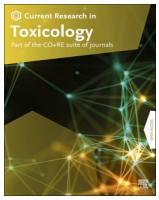
Current Research in Toxicology 8 (2025) 100219

Contents lists available at ScienceDirect

Current Research in Toxicology

journal homepage: www.journals.elsevier.com/current-research-in-toxicology



Research Paper

Evaluation of a hypothesized Sertoli cell-based adverse outcome pathway for effects of diisononyl phthalate on the developing testis

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ARTICLE INFO

Keywords:
Sertoli cells
Testes development
Phthalates
Adverse outcome pathway

ABSTRACT

Exposure of pregnant rats to some phthalates during the masculinization programming window (MPW) can lower fetal testis testosterone production and adversely affect development of the fetal male reproductive tract. Some of the effects in rats are androgen-dependent, while others also occur in mice without lower testosterone production. An adverse outcome pathway (AOP) network has been proposed for these developmental effects that includes both androgen-dependent and androgen-independent pathways, the latter of which includes a short list of putative molecular initiating events (MIEs) including peroxisome proliferator activated receptor (PPAR) activation, and effects on Sertoli cells in the developing testes as early key events (KEs) (PMID 34314370). Data from peer-reviewed literature, publicly cited toxicology reports, and EPA's Toxicity Forecaster (ToxCast) were evaluated in the context of this hypothesized Sertoli cell-based AOP and exposure to diisononyl phthalate (DINP). Each of the fifteen identified studies underwent a risk of bias (RoB) assessment, which revealed a high risk of bias for all but one study endpoint. *In vitro* evidence in kidney, liver, and fibroblast-like cell lines indicates that the DINP metabolites mono-isononyl phthalate (MINP) and mono-hydroxyisononyl phthalate (MHINP) activate PPARα/γ and that mouse PPARα/γ are more sensitive than human PPARα/γ. However, DINP did not activate PPARα-related genes in rat fetal testes at high maternal dosages (PMID 22112501), and it remains unknown whether PPARs are expressed in fetal Sertoli cells. Overall, there is insufficient evidence to evaluate whether PPAR activation in the developing male reproductive tract is causally linked to the KEs in the hypothesized AOP. Regarding the KEs, no *in vivo* studies were identified that examined the effects of DINP on Sertoli cell proliferation or cytoskeleton; a single *in vitro* study found no effect of DINP on Sertoli cell proliferation. There was limited and conflicting evidence for the effects of DINP on tubulogenesis, but strong *in vivo* evidence for increased multinucleated germ (MNG) cells. No evidence was found concerning germ cell apoptosis. For the adverse outcomes (AOs), there was limited *in vivo* evidence for testicular dysgenesis following altered tubulogenesis, and impaired spermatogenesis following increased MNGs. There was strong evidence against reduced fertility, but this is not a sensitive endpoint in rats given their robust sperm production and excess capacity. In conclusion, following *in utero* DINP exposure, while PPAR activation (MIE) is plausible, linkage to effects on Sertoli cells and downstream AOPs is lacking. The sparse evidence currently available is insufficient to support the applicability of the hypothesized Sertoli cell-based AOP to DINP.

1. Introduction

Phthalates are diesters of phthalic acid that, after oral exposure, are metabolized rapidly to monoester metabolites and excreted in the urine (Dutta et al., 2020). *In utero* exposure to some phthalates during the masculinization programming window (MPW, gestation days [GDs] 15.5–18.5 in rats) (Sharpe et al., 2024) can result in a spectrum of effects on the developing male reproductive tract known as the rat phthalate

Abbreviations: AGD, anogenital distance; AO, adverse outcome; AOP, adverse outcome pathway; DINP, diisononyl phthalate; GD, gestational day; INSL3, insulin-like factor 3; KE, key event; KER, key event relationship; LOAEL, lowest observed adverse effect level; MHINP, mono-hydroxyisononyl phthalate; MIE, molecular initiating event; MINP, mono-isononyl phthalate; MNG, multinucleated germ cell; MoA, mode of action; NOAEL, no observed adverse effect level; OHAT, Office of Health Assessment and Translation; PND, postnatal day; PPAR, peroxisome proliferator activated receptor; RoB, risk of bias; WoE, weight of evidence.

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https://doi.org/10.1016/j.crtox.2025.100219
Received 2 October 2024; Received in revised form 21 January 2025; Accepted 23 January 2025
Available online 1 February 2025

syndrome (Foster et al., 2006; NRC, 2008). Apical effects encompassed in rat phthalate syndrome include epididymis, vas deferens, seminal vesicles, prostate, and external genitalia (hypospadias) malformations, cryptorchidism, testicular injury, permanent nipple retention, and reduced anogenital distance (AGD) (Foster et al., 2006). Phthalate syndrome also includes earlier indicators of effect, including lower expression of genes related to steroidogenesis and insulin-like factor 3 (INSL3) production, lower fetal testicular testosterone production or content, increased large Leydig cell aggregations, and increased presence of multinucleated germ cells (MNGs) (NASEM, 2017; Arzuaga et al., 2020; Gray, 2021). The effects on the male reproductive tract induced *in utero* in rats have been attributed largely to impaired testosterone and INSL3 synthesis (Gray et al., 2021; Howdeshell et al., 2008; Li and Spade, 2021). In 2017, NASEM reviewed the available literature and calculated rat maternal benchmark doses (BMDs) for elicitation of a 40 % decrease in fetal testosterone content and/or production (approximately the reduction needed to elicit downstream apical effects) of from 140 to 160 mg/kg/day for DEHP (most potent) to 701 mg/kg/day for DINP (least potent) of those tested (NASEM, 2017). However, differences in testosterone responses and apical effects observed in mice, humans, and non-human primates, compared to the rat, indicate that these effects arise from both androgen-dependent and androgen-independent perturbations in male reproductive tract development (Li and Spade, 2021; Johnson et al., 2012; Veeramachaneni and Klinefelter, 2014).

A hypothesized adverse outcome pathway (AOP) network consisting of two AOPs for the rat phthalate syndrome has been proposed by Li and Spade (Li and Spade, 2021), comprising an antiandrogenic mode of action (MoA) based on Leydig cell effects and an androgen-independent arm mediated through effects on Sertoli cell proliferation and cytoskeletal structure leading to MNGs, testicular dysgenesis, impaired spermatogenesis, and lower fertility. The evaluation of data performed by Li and Spade (Li and Spade, 2021) led them to conclude that the Leydig cell-based arm of their hypothesized AOP is specific to rats, while the hypothesized Sertoli cell-based arm was considered more broadly relevant to humans and mice, as well as rats.

Sertoli cells play critical roles in male reproductive system development in both rodents and humans. In male embryos, expression of the Y chromosome *Sry* gene in gonadal somatic support cells triggers male primary sex determination. These support cells differentiate into pre-Sertoli cells that recruit cells from the mesonephros (embryonic kidney) to become precursors of Leydig cells, peritubular myoepithelial cells, and endothelial cells of the testis. Differentiated Sertoli cells surround the primordial germ cells, and interactions of Sertoli cells with germ cells are critical for development of the sex cords and later for seminiferous tubules and spermatogenesis. Leydig cell progenitor interactions with Sertoli cells facilitate fetal Leydig cell differentiation. Thus, Sertoli cells are critical organizers of the developing sex cords and testes. Sertoli cells proliferate during the fetal period and through postnatal day (PND) 15 in mice and PND 17 in rats. Extension of the period of Sertoli cell proliferation in the rat, thereby increasing their number, has been shown to result in increased size of the testis and increased numbers of germ cells (de Franca et al., 1995). Sertoli cells directly provide soluble growth factors and membrane-bound signals to the developing germ cells (de Franca et al., 2016); these growth factors are critical for maintenance of spermatogonial stem cells, self-renewal of stem cells after birth, and germ cell differentiation.

Effects associated with both the Leydig cell-based arm and the Sertoli cell-based arm of the hypothesized AOP network have been observed in male fetuses following exposure of pregnant rats to high doses (i.e., 288 to 1500 mg/kg/day) of the long-chain phthalate diisononyl phthalate (DINP), 1,2-benzenedicarboxylic acid esterified with branched alcohols consisting of C8-C10 (C9 rich) alkyl side chains (primarily C7 carbon backbone) or esterified with isononyl alcohols, during the MPW (Gray et al., 2000; Boberg et al., 2011; Hannas et al., 2011; Clewell et al., 2013; Clewell et al., 2013; Gray et al., 2023). While perinatal exposure to some phthalates, including di(2-ethylhexyl) phthalate (DEHP) and di(n-butyl) phthalate (DBP), has been demonstrated to induce effects on Sertoli cells (Dostal et al., 1988; Wang et al., 2016; Kleymenova et al., 2005), there is a paucity of studies designed to assess DINP's effects on fetal Sertoli cells. Given that there is some evidence that *in utero* DINP treatment is associated with the non-androgen-dependent effects of rat phthalate syndrome (i.e., increased MNGs and seminiferous tubule effects), a Sertoli cell-based MoA for effects of DINP on the developing testis is plausible. Herein, we evaluate the evidence supporting the potential for DINP to act through a hypothesized Sertoli cell-based AOP, as proposed by Li and Spade (Li and Spade, 2021). An evaluation of the applicability of the hypothesized anti-androgenic Leydig cell-based AOP proposed by Li and Spade (Li and Spade, 2021) (see also (Gray et al., 2021) for effects of DINP is presented in a companion paper by Lea et al. (Lea et al., 2025) as part of an assessment of the endocrine disrupting potential of DINP through the estrogen, androgen, testosterone, and steroidogenesis (EATS) pathways.

2. Methods

2.1. Data identification & extraction

A comprehensive literature review was conducted by Lea et al. (Lea et al., 2025) in PubMed, Embase, and the U.S. Environmental Protection Agency (EPA) Health and Environmental Research Online (HERO) databases for DINP (CASRN 68515-48-0 and CASRN 28553-12-0) and its metabolites mono-isononyl-phthalate (MiNP), mono-oxoisononyl phthalate (MOiNP), mono-carboxyisooctyl phthalate (MCiOP), and mono-hydroxyisononyl phthalate (MHiNP). Additional data were identified through citation mining of authoritative documents, publicly cited stakeholder toxicology reports, and the EPA Toxicity Forecaster (ToxCast).[1] Information regarding the syntax, dates of searches, and inclusion/exclusion criteria are described by Lea et al. (Lea et al., 2025). Through this literature search, data germane to applying the hypothesized Sertoli cell-based AOP to DINP were identified. To ensure that the literature corpus for the hypothesized Sertoli cell AOP was comprehensive, targeted literature searches were conducted in PubMed on April 9, 2024 regarding the MIEs and KEs (Supplement — A. Literature Search Syntax). The data (including aspects related to study design and observed effects) relevant to each MIE, KE, and adverse outcome (AO) in the Li and Spade (Li and Spade, 2021) hypothesized Sertoli cell-based AOP were extracted and collated prior to analysis.

2.2. Study quality assessment and weight of evidence integration

A risk of bias (RoB) assessment was conducted on all studies used in assessing the hypothesized Sertoli cell-based AOP. The RoB assessment was performed with the Office of Health Assessment and Translation (OHAT) RoB tool (NTP-OHAT, 2019). For each endpoint in each study, the following ratings were used for each question: (1) definitely low RoB, (2) probably low RoB, (3) probably high RoB or not reported, and (4) definitely high RoB. An overall RoB tier was determined for each endpoint in each study based on consideration of the ratings for key and non-key questions, as adapted from EFSA (EFSA, 2020): low RoB (Tier 1), medium RoB (Tier 2), or high RoB (Tier 3).

The RoB assessment was performed separately by two scientists with expertise in toxicology, developmental and reproductive toxicology, animal studies, and/or *in vitro* studies. Each RoB assignment underwent quality control by the second scientist to ensure consistency in approach across all studies and endpoints.

A weight of evidence (WoE) approach was used to evaluate whether DINP exposure was associated with each MIE, KE, and AO in the

[1] https://clowder.edap-cluster.com/spaces/647f710ee4b08a6b394e426b (Downloaded May 29, 2024).

hypothesized Sertoli cell AOP proposed by Li and Spade (Li and Spade, 2021). This WoE approach considered the consistency of the effects across studies, such that the overall level of evidence for each MIE, KE, and AO was determined by the proportion of studies that reported each effect following DINP exposure during the MPW, regardless of the RoB evaluation.

3. Results

Fifteen studies were identified that evaluated one or more of the MIEs, KEs, or AOs in the hypothesized Sertoli cell AOP for DINP; the limited availability of data did not support evaluation of KE relationships (KERs), which are the causal links in AOPs. A medium (Tier 2) RoB was determined for PPARγ binding, as measured by surface plasmon resonance spectroscopy (Schaffert et al., 2022). A high (Tier 3) RoB was determined for all other study endpoints considered in the evaluation (Supplement — B. RoB Assessment). The most common sources of potential bias were not concealing the allocation to study groups, not blinding researchers who evaluated the endpoint, not characterizing the test chemical and/or exposure, using inadequate or small sample sizes, using inappropriate statistical methods or none at all, and not evaluating endpoints at the most sensitive time point. No studies were excluded from the AOP assessment based on RoB determination (Supplement — C: Sertoli Cell Mediated AOP; D: AOP Supporting Evidence). Available evidence for DINP for each MIE, KE, and AO in the hypothesized Sertoli cell-based AOP (Fig. 1) is presented.

3.1. Molecular initiating events (MIEs)

The MIE(s) that initiate(s) the hypothesized Sertoli cell-mediated AOP in the fetal testis is (are) unknown, but several have been proposed, including activation or inhibition of peroxisome proliferator-activated receptors (PPARs), prolonged chicken ovalbumin upstream promotor transcription factor 2 (COUP TFII) expression, and decreased arachidonic acid release (Li and Spade, 2021). No DINP-specific data were identified to evaluate the applicability of prolonged COUP TFII expression or decreased arachidonic acid release, so these putative MIEs will not be discussed further. DINP-specific data regarding PPAR activation are available and are discussed below.

Several *in vitro* assays have demonstrated that DINP (which is rapidly metabolized in the mammalian gastrointestinal system to MINP), MINP, and/or MHINP can activate PPARs, with differences in species and isoform specificity and sensitivity. Two transactivation assays demonstrated that DINP and MINP activate human PPARα (hPPARα) and/or mouse PPARα (mPPARα); no data were identified for rat PPARα. MINP activated both mPPARα and hPPARα in transfected mouse 3T3-L1 fibroblasts; mPPARα was more sensitive, with activation at ≥3 μM compared to ≥10 μM for hPPARα, and a higher maximum fold-induction of 27.1 ± 1.9 in mPPARα compared to 5.8 ± 2.1 in hPPARα (Bility et al., 2004). In transfected monkey COS-1 cells, MINP activated hPPARα at 100 μM (highest concentration tested), while DINP did not activate hPPARα (however, only a 1 μM concentration was tested) (Laurenzana et al., 2016). Bioactivity data for MINP were not available from the EPA's ToxCast program; DINP (branched and unbranched) was inactive in one hPPARα transactivation assay.

Similar to the trends for PPARα, mPPARγ was more sensitive to MINP than was hPPARγ, with activation at 3 μM and 30 μM, respectively, and a maximum fold induction of 14.1 ± 1.0 at 200 μM for mPPARγ versus a maximum fold induction at 200 μM for hPPAR of 9.3 ± 1.2 (Bility et al., 2004). In transfected COS-1 cells, hPPARγ was activated at 100 μM MINP (highest dose tested), but no activation occurred at 1 μM DINP (only concentration tested) (Laurenzana et al., 2016). In another assay, concentrations of DINP ≥ 10 μM activated hPPARγ in transfected HepG2 cells (Pomatto et al., 2018). However, in a HEK 293 cell-based reporter gene assay, DINP concentrations of up to 100 μM did not activate hPPARγ, whereas the metabolite MHINP activated hPPARγ at concentrations ≥1 μM (Schaffert et al., 2022). In this same study, binding of recombinant hPPARγ ligand binding domain to DINP or MHINP was measured. DINP did not bind to hPPARγ at concentrations up to 400 μM, while MHINP bound to hPPARγ at concentrations ≥100 μM (Schaffert et al., 2022). DINP (branched and unbranched) bioactivity data from the EPA's ToxCast program were negative for hPPARγ.

The PPARβ isoform is less sensitive to MINP than either PPARα or PPARγ. In transfected 3 T3-L1 fibroblasts, MINP did not activate mPPARβ or hPPARβ at concentrations up to 200 μM (Bility et al., 2004). COS-1 cells transfected with hPPARβ showed significant activation at 100 μM MINP; no activation was observed for DINP at 1 μM (only concentration tested) (Laurenzana et al., 2016). DINP (branched and unbranched) bioactivity data from the EPA's ToxCast program were negative for hPPARβ.

Collectively, these limited data indicate that both mouse and human PPARα and PPARγ isoforms are activated by the DINP metabolites, MINP and MHINP; data are limited for DINP, but one study found activation of hPPARγ by DINP. Given the rapid metabolism of DINP *in vivo*, these *in vitro* assays indicate the potential for activation of PPARα/γ in mice and humans, although with differences in sensitivity between isoforms and species. It appears that mPPARβ and hPPARβ are only weakly activated or not activated at all by DINP or its metabolites.

The WoE indicates that DINP's metabolites MINP and MHINP activate hPPARα/γ *in vitro* (Table 1) and that mPPARα/γ are more sensitive than hPPARα/γ *in vitro;* however, the lack of effect of DINP on PPARα target genes in the fetal testes on GD 18 (Hannas et al., 2012) casts doubt on the likelihood that PPARα activation is an MIE for the hypothesized Sertoli cell-based AOP. Neither the *in vivo* nor the *in vitro* data inform whether PPARs are activated by DINP in the developing male reproductive tract and whether such activation is causally associated with the subsequent Sertoli cell-based KEs.

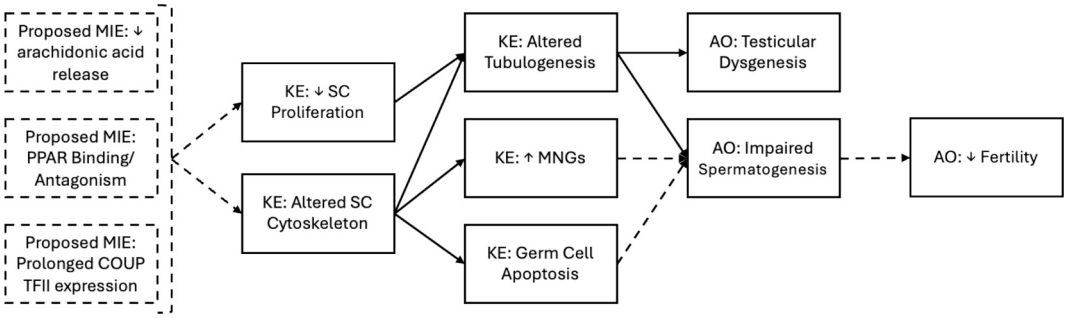

Fig. 1. The hypothesized Sertoli cell-based AOP proposed by Li and Spade (Li and Spade, 2021). AO, adverse outcome; COUP TFII, chicken ovalbumin upstream promotor transcription factor 2; KE, key event; MIE, molecular initiating event; MNG, multinucleated germ cell; PPAR, peroxisome proliferator activated receptor; SC, Sertoli cell.

Table 1
Summary of *in vitro* data pertaining to the proposed MIE and one KE for DINP, MINP, and MHINP.

	Li and Spade (2021) Proposed MIE PPAR Binding/ Antagonism[A]					KE Decreased Sertoli Cell Proliferation
	↑	↔	↑	↔	↑	↔
Concentration (uM)	Evidence that DINP or its metabolites induce this MIE					No evidence that DINP induces KE
	DINP		MINP		MHINP	DINP
1		−, −, −				
3			+, +			
10	+		+			−
30			+			
50		−, −, −				
100			+, +, +		+	
200				−, −		
400		−				

[A]Data shown for PPAR Binding/ Antagonism includes NO(A)EC/LO(A)EC data for both human and mouse PPARs, including all three isoforms (α, β, γ).
+ Each '+' represents data for a PPAR isoform from a study reporting effects for the MIE or KE following *in vitro* exposure, therefore studies that evaluated more than one PPAR isoform will be represented by more than 1 entry in the table; effects are reported at the LO(A)EC.
− Each '−' represents data for a PPAR isoform from a study reporting no effects for the MIE or KE following *in vitro* exposure, therefore studies that evaluated more than one PPAR isoform will be represented by more than 1 entry in the table; a lack of effects (i.e., NO(A)ECs) is reported at the highest concentration used in the study.

3.2. KEs: effects on sertoli cell proliferation and cytoskeleton

No studies were found that examined the effects of DINP on Sertoli cell proliferation or cytoskeleton *in vivo*. Tardif et al. (Tardif et al., 2023) exposed fetal rat testes grown *ex vivo* for three days to multiple chemicals at 10 μM, including DINP and MEHP. While MEHP reduced Sertoli-cell proliferation, showing the ability to detect this effect under the conditions of the experiment, DINP at 10 μM did not. Sertoli cell density was not affected by either MEHP or DINP. While there is no evidence that DINP causes adverse effects on Sertoli cell proliferation, Sertoli cell number, or cytoskeletal organization, the evidence against this KE was not considered robust, because it is derived from a single *ex vivo* study that tested only one concentration.

3.3. KE: altered tubulogenesis

There was limited and conflicting evidence for the effects of DINP on tubulogenesis, as indicated by altered tubule diameter. Boberg et al. (Boberg et al., 2011) dosed Wistar rats from GD 7 to PND 17 by oral gavage with 0, 300, 600, 750, or 900 mg/kg/day DINP. Four dams per group were euthanized on GD 21 for analysis of fetal testicular testosterone production and content and testis histopathology. There was a dose-related increase in testicular histopathological findings, including enlarged seminiferous tubules and multiple gonocytes with central location in tubules at ≥750 mg/kg/day. When all histopathological findings were considered, the percent of animals with testicular histological findings was higher in all DINP-treated groups—statistically significant at ≥600 mg/kg/day—and all fetuses were affected at 750 and 900 mg/kg/day. No maternal toxicity was noted, and birth weight in the postnatal cohort was not affected by DINP; however, pup weight at PND 13 was lower in the 900 mg/kg/day group. While the dose response for these effects is clear, the authors note that only one section from each of five to seven testes from three to four litters was examined. Further, no changes in seminiferous tubule structure were observed when testis histopathology was evaluated at PND 90. Clewell et al. (Clewell et al., 2013) dosed Sprague Dawley rats by oral gavage from GD 12 to 19 with 0, 50, 250, or 750 mg/kg/day DINP. Testis histopathology was evaluated on GD 20, 24 h after the last administered dose. Fetal weight was not affected by DINP. One pair of testes per litter was evaluated histologically and morphometrically. Mean seminiferous tubule diameter was not affected by DINP. Adamsson et al. (Adamsson et al., 2009) administered DINP by oral gavage at 0, 250, or 750 mg/kg/day from GD 13.5 to 17.5 and evaluated testis histopathology on GD 19.5. No effects of DINP on testicular histopathology, including "testicular cords," were observed.

The histopathological findings of the seminiferous tubules following DINP exposure during the MPW are inconsistent. The differences may be attributed to study design differences, including the rat strain used (Wistar in Boberg et al. (Boberg et al., 2011) vs. Sprague Dawley in Clewell et al. (Clewell et al., 2013); duration of dosing (GD 7-17 in Boberg et al. (Boberg et al., 2011) vs. GD 12-19 in Clewell et al. (Clewell et al., 2013); time of evaluation (GD 21 in Boberg et al. (Boberg et al., 2011) vs. GD 20 in Clewell et al. (Clewell et al., 2013) with associated difference in fetal weight at time of assessment (~6 g in Boberg et al. (Boberg et al., 2011) vs. ~4 g in Clewell et al. (Clewell et al., 2013); and different methods for determining changes to seminiferous tubule diameter. Boberg et al. (Boberg et al., 2011) did not describe their method for determining seminiferous tubule diameter (presumed visual inspection, measurements not provided), while Clewell et al. (Clewell et al., 2013) used microscopy image analysis software to assess tubule morphometry and reported actual tubule measurements. No positive controls or historical background data (not required in test guidelines) were presented in these papers. Thus, there is limited evidence that DINP alters tubulogenesis in the developing male rat.

3.4. KE: increased MNGs

There was strong evidence that *in utero* DINP exposure causes increased MNGs in rats. In all four studies evaluated, the incidence or percent of animals or seminiferous tubules with MNGs, and/or the number of MNGs per testis section or tubule section, were increased. Clewell et al. (Clewell et al., 2013) reported a significant increase in the number of animals with MNGs at 750 mg/kg/day DINP, and higher numbers of MNGs per testis section and per seminiferous tubule section at 250 and 750 mg/kg/day DINP. Thus, the no-observed-adverse-effect level (NOAEL) for MNG induction in Clewell et al. (Clewell et al., 2013) was 50 mg/kg/day. Boberg et al. (Boberg et al., 2011) reported increased numbers of fetuses and litters with MNGs at ≥600 mg/kg/day DINP, and a non-statistically significant increase at 300 mg/kg/day, which is the NOAEL for MNG induction in that study.

Li et al. (Li et al., 2015) exposed Sprague Dawley rats by oral gavage to 0, 10, 100, 500, or 1000 mg/kg/day DINP from GD 12 to 21. Litters were collected on GD 21.5. Male fetal weight was significantly lower than controls at all maternal dosages, and there was a clear dose-related increase in the percentage of seminiferous tubules with MNGs at 100 mg/kg/day. Less than 1 % of control seminiferous tubules contained MNGs, while the incidence of tubules with MNGs rose to close to 10 % at the high dose of 1000 mg/kg/day. The NOAEL for MNG increase in this study was 10 mg/kg/day DINP.

Clewell et al. (Clewell et al., 2013) fed Sprague Dawley rats diets containing DINP at 0, 760, 3,800, or 11,400 ppm (reported to be equivalent to 0, 56–109, 288–555, or 720–1,513 mg/kg/day) from GD 12 to PND 14. Maternal body weight was lower than control on GD 20, PND 2, and PND 14 at 11,400 ppm, and male pup weight was lower than control on PND 2 and PND 14 at 11,400 ppm. The number of animals with MNGs was higher at ≥3800 ppm (288–555 mg/kg/day), increasing with dose. The NOAEL for MNG induction in this study was 760 ppm

(56–109 mg/kg/day) DINP in the maternal diet.

The WoE for induction of MNGs is strong, somewhat weakened by the observation that in three of four studies, lower male offspring body weight indicated perinatal systemic toxicity coincident with increased MNGs (Boberg et al., 2011; Clewell et al., 2013; Li et al., 2015). Maternal toxicity was only observed in a single study (Clewell et al., 2013).

3.5. KE: germ cell apoptosis

No evidence was found to evaluate the relationship of developmental DINP exposure with germ cell apoptosis.

3.6. Adverse outcomes (AOs): testicular dysgenesis and impaired spermatogenesis

Only limited evidence supported DINP causing testicular dysgenesis and impaired spermatogenesis. Testicular dysgenesis was defined by studies examining testicular histopathology, agenesis, and atrophy; studies were considered positive if an increased incidence of any of these findings was reported. In three of seven studies, evidence of testicular dysgenesis was reported, although the evidence was weakened based on inappropriate statistical analysis in a few studies, and fetal/postnatal toxicity in some studies (Supplement — C. SC Mediated AOP).

Li et al. (Li et al., 2015) dosed Sprague Dawley rats by gavage with 0, 10, 100, 500, or 1000 mg/kg/day DINP from GD 12 to 21. Fetal weight was significantly lower than controls at all doses, while no maternal toxicity was observed. Testicular dysgenesis was assessed in fetuses at GD 21.5 using desmin staining of peritubular myoid cells. The number of males with focal testis dysgenesis (indicated by disruption of circular tubule cross sections, as seen with desmin staining in controls) was increased at 100 mg/kg/day, so the NOAEL for focal testis dysgenesis was 10 mg/kg/day.

Gray et al. (Gray et al., 2000) dosed Sprague Dawley rats with 0 or 750 mg/kg/day DINP from GD 14 to PND 3 and assessed effects on testis anatomy and histology at PND 2 and 3–7 months of age. DINP had no effect on maternal or male offspring body weights. At PND 2, there were no testicular histopathological findings. However, at 3–7 months, these investigators reported one male with bilateral testicular atrophy (described as small and malformed testes with atrophic tubules devoid of spermatids) and another male with fluid-filled testis and epididymal agenesis. Statistical analysis was done for combined malformations of the male reproductive tract, so no separate statistical analyses of these low-incidence findings were reported. It was noted that these effects had never been observed in control animals in this laboratory over several decades. In the study by Gray (Gray, 2023), Sprague Dawley rats were dosed by oral gavage with 0, 1,000, or 1,500 mg/kg/day DINP from GD 14 to PND 3. Maternal body weight and male offspring body weight at PND 210-240 were not affected. A higher number of males with seminiferous tubule hypospermatogenesis/atrophy and epididymal atrophy was observed at PNDs 210–240 at both dose levels.

van den Driesche et al. (van den Driesche et al., 2020) dosed Wistar rats with 0, 125, or 750 mg/kg/day DINP from GD 15.5 to 18.5. Fetuses were evaluated at GD 17.5 and 21.5, and there was no evidence of testicular dysgenesis, as characterized by fetal Leydig cell aggregations and/or ectopically localized Sertoli cells. Adamsson et al. (Adamsson et al., 2009) dosed Sprague Dawley rats with 0, 250, or 750 mg/kg/day DINP by oral gavage from GD 13.5 to 17.5. Fetuses were collected on GD 19.5 for histological and ultrastructural evaluation of the testes. No effects of DINP on these endpoints were observed.

Boberg et al. (Boberg et al., 2011) dosed Wistar rats with 0, 300, 600, 750, or 900 mg/kg/day DINP from GD 7 to PND 17. At PND 90, there were no histological changes indicative of testicular dysgenesis at any dose.

Waterman et al. (Waterman et al., 2000) dosed Sprague Dawley rats with 0, 2000, 4000, or 8000 ppm DINP in the diet from GD 0 to PND 21 and examined male offspring at young adulthood. No changes consistent with testicular dysgenesis were reported (NOAEL of ≥8000 ppm or approximately 500 mg/kg/day).

In two of three studies, there was some evidence of changes in spermatogenesis from *in utero* exposure to DINP. Masutomi et al. (Masutomi et al., 2003) exposed Sprague Dawley rats to DINP in the diet at 0, 400, 4000, or 20,000 ppm from GD 15 to PND 10. Maternal body weight during gestation and lactation and male pup weight from PND 2 to 10 were significantly lower than controls at 20,000 ppm (calculated dose 1164 mg/kg/day during gestation and 2658 mg/kg/day during lactation). Testes were evaluated at PND 27, and degeneration of stage XIV spermatocytes and Sertoli cells was observed at 20,000 ppm DINP; the changes were described as minimal to slight, but four of five animals were affected. Based on this finding, the NOAEL was 4000 ppm DINP in the diet (calculated dosage 307 mg/kg/day in gestation, 657 mg/kg/day in lactation). In the Gray studies (Gray et al., 2000; Gray, 2023), evidence for effects on spermatogenesis was limited; only one male from the 750 mg/kg/day DINP group had hypospermatogenesis (Gray et al., 2000), and there were one, three, and three animals with hypospermatogenesis at 0, 1000, and 1500 mg/kg DINP, respectively (Gray, 2023).

Overall, there is some evidence that DINP can induce testicular dysgenesis and impaired spermatogenesis, although the evidence is more consistent for the latter. Because some of the AOs were observed at very low incidence, inconsistent findings may be due to differences in sample sizes and power to detect rare events in the studies. Masutomi et al. (Masutomi et al., 2003) examined only five males per control and DINP treated groups; van den Driesch et al. (van den Driesche et al., 2020) examined 38 males per group; and Gray et al. (Gray et al., 2000; Gray, 2023) examined a combined 110–161 controls (varied by endpoint) and 34–52 DINP-treated males per group.

3.7. AO: reduced fertility

In a two-generation study in rats by Waterman et al. (Waterman et al., 2000), there was strong evidence for no effect of DINP exposure on fertility up to 8000 ppm in the diet (highest concentration tested, approximately 500 mg/kg/day). In support of these findings, there were no consistent changes in sperm count, percent progressive motility, or velocity in either the Boberg (Boberg et al., 2011) or van den Driesche (van den Driesche et al., 2020) studies, with highest administered doses of 900 and 750 mg/kg/day, respectively. (Supplement — D. AOP Supporting Evidence).

4. Overall WoE for applicability of the hypothesized sertoli cell-based AOP to DINP

The *in vitro* evidence in kidney, liver, and fibroblast-like cell lines indicates that DINP metabolites MINP and MHINP (and to a lesser extent, DINP) can bind or activate both human and rodent PPARs (Table 1). While DINP/MINP activate PPARs *in vitro*, no *in vitro* or *in vivo* evidence for DINP or its metabolites was identified to assess the potential for a relationship between this putative MIE in fetal Sertoli cells and the first two KEs (reduced Sertoli cell proliferation and Sertoli cell cytoskeletal changes). Potential effects of DINP on Sertoli cell proliferation have been inadequately studied, so evidence is lacking to determine if DINP exposure *in vivo* causes these effects. While Masutomi et al. (Masutomi et al., 2003) reported a “minimal to mild” vacuolar degeneration of Sertoli cells at a high dietary concentration (20,000 ppm) of DINP, Sertoli cell proliferation and/or Sertoli cell number were not assessed. Sertoli cell number and cytoskeletal organization were unaffected by exposure of fetal testes to 10 mM DINP *in vitro*, but other concentrations were not tested (Tardif et al., 2023).

The three studies that examined the KE, altered tubulogenesis, had conflicting findings concerning testis histopathology, so the evidence for DINP causing altered tubulogenesis is limited. The studies used two different rat strains, different durations of dosing, and different ages of

the animals at evaluation, as well as different techniques for assessing the key finding of enlarged seminiferous tubule diameter. Thus, further research would be needed to evaluate the potential effect of DINP on this KE.

The strongest evidence for any DINP-induced KE in the hypothesized Sertoli cell-based AOP is for induction of MNGs. All four studies that evaluated MNGs after DINP exposure reported increases in the percent of animals or seminiferous tubules with MNGs and/or the number of MNGs per testis or tubule section. The WoE for induction of MNGs is slightly weakened by the observation that in three of four studies, lower

Table 2
Summary of events from DINP studies with *in utero* exposure in support of a hypothesized Sertoli cell-based AOP.

Changes in Sertoli cell development leading to altered seminiferous cord development and decreased fertility.[1]
Altered Sertoli cell proliferation and cytoskeletal processes leading to seminiferous tubule development and germ cell outcomes that result in impaired spermatogenesis and reduced fertility.

	Description	Lines of evidence[2]
MIE (proposed)[3]	PPAR binding / antagonism	**Evidence that DINP's metabolites, MINP and MHINP, induce this proposed MIE** **Limited evidence that DINP induces this proposed MIE** • DINP metabolites, MINP and MHINP, activate PPARα, PPARβ, and/or PPARγ in transfected kidney cell lines (Schaffert, 2022; Laurenzana, 2016) • DINP metabolite MHINP, but not DINP, bound the recombinant hPPARγ ligand-binding domain (Schaffert, 2022) • DINP metabolite MINP activates PPARα and PPARγ in a transfected adipocyte cell line (Bility, 2004) • Limited evidence that DINP activates PPARs in kidney cell lines; one study showed PPARγ activation by DINP in HepG2 cells (Pomatto, 2018) but other *in vitro* and ToxCast assays consistently demonstrated no activity (Schaffert, 2022; Laurenzana, 2016)
KE	Decreased Sertoli cell proliferation	**No evidence that DINP induces this KE** • No effect on Sertoli cell proliferation in cultured fetal rat testis (GD 15.5) exposed to a single dose of DINP (10 μM) for 3 days (Tardif, 2023)
KE	Altered Sertoli cell cytoskeleton	• No data to evaluate this KE
KE	Altered tubulogenesis	**Limited evidence that DINP induces this KE** • Limited and weak evidence for increased seminiferous tubule diameter in one of three studies • Tubule diameter increased in GD 21 testis at ≥ 750 mg/kg/day DINP (Boberg, 2011) but not in another study of GD 20 testis conducted using similar dose levels (Clewell, 2013) • No histopathological changes in testicular cords were observed on GD 19.5 at up to the highest DINP dose tested, 750 mg/kg/day (Adamsson, 2009) • In the study in which increased tubule diameter was observed, there was no maternal toxicity, but F1 male offspring had significantly decreased body weight at PND 13 at 900 mg/kg/day (Boberg, 2011) • Inconsistency between studies may be due to the different exposure windows or different measurement methodology
KE	Increased MNGs[4]	**Evidence that DINP induces this KE** • Evidence for increased incidence of MNGs in fetal and neonatal rat testis (GD 19, 21, and PND 2) at doses ≥100 mg/kg/day (Boberg, 2011; Clewell, 2013; Clewell, 2013; Li, 2015)
KE	Germ cell apoptosis	• No data to evaluate this KE
AO	Testicular dysgenesis	**Limited evidence that DINP induces this AO** • Limited evidence of testicular dysgenesis, as measured by changes in testicular histopathology, agenesis, and/or atrophy • Testicular dysgenesis reported in three of seven studies, although the evidence was weakened by the lack of statistical analysis and potential for fetal toxicity • Effects occurred in neonates (GD 21.5) and F1 adult animals at doses ≥ 100 mg/kg/day (Gray et al., 2000; Gray, 2023; Li, 2015) • No effects in studies sampled at similar timepoints (Boberg, 2011; Adamsson, 2009; van den Driesche, 2020; Waterman, 2000) • Inconsistency between studies may be due to differences in statistical power, exposure periods, measured endpoints, or measuring sensitivity
AO	Impaired spermatogenesis	**Some evidence that DINP induces this AO** • Some but weak evidence of impaired spermatogenesis • Minimal/slight degeneration of stage XIV spermatocytes in PND 27 males at the highest concentration of DINP of 20,000 ppm (~1165–2657 mg/kg/day DINP), the dose at which there was reduced maternal and fetal body weight (Masutomi, 2003) • Increased number of males with hypospermatogenesis / atrophy and epididymal atrophy (LO(A)EL 1,000 mg/kg/day) in adult F1 males; no statistical analysis performed (Gray, 2023) • No changes in incidence of F1 adult males with impaired spermatogenesis in a single dose study (750 mg/kg/day) (Gray et al., 2000) • Inconsistency between studies may be due to different exposure periods, measured endpoints, sensitivity and precision of measurements, chance, confounding, or bias
AO	Decreased fertility	**No evidence that DINP induces this AO** • No evidence that exposure to DINP at dose levels up to 8,000 ppm (~500 mg/kg/day) results in decreased fertility (Waterman, 2000)

[1] Events and outcomes associated with phthalate toxicity proposed by Li and Spade (Li and Spade, 2021).

[2] Lines of evidence were categorized as follows: 'Evidence' = all studies provide consistent evidence of an effect; 'Some' = majority, but not all, studies provide consistent evidence of an effect (>50 % and < 100 % of studies); 'Limited' = a few studies provide consistent evidence of an effect (>1 study but ≤ 50 % studies); "Very limited' = evidence of an effect observed in a single study; 'No evidence' = none of the studies provided evidence of an effect.

[3] PPAR activation is one of the three proposed MIEs for this hypothesized AOP by Li and Spade (Li and Spade, 2021); however, no biological evidence has been identified that links this putative MIE with the KEs in this hypothesized AOP.

[4] Increased MNGs and germ cell apoptosis are hypothesized to contribute to impaired spermatogenesis and ultimately decreased fertility in this hypothesized AOP; however, data directly evaluating the KERs between these KEs and AOs were not evaluated.

male offspring body weight was coincident with increased MNGs, indicating that systemic toxicity may contribute to this testes-specific finding.

No data were found on which to base an evaluation of the potential for DINP to cause germ cell apoptosis, the other KE in this hypothesized AOP.

For the AO testicular dysgenesis, studies had inconsistent findings, at least in part due to different group sizes, different approaches to evaluate testis histology, and different ages at which this endpoint was evaluated, ranging from the perinatal period to adulthood. There was limited evidence that DINP caused testicular dysgenesis, with three of seven studies reporting significant testicular histopathology consistent with testicular dysgenesis. In general, it is difficult to assess consistency of study outcomes for this endpoint with DINP, simply because of a lack of standardization of what and when observations are relevant for characterizing testicular dysgenesis. Further, differences in study design exacerbate the difficulty of comparing findings of the positive and negative studies.

Regarding impaired spermatogenesis, there was some support for this AO; two of three studies reported adverse effects on seminiferous tubules, indicating reduced spermatogenesis. However, the histopathological analyses were limited and did not include quantitative evaluations. Finally, the last AO, decreased fertility, was not caused by DINP, although only one study was identified that directly evaluated fertility in adult offspring exposed *in utero* (Waterman et al., 2000). Two additional studies (Boberg et al., 2011; van den Driesche et al., 2020) were identified that evaluated sperm parameters (i.e., motility, velocity, count, head count) and were considered supportive evidence relevant to, but not direct evidence of, effects on fertility (Supplement — D: AOP Supporting Evidence), because in rodents, fertility can be maintained in the face of significant reductions in sperm production (Amann et al., 1986).

Relevant findings of studies providing data applicable to the hypothesized Sertoli cell-based AOP are summarized in Table 2; the overall WoE for each proposed MIE, KE, and AO in the hypothesized AOP is illustrated in Fig. 2; and a comparison of available NOAELs and LOAELs for effects of *in vivo* exposure to DINP on KEs and AOs is presented in Table 3.

5. Discussion

Evidence from fifteen studies was assessed for the applicability of the hypothesized Sertoli cell-based AOP of Li and Spade (Li and Spade, 2021) to DINP. Critical appraisal of these studies showed all but one to have a high risk of bias (Tier 3), lowering confidence in the findings. There was little evidence that DINP produces adverse effects through the hypothesized Sertoli cell-based AOP.

Regarding the proposed MIEs, there was evidence that the DINP metabolites MINP and MHINP activate PPARs *in vitro* in kidney, liver, and fibroblast-like cell lines; however, no studies directly evaluated the relationship between PPAR activation in fetal Sertoli cells by DINP or its metabolites and the Sertoli cell KEs. While PPARα and PPARβ are expressed in adult rat Sertoli cells and PPARγ is weakly or not expressed (reviewed by Corton and Lapinskas (Corton and Lapinskas, 2005), only a few studies have evaluated whether PPARs are expressed in the fetal testis during the MPW, and to our knowledge, none have evaluated PPAR expression specifically in fetal Sertoli cells during the MPW. Although other phthalates (i.e., DEHP, diisobutyl phthalate [DiBP]) may affect the expression of some PPARs in the fetal testis (Borch et al., 2006; Boberg et al., 2008); Hannas et al. (Hannas et al., 2012) examined expression of PPARs and PPAR-related genes in the fetal rat testes after maternal exposure to several phthalates, including DINP, and found a general lack of changes in gene expression, with a single PPAR-related gene (*Acox1*) downregulated by DINP at the high dose of 1500 mg/kg/day. Thus, these data support that DINP does not affect PPAR expression in the fetal testis and has no to minimal effects on PPAR-associated target genes. Thus, although *in vitro* studies in kidney, liver, and fibroblast-like cell lines support DINP, MINP, and MHINP activation of PPARs, the *in vivo* biological relevance of this putative MIE in fetal Sertoli cells is unknown.

Regarding the first two KEs, there was a lack of evidence concerning how DINP exposure affects fetal Sertoli cell proliferation or cytoskeletal structure. As discussed above, Matsutomi et al. (Masutomi et al., 2003) reported degeneration of Sertoli cells in 27-day-old offspring of Sprague Dawley rats exposed to DINP in the diet at 20,000 ppm from GD 15 to PND 10, but fetal testes were not examined, and the changes were described as minimal to slight. While evidence is lacking for DINP, there is evidence that some phthalates with shorter chain lengths than DINP, such as DBP, disrupt the development of the male reproductive tract, including limited evidence for effects on Sertoli cell proliferation and/or cytoskeleton. Kleymenova et al. (Kleymenova et al., 2005) dosed Sprague-Dawley rats with 500 mg/kg/day DBP orally from GD 12 to GD 16–20 and observed Sertoli cells with retracted apical processes, altered vimentin cytoskeleton, and abnormal contacts with gonocytes. These authors hypothesized that abnormal contacts between Sertoli cells and gonocytes played a role in the observed increase in MNGs. Van den Driesche et al. (van den Driesche et al., 2015) treated Wistar rats with DBP from GD 13.5 to termination on GD 17.5, GD 21.5, or PND 4, and studied the effects of DBP on human fetal testis xenografts in nude mice.

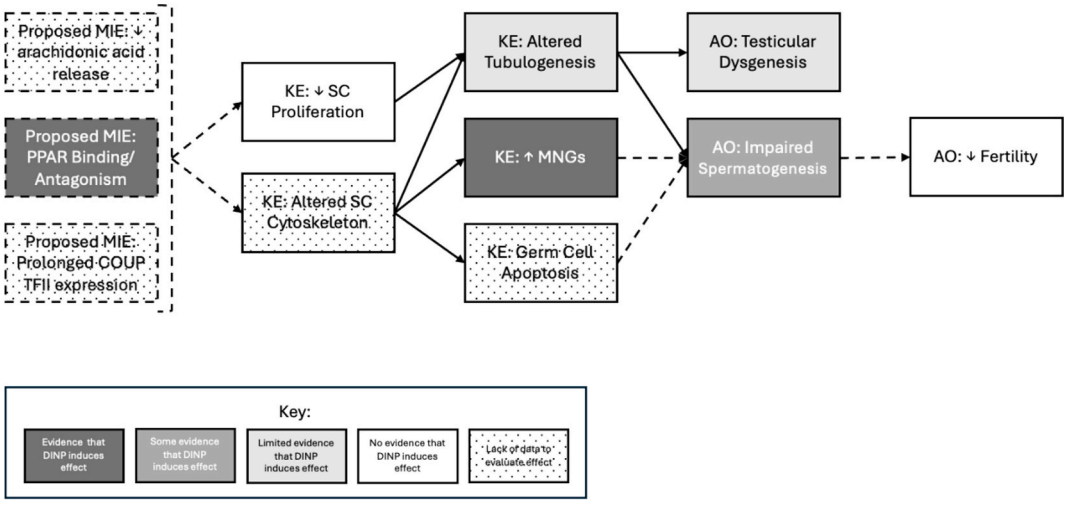

Fig. 2. WoE for each MIE, KE, and AO in the hypothesized Sertoli cell-based AOP. The dashed lines indicate the uncertainty surround the MIE and the KERs for this AOP.

Table 3
Dose concordance of *in vivo* evidence for DINP for KEs and AOs of the hypothesized Sertoli cell-based AOP.

	KEs*		AOs		
	Altered Tubulogenesis Tubule diameter	**Increased MNGs**	**Testicular Dysgenesis**	**Impaired Spermatogenesis**	**Decreased Fertility**
Dose (mg/kg/day)	↑ ↔ **Limited Evidence that DINP induces KE**	↑ **Evidence that DINP induces KE**	↑ ↔ **Limited Evidence that DINP induces AO**	↑ ↔ **Some evidence that DINP induces AO**	↔ **No evidence that DINP induces AO**
100		(+)	(+)		
250		+			
422†		+			
500			–		–
600		+			
750	+ –,–		+ –,–,–	–	
900	–		–		
1000			+	+	
1911‡				(+)	

Each '+' represents a study reporting effects for the KE or AO following exposure *in vivo* exposure during the MPW; effects are reported at the LO(A)EL.
Each '−' represents a study reporting no effects for the KE or AO following exposure *in vivo* exposure during the MPW; lack of effects (i.e., NO(A)ELs) are reported at the highest concentration used in the study.
Symbols in parentheses () show studies also reporting loss of maternal and/or fetal body weight at the same dose.
* Only KEs for which there are *in* vivo data are included in this table.
† Dose reported represents the mid-point of the dose range provided by the author that corresponded to the 3,800 ppm dietary concentration at this LOAEL.
‡ Dose reported represents the mid-point of the dose range provided by the author that corresponded to the 20,000 ppm dietary concentration at this LOAEL.

In rats, germ cell aggregation on GD 21.5 was observed at maternal doses ≥20 mg DBP/kg/day. Staining for cell–cell adhesion molecules revealed an absence of direct Sertoli cell–germ cell contact coincident with germ cell aggregation and staining for vimentin, and N-cadherin showed disruption of the Sertoli cell cytoskeleton. Effects on human fetal testis xenograft Sertoli cell structure and germ cell aggregation were minimal, but both rat fetal testis and human fetal testis xenografts showed increased MNGs. These studies support the relationship between Sertoli cells effects and MNGs for DBP, but the potential for DINP to act via this hypothesized AOP has not been adequately explored.

Integration of evidence to support MIEs, KEs, and AOs (Fig. 2 and Supplement — C. SC Mediated AOP) showed no or only limited evidence for DINP causing most KEs (i.e., decreased Sertoli cell proliferation, altered Sertoli cell cytoskeleton, and altered tubulogenesis), strong evidence for DINP eliciting one KE (increased MNGs), some or limited evidence for the AOs testicular dysgenesis and impaired spermatogenesis, and no evidence that DINP causes decreased male fertility (the final AO). There was insufficient evidence for DINP to plausibly link early KEs to AOs on the male reproductive tract and fertility through the hypothesized testosterone-independent, Sertoli cell-based AOP (e.g., uncertainty regarding the KERs).

Most proposed AOP networks related to rat phthalate syndrome include KEs involving changes in testicular gene expression, structural fetal Leydig cell effects, and lower testicular androgen production (Arzuaga et al., 2020; Gray et al., 2021; Howdeshell et al., 2017; Howdeshell et al., 2015; Kortenkamp and Faust, 2010); however, other proposed AOP networks incorporate changes to Sertoli cells and histopathological lesions in the testis (Arzuaga et al., 2020). A commonality between these latter proposed AOPs is a degree of reliance on KEs that aggregate multiple biological, cellular, or target organ effects into a single event (Palermo et al., 2021), thereby insufficiently reflecting the mechanistic granularity in the collective research on phthalate syndrome. Li and Spade (Li and Spade, 2021) have compiled a hypothesized AOP network that greatly improves the refinement of KEs, pathway bifurcations, and cellular targets during this critical window of development (i.e., the MPW). While many uncertainties remain (including those related to the KERs), this hypothesized AOP provides a framework for organizing and assessing evidence in a more biologically precise manner, providing confidence in understanding a chemical's potential to induce AOs. It remains to be determined whether refinement between androgen- and non-androgen-dependent phthalate syndrome-associated effects will facilitate identifying improved biomarkers of effect or chemical management decisions that rely on improved quantitative risk assessment. However, doing so does have clear implications for chemical management decisions that depend on hazard classification paradigms in which endocrine disruption exists as a unique hazard class, such as that recently implemented in the European Union (per the Commission Delegated Regulation (EU) 2023/707 amending the Classification, Labelling and Packaging (CLP) Regulation ((EC) No 1272/2008). The hypothesized Sertoli cell-based AOP, in which lower Sertoli cell proliferation and cytoskeletal changes result in MNGs, tubular dysgenesis, germ cell loss, and decreased fertility is largely non-endocrine; however, androgen insufficiency has been reported to contribute to germ cell loss (Li and Spade, 2021; Benbrahim-Tallaa et al., 2008). The evidence is strong that exposure to DINP during the MPW leads to an increased incidence of MNGs in rats around the time of birth. The long-term consequences of this finding are unclear, because MNGs in the testis, likely formed from fusion of gonocytes via intercellular bridges, may be associated with spermatogonial apoptosis, a normal feature of testis development, and few if any MNGs persist beyond the neonatal period. There was some evidence for impaired spermatogenesis by DINP, but the relationship of this and induction of MNGs is unknown. There was also some evidence that developmental exposure to DINP induced testicular dysgenesis in fetal (GD 21.5; (Li et al., 2015) or adult (~210 days old; (Gray, 2023) rats. It is important to note that the focal dysgenesis reported by Li et al. was limited and did not include malformed seminiferous cords or altered cord diameter. DINP did not affect fertility in a two-generation study up to approximately 500 mg/kg/day, albeit at lower doses than were used in other studies. These results were supported by two other studies that showed limited evidence of effects on sperm parameters at doses up to 900 mg/kg/day (Boberg et al., 2011; van den Driesche et al., 2020).

Overall, a combination of data gaps and negative findings for most KEs and AOs means that the evidence that DINP causes toxicity through the hypothesized Sertoli cell-based AOP is insufficient. Where there is evidence for an effect of DINP on elements of the hypothesized Sertoli cell-based AOP, the *in vivo* dose levels are typically high (e.g., 750 mg/kg/day by oral dosing during the MPW), and the incidence of adverse effects is low. Applicability of such high testing doses was a limitation of this dataset. Large studies, focused on the MIEs, KEs, and KERs of the

hypothesized Sertoli cell-based AOP, would be needed to clarify the applicability of this hypothesized AOP to DINP.

Funding

This work was supported by ExxonMobil Biomedical Sciences, Inc. The contents of this manuscript solely reflect the view of the authors. Contribution to this manuscript by Dr. Christine Palermo was made while employed by a producer of DINP.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Dr. Neepa Choksi for support performing the RoB assessments, Dr. Susan Borghoff for contribution to the conceptualization of this manuscript, Sarah Rogers for reference management, and Rick Nelson for editorial review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crtox.2025.100219.

Data availability

No new data were generated in producing this manuscript; all data are from papers published in the open literature.

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