Di-n-pentylphthalate (DPP), CAS no.131-18-0

Synonyms: Dipentyl phthalate (DPeP), Diamyl phthalate, Amyl phthalate, Amoil, di-n-Amyl phthalate

Di-n-pentyl phthalate (C_{16} H_{26} O_4) is a phthalate ester used as a plasticizer (Figure 1). Since no registration dossiers in REACH on DPP are available, it is assumed that it is produced and/or imported to EU in tonnages less than 100 tpa. In EU (classification) the substance is classified as Rep. Cat. 1B (H360: May damage fertility or the unborn child) and as hazardous to the environment/ acute hazard category 1 (H400: very toxic to aquatic life).

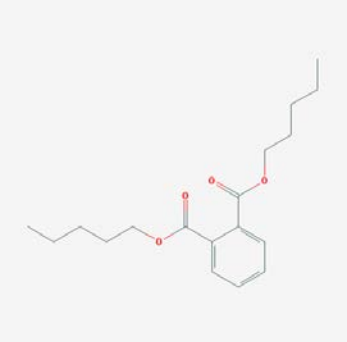

Figure 1: 2D structure from PubChem

4. Human health hazard assessment

4.10.3 Endocrine disruption

4.10.3.1 General approach – human health

4.10.3.2 In vitro information indicative of endocrine activity

Yuan et al. (2012)

Summary: In this study, 14 phthalates were tested for their inhibitory action on human and rat testicular 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3enzymes (17β-HSD3) (with exposure to 1mM phthalate) and their activity was compared with their structure. The inhibitory effect of a range of concentrations of the phthalate (10^{-7}-10^{-3} M) was tested on the microsomal enzymes prepared from human and rat testis. The enzymes are important in testosterone synthesis in Leydig cells. The enzyme 3β-HSD catalyses conversion of pregnenolone to progesterone and 17β-HSD3 catalyses conversion of androstenedione to testosterone.

Activity of 3β-HSD was reduced by more than 50% in both rat and human testis and 17β-HSD3 was also reduced in rat and human testis by DPP. Altogether, the results demonstrated that phthalates with ethanol moieties consisting of 1-2 or 7-8 carbon atoms had no effects on the enzyme activities.

Study quality and assessment: The study is assessed to be of high quality and the study provides moderate evidence of an endocrine mode of action of DPP on testosterone synthesis.

Christen et al. (2010)

Summary: In this study *in vitro* androgen receptor reporter gene assays for androgenic and anti-androgenic activity was performed. Partial inhibition curves were observed for DPP whereas no notable androgenic activity was seen at the concentrations tested (3.3, 67 and 334 µM DPP).

Study quality and assessment: The study is well described, included triplicates of the assays, included controls and tested concentrations of DPP below cytotoxic levels. This study is assessed to be of high quality. The study provides strong evidence for anti-androgenic mode of action of DPP *in vitro*.

Creasy et al. (1988)
Summary: The effects of mono-*n*-pentyl phthalate (MPP) on Sertoli cell ultrastructure and germ cells *in vitro* was investigated and compared with the effects of DPP on these cell types *in vivo*. Sertoli and germ cell cultures from Sprague-Dawley rat testis was exposed to MPP at 0, 100, 200, 500 or 1000 µM for 3 or 24 hours. Cell morphology was examined under transmission electron microscope and scanning electron microscope and evaluation of the distribution of actin filaments was performed on the cultured cells.

Sertoli cells *in vitro* changed in shape and developed microprocesses between Sertoli cells and germ cells after MPP exposure (200-1000 µM). Reorganization of actin filaments was seen *in vitro* after MPP exposure (500-1000 µM), a change that correlated well with changes in Sertoli cell shape *in vivo* after DPP exposure. Rarefaction of Sertoli cell cytoplasm *in vivo* correlated with mitochondrial hypertrophy *in vitro*.

Study quality and assessment: The study is old and the *in vivo* part of the study is relatively superficially described. The *in vitro* part of the study is described in more details than the *in vivo* part, yet some information on the number of samples and replicates used per group and assessment of cytotoxicity is not described. The *in vitro* part of the study is assessed to be of low quality. The data provide weak evidence that MPP can lead to adverse effects on the testicular Sertoli cells.

4.10.3.3 In vivo effects with regard to an endocrine mode of action

Gray Jr et al. (2016)
Summary: The aims of this study were to (1) determine the reductions necessary in testosterone production, testicular testosterone levels and foetal rat plasma testosterone to induce reproductive malformations and (2) determine the reduction in foetal testosterone necessary to induce postnatal adverse effects in the offspring. In this paper two animal studies are described with exposure to DPP by oral gavage of time-mated rats (0, 11, 33, 100, 300 mg/kg/day). In the foetal experiment dams were dosed GD14-18 and animals were euthanized on GD18 (n=7-9 litters per group). Testes and blood samples from foetuses were collected and endpoints for assessment of maternal toxicity was recorded (maternal weight gain, number of resorptions, live foetuses etc.). Testis weight was recorded (n=up to 3 males from 5-6 litters/group), foetal testosterone levels in plasma and testis was measured, the descent of testis was evaluated, the *ex vivo* testosterone production in testis and gene expression of foetal testes was investigated. In the postnatal experiment, dams (n=5 litters per group) were dosed GD 8-18 and necropsy of the offspring was performed when they were 120 days old. Age and weight at sexual maturation of male and female offspring were recorded. At necropsy of female offspring, gross malformations and weights of pituitary, uterus, ovaries, kidneys and livers were recorded. In male offspring gross malformations, length or gubernacular cord and weight of glans penis, seminal vesicle, ventral prostate, testis, epididymis, levator ani-bulbocavernosus muscles, Cowper's glands, kidney and liver were assessed. Testis and epididymis were processed for histopathology and male and female skulls were processed for morphometric analysis.

Assessment of maternal toxicity in the postnatal study showed decreased maternal weight gain and lower pup survival in the highest exposure group (300 mg/kg/day). In the foetal study, delayed testis descent (1 and 13 male pups with undescended testis out of 14 and 13 examined males from the 100 and 300 mg/kg dose-groups, respectively), decreased foetal testis weight (at 300 mg/kg/day) and decreased testosterone levels in plasma and testis (at 33-300 mg/kg/day) and decreased *ex vivo* testicular testosterone production (at 33-300 mg/kg/day) was found. Decreased male anogenital

distance (AGD) and nipple retention was observed in offspring from the two highest exposure groups in the postnatal experiment (2 out of 14 and 9 out of 13 examined males from each 4 litters, in 100 and 300 mg/kg/day, for nipple retention respectively). Malformations of adult male reproductive organs were seen in high numbers in the highest exposure group (50% of examined animals or more; n=13 males examined from 4 litters in the highest exposure group compared to n=30 males examined from 5 litters in controls). The malformations observed were hypospadias, agenesis of vas deferens, agenesis or abnormal seminal vesicle, abnormal testis, agenesis of epididymis, agenesis or elongated gubernaculums and undescended testis. Few males (n=2) had small ventral prostates in the highest exposure group. Weights of reproductive organs (seminal vesicle, epididymis, testes, ventral prostate, levator ani-bulbocavernosus muscles, Cowper's glands and glans penis) were decreased in 120 days old offspring. Decreased body weight and liver weight were found in adult males. Altogether, this study shows that DPP disrupts foetal testicular endocrine function and induces malformations in reproductive organs of male rats.

Study quality and assessment: The study is well-described and thorough. The number of test and control samples (i.e., n=5-9 litters) is adequate for showing clear effects and the study design is relevant. The study is assessed to be of high quality. The study provides strong evidence for an endocrine mode of action on testosterone steroidogenesis. Moreover, the study provides strong evidence for irreversible adverse effects on male reproductive system such as malformations of male reproductive organs, decreased weight of male reproductive organs, decreased AGD and nipple retention in males.

Beverly et al. (2014)

Summary: The objective of the study was to test two hypotheses: (1) that Simvastatin exposure *in utero* leads to lower foetal testicular testosterone production without changes in the expression of genes involved in cholesterol and androgen synthesis and transport; (2) that Simvastatin and DPP in a mixture reduces testosterone levels in an additive manner as they may have different modes of action. Two animal studies were performed, and one of the studies investigated the effects of DPP. In this study, pregnant rat dams were exposed by oral gavage on GD 14-18 to 50 mg/kg/day of DPP (3-4 dams/group) and euthanized GD 18. Samples from dams included blood (for clinical chemistry, e.g. cholesterol, creatinine and bilirubin), weights and gene expression of liver, adrenals and ovaries and histopathology of livers. Samples from offspring included blood (for clinical chemistry), testis for *ex vivo* foetal testicular testosterone production and gene expression of adrenals and testis.

Ex vivo foetal testicular testosterone production was measured on GD 18 and was found to be decreased. Genes involved in cholesterol transport and synthesis, sex differentiation and steroidogenesis were downregulated in the foetal testis.

Study quality and assessment: The study is well-described with few uncertainties on duration of dosing. The number of test and control samples (i.e., n=3-4 litters) is adequate based on power analysis. The study is assessed to be of high quality. The study provides strong evidence for an endocrine mode of action on testosterone steroidogenesis.

Hannas et al. (2012)

Summary: This study had three objectives. Firstly, to determine if phthalates with an anti-androgenic mode of action act through similar mechanisms on the gene expression level, secondly, to determine if the potency for the individual phthalates to reduce testosterone production correlates with their potency to reduce gene expression. Third, test a model prediction of the ability of a mixture of 9 phthalates to reduce gene expression. Several phthalates were tested in this study (DPP, diisobutyl, dihexyl, diheptyl, diisononyl and diisodecyl phthalate) for effects in testis after gestational exposure and evaluation of the relative potency of the tested phthalates. Pregnant rat dams were exposed by oral gavage GD 14-18. Animals exposed to DPP were dosed with 0, 11, 22, 100 or 300 mg/kg/day. A gene array screen on testis from GD18 foetuses was performed for DPP and downregulation was found for several genes involved in steroid synthesis (in testis and adrenals), steroid regulation and steroid transport. DPP was found to be more potent in downregulation of steroid-related genes than the other phthalates tested. This was in good coherence with the high potency of DPP to decrease testosterone production and inducing malformations in the reproductive tract postnatally, as reported in a previous study (Hannas et al. 2011). The data demonstrated that the effects of phthalates on gene expression of androgen-related genes were linked to the reduced testosterone production.

Study quality and assessment: The study is well-described and thorough. The number of test and control samples (one testis from every male foetus in a litter were pooled; n=3-4 litters per group) is adequate. The study is assessed to be of high quality. The study provides moderate evidence for an endocrine mode of action on testosterone steroidogenesis, but when these effects at gene expression level are taken together with data on testosterone synthesis reported in the previous study by Hannas et al. (Hannas et al. 2012), these data provide strong evidence for an anti-androgenic mode of action of DPP.

Hannas et al. (2011)

Summary: The objective was to establish comprehensive dose-response and potency data on foetal and postnatal male reproductive endpoints for DPP. Four different *in vivo* experiments are described. In the first experiment pregnant rat dams were exposed to a single dose of 500 mg/kg DPP on GD 17 (6 dams per group). In the second study, pregnant rat dams were exposed orally to a single dose of 0, 300, 600, 900 or 1200 mg/kg DPP on GD 17 (3 dams per group). In these two first studies *ex vivo* testicular testosterone production in foetuses GD 17 was measured in testis dissected 5-6 hours after maternal exposure. In the third study pregnant rat dams were dosed by oral gavage to 0, 11, 33, 100, 300 mg/kg/day of DPP from GD 14-18 (3 dams per group). Gene expression (of StAR, insl3 and Cyp11a) and testosterone production on GD 18 were assessed in this 5-days repeated-dose study. In the final study on postnatal development pregnant rat dams were dosed by gavage to 0, 11, 33, 100, 300 mg/kg/day of DPP from GD 8-18 (5 dams per group). AGD and NR were evaluated in the last study. Foetal testicular testosterone production was decreased in a dose-related manner and in 5-days repeated-dose study the gene expression levels of the androgen synthesis related genes StAR and Cyp11a and of insl3 were decreased (from 100 mg/kg/day). Decreased AGD (at 100mg/kg/day or more) and increased NR (at 300 mg/kg/day) was found in male offspring exposed to DPP for 10 days *in utero.*

Study quality and assessment: The study is well-described and thorough, although few details on the number of exposed dams in the first study are missing. The study is assessed to be of high quality. The study provides strong evidence for an endocrine mode of action on testicular testosterone

steroidogenesis. The study also provides strong evidence for endocrine-related adverse effects on male sexual differentiation.

Howdeshell et al. (2008)
Summary: The aim was to provide dose-response information on the effects of several phthalates on foetal testosterone production and to investigate if the data could be used to predict effects of a mixture of phthalates on testosterone production. Time-mated rat dams were dosed by oral gavage from GD8-18 with 0, 25, 50, 100, 200, 300, 600 or 900 mg/kg/day of DPP. At study start the number of mated dams was 2-6 per dose-group. On GD18, there were 5, 5, 4, 6, 4, 2, 1 and 2 pregnant dams, respectively. DPP reduced maternal body weight gain GD8-18 at 300, 600 and 900 mg/kg/day and in these dose-groups 100% foetal mortality (resorptions/implantations) was seen. Testosterone production measured in *ex vivo* testis from foetuses exposed to DPP during gestation (GD 8-18) was reduced at 100 and 200 mg/kg/day. The study showed inhibition of foetal testosterone production

Study quality and assessment: This study is well described and includes necessary information such as compound, dosing period and number of animals. The study is assessed to be of high quality. The data provide strong evidence on an anti-androgenic mode of action of DPP on foetal testosterone production.

Hild et al. (2007)
Summary: This study evaluates the time course of acute ultrastructural changes occurring in Sertoli and germ cells after a single dose of CDB-4022 with DPP as a positive control. Four adult male rats per group were exposed orally to a single dose of 0 or 2200 mg/kg DPP and killed after 0, 3, 6, 9, 12, 18 or 24 h after dosing. Controls were killed after 0 or 24 h after dosing. Testes were examined morphologically under light microscope and ultrastructurally in a transmission electron microscope.

Degenerative changes in Sertoli cells, spermatocytes and spermatids were seen after DPP exposure. Spermatogonia appeared unaffected.

Study quality and assessment: The present study is well described and included important information such as animal number, life stage of animals and time point at euthanasia, although more details on CAS-number, exact age of the animals and housing conditions would be preferred. The study is assessed to be of moderate quality. The data from the study provides strong evidence of adverse effects on testes at the cellular level but does not study the underlying modes of action and it is therefore not clear if the adverse effects are due to an endocrine mode of action.

Liu et al. (2005)
Summary: The aim of the study was to identify genes involved in the development of testicular dysgenesis. A selection of phthalates known to affect male reproductive development after *in utero* exposure was used to induce such changes, including DPP. A range of phthalates known not to affect male reproductive development was used as negative controls. Time-mated rat dams (10 controls and 5 per exposed group) were dosed by oral gavage from GD 12-19 to 500 mg/kg/day DPP. Caesarean section of foetuses was performed on GD 19, where AGD was measured and testes were used for gene expression analysis (testis from 1 foetus per litter per group was used for microarray analysis.

For RT-QPCR, 1 testis per litter from 6 litters in the control group and 1 testis per litter from 3 litters in the treated group were used). Immunohistochemistry of testes from control and DBP exposed foetuses was performed.

Male foetuses exposed to DPP *in utero* had reduced AGD compared to controls on GD19. Gene expression levels in GD19 foetal testes exposed to DPP *in utero* was changed for genes involved in regulating steroidogenesis and insulin signalling (e.g. downregulation of Luteinizing hormone/choriogonadotropin receptor (*Lhcgr*), Low-density lipoprotein receptor (*Ldlr*) and Insulin induced gene (*Insig1*)). The phthalates appeared to target Sertoli cells and gonocytes and the interaction between the two.

Study quality and assessment: The study is well described and important information on sample sized, exposure period and age at tissue sampling are described. Although no CAS-number for DPP is mentioned, the study is assessed to be of high quality. The data provide strong evidence of adverse effects on male AGD. The changes in gene expression levels of genes involved in steroidogenesis provide moderate evidence of a mode of action of DPP on steroidogenesis.

Hild et al. (2001)

Summary: In this study, the testicular cell types affected by CDB-4022 were determined. DPP was used as a known Sertoli cell toxicant and estradiol-3-benzoate was used as a positive control of germ dell apoptosis. To study the acute effects on testicular germ cell apoptosis and Sertoli cell function and testicular morphology, fertile male rats were exposed to a single oral dose of DPP (2200 mg/kg, 3 per group) and killed after 3, 6 or 12 hours after dosing. A second study was performed to investigate the acute effects on Sertoli cell function in prepubertal male rats (10 per group) dosed orally with a single dose of 2200 mg/kg DPP and euthanized 24 or 48 hours after dosing. An indicator of the seminiferous tubule fluid (STF) was assessed. Serum inhibin B and epididymal androgen binding protein (ABP) content were assessed in the two studies to evaluate Sertoli cell function. A third study was performed to determine the effects on Leydig cell function and fertility but only exposure to CDB-4022 was investigated.

In the first study (in adult males), an increased percentage of seminiferous tubules with a higher number of apoptotic germ cells (early spermatocytes and spermatogonia) per tubule compared to controls was seen. Morphologically, vacuolization of Sertoli cells and detachment of germ cells was observed. Serum inhibin B was decreased. In the second study (in prepubertal males), STF secretion (by Sertoli cells) was suppressed and serum inhibin B levels and epididymal ABP were decreased. The data indicate that DPP interferes with early stages of spermatogenesis by inducing apoptosis of early stages of germ cells via disruption of Sertoli cell structure and function.

Study quality and assessment: The study is well-described, but more details on housing conditions, age of adult males, CAS-number and purity of DPP would be preferred. The study is assessed to be of moderate quality. The data show decreased Sertoli cell function, disrupted Sertoli cell morphology and apoptosis of germ cells. The study provides strong evidence of adverse effects on testis function and morphology in adult males and decreased function of Sertoli cells in prepubertal male rats after exposure to DPP.

Jones et al. (1993)
Summary: The aim of this study was to investigate phthalate toxicity on Leydig cells structure and functionality. Four different phthalates, including DPP, were investigated *in vivo* and their monoesters, including MPP, were investigated *in vitro*. Adult (6-8 weeks old) male rats were exposed to DPP by daily oral gavage for 2 days (n=3/group). Rats were euthanized 24h after the last dose. Rats were killed and both testes from each rat were used for pathological and ultrastructural examination. *In vitro* tests on isolated Leydig cells from 10 male rats were performed to measure the effects of MPP on testosterone production.

Exposure to DPP showed rarefaction and vacuolization of Sertoli cells, necrosis of germ cells and shedding of spermatocytes and spermatids. No changes were seen in Leydig cell morphology or ultrastructure and *in vitro* secretion of testosterone from Leydig cells was not affected by MPP.

Study quality and assessment: Although the study is old, some important information on number and age of animals and viability of cultured cells *in vitro* is provided. No information on CAS-number or purity is provided and more details on housing of animals would be preferred. The quality of this study is assessed to be of moderate quality. The data provide strong evidence on adverse effects of DPP on the structure of testicular tubular cells (Sertoli cells and spermatogonia) but not on Leydig cells *in vivo*. The study provides moderate evidence that the mode of action of DPP behind the effects on testosterone production is not through the metabolite MPP on Leydig cell function.

Granholm et al. (1992)
Summary: The testicular inflammatory reaction after DPP exposure and correlation with morphological inflammatory changes in the testes was investigated in rats and mice in this study. Rat testis weight and morphology were also assessed. Male rats (40 days old) were dosed by oral gavage to 2.2 g/kg DPP. Rats were killed after 0, 3, 6, 9, 12, 18 or 24 hours after dosing. Control animals were killed 0 or 24 h after dosing.

No changes were seen in testis weight but histological examination showed slight morphological changes in Sertoli cells with rarefaction of the basal cytoplasm.

Study quality and assessment: The present study is poorly described and it is not always readily obvious whether data are assessed from mice or rats. The study is old and there is missing information on CAS-no, number of animals used per treatment groups and animal housing conditions. The study is assessed to be of low quality. The reported data provide weak evidence for adverse effects of DPP on male testis.

Heindel et al. (1989)
Summary: The reproductive toxicity of three phthalates was investigated in this study, where a continuous breeding protocol was followed. The reproductive effects of DPP were investigated in a breeding study and in a cross-over breeding study. In the breeding study, 40 control pairs and 20 pairs/treatment group was used. Male and female mice (11 weeks old) were exposed to 0, 0.5, 1.25 or 2.5% DPP in the feed for 7 days prior mating. The breeding pairs were evaluated for clinical signs, body weight, fertility (based on the number of pairs producing litters), number of litters per pair, live pups per litter, proportion of pups born alive, sex of live pups, pup body weight and food and water consumption. In the 1-week cross-over breeding study, exposed mice (20 pairs/group) were paired

with control mice of the opposite sex to determine the sex affected by DPP. Mice were exposed to 0 or 2.5% DPP in the food during the whole period after mating. Decreased fertility was seen in the breeding study in the 0.5% treatment group; fewer litters/pair, fewer live pups per litter and fewer pups born alive were seen. Breeding pairs exposed to higher levels of DPP were infertile. In the cross-over breeding study, both male and female mice were infertile after treatment with 2.5% DPP. Decreased body weight was seen for male and female mice and decreased absolute and relative organ weights were found for testis, epididymis, seminal vesicles and kidneys. Epididymal sperm count was decreased to a non-detectable level. Prostate weight was comparable to controls and liver weight was increased (absolute and relative). Histopathological changes were seen in testis, where degeneration of seminiferous tubules and interstitial cell hyperplasia was seen. In epididymis, accumulation of fluid and degenerated cells was seen. No effects were seen on oestrous cycle length and no histopathological changes were found in female reproductive organs (ovaries, oviduct, uterus and vagina). Infertility induced by DPP on mating mice was shown to be related to effects in both the male and the female mice, although no morphological or weight-related changes were found in the reproductive organs of exposed female mice. Several changes in male reproductive organs were found.

Study quality and assessment: Although the study is old and the study protocol is complicated, it is well-described and includes important information such as number and age of animals. More information on housing conditions and CAS-number would have been preferred. A large number of animals per group were used. The study is assessed to be of high quality. This study provides strong evidence on adverse effects on reproductive organs and fertility of mice exposed to DPP.

Creasy et al. (1988)

Summary: The effects of MPP on Sertoli cell ultrastructure and germ cells *in vitro* was investigated and compared with the effects of DPP on these cell types *in vivo*. An *in vivo* study with 3 adult (28 days old) male rats per group exposed orally to a single dose of 2.2 g/kg DPP were killed 1, 3, 6, 12 or 24 hours after dosing. Morphology of Sertoli- and germ cells was evaluated under transmission electron microscopy and compared with the findings from the *in vitro* tests with MPP.

Generally seen, Sertoli cell shape resulted in direct apposition of germ cells 6 hours after dosing and necrotic germ cells 24 hours after dosing. Interstitial neutrophil infiltrates were apparent after 12 hours of dosing. Rarefaction of Sertoli cell cytoplasm *in vivo* correlated with mitochondrial hypertrophy *in vitro* and changes in Sertoli cell shape *in vivo* after DPP exposure correlated well with Sertoli cell shape and actin filament organisation changes seen *in vitro* after MPP exposure.

Study quality and assessment: The study is old and the *in vivo* part of the study is relatively superficially described. The most important information such as age and number of animals used, the doses and timing of termination is described, but no details on housing conditions, source of the animals or whether one or two testes per animal was used for the assessment of testicular changes was described. The *in vivo* part of the study is assessed to be of low quality. The data provide moderate evidence that DPP can lead to adverse effects on the testicular Sertoli cells.

Lindström et al. (1988)
Summary: The relationship between spermatogenesis and serum levels of androgen binding protein were investigated in this study by testing if testicular effects are reflected by altered serum levels of androgen binding protein. This was performed in two animal studies with single dose-exposure to DPP at 0, 0.25, 1 or 2g/kg by oral gavage. In the first study, 10 adult Fisher 344 rats per group (approximately 6 weeks of age) were killed 2 days after exposure or each week for 10 weeks. At necropsy, body weight and weight of testis, epididymis, prostate, seminal vesicle, liver and kidney were assessed. Epididymal sperm density and morphology were measured and histological morphology of testis and epididymis was evaluated. Serum androgen binding protein was measured.

In the second study (20 males per group), male fertility after DPP exposure was evaluated by testing their ability to impregnate female rats and assessment of the number of live pups and preimplantation loss. After fertility assessment, the males were killed 14, 18 and 30 weeks after dosing (5 controls and 5 high-dose males at each time-point) to assess testicular recovery histologically.

In the first study Lindström and co-workers found effects on body weight and male reproductive organs. Lower body weight in the two highest dose-groups was seen during the first week after dosing. Testicular weight and epididymal weight were lower than controls in the 1 and 2g/kg dose-groups and remained below controls for all 10 weeks they were studied. Sperm density was lower than controls in all dose-groups and sperm morphology was abnormal in the highest dose-group. Histological evaluation of testis showed effects on germ cells and intertubular spaces. Liver weights were increased 2 days after dosing but returned to control levels after 2 weeks. In the second study, fewer fertile males were found in the high-dose group compared to controls; they had less ability to impregnate female rats, there was an increased number of preimplantation losses after mating and an increased number of dead pups. Finally, 30 weeks after dosing no signs of recovery of the testicular damage was seen.

Study quality and assessment: Although this is an old study much important information is included such as purity of the chemical, rat strain, details on housing conditions and number of animals for each study performed and for the endpoints assessed. However, more information on CAS-number would be preferred. The study is assessed to be of moderate quality. The data from the present study provide strong evidence for adverse effects on testis in adult males after exposure to a single dose of DPP without obvious recovery at the cellular level 30 days after exposure.

Creasy et al. (1987)
Summary: The study investigates the effects of 2.2 g/kg DPP on sexually mature rat testis at different time-points after oral exposure. Two control and three exposed animals (15 weeks of age) were killed 3, 6, 12, 18, 24 or 48 h after dosing and testis were examined under light microscope, electron microscope and transmission electron microscope.

Creasy and co-workers found stage-specific changes in the testicular tubules (morphologically observed as vacuolization and closure of the lumen, increased affinity for stain and vacuolization of tail cytoplasm of elongating spermatids and necrosis of spermatocytes) and degeneration of Sertoli cells. Interstitial inflammatory infiltrate was seen 12 hours after dosing or later. The changes appeared to be reversible.

Study quality and assessment: Although this is an old study, it is well described and includes much important information such as number of animals used, age of animals at study start and detailed

descriptions of the changes observed. Few control animals were included (2 control rats per experiment) and no CAS number or purity was stated for the chemical. All in all the study is assessed to be of moderate quality. This study provides strong evidence for testicular adverse effects in sexually immature rats in the case of continuous exposure.

Gray and Gangolli (1986)
Summary: This paper investigates some features of testicular effects of some phthalates in rats, including effects on Sertoli cells, and effects of phthalate monoesters on testicular germ cell cultures. Some investigations, such as the effects on reproductive organs with co-administration of gonadotropins or the effects of metabolites on testicular cell cultures of Sertoli cells and germ cells were performed on other phthalates but not on DPP or the metabolites of DPP.

Tubular atrophy was seen in testes from 15 weeks old males 24 hours after exposure to 2200 mg/kg/day DPP, but was less severe and developed slower than in immature rats. A single dose of 2200 mg/kg DPP in immature 4-5 weeks old rats (5 per group) showed a complete or close to complete depression of androgen binding protein (ABP) and fluid in rete testis, respectively. The lower single dose of 440 mg/kg DPP decreased fluid and ABP, but not 220 mg/kg. In older rats 10 weeks of age, the highest dose (2200 mg/kg) also decreased fluid and ABP, but not in the same dramatic extent as in the younger rats (reduced to 60% of control values). Repeated dosing of 220 mg/kg for 3 days did not change fluid or ABP production significantly. Mono-*n*-pentyl phthalate (MPP) was shown to cross the blood-testis barrier in very little amounts (levels in rete testis was 5% of plasma levels 25 minutes after phthalate administration, n=3 controls and 2 dosed with MPP). MPP administered *i.v.* for 4 days showed histological testicular changes characteristic of phthalate changes at 50 mg/kg/day but not at 25 mg/kg/day.

Study quality and assessment: The methods used in the study are described in an unstructured way and are confusing. Dosing levels, duration of dosing and number and age of animals used is not described in the methods section and the number of animals is not accessible, if the data are not presented in a table (e.g. for the data on testicular atrophy in males exposed to DPP). The methods used for cell culture are poorly or hardly described. It is unclear whether some of the data described are from the present study or are a description of findings from a previously published study. The study is assessed to be of low quality. The data provide weak evidence of adverse effects of DPP on male reproductive tract.

Creasy et al. (1983)
Summary: The study was performed to identify the primary site of DPP damage and follow the development of the lesion. Moreover, the study aimed at providing indications of the functional integrity of the affected Sertoli cells. Single dose studies and repeated dose studies on DPP are described with focus on testicular effects in sexually immature male Sprague-Dawley rats (3-4 weeks of age). Necropsies of rats were performed 1, 3, 6 or 24 hours after a single dose of 2.2 g/kg DPP or after 3, 4 or 5 days of daily dosing of 2.2 g/kg DPP. One control animal and 3 exposed animals were used for each experiment.

Early degenerative vacuolization of Sertoli cells 3 to 6 hours after oral dosing was seen. After 2-4 days of repeated dosing, degeneration of spermatocytes and spermatids as well as depletion of spermatogonia and germinal cells was seen.

Study quality and assessment: Although this is an old study, it is well described and includes much important information such as purity of the chemical, age of animals at study start and at necropsy and number of animals used. Few control animals were included (1 control rat per experiment) and no CAS-number was stated for the chemical. All in all the study is assessed to be of moderate quality.

This study provides moderate evidence for testicular adverse effects in sexually immature rats.

Foster et al. (1983)
Summary: Steroidogenic enzyme activity was measured in testis and livers from male rats (4 weeks old) exposed orally to DPP or diethyl phthalate daily or as a single dose. Male rats were dosed with a single dose of 7.2 mmol/kg of DPP and killed 0, 1, 3, 6 or 24 h after dosing. Other male rats were dosed daily with 7.2 mmol/kg/day of DPP and killed after 2, 3 or 4 days. Microsomes and cytochrome P450 from testes and livers were extracted. Single doses of DPP decreased enzyme activity of 17-α-hydroxylase and 17-20 lyase in testes. Repeated dosing of DPP for 2 or 4 days increased the activity of 17-β-dehydrogenase in testicular microsomes. 17-α-hydroxylase, 17-20 lyase and 17-β-dehydrogenase are enzymes involved in conversion of progesterone to testosterone. Cytochrome P450 in testes was also decreased by DPP.

Study quality and assessment: The present study is old and there is missing information on CAS-number, number of animals used per treatment groups and animal housing conditions. The study is assessed to be of weak quality. The reported data provide moderate evidence for an endocrine mode of action of DPP through action on the steroidogenic enzymes in testes.

Foster et al. (1982)
Summary: The aim of this study was to determine the localization of zinc in the normal testis and in testis exposed to DPP until reaching atrophy of testis. Single dose and repeated dose studies of 2.2 g/kg DPP by oral gavage in male rats were described by Foster et al (1982). In the single-dose experiments, 3 controls and 3 exposed animals were killed at 0, 1, 3, 6 or 24 h after dosing. In the repeated-dose studies, 3 controls and 3 exposed animals were killed after 2, 3 or 4 days of daily exposure. The weight of testis, prostate and seminal vesicle was measured and a significant decrease in testis weight was found. Morphology of testes was assessed and vacuolization of Sertoli cells and necrosis of spermatids and spermatocytes was found. Analysis of zinc deposits in testicular cells indicated that the testicular damage may be associated to zinc depletion.

Study quality and assessment: Although this is an old study, it is well described and includes much important information such as purity of the chemical, weight of animals at study start and number of animals used. The relative organ weights were reported as % of controls and the life stage (sexually mature or immature) of the animals is unclear. The study is assessed to be of moderate quality. This study provides strong evidence of testicular adverse effects. The data indicate a relation to zinc depletion which may be affect gonadotropins and androgen production in testis, but it is not clear if the adverse effects are due to an endocrine mode of action.

Foster et al. (1980)
Summary: This study was performed to compare the testicular effects of some phthalates with their effects on zinc excretion in the rat. Young adult male rats (70-90 g, 12/group) were exposed to 7.2 mmol/kg/day (2.1 g/kg/day) of DPP by oral intubation for 4 days. Body weight gain, food intake, testis weight, histological testicular morphology and zinc levels in urine, faeces, testis, liver and kidney were assessed. Relative testis weight was decreased (data on absolute weight is neither shown nor mentioned) and histological evaluation of testis showed atrophy of seminiferous tubules with loss of spermatocytes and spermatids. Urinary excretion of zinc-65 was increased. Zinc content in testis was decreased whereas the content in liver and kidney was increased. The animals showing testicular atrophy also had increased urinary excretion of zinc and a putative link between low zinc levels in testis and testicular atrophy was established.

Study quality and assessment: Although no CAS-number or information on housing conditions is provided in this early study, other important information is given, such as number and weight of animals used, dose level and method and duration of dosing. Therefore the study is assessed to be of moderate quality. The data from the present study provide strong evidence of testicular adverse effects of DPP. Moreover, the study provides some evidence of a mode of action of DPP involving zinc, but it is not clear if the adverse effects are due to an endocrine disrupting mode of action. Therefore the study is assessed to provide weak evidence of an endocrine-related mode of action of DPP.

4.10.3.4 Summary of the plausible link between adverse effects and endocrine mode of action

Several *in vivo* studies investigating the endocrine mode of action of DPP showed an anti-androgenic mode of action of DPP (Table 1). Many studies showed decreased testosterone levels in foetuses either in plasma, testis or ex vivo testicular testosterone production (Gray et al. 2016; Beverly et al. 2014; Hannas et al. 2011; Howdeshell et al. 2008). Genes involved in steroid synthesis, regulation or transport have also been shown to be downregulated by DPP exposure (Hannas et al. 2012; Hannas et al. 2011; Liu et al. 2005). Finally, microsomal enzymes involved in steroid synthesis were affected by DPP exposure *in vivo* and *in vitro* (Yuan et al. 2012; Foster et al. 1983). Other *in vitro* studies investigating the endocrine mode of action of DPP and the monoester mono-n-pentyl phthalate (MPP) showed weak signs of anti-androgenic activity of DPP and no effects of MPP on testosterone production in Leydig cells (Christen et al. 2010; Jones et al. 1993). Some studies investigating zinc excretion and deposition in testis showed that low zinc levels may be involved in DPP related testis damage (Foster et al. 1982; Foster et al. 1980). Altogether, several rodent studies have demonstrated strong evidence of an anti-androgenic mode of action of DPP *in vivo*.

DPP is classified as a reproductive toxicant and adverse effects on the reproductive system in rodents that may be related to an endocrine disrupting mode of action have been shown in a large number of *in vivo* studies (Table 2). Effects on the male reproductive system that are known to be able to be induced via endocrine disrupting modes of action have been reported, e.g. decreased male AGD, increased nipple retention in males, decreased number of spermatocytes (decreased sperm count), malformations of male reproductive organs (hypospadias, malformations of vas deferens, epididymis and gubernacular cord), decreased weight of male reproductive organs in adulthood, decreased foetal testis weight and delayed descent of testis. Other changes in the male reproductive system were also seen although it is not clear if the adverse effects are due to an endocrine mode of action. The changes

include degenerative changes of testicular Sertoli cells and degeneration or loss of spermatocytes (Hild et al. 2007; Hild et al. 2001; Jones et al. 1993; Granholm et al. 1992; Creasy et al. 1988; Creasy et al. 1987; Creasy et al. 1983; Foster et al. 1982; Foster et al. 1980).

Effects of DPP were also seen on female fertility without effects on weights of female reproductive organs (Heindel et al. 1989), but the modes of action are unclear. All in all the evidence for adverse effects of DPP in males is strong but the evidence for adverse effects in the female reproductive system is moderate.

Summary and conclusions

Overall, several rodent studies have shown adverse effects on the male reproductive system and male reproductive development. Many of the effects observed are generally known to be androgen dependent and changes such as decreased AGD and increased nipple retention in males is associated with an anti-androgenic mode of action and low androgen levels. Studies on the endocrine-related modes of action of DPP showed effects on steroid production at several levels including downregulation of steroid-related genes, reduced activity of enzymes involved in steroid synthesis and decreased levels of testosterone in plasma and testis. The adverse effects observed for DPP can be attributed to an anti-androgenic mode of action, and it is highly biologically plausible, that there is a link between the decreased testosterone synthesis found for DPP and the adverse effects observed on male reproductive organs and male reproductive development. The evidence for an endocrine mode of action of DPP is strong, the evidence for adverse effects on male reproductive system is strong and there is strong evidence for a causal link. In conclusion, DPP meets the WHO definition of an endocrine disruptor.

Additional literature not included in the evaluation

Reviews on DPP have been used to check for additional literature not found in our literature search (Gangolli et al. 1982; Gray et al. 1982; NTP 1985).

Table 1. Overview of *in vitro* and *in vivo* endocrine disrupting (ED) mode(s) of action (MoA(s)) of di-n-pentylphthalate (DPP)

Reference	**MoA**		**Quality of study**	**Evidence for ED MoA**
	In vitro	*In vivo*		
Gray et al. 2016		Decreased testosterone levels in plasma, testis and *ex vivo* testicular testosterone production.	High	Strong
Beverly et al. 2014		Decreased *ex vivo* testicular testosterone production.	Medium	Strong
Hannas et al. 2012		Downregulation of several genes in GD18 testis involved in steroid synthesis, steroid regulation and steroid transport.	High	Moderate
Yuan et al. 2012	Reduced activity of microsomal enzymes related to testicular testosterone synthesis.		High	Moderate
Hannas et al. 2011		Decreased *ex vivo* testicular testosterone production in a dose-related manner in testis GD 17 and 18 and downregulation of gene expression levels of androgen synthesis genes StAR and cyp11a and of insl3 in testis GD 18.	High	Strong
Christen et al. 2010	Androgen receptor reporter gene assay: Partial inhibition curves (anti-androgenic activity) were observed for DPP. No notable androgenic activity was seen.		High	Moderate
Howdeshell et al. 2008		Reduced testosterone production in *ex vivo* foetal testis exposed to DPP during foetal development (GD 8-18).	High	Strong
Liu et al. 2005		Gene expression levels in GD 19 foetal testes was changed for genes involved in regulating steroidogenesis and insulin signaling. DPP appeared to target Sertoli cells and gonocytes and the interaction between the two.	High	Moderate
Jones et al. 1993	Secretion of testosterone from cultured Leydig cells was not affected by MPP.		Medium	Moderate

Reference	MoA		Quality of study	Evidence for ED MoA
	In vitro	*In vivo*		
Creasy et al. 1988	An *in vitro* study on effects of MPP on Sertoli cells showed changes in Sertoli cell shape, development of microprocesses between Sertoli cells and germ cells and reorganization of actin filaments.		Low	Weak
Foster et al. 1983		Single doses of DPP decreased microsomal enzyme activity involved in testosterone synthesis in testes. Repeated dosing of DPP for 2 or 4 days increased the activity the enzymes. Cytochrome P450 in testes was also decreased by DPP.	Low	Moderate
Foster et al. 1982		Analysis of zinc deposits in testicular cells indicated that the testicular damage may be associated to zinc depletion.	Medium	Weak
Foster et al. 1980		Animals showing testicular atrophy had increased urinary excretion of zinc and decreased zinc levels in testis.	Medium	Weak

MPP: Mono-n-pentyl phthalate, the monoester of DPP, gestation day (GD)

Table 2. Overview of potential endocrine-related adverse effects of di-n-pentylphthalate (DPP)

Reference	Species, n	Adverse effects	Quality of study	Evidence for adverse effects
Gray Jr et al 2016	Rats, n=5 litters/ group	Decreased male AGD PND2, malformations of male reproductive organs in adult offspring (hypospadias, malformations of vas deferens, epididymis and gubernacular cord), decreased weight of reproductive organs in adult offspring. Decreased foetal testis weight and delayed testis descent GD 18.	Medium	Strong
Hannas et al. 2011a	Rats, n=5 dams/group	Decreased male AGD PND2 and increased male NR PND13.	High	Strong
Hild et al. 2007	Rats, n=4/ group	Degenerative changes in Sertoli cells, spermatocytes and spermatids were seen after DPP exposure. Spermatogonia appeared unaffected.	High	Strong
Liu et al 2005	Rats; n=10 control dams and n=5 exposed dams	AGD in GD 19 foetuses was decreased after DPP exposure GD12-19.	High	Strong
Hild et al. 2001	Rats, n= 3 adults/group and 10 prepubertal / group	An increased % of seminiferous tubules with a higher number of apoptotic germ cells per tubule compared to controls was seen. Morphologically, vacuolization of Sertoli cells and detachment of germ cells was observed. Serum inhibin B was decreased. In the second study (in prepubertal males), STF secretion (by Sertoli cells) was suppressed and serum inhibin B levels and epididymal ABP were decreased.	Medium	Strong
Jones et al. 1993	Rats; n=3/group	Rarefaction and vacuolization of Sertoli cells, necrosis of germ cells and shedding of spermatocytes and spermatids after DPP exposure. No changes were seen in Leydig cell morphology or ultrastructure after DPP exposure.	Medium	Strong
Granholm et al. 1992	Rats, n=not described.	No changes were seen in rat testis weight but histological examination showed slight morphological changes in Sertoli cells with rarefaction of the basal cytoplasm.	Low	Weak
Heindel et al. 1989	Mice; study A: n=40 control and 20 treated	Fewer litters/pair, fewer live pups per litter and fewer pups born alive were seen in the 0.5% treatment group. Breeding pairs exposed to higher levels of DPP (1.25 or 2.5%) were infertile. In the cross-over breeding study, both male and female mice were infertile after treatment with 2.5%	High	Strong for males, moderate for females

Reference	Species, n	Adverse effects	Quality of study	Evidence for adverse effects
	breeding pairs/group; study B: n=20 cross-over breeding pairs/group;	DPP. Decreased body weight was seen for male and female mice and decreased absolute and relative organ weights were found for testis, epididymis and seminal vesicles. Epididymal sperm count was decreased to a non-detectable level. Prostate weight was comparable to controls and liver weight was increased (absolute and relative). Histopathological changes were seen in testis, where degeneration of seminiferous tubules and interstitial cell hyperplasia was seen. In epididymis, accumulation of fluid and degenerated cells was seen. No effects were seen on estrous cycle length and no histopathological changes were found in female reproductive organs.		
Creasy et al. 1988	Rats; n=3/group	Changes in Sertoli cell shape and rarefaction of the cytoplasm was seen after DPP exposure.	Low	Moderate
Lindström et al. 1988	Rats, n=10/ group, n=20 in fertility study, n=5 in recovery study	Study on reproductive organs: lower body weight in the two highest dose-groups (1 and 2 g/kg) was seen during the first week after dosing. Testicular weight and epididymal weight were lower than controls in the 1 and 2g/kg dose-groups and remained below controls for all 10 weeks they were studied. Sperm density was lower than controls in all dose-groups. Sperm morphology was abnormal in the highest dose-group. Histological assessment of testis showed effects on germ cells and intertubular spaces. Liver weights were increased 2 days after dosing but returned to control levels after 2 weeks. Prostate and seminal vesicle weights were never different from controls. Fertility study: less fertile males were found in the high-dose group compared to controls – they had less ability to impregnate female rats, there was an increased number of preimplantation losses after mating and an increased number of dead pups. Recovery study: no signs of recovery on germinal epithelium were seen 30 weeks after dosing.	Low-medium	Strong
Creasy et al. 1987	Rats, n= 2 control and 3 exposed	Stage-specific changes in the testicular tubules and degeneration of Sertoli cells. The changes appeared to be reversible.	Medium	Strong

Reference	Species, n	Adverse effects	Quality of study	Evidence for adverse effects
Gray and Gangolli 1986	Rats, n= 5/group unless otherwise mentioned	Testicular tubular atrophy (in 15 weeks old males) 24 hours after exposure to 2200 mg/kg/day DPP, but was less severe and developed slower than in immature rats. A single dose of 2200 mg/kg DPP in immature 4-5 weeks old rats showed a complete or close to complete depression of ABP and fluid in rete testis, respectively. 440 mg/kg DPP decreased fluid and ABP. In rats 10 weeks of age, the highest dose (2200 mg/kg) also decreased fluid and ABP, but not in the same dramatic extent as in the younger rats. Repeated dosing of 220 mg/kg for 3 days did not change fluid or ABP production significantly. Mono-n-pentyl phthalate (MPP) was shown to cross the blood-testis barrier in very little amounts (n=3 controls and 2 dosed with MPP). MPP administered i.v. for 4 days showed histological testicular changes characteristic of phthalate changes at 50 mg/kg/day but not at 25 mg/kg/day	Low	Weak
Creasy et al. 1983	Rats, n=1 control and 3 exposed/ time-point	Degenerative vacuolization of Sertoli cells 3 to 6 hours after oral dosing. Degeneration of spermatocytes and spermatids as well as depletion of spermatogonia and germinal cells was seen with repeated dosing.	Medium	Moderate
Foster et al. 1982	Rats, n=3/group	Vacuolization of Sertoli cells and necrosis of spermatids and spermatocytes was found.	Medium	Strong
Foster et al. 1980	Rats, n=12/group	Relative testis weight was decreased and histological evaluation of testis showed atrophy of seminiferous tubules with loss of spermatocytes and spermatids.	Medium	Strong

ABP: androgen binding protein. AGD: ano-genital distance. GD: gestation day. PND: postnatal day. STF: Seminiferous tubule fluid, MPP: Mono-n-pentyl phthalate, the monoester of DPP.

References

Beverly, B.E., Lambright, C.S., Furr, J.R., Sampson, H.2., Wilson, V.S., McIntyre, B.S., Foster, P.M., Travlos, G.3. and Gray, L.E. Jr. (2014) 'Simvastatin and dipentyl phthalate lower ex vivo testicular testosterone production and exhibit additive effects on testicular testosterone and gene expression via distinct mechanistic pathways in the fetal rat.', *Toxicological Sciences.141(2), pp. 524-537*, Doi: 10.1093/toxsci/kfu149

Christen, V., Crettaz, P., Oberli-Schrämmli, A. and Fent, K. (2010) 'Some flame retardants and the antimicrobials triclosan and triclocarban enhance the androgenic activity in vitro.', *Chemsphere.81(10), pp.1245-1252*, Doi: 10.1016/j.chemosphere.2010.09.031

Creasy, D.M., Beech, L.M. and Gray, T.J. (1988) 'Effects of mono-(2-ethylhexyl) phthalate and mono-n-pentyl phthalate on the ultrastructural morphology of rat Sertoli cells in Sertoli/germ cell co-cultures: Correlation with the in vivo effects of di-n-pentyl phthalate.', *Toxicol.In Vitro.2(2), pp.83-95*

Creasy, D.M., Beech, L.M., Gray, T.J. and Butler, W.H. (1987) The ultrastructural effects of di-n-pentyl phthalate on the testis of the mature rat.', *Exp.Mol.Pathology.46(3), pp.357-71*

Creasy, D.M., Foster, J.R. and Foster, P.M. (1983) 'The morphological development of di-N-pentyl phthalate induced testicular atrophy in the rat.', *J.Pathology.139(3), pp.309-321*, Doi: 10.1002/path.1711390307

Foster, P.M., Thomas, L.V., Cook, M.W. and Walters, D.G. (1983) 'Effect of DI-n-pentyl phthalate treatment on testicular steroidogenic enzymes and cytochrome P-450 in the rat.', *Tocicol.Lett.15(2-3), pp.265-271*

Foster, P.M., Foster, J.R., Cook, M.W., Thomas, L.V. and Gangolli, S.D. (1982) 'Changes in ultrastructure and cytochemical localization of zinc in rat testis following the administration of di-n-pentyl phthalate.', *Toxicol.Appl.Pharmacol.63(1), pp.120-132.* Doi: 10.1016/0041-008X(82)90031-X

Foster, P.M., Thomas, L.V., Cook, M.W. and Gangolli, S.D. (1980) 'Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat.', *Toxicol.Appl.Pharmacol.54(3), pp.392-398*, Doi: 10.1016/0041-008X(80)90165-9

Gangolli, S.D. (1982) 'Testicular effects of phthalate esters', *Environ.Health.Perspect.45, pp.77-84*

Granholm, T., Creasy, D.M., Pöllänen, P. and Söder, O. (1992) 'Di-n-pentyl phthalate-induced inflammatory changes in the rat testis are accompanied by local production of a novel lymphocyte activating factor.', *J.Reprod.Immunol.21(1), pp.1-14.*

Gray, L.E.Jr., Furr , J., Tatum-Gibbs, K.R., Lambright, C., Sampson ,H., Hannas, B.R., Wilson, V.S., Hotchkiss, A. and Foster, P.M. (2016) 'Establishing the "Biological Relevance" of Dipentyl Phthalate Reductions in Fetal Rat Testosterone Production and Plasma and Testis Testosterone Levels.', *Toxicol.Sci.149(1), pp.178*-191, Doi: 10.1093/toxsci/kfv224.

Gray, T.J. and Gangolli, S.D. (1986) 'Aspects of the testicular toxicity of phthalate esters.', *Environ.Health.Perspect.65, pp.229-235*

Gray, T.J., Rowland, I.R., Foster, P.M. and Gangolli, S.D. (1982) 'Species differences in the testicular toxicity of phthalate esters.', *Toxicol.Lett.11(1-2), pp.141-147*

Hannas, B.R., Lambright, C.S., Furr, J., Evans, N., Foster, P.M., Gray, E.L. and Wilson, V.S. (2012) 'Genomic biomarkers of phthalate-induced male reproductive developmental toxicity: a targeted RT-PCR array approach for defining relative potency.', *Toxicol.Sci.125(2), pp.544-557,* Doi:10.1093/toxsci/kfr315

Hannas, B.R., Furr, J., Lambright, C.S., Wilson, V.S., Foster, P.M. and Gray, L.E.Jr. (2011a) 'Dipentyl phthalate dosing during sexual differentiation disrupts fetal testis function and postnatal development of the male Sprague-Dawley rat with greater relative potency than other phthalates.', *Toxicol.Sci.120(1), pp.184-193,* Doi: 10.1093/toxsci/kfq386

Hannas, B.R., Lambright, C.S., Furr, J., Howdeshell, K.L., Wilson, V.S. and Gray, L.E.Jr. (2011b) 'Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following in utero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate', *Toxicol.Sci.123(1), pp.206*-216, Doi: 10.1093/toxsci/kfr146

Heindel, J.J., Gulati, D.K., Mounce, R.C., Russell, S.R. and Lamb, J.C.4th. (1989) 'Reproductive toxicity of three phthalic acid esters in a continuous breeding protocol.', *Fundam Appl Toxicol.12(3), pp.508-18*

Hild, S.A., Reel, J.R., Dykstra, M.J., Mann, P.C. and Marshall, G.R. (2007) 'Acute adverse effects of the indenopyridine CDB-4022 on the ultrastructure of sertoli cells, spermatocytes, and spermatids in rat testes: comparison to the known sertoli cell toxicant Di-n-pentylphthalate (DPP).', *J.Androl.28(4), pp.621-629,* Doi: 10.2164/jandrol.106.002295

Hild, S.A., Reel, J.R., Larner, J.M. and Blye, R.P. (2001) 'Disruption of spermatogenesis and Sertoli cell structure and function by the indenopyridine CDB-4022 in rats', *Biol.Reprod.65(6), pp.1771-1779,* Doi: 10.1095/biolreprod65.6.1771

Howdeshell, K.L., Wilson, V.S., Furr, J., Lambright, C.R., Rider, C.V., Blystone, C.R., Hotchkiss, A.K. and Gray, L.E.Jr. (2008) 'A mixture of five phthalate esters inhibits fetal testicular testosterone production in the sprague-dawley rat in a cumulative, dose-additive manner.', *Toxicol.Sci.105(1), pp.153-16,* Doi:10.1093/toxsci/kfn077

Jones, H.B., Garside, D.A., Liu, R. and Roberts, J.C. (1993). 'The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo.', *Exp.Mol.Pathol. 58(3), pp.179-193,* Doi:10.1006/exmp.1993.1016

Lindström, P., Harris, M., Ross, M., Lamb, J.C. 4th. and Chapin, R.E. (1988) 'Comparison of changes in serum androgen binding protein with germinal epithelial damage and infertility induced by di-n-pentyl phthalate.', *Fundam.Appl.Toxicol.11(3), pp.528-539*

Liu, K., Lehmann, K.P., Sar, M., Young, S.S. and Gaido, K.W. (2005) 'Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis.', *Biol.Reprod.73(1), pp.180-192,* Doi: 10.1095/biolreprod.104.039404

NTP Reproduction and Fertility Assessment in CD-1 mice when administered in feed (1985), *Environmental Health Perspective, Vol.105, Supp.1,pp.255-256*

Yuan, K., Zhao, B., Li, X.W., Hu, G.X., Su, Y., Chu, Y., Akingbemi, B.T., Lian, Q.Q. and Ge, R.S. (2012) 'Effects of phthalates on 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid

dehydrogenase 3 activities in human and rat testes.', *Chem.Biol.Interact.195(3). pp.180-188*, Doi: 10.1016/j.cbi.2011.12.008