	78.23	0.7930	303.85	Viable – Alternate		100.39	37.06
Multistage Degree 3	Restricted	162.13	85.74	0.7420	303.89	Viable – Alternate		94.76	41.74
Multistage Degree 2	Restricted	162.13	85.74	0.7420	303.89	Viable – Alternate		94.76	41.74
Multistage Degree 1	Restricted	126.33	84.11	0.8212	302.17	Viable – Alternate		61.50	40.94
Weibull	Restricted	161.48	85.94	0.7832	303.86	Viable – Alternate		98.74	41.84
Logistic	Unrestricted	158.52	124.56	0.9417	301.90	Viable – Recommended	Lowest AIC	88.34	69.47
Log-Probit	Unrestricted	159.84	46.47	0.8230	303.83	Viable – Alternate	BMD/BMDL ratio > 3	104.60	12.63
Probit	Unrestricted	153.31	118.45	0.9368	301.91	Viable – Alternate		83.82	64.96
Quantal Linear	Unrestricted	126.33	84.11	0.8212	302.17	Viable – Alternate		61.50	40.95

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

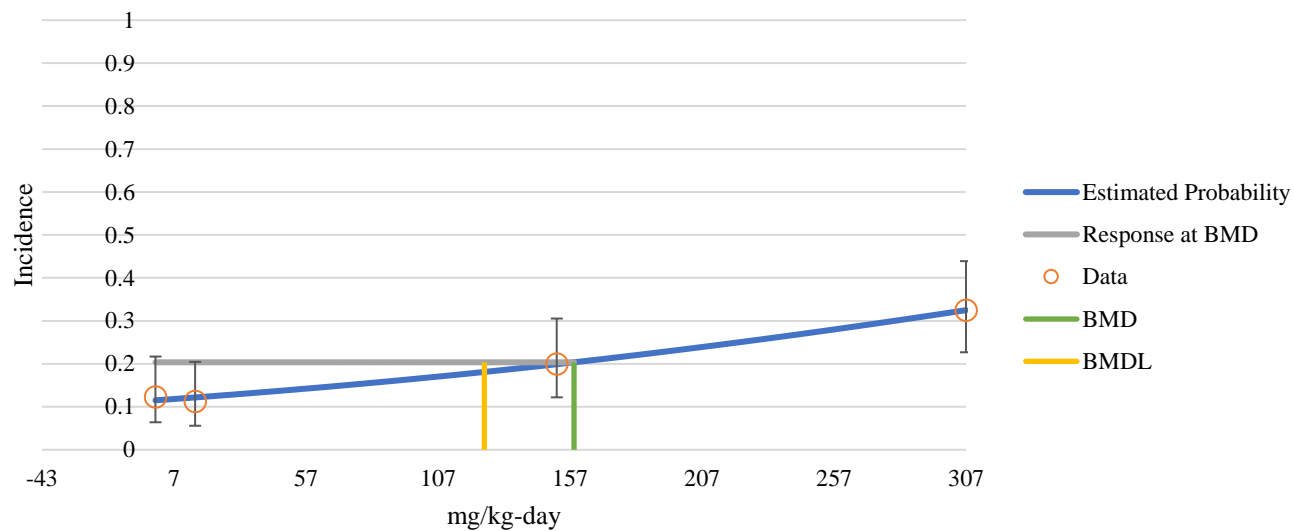
^a Selected Model is bolded and shaded gray; residuals for doses 0, 15, 152 and 307 were 0.2347, -0.2546, 0.0189 and 0.0007, respectively.

^b Restrictions defined in the BMDS 3.3 User Guide.

Model Summary with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



Frequentist Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



Results for Selected Model - Logistic (Unrestricted) - Extra Risk, BMR = 0.1

User Input					
Info		Options		Model Data	
Model	Logistic	Risk Type	Extra Risk	Dependent Variable	mg/kg-day
Dataset Name	Male F344 Rats -	BMR	0.1	Independent Variable	Incidence
Formula	$P[\text{dose}] = 1/[1+\exp(-a-b \cdot \text{dc})]$	Confidence Level	0.95	Total # of Observation	4
		Background	Estimated		

Model Results

Benchmark Dose	
BMD	158.52
BMDL	124.56
BMDU	239.50
AIC	301.90
P-value	0.94
D.O.F.	2.00
Chi²	0.12
Slope Factor	158.52

Model Parameters	
# of Parameters	2
Variable	Estimate
a	-2.0393
b	0.00426

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.115134137	9.32586507	10	81	0.2347
15	0.121808403	9.744672223	9	80	-0.2546
152	0.199154436	15.93235488	16	80	0.0189
307	0.324963847	25.99710772	26	80	0.0007

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-148.8897738	4	-	-	NA
Fitted Model	-148.950072	2	0.12059642	2	0.9414837
Reduced Model	-156.0920707	1	14.4045939	3	0.0024031

User Input

Info		Options		Model Data	
Model	Logistic	Risk Type	Extra Risk	Dependent Variable	mg/kg-day
Dataset Name	Male F344 Rats -	BMR	0.1	Independent Variable	Incidence
Formula	$P[\text{dose}] = \frac{1}{1 + \exp(-a - b \cdot \text{dose})}$	Confidence Level	0.95	Total # of Observation	4
		Background	Estimated		

Model Results

Benchmark Dose	
BMD	158.52
BMDL	124.56
BMDU	239.50
AIC	301.90
P-value	0.94
D.O.F.	2.00
Chi²	0.12
Slope Factor	158.52

Model Parameters	
# of Parameters	2
Variable	Estimate
a	-2.0393
b	0.00426

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.115134137	9.32586507	10	81	0.2347
15	0.121808403	9.744672223	9	80	-0.2546
152	0.199154436	15.93235488	16	80	0.0189
307	0.324963847	25.99710772	26	80	0.0007

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-148.8897738	4	-	-	NA
Fitted Model	-148.950072	2	0.12059642	2	0.9414837
Reduced Model	-156.0920707	1	14.4045939	3	0.0024031

E.5.1.2 Female F344 Rats

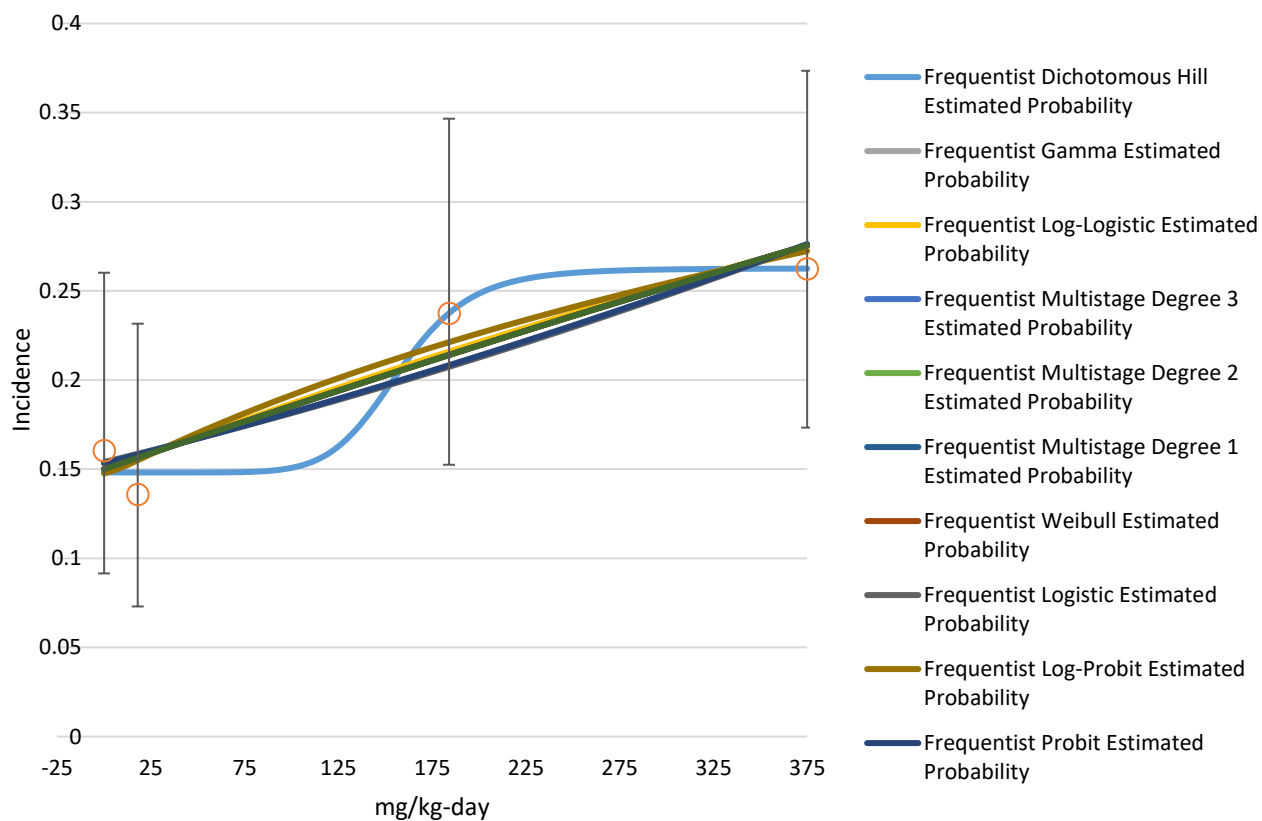
Table_Apx E-12. Dose-Response Modeling Data for Focal Necrosis of the Liver in Female F344 Rats Following a 2-Year Exposure to DINP ([Lington et al., 1997](#))

Dose (mg/kg-day)	Number per Group	Incidence
0	81	13
18	81	11
184	80	19
375	80	21

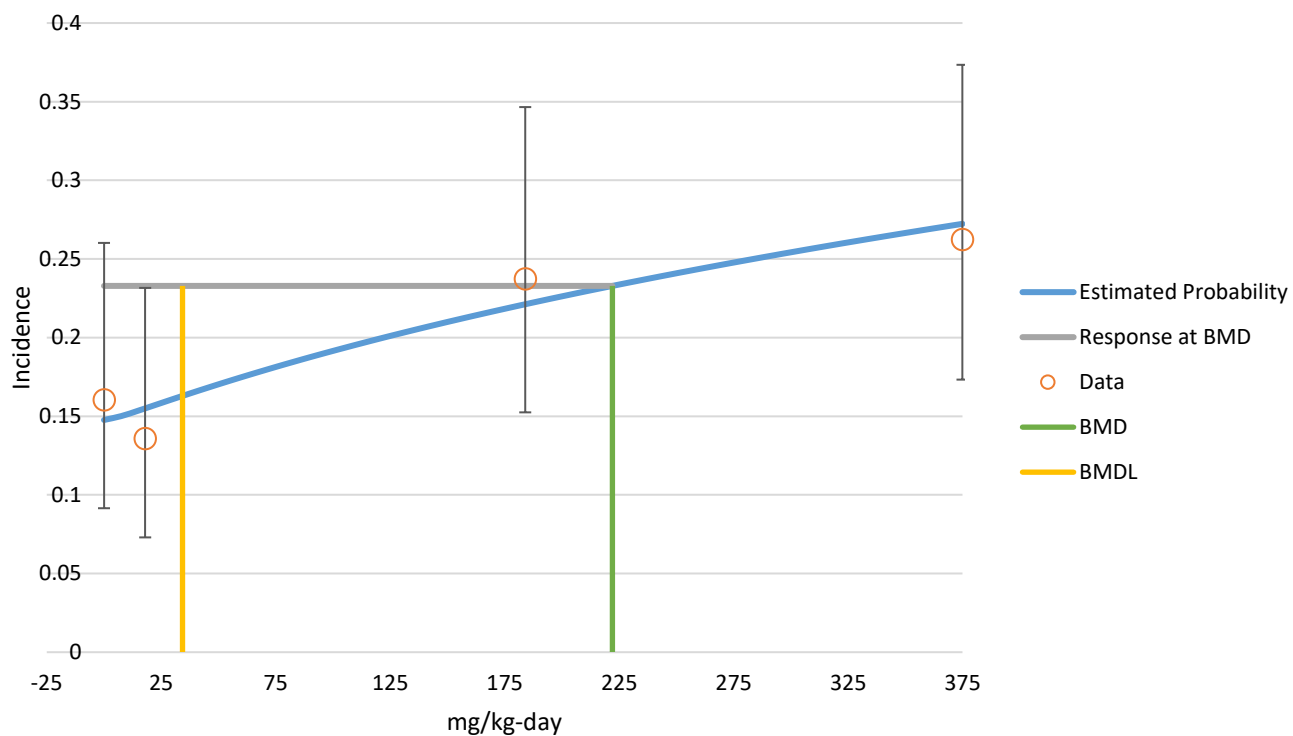
Table_Apx E-13. Summary of Benchmark Dose Modeling Results for Focal Necrosis of the Liver in Female F344 Rats Following a 2-Year Exposure to DINP (Lington et al., 1997)

Models ^a	Restriction ^b	BMR=10%		P Value	AIC	BMDS Recommends	BMDS Recommendation Notes	BMR = 5%	
		BMD	BMDL					BMD	BMDL
Dichotomous Hill	Restricted	179.57	19.90	NA	323.73	Questionable	BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)	148.09	7.87
Gamma	Restricted	247.12	136.68	0.7185	320.19	Viable – Alternate		120.31	66.54
Log-Logistic	Restricted	239.78	125.46	0.7335	320.15	Viable – Alternate		113.58	59.43
Multistage Degree 3	Restricted	247.12	136.68	0.7185	320.19	Viable – Alternate		120.31	66.53
Multistage Degree 2	Restricted	247.12	136.68	0.7185	320.19	Viable – Alternate		120.31	66.54
Multistage Degree 1	Restricted	247.12	136.68	0.7185	320.19	Viable – Alternate		120.31	66.54
Weibull	Restricted	247.12	136.68	0.7185	320.19	Viable – Alternate		120.31	66.54
Logistic	Unrestricted	275.16	179.48	0.6509	320.39	Viable – Alternate		148.92	98.02
Log-Probit	Unrestricted	222.08	34.30	0.4809	322.03	Viable – Recommended	Lowest BMDL BMD/BMDL ratio > 3	96.76	0.90
Probit	Unrestricted	271.03	173.31	0.6617	320.36	Viable – Alternate		144.53	93.23
Quantal Linear	Unrestricted	247.12	136.68	0.7185	320.19	Viable – Alternate		120.31	66.54
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable ^a Selected Model is bolded and shaded gray; residuals for doses 0, 18, 184 and 375 were 0.3259, -0.4779, 0.3508 and -0.1977, respectively. ^b Restrictions defined in the BMDS 3.3 User Guide.									

Model Summary with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



**Female F344 Relative Liver Weight vs mg/kg-day; LogProbit model
with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence
Limit for the BMDL**



Results for Selected Model - LogProbit (Unrestricted) - Extra Risk, BMR = 0.1

User Input					
Info		Options		Model Data	
Model	Log-Probit	Risk Type	Extra Risk	Dependent Variable	mg/kg-day
Dataset Name	Female F344 Rats - focal necrosis	BMR	0.1	Independent Variable	Incidence
Formula	$P[\text{dose}] = \frac{g}{g + (1-g) * \text{CumNorm}(a+b*\text{Log}(\text{Dose}))}$	Confidence Level	0.95	Total # of Observation	4
		Background	Estimated		

Model Results

Benchmark Dose	
BMD	222.0806266
BMDL	34.3001408
BMDU	Infinity
AIC	322.0314517
P-value	0.48091731
D.O.F.	1
Chi²	0.496782444

Model Parameters	
# of Parameters	3
Variable	Estimate
Background (g)	0.147649782
a	-3.644150287
b	0.437272073

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.147649782	11.95963234	13	81	0.3258509
18	0.155022564	12.55682771	11	81	-0.477945
184	0.221220007	17.69760055	19	80	0.3508162
375	0.27234158	21.7873264	21	80	-0.197738

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-157.7653174	4	-	-	NA
Fitted Model	-158.0157259	3	0.50081701	1	0.4791414
Reduced Model	-160.5735074	1	5.61638012	3	0.1318411

E.5.2 Spongiosis Hepatis in the Liver – Male F344 Rats

Table_Apx E-14. Dose-Response Modeling Data for Spongiosis Hepatis of the Liver in Male F344 Rats Following 2-Year Exposure to DINP ([Lington et al., 1997](#))

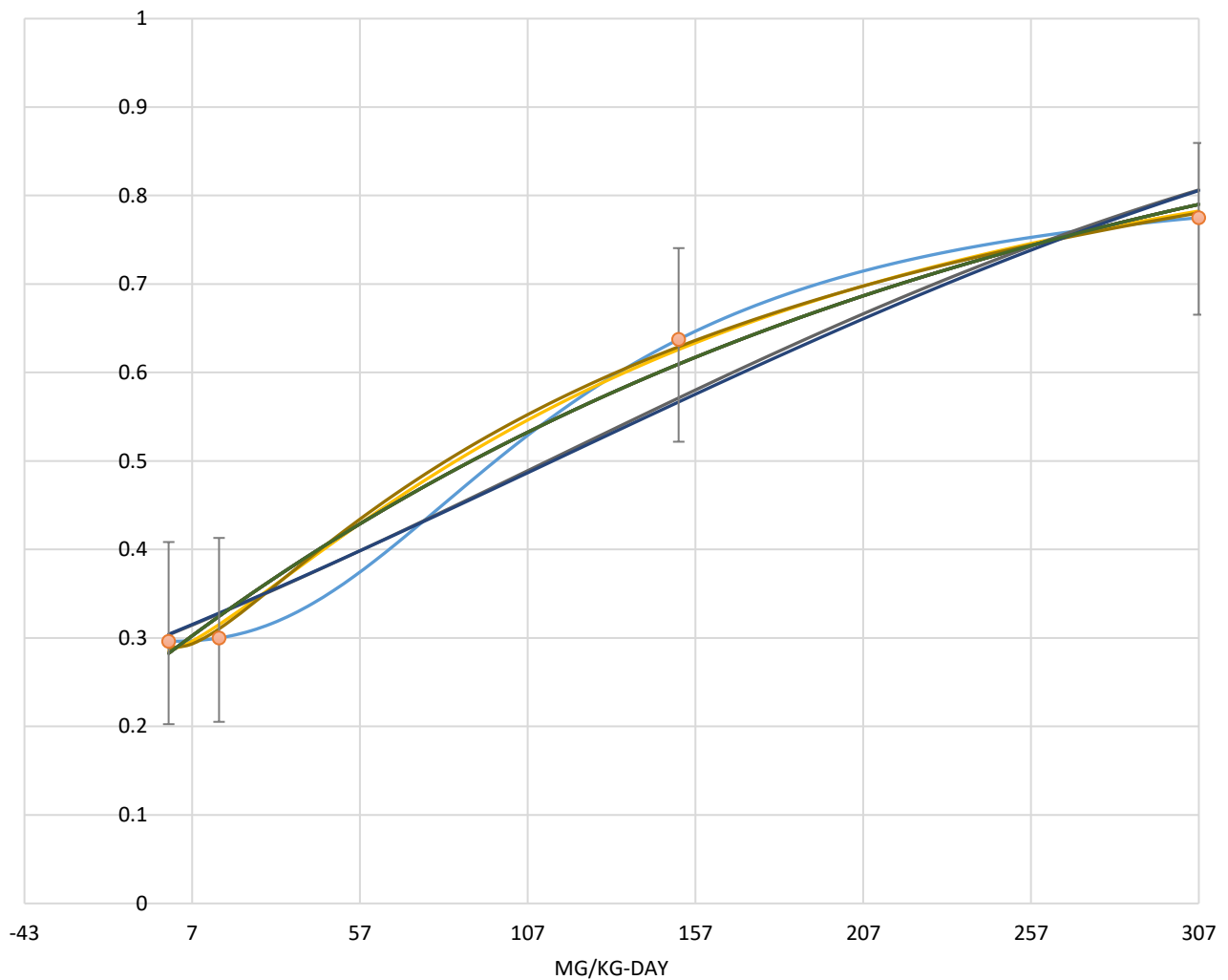
Dose (mg/kg-day)	Number per Group	Incidence
0	81	24
15	80	24
152	80	51
307	80	62

Table_Apx E-15. Summary of Benchmark Dose Modeling Results for Spongiosis Hepatis of the Liver in Male F344 Rats Following a 2-Year Exposure to DINP ([Lington et al., 1997](#))

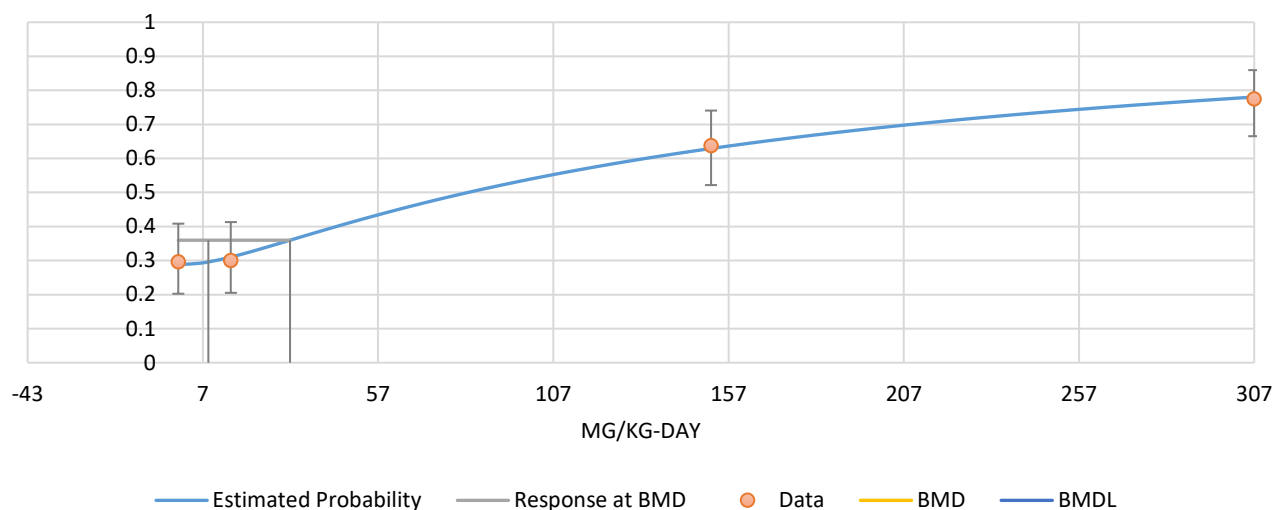
Models ^a	Restriction ^b	BMR = 10%		P Value	AIC	BMDS Recommends	BMDS Recommendation Notes	BMR = 5%	
		BMD	BMDL					BMD	BMDL
Dichotomous Hill	Restricted	53.05	9.92	1	394.27	Viable – Alternate	BMD/BMDL ratio > 3	37.76	4.81
Gamma	Restricted	26.33	20.77	0.8496	390.93	Viable – Alternate		12.82	10.11
Log-Logistic	Restricted	30.45	11.96	0.7322	392.47	Viable – Alternate		17.20	5.67
Multistage Degree 3	Restricted	26.33	20.77	1	–9999	Unusable	AIC not estimated	12.82	10.11
Multistage Degree 2	Restricted	26.33	20.77	1	–9999	Unusable	AIC not estimated	12.82	10.11
Multistage Degree 1	Restricted	26.33	20.77	0.8496	390.93	Viable – Alternate		12.82	10.11
Weibull	Restricted	26.33	20.77	0.8496	390.93	Viable – Alternate		12.82	10.11
Logistic	Unrestricted	42.42	35.87	0.6349	392.50	Viable – Alternate		21.74	18.35
Log-Probit	Unrestricted	31.88	8.57	0.8137	392.37	Viable – Recommended	Lowest BMDL; BMD/BMDL ratio > 3	20.08	4.03
Probit	Unrestricted	42.55	36.41	0.6037	392.70			21.70	18.55
Quantal Linear	Unrestricted	26.33	20.77	0.8496	390.93			12.82	10.11

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit
^a Selected Model is bolded; residuals for doses 0, 15, 152, and 307 were 0.1279, -0.1656, 0.0941, and -0.0539, respectively.
^b Restrictions defined in the BMDS 3.3 User Guide

Model Summary with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



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



Results for Selected Model - LogProbit (Unrestricted) - Extra Risk, BMR = 0.1

User Input

Info		Options		Model Data	
Model	Log-Probit	Risk Type	Extra Risk	Dependent Variable	mg/kg-day
Dataset Name	Male F344 Rats_spongiosis hepatitis	BMR	0.1	Independent Variable	Incidence
Formula	$P[\text{dose}] = g + (1-g) * \text{CumNorm}(a+b*\text{Log}(\text{Dose}))$	Confidence Level	0.95	Total # of Observation	4
		Background	Estimated		

Model Results

Benchmark Dose	
BMD	31.87966632
BMDL	8.566931336
BMDU	77.63938389
AIC	392.3657526
P-value	0.813651618
D.O.F.	1
Chi2	0.055562904

Model Parameters	
# of Parameters	3
Variable	Estimate
Background (g)	0.288658724
a	-4.003497521
b	0.786242291

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.288658724	23.38135661	24	81	0.1279398
15	0.310314502	24.82516015	24	80	-0.1656122
152	0.629151263	50.33210107	51	80	0.094143
307	0.780322211	62.4257769	62	80	-0.053889

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-193.1328632	4	-	-	NA
Fitted Model	-193.1828763	3	0.10002618	1	0.7517982
Reduced Model	-222.4986873	1	58.6316221	3	0.7517982

E.5.3 Sinusoid Ectasia in the Liver Male F344 Rats

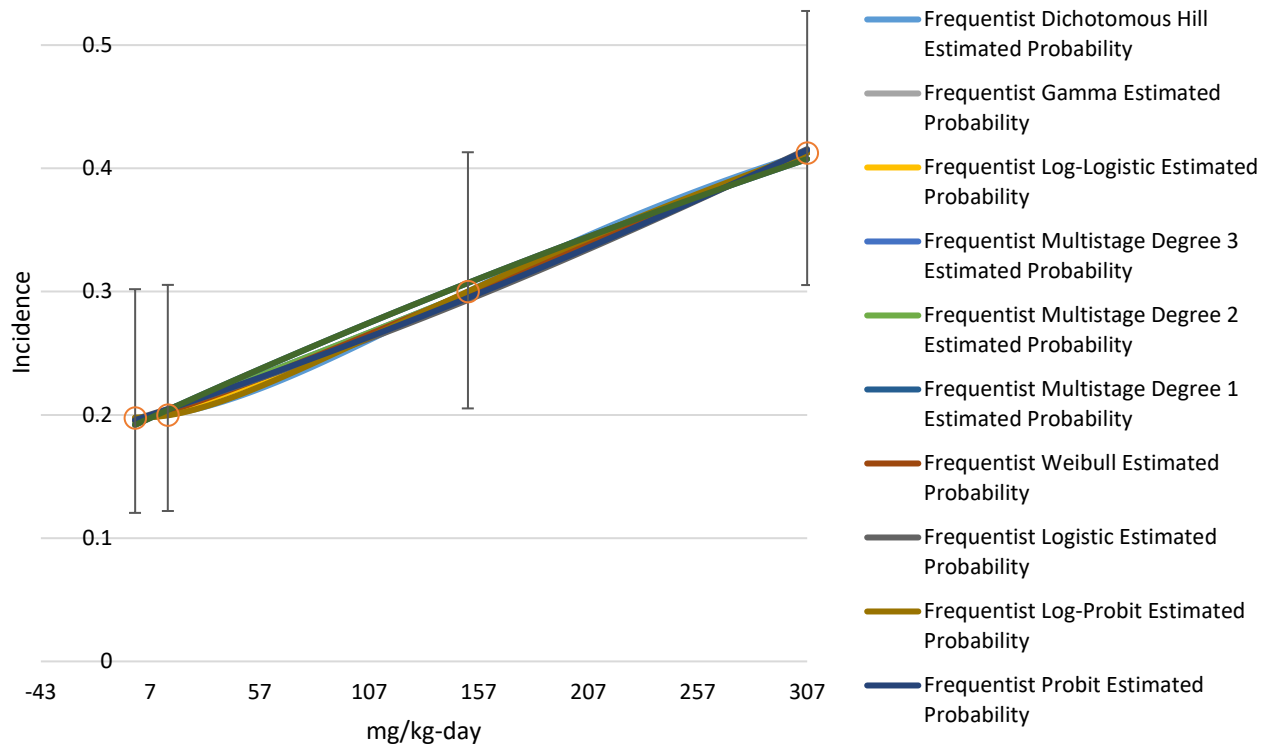
Table_Apx E-16. Dose-Response Modeling Data for Sinusoid Ectasia of the Liver in Male F344 Rats Following a 2-Year Exposure to DINP ([Lington et al., 1997](#))

Dose (mg/kg-day)	Number per Group	Incidence
0	81	16
15	80	16
152	80	24
307	80	33

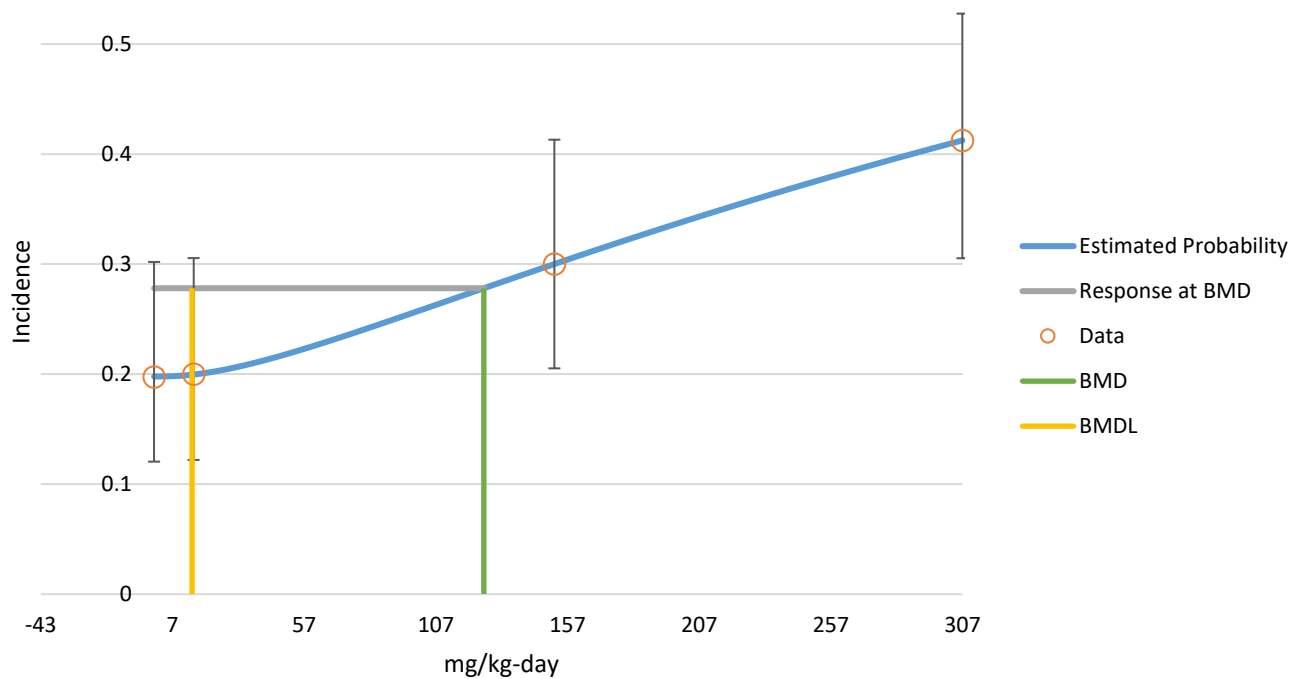
Table_Apx E-17. Summary of Benchmark Dose Modeling Results for Sinusoid Ectasia of the Liver in Male F344 Rats Following a 2-Year Exposure to DINP (Lington et al., 1997)

Models ^a	Restriction ^b	BMR=10%		P Value	AIC	BMDS Recommends	BMDS Recommendation Notes	BMR = 5%	
		BMD	BMDL					BMD	BMDL
Dichotomous Hill	Restricted	126.62	19.59	NA	374.75	Questionable	BMD/BMDL ratio > 3 d.f.=0, saturated model (Goodness of fit test cannot be calculated)	79.29	7.58
Gamma	Restricted	121.73	68.52	0.9441	372.76	Viable – Alternate		66.95	33.36
Log-Logistic	Restricted	122.39	58.96	0.9572	372.75	Viable – Alternate		69.06	27.93
Multistage Degree 3	Restricted	118.39	68.47	0.9930	370.77	Viable – Alternate		60.57	33.33
Multistage Degree 2	Restricted	118.39	68.47	0.9930	370.77	Viable – Alternate		60.57	33.33
Multistage Degree 1	Restricted	104.19	68.30	0.9746	370.80	Viable – Alternate		50.72	33.25
Weibull	Restricted	121.20	68.51	0.9372	372.76	Viable – Alternate		65.82	33.35
Logistic	Unrestricted	128.86	97.30	0.9836	370.78	Viable – Alternate		68.24	51.73
Log-Probit	Unrestricted	125.23	14.42	0.9911	372.75	Viable – Recommended	Lowest BMDL BMD/BMDL ratio > 3	76.52	2.40
Probit	Unrestricted	125.62	93.71	0.9883	370.77	Viable – Alternate		65.79	49.29
Quantal Linear	Unrestricted	104.19	68.30	0.9746	370.80	Viable – Alternate		50.72	33.25
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model is bolded; residuals for doses 0, 15, 152 and 307 were -0.0075, 0.0082, -0.0013 and 0.0007, respectively.									
^b Restrictions defined in the BMDS 3.3 User Guide									

Model Summary with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



**Male F344 Relative Liver Weight vs mg/kg-day; LogProbit model
with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence
Limit for the BMDL**



Results for Selected Model - LogProbit (Unrestricted) - Extra Risk, BMR = 0.1

User Input

Info		Options		Model Data	
Model	Log-Probit	Risk Type	Extra Risk	Dependent Variable	mg/kg-day
Dataset Name	Sinusoid Ectasia -	BMR	0.1	Independent Variable	Incidence
Formula	$P[\text{dose}] = g + (1-g)$	Confidence Level	0.95	Total # of Observation	4
		Background	Estimated		

Model Results

Benchmark Dose	
BMD	125.23
BMDL	14.42
BMDU	247.62
AIC	372.75
P-value	0.99
D.O.F.	1.00
Chi ²	0.00

Model Parameters	
# of Parameters	
Variable	Estimate
g	0.197861854
a	-4.843490179
b	0.73743948

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.197861854	16.02681018	16	81	-0.0075
15	0.199634872	15.97078978	16	80	0.0082
152	0.300068561	24.00548484	24	80	-0.0013
307	0.412461541	32.99692324	33	80	0.0007

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	Full Model	-183.3755714	4	-	-
Fitted Model	Fitted Model	-183.3756339	3	0.00012493	1
Reduced Model	Reduced Model	-189.5008934	1	12.2506439	3

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

For DINP, all data considered for PODs are obtained from oral animal toxicity studies in rats, mice, or beagles. Because toxicity values for DINP are from oral animal studies, EPA must use an extrapolation method to estimate HEDs. The preferred method would be to use chemical-specific information for such an extrapolation. EPA identified one study reporting a physiologically based pharmacokinetic model for DINP based on humanized liver mice ([Miura et al., 2018](#)). Since the study made use of genetically modified animals and has not been validated by the Agency, it was not considered fit-for-purpose or used to calculate HEDs. EPA did not locate other DINP information to conduct a chemical-specific quantitative extrapolation. In the absence of such data, EPA 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 F-1.

Equation_Apx F-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 DINP risk evaluation ([U.S. EPA, 2011a](#)). EPA 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 UF (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 F-1, EPA adjusts the POD to obtain the HED using Equation_Apx F-2:

Equation_Apx F-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 the risk evaluation of DINP, 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, 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, as follows:

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

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

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 /hr) = 0.6125
ED_C	=	Exposure duration for a continuous exposure (hr/day) = 24

Based on information from U.S. EPA (2011a), EPA assumes an at rest breathing rate of 0.6125 m³/hr. 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/m³ 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 F-4 presents the conversion of the HEC from mg/m³ to ppm.

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

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

Where:

24.45	=	Molar volume of a gas at standard temperature and pressure (L/mol), default
MW	=	Molecular weight of the chemical (MW of DINP = 418.62 g/mol)

F.1 DINP Non-cancer HED and HEC Calculations for Acute and Intermediate Duration Exposures

The acute and intermediate duration non-cancer POD is based on a BMDL₅ of 49 mg/kg-day, and the critical effect is decreased fetal testicular testosterone. The BMDL₅ was derived by NASEM (2017) through meta-regression and BMD modeling of fetal testicular testosterone data from two studies of DINP with rats (Boberg et al., 2011; Hannas et al., 2011). R code supporting NASEM's meta-regression and BMD analysis of DINP is publicly available through [GitHub](#). This non-cancer POD is considered protective of effects observed following acute and intermediate duration exposures to DINP. EPA used Equation_Apx F-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 F-2:

$$11.6 \frac{mg}{kg - day} = 49 \frac{mg}{kg - day} \times 0.236$$

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

$$63.0 \frac{mg}{m^3} = 11.6 \frac{mg}{kg - day} \times \left(\frac{80 kg}{0.6125 \frac{m^3}{hr} * 24 hr} \right)$$

Equation_Apx F-4 was used to convert the HEC from mg/m³ to ppm:

$$3.68 ppm = 63.0 \frac{mg}{m^3} \times \frac{24.45}{418.62}$$

F.2 DINP Non-cancer HED and HEC Calculations for Chronic Exposures

The chronic duration non-cancer POD is based on a NOAEL of 15 mg/kg-day, and the critical effect is liver toxicity (*i.e.*, increased relative liver weight, increased serum chemistry (AST, ALT, ALP), histopathologic findings (*e.g.*, focal necrosis, spongiosis hepatitis) in F344 rats following 2 years of dietary exposure to DINP ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)). EPA used Equation_Apx F-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 F-2:

$$3.54 \frac{mg}{kg - day} = 15 \frac{mg}{kg - day} \times 0.236$$

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

$$19.3 \frac{mg}{m^3} = 3.54 \frac{mg}{kg - day} \times \left(\frac{80 kg}{0.6125 \frac{m^3}{hr} * 24 hr} \right)$$

Equation_Apx F-4 was used to convert the HEC from mg/m³ to ppm:

$$1.13 ppm = 19.3 \frac{mg}{m^3} \times \frac{24.45}{418.62}$$

Appendix G CONSIDERATIONS FOR BENCHMARK RESPONSE (BMR) SELECTION FOR REDUCED FETAL TESTICULAR TESTOSTERONE

G.1 Purpose

EPA has conducted an updated meta-analysis and benchmark dose modeling (BMD) analysis of decreased fetal rat testicular testosterone. During the July 2024 SACC peer review meeting of the Draft *Risk Evaluation of Diisodecyl Phthalate (DIDP)* and Draft *Human Health Hazard Assessment for Diisononyl Phthalate (DINP)*, the SACC recommended that EPA should clearly state its rationale for selection of benchmark response (BMR) levels evaluated for decreases in fetal testicular testosterone relevant to the single chemical assessments ([U.S. EPA, 2024g](#)). This appendix describes EPA's rationale for evaluating BMRs of 5, 10, and 40 percent for decreases in fetal testicular testosterone. (*Note: EPA will assess the relevant BMR for deriving relative potency factors to be used in the draft cumulative risk assessment separately from this analysis.*)

G.2 Methods

As described in EPA's *Benchmark Dose Technical Guidance* ([U.S. EPA, 2012](#)), "Selecting a BMR(s) involves making judgments about the statistical and biological characteristics of the dataset and about the applications for which the resulting BMDs/BMDLs will be used." For the updated meta-analysis and BMD modeling analysis of fetal rat testicular testosterone, EPA evaluated BMR values of 5, 10, and 40 percent based on both statistical and biological considerations.

In 2017, NASEM ([2017](#)) modeled BMRs of 5 and 40 percent for decreases in fetal testicular testosterone. NASEM did not provide explicit justification for selection of a BMR of 5 percent. However, justification for the BMR of 5 can be found elsewhere. As discussed in EPA's *Benchmark Dose Technical Guidance* ([U.S. EPA, 2012](#)), a BMR of 5 percent is supported in most developmental and reproductive studies. Comparative analyses of a large database of developmental toxicity studies demonstrated that developmental NOAELs are approximately equal to the BMDL₅ ([Allen et al., 1994a, b](#); [Faustman et al., 1994](#)).

EPA also evaluated a BMR of 10 percent as part of the updated BMD analysis. BMD modeling of fetal testosterone conducted by NASEM ([2017](#)) indicated that BMD₅ estimates are below the lowest dose with empirical testosterone data for several of the phthalates (*e.g.*, DIBP). As discussed in EPA's *Benchmark Dose Technical Guidance* ([U.S. EPA, 2012](#)) "For some datasets the observations may correspond to response levels far in excess of a selected BMR and extrapolation sufficiently below the observable range may be too uncertain to reliably estimate BMDs/BMDLs for the selected BMR." Therefore, EPA modeled a BMR of 10 percent because data sets for some of the phthalates may not include sufficiently low doses to support modeling of a 5 percent response level.

NASEM ([2017](#)) also modeled a BMR of 40 percent using the following justification: "previous studies have shown that reproductive-tract malformations were seen in male rats when fetal testosterone production was reduced by about 40% ([Gray et al., 2016](#); [Howdeshell et al., 2015](#))."

Further description of methods and results for the updated meta-analysis and BMD modeling analysis that evaluated BMRs of 5, 10, and 40 percent for decreased fetal testicular testosterone are provided in EPA's *Draft Meta-analysis and Benchmark Dose Modeling of Fetal Testicular Testosterone for Di(2-*

ethylhexyl) Phthalate (DEHP), Dibutyl Phthalate (DBP), Butyl Benzyl Phthalate (BBP), Diisobutyl Phthalate (DIBP), and Dicyclohexyl Phthalate (DCHP) ([U.S. EPA, 2024a](#)).

G.3 Results

BMD estimates, as well as 95 percent upper and lower confidence limits, for decreased fetal testicular testosterone for the evaluated BMRs of 5, 10, and 40 percent are shown in Table_Apx G-1. BMD₅ estimates ranged from 8.4 to 74 mg/kg-day for DEHP, DBP, DCHP, and DINP; however, a BMD₅ estimate could not be derived for BBP or DIBP. Similarly, BMD₁₀ estimates ranged from 17 to 152 for DEHP, DBP, DCHP, DIBP and DINP; however, a BMD₁₀ estimate could not be derived for BBP. BMD₄₀ estimates were derived for all phthalates (*i.e.*, DEHP, DBP, DCHP, DIBP, BBP, and DINP) and ranged from 90 to 699 mg/kg-day.

In the mode of action (MOA) for phthalate syndrome, which is described elsewhere ([U.S. EPA, 2023a](#)) and in Section 3.1.2 of this document, decreased fetal testicular testosterone is an early, upstream event in the MOA that precedes downstream apical outcomes such as male nipple retention, decrease anogenital distance, and reproductive tract malformations. Decreased fetal testicular testosterone should occur at lower or equal doses than downstream apical outcomes associated with a disruption of androgen action. Because the lower 95 percent confidence limit on the BMD, or BMDL, is used for deriving a point of departure (POD), EPA compared BMDL estimates at the 5, 10, and 40 percent response levels for each phthalate (DEHP, DBP, DCHP, DIBP, BBP, DINP) to the lowest identified apical outcomes associated with phthalate syndrome to determine which response level is protective of downstream apical outcomes.

Table_Apx G-1 provides a comparison of BMD and BMDL estimates for decreased fetal testicular testosterone at BMRs of 5, 10, and 40 percent, the lowest LOAEL(s) for apical outcomes associated with phthalate syndrome, and the POD selected for each phthalate for use in risk characterization. As can be seen from Table_Apx G-1, BMDL₄₀ values for DEHP, DBP, DIBP, BBP, DCHP, and DINP are all well above the PODs selected for use in risk characterization for each phthalate by 3× (for BBP) to 25.4× (for DEHP). Further, BMDL₄₀ values for DEHP, DBP, DIBP, BBP, and DCHP, but not DINP, are above the lowest LOAELs identified for apical outcomes on the developing male reproductive system. *These results clearly demonstrate that a BMR of 40 percent is not appropriate for use in human health risk assessment.*

As can be seen from Table_Apx G-1, BMDL₁₀ values for DBP (BMDL₁₀, POD, LOAEL = 20, 9, 30 mg/kg-day, respectively) and DCHP (BMDL₁₀, POD, LOAEL = 12, 10, 20 mg/kg-day, respectively) are slightly higher than the PODs selected for use in risk characterization and slightly less than the lowest LOAELs identified based on apical outcomes associated with the developing male reproductive system. This indicates that a BMR of 10 percent may be protective of apical outcomes evaluated in available studies for both DBP and DCHP. BMDL₁₀ values could not be derived for DIBP or BBP (Table_Apx G-1). Therefore, no comparisons to the POD or lowest LOAEL for apical outcomes could be made for either of these phthalates at the 10 percent response level.

For DEHP, the BMDL₁₀ is greater than the POD selected for use in risk characterization by 5X (BMDL₁₀ and POD = 24 and 4.8 mg/kg-day, respectively) and is greater than the lowest LOAEL identified for apical outcomes on the developing male reproductive system by 2.4X (BMDL₁₀ and LOAEL = 24 and 10 mg/kg-day, respectively). *This indicates that a BMR of 10 percent for decreased fetal testicular testosterone is not health protective for DEHP.* For DEHP, the BMDL₅ (11 mg/kg-day) is similar to the selected POD (NOAEL of 4.8 mg/kg-day) and the lowest LOAEL identified for apical outcomes on the developing male reproductive system (10 mg/kg-day).

G.4 Weight of Scientific Evidence Conclusion

As discussed elsewhere ([U.S. EPA, 2023a](#)), DEHP, DBP, BBP, DIBP, DCHP, and DINP are toxicologically similar and induce effects on the developing male reproductive system consistent with a disruption of androgen action. Because these phthalates are toxicologically similar, it is more appropriate to select a single BMR for decreased fetal testicular testosterone to provide a consistent basis for dose response analysis and for deriving PODs relevant to the single chemical assessments. *EPA has reached the conclusion that a BMR of 5 percent is the most appropriate and health protective response level for evaluating decreased fetal testicular testosterone* when sufficient dose-response data are available to support modeling of fetal testicular testosterone in the low-end range of the dose-response curve. This conclusion is supported by the following weight of scientific evidence considerations.

- For DEHP, the BMDL₁₀ estimate is greater than the POD selected for use in risk characterization by 5× and is greater than the lowest LOAEL identified for apical outcomes on the developing male reproductive system by 2.4×. *This indicates that a BMR of 10 percent is not protective for DEHP.*
- The BMDL₅ estimate for DEHP is similar to the selected POD and lowest LOAEL for apical outcomes on the developing male reproductive system.
- BMDL₁₀ estimates for DBP (BMDL₁₀, POD, LOAEL = 20, 9, 30 mg/kg-day, respectively) and DCHP (BMDL₁₀, POD, LOAEL = 12, 10, 20 mg/kg-day, respectively) are slightly higher than the PODs selected for use in risk characterization and slightly less than the lowest LOAELs identified based on apical outcomes associated with the developing male reproductive system. This indicates that a BMR of 10 percent may be protective of apical outcomes evaluated in available studies for both DBP and DCHP. However, this may be a reflection of the larger database of studies and wider range of endpoints evaluated for DEHP, compared to DBP and DCHP.
- NASEM ([2017](#)) modeled a BMR of 40 percent using the following justification: “previous studies have shown that reproductive-tract malformations were seen in male rats when fetal testosterone production was reduced by about 40% ([Gray et al., 2016](#); [Howdeshell et al., 2015](#)).” However, publications supporting a 40 percent response level are relatively narrow in scope and assessed the link between reduced fetal testicular testosterone in SD rats on GD18 and later life reproductive tract malformations in F1 males. More specifically, Howdeshell et al. ([2015](#)) found reproductive tract malformations in 17 to 100 percent of F1 males when fetal testosterone on GD18 was reduced by approximately 25 to 72 percent, while Gray et al. ([2016](#)) found dose-related reproductive alterations in F1 males treated with dipentyl phthalate (a phthalate not currently being evaluated under TSCA) when fetal testosterone was reduced by about 45 percent on GD18. Although NASEM modeled a BMR of 40 percent based on biological considerations, there is no scientific consensus on the biologically significant response level and no other authoritative or regulatory agencies have endorsed the 40 percent response level as biologically significant for reductions in fetal testosterone.
- BMDL₄₀ values for DEHP, DBP, DIBP, BBP, DCHP, and DINP are above the PODs selected for use in risk characterization for each phthalate by 3× to 25.4× (Table_Apx G-1). BMDL₄₀ values for DEHP, DBP, DIBP, BBP, and DCHP, but not DINP, are above the lowest LOAELs identified for apical outcomes on the developing male reproductive system. These results clearly demonstrate that a BMR of 40 percent is not health protective.

Table_Apx G-1. Comparison of BMD/BMDL Values across BMRs of 5%, 10%, and 40% with PODs and LOAELs for Apical Outcomes for DEHP, DBP, DIBP, BBP, DCHP, and DINP

Phthalate	POD (mg/kg-day) Selected for use in Risk Characterization (Effect)	Lowest LOAEL(s) (mg/kg-day) for Apical Effects on the Male Reproductive System	BMD ₅ Estimate ^a (mg/kg-day) [95% CI]	BMD ₁₀ Estimate ^a (mg/kg-day) [95% CI]	BMD ₄₀ Estimate ^a (mg/kg-day) [95% CI]	Reference For Further Details on the Selected POD and Lowest Identified LOAEL
DEHP	NOAEL = 4.8 (↑ male RTM in F1 and F2 males)	10 to 15 (NR, ↓ AGD, RTMs)	17 [11, 31]	35 [24, 63]	178 [122, 284]	(U.S. EPA, 2024e)
DBP	BMDL ₅ = 9 (↓ fetal testicular testosterone)	30 (↑ Testicular Pathology)	14 [9, 27]	29 [20, 54]	149 [101, 247]	(U.S. EPA, 2024c)
DIBP	BMDL ₅ = 24 (↓ fetal testicular testosterone)	125 (↑ Testicular Pathology)	— ^b	55 [NA, 266] ^b	279 [136, 517]	(U.S. EPA, 2024f)
BBP	NOAEL = 50 (phthalate syndrome-related effects)	100 (↓ AGD)	— ^b	— ^b	284 [150, 481]	(U.S. EPA, 2024b)
DCHP	NOAEL = 10 (phthalate syndrome-related effects)	20 (↑ Testicular Pathology)	8.4 [6.0, 14]	17 [12, 29]	90 [63, 151]	(U.S. EPA, 2024d)
DINP	BMDL ₅ = 49 (↓ fetal testicular testosterone)	600 (↓ sperm motility)	74 [47, 158]	152 [97, 278]	699 [539, 858]	(U.S. EPA, 2025e)
AGD = anogenital distance; BMD = benchmark dose; BMDL = lower 95% confidence limit on BMD; CI = 95% confidence interval; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; RTM = reproductive tract malformations ^a The linear-quadratic model provided the best fit (based on lowest AIC) for DEHP, DBP, DIBP, BBP, DCHP, and DINP. ^b BMD and/or BMDL estimate could not be derived.						

Appendix H UPDATED META-ANALYSIS AND BMD MODELING OF FETAL TESTICULAR TESTOSTERONE

H.1 Purpose

EPA has conducted an updated meta-analysis and benchmark dose modeling (BMD) analysis of decreased fetal rat testicular testosterone. During the July 2024 Science Advisory Committee on Chemicals (SACC) peer-review meeting of the draft risk evaluation of diisodecyl phthalate (DIDP) and draft human health hazard assessments for diisononyl phthalate (DINP), the SACC recommended that EPA should conduct a new BMD modeling analysis that should consider new experimental studies ([U.S. EPA, 2024g](#)). This appendix describes EPA's updated meta-analysis and BMD modeling analysis of fetal testicular testosterone for DINP.

H.2 Methods

In 2017, NASEM demonstrated the utility of meta-analysis and meta-regression to summarize several outcomes from experimental animal studies ([NASEM, 2017](#)). The 2017 NASEM analysis included reduced fetal testicular testosterone, reduced male anogenital distance (AGD), and increased incidence of hypospadias in rodents following oral exposure to DEHP, DBP, BBP, DIBP, and DINP. Boxes 3-3 and 3-4 in ([NASEM, 2017](#)) provide detailed descriptions of the meta-analysis approach employed by NASEM. Briefly, NASEM conducted meta-analyses using the Metafor (Version 2.0.0) meta-analysis package for R (<https://wviechtb.github.io/metafor/index.html>), which employs a standard random effects model using the Restricted Maximum Likelihood Estimate. The meta-analyses conducted by NASEM focused on the dose-response relationship and employed three models, including the linear, log-linear, and linear-quadratic models. For the linear and linear-quadratic models, BMD values were estimated based on benchmark response (BMR) levels of 5 and 40 percent (BMR selection rationale is provided in Appendix G). R code used by NASEM to conduct all meta-analyses is publicly available (<https://github.com/wachiuphd/NASEM-2017-Endocrine-Low-Dose>).

As part of its updated analysis, EPA used a similar meta-analysis and BMD modeling approach as employed by NASEM ([2017](#)), with several notable differences. First, EPA used the most recent version of the R Metafor package (Version 4.6.0) available at the time of the updated analysis, while NASEM used Metafor Version 2.0.0. However, EPA also conducted the updated analysis with Metafor Version 2.0.0 so that results from the two different versions of Metafor could be compared. Similar to the NASEM approach, EPA's updated meta-analysis focused on the dose-response relationship and employed the linear, log-linear, and linear-quadratic models. Another notable difference between the NASEM analysis and EPA's updated analysis is that EPA evaluated BMRs of 5, 10, and 40 percent, while NASEM evaluated BMRs of 5 and 40 percent (BMR selection rationale is provided in Appendix G). As part of the updated meta-analysis, EPA utilized all of the same fetal rat testicular testosterone data included in the original NASEM ([2017](#)) analysis, as well as new fetal rat testosterone data identified through the 2024 TSCA literature update for DINP, as described in the systematic review protocol for DINP ([U.S. EPA, 2025h](#)). EPA also considered new literature identified outside of the 2019 TSCA literature searches that was identified through the literature searches conducted in support of EPA's *Draft Proposed Approach for Cumulative Risk Assessment of High-Priority Phthalates and a Manufacturer-Requested Phthalate under the Toxic Substances Control Act* ([U.S. EPA, 2023a](#)).

Consistent with the meta-analysis and BMD modeling approach employed by NASEM ([2017](#)), new fetal rat testicular testosterone data were included in the updated meta-analysis if the following criteria were met:

- Study conducted with pregnant rats (all strains considered relevant, including Sprague-Dawley, Wistar, Long Evans, F344, etc.). For the updated analysis, studies of mice were excluded, because rats are considered for the more sensitive species.
- Study exposed rats via the oral route.
- Study measured fetal testis testosterone content or *ex vivo* fetal testicular testosterone production. Studies measuring only serum or plasma testosterone not included. Studies measuring testosterone at non-fetal lifestages were excluded. Studies measuring testosterone production following stimulation with luteinizing hormone were excluded.
- Study should include an exposure that covers the male programming window (defined by NASEM as GD16–18).
- Study fully reports data (*i.e.*, mean, standard deviation or standard error, and sample size) to support inclusion in the meta-analysis.

H.3 Results

In 2017, NASEM included fetal rat testicular testosterone data from two studies ([Boberg et al., 2011](#); [Hannas et al., 2011](#)) as part of its meta-analysis and BMD modeling analysis for DINP. Fetal testosterone data from Boberg et al. and Hannas et al. was included as part of EPA's updated analysis. EPA identified new fetal rat testicular testosterone data from two studies ([Gray et al., 2024](#); [Furr et al., 2014](#)), which was included as part of the updated meta-analysis and BMD modeling analysis for DINP. Table_Apx H-1 provides an overview of the four studies included in the updated analysis.

EPA identified testosterone data from six other studies of DINP ([Gray, 2023](#); [Gray et al., 2021](#); [Li et al., 2015](#); [Clewell et al., 2013a](#); [Clewell et al., 2013b](#); [Adamsson et al., 2009](#)). Testosterone data from these studies was not included in the updated analysis for various reasons. Studies by Li et al. (2015) and Adamson et al. (2009), which were previously considered by NASEM (2017), were excluded because Li et al. evaluated testicular testosterone on PND1 (not a fetal lifestage), while Adamson et al. did not sufficiently report data to support its inclusion (*i.e.*, the exact number of litters per dose group was not report, the number of litters were dose group was reported as a range). Studies by Gray et al. (2023; 2021) were not included because these publications re-report fetal testosterone data previously reported by the same research group in publications by Hannas et al. (2011) and Furr et al. (2014), which are both studies included in the updated analysis. Testosterone data from Clewell et al. (2013b) was not included because testicular testosterone was measured in F1 males on PND49 (not a fetal lifestage). Finally, testosterone data from Clewell et al. (2013a) was not included because of data reporting limitations (data reported as percent control with number of litters per dose group reported; however, no measure of variability provided [*i.e.*, standard deviation or standard error]).

EPA conducted the updated meta-analysis using random effects models, as implemented in the R Metafor package. Metafor versions 2.0.0 and 4.6.0 were used so that results could be compared. Additionally, the updated analysis included a sensitivity analysis to determine if the meta-analysis was sensitive to leaving out results from individual studies. In 2017, NASEM did not conduct a sensitivity analysis because there were too few studies available to do so.

Table_Apx H-1. Summary of Studies Included in EPA’s Meta-analysis and BMD Modeling Analysis for DINP

Reference (TSCA Study Quality Rating)	Included in NASEM Meta-analysis and BMD Modeling Analysis?	Brief Study Description	Measured Outcome
(Hannas et al., 2011) (Medium)	Yes	Pregnant SD rats (5–9 dams/group) gavaged with 0, 500, 750, 1,000, 1,500 mg/kg-day DINP on GD14–18	<i>Ex vivo</i> fetal testicular testosterone production (3-hour incubation) on GD18
(Boberg et al., 2011) (Medium)	Yes	Pregnant Wistar rats (9-10 dams/group) gavaged with 0, 300, 600, 750, 900 mg/kg-day DINP on GD7–21.	<i>Ex vivo</i> fetal testicular testosterone production and testes testosterone on GD21
(Furr et al., 2014) (High)	No	Pregnant SD rats (3–5 dams/group) gavaged with 0, 750 mg/kg-day DINP on GD14–18 (Block 1).	<i>Ex vivo</i> fetal testicular testosterone production (3-hour incubation) on GD18
	No	Pregnant SD rats (3–5 dams/group) gavaged with 0, 750 mg/kg-day DINP on GD14–18 (Block 5).	
	No	Pregnant SD rats (3–5 dams/group) gavaged with 0, 750 mg/kg-day DINP on GD14–18 (Block 7).	
(Gray et al., 2024) (Medium)	No	Pregnant SD rats (4 dams/group) gavaged with 0, 750 mg/kg-day DINP on GD14–18 (Block 166).	<i>Ex vivo</i> fetal testicular testosterone production (3-hour incubation) on GD18
	No	Pregnant SD rats (3–4 dams/group) gavaged with 0, 750 mg/kg-day DINP on GD14–18 (Block 167).	

Overall meta-analyses and sensitivity analyses results obtained using Metafor Versions 2.0.0 and 4.6.0 are shown in Table_Apx H-2 and Table_Apx H-3, respectively. A comparison of BMD estimates obtained by NASEM ([2017](#)) and as part of EPA’s updated analysis are shown in Table_Apx H-4. Additional meta-analysis results (*i.e.*, forest plots) and BMD model fit curves are shown in Figure_Apx H-1 through Figure_Apx H-4. For meta-analyses conducted using both versions of Metafor, there was a statistically significant overall effect and linear trends in $\log_{10}(\text{dose})$ and dose, with an overall effect that is large in magnitude ($>50\%$ change). For both meta-analyses, there was substantial, statistically significant heterogeneity in all cases ($I^2 > 40\%$ for Metafor v.2.0.0; $I^2 > 50\%$ for Metafor v.4.6.0). The statistical significance of these effects was robust to leaving out individual studies for analyses conducted with both versions of Metafor. Although there was substantial heterogeneity, standard deviation of the random effect (tau) was less than the estimated size of the effect at higher doses.

Therefore, the heterogeneity does not alter the conclusion that gestational exposure to DINP reduces fetal testicular testosterone in the rat.

For meta-analyses conducted using both versions of Metafor, the linear-quadratic model provided the best fit (*i.e.*, had the lowest AIC) (Table_Apx H-4). BMD estimates from the linear-quadratic model were 79 mg/kg-day [95% CI: 52, 145] for a 5 percent change (BMR = 5%), 160 mg/kg-day [108, 262] for a 10 percent change (BMR = 10%), and 715 mg/kg-day [584, 842] for a 40 percent change (BMR = 40%) when Metafor Version 2.0.0 was used. Similarly, BMD estimates were 74 mg/kg-day [47, 158] for a 5 percent change (BMR = 5%), 152 mg/kg-day [97, 278] for a 10 percent change (BMR = 10%), and 699 mg/kg-day [539, 858] for a 40 percent change (BMR = 40%) when Metafor Version 4.6.0 was used.

Notably, Metafor versions 2.0.0 and 4.6.0 provided similar BMD₅ (79 vs. 74 mg/kg-day), BMD₁₀ (160 versus 152 mg/kg-day), and BMD₄₀ (715 vs. 699 mg/kg-day) estimates for the best fitting, linear-quadratic model (Table_Apx H-4), and these results are similar to those obtained in the 2017 NASEM meta-analysis (*i.e.*, BMD₅ and BMD₄₀ estimates of 76 and 701 mg/kg-day, respectively, based on the best fitting linear quadratic model).

Table_Apx H-2. Updated Overall Meta-Analyses and Sensitivity Analyses of Rat Studies of DINP and Fetal Testosterone (Metafor Version 2.0.0)

Analysis	Estimate	Beta	CI, Lower Bound	CI, Upper Bound	P value	Tau	I ²	P value for Heterogeneity	AICs
Primary Analysis									
Overall	intcpt	-58.82	-73.97	-43.67	2.76E-14	25.23	79.73	1.78E-10	162.76
Trend in log10(dose)	log10(dose)	-124.31	-186.04	-62.59	7.91E-05	14.10	53.75	3.50E-03	148.19
Linear in dose100	dose100	-7.37	-8.49	-6.26	1.21E-38	11.37	44.83	2.33E-02	150.63
Linear Quadratic in dose100	dose100	-6.45	-9.98	-2.92	3.44E-04	11.57	44.90	2.32E-02	145.92*
Linear Quadratic in dose100	I(dose100^2)	-0.10	-0.44	0.25	5.87E-01	11.57	44.90	2.32E-02	145.92
Sensitivity analysis									
Overall minus Boberg et al. 2011	intcpt	-62.16	-80.41	-43.90	2.50E-11	25.21	85.62	8.04E-10	82.20
Overall minus Hannas et al. 2011b	intcpt	-49.60	-63.79	-35.41	7.35E-12	17.31	57.55	3.22E-03	121.50
Overall minus Furr et al. 2014	intcpt	-62.58	-81.06	-44.11	3.15E-11	27.43	79.60	3.95E-09	135.63
Overall minus Gray et al. 2024	intcpt	-59.12	-76.62	-41.61	3.62E-11	27.72	82.87	4.19E-11	145.49
* Indicates lowest AIC.									

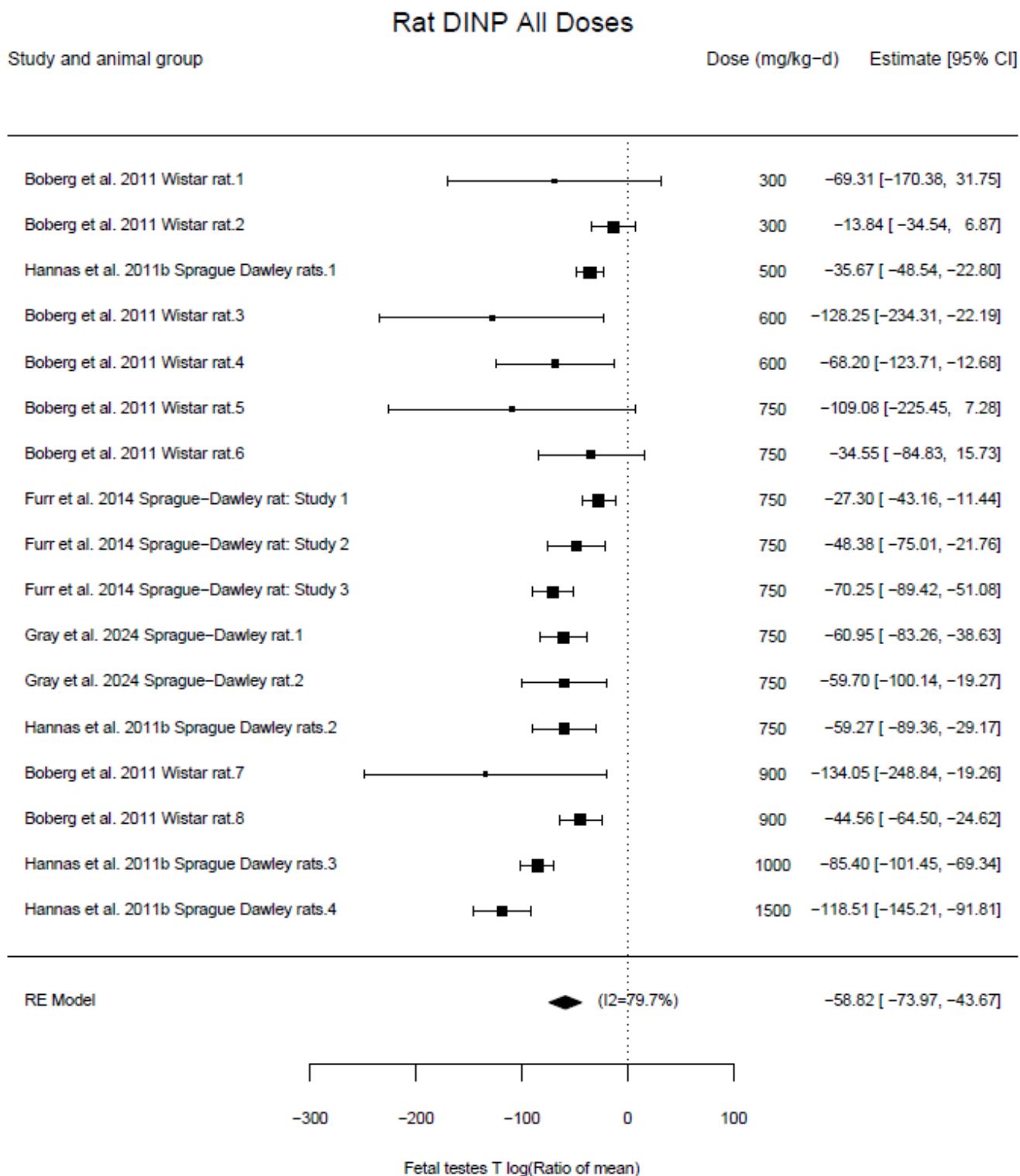
Table_Apx H-3. Updated Overall Meta-Analyses and Sensitivity Analyses of Rat Studies of DINP and Fetal Testosterone (Metafor Version 4.6.0)

Analysis	Estimate	Beta	CI, Lower Bound	CI, Upper Bound	P value	Tau	I ²	P value for Heterogeneity	AICs
Primary analysis									
Overall	intrcpt	-58.82	-73.97	-43.67	2.76E-14	25.23	79.73	1.78E-10	162.76
Trend in log10(dose)	log10(dose)	-124.31	-186.04	-62.59	7.91E-05	14.10	53.75	3.50E-03	148.19
Linear in dose100	dose100	-7.72	-9.73	-5.71	5.36E-14	27.81	82.93	1.54E-14	157.08
Linear Quadratic in dose100	dose100	-6.83	-11.16	-2.51	1.97E-03	16.65	62.81	3.49E-04	146.87*
Linear Quadratic in dose100	I(dose100^2)	-0.07	-0.49	0.36	7.56E-01	16.65	62.81	3.49E-04	146.87
Sensitivity analysis									
Overall minus Boberg et al. 2011	intrcpt	-62.16	-80.41	-43.90	2.50E-11	25.21	85.62	8.04E-10	82.20
Overall minus Hannas et al. 2011b	intrcpt	-49.60	-63.79	-35.41	7.35E-12	17.31	57.55	3.22E-03	121.50
Overall minus Furr et al. 2014	intrcpt	-62.58	-81.06	-44.11	3.15E-11	27.43	79.60	3.95E-09	135.63
Overall minus Gray et al. 2024	intrcpt	-59.12	-76.62	-41.61	3.62E-11	27.72	82.87	4.19E-11	145.49
* Indicates lowest AIC.									

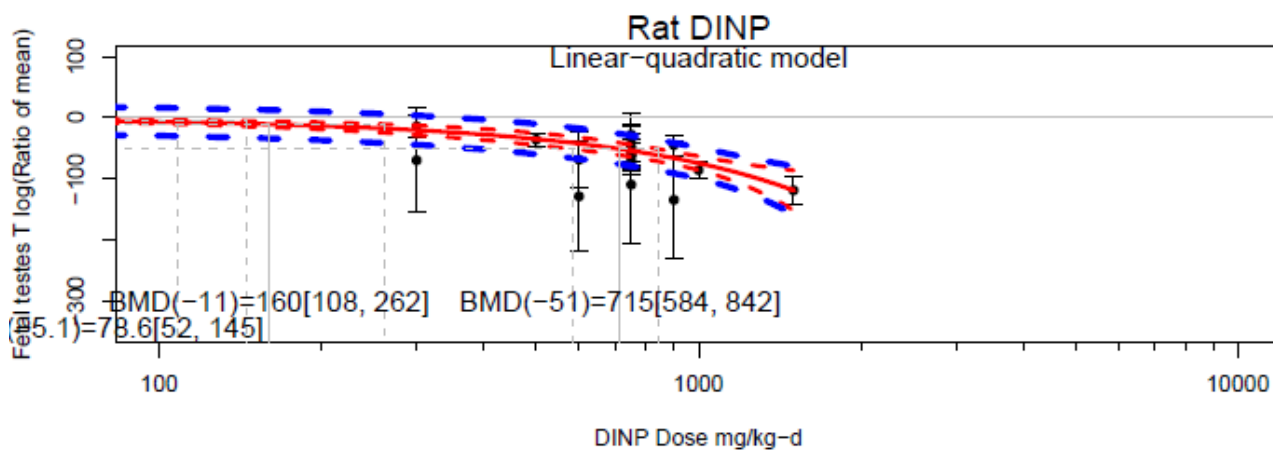
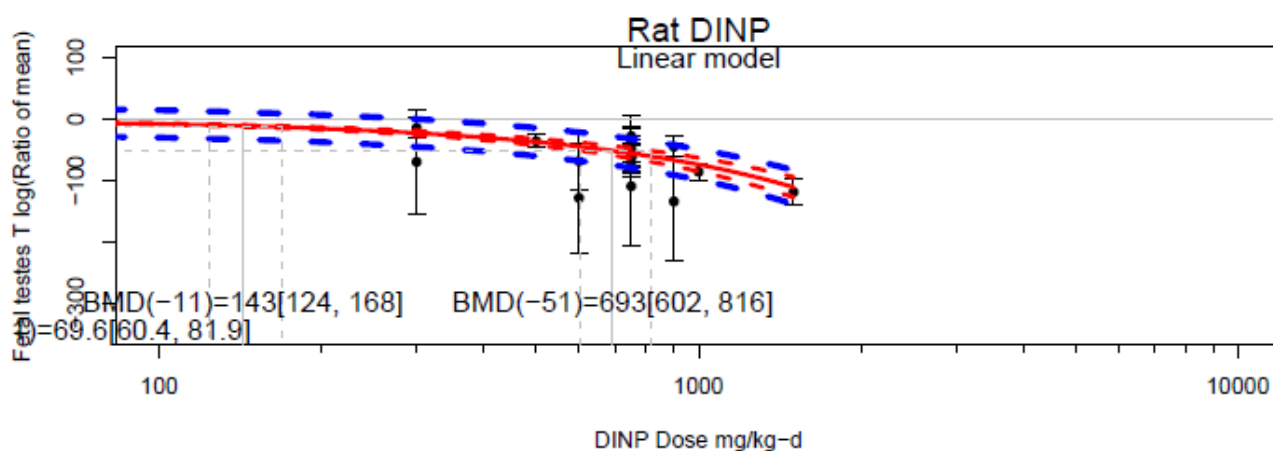
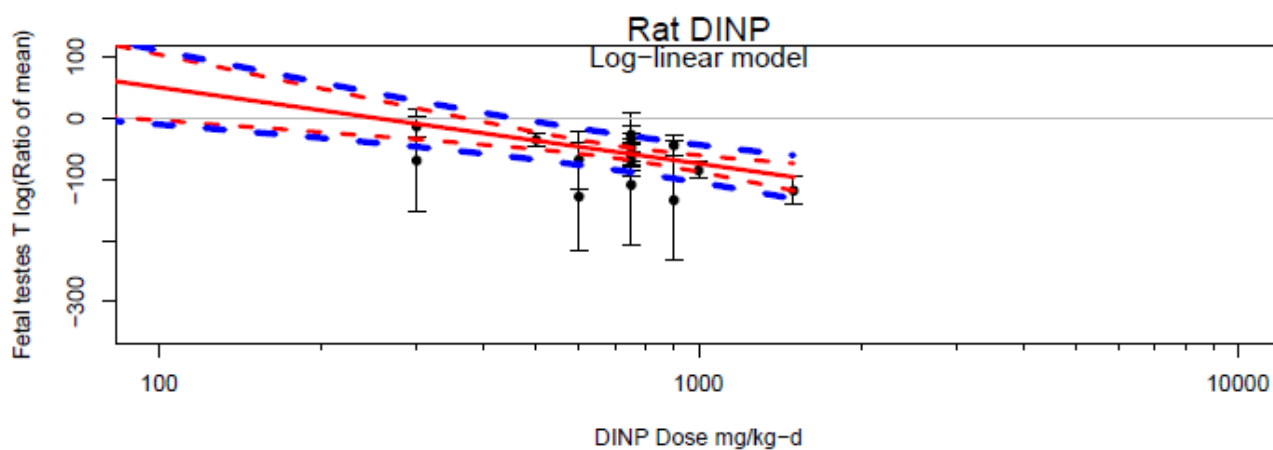
Table_Apx H-4. Comparison of Benchmark Dose Estimates for DINP and Fetal Testosterone in Rats

Analysis	BMR	BMD	CI, Lower Bound	CI, Upper Bound
2017 NASEM analysis using Metafor Version 2.0.0 (as reported in Table C6-16 of NASEM, 2017)				
Linear in dose100	5%	68	59	80
Linear in dose100	40%	676	588	795
Linear Quadratic in dose100*	5%	76	49	145
Linear Quadratic in dose100*	40%	701	552	847
Updated analysis using Metafor Version 2.0.0 including study by Furr et al. (2014) & Gray et al. (2024)				
Linear in dose100	5%	70	60	82
Linear in dose100	10%	143	124	168
Linear in dose100	40%	693	602	816
Linear Quadratic in dose100*	5%	79	52	145
Linear Quadratic in dose100*	10%	160	108	262
Linear Quadratic in dose100*	40%	715	584	842

Analysis	BMR	BMD	CI, Lower Bound	CI, Upper Bound
Updated Analysis using Metafor Version 4.6.0 including study by Furr et al. (2014) & Gray et al. (2024)				
Linear in dose100	5%	66	53	90
Linear in dose100	10%	136	108	185
Linear in dose100	40%	662	525	895
Linear Quadratic in dose100*	5%	74	47	158
Linear Quadratic in dose100*	10%	152	97	278
Linear Quadratic in dose100*	40%	699	539	858
* Indicates model with lowest AIC.				



Figure_Apx H-1. Meta-analysis of Studies of DINP and Fetal Testosterone in Rats (Metafor Version 2.0.0)



Figure_Apx H-2. Benchmark Dose Estimates from Rat Studies of DINP and Fetal Testosterone (Metafor Version 2.0.0)

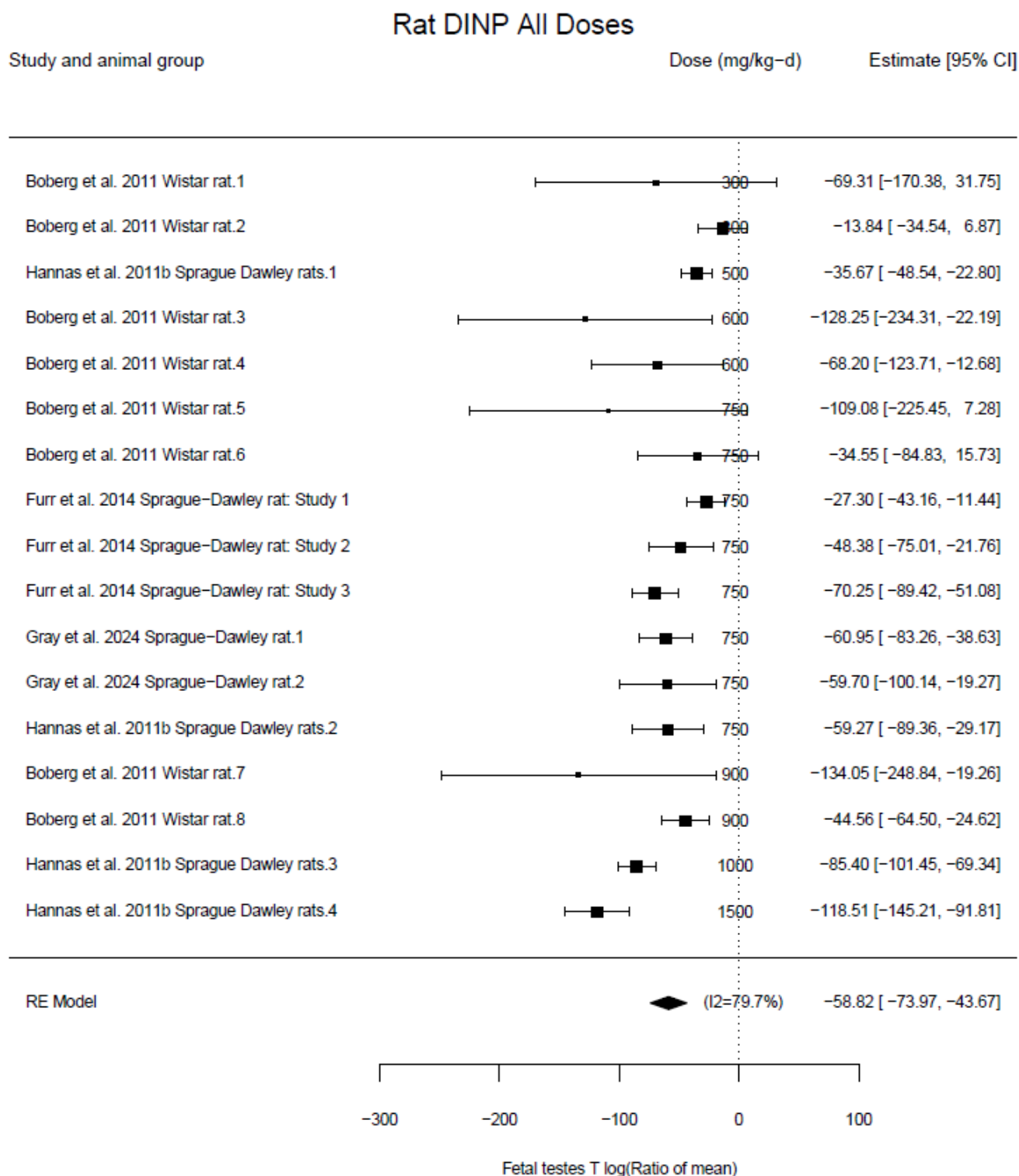
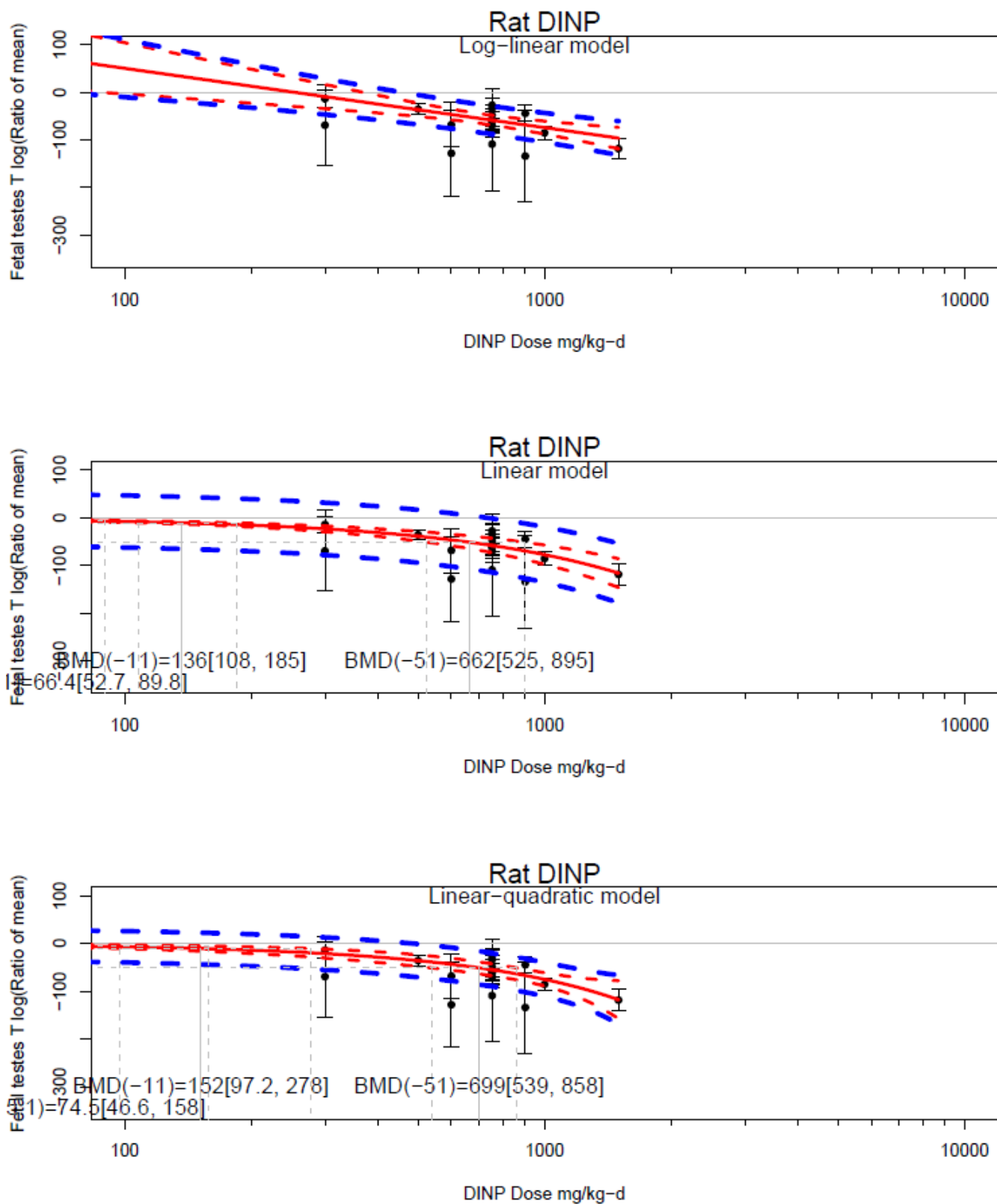


Figure Apx H-3. Meta-analysis of Studies of DINP and Fetal Testosterone in Rats (Metafor Version 4.6.0)



Figure_Apx H-4. Updated Benchmark Dose Estimates from Rat Studies of DINP and Fetal Testosterone (Metafor Version 4.6.0)

Appendix I **CONSIDERATION OF APPLICABILITY OF CHRONIC POD AND BENCHMARK MOE FOR DIFFERENT LIFESTAGES**

Background

As described Section 4.2.2, EPA selected a chronic POD of 3.5 mg/kg-day (based on a NOAEL) from the 2-year dietary study of F344 rats based on liver toxicity ([Lington et al., 1997](#); [Bio/dynamics, 1986](#)) to calculate risk for chronic exposure durations. A total UF of 30 was selected for use as the benchmark MOE (based on an interspecies UF (UF_A) of 3 and an intraspecies UF (UF_H) of 10). Consistent with EPA guidance ([2022](#), [2002b](#), [1993](#)), EPA 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 F).

During the SACC peer-review and the public comment period for the draft DINP hazard assessment, EPA received comments (see [EPA-HQ-OPPT-2018-0096](#) and [EPA-HQ-OPPT-2024-0069](#)) suggesting the following:

- The toxicodynamics portion of the interspecies UF (UF_A) of 3 should be reduced to 1 based on toxicodynamic differences between rats and humans, with rats being more sensitive than humans to liver toxicity associated with PPAR α activation.
- The POD based on chronic liver toxicity is not appropriate for characterizing risk from chronic exposure to infants and children because spongiosis hepatitis is lesion prevalent in aging rats.

EPA addresses these points raised by stakeholders below.

Consideration of the Toxicodynamics Component of the interspecies UF (UF_A)

- EPA considered whether the toxicodynamics component of the UF_A should be reduced from 3 to 1 based on differences in species sensitivity to the liver effects that form the basis of the chronic POD.
- As described in EPA's *Cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP)* ([U.S. EPA, 2025a](#)), the weight of evidence indicates that humans are less sensitive than rodents to liver effects associated with PPAR α activation, which could support a reduction in the toxicodynamics component of the UF_A from 3 to 1.
- However, the chronic POD of 3.5 mg/kg-day is based on a spectrum of liver effects, some of which are related to PPAR α activation (*e.g.*, \uparrow liver weight, hypertrophy, necrosis) and some of which are PPAR α -independent (*i.e.*, spongiosis hepatitis).
- The mode of action underlying spongiosis hepatitis is unknown but is not believed to be related to peroxisome proliferation. Further, as discussed by ECHA ([2013b](#)), spongiosis hepatitis has been observed in the livers of some strains of rats and certain species of fish (*e.g.*, medaka), but is less common in mice, has not been observed in non-human primates or dogs, and with the exception of two case reports, has not been described in humans. These findings raise some uncertainty as to the human relevance of spongiosis hepatitis ([Karbe and Kerlin, 2002](#)).
- Spongiosis hepatitis is considered independent of mononuclear cell leukemia (MNCL). This is based on the Pathology Working Groups re-analysis of liver histopathology data from Lington et al. ([1997](#)) and Covance Labs ([1998c](#)) two-year dietary studies of DINP with F344 rats that demonstrated that MNCL and spongiosis hepatitis co-occurred in rats only about 50% of the time ([EPL, 1999](#)).

- Given that the chronic POD is based on liver effects that are both dependent and independent of PPAR α and the uncertainty in mode of action associated with spongiosis hepatitis, *EPA concluded that a reduction in the toxicodynamics component of the UF_A from 3 to 1 is not warranted.*

Applicability of the Chronic POD to Adults

- The chronic POD of 3.5 mg/kg-day is based on a spectrum of liver effects, some of which are related to PPAR α activation (*e.g.*, \uparrow liver weight, hypertrophy, necrosis) and some of which are considered PPAR α -independent (*i.e.*, spongiosis hepatitis).
- *EPA considers the chronic POD applicable for characterization of risk from exposure to DINP for male and female adult workers, consumers and members of the general population that may be exposed to DINP through TSCA releases.* It is plausible that these populations may be exposed chronically to DINP through work, regular contact with consumer products and/or articles containing DINP, or through TSCA releases of DINP to the environment.

Applicability of the Chronic POD to Infants and Children

- As discussed above, humans are less sensitive than rodents to liver effects associated with PPAR α activation, while spongiosis hepatitis is most common in the livers of aging rats. Given that spongiosis hepatitis is common in aging rats, the applicability of using this lesion to characterizing risk to infants and children is questionable.
- EPA considered whether gestational and/or perinatal exposure to DINP might result in increased incidence of spongiosis hepatitis later in life. Of the gestational/perinatal studies listed in Table 3-1, four studies evaluated liver outcomes in adult SD and Wistar rat following gestational and/or perinatal exposure to DINP ([Gray, 2023](#); [Clewett et al., 2013b](#); [Boberg et al., 2011](#); [Gray et al., 2000](#)). All four studies evaluated liver weight in F1 offspring between approximately 3.5 to 8 months of age; however, none of the available studies evaluated liver histopathology precluding conclusions pertaining to the effect of gestational/perinatal exposure on incidence of spongiosis hepatitis later in life for this study type.
- EPA also considered whether continuous exposure to DINP for two-generations (including gestational/perinatal exposures for F1 and F2 offspring) may increase the incidence of spongiosis hepatitis. In an initial dose-range finding one-generation study of DINP with SD rats, histopathologic examinations were not included ([Waterman et al., 2000](#); [Exxon Biomedical, 1996a](#)). In a subsequent two-generation study of SD rats ([Waterman et al., 2000](#); [Exxon Biomedical, 1996b](#)), liver histopathologic examinations were included for P1 and P2 adults. No significant increase in spongiosis hepatitis was observed in male or female P1 or P2 rats. These findings indicate that gestational/perinatal exposure to DINP may not significantly increase the incidence of spongiosis hepatitis later in life. However, some uncertainty remains, as spongiosis hepatitis was observed in F344 rats after up to two-years of oral exposure in the study by Lington et al. ([1997](#)), while liver histopathology in P1 and P2 SD rats in the two-generation study was examined after exposure to DINP for approximately 15 to 24 weeks of exposure to DINP. Given that spongiosis hepatitis is most prevalent in aging rats, the two-generation study may not have examined liver histopathology in old enough rats to detect this lesion.
- *Given that spongiosis hepatitis is most prevalent in aging rats, use of the chronic POD of 3.5 mg/kg-day to assess risk from chronic exposure to DINP for infants and children may be conservative and may not be relevant.*
- EPA considered whether other candidate intermediate and chronic PODs may be more appropriate for assessing risk to infants and children from chronic exposure to DINP.

- As discussed in Sections 4.1.1 and 4.1.2, EPA selected an acute/intermediate POD of 12 mg/kg-day based on reduced fetal testicular testosterone. Use of this POD to calculate chronic risks from exposure to DINP for infants and children would result in MOEs above the benchmark of 30 for all consumer exposure scenarios discussed in Section 4.3.3 of the risk evaluation of DINP ([U.S. EPA, 2025g](#)).
- Of the candidate PODs listed in Table 4-5, the most sensitive candidate POD most directly applicable to the infant and children lifestages is a LOAEL of 133 mg/kg-day (HED of 31 mg/kg-day) based on reduced F1 and F2 male and female offspring body weight on PND7, 14, and 21 in a two-generation study of SD rats ([Waterman et al., 2000](#); [Exxon Biomedical, 1996b](#)). Given that no NOAEL could be identified, this study supports a benchmark MOE of 300, based on an intraspecies UF (UF_H) of 10, interspecies UF (UF_A) of 3, and a LOAEL-to-NOAEL UF (UF_L) of 10. Given the additional UF_L of 10, this candidate POD and benchmark (HED of 31 and benchmark MOE of 300) would lead to nearly identical risk conclusions for infants and children as were obtained using the current chronic POD based on liver toxicity (HED of 3.5 and benchmark MOE of 30).
- The magnitude of the effect of DINP on offspring body weight was relative small at the LOAEL, with statistically significant decreases of 8.9% for F1 females on PND21 to 10% for F1 males on PND21 and F2 males on PND7 ([Waterman et al., 2000](#); [Exxon Biomedical, 1996b](#)). Given the magnitude of the effect, a full UF_L of 10 for this LOAEL may be over-conservative.
- To refine the LOAEL based on reduced F1 and F2 offspring bodyweight from the two-generation study of reproduction, EPA conducted benchmark dose (BMD) modeling of F1 and F2 male and female offspring body weights on PND7, PND14, and PND21. BMD modeling results are provided in Appendix J. The lowest BMDL₅ derived was 65 mg/kg-day (HED of 15 mg/kg-day) based on reduced F1 male body weight on PND21 (Table_Apx J-1). Consistent with U.S. EPA guidance ([U.S. EPA, 2022](#), [2002b](#), [1993](#)), since the LOAEL was refined to a BMDL₅, the UF_L of 10 was no longer necessary.
- Overall, this analysis supports an HED of 15 mg/kg-day and a total UF of 30, based on an intraspecies UF (UF_H) of 10, and an interspecies UF (UF_A) of 3. This HED is less sensitive than the acute/intermediate POD of 12 mg/kg-day and total UF of 30. Use of this HED to calculate chronic risks from exposure to DINP for infants and children would result in MOEs above the benchmark of 30 for all consumer exposure scenarios discussed in Section 4.3.3 of the risk evaluation of DINP ([U.S. EPA, 2025g](#)).

Appendix J BENCHMARK DOSE MODELING OF PUP BODYWEIGHT DATA FROM WATERMAN ET AL. (2000)

The two-generation study of reproduction of DINP with SD rats by Waterman et al. supports a LOAEL of 133 mg/kg-day based on reduced F1 and F2 male and female offspring body weight on PND7, 14, and 21 ([Waterman et al., 2000](#); [Exxon Biomedical, 1996b](#)). The magnitude of the effect of DINP on offspring body weight was relative small at the LOAEL, with statistically significant decreases of 8.9% for F1 females on PND21 to 10% for F1 males on PND21 and F2 males on PND7 ([Waterman et al., 2000](#); [Exxon Biomedical, 1996b](#)). EPA conducted benchmark dose (BMD) modeling of decreased F1 and F2 male and female bodyweight on PND7, PND14, and PND21 to refine the LOAEL.

The BMD modeling for continuous data was conducted with EPA's BMD software (BMDS 3.3.2). All standard BMDS 3.3.2 continuous 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 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.

Standard BMDS 3.3.2 Models Applied to Continuous Endpoints:

- Exponential 3-restricted (exp3-r)
- Exponential 5-restricted (exp5-r)
- Hill-restricted (hil-r)
- Polynomial Degree 3-restricted (ply3-r)
- Polynomial Degree 2-restricted (ply2-r)
- Power-restricted (pow-r)
- Linear-unrestricted (lin-ur)

EPA evaluated benchmark response (BMR) levels of 1 control standard deviation (1 SD) and 5% relative deviation. Model fit was judged consistent with EPA's benchmark Dose Technical Guidance ([U.S. EPA, 2012](#)). An adequate fit was judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (*i.e.*, Test 2; $p\text{-value} > 0.05$ [note: this is a change from previous versions of BMDS, which required variance $p\text{-value} > 0.10$ for adequate fit]), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected (*i.e.*, $p\text{-value} < 0.05$), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model did not adequately fit the data (*i.e.*, Test 3; $p\text{-value} < 0.05$), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different adequately fitting models varied >3 -fold; otherwise, the BMDL from the model with the lowest AIC was selected.

If no model adequately fit the data set using the approach described above, EPA removed the highest dose group and modelled the data again using the approach described above.

Table_Apx J-1 summarizes BMD modeling results for reduced F1 and F2 male and female bodyweight on PND7, PND14, and PND21, while more detailed BMD model results for F1 and F2 offspring are provided in Appendices J.1 and J.2, respectively.

Table_Apx J-1. Summary of BMD Model Results for Reduced F1 and F2 Offspring Bodyweight
([Waterman et al., 2000](#))

Data Set	BMR	Best-Fit Model (Variance)	BMD (mg/kg-day)	BMDL (mg/kg-day)	Notes	Appendix Containing Results
F1 Males PND7	5%	—	—	—	No models adequately fit the data set	J.1.1
F1 Males PND14	5%	Linear (Constant)	106	87	Adequate fit with highest dose group removed	J.1.2
F1 Males PND21	5%	Exponential 3	78	65	Adequate fit with highest dose group removed	J.1.3
F1 Females PND7	5%	—	—	—	No models adequately fit the data set	J.1.4
F1 Females PND14	5%	Linear (Constant)	106	87	Adequate fit with highest dose group removed	J.1.5
F1 Females PND21	5%	Exponential 3 (Constant)	83	69	Adequate fit with highest dose group removed	J.1.6
F2 Males PND7	5%	—	—	—	No models adequately fit the data set	J.2.1
F2 Males PND14	5%	—	—	—	No models adequately fit the data set	J.2.2
F2 Males PND21	5%	Exponential 3 (Constant)	118	102		J.2.3
F2 Females PND7	5%	—	—	—	No models adequately fit the data set	J.2.4
F2 Females PND14	5%	Linear (Constant)	104	85	Adequate fit with highest dose group removed	J.2.5
F2 Females PND21	5%	Exponential 3 (Constant)	111	98		J.2.6

J.1 F1 Offspring Bodyweight

J.1.1 F1 Male Offspring Bodyweight on PND7

Table_Apx J-2. F1 Male Offspring Bodyweight on PND7

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	97	17.62	2.35		Table 8 in (Waterman et al., 2000); Table 19 in (Exxon Biomedical, 1996b)
0.2	139	95	16.44	2.85		
0.4	274	90	15.28	3.19	*	
0.8	543	94	15.67	1.74	*	

Table_Apx J-3. BMD Model Results for F1 Male Offspring Bodyweight on PND7 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BM R	BMD	BMDL	P Value	AIC	BMD S Recommends	BMD S Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	749.8948	556.508	0.000257	1799.008897	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose
Exponential 5	Restricted	Constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Hill	Restricted	Constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Polynomial Degree 3	Restricted	Constant	1 SD	754.8555	570.8735	0.0001778	1799.746131	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose
Polynomial Degree 2	Restricted	Constant	1 SD	762.1407	570.6021	0.0001776	1799.748087	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose
Power	Restricted	Constant	1 SD	755.59	570.8596	0.0001778	1799.746106	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose
Linear	Unrestricted	Constant	1 SD	755.5899	570.8596	0.0001778	1799.746106	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose
Exponential 3	Restricted	Constant	5%	231.2154	175.338	0.000257	1799.008897	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Exponential 5	Restricted	Constant	5%	127.1849	61.77582	NA	1787.534509	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	134.7184	109.4018	0.3035716	1785.534556	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 3	Restricted	Constant	5%	245.5176	189.1077	0.0001778	1799.746131	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Polynomial Degree 2	Restricted	Constant	5%	247.7713	189.0559	0.0001776	1799.748087	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Power	Restricted	Constant	5%	245.745	189.102	0.0001778	1799.746106	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Linear	Unrestricted	Constant	5%	245.745	189.0999	0.0001778	1799.746106	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Exponential 3	Restricted	Non-constant	1 SD	1013.702	851.8207	0.0009372	1794.28836	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Exponential 5	Restricted	Non-constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Hill	Restricted	Non-constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Polynomial Degree 3	Restricted	Non-constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Polynomial Degree 2	Restricted	Non-constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Power	Restricted	Non-constant	1 SD	952.6842	705.9678	0.0008987	1794.372126	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Linear	Unrestricted	Non-constant	1 SD	1002.876	708.8889	0.0009355	1794.291951	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Non-constant	5%	302.3829	197.5137	0.0009372	1794.28836	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Exponential 5	Restricted	Non-constant	5%	128.6863	61.77299	0.0226984	1787.534509	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Hill	Restricted	Non-constant	5%	133.935	107.1847	0.0300325	1787.050504	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 3	Restricted	Non-constant	5%	—	—	—	—	Unusable	BMD computation failed
Polynomial Degree 2	Restricted	Non-constant	5%	243.9276	243.2026	<0.0001	1799.962907	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Power	Restricted	Non-constant	5%	272.7174	210.5346	0.0008987	1794.372126	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Linear	Unrestricted	Non-constant	5%	282.7436	211.2587	0.0009355	1794.291951	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable ^a Selected Model (bolded and shaded gray). ^b Restrictions defined in the BMDs 3.3 User Guide .									

Table_Apx J-4. BMD Model Results for F1 Male Offspring Bodyweight on PND7 (Highest Dose Group Removed)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	328.1537	251.186	NA	1387.511903	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	1 SD	332.9178	148.3848	NA	1389.511903	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	330.0178	158.5132	NA	1389.511903	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	326.1608	252.4184	0.99564	1385.511933	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose
Power	Restricted	Constant	1 SD	326.461	252.4097	NA	1387.511906	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	1 SD	327.042	252.3835	0.9839528	1385.512308	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose
Exponential 3	Restricted	Constant	5%	104.6753	76.73575	NA	1387.511903	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	5%	106.1192	51.88479	NA	1389.511903	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	105.3628	48.60752	NA	1389.511903	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	104.1664	81.52328	0.99564	1385.511933	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	5%	104.1108	81.52331	NA	1387.511906	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	5%	103.184	81.54133	0.9839528	1385.512308	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	1 SD	328.1538	251.1877	0.0035502	1387.511903	Questionable	Goodness of fit p-value < 0.1 BMD higher than maximum dose

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 5	Restricted	Non-constant	1 SD	328.4096	251.1132	NA	1389.511903	Questionable	BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	1 SD	280.8265	147.1053	NA	1383.011249	Questionable	BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	1 SD	326.2536	252.4134	0.0142597	1385.511903	Questionable	Goodness of fit p-value < 0.1 BMD higher than maximum dose
Power	Restricted	Non-constant	1 SD	326.445	252.4109	0.0035502	1387.511908	Questionable	Goodness of fit p-value < 0.1 BMD higher than maximum dose
Linear	Unrestricted	Non-constant	1 SD	327.042	252.3792	0.0142568	1385.512308	Questionable	Goodness of fit p-value < 0.1 BMD higher than maximum dose
Exponential 3	Restricted	Non-constant	5%	104.6752	76.73574	0.0035502	1387.511903	Questionable	Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	5%	104.7656	51.88902	NA	1389.511903	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	100.4919	46.75259	NA	1383.011249	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	5%	103.9411	81.52232	0.0142597	1385.511903	Questionable	Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	5%	104.1388	81.52362	0.0035502	1387.511908	Questionable	Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	5%	103.184	81.54133	0.0142568	1385.512308	Questionable	Goodness of fit p-value < 0.1
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded).									
^b Restrictions defined in the BMDs 3.3 User Guide .									

J.1.2 F1 Male Offspring Bodyweight on PND14

Table_Apx J-5. F1 Male Offspring Bodyweight on PND14

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	97	35.01	3.94		Table 8 in (Waterman et al., 2000); Table 19 in (Exxon Biomedical, 1996b)
0.2	139	94	33.28	4.82		
0.4	274	90	30.43	4.36	*	
0.8	543	92	29.66	2.55	*	

Table_Apx J-6. BMD Model Results for F1 Male Offspring Bodyweight on PND14 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMSD Recommends	BMSD Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	385.4421	321.5715	0.002458	2108.61844	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Exponential 5	Restricted	Constant	1 SD	236.4395	146.3635	NA	2100.601621	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	227.8099	158.521	NA	2100.601621	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	398.0597	341.1634	0.0011488	2110.139761	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Polynomial Degree 2	Restricted	Constant	1 SD	464.2469	322.2132	<0.0001	2114.546805	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	1 SD	402.9291	341.0565	0.0011577	2110.124342	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Linear	Unrestricted	Constant	1 SD	403.2053	341.0542	0.0011578	2110.124084	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Exponential 3	Restricted	Constant	5%	158.7313	135.2375	0.002458	2108.61844	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Constant	5%	139.8526	99.88388	NA	2100.601621	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	139.6717	116.9422	NA	2100.601621	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	5%	169.2801	147.7802	0.0011488	2110.139761	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	5%	201.8421	140.6115	<0.0001	2114.546805	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	5%	171.1518	147.8322	0.0011577	2110.124342	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	5%	171.3268	147.8477	0.0011578	2110.124084	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Non-constant	1 SD	513.9093	419.4072	0.064527	2093.256241	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	1 SD	356.4487	256.1837	NA	2091.771992	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	1 SD	388.605	251.3757	NA	2091.771992	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	1 SD	555.656	544.2749	0.0030411	2098.557523	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Polynomial Degree 2	Restricted	Non-constant	1 SD	519.0093	437.047	0.0520345	2093.686597	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	1 SD	518.6872	437.0171	0.0150328	2095.687521	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	1 SD	489.2304	426.7211	0.0361383	2094.415703	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Non-constant	5%	172.1766	147.9772	0.064527	2093.256241	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	5%	178.7382	144.6059	NA	2091.771992	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	174.3258	141.3404	NA	2091.771992	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	5%	237.7356	232.8004	0.0030411	2098.557523	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1

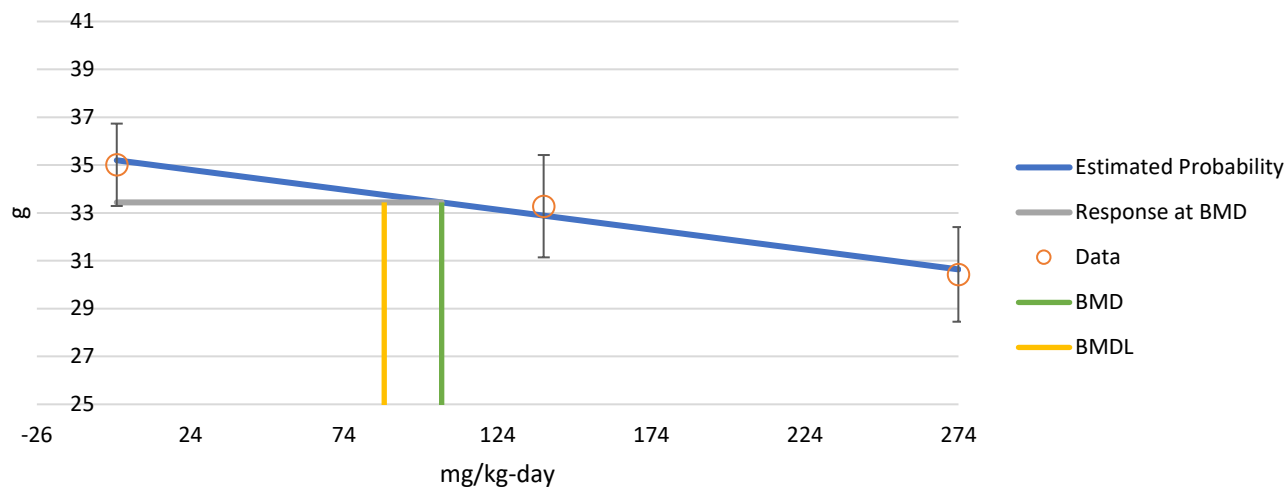
Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
									Residual for Dose Group Near BMD > 2 Residual at control > 2
Polynomial Degree 2	Restricted	Non-constant	5%	183.4337	160.7366	0.0520345	2093.686597	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	5%	183.4279	160.7333	0.0150328	2095.687521	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	5%	177.9682	157.9424	0.0361383	2094.415703	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded).									
^b Restrictions defined in the BMDS 3.3 User Guide .									

Table_Apx J-7. BMD Model Results for F1 Male Offspring Bodyweight on PND14 (Highest Dose Group Removed)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	264.5474	219.5794	NA	1633.027224	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	1 SD	264.5445	219.5679	NA	1635.027224	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	168.7185	149.9951	NA	1633.027224	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	265.6614	221.2077	NA	1633.027546	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Power	Restricted	Constant	1 SD	264.7751	252.1797	NA	1633.027224	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	1 SD	262.5855	211.4703	0.2824621	1632.18243	Viable – Recommended	Lowest AIC
Exponential 3	Restricted	Constant	5%	140.1249	88.30174	NA	1633.027224	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 5	Restricted	Constant	5%	140.1246	85.99834	NA	1635.027224	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	139.1467	124.0619	NA	1633.027224	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	140.7787	90.98064	NA	1633.027546	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Power	Restricted	Constant	5%	140.1461	90.99624	NA	1633.027224	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	5%	105.765	87.06488	0.2824621	1632.18243	Viable – Recommended	Lowest AIC
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable ^a Selected Model (bolded). ^b Restrictions defined in the BMDs 3.3 User Guide .									

Frequentist Linear Model with BMR of 0.05 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



User Input

Info	
Model	frequentist Linear
Model Restriction	Unrestricted
Dataset Name	Waterman-2000_F1-BW-M-PND14_HDD
User notes	[Add user notes here]
Dose-Response Model	$M[\text{dose}] = g + b \cdot \text{dose}$
Variance Model	$\text{Var}(i) = \alpha$

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	g
Total # of Observation	3
Adverse Direction	Downward

Model Results

Benchmark Dose	
BMD	105.7650391
BMDL	87.06487914
BMDU	135.5891999
AIC	1632.18243
Test 4 P-value	0.282462111
D.O.F.	1

Model Parameters				
# of Parameters	3			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	35.198387	0.40748413	34.39973	35.99704
beta	-0.016639897	2.33E-03	-0.02121	-0.01207
alpha	19.09161088	3.08E+01	-41.1776	79.36081

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	97	35.198387	35.01	35.01	4.369395	3.94	3.94	-0.424635
139	94	32.8854413	33.28	33.28	4.369395	4.82	4.82	0.8754962
274	90	30.6390552	30.43	30.43	4.369395	4.36	4.36	-0.453901

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-812.5136122	4	1633.027
A2	-810.5814201	6	1633.163
A3	-812.5136122	4	1633.027
fitted	-813.0912149	3	1632.182
R	-836.4720145	2	1676.944

* Includes additive constant of -258.22173. This constant was not included in the LL derivation prior to BMDs 3.0.

Tests of Interest			
Test	2*Log(Likelihood Ratio)	Test df	p-value
1	51.78118886	4	<0.0001
2	3.864384145	2	0.14483
3	3.864384145	2	0.14483
4	1.155205454	1	0.282462

J.1.3 F1 Male Offspring Bodyweight on PND21

Table_Apx J-8. F1 Male Offspring Bodyweight on PND21

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	96	57.25	6.73		Table 8 in (Waterman et al., 2000); Table 19 in (Exxon Biomedical, 1996b)
0.2	139	94	51.4	8.52	*	
0.4	274	90	47.95	7.94	*	
0.8	543	92	46.52	5.15	*	

Table_Apx J-9. BMD Model Results for F1 Male Offspring Bodyweight on PND21 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	367.0454	306.7887	<0.0001	2545.274942	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Exponential 5	Restricted	Constant	1 SD	177.2542	125.8513	NA	2530.405182	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	173.107	125.1314	NA	2530.405182	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	393.4128	333.2567	<0.0001	2548.05993	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Polynomial Degree 2	Restricted	Constant	1 SD	399.7122	333.1663	<0.0001	2548.089361	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Power	Restricted	Constant	1 SD	392.6991	333.2785	<0.0001	2548.05962	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Linear	Unrestricted	Constant	1 SD	392.6991	333.2779	<0.0001	2548.05962	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Exponential 3	Restricted	Constant	5%	132.7414	113.5851	<0.0001	2545.274942	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 5	Restricted	Constant	5%	70.4488	37.76684	NA	2530.405182	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	82.09051	35.8347	NA	2530.405182	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)

Models ^a	Restriction ^b	Variance	BM _R	BM _D	BM _D L	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Polynomial Degree 3	Restricted	Constant	5%	147.4963	127.677	<0.0001	2548.05993	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Polynomial Degree 2	Restricted	Constant	5%	149.6593	127.6308	<0.0001	2548.089361	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Power	Restricted	Constant	5%	147.2538	127.6716	<0.0001	2548.05962	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Linear	Unrestricted	Constant	5%	147.2538	127.6716	<0.0001	2548.05962	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 3	Restricted	Non-constant	1 SD	477.7192	377.3672	0.004205	2538.203338	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 5	Restricted	Non-constant	1 SD	213.5096	138.4185	NA	2531.26037	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	1 SD	209.6099	138.4402	NA	2531.26037	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	1 SD	503.5426	406.2491	0.0019455	2539.744912	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Polynomial Degree 2	Restricted	Non-constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Power	Restricted	Non-constant	1 SD	497.1371	407.0933	0.001981	2539.708698	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Linear	Unrestricted	Non-constant	1 SD	503.0601	407.0398	0.0019937	2539.695929	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 3	Restricted	Non-constant	5%	146.3514	123.8637	0.004205	2538.203338	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 5	Restricted	Non-constant	5%	74.63699	40.39051	NA	2531.26037	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	83.13899	35.5995	NA	2531.26037	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	5%	162.0372	138.6099	0.0019455	2539.744912	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Polynomial Degree 2	Restricted	Non-constant	5%	—	—	—	—	Unusable	BMD computation failed
Power	Restricted	Non-constant	5%	160.2951	138.658	0.001981	2539.708698	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Linear	Unrestricted	Non-constant	5%	161.2557	138.8609	0.0019937	2539.695929	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded and shaded gray).									
^b Restrictions defined in the BMDS 3.3 User Guide .									

Table_Apx J-10. BMD Model Results for F1 Male Offspring Bodyweight on PND21 (Highest Dose Group Removed)

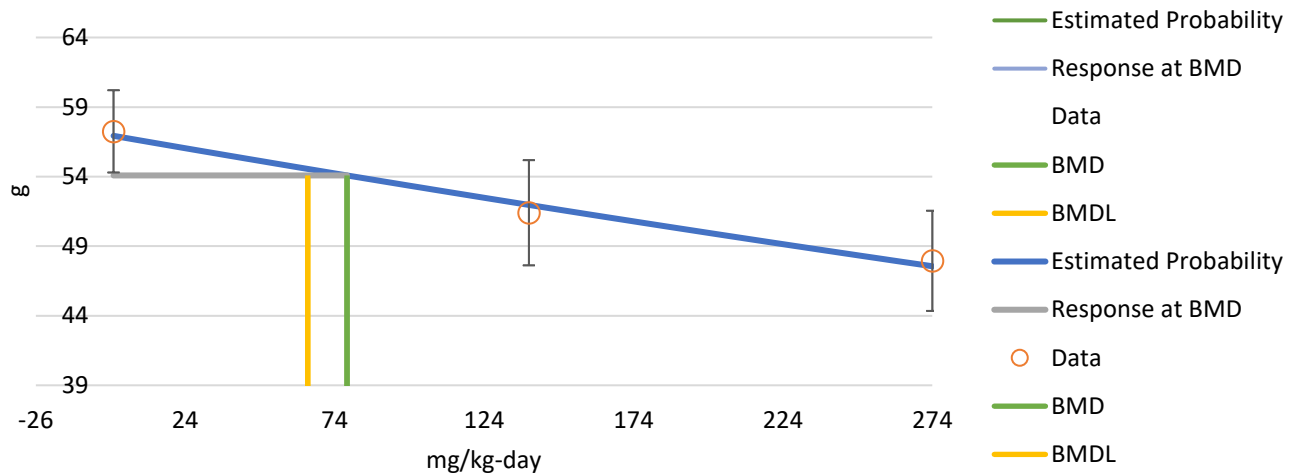
Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	222.0347	179.7111	0.3426081	1945.657114	Viable – Recommended	Lowest AIC
Exponential 5	Restricted	Constant	1 SD	203.5517	138.8042	NA	1948.756466	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	204.7198	138.6643	NA	1946.756466	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	227.9437	187.1874	0.2467275	1946.098206	Viable – Alternate	
Power	Restricted	Constant	1 SD	226.9683	187.1713	0.2469453	1946.096969	Viable – Alternate	
Linear	Unrestricted	Constant	1 SD	226.9683	187.1707	0.2469453	1946.096969	Viable – Alternate	
Exponential 3	Restricted	Constant	5%	78.14108	64.98007	0.3426081	1945.657114	Viable – Recommended	Lowest AIC
Exponential 5	Restricted	Constant	5%	59.6461	34.40916	NA	1948.756466	Questionable	BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	58.25307	28.57678	NA	1946.756466	Questionable	BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	83.8116	70.57765	0.2467275	1946.098206	Viable – Alternate	
Power	Restricted	Constant	5%	83.48213	70.56802	0.2469453	1946.096969	Viable – Alternate	
Linear	Unrestricted	Constant	5%	83.48212	70.56801	0.2469453	1946.096969	Viable – Alternate	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

**Frequentist Exponential Degree 3 Model with BMR of 0.05 Added Risk for the
BMD and 0.95 Lower Confidence Limit for the BMDL**



User Input

Info	
Model	frequentist Exponential degree 3
Model Restriction	Restricted
Dataset Name	Waterman-2000_F1-BW-M-PND2LHDD
User notes	[Add user notes here]
Dose-Response Model	$M(\text{dose}) = a * \exp(\pm 1 * (b * \text{dose})^d)$
Variance Model	Var(i) = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	g
Total # of Observation	3
Adverse Direction	Downward

Model Results

Benchmark Dose	
BMD	78.14108041
BMDL	64.9800676
BMDU	110.6809979
AIC	1945.657114
Test 4 P-value	0.342608058
D.O.F.	1

Model Parameters				
# of Parameters	4			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
a	56.93026098	0.73570973	55.4883	58.37223
b	0.000656419	7.99E-05	0.0005	0.000813
d	Bounded	NA	NA	NA
log-alpha	4.087921065	8.45E-02	3.922396	4.253446

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	96	56.930261	57.25	57.25	7.721129	6.73	6.73	0.4057425
139	94	51.9657463	51.4	51.4	7.721129	8.52	8.52	-0.710403
274	90	47.5588648	47.95	47.95	7.721129	7.94	7.94	0.4805819

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-969.3782332	4	1946.756
A2	-966.6852344	6	1945.37
A3	-969.3782332	4	1946.756
fitted	-969.8285572	3	1945.657
R	-1000.384579	2	2004.769

* Includes additive constant of -257.30279. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	2*Log(Likelihood Ratio)	Test df	p-value
1	67.39868874	4	<0.0001
2	5.385997686	2	0.067678
3	5.385997686	2	0.067678
4	0.900647851	1	0.342608

J.1.4 F1 Female Offspring Bodyweight on PND7

Table_Apx J-11. F1 Female Offspring Bodyweight on PND7

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	96	16.7	2.15		Table 8 in (Waterman et al., 2000); Table 19 in (Exxon Biomedical, 1996b)
0.2	139	94	15.54	2.79		
0.4	274	95	14.21	3.21	*	
0.8	543	97	15.03	1.72	*	

Table_Apx J-12. BMD Model Results for F1 Female Offspring Bodyweight on PND7 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	1	873.3122	<0.0001	1820.613354	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Exponential 5	Restricted	Constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Hill	Restricted	Constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Polynomial Degree 3	Restricted	Constant	1 SD	1	871.7201	<0.0001	1821.327416	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Polynomial Degree 2	Restricted	Constant	1 SD	1	880.3641	<0.0001	1821.326564	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Power	Restricted	Constant	1 SD	1	877.7875	<0.0001	1821.326396	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Linear	Unrestricted	Constant	1 SD	1	877.7877	<0.0001	1821.326396	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Exponential 3	Restricted	Constant	5%	254.4842	186.3013	<0.0001	1820.613354	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Exponential 5	Restricted	Constant	5%	126.7009	67.24957	NA	1803.662419	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Hill	Restricted	Constant	5%	134.2006	107.7629	0.0243488	1801.662519	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 3	Restricted	Constant	5%	269.2419	201.2969	<0.0001	1821.327416	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Polynomial Degree 2	Restricted	Constant	5%	271.806	201.4052	<0.0001	1821.326564	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Power	Restricted	Constant	5%	271.0633	201.3112	<0.0001	1821.326396	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Linear	Unrestricted	Constant	5%	271.0633	201.3093	<0.0001	1821.326396	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Exponential 3	Restricted	Non-constant	1 SD	1183.284	1008.202	<0.0001	1818.804639	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Exponential 5	Restricted	Non-constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Hill	Restricted	Non-constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Polynomial Degree 3	Restricted	Non-constant	1 SD	1171.144	705.7232	<0.0001	1818.783138	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Polynomial Degree 2	Restricted	Non-constant	1 SD	948.1995	666.0418	<0.0001	1821.732072	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Power	Restricted	Non-constant	1 SD	1097.551	771.3424	<0.0001	1818.870369	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Linear	Unrestricted	Non-constant	1 SD	1171.145	775.6192	<0.0001	1818.783138	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD higher than maximum dose BMDL higher than maximum dose Residual at control > 2
Exponential 3	Restricted	Non-constant	5%	358.6459	208.7593	<0.0001	1818.804639	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Exponential 5	Restricted	Non-constant	5%	134.2801	67.24804	<0.0001	1803.662419	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Hill	Restricted	Non-constant	5%	131.732	99.5847	<0.0001	1800.18868	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 3	Restricted	Non-constant	5%	320.1139	225.7198	<0.0001	1818.783138	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Polynomial Degree 2	Restricted	Non-constant	5%	284.8783	199.4796	<0.0001	1821.732072	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Power	Restricted	Non-constant	5%	305.2412	224.6379	<0.0001	1818.870369	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Linear	Unrestricted	Non-constant	5%	320.1141	225.718	<0.0001	1818.783138	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

Table_Apx J-13. BMD Model Results for F1 Female Offspring Bodyweight on PND7 (Highest Dose Group Removed)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	298.5781	236.6102	NA	1390.26048	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	1 SD	299.0891	146.5579	NA	1392.26048	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	302.2253	153.6427	NA	1392.26048	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	296.9767	237.1552	0.7810776	1388.337717	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose
Power	Restricted	Constant	1 SD	297.812	237.8871	NA	1390.26048	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	1 SD	301.1549	236.8887	0.7646431	1388.350114	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose
Exponential 3	Restricted	Constant	5%	104.5492	69.92462	NA	1390.26048	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	5%	105.0005	53.80445	NA	1392.26048	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	107.5409	51.41115	NA	1392.26048	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	92.13786	74.34106	0.7810776	1388.337717	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	5%	103.7932	74.58938	NA	1390.26048	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	5%	92.11699	74.3079	0.7646431	1388.350114	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	1 SD	298.5781	236.6077	0.0001469	1390.26048	Questionable	Goodness of fit p-value < 0.1 BMD higher than maximum dose
Exponential 5	Restricted	Non-constant	1 SD	299.0267	146.5157	NA	1392.26048	Questionable	BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Hill	Restricted	Non-constant	1 SD	224.0718	143.2736	NA	1379.84887	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	1 SD	296.842	237.7491	0.0001469	1390.26048	Questionable	Goodness of fit p-value < 0.1 BMD higher than maximum dose
Power	Restricted	Non-constant	1 SD	297.812	237.8872	0.0001469	1390.26048	Questionable	Goodness of fit p-value < 0.1 BMD higher than maximum dose
Linear	Unrestricted	Non-constant	1 SD	301.1549	236.888	0.0007097	1388.350114	Questionable	Goodness of fit p-value < 0.1 BMD higher than maximum dose
Exponential 3	Restricted	Non-constant	5%	104.5494	69.92475	0.0001469	1390.26048	Questionable	Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	5%	104.9473	53.80475	NA	1392.26048	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	109.5812	47.40981	NA	1379.84887	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	5%	102.5201	74.54359	0.0001469	1390.26048	Questionable	Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	5%	103.7928	74.58906	0.0001469	1390.26048	Questionable	Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	5%	92.11699	74.3079	0.0007097	1388.350114	Questionable	Goodness of fit p-value < 0.1

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

J.1.5 F1 Female Offspring Bodyweight on PND14

Table_Apx J-14. F1 Female Offspring Bodyweight on PND14

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	96	33.52	3.7		Table 8 in (Waterman et al., 2000); Table 19 in (Exxon Biomedical, 1996b)
0.2	139	93	31.89	4.57		
0.4	274	94	29.14	4.5	*	
0.8	543	97	28.41	3.1	*	

Table_Apx J-15. BMD Model Results for F1 Female Offspring Bodyweight on PND14 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	405.3895	336.35	0.0030515	2146.282814	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2
Exponential 5	Restricted	Constant	1 SD	246.5212	153.6476	NA	2138.698573	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	239.1764	160.0051	NA	2138.698573	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	426.8875	356.1585	0.0014995	2147.703832	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	1 SD	421.1136	356.0104	0.0015043	2147.697443	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	1 SD	422.8253	356.0833	0.0015054	2147.696003	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	1 SD	422.8253	356.0834	0.0015054	2147.696003	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Constant	5%	159.7284	135.3876	0.0030515	2146.282814	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Constant	5%	140.9941	99.25743	NA	2138.698573	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	140.5682	116.8619	NA	2138.698573	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	5%	173.9302	148.0675	0.0014995	2147.703832	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	5%	171.739	148.1089	0.0015043	2147.697443	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	5%	172.391	148.2262	0.0015054	2147.696003	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	5%	172.391	148.2262	0.0015054	2147.696003	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Non-constant	1 SD	470.6668	375.5155	0.0139371	2144.223772	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	1 SD	276.0887	150.6508	NA	2139.677365	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

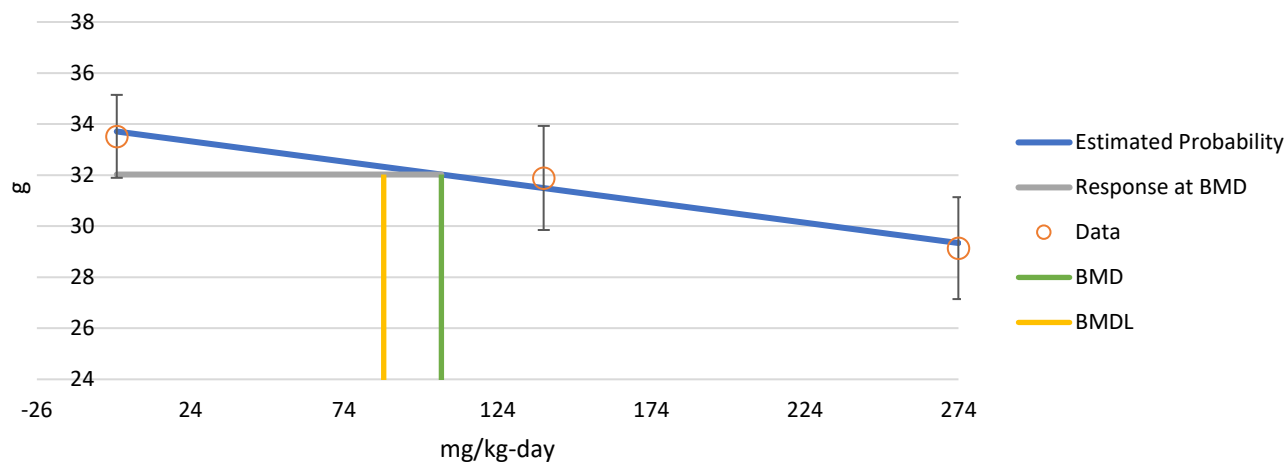
Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
									d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	1 SD	276.738	167.5475	NA	2139.677365	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	1 SD	502.5831	396.2205	0.0086929	2145.167866	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Non-constant	1 SD	488.4594	397.0909	0.0089226	2145.115705	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	1 SD	486.0556	397.154	0.0089092	2145.118707	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	1 SD	488.4594	397.0906	0.0089226	2145.115705	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Non-constant	5%	166.6613	140.7766	0.0139371	2144.223772	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	5%	148.574	102.5272	NA	2139.677365	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	146.8637	119.7526	NA	2139.677365	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	5%	183.2216	153.8292	0.0086929	2145.167866	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Non-constant	5%	179.7093	154.0859	0.0089226	2145.115705	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	5%	179.3159	154.1033	0.0089092	2145.118707	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	5%	179.7093	154.0868	0.0089226	2145.115705	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded).									
^b Restrictions defined in the BMDS 3.3 User Guide .									

Table_Apx J-16. BMD Model Results for F1 Female Offspring Bodyweight on PND14 (Highest Dose Group Removed)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	268.1257	255.3466	NA	1629.716555	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	1 SD	268.1257	223.1241	NA	1631.716555	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	255.0912	150.8382	NA	1631.716555	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	266.4022	223.9918	NA	1629.821805	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Power	Restricted	Constant	1 SD	268.2731	223.9493	NA	1629.716555	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	1 SD	266.8633	214.6232	0.2713215	1628.926609	Viable – Recommended	Lowest AIC
Exponential 3	Restricted	Constant	5%	141.6317	88.39683	NA	1629.716555	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	5%	141.6317	88.39683	NA	1631.716555	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	140.3123	116.9823	NA	1631.716555	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	130.2598	90.61312	NA	1629.821805	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Power	Restricted	Constant	5%	141.6808	91.03917	NA	1629.716555	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	5%	105.6747	86.89817	0.2713215	1628.926609	Viable – Recommended	Lowest AIC
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded).									
^b Restrictions defined in the BMDS 3.3 User Guide .									

Frequentist Linear Model with BMR of 0.05 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



User Input

Info	
Model	frequentist Linear
Model Restriction	Unrestricted
Dataset Name	Waterman-2000_F1-BW-F-PND14_HDD
User notes	[Add user notes here]
Dose-Response Model	$M[\text{dose}] = g + b1 * \text{dose}$
Variance Model	$\text{Var}(i) = \text{alpha}$

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	g
Total # of Observation	3
Adverse Direction	Downward

Model Results

Benchmark Dose	
BMD	105.6747086
BMDL	86.89816993
BMDU	135.7553767
AIC	1628.926609
Test 4 P-value	0.271321544
D.O.F.	1

Model Parameters				
# of Parameters	3			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	33.70965857	0.39867194	32.92828	34.49104
beta	-0.015949729	2.25E-03	-0.02037	-0.01153
alpha	18.11691691	2.76E+01	-35.9638	72.19765

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	96	33.7096586	33.52	33.52	4.256397	3.7	3.7	-0.436582
139	93	31.4926462	31.89	31.89	4.256397	4.57	4.57	0.900278
274	94	29.3394328	29.14	29.14	4.256397	4.5	4.5	-0.454275

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-810.8582776	4	1629.717
A2	-808.3495504	6	1628.699
A3	-810.8582776	4	1629.717
fitted	-811.4633043	3	1628.927
R	-834.5184646	2	1673.037

* Includes additive constant of -260.0596. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	2*Log(Likelihood Ratio)	Test df	p-value
1	52.33782852	4	<0.0001
2	5.017454444	2	0.081372
3	5.017454444	2	0.081372
4	1.210053412	1	0.271322

J.1.6 F1 Female Offspring Bodyweight on PND21

Table_Apx J-17. F1 Female Offspring Bodyweight on PND21

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	96	53.99	6.17		Table 8 in (Waterman et al., 2000); Table 19 in (Exxon Biomedical, 1996b)
0.2	139	93	49.19	7.54	*	
0.4	274	94	45.63	7.31	*	
0.8	543	97	44.68	5.68	*	

Table_Apx J-18. BMD Model Results for F1 Female Offspring Bodyweight on PND21 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	393.9975	327.3418	0.0001081	2545.025349	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Exponential 5	Restricted	Constant	1 SD	192.929	180.0446	NA	2530.760975	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	185.2249	144.9092	NA	2530.760975	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	418.2624	352.1953	<0.0001	2547.393332	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Polynomial Degree 2	Restricted	Constant	1 SD	455.8233	346.4451	<0.0001	2547.998204	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Power	Restricted	Constant	1 SD	417.4673	352.1606	<0.0001	2547.392998	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Linear	Unrestricted	Constant	1 SD	417.4673	352.1597	<0.0001	2547.392998	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 3	Restricted	Constant	5%	145.0004	123.2199	0.0001081	2545.025349	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 5	Restricted	Constant	5%	89.14845	44.68508	NA	2530.760975	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	100.591	81.65762	NA	2530.760975	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	5%	159.5909	137.3508	<0.0001	2547.393332	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Polynomial Degree 2	Restricted	Constant	5%	172.5487	135.1877	<0.0001	2547.998204	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Power	Restricted	Constant	5%	159.3053	137.2556	<0.0001	2547.392998	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Linear	Unrestricted	Constant	5%	159.3053	137.2556	<0.0001	2547.392998	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 3	Restricted	Non-constant	1 SD	439.3371	347.1973	0.0002438	2545.398207	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 5	Restricted	Non-constant	1 SD	192.929	146.2211	0.9722902	2530.760975	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Hill	Restricted	Non-constant	1 SD	184.4397	140.2982	NA	2532.759768	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	1 SD	466.1738	375.9204	<0.0001	2547.335844	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Polynomial Degree 2	Restricted	Non-constant	1 SD	465.929	375.9273	<0.0001	2547.335807	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Power	Restricted	Non-constant	1 SD	465.6475	375.9364	<0.0001	2547.335844	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Linear	Unrestricted	Non-constant	1 SD	462.4941	375.9167	<0.0001	2547.346425	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 3	Restricted	Non-constant	5%	150.8913	127.0587	0.0002438	2545.398207	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Exponential 5	Restricted	Non-constant	5%	89.14798	44.68552	0.9722902	2530.760975	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMDL 3× lower than lowest non-zero dose
Hill	Restricted	Non-constant	5%	100.5987	73.2584	NA	2532.759768	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
									d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	5%	165.868	141.7386	<0.0001	2547.335844	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Polynomial Degree 2	Restricted	Non-constant	5%	165.8318	141.7415	<0.0001	2547.335807	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Power	Restricted	Non-constant	5%	165.7909	141.7454	<0.0001	2547.335844	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Linear	Unrestricted	Non-constant	5%	165.5491	141.7212	<0.0001	2547.346425	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

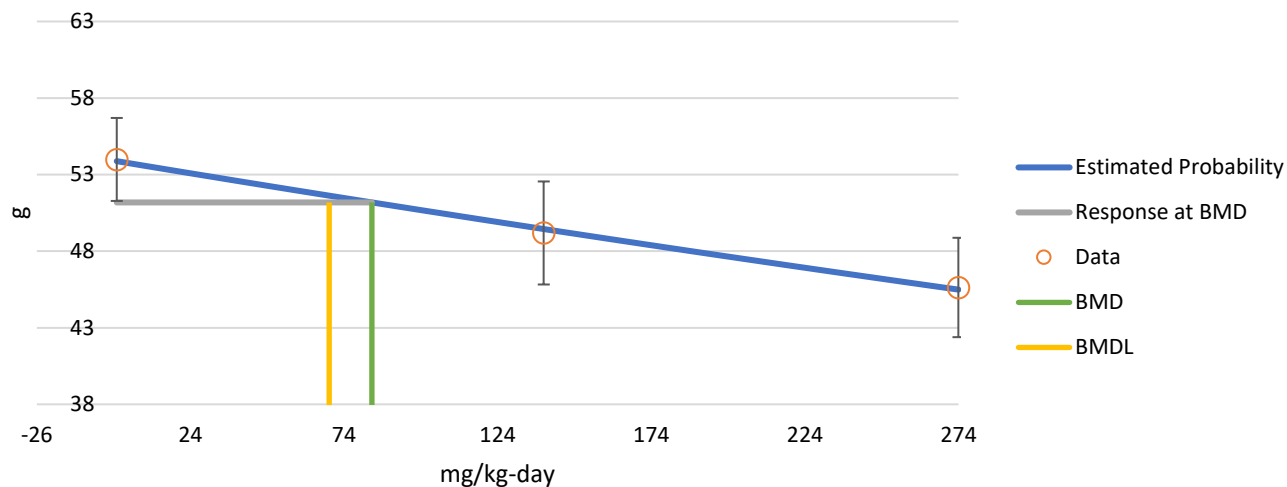
^b Restrictions defined in the [BMDS 3.3 User Guide](#).

Table_Apx J-19. BMD Model Results for F1 Female Offspring Bodyweight on PND21 (Highest Dose Group Removed)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	224.9896	182.5315	0.6656393	1909.657582	Viable – Recommended	Lowest AIC
Exponential 5	Restricted	Constant	1 SD	217.6212	139.8341	NA	1911.470837	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	207.4601	190.2318	NA	1913.470837	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	228.849	188.8653	0.5274787	1909.870083	Viable – Alternate	
Power	Restricted	Constant	1 SD	228.9162	188.8651	0.5274824	1909.870076	Viable – Alternate	
Linear	Unrestricted	Constant	1 SD	228.9162	188.8645	0.5274824	1909.870076	Viable – Alternate	
Exponential 3	Restricted	Constant	5%	83.05437	69.15258	0.6656393	1909.657582	Viable – Recommended	Lowest AIC

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 5	Restricted	Constant	5%	73.45746	43.55987	NA	1911.470837	Questionable	BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	89.14739	39.34772	NA	1913.470837	Questionable	BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	88.0599	74.44438	0.5274787	1909.870083	Viable – Alternate	
Power	Restricted	Constant	5%	88.08571	74.44467	0.5274824	1909.870076	Viable – Alternate	
Linear	Unrestricted	Constant	5%	88.08571	74.44467	0.5274824	1909.870076	Viable – Alternate	
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable ^a Selected Model (bolded). ^b Restrictions defined in the BMDs 3.3 User Guide .									

Frequentist Exponential Degree 3 Model with BMR of 0.05 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



User Input

Info	
Model	frequentist Exponential degree 3
Model Restriction	Restricted
Dataset Name	Waterman-2000_F1-BW-F-PND21_HDD
User notes	[Add user notes here]
Dose-Response Model	$M[\text{dose}] = a * \exp(\pm 1 * (b * \text{dose})^d)$
Variance Model	$\text{Var}(i) = \text{alpha}$

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	g
Total # of Observation	3
Adverse Direction	Downward

Model Results

Benchmark Dose	
BMD	83.05437022
BMDL	69.15258454
BMDU	125.1576767
AIC	1909.657582
Test 4 P-value	0.665639298
D.O.F.	1

Model Parameters				
# of Parameters	4			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
a	53.87795597	0.66498723	52.5746	55.18131
b	0.000617587	7.50E-05	0.000471	0.000765
d	Bounded	NA	NA	NA
log-alpha	3.888828168	8.41E-02	3.724062	4.053595

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	96	53.877956	53.99	53.99	6.989535	6.17	6.17	0.1570638
139	93	49.4457855	49.19	49.19	6.989535	7.54	7.54	-0.352914
274	94	45.4904579	45.63	45.63	6.989535	7.31	7.31	0.1935623

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-951.7354183	4	1911.471
A2	-949.6122154	6	1911.224
A3	-951.7354183	4	1911.471
fitted	-951.828791	3	1909.658
R	-982.4292261	2	1968.858

* Includes additive constant of -260.0596. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	65.63402137	4	<0.0001
2	4.24640576	2	0.119648
3	4.24640576	2	0.119648
4	0.186745415	1	0.665639

J.2 F2 Offspring Bodyweight

J.2.1 F2 Male Offspring Bodyweight on PND7

Table_Apx J-20. F2 Male Offspring Bodyweight on PND7

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	87	18.08	3.18		Table 11 in (Waterman et al., 2000); Table 39 in (Exxon Biomedical, 1996b)
0.2	133	79	16.43	2.34		
0.4	271	83	15.48	2.90	*	
0.8	544	72	14.70	3.00	*	

Table_Apx J-21. BMD Model Results for F2 Male Offspring Bodyweight on PND7 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	461.6142	366.133	0.046876	1597.673704	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Constant	1 SD	330.5471	198.1884	NA	1595.553206	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	333.4077	190.6706	NA	1595.553206	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	478.1074	390.0737	0.0254152	1598.898024	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	1 SD	497.7536	388.9764	0.0246232	1598.961341	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	1 SD	480.7334	389.9598	0.0254369	1598.896317	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	1 SD	480.7334	389.9593	0.0254369	1598.896317	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Constant	5%	132.6866	108.0924	0.046876	1597.673704	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Constant	5%	63.67049	38.79517	NA	1595.553206	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	67.92507	29.57314	NA	1595.553206	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	5%	145.4626	121.4488	0.0254152	1598.898024	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	5%	151.0412	121.1417	0.0246232	1598.961341	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	5%	146.213	121.4082	0.0254369	1598.896317	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	5%	146.213	121.4085	0.0254369	1598.896317	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Non-constant	1 SD	461.6142	366.1331	0.0929516	1597.673704	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	1 SD	349.6853	202.3994	0.9340853	1595.262507	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Hill	Restricted	Non-constant	1 SD	358.4047	195.4941	NA	1597.255666	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	1 SD	480.7334	389.9548	0.0540526	1598.896317	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Non-constant	1 SD	480.7723	389.9577	0.0219198	1600.896392	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	1 SD	480.7334	389.9598	0.0540526	1598.896317	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	1 SD	480.7334	389.9598	0.0540526	1598.896317	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Non-constant	5%	132.6866	108.0924	0.0929516	1597.673704	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	5%	60.71248	36.62418	0.9340853	1595.262507	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMDL 3× lower than lowest non-zero dose
Hill	Restricted	Non-constant	5%	60.89926	26.79399	NA	1597.255666	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	5%	146.213	121.4083	0.0540526	1598.896317	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Non-constant	5%	146.2209	121.4081	0.0219198	1600.896392	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	5%	146.213	121.4082	0.0540526	1598.896317	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	5%	146.213	121.4083	0.0540526	1598.896317	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

Table_Apx J-22. BMD Model Results for F2 Male Offspring Bodyweight on PND7 (Highest Dose Group Removed)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	294.8547	224.4383	0.4014984	1230.413076	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose
Exponential 5	Restricted	Constant	1 SD	320.706	136.3415	NA	1233.709242	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	1144.681	140.9213	NA	1233.709242	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD/BMDL ratio > 3 BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	295.3284	229.5497	0.3312634	1230.653194	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose
Power	Restricted	Constant	1 SD	294.8149	229.5624	0.3312941	1230.653074	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose
Linear	Unrestricted	Constant	1 SD	294.8149	229.5624	0.3312941	1230.653074	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMD higher than maximum dose
Exponential 3	Restricted	Constant	5%	88.39555	69.21579	0.4014984	1230.413076	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	5%	63.28412	27.97896	NA	1233.709242	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	91.79895	60.42561	NA	1233.709242	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	93.72347	74.7514	0.3312634	1230.653194	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	5%	93.56592	74.75603	0.3312941	1230.653074	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	5%	93.56592	74.7555	0.3312941	1230.653074	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	1 SD	311.7912	232.4983	0.2051934	1231.574104	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD higher than maximum dose
Exponential 5	Restricted	Non-constant	1 SD	—	—	—	—	Unusable	BMD computation failed

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Hill	Restricted	Non-constant	1 SD	460.2859	144.0986	NA	1233.969021	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD/BMDL ratio > 3 BMD higher than maximum dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	1 SD	299.217	233.5823	0.1368367	1232.182261	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD higher than maximum dose
Power	Restricted	Non-constant	1 SD	296.895	231.7951	0.1215844	1232.365876	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD higher than maximum dose
Linear	Unrestricted	Non-constant	1 SD	310.049	236.4869	0.1647878	1231.898816	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD higher than maximum dose
Exponential 3	Restricted	Non-constant	5%	89.0521	69.69518	0.2051934	1231.574104	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	5%	71.81779	16.30153	NA	1233.969021	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD/BMDL ratio > 3 BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	49.78012	3.140952	NA	1233.969021	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMD/BMDL ratio > 3 BMDL 3× lower than lowest non-zero dose BMDL 10× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	5%	93.50841	74.58525	0.1368367	1232.182261	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	5%	93.43043	74.09874	0.1215844	1232.365876	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Linear	Unrestricted	Non-constant	5%	94.37421	75.34447	0.1647878	1231.898816	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
^b Restrictions defined in the BMDS 3.3 User Guide .									

J.2.2 F2 Male Offspring Bodyweight on PND14

Table_Apx J-23. F2 Male Offspring Bodyweight on PND14

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	87	37.09	4.68		Table 11 in (Waterman et al., 2000); Table 39 in (Exxon Biomedical, 1996b)
0.2	133	82	34.80	3.47		
0.4	271	83	32.51	4.85	*	
0.8	544	72	29.88	4.00	*	

Table_Apx J-24. BMD Model Results for F2 Male Offspring Bodyweight on PND14 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDs Recommends	BMDs Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	310.9715	256.7945	0.3516382	1869.101249	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	1 SD	254.0299	185.7105	0.6721636	1869.190026	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Hill	Restricted	Constant	1 SD	249.6883	186.3998	NA	1871.010944	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	325.1459	278.5947	0.2210705	1870.029491	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 2	Restricted	Constant	1 SD	346.8172	271.7199	0.0449369	1873.031951	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	1 SD	324.2721	278.6708	0.2211645	1870.028641	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	1 SD	324.2721	278.6708	0.2211645	1870.028641	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Constant	5%	126.8871	109.2117	0.3516382	1869.101249	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	5%	96.50161	68.47018	0.6721636	1869.190026	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Hill	Restricted	Constant	5%	110.4933	66.3493	NA	1871.010944	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	5%	138.8628	121.73	0.2210705	1870.029491	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 2	Restricted	Constant	5%	151.2085	119.1812	0.0449369	1873.031951	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	5%	138.5227	121.772	0.2211645	1870.028641	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	5%	138.5227	121.772	0.2211645	1870.028641	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	1 SD	316.2307	261.0941	0.4073079	1870.545289	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	1 SD	258.9523	190.356	NA	1872.748917	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	1 SD	304.6504	178.2839	0.2920302	1871.859155	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Polynomial Degree 3	Restricted	Non-constant	1 SD	335.0496	281.3713	0.233684	1871.656489	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Polynomial Degree 2	Restricted	Non-constant	1 SD	334.8946	281.3551	0.233678	1871.65654	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	1 SD	334.8787	281.3555	0.2336786	1871.656535	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Linear	Unrestricted	Non-constant	1 SD	335.0402	281.3703	0.2336844	1871.656485	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 3	Restricted	Non-constant	5%	127.2935	110.1911	0.4073079	1870.545289	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	5%	105.3862	68.36967	NA	1872.748917	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	119.3041	59.85716	0.2920302	1871.859155	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Polynomial Degree 3	Restricted	Non-constant	5%	139.3297	122.4641	0.233684	1871.656489	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Polynomial Degree 2	Restricted	Non-constant	5%	139.318	122.4646	0.233678	1871.65654	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	5%	139.3187	122.4652	0.2336786	1871.656535	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Linear	Unrestricted	Non-constant	5%	139.3322	122.4638	0.2336844	1871.656485	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

Table_Apx J-25. BMD Model Results for F2 Male Offspring Bodyweight on PND14 (Highest Dose Group Removed)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	257.3344	202.0522	NA	1465.131238	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	1 SD	257.0731	136.7782	NA	1467.131238	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	256.2765	139.8734	NA	1467.131238	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	258.8061	206.1186	0.9358909	1463.137708	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	1 SD	257.9058	206.0472	0.9425274	1463.136435	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	1 SD	257.9058	206.0469	0.9425274	1463.136435	Questionable	Constant variance test failed (Test 2 p-value < 0.05)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	5%	107.5215	84.93791	NA	1465.131238	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	5%	107.9591	60.49319	NA	1467.131238	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	109.2554	57.44817	NA	1467.131238	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	110.1849	89.62363	0.9358909	1463.137708	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	5%	109.6875	89.65173	0.9425274	1463.136435	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	5%	109.6875	89.65173	0.9425274	1463.136435	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	1 SD	257.3345	202.0522	0.6887133	1465.131238	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	1 SD	256.8383	136.6679	NA	1467.131238	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	1 SD	154.4324	139.7685	NA	1468.970755	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	1 SD	257.9056	206.047	0.9204987	1463.136435	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	1 SD	257.9058	206.0471	0.9204987	1463.136435	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Linear	Unrestricted	Non-constant	1 SD	257.9058	206.0471	0.9204987	1463.136435	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 3	Restricted	Non-constant	5%	107.5217	84.93756	0.6887133	1465.131238	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	5%	108.3242	60.0712	NA	1467.131238	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	130.7914	124.4926	NA	1468.970755	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Polynomial Degree 2	Restricted	Non-constant	5%	109.6871	89.65201	0.9204987	1463.136435	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	5%	109.6875	89.65172	0.9204987	1463.136435	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Linear	Unrestricted	Non-constant	5%	109.6875	89.65172	0.9204987	1463.136435	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

J.2.3 F2 Male Offspring Bodyweight on PND21

Table_Apx J-26. F2 Male Offspring Bodyweight on PND21

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	87	62.34	7.68		Table 11 in (Waterman et al., 2000); Table 39 in (Exxon Biomedical, 1996b)
0.2	133	79	57.89	6.56		
0.4	271	82	54.82	7.45	*	
0.8	544	72	49.12	7.38	*	

Table_Apx J-27. BMD Model Results for F2 Male Offspring Bodyweight on PND21 (All Dose Groups Included)

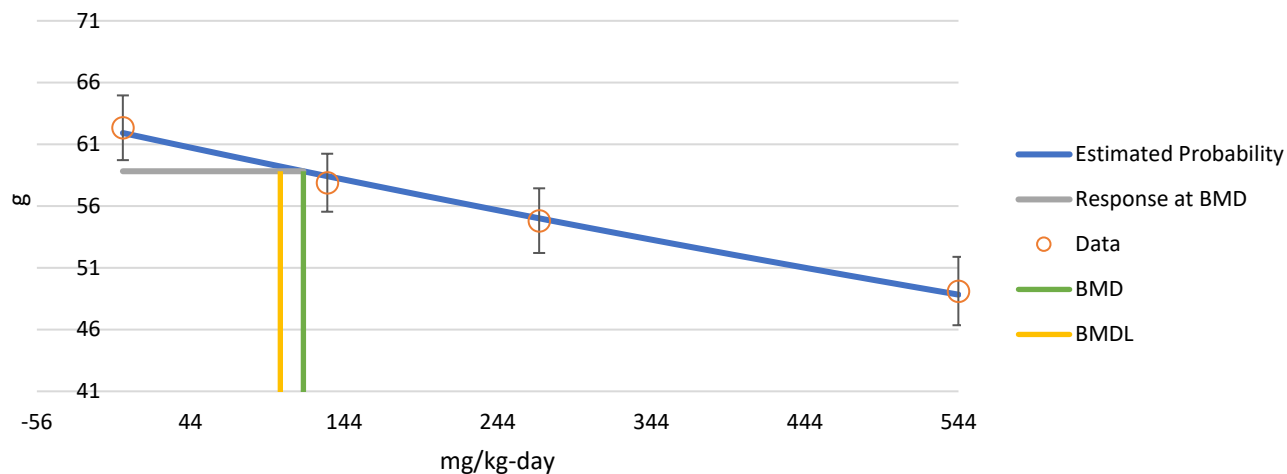
Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	285.4789	242.8247	0.6383876	2182.321925	Viable – Recommended	Lowest AIC
Exponential 5	Restricted	Constant	1 SD	253.8703	187.7986	0.6513583	2183.628498	Viable – Alternate	
Hill	Restricted	Constant	1 SD	253.3356	185.5706	0.6705813	2183.605229	Viable – Alternate	
Polynomial Degree 3	Restricted	Constant	1 SD	304.7243	263.0126	0.4122808	2183.196407	Viable – Alternate	
Polynomial Degree 2	Restricted	Constant	1 SD	—	—	—	—	Unusable	BMD computation failed
Power	Restricted	Constant	1 SD	304.1596	262.977	0.4124379	2183.195645	Viable – Alternate	
Linear	Unrestricted	Constant	1 SD	304.1596	262.977	0.4124379	2183.195645	Viable – Alternate	
Exponential 3	Restricted	Constant	5%	117.5098	102.4965	0.6383876	2182.321925	Viable – Recommended	Lowest AIC
Exponential 5	Restricted	Constant	5%	99.05525	70.38601	0.6513583	2183.628498	Viable – Alternate	
Hill	Restricted	Constant	5%	97.87309	69.56036	0.6705813	2183.605229	Viable – Alternate	
Polynomial Degree 3	Restricted	Constant	5%	129.4509	114.4269	0.4122808	2183.196407	Viable – Alternate	
Polynomial Degree 2	Restricted	Constant	5%	253.9388	248.7375	<0.0001	2200.267654	Questionable	Goodness of fit p-value < 0.1 Residual for Dose Group Near BMD > 2 Residual at control > 2
Power	Restricted	Constant	5%	129.1878	114.3972	0.4124379	2183.195645	Viable – Alternate	
Linear	Unrestricted	Constant	5%	129.1878	114.3972	0.4124379	2183.195645	Viable – Alternate	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

Frequentist Exponential Degree 3 Model with BMR of 0.05 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



User Input

Info	
Model	frequentist Exponential degree 3
Model Restriction	Restricted
Dataset Name	Waterman-2000_F2-BW-M-PND21
User notes	[Add user notes here]
Dose-Response Model	$M[dose] = a * \exp(\pm 1 * (b * dose)^d)$
Variance Model	$Var(i) = \alpha$

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	g
Total # of Observation	4
Adverse Direction	Downward

Model Results

Benchmark Dose	
BMD	117.509799
BMDL	102.4965127
BMDU	154.9025277
AIC	2182.321925
Test 4 P-value	0.638387563
D.O.F.	2

Model Parameters				
# of Parameters	4			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
a	61.91561838	0.63620624	60.66868	63.16256
b	0.000436502	3.83E-05	0.000361	0.000512
d	Bounded	NA	NA	NA
log-alpha	3.963128954	7.91E-02	3.80818	4.118078

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	87	61.9156184	62.34	62.34	7.254083	7.68	7.68	0.5456745
133	79	58.4234682	57.89	57.89	7.254083	6.56	6.56	-0.653641
271	82	55.0080945	54.82	54.82	7.254083	7.45	7.45	-0.234801
544	72	48.8285521	49.12	49.12	7.254083	7.38	7.38	0.3409138

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-1087.712153	5	2185.424
A2	-1086.591076	8	2189.182
A3	-1087.712153	5	2185.424
fitted	-1088.160963	3	2182.322
R	-1145.233171	2	2294.466

* Includes additive constant of -294.06033. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	2*Log(Likelihood Ratio)	Test df	p-value
1	117.2841904	6	<0.0001
2	2.2421544	3	0.523693
3	2.2421544	3	0.523693
4	0.897619428	2	0.638388

J.2.4 F2 Female Offspring Bodyweight on PND7

Table_Apx J-28. F2 Female Offspring Bodyweight on PND7

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	84	17.47	2.88		Table 11 in (Waterman et al., 2000); Table 39 in (Exxon Biomedical, 1996b)
0.2	133	80	15.72	2.22	*	
0.4	271	73	14.56	3.03	*	
0.8	544	78	13.76	2.49	*	

Table_Apx J-29. BMD Model Results for F2 Female Offspring Bodyweight on PND7 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMF	BMD	BMDL	P Value	AIC	BMDL Recommends	BMDL Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	386.1792	315.1079	0.017201	1522.616108	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Constant	1 SD	231.33	156.711	NA	1518.490529	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	229.2439	155.0224	NA	1518.490529	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	412.9036	343.1368	0.0067363	1524.491032	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	1 SD	410.103	343.3094	0.0067351	1524.491367	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	1 SD	411.6254	343.2307	0.0067387	1524.490298	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	1 SD	411.6254	343.2304	0.0067387	1524.490298	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Constant	5%	115.3871	96.82845	0.017201	1522.616108	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Constant	5%	64.38746	36.81541	NA	1518.490529	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	70.58941	30.0512	NA	1518.490529	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	5%	129.7898	110.6013	0.0067363	1524.491032	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Constant	5%	128.9872	110.6659	0.0067351	1524.491367	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Constant	5%	129.4239	110.6332	0.0067387	1524.490298	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Constant	5%	129.4239	110.6331	0.0067387	1524.490298	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Non-constant	1 SD	404.4602	319.4208	0.0179951	1524.23099	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	1 SD	246.4343	160.2465	NA	1520.19568	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Hill	Restricted	Non-constant	1 SD	333.6476	128.9798	0.0948987	1520.984929	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 3	Restricted	Non-constant	1 SD	432.9737	348.2139	0.0073081	1526.033221	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Non-constant	1 SD	430.775	348.2967	0.0073205	1526.029846	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	1 SD	432.128	348.2421	0.0073146	1526.031449	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	1 SD	430.8024	348.2957	0.0073205	1526.029846	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 3	Restricted	Non-constant	5%	116.9971	97.77591	0.0179951	1524.23099	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Exponential 5	Restricted	Non-constant	5%	58.3255	36.26001	NA	1520.19568	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	80.94619	21.80169	0.0948987	1520.984929	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1 BMD/BMDL ratio > 3 BMDL 3× lower than lowest non-zero dose
Polynomial Degree 3	Restricted	Non-constant	5%	131.5494	111.7242	0.0073081	1526.033221	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Polynomial Degree 2	Restricted	Non-constant	5%	131.2504	111.7513	0.0073205	1526.029846	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Power	Restricted	Non-constant	5%	131.4056	111.7371	0.0073146	1526.031449	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
Linear	Unrestricted	Non-constant	5%	131.2537	111.7512	0.0073205	1526.029846	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) Goodness of fit p-value < 0.1
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded).									
^b Restrictions defined in the BMDS 3.3 User Guide .									

Table_Apx J-30. BMD Model Results for F2 Female Offspring Bodyweight on PND7 (Highest Dose Group Removed)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	248.5057	193.4175	0.4944471	1151.359205	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	1 SD	242.0645	134.3484	NA	1152.89237	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	242.3856	136.5245	NA	1154.89237	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	251.9734	200.3953	0.3876062	1151.638809	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	1 SD	251.6715	200.4059	0.3876267	1151.638744	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	1 SD	251.6715	200.4059	0.3876267	1151.638744	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Constant	5%	75.22937	60.2912	0.4944471	1151.359205	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	5%	59.12399	30.58226	NA	1152.89237	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	59.35102	23.61017	NA	1154.89237	Questionable	Constant variance test failed (Test 2 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	80.64244	65.91709	0.3876062	1151.638809	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Power	Restricted	Constant	5%	80.55384	65.91988	0.3876267	1151.638744	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	5%	80.55384	65.91988	0.3876267	1151.638744	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	1 SD	248.5057	193.4175	0.7807922	1151.359205	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	1 SD	241.742	134.4873	NA	1154.89237	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	1 SD	238.6763	135.7701	NA	1156.864312	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	1 SD	255.768	198.732	0.3164059	1153.868058	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	1 SD	251.6715	200.4059	0.6789445	1151.638744	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Linear	Unrestricted	Non-constant	1 SD	251.6715	200.4059	0.6789445	1151.638744	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 3	Restricted	Non-constant	5%	75.22939	60.29121	0.7807922	1151.359205	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	5%	59.83184	29.8113	NA	1154.89237	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	62.43963	22.61827	NA	1156.864312	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) BMDL 3× lower than lowest non-zero dose d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Non-constant	5%	80.99489	65.37813	0.3164059	1153.868058	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	5%	80.55385	65.91989	0.6789445	1151.638744	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Linear	Unrestricted	Non-constant	5%	80.55385	65.91989	0.6789445	1151.638744	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded).									
^b Restrictions defined in the BMDS 3.3 User Guide .									

J.2.5 F2 Female Offspring Bodyweight on PND14

Table_Apx J-31. F2 Female Offspring Bodyweight on PND14

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	84	35.89	4.12		Table 11 in (Waterman et al., 2000); Table 39 in (Exxon Biomedical, 1996b)
0.2	133	85	33.64	3.66		
0.4	271	73	31.22	4.81	*	
0.8	544	78	28.20	3.32	*	

Table_Apx J-32. BMD Model Results for F2 Female Offspring Bodyweight on PND14 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	266.1234	225.7549	0.5462158	1798.066503	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	1 SD	228.0086	173.9014	NA	1800.857021	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	227.3191	173.3892	NA	1800.857021	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	284.1348	246.7919	0.3186125	1799.14458	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 2	Restricted	Constant	1 SD	394.9127	386.7879	0.0005599	1810.761746	Questionable	Constant variance test failed (Test 2 p-value < 0.05) Goodness of fit p-value < 0.1 Residual at control > 2
Power	Restricted	Constant	1 SD	282.7796	246.879	0.3192175	1799.140786	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	1 SD	282.7795	246.879	0.3192175	1799.140786	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Constant	5%	115.6055	100.6113	0.5462158	1798.066503	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 5	Restricted	Constant	5%	109.0935	70.33997	NA	1800.857021	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	109.7149	68.27143	NA	1800.857021	Questionable	Constant variance test failed (Test 2 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	5%	126.7898	112.8911	0.3186125	1799.14458	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Polynomial Degree 2	Restricted	Constant	5%	-	-	-	-	Unusable	BMD computation failed
Power	Restricted	Constant	5%	126.2408	112.9247	0.3192175	1799.140786	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Linear	Unrestricted	Constant	5%	126.2408	112.9244	0.3192175	1799.140786	Questionable	Constant variance test failed (Test 2 p-value < 0.05)
Exponential 3	Restricted	Non-constant	1 SD	282.6982	236.805	0.7259715	1798.334396	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	1 SD	248.9075	183.6547	NA	1801.693906	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	1 SD	248.7313	183.1052	NA	1801.693906	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
									d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	1 SD	287.3642	251.4546	0.2714972	1800.301513	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Polynomial Degree 2	Restricted	Non-constant	1 SD	304.509	258.5391	0.4623337	1799.236843	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	1 SD	304.4458	258.5427	0.4623842	1799.236625	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Linear	Unrestricted	Non-constant	1 SD	303.9056	258.5765	0.46257	1799.235821	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 3	Restricted	Non-constant	5%	115.0306	101.7281	0.7259715	1798.334396	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Exponential 5	Restricted	Non-constant	5%	105.1363	70.99505	NA	1801.693906	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Non-constant	5%	105.4723	69.52159	NA	1801.693906	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05) d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Non-constant	5%	126.3314	112.0662	0.2714972	1800.301513	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Polynomial Degree 2	Restricted	Non-constant	5%	127.4205	114.2369	0.4623337	1799.236843	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Power	Restricted	Non-constant	5%	127.4102	114.2391	0.4623842	1799.236625	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
Linear	Unrestricted	Non-constant	5%	127.3599	114.2466	0.46257	1799.235821	Questionable	Non-constant variance test failed (Test 3 p-value < 0.05)
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded).									
^b Restrictions defined in the BMDS 3.3 User Guide .									

Table_Apx J-33. BMD Model Results for F2 Female Offspring Bodyweight on PND14 (Highest Dose Group Removed)

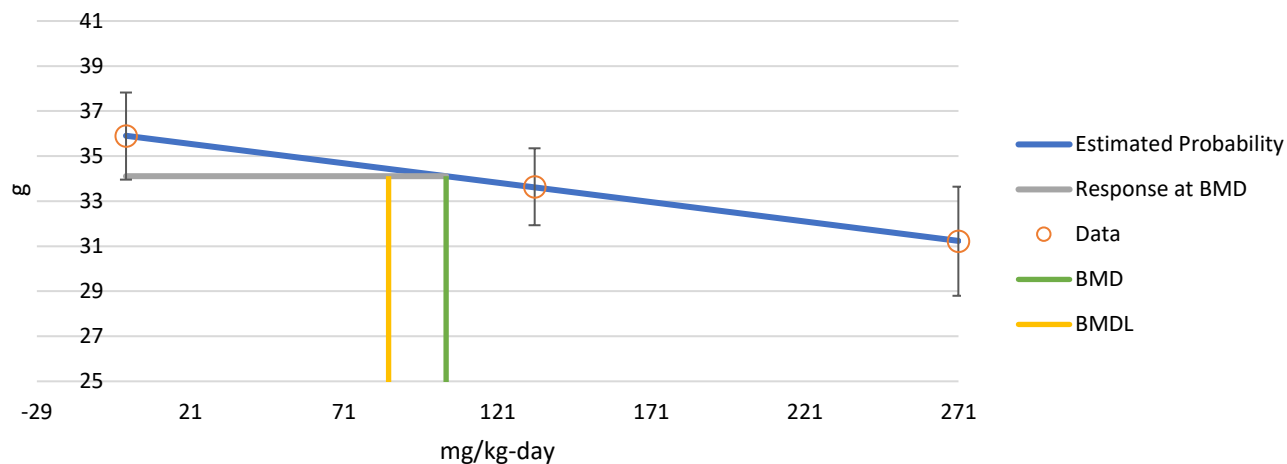
Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	241.9772	189.9277	NA	1385.405068	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	1 SD	241.5843	135.662	NA	1387.405068	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	1 SD	199.0526	181.6711	NA	1387.405068	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	1 SD	245.1661	193.9458	NA	1385.410712	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Power	Restricted	Constant	1 SD	242.448	193.9561	NA	1385.405068	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	1 SD	241.8307	193.8945	0.9404642	1383.410646	Viable – Recommended	Lowest AIC
Exponential 3	Restricted	Constant	5%	107.1502	81.01798	NA	1385.405068	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Exponential 5	Restricted	Constant	5%	107.515	62.22856	NA	1387.405068	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Hill	Restricted	Constant	5%	122.8124	99.34293	NA	1387.405068	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 2	Restricted	Constant	5%	108.6735	85.47927	NA	1385.410712	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Power	Restricted	Constant	5%	106.6836	85.49744	NA	1385.405068	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Linear	Unrestricted	Constant	5%	104.2095	85.49203	0.9404642	1383.410646	Viable – Recommended	Lowest AIC

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable

^a Selected Model (bolded).

^b Restrictions defined in the [BMDS 3.3 User Guide](#).

Frequentist Linear Model with BMR of 0.05 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



User Input

Info	
Model	frequentist Linear
Model Restriction	Unrestricted
Dataset Name	Waterman-2000_F2-BW-F-PND14_HDD
User notes	[Add user notes here]
Dose-Response Model	$M[\text{dose}] = g + b1 \cdot \text{dose}$
Variance Model	$\text{Var}[i] = \text{alpha}$

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	g
Total # of Observation	3
Adverse Direction	Downward

Model Results

Benchmark Dose	
BMD	104.2094736
BMDL	85.49203164
BMDU	134.3993288
AIC	1383.410646
Test 4 P-value	0.940464162
D.O.F.	1

Model Parameters				
# of Parameters	3			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	35.90399974	0.41409874	35.09238	36.71562
beta	-0.01722684	2.46E-03	-0.02205	-0.01241
alpha	17.35537836	2.74E+01	-36.3137	71.02445

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	84	35.9039997	35.89	35.89	4.165979	4.12	4.12	-0.030799
133	85	33.61283	33.64	33.64	4.165979	3.66	3.66	0.0601286
271	73	31.2355262	31.22	31.22	4.165979	4.81	4.81	-0.031843

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-688.7025338	4	1385.405
A2	-685.7506247	6	1383.501
A3	-688.7025338	4	1385.405
fitted	-688.7053228	3	1383.411
R	-711.0549619	2	1426.11

* Includes additive constant of -222.38313. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	2*Log(Likelihood Ratio)	Test df	p-value
1	50.60867432	4	<0.0001
2	5.903818186	2	0.05224
3	5.903818186	2	0.05224
4	0.005578071	1	0.940464

J.2.6 F2 Female Offspring Bodyweight on PND21

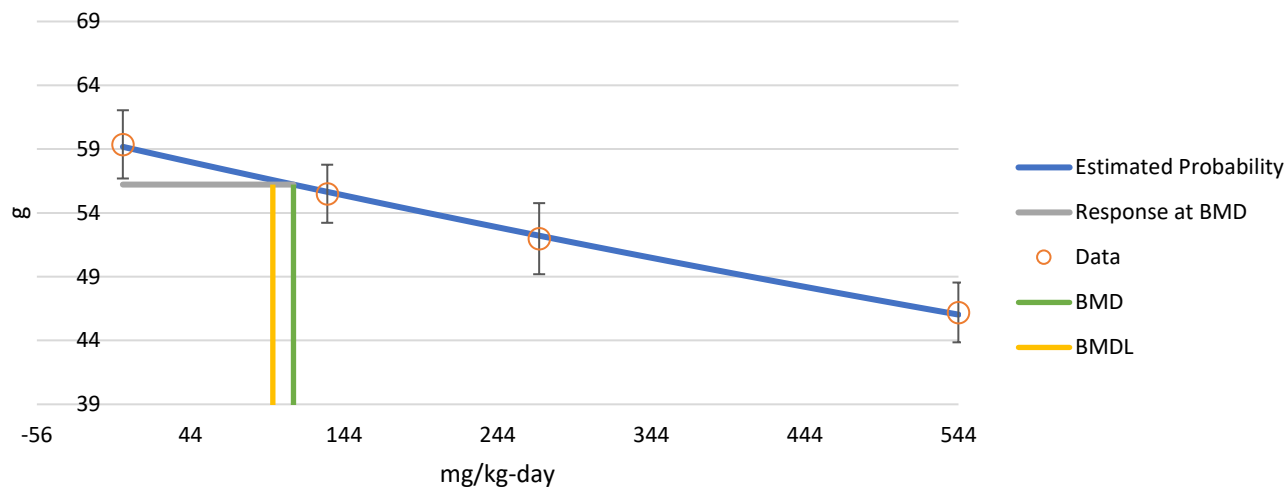
Table_Apx J-34. F2 Female Offspring Bodyweight on PND21

Dietary Dose (%)	Received Dose (mg/kg-day)	N	Mean	SD	Statistical Significance	Location of Data
0	0	84	59.37	7.70		Table 11 in (Waterman et al., 2000); Table 39 in (Exxon Biomedical, 1996b)
0.2	133	79	55.50	6.36		
0.4	271	73	51.98	7.48	*	
0.8	544	78	46.20	6.50	*	

Table_Apx J-35. BMD Model Results for F2 Female Offspring Bodyweight on PND21 (All Dose Groups Included)

Models ^a	Restriction ^b	Variance	BMR	BMD	BMDL	P Value	AIC	BMDS Recommends	BMDS Recommendation Notes
Exponential 3	Restricted	Constant	1 SD	272.4073	233.1442	0.891522	2118.778944	Viable – Recommended	Lowest AIC
Exponential 5	Restricted	Constant	1 SD	254.4618	189.4706	0.9922188	2120.549389	Viable – Alternate	
Hill	Restricted	Constant	1 SD	254.5222	188.6821	NA	2122.549294	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	1 SD	291.846	253.7424	0.643646	2119.430507	Viable – Alternate	
Polynomial Degree 2	Restricted	Constant	1 SD	291.2796	253.7626	0.6435126	2119.430921	Viable – Alternate	
Power	Restricted	Constant	1 SD	291.7664	253.7459	0.6436504	2119.430493	Viable – Alternate	
Linear	Unrestricted	Constant	1 SD	291.7664	253.7459	0.6436504	2119.430493	Viable – Alternate	
Exponential 3	Restricted	Constant	5%	111.0568	97.60821	0.891522	2118.778944	Viable – Recommended	Lowest AIC
Exponential 5	Restricted	Constant	5%	100.7087	71.77118	0.9922188	2120.549389	Viable – Alternate	
Hill	Restricted	Constant	5%	100.4403	69.74917	NA	2122.549294	Questionable	d.f.=0, saturated model (Goodness of fit test cannot be calculated)
Polynomial Degree 3	Restricted	Constant	5%	122.8587	109.5126	0.643646	2119.430507	Viable – Alternate	
Polynomial Degree 2	Restricted	Constant	5%	122.6421	109.5202	0.6435126	2119.430921	Viable – Alternate	
Power	Restricted	Constant	5%	122.8279	109.514	0.6436504	2119.430493	Viable – Alternate	
Linear	Unrestricted	Constant	5%	122.8279	109.514	0.6436504	2119.430493	Viable – Alternate	
AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; NA = not applicable									
^a Selected Model (bolded).									
^b Restrictions defined in the BMDS 3.3 User Guide .									

Frequentist Exponential Degree 3 Model with BMR of 0.05 Added Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



User Input

Info	
Model	frequentist Exponential degree 3
Model Restriction	Restricted
Dataset Name	Waterman-2000_F2-BW-F-PND21
User notes	[Add user notes here]
Dose-Response Model	$M[\text{dose}] = a * \exp(\pm 1 * (b * \text{dose})^d)$
Variance Model	Var(i) = alpha

Model Options	
BMR Type	Rel. Dev.
BMRF	0.05
Tail Probability	-
Confidence Level	0.95
Distribution Type	Normal
Variance Type	Constant

Model Data	
Dependent Variable	mg/kg-day
Independent Variable	g
Total # of Observation	4
Adverse Direction	Downward

Model Results

Benchmark Dose	
BMD	111.0567927
BMDL	97.60820616
BMDU	155.3477035
AIC	2118.778944
Test 4 P-value	0.891522029
D.O.F.	2

Model Parameters				
# of Parameters	4			
Variable	Estimate	Std Error	Lower Conf	Upper Conf
a	59.17778146	0.62136336	57.95993	60.39563
b	0.000461865	3.83E-05	0.000387	0.000537
d	Bounded	NA	NA	NA
log-alpha	3.890718337	7.98E-02	3.734296	4.047141

Goodness of Fit								
Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	84	59.1777815	59.37	59.37	6.996144	7.7	7.7	0.2518119
133	79	55.6520022	55.5	55.5	6.996144	6.36	6.36	-0.19311
271	73	52.2155647	51.98	51.98	6.996144	7.48	7.48	-0.287682
544	78	46.0299081	46.2	46.2	6.996144	6.5	6.5	0.2147198

Likelihoods of Interest			
Model	Log Likelihood*	# of Parameters	AIC
A1	-1056.274647	5	2122.549
A2	-1054.042132	8	2124.084
A3	-1056.274647	5	2122.549
fitted	-1056.389472	3	2118.779
R	-1118.934618	2	2241.869

* Includes additive constant of -288.5467. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest			
Test	2*Log(Likelihood Ratio)	Test df	p-value
1	129.7849717	6	<0.0001
2	4.465030273	3	0.215431
3	4.465030273	3	0.215431
4	0.229650264	2	0.891522