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REVIEW

# Peroxisome Proliferator-Activated Receptors: Mediators of Phthalate Ester-Induced Effects in the Male Reproductive Tract?

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**Many phthalate ester plasticizers are classified as peroxisome proliferators (PP), a large group of industrial and pharmaceutical chemicals. Like PP, exposure to some phthalates increases hepatocyte peroxisome and cellular proliferation, as well as the incidence of hepatocellular adenomas in mice and rats. Most effects of PP are mediated by three nuclear receptors called peroxisome proliferator-activated receptors (PPARα,β,γ). An obligate role for PPARα in PP-induced events leading to liver cancer is well-established. Exposure of rats *in utero* or in the neonate to a subset of phthalate esters causes profound, sometimes irreversible malformations in the male reproductive tract. We review here the data that supports or discounts roles for PPARs in phthalate-induced testis toxicity including (1) toxic effects of phthalates on the male reproductive tract, (2) expression of PPARs in the testis, (3) activation of PPARs by phthalates, (4) role of PPARα in testis toxicity, (5) gene targets of phthalates involved in steroid biosynthesis and catabolism, and (6) interactions between PPARs and other nuclear receptors that play roles in testis development and homeostasis. Critical research needs are identified that will help determine the significance of PPARs in phthalate-induced effects in the rat male reproductive tract and the relevance of toxicity to humans.**

*Key Words:* **phthalate ester; PPAR; testis; peroxisome proliferator; male reproductive tract.**

Most animal cells contain peroxisomes, subcellular organelles that perform diverse metabolic functions including $H_2O_2$-derived respiration, β-oxidation of fatty acids, and cholesterol metabolism (Lock *et al*., 1989). Peroxisome proliferators (PP) are a large group of structurally dissimilar industrial and pharmaceutical chemicals that were identified as inducers of both the size and number of peroxisomes after *in vivo* exposure. PP include a large number of phthalate ester plasticizers which are, through their widespread use in various industries, widely distributed in the environment (Staples *et al*., 1997). Unlike many PP, phthalates are metabolized to active species by esterases found in the gut and other tissues. These esterases cleave one of the two side chains from the parent diester phthalate producing an active monoester phthalate. Rodent exposure to PP, including many phthalate esters leads to predictable adaptations in the liver consisting of hepatocellular hypertrophy and hyperplasia and transcriptional induction of fatty acid metabolizing enzymes (Lock *et al*., 1989). Chronic exposure to some PP causes an increased incidence of liver tumors in male and female mice and rats (Klaunig *et al*., 2003). Many studies also show that exposure to some phthalates results in profound, irreversible changes in the development of the male reproductive tract (Foster *et al*., 2001; Sharpe, 2001).

Many of the adaptive consequences of PP exposure are mediated by a subset of nuclear receptor superfamily members called peroxisome proliferator-activated receptors (PPARs). The PPAR family includes three distinct subtypes, PPARα, PPARβ (also known as NUC1 or PPARδ), and PPARγ encoded by separate genes (Lemberger *et al*., 1996). PP activate PPARs leading to altered expression of genes with roles in metabolism, cell growth, and stress responses (Corton *et al*., 2000). Most, if not all responses to PP including some phthalates in the liver are dependant on PPARα (Klaunig *et al*., 2003). Given recent findings describing novel aspects of phthalate-PPAR interactions, we review here evidence supporting or discounting roles for PPARs in mediating the toxic effects of phthalates on the male reproductive tract.

## *Effects of Phthalates on the Developing Male Reproductive Tract* in Utero

Exposure of male rats to some diester and monoester phthalates *in utero* results in profound changes in the testis and other androgen-dependent structures. The extent and severity of the changes depend in large part on the timing and duration of the exposure and how the compound is administered. Studies in which reproductive tract defects have been observed involve exposure of pregnant dams during the time of androgen-dependent sexual differentiation, estimated to begin in the

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**TABLE 1**
**Summary of Effects of *in Utero* Exposure to Phthalates on the Developing Male Reproductive Tract**

| Endpoint measured[a] | DBP | DEHP | BBP | DINP |
|---|---|---|---|---|
| Testis | | | | |
| ↓ Weight | + | + | + | – |
| ↓ Sperm number | + | + | + | |
| Degeneration/atrophy of seminiferous tubules | + | + | + | |
| Leydig cell hyperplasia/aggregates | + | + | + | |
| Leydig cell adenoma | + | + | | |
| Cryptorchidism | + | + | + | |
| Sex organs | | | | |
| Epididymis: ↓ wt, agenesis/malformed | + | + | + | – |
| Penis: delayed/incomplete preputial separation, hypospadias, ↓ wt of glans | + | + | + | – |
| Prostate: ↓ wt, agenesis | + | + | + | – |
| Seminal vesicle: ↓ wt | + | + | + | – |
| Vas deferens: ↓ wt, malformed/agenesis | + | | | |
| Miscellaneous | | | | |
| Anogenital distance (↓) | + | + | + | + |
| Nipple retention | + | + | + | |

[a]Endpoints observed in the indicated tissues from male rats exposed *in utero* to DBP (Barlow and Foster, 2003; Barlow *et al*., 2004; Fisher *et al*., 2003; Mylcreest *et al*., 1999; Wine *et al*., 1997), DEHP (Gray *et al*., 2000; Moore *et al*., 2001; Parks *et al*., 2000), BBP (Gray *et al*., 2000; Nagao *et al*., 2000; Piersma *et al*., 2000), or DINP (Gray *et al*., 2000).

rat ∼ gestation day 12 (GD 12). Studies in which di-*n*-butyl phthalate (DBP) or butylbenzyl phthalate (BBP) were dosed before GD 12 had no effect on reproductive tract development, as expected, but did produce other malformations (Ema *et al*., 2000; Ema and Miyawaki, 2002). Exposure regimens that resulted in the most consistent and robust effects involved administration of the phthalate by daily gavage throughout development of the reproductive tract until birth or shortly thereafter (Gray *et al*., 2000; Mylchreest *et al*., 1999). A number of studies showed common defects in the male rat reproductive tract after *in utero* exposure to DBP (Barlow and Foster, 2003; Barlow *et al*., 2004; Fisher *et al*., 2003; Mylchreest *et al*., 1999, 2000, 2002; Wine *et al*., 1997), di-(2-ethylhexyl) phthalate (DEHP) (Moore *et al*., 2001; Parks *et al*., 2000), and BBP (Nagao *et al*., 2000; Piersma *et al*., 2000; Tyl *et al*., 2004). These effects included those in the testis as well as other organs that depend on testosterone for proper development (Table 1). Some effects caused by DBP were found to be permanent including gross lesions in the testis, vasa deferentia, seminal vesicles, prostate, and penis, as well as decreases in anogenital distance and nipple retention (Barlow *et al*., 2004). In addition, DBP at 1% in the diet (Wine *et al*., 1997) and BBP at 750 mg/kg/day (Tyl *et al*., 2004) but not 500 mg/kg/day (Nagao *et al*., 2000) decreased total number of sperm and indices for mating, fertility, and pregnancy in F1 generation rats in multi-generation exposure studies. In contrast, di-isononyl phthalate (DINP) was a weak reproductive toxicant as *in utero* exposure led only to increased nipple retention (Gray *et al*., 2000). Dimethyl phthalate and diethyl phthalate had no adverse effects on the development of the male reproductive tract (Gray *et al*., 2000). Monobutyl phthalate (MBP), the principle metabolite of DBP, disrupted the descent of the fetal testis (Imajima *et al*., 2001; Shono and Suita, 2003).

Reproductive effects of DBP are not exclusively found in rats. Exposure of male tadpoles of the frog species *Rana rugosa* increased the incidence of undifferentiated gonads developing into those having complete or partial ovarian structure (Ohtani *et al*., 2000). Male rabbits exposed *in utero* exhibited decreased weights of testis and accessory sex glands, decreases in ejaculated sperm, and increases in abnormal sperm six weeks after exposure (Higuchi *et al*., 2003).

The effects of the active phthalates on the male rat reproductive tract share similarities with a condition in humans termed testicular dysgenesis syndrome (TDS). As proposed by Skakkebaek *et al*. (2001), TDS includes testicular germ cell cancers, poor semen quality, crytorchidism and hypospadias, all thought to arise from disruption of embryonic gonadal development. That TDS is increasing in developing countries and that environmental exposure to man-made male reproductive toxicants are responsible for TDS is unresolved, partly because of the lack of high quality data allowing comparison of studies (Sharpe, 2001). Only increases in testicular cancer over the last 50–90 years have been established (Bergstrom *et al*., 1996; Toppari *et al*., 1996). TDS, like exposure to phthalates is associated with abnormal function in both Leydig and Sertoli cells, resulting in downstream consequences in the testis and sex organs (Fig. 1). Exposure of rats to DBP *in utero* increased the proportion of immature Sertoli cells unable to support spermatogenesis and the number of multinucleated gonocytes, possibly through alterations in Sertoli cell-gonocyte interactions (Fisher *et al*., 2003). How DBP treatment interferes with these interactions and in particular, gonocyte cytokinesis is not understood.

Phthalates which produce changes in male sex organs may be operating through an anti-androgen-like mechanism. The effects of DBP and DEHP overlapped with, but were distinct from androgen receptor antagonists including linuron (Gray *et al*., 1999) and flutamide (Mylchreest *et al*., 1999). Unlike other anti-androgens, some active phthalates target the Leydig cell testosterone biosynthetic machinery (discussed in detail below). Decreases in fetal testicular testosterone levels or serum testosterone in young postnatal male rats ($\leq$ PND 35) were observed after *in utero* exposure to DBP (Fisher *et al*., 2003; Mylchreest *et al*., 2002; Shultz *et al*., 2001; Wilson *et al*., 2004), DEHP (Akingbemi *et al*., 2001; Parks *et al*., 2000; Wilson *et al*., 2004), and BBP (Nagao *et al*., 2000; Wilson *et al*., 2004). When exposed only *in utero*, testosterone levels returned to normal after PND 35 (Akingbemi *et al*., 2001; Fisher *et al*., 2003). Male rabbits exposed *in utero* to DBP also exhibited decreases in testosterone six weeks after exposure that were reversible at 12 weeks (Higuchi *et al*., 2003). In contrast, testicular descent is at least partially testosterone-independent and

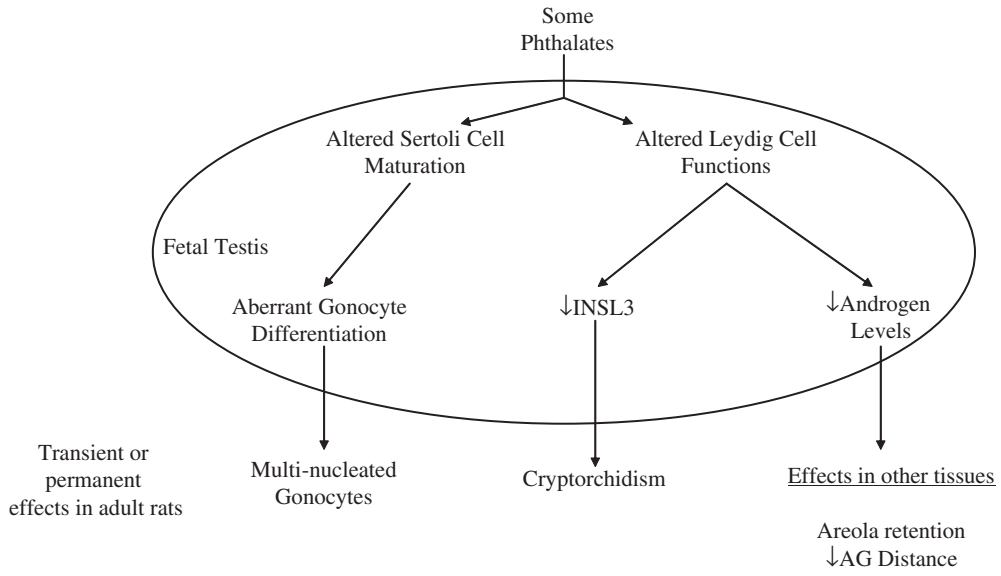


**FIG. 1.** Effects in the male reproductive tract after *in utero* exposure to toxic phthalates. Some phthalates can alter the fetal testis through effects on Sertoli and Leydig cells. A number of permanent or transient downstream effects are observed in the adult rat. Decreases in testosterone levels could also impair spermatogenesis. See text for details. AG, anogenital distance.

may be disrupted through an alternate mechanism involving decreases in insulin-like hormone 3 (*INSL3)* expression observed in fetal testis after exposure to DBP, DEHP, and BBP (Wilson *et al*., 2004). Overall, the data is consistent with some phthalates indirectly down-regulating the activity of the androgen receptor through decreases in testosterone levels, resulting in delayed or absent development of androgen-dependent male reproductive organs.

### *Alteration of Testis Structure and Function by Phthalates in Postnatal and Adult Rats*

Exposure of postnatal, preadolescent, and adult rats to some phthalates produces a different set of sequelae in testis compared to that of male rats exposed *in utero*. The age of the rat at the time of exposure has a dramatic effect on the testis as younger animals are more sensitive to the effects of phthalates (Gray and Gangolli, 1986). Compared to rats, mice are relatively resistant at any age. Some phthalates induce testicular toxicity in guinea pigs (Gray *et al*., 1982) and ferrets (Lake *et al*., 1976). Resistance to phthalate toxicity in Syrian hamsters is likely due to inefficient metabolic activation of diesters to monoesters by gut esterases compared to that in rats (Gray *et al*., 1982). Exposure to phthalates in responsive species leads to decreases in seminiferous tubule diameter and testis weight. At least in rats, this occurs through increased germ cell apoptosis and necrosis preceding the sloughing of germ cells into the tubular lumen (Gangolli, 1982).

The primary cellular target of toxic phthalates is the Sertoli cell which exhibits biochemical and morphological changes after exposure. FSH-stimulated cAMP accumulation in primary Sertoli cells was inhibited by Mon-(2-ethyl)-hexlphthalate (Heindel and Chapin, 1989; Lloyd and Foster, 1988), MBP and monopentyl phthalate, but not monomethyl or monoethyl phthalate (Heindel and Powell, 1992). Decreases in proliferation of Sertoli cells were observed after DEHP exposure *in vivo* (Li *et al*., 2000) or after exposure to MEHP *in vitro* (Li *et al*., 1998; Li and Kim, 2003), and may occur through a block in FSH-stimulated Sertoli cell proliferation (Li *et al*., 1998). Two genes which play roles in Sertoli cell proliferation and differentiation, Müllerian inhibiting substance and GATA-4, were decreased by MEHP in Sertoli cells within cultured testis from GD 18 and PND 3 rats (Li and Kim, 2003). Sertoli cell numbers recover somewhat during the course of exposure, as the decreases observed at one week were no longer evident after two to three weeks exposure to DEHP (Dostal *et al*., 1988). In rats exposed to MEHP, collapse in the Sertoli cell intermediate filament vimentin structure (indicative of changes in cell morphology) was observed as early as 3 h, followed by increases in perinuclear condensation of vimentin at 6–12 h. These changes preceded or were coincident with increased apoptosis in germ cells (Richburg and Boekelheide, 1996). Immunostaining for flamingo1/Celsr2, a G protein coupled receptor family member that may link cell-cell adhesion to G-protein dependent signaling was redistributed within 2 h of MEHP exposure and disappeared by 12 h, indicating that this protein is a proximate target of MEHP (Richburg *et al*., 2002).

Germ cells exhibit changes that may be secondary to effects on Sertoli cells. In the intact testis DEHP increased the generation of reactive oxygen species with concomitant decreases in the concentration of glutathione and ascorbic acid, and in primary cultures, oxidative stress was selectively induced in germ cells but

not in Sertoli cells treated with MEHP (Kasahara *et al*., 2002). MEHP may specifically target the stages IX–XI of the mouse seminiferous cycle as apoptosis was increased in cells of these timed tubule segments treated *in vitro* (Suominen *et al*., 2003). Sertoli cells from mice treated with DEHP express the Fas ligand (FasL) while associated spermatocytes express Fas, the receptor for FasL, indicating that activation of the Fas system originating in Sertoli cells could lead to subsequent apoptosis in spermatocytes (Ichimura *et al*., 2003). Experiments in *gld* mice which carry a defective *FasL* gene revealed that germ cell apoptosis after MEHP exposure had both Fas-dependent (Richburg *et al*., 2000) and Fas-independent components (Giammona *et al*., 2002). Additionally, MEHP alters the expression and activity of death receptors 4,5,6 in the testis which may act in concert with the Fas-signaling pathway to initiate germ cell apoptosis (Giammona *et al*., 2002).

Leydig cells in postnatal and adult rats are also targeted by some active phthalates. DEHP (Jones *et al*., 1993; Kim *et al*., 2003) and di-n-octyl phthalate (Jones *et al*., 1993) decreased testosterone serum levels. Primary Leydig cells treated with MEHP or mono-octyl phthalate also exhibited decreases in testosterone secretion (Jones *et al*., 1993) although the concentrations used to achieve these effects were very high ($10^{-3}$ M). Human chorionic gonadotropin-induced testosterone secretion was suppressed by prior exposure of Leydig cells in culture to PP (Biegel *et al*., 1995; Cook *et al*., 1992; Liu *et al*., 1996). No phthalates were tested in these studies, however. Decreases in testosterone levels may explain the decreased weights of accessory sex organs (seminal vesicle and prostate) in male rats exposed to DEHP that were partially reversed by co-administration of testosterone or gonadotropins (Gray and Gangolli, 1986). Decreased testosterone levels may also play a role in the genesis of Leydig cell tumors (Klaunig *et al*., 2003) through increases in compensatory Leydig cell proliferation observed with DEHP (Akingbemi *et al*., 2004) and the PP, WY-14,643 (Biegel *et al*., 1992). Two studies have shown increases in serum testosterone levels in male rats after exposure to DEHP at 200 mg/kg from PND 21–48 (Akingbemi *et al*., 2001) or relatively low doses of DEHP (10 or 100 mg/kg) for PND 21–90 (Akingbemi *et al*., 2004), which the authors attribute to an observed increase in Leydig cell numbers. Thus, short-term exposure to phthalates may lead to decreases in testosterone while longer exposures may stimulate Leydig cell proliferation resulting in consequent increases in testosterone. A more comprehensive time course would be useful to confirm these events for DEHP and other phthalates.

*Expression of PPARs in the Testes*

PPAR subtypes are expressed in the adult rat testes (Table 2). PPARα was expressed in Leydig and Sertoli cells of the adult rat (Braissant *et al*., 1996; Schultz *et al*., 1999). Conflicting reports exist as to the expression of PPARα in spermatocytes, with one report detecting expression (Schultz *et al*., 1999) and the other negative for expression (Braissant *et al*., 1996). PPARβ is expressed in Leydig and Sertoli cells but not in spermatocytes (Braissant *et al*., 1996). PPARγ, in contrast, is either weakly expressed or not expressed at all in these cell types (Braissant *et al*., 1996). The spectrum of PPAR subtype expression in rat Leydig cells is similar to that in a Leydig mouse cell line (MA-10); PPARα and PPARβ, but not PPARγ were expressed (Gazouli *et al*., 2002). Although there are no reports, that we know of, that examine PPARα target gene expression specifically in Sertoli cells or spermatocytes, there were increased levels of two PPARα gene targets involved in peroxisomal β-oxidation (acyl-CoA oxidase, multifunctional protein-1) in Leydig cells after adult rats were fed the PP ciprofibrate in the diet for two weeks (Nemali *et al*., 1988) indicating that PPARα expressed in Leydig cells is responsive to PP. In contrast, neither WY or the PP ammonium perfluorooctanoate increased peroxisomal β-oxidation in Leydig cells from chronically treated rats (Biegel *et al*., 2001).

The PPARα gene exhibited stage-specific expression during the spermatocyte differentiation cycle, peaking during stages II–VI and having the lowest expression at stages VII–VIII and IX–XII as assessed in isolated rat seminiferous tubules. Strong PPARα expression was observed in Sertoli cell nuclei during stages XIII–I (Schultz *et al*., 1999), a time when cells are most sensitive to FSH (Parvinen, 1982). Consistent with this, PPARα expression was increased in cultured seminiferous tubules after FSH treatment during all stages of the cycle (Schultz *et al*., 1999). These results indicate that PPARα expression is controlled in part by FSH, and that PPARα carries out a specific functional role during different stages of the differentiation cycle.

Only expression of PPARα has been examined in the early postnatal testis (Schultz *et al*., 1999). Expression of PPARα was

**TABLE 2**
**mRNA Expression of PPAR Subtypes in Rat and Human Testis**

| | Rat PPAR subtype | | | Human PPAR subtype | | |
|---|---|---|---|---|---|---|
| Testis cell type | α | β | γ | α | β | γ |
| Leydig cells | $+^{a,b,c}$ | $+^{a,b}$ | $-^{a,b}$ | $+^{c}$ | | |
| Sertoli cells | $+^{b,c}$ | $+^{b}$ | $+/-^{b}$ | $-^{c}$ | | |
| Spermatocytes | $+/-^{b,c}$ | $-^{b}$ | $-^{b}$ | $+^{c}$ | | |
| Whole testis | $+^{c}$ | $+^{d}$ | | $+^{e}$ | $+^{e}$ | $+/-^{e,f}$ |

*Note.* Blank spaces indicate no information available. −, no expression; +, weak to strong expression; +/−, either barely detectable expression or conflicting data (see text).

[a]Gazouli *et al*., 2002 (expression determined in the mouse Leydig cell line MA-10).

[b]Braissant *et al*., 1996.

[c]Schultz *et al*., 1999.

[d]Xing *et al*., 1995.

[e]Hase *et al*., 2002.

[f]Elbrecht *et al*., 1996.

high in rat seminiferous tubules at PND 1 followed by a steady decline until PND 30 and an increase at PND 60. Information about expression of PPAR subtypes in the rat testis *in utero* is lacking but PPARα-null (Lee *et al*., 1995) and PPARβ-null (Peters *et al*., 2000) mice remain viable and fertile indicating that these receptors are not essential for mouse testicular development and fertility. Compensatory mechanisms have not been ruled out (e.g., increased expression of another PPAR subtype) that would allow proper testicular development.

PPARα was expressed in human Leydig cells and spermatocytes, but not in Sertoli cells (Schultz *et al*., 1999). PPARα and PPARβ were expressed in human testis homogenates (Hase *et al*., 2002). PPARγ may also be expressed although conflicting data exists (Elbrecht *et al*., 1996; Hase *et al*., 2002). Overall, these results indicate that PPAR subtypes are expressed in some types of adult cells in both rat and human testis. Further work is needed to determine if PPAR subtypes are expressed at both the transcript and protein levels in the testis throughout development from humans as well as a range of species susceptible to phthalate-induced testicular toxicity.

### *Activation of PPARs by Phthalate Esters*

Despite their structural diversity compared to ligands that activate other nuclear receptors, PP do have similar structural requirements for activating PPARs *in vitro* and for initiating biological effects in animals. Most PP are amphipathic molecules containing a hydrophobic backbone (aliphatic or aromatic) linked to an acidic group or a moiety that can be metabolized to an acidic group. The acidic group is essential for ligand activity and typically consists of a carboxylic acid present in the parent compound. In the case of phthalates, esterases cleave a side chain from the diester resulting in a monoester containing a carboxylic acid. Some PP resemble endogenous lipid activators of PPARs such as long-chain saturated and unsaturated fatty acids (Xu *et al*., 1999).

Many phthalates activate PPARs in *in vitro* transactivation assays. In these assays either full length PPAR (Hurst and Waxman, 2003; Lampen *et al*., 2003; Lapinskas *et al*., 2004; Maloney and Waxman, 1999) or hybrid transcription factors consisting of the PPAR ligand binding domain cloned to the glucocorticoid receptor (Lampen *et al*., 2003) or GAL4 (Bility *et al*., 2004) DNA binding domains were co-transfected into a mammalian cell line with a reporter gene (e.g., luciferase) under control of either PPAR binding sites called peroxisome proliferator responsive elements (PPREs) (Hurst and Waxman, 2003; Lapinskas *et al*., 2004; Maloney and Waxman, 1999), glucocorticoid responsive elements (Lampen *et al*., 2003) or the binding site for GAL4, $UAS_G$ (Bility *et al*., 2004). After time to express the receptor, the cells are treated with the phthalate esters. The reporter gene activity is then normalized to the activity of an additional reporter gene used as a transfection control and results are usually reported as a fold-change relative to a solvent control.

Mouse PPARα and PPARγ are activated by a large number of phthalate monoesters (Table 3). Monoester phthalates with longer aliphatic or aromatic side chains tended to be more potent activators. Monoesters with short straight chains either do not activate at all or activate weakly. Monooctyl phthalate possessing a longer side chain activated all three subtypes (Bility *et al*., 2004), consistent with the observation that the longer the length of the fatty acid (up to 21 carbons), the better the fatty acid is at activating PPARs *in vitro* (Xu *et al*., 1999). Monooctyl and monoheptyl phthalates are unique in that they can activate PPARs *in vitro* but do not act as PP because the aliphatic side chains are rapidly cleaved *in vivo* (Albro and Moore, 1974). Steric restrictions appear to play a part in the ability of some monoesters to activate. Monoesters with the bulkiest side chains were less potent activators than MEHP or were completely inactive (e.g., mono-(1-methyl)-2-norbornyl phthalate, mono-(2,2-dimethyl-1-phenylpropyl) phthalate). The DEHP metabolite 2-ethylhexanoic acid weakly activated PPARα and γ compared to MEHP. In two studies the diesters DEHP and BBP were able to activate PPARα and PPARγ, albeit weakly (Lampen *et al*., 2003; Lapinskas *et al*., 2004) despite the fact that the monoesters are considered to be the active toxic species. However, it is possible that the activation reflects a low level of esterase activity in the cell lines used.

Mouse PPARβ was activated by only a small number of phthalates and in general, higher concentrations of those phthalates were required to activate to the same level as PPARα and PPARγ. PPARβ was activated by monoester phthalates with longer or bulkier side chains such as MEHP, mono-isohexyl phthalate, mono-(1-methyl)-heptyl phthalate, mono-isodecyl phthalate and monobenzyl phthalate but not monoesters with shorter side chains (Bility *et al*., 2004; Lampen *et al*., 2003).

Human PPARs were also activated by phthalates. All human PPAR subtypes were activated by MEHP, mono-(1-methyl)-heptyl phthalate and monobenzyl phthalate. Mono-*sec*-butyl phthalate, DL-mono-(1-methyl)-hexyl phthalate, mono-isoheptyl phthalate, mono-isodecyl phthalate, mono-isohexyl phthalate and mono-2-(methacryloyloxy)ethyl phthalate were more selective, activating only one or two of the subtypes. Importantly, the human PPARs required higher concentrations of the monoesters to be activated to the same levels as the corresponding mouse receptor (Bility *et al*., 2004; Hurst and Waxman, 2003; Maloney and Waxman, 1999).

Activation of PPARs comes about through two distinct mechanisms. Most PP are thought to bind directly to PPAR leading to activation. However, there is evidence that some PP may activate PPARs indirectly, through increasing the pool of endogenous activators, e.g., by displacing fatty acids from carrier proteins (Luebker *et al*., 2002). To determine whether phthalate esters interact directly with PPARs, the scintillation proximity assay (Nichols *et al*., 1998) was used to assess the ability of phthalate esters to bind to human PPARα and PPARγ (Lapinskas *et al*., 2004). Some monoester phthalates interacted with both receptors, including, monohexyl

**TABLE 3**
**Binding and Activation of PPARs by Phthalate Esters**

| | PPARα | | | PPARγ | | | PPARβ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mouse activation | Human activation | Human binding[c] | Mouse activation | Human activation | Human binding[c] | Mouse activation | Human activation | Reproductive tract defects in males[f] |
| Mono-substituted straight chain phthalate | | | | | | | | | |
| Monomethyl | +/−[a,b] | −[a] | | −[a,b] | −[a] | | −[b] | | −[g] |
| Monoethyl | −[b] | −[r] | 83 | −[b] | −[r] | 132 | −[b] | +[r] | −[g] |
| Monopropyl | | | − | | | − | | | |
| Mono-*n*-butyl | −[a,d]; +[r] | −[a]; +[r] | − | −[a]; +[d,r] | −[a,r] | − | −[d,r] | −[r] | +[h–m] |
| Monopentyl | | | − | | | − | | | |
| Monohexyl | | | 41 | | | 54 | | | |
| Monooctyl | +[r] | −[r] | 44 | +[r] | +[r] | 19 | +[r] | −[r] | |
| Mono-substituted branched chain phthalate | | | | | | | | | |
| Mono-*sec*-butyl | +[a] | +[a] | | +[a] | −[a] | | | | |
| Mono-(1,2-dimethyl)-propyl | +[b] | | | +[b] | | | −[b] | | |
| Mono-(2,2-dimethyl-1-isopropyl)-propyl | ++[b] | | | +[b] | | | −[b] | | |
| Mono-(2,2-dimethyl-1-ethyl)-propyl | ++[b] | | | ++[b] | | | −[b] | | |
| Mono-(1-tert-butyl-3-methyl)-butyl | +[b] | | | +[b] | | | −[b] | | |
| (+)-Mono-(1-tert-butyl-3-methyl)-butyl | +[b] | | | +[b] | | | −[b] | | |
| Mono-(1-methyl)-2-norbornyl | +[b] | | | +[b] | | | −[b] | | |
| dl-Mono-(1-methyl)-hexyl | ++[b] | ++[b] | | ++[b] | ++[b] | | −[b] | −[b] | |
| Mono-(2-ethyl)-hexyl (MEHP) | ++[a,b,d,e,r] | +[a,b,e,r] | 15 | ++[a,b,d,e,r] | +[a,b,e,r] | 12 | +[b,d] | +[b–r] | +[g,i,n,o] |
| Mono-(1-methyl)-heptyl (MHP) | ++[b] | +[b] | | ++[b] | +[b] | | ++[b] | +[b] | |
| Mono-2-(methacryloyloxy) ethyl | +[b] | +[b] | | ++[b] | ++[b] | | −[b] | −[b] | |
| Mono-isohexyl | +[r] | +[r] | | +[r] | −[r] | | +[r] | −[r] | |
| Mono-isoheptyl | +[r] | +[r] | | +[r] | +[r] | | −[r] | −[r] | |
| Mono-isononyl | +[r] | −[r] | | +[r] | +[r] | | −[r] | −[r] | +[g] |
| Mono-isodecyl | +[r] | −[r] | | +[r] | +[r] | | +[r] | −[r] | |
| Mono-substituted aromatic chain phthalate | | | | | | | | | |
| Mono-benzyl | ++[a,b,r] | +[a–r] | | ++[a,b,r] | +[a,r] | | ++[b,r] | +[b,r] | +[g,p,q] |
| Mono-(1-ethyl-1-methyl)-benzyl | +[b] | | | +[b] | | | −[b] | | |
| Mono-(3-chlorophenyl)-phenylmethyl | +[b] | | | ++[b] | | | −[b] | | |
| (-)-Mono-(2,2-dimethyl-1-phenylpropyl) | −[b] | | | −[b] | | | −[b] | | |
| (+)-Mono-(2,2-dimethyl-1-phenylpropyl) | −[b] | | | −[b] | | | −[b] | | |
| Di-substituted phthalate | | | | | | | | | |
| Dimethyl phthalate | −[a,b] | −[a] | | −[b] | | | −[b] | | |
| Diethyl phthalate | −[a] | −[a] | | −[a] | −[a] | | | | |
| Di-*n*-butyl phthalate (DBP) | +[d] | | 34 | +[d] | | 10 | +[d] | | |
| Di-(2-ethyl-hexyl) phthalate (DEHP) | +/−[b,d,e] | −[e] | − | +/−[b,d] | −[e] | − | −[b,d] | | |
| Benzyl-butyl phthalate (BBP) | ++[b,d] | | 27 | ++[b,d] | | 10 | +[b,d] | +[b] | |
| Miscellaneous | | | | | | | | | |
| WY-14,643 | ++[d,e] | +[e] | 9 | +[d] | + | 44 | +[d] | | |
| 2-Ethylhexanoic acid | +[b,d] | +[e] | | +[b,d]_[e] | −[e] | | +[b,d] | | |

*Note.* The transactivation data from the Lampen *et al.* (2003) study has been simplified: ++, < 150 μMol, effective concentration to induce reporter gene expression two-fold; +, > 150 μMol, effective concentration to induce reporter gene expression two-fold.

[a]Hurst and Waxman, 2003. [b]Lampen *et al.*, 2003. [c]Binding data (Ki) using scintillation proximity assays for human PPARα and PPARγ in μM from Lapinskas *et al.*, 2004. [d]Lapinskas *et al.*, 2004. [e]Maloney and Waxman, 1999. [f]The ability to induce male reproductive tract defects through *in utero* exposure is indicated for the putative phthalate monoester to facilitate comparison with the PPAR activation data. [g]Gray *et al.*, 2000. [h]Wine *et al.*, 1997. [i]Gray *et al.*, 1999. [j]Mylchreest *et al.*, 1999. [k]Higuchi *et al.*, 2003. [l]Ohtani *et al.*, 2000. [m]Imajima *et al.*, 2001. [n]Moore *et al.*, 2001. [o]Parks *et al.*, 2000. [p]Nagao *et al.*, 2000. [q]Piersma *et al.*, 2000. [r]Bility *et al.*, 2004.

phthalate, monooctyl phthalate, monobenzyl phthalate, but not shorter chain phthalates (monopropyl phthalate, monopentyl phthalate). Despite the short length of the side chain, monoethyl phthalate binds with low affinity to both PPARα and PPARγ. BBP and DBP also bind weakly to both subtypes and this could explain why these diesters were able to weakly activate in transactivation assays. The data supports phthalate-induced changes in gene expression through binding to and activation of PPAR by monoester phthalates.

### *Activation of Other Nuclear Receptors by Phthalates*

Phthalate esters have been tested for their ability to interact with sex hormone receptors. In transactivation assays, a wide range of diesters and monoesters did not act as androgen receptor agonists or antagonists in HepG2 cells (Gaido, personal communication). In addition, DEHP and its active metabolite, MEHP, do not bind to the human androgen receptor (Parks *et al*., 2000). Some phthalates behave like estrogen receptor ligands in that the phthalates inhibited binding of estrogen to isolated estrogen receptors and induced estrogen-responsive endpoints *in vitro* (summarized in Moore, 2000). The relevance of these findings is questioned as monoesters, considered to be the proximate metabolites, were inactive in these *in vitro* assays and the phthalate diesters that do alter estrogen receptor activity only do so at concentrations that approach solubility of the compound, which could lead to nonspecific effects. Importantly, the phthalates were uniformly negative in a number of *in vivo* tests for estrogenicity including the uterotrophic assay (Moore, 2000). Taken together, these studies indicate that phthalates do not mediate their effects through estrogen or androgen receptors, but rather, support PPAR subtypes as primary nuclear receptor targets for phthalate esters.

### *Correlation of PPAR Activation to Male Reproductive Tract Malformations*

The strength of the monoester as a PPAR activator partially correlates with the ability of the parent phthalate to act as a male reproductive toxicant (Table 3). Monomethyl and monoethyl phthalate were either inactive or only weakly active as PPAR activators. The parent phthalates dimethyl and diethyl phthalate were inactive as *in utero* reproductive toxicants (Gray *et al*., 2000). In contrast, MEHP was an activator of all three mouse PPAR subtypes and DEHP exposure led to a spectrum of male reproductive tract changes in rats (Gray *et al*., 2000; Moore *et al*., 2001; Parks *et al*., 2000). BBP is hydrolyzed to either monobenzyl phthalate or monobutyl phthalate with the ratio of hydrolysis products in rats being approximately 3:5, respectively (Mikuriya *et al*., 1988). Monobenzyl phthalate activated all PPAR subtypes and BBP was positive as a reproductive toxicant (Gray *et al*., 2000; Nagao *et al*., 2000; Tyl *et al*., 2004).

There are two outliers in this comparison. DINP was a very weak reproductive toxicant while MINP was a moderate activator of mouse PPARα and PPARγ (Bility *et al*., 2004). DBP is a reproductive toxicant in rats despite the fact that MBP was only weakly active in PPAR transactivation assays (Bility *et al*., 2004; Hurst and Waxman, 2003; Lapinskas *et al*., 2004). The weak activity of MBP in PPAR transactivation assays was surprising given that DBP, like DEHP and BBP, acts like a typical PP in the rat liver (Corton *et al*., 1996; Marsman, 1995) and effects in the mouse liver induced by DBP as well as DEHP and DINP are dependent on PPARα (Anderson *et al*., 1999; Lapinskas *et al*., 2004; Valles *et al*., 2003; Ward *et al*., 1998). Although treatment with MBP induced the same effects as DBP in the juvenile rat testis (Foster *et al*., 1981; Oishi and Hiraga, 1980) indicating that MBP is the proximate metabolite, no studies have been carried out in which MBP metabolites were comprehensively examined for toxic effects in the testis as well as abilty to activate PPARs. Candidate metabolites include MBP-glucuronide, a dominant MBP metabolite in adult rats (Foster *et al*., 1983; Tanaka *et al*., 1978) and omega and omega-1 products of MBP (Tanaka *et al*., 1978). It is also possible that a metabolite of DBP or DBP itself activates PPARs indirectly, i.e., by releasing a lipid activator of PPAR as discussed above a process which would not necessarily be detectable using transactivation assays. The fact that two out of six phthalates are outliers in this comparison weakens the hypothesis that PPARs are generally involved in phthalate-induced male reproductive tract defects.

### *Regulation of Sex Hormone Synthesis and Catabolism Genes by Phthalate Esters*

As discussed above, BBP, DBP, and DEHP decrease testosterone levels in the fetus and in the young male rat. Consistent with this, genes involved in testosterone biosynthesis were uniformly down-regulated by DBP exposure in the fetal testis (Barlow *et al*., 2003; Shultz *et al*., 2001; Thompson *et al*., 2004) (Fig. 2A). The genes include Scavenger Receptor Class B, type 1 (SR-B1) and steroidogenic acute regulatory protein (StAR) (Barlow *et al*., 2003; Shultz *et al*., 2001). SR-B1 mediates the selective uptake of cholesterol esters from high-density lipoproteins, and StAR carries cholesterol from the outer to the inner mitochondrial membrane. The peripheral benzodiazepine receptor (PBR), which also carries cholesterol into the mitochondria was down-regulated by WY-14,643 and DEHP in adult mouse testes in a PPARα-dependent manner (Gazouli *et al*., 2002). In the fetal rat testis, PBR mRNA was up-regulated by DBP but by immunohistochemistry the protein was decreased in Leydig cells (Lehmann *et al*., 2004). Leydig cell mitochondria isolated from fetuses exposed to DBP *in utero*, exhibited decreased uptake of cholesterol supporting altered cholesterol handling for the decreases in testosterone synthesis (Thompson *et al*., 2004). Other genes involved in steroid biosynthesis that were down-regulated by DBP included P450 side chain cleavage enzyme (P450scc) thought to be the limiting enzymatic step in testosterone biosynthesis, 3β-hydroxysteroid dehydrogenase

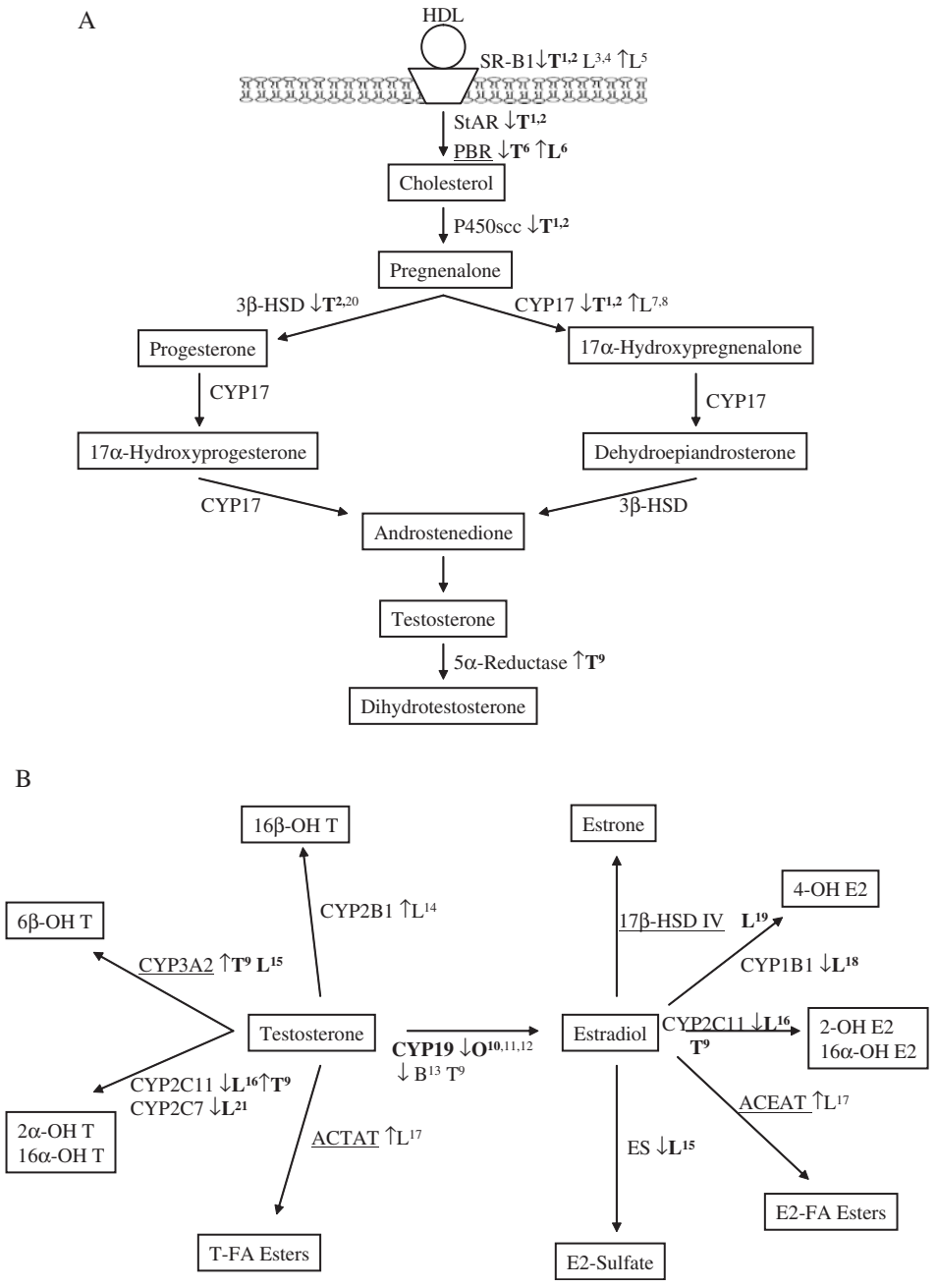


**FIG. 2.** Alteration of genes involved in steroid hormone metabolism by peroxisome proliferators. (A) Genes involved in testosterone biosynthesis. (B) Genes involved in estrogen and testosterone catabolism. Up- or down-regulated genes are indicated by arrows. Genes known to be regulated by PPARα are underlined. Studies which showed the genes to be regulated by phthalates are shown in bold. Organs in which the genes are altered by PP are footnoted: liver [L], kidneys [K], testes [T], breast [B] and ovaries [°]. References to literature are also footnoted (compounds used in the studies are indicated in parenthesis): [1]Shultz *et al*., 2001 (DBP); [2]Barlow *et al*., 2003 (DBP); [3]Fu *et al*., 2003 (clofibrate); [4]Mardones *et al*., 2003 (fibrates); [5]Malerod *et al*., 2003 (WY, BRL49653); [6]Gazouli *et al*., 2002 (WY, DEHP); [7]Gu *et al*., 2003 (nafenopin); [8]Richert *et al*., 2003 (clofibric acid); [9]Kim *et al*., 2003 (DEHP); [10]Lovekamp *et al*., 2001 (MEHP); [11]Toda *et al*., 2003 (fenofibrate); [12]Mu *et al*., 2001 (troglitazone); [13]Rubin *et al*., 2000 (troglitazone); [14]Bars *et al*., 1993 (clofibric acid); [15]Fan *et al*., 2004-b (WY, gemfibrozil, DBP); [16]Corton *et al*., 1998 (WY, gemfibrozil, DBP); [17]Xu *et al*., 2001 (WY); [18]Seo *et al*., 2004 (DBP, DEHP, BBP); [19]Corton *et al*., 1996 (WY, gemfibrozil, DBP); [20]BLAST sequence homology searches were carried out using the published sequence of the oligonucleotides used to determine the expression of 3β-HSD; there was an exact match for rat Types 1 and 2 (forward primer) and an exact match for rat Type 1 (one example), or one mismatch out of 21 nucleotides for rat Type 1 (two examples), type II, type IV, and type V (reverse primer) indicating multiple 3β-HSD subtypes may be queried in these studies. [21]Fan *et al*., 2004-a (WY, gemfibrozil, DBP). Abbreviations: ACEAT, acyl-coenzyme A:estradiol acyltransferase; ACTAT, acyl-coenzyme A:testosterone acyltransferase; E2-FA, estrogen-fatty acid; ES, estrogen sulfotransferase; HDL, high-density lipoprotein; SR-B1, Scavenger Receptor Class B, Type 1; StAR, steroidogenic acute regulatory protein; P450scc or CYP11a, cholesterol side-chain cleavage; DHEA, dehydroepiandrosterone; HSD, hydroxysteroid dehydrogenase; DHT, dihydrotestosterone; PBR, peripheral benzodiazepine receptor. (A) was adapted from Wong and Gill (2002).

(3β-HSD) and CYP17α (Barlow *et al*., 2003; Shultz *et al*., 2001). Uniform decreases in the expression of these steroidogenic genes especially in a dose-dependent manner (Lehmann *et al*., 2004) is consistent with decreased levels of testicular testosterone.

The nuclear receptor steroidogenic factor 1 (SF-1) plays a prominant role in the development and differentiation of steroidogenic tissues and controls the expression of steroidogenic enzymes and cholesterol transporters required for steroidogenesis (Val *et al*., 2003). INSL3 (Emmen *et al*., 2000) and receptors for follicle-stimulating and leutinizing hormones (Val *et al*., 2003) are also regulated by SF-1. Genes altered by DBP exposure including SR-B1, StAR, P450scc, CYP17 and 3β-HSD, Type II (Barlow *et al*., 2003; Shultz *et al*., 2001) require SF-1 for basal promoter activity and for cyclic AMP induction. The hypothesis that phthalate ester exposure leads to suppression of SF-1 activity was tested in the Leydig cell line MA-10 in which expression of a reporter gene linked to the promoters of SR-B1, StAR, and CYP17 genes was measured after MBP exposure (Thompson *et al*., 2004). However, MBP had no effect on reporter gene expression. Although this finding indicates that phthalates inhibit steroidogenic gene expression independently of effects on SF-1 activity additional monoesters should be tested in this system before any definitive conclusion can be made of SF-1 involvement. The mechanism by which these genes are down-regulated by DBP and whether other phthalates target the same genes requires further study.

Phthalate esters along with other PP, also alter the expression of testosterone and estrogen metabolism genes (Figs. 2A and 2B). Among these, 5α-reductase which converts testosterone to the more potent androgen, dihydrotestosterone was up-regulated in the prepubertal rat testis by DEHP (Kim *et al*., 2003). Multiple cytochrome P450 family members which hydroxylate testosterone were altered by PP exposure but in ways that do not clearly indicate a role in decreasing testosterone levels. CYP2C7 and CYP2C11 were down-regulated while CYP3A2 was up-regulated in the male rat liver (Corton *et al*., 1998; Fan *et al*., 2004-a,b). CYP2C11 and CYP3A were both up-regulated in the testes (Kim *et al*., 2003). WY-14,643 increased expression of fatty acyl-CoA enzyme A:testosterone acyl transferase in the liver (Xu *et al*., 2001). The down-regulation of CYP2C11 by a PP required PPARα; the site within the CYP2C11 promoter required for down-regulation by PP was identified and likely interacts with transcription factors other than PPARα implying that PPARα down-regulates CYP2C11 through an indirect mechanism (Ripp *et al*., 2003).

Serum estrogen levels were increased in male rats after DEHP exposure (Akingbemi *et al*., 2004; Eagon *et al*., 1994) and after exposure to a number of PP (Biegel *et al*., 2001; Liu *et al*., 1996). Phthalates alter the expression of many estrogen metabolizing enzymes (Fig. 2B). Aromatase exhibits both up-and down-regulation by PP depending on the tissue and compound (Biegel *et al*., 2001; Kim *et al*., 2003; Lovekamp and Davis, 2001; Lovekamp-Swan *et al*., 2003; Mu *et al*., 2001; Rubin *et al*., 2000; Toda *et al*., 2003). Genes that decrease estrogen levels were increased after PP exposure including 17β-hydroxysteroid dehydrogenase, type IV (Corton *et al*., 1996) and fatty acyl-CoA enzyme A:estradiol acyl transferase (Xu *et al*., 2001). However, these changes may be offset by decreases in the estrogen hydroxylases CYP1B1 after exposure to DBP, DEHP, and BBP in the testis (Seo *et al*., 2004) or CYP2C11 (Corton *et al*., 1998) and estrogen sulfotransferase (Fan *et al*., 2004-b) after WY, GEM, or DBP exposure in rat livers. A handful of these genes involved in testosterone or estrogen metabolism are directly regulated by PPARα either in the liver or testis as determined by studies in PPARα-null mice. These include 17β-HSD, type IV (Corton *et al*., 1996) and the CYP3A2 ortholog *Cyp3a11* (Fan *et al*., 2004-b). These results point to a role for PPARα in regulating expression of some sex steroid metabolism genes upon phthalate exposure in the adult animal. Although these studies help us appreciate the gene targets of phthalates involved in regulation of steroid metabolism, more work is needed to determine the mechanism by which phthalates alter the expression of these genes, whether these genes are also regulated in parallel at the protein level and the contribution of these collective changes to phthalate toxicity in the postnatal testis.

### *PPARα and Phthalate-Induced Testicular Toxicity*

The role of PPARα in phthalate-induced developmental and testicular toxicity has been determined directly in wild-type and PPARα-null mice in two studies. In the first study (Peters *et al*., 1997), pregnant dams were dosed with DEHP at GD 8 and GD 9 (before development of the male reproductive tract) and malformations of the fetuses were evaluated on GD 10 and GD 18. DEHP exposure decreased crown-rump length and increased the incidence of open neural tubes (failure of the hind- and mid-brain to close) in both DEHP-treated wild-type and PPARα-null mice compared to controls, demonstrating that DEHP-induced malformations are PPARα-independent. However, this study cannot be used to determine the role for PPARα in phthalate-induced male reproductive tract defects from *in utero* exposure, because DEHP treatment did not occur during the critical window of development of the male reproductive tract and to our knowledge, the rat model of *in utero* male reproductive tract defects has not been successfully recapitulated in mice.

In the second study (Ward *et al*., 1998), male wild-type and PPARα-null mice were fed a diet containing 12,000 ppm DEHP and lesions in the liver and testis were examined. In the liver all of the expected effects of DEHP were dependent on PPARα. After four and eight weeks of exposure, testis from wild-type mice exhibited mild or moderate toxic effects including focal tubular degenerative lesions, decreased spermatogenesis, and giant cells within the epididymis. In sharp contrast, the testis from PPARα-null mice at these time points were predominantly normal except for a few tubules that lacked normal indicators of spermatogenesis. PPARα-null mice at 24 weeks exhibited moderate testicular effects. However, all of the wild-type mice were sacrificed at

12–16 weeks due to toxicity when there was severe testicular atrophy making it impossible to directly compare the effects in the two strains at the 24 week time point. The authors concluded that DEHP effects in the testis were the result of both PPARα-dependent and -independent mechanisms. This study indicates that PPARα determines the timing and severity of testicular toxicity by DEHP. To better characterize the role of PPARα in phthalate-induced testicular toxicity, additional studies using other phthalates should be carried out in wild-type and PPARα-null mice. Given that PPARβ is expressed in the testis, PPARβ-null mice could also be used to determine any role played by this receptor in phthalate-induced testicular toxicity. Determining a role for PPARγ will require construction of testis-specific nullizygous mice as the PPARγ-null mutation is embryonically lethal (Barak *et al*., 1999).

### *Cross-Talk between PPAR and Nuclear Receptor Signaling: Potential Impact on the Male Reproductive Tract*

Several studies have demonstrated 'cross-talk' between PPAR and other nuclear receptors. Cross-talk occurs when signaling pathways other than the primary pathway are activated or repressed through interactions with components of the primary pathway. PPAR-induced interference of other nuclear receptor pathways occurs through competition between PPARs and other receptors for binding to (1) the same DNA response element or (2) a common heterodimerization partner (Fig. 3). In the Supplementary Materials we examine cross-talk between PPARs and nuclear receptors for estrogen, thyroid hormone, and retinoic acid that play roles in the development or homeostasis of the testis. Many of these studies supporting interactions between PPAR and other nuclear receptors were either performed with PP other than phthalates in *in vitro* cell models allowing only conjecture as to how PPAR-phthalate interactions may disrupt male reproductive tract function *in vivo*. Further work is needed before these proposed mechanisms should be considered to play a role in phthalate-induced effects in the male reproductive tract.

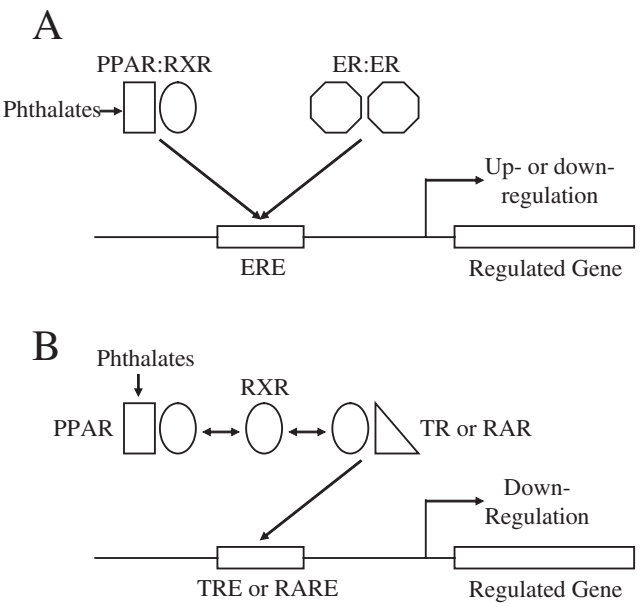


**FIG. 3.** Modes of cross-talk between PPARs and other nuclear receptors. (A) Cross-talk between PPAR and estrogen receptor. Heterodimers of PPAR and RXR compete with estrogen receptor (ER) homodimers for binding to an estrogen response element (ERE). The result is either up- or down-regulation by phthalates depending on the promoter context of the ERE. (B) Cross-talk between PPAR and thyroid hormone receptor or retinoic acid receptor. PPAR can prevent activation by thyroid hormone receptor (TR)—RXR heterodimers or retinoic acid receptor (RAR)—RXR heterodimers by sequestering a limiting amount of RXR. This results in down-regulation of genes under control of thyroid hormone response elements (TRE) or retinoic acid response elements (RARE).

### *Conclusions*

In this review we have assessed the involvement of PPAR subtypes in phthalate-induced effects on the male reproductive tract. Much of the evidence supporting a role for PPARs is correlative. A high level of PPAR activation and testicular toxicity requires metabolic conversion of the diester to the monoester. PPAR subtypes are activated by structurally diverse phthalate monoesters. Phthalate diesters activate weakly or not at all. PPARα and PPARγ are usually more sensitive to monoester activation than PPARβ, and mouse PPARs are more sensitive than human PPARs. PPARs, principally the α and β subtypes are expressed in neonatal or adult Sertoli and Leydig cells, the target cells of phthalate ester effects. The testicular toxicity of DEHP occurred later and with diminished severity in PPARα-null mice, indicating PPARα acts as a modifier gene regulating the timing and severity of toxicity. There is a correlation between the ability of four of six diesters to induce reproductive tract changes and the corresponding monoesters to activate PPAR subtypes. Phthalates alter the expression of genes encoding sex steroid metabolizing enzymes and enzymes involved in testosterone biosynthesis, some in a PPARα-dependent manner. PPARs interact with and down-regulate the activities of other nuclear receptors that play roles in the developing testis including receptors for estrogen, thyroid hormone, and retinoic acid, leading to plausible mechanisms by which PPAR activation by phthalates could alter testicular function *in vivo*.

Other evidence exists to argue for little, if any participation of PPARs in phthalate-induced testicular effects. Although phthalates and PP can activate PPARs, only the phthalates are generally considered testicular toxicants. However, it is not unprecedented that structurally distinct classes of nuclear receptor ligands (e.g., ER ligands) have unique properties as agonists, partial agonists, or antagonists depending on receptor-ligand conformations and subsequent interactions with tissue-specific co-activators/co-repressors (McDonnell *et al*., 2002). With this in mind, it is interesting to note that many genes normally up-regulated by PP in the adult rat liver were down-regulated by DBP in the fetal testis and include those that are known targets of

PPARα including fatty acid β–oxidation genes (Shultz *et al.*, 2001). Phthalate effects in the adult testis can occur rapidly (within 2 h), likely before any phenotypic effects of PPAR-mediated gene regulation would be observed. Two phthalates, DBP and DINP, do not neatly fit into a relationship of inducing male reproductive tract malformations while the corresponding monoester induces PPAR activation. DBP is a strong male reproductive toxicant but MBP only weakly activates PPARs; DINP is a weak toxicant but MINP is a moderately strong PPAR activator. The fact that phthalate-induced testicular toxicity occurs in species that do not respond to PP-induced liver effects has been used to invoke a PPARα-independent mechanism, but comprehensive information on PPARα expression in the testis of responsive vs. nonresponsive species is lacking.

One way to rationalize these seemingly disparate findings is to invoke a mechanism that at least in the neonatal testis, includes both PPAR-dependent and -independent events leading to testicular toxicity after phthalate exposure. Future investigations should be designed to directly determine the role of PPARs as potential mediators of phthalate effects. Resolving a role for PPARs will be expedited by a comprehensive determination of PPAR expression in the developing testis and testing the effects of multiple phthalates in established mouse models nullizygous for each PPAR gene during male reproductive tract development.

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## SUPPLEMENTARY DATA

Supplementary data are available online at www.toxsci.oupjournals.org.

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