

# The Environmental Pollutant 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene Induces Rat Hepatic Cytochrome P450 2B and 3A Expression through the Constitutive Androstane Receptor and Pregnane X Receptor

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## ABSTRACT

1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), a persistent environmental contaminant, is a metabolite of the pesticide 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT). DDE is similar to phenobarbital (PB) in that both compounds are inducers of rat hepatic cytochrome P450 2B and 3A (CYP 2B and 3A). The induction of CYP 2B and 3A by PB is known to be regulated through the nuclear receptors constitutive androstane receptor (CAR) and pregnane X receptor (PXR), respectively. In the current study, the induction of hepatic CYP 3A1 and 2B1 by DDE was correlated with CAR and PXR activity. Induction of 3A1 and 2B1 was observed in the livers of adult and developing male Sprague-Dawley rats following exposure

to DDE. Increased hepatic expression of 3A1, but not 2B1, in developing rats exposed during gestation and lactation persisted into adulthood. In receptor transactivation assays, both CAR and PXR transcriptional activities were significantly enhanced by DDE. Nuclear accumulation of CAR, but not PXR, was observed in the liver tissue following DDE and PB treatment. These data support the idea that induction of hepatic 3A1 and 2B1 by DDE is mediated through the activation of CAR and PXR. This study suggests that regulation by environmental compounds of hepatic enzymes via CAR and PXR may have impact on the metabolism of endogenous and exogenous substrates.

1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), a persistent metabolite of the pesticide 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) (Fig. 1), is a developmental toxicant that induces sexual and reproductive abnormalities in male rats (Kelce et al., 1995; You et al., 1998). DDE binds with low affinity to the androgen receptor (AR) in vitro and inhibits 5 $\alpha$ -dihydroxytestosterone-induced transcriptional activity (Kelce et al., 1995). Since activation of the AR is critical for male sexual differentiation and reproductive development, the reproductive malformations in the male reproductive tract associated with DDE exposure are primarily attributed to AR antagonism.

DDE induces the expression of hepatic cytochrome P450 (P450) enzymes, including the P450 isozymes CYP 2B1 and 3A1 (Nims and Lubet, 1995; You et al., 1999). In rodent liver,

CYP 3A1 catalyzes 6 $\beta$ -hydroxylation of testosterone, and 2B1 catalyzes 16 $\alpha$ - and 16 $\beta$ -hydroxylation, and 17 $\beta$ -hydroxysteroid dehydrogenation of testosterone (Arlotto et al., 1991). The activities of hepatic testosterone 16 $\beta$ -hydroxylase and 17 $\beta$ -hydroxysteroid dehydrogenase, which oxidizes testosterone to produce androstenedione, are increased in DDE-treated rats (You et al., 1999). Treatment with DDE in utero also significantly induces hepatic 2B1- and 3A1-mediated 6 $\beta$ -, 16 $\alpha$ -, and 16 $\beta$ -hydroxylase activities and the activity of 17 $\beta$ -hydroxysteroid dehydrogenation in 21-day-old male rats (You et al., 1999). These increases in enzyme activities can be expected to alter the metabolism of their substrates, including endogenous androgens.

Similar to DDE, exposure to phenobarbital (PB) increases the expression of hepatic CYP 3A1 and 2B1 and induces reproductive dysfunction (Gupta et al., 1982; Waxman and Azaroff, 1992). The effects of PB on the male reproductive tract are similar to those caused by DDE and include reduced

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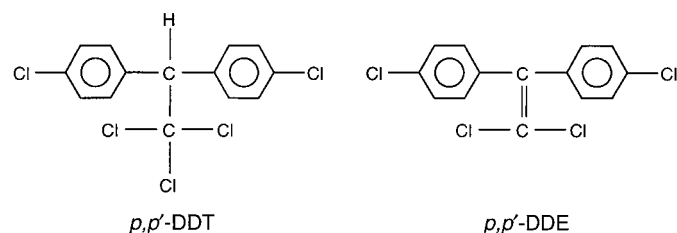
**ABBREVIATIONS:** DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; AR, androgen receptor; P450, cytochrome P450; PB, phenobarbital; PXR, pregnane X receptor; CAR, constitutive androstane receptor; NR, nuclear receptor; PCN, pregnenolone 16- $\alpha$ -carbonitrile; PND, postnatal day; LDH, lactate dehydrogenase; DMSO, dimethyl sulfoxide; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; bp, base pair(s); ANOVA, analysis of variance; DEX, dexamethasone.

anogenital distance, delayed testicular descent, delayed onset of puberty, and reduced seminal vesicle weights (Gupta et al., 1980, 1982). These antiandrogen-like effects are believed to be a consequence of decreased plasma testosterone concentrations, resulting from increased hepatic steroid metabolism and inhibition of gonadal testosterone synthesis (Elias and Gwinup, 1980; Gupta et al., 1982). In contrast to DDE, PB does not inhibit AR transactivation (L. You, unpublished observation). Thus, alterations in the activities of steroid-metabolizing enzymes, including CYP 3A1 and 2B1, may change the bioavailability of androgens and contribute to the reproductive toxicity of DDE and PB.

The expression of CYP 3A1 and 2B1 is regulated by the nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR), respectively (Honkakoski et al., 1998a,b; Kliewer et al., 1998). PXR and CAR are members of the nuclear receptor (NR) 1I subfamily of nuclear receptors (Moore et al., 2002). When activated, both PXR and CAR form heterodimers with the retinoid X receptor, bind to specific DNA sites, and function as transcriptional factors for target gene regulation (Honkakoski et al., 1998a,b; Kliewer et al., 1998). PXR, once activated, binds to xenobiotic response elements in the promoter region of the CYP 3A gene, and the potency of PXR activation is the main determinant for the degree of CYP3A induction (Kliewer et al., 1998; Moore and Kliewer, 2000). CAR binds to NR binding sites in the phenobarbital-responsive enhancer region in the CYP 2B gene (Honkakoski et al., 1998a,b). CAR, which can transactivate its target genes in the absence of a ligand (Baes et al., 1994), is known to reside in the cytoplasm and undergoes nuclear translocation upon activation (Kawamoto et al., 1999). Although the expression of CYP 2B is primarily regulated by CAR and 3A by PXR, CAR and PXR possess cross-regulation for their target genes, and some compounds, such as PB, can activate both CAR and PXR (Wei et al., 2002; Pascucci et al., 2003). Based on previous observations of CYP 3A1 and 2B1 induction by DDE (You et al., 1999), we hypothesize that DDE, like PB, induces the expression of cytochrome P450 via interaction with PXR and CAR. The aim of the current study was to characterize the induction of CYP 3A1 and 2B1 by DDE in relation to activation of CAR and PXR. The effects of DDE on hepatic CYP 3A1 and 2B1 expression were characterized in male rats at prepubertal and adult stages. The pharmacological action of DDE was investigated in reference to PB, a known CAR and PXR activator, and pregnenolone 16- $\alpha$ -carbonitrile (PCN), a known PXR activator.

## Materials and Methods

**Animals and Treatment.** Adult male Sprague-Dawley rats (310–330 g) were obtained from Charles River (Raleigh, NC). Rats



**Fig. 1.** Structures of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its metabolite 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE).

were housed individually under conditions of controlled temperature (22–25°C), humidity (40–60%), and lighting (12-h light/dark cycle), and received NIH-07 certified feed (Zeigler Bros. Inc., Gardner, PA) and water ad libitum. Animals were randomly distributed into four treatment groups ( $n = 8$ ). Each rat received daily gavage doses of corn oil vehicle (controls), 100 mg of DDE, 40 mg of PCN, or 40 mg of PB per kg body weight for 7 consecutive days. All animals were decapitated 24 h after the last dosing, trunk blood was collected, and livers were removed, weighed, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$ . Adult male Sprague-Dawley rats used for the isolation of primary hepatocytes were housed under the same conditions.

To determine nuclear translocation of CAR and PXR, adult male Sprague-Dawley rats received a single i.p. injection of 40 mg of DDE/kg body weight or 100 mg of PB/kg body weight. Animals were killed by asphyxiation with  $\text{CO}_2$  at 3, 6, or 24 h after DDE treatment and 6 h after PB treatment. Control animals received no treatment. Livers were removed and weighed, and nuclear extracts were isolated as described below.

The effect of gestational and lactational DDE exposure on perinatal hepatic enzyme expression was determined in a separate experiment. Female Sprague-Dawley rats (28 days old) were fed diets containing nominal concentrations of DDE at 0, 2.5, 25, or 250 mg/kg feed, or ppm. Diets were prepared by mixing powdered NIH-07 diet and the appropriate amount of DDE. Feed concentrations of DDE were verified in hexane-extracts of the finished dietary samples by gas chromatography. After 8 weeks on the treatment diet, the female rats were mated with untreated young male Sprague-Dawley rats maintained on regular NIH-07 diet. The pregnant  $F_0$  females continued to receive DDE in the diet throughout gestation and lactation. On postnatal day (PND) 14, three  $F_1$  male nonlittermate pups from each group were killed by asphyxiation with  $\text{CO}_2$ . The livers were removed, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until subsequent analysis. The remaining pups were all weaned on PND 21 and shifted onto regular NIH-07 diet without DDE. On PND 90,  $F_1$  male rats were killed by asphyxiation with  $\text{CO}_2$ , and livers ( $n = 4$ ) were removed and frozen in liquid nitrogen.

**Primary Hepatocyte Culture and Treatment.** Hepatocytes were isolated by a two-step perfusion procedure as previously described (You et al., 2001). Hepatocyte viability was determined by lactate dehydrogenase (LDH) release using Roche Kit 43622 and a Cobas Fara II AutoAnalyzer (Roche Diagnostics, Branchburg, NJ). Total LDH activity was determined by comparing an aliquot of cells solubilized with 1% Triton X-100 to the amount of LDH leaking into the medium from the cells. Only cell preparations with initial viability  $>90\%$  ( $<10\%$  LDH leakage) were used for experiments.

The isolated hepatocytes were cultured at a density of  $4.8 \times 10^4$  cells/cm in collagen-coated 6-cm plastic culture plates. Cells attached to culture plates in the presence of 5% fetal bovine serum for 90 min at  $37^{\circ}\text{C}$  in air/ $\text{CO}_2$  (95:5%, v/v). Cells were washed and cultured under the same incubation conditions in medium containing 3 ml of Williams' medium E supplemented with 2 mM L-glutamine, 10 ng/ml dexamethasone (DEX), 50  $\mu\text{g}/\text{ml}$  gentamicin, 5  $\mu\text{g}/\text{ml}$  insulin, 5  $\mu\text{g}/\text{ml}$  transferrin, and 5 ng/ml selenium. At 24 h, the culture medium was removed and replaced with 3 ml of fresh medium of the same composition; at 48 h, the medium was removed again and replaced with 3 ml of the medium containing 0.05, 0.5, 5.0, or 50  $\mu\text{M}$  DDE or 0.1 or 1.0  $\mu\text{M}$  PB in dimethyl sulfoxide (DMSO). The final concentration of DMSO was 0.1% (v/v) in all groups. At 72 h, after 24 h of incubation with DDE or PB, the medium was removed, and the cells were harvested with a plastic spatula. Cells from three plates within the same treatment group were pooled and pelleted by centrifugation at approximately 1000g at  $4^{\circ}\text{C}$ . The cell pellet was washed twice with 1 ml of phosphate-buffered saline before being pelleted and frozen in microcentrifuge tubes at  $-20^{\circ}\text{C}$ .

**Preparation of Total Protein, Microsomes, and Nuclear Extracts.** Total protein and microsomal protein were prepared from cultured hepatocytes and liver tissue as previously described (You et al., 1999, 2001). Frozen cultured hepatocytes were resuspended in

NP40 lysis buffer (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK) and homogenized in a glass and Teflon homogenizer. Nuclear extracts were prepared by homogenizing 2 g of liver tissue in 30 ml of homogenization buffer (10 mM Hepes, pH 7.6, 2 M sucrose, 25 mM KCl, 1 mM EDTA, 10% glycerol, 0.15 M spermidine, and 0.5 mM spermine). Homogenized tissue was layered on a 10-ml cushion of homogenization buffer and centrifuged at 15,000g at 4°C for 45 min. The nuclear pellet was resuspended in lysis buffer (10 mM Hepes, pH 7.6, 10% glycerol, 0.1 M potassium chloride, 3 mM magnesium chloride, 0.1 mM EDTA, 1 mM sodium orthovanadate, 1 µg/ml pepstatin, 1 µg/ml leupeptin, 0.5 µg/ml E-64, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM DTT). Sodium chloride (5 M) was added, and the sample was mixed continuously at 4°C for 1 h. Samples were centrifuged in an ultracentrifuge at 15,000g at 4°C for 1 h. Supernatant was dialyzed against 1 liter of dialysis buffer (10 mM Hepes, pH 7.6, 10% glycerol, 0.1 M potassium chloride, 3 mM magnesium chloride, 0.1 mM EDTA, 1 mM sodium orthovanadate, 1 µg/ml pepstatin, 1 µg/ml leupeptin, 0.5 µg/ml E-64, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM DTT) overnight at 4°C. Samples were centrifuged in an ultracentrifuge at 15,000g at 4°C for 30 min, and the nuclear extract supernatant was stored in liquid nitrogen.

**Protein Immunoblotting for CYP 3A1, 2B1, PXR, and CAR.** Immunoblotting for cytochrome P450 enzymes and nuclear receptors was performed as previously described (You et al., 1999). Liver microsomes or total protein from cultured cells or liver tissue were denatured and separated by SDS-polyacrylamide gel electrophoresis (PAGE) with 12% polyacrylamide. Proteins were transferred to nitrocellulose membranes, blocked for nonspecific binding, and incubated with polyclonal primary antibodies for CYP3A1, CYP2B1, CAR, or PXR. After incubation with primary antibody, membranes were incubated with horseradish peroxidase-linked anti-rabbit (CYP3A1, PXR, and CAR) or anti-goat (CYP2B1) IgG secondary antibodies and visualized on film exposed to enhanced chemiluminescence (Hyperfilm-ECL; Amersham Biosciences UK, Ltd.). Goat anti-rat CYP2B1 polyclonal antibodies were obtained from Daiichi Pure Chemical Company (Tokyo, Japan). CYP3A1 antibodies were obtained from Research Diagnostics (Flanders, NJ). PXR anti-peptide antibody was prepared and purified as previously described (Zhu et al., 2000). Rabbit anti-CAR peptide antibodies were prepared as previously described (Yoshinari et al., 2001).

**Quantitative Reverse Transcriptase-PCR.** To quantitate the amount of CYP 2B1 and 3A1 mRNA, cDNA was synthesized from total RNA isolated from liver tissue or cultured primary hepatocytes. Random hexamers and the TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA) were used according to the manufacturer's suggested protocol. The PCR primers were designed using Primer Express software (Applied Biosystems). Rat 3A1 (GenBank accession no. X64401) forward (ctcttcaccgtgatccacagcact) and reverse (atgctgccttggtctccttgc) primers and 2B1 (GenBank accession no. J00719) forward (tgagaacctcatgatctcctgc) and reverse (aggaaacatagcggagtgtgg) primers were designed with the following parameters: low  $T_m$  = 60°C, high  $T_m$  = 64°C, optimum  $T_m$  = 62°C, amplicon length = 80 to 150 bp, and primer length 20 to 24 bp with an optimum of 22 bp. The production of a single PCR product was confirmed by gel electrophoresis for each pair of PCR primers before quantification. Primer efficiency was determined according to the manufacturer's suggested protocol. Real-time quantitative PCR (Taqman) was performed on a 7700 PRISM Sequence Detector (Applied Biosystems) using SYBR Green according to the manufacturer's instructions for quantification of relative gene expression (User Bulletin 2: P/N 4303859).

**Nuclear Receptor Transcriptional Activation Assays.** The expression plasmids encoding full-length rat PXR were prepared by a cDNA-trapping method as described previously (Zhang et al., 1999). The reporter plasmids were constructed by inserting two copies of rat PXR response element (IR6 and DR3) into the pGL-3 SV40 promoter vector driving a firefly luciferase gene. The pRL-TK

plasmid encoding a *Renilla* luciferase was purchased from Promega (Madison, WI). Activation of rat PXR was determined by cotransfection experiments. Cells (CV-1) were plated in 24-well plates in Dulbecco's modified Eagle's medium supplemented with 10% dilapidated fetal calf serum at a density of  $8 \times 10^4$  cells per well. Transfection was conducted by lipofection with LipofectAMINE (Invitrogen, Carlsbad, CA) with 100 ng of PXR plasmid, 100 ng of reporter plasmid, and 10 ng of pRL-TK plasmid. After a 12-h incubation, the transfected cells were treated with DDE at various concentrations for an additional 24 h, washed once with phosphate-buffered saline, and collected. The collected cells were subjected to two freeze-thaw cycles. The reporter enzyme activities were assayed with a Dual-Luciferase Reporter Assay System (Promega). The luminescence signal was internally normalized, and the ratio of the normalized signal from DDE-treated cells over that from solvent-treated cells was calculated and reported as a fold of activation. Assays to determine the activation of CAR were performed as previously described by Kawamoto et al. (2000). HepG2 cells were cultured in minimal essential medium (Sigma-Aldrich, St. Louis, Mo.) with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA). (NR1)<sub>5</sub>-tk-luciferase plasmid, pRL-SV40 and rat CAR plasmids were cotransfected using the CellPfect Transfection Kit calcium phosphate transfection method (Amersham Biosciences Inc., Piscataway, NJ). Cells were treated with 1, 10, 25, or 50 µM DDE for 1 h, lysed, collected, and measured for luciferase activity. Increased CAR activation resulted in increased luciferase expression through increased NR1 enhancer element activity. Luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega). Data were normalized to DMSO control values.

**Statistics.** All data are presented as means  $\pm$  standard deviation. Significant differences were determined by analysis of variance (ANOVA) and pairwise comparisons by Fisher's least significant difference ( $p < 0.05$ ). Significant differences between treated and control groups for PXR transcriptional transactivation activity were determined by Dunnett's test ( $p < 0.05$ ).

## Results

In young adult male Sprague-Dawley rats exposed to daily gavage doses of DDE (100 mg/kg), PB (40 mg/kg), or PCN (40 mg/kg) for 7 days, no differences in body weight were observed between the control and the treated. The liver/body weight ratio was greater in DDE- and PB-treated rats (6.3 and 5 g liver weight/100 g body weight, respectively) compared with the controls (4 g liver weight/100 g body weight). No difference was observed in liver weights between rats exposed to PCN and controls.

Immunoblot analysis demonstrated basal expression of CYP 3A1 and 2B1 proteins in the livers of adult male Sprague-Dawley rats (Fig. 2). Hepatic CYP 3A1 protein was higher in rats exposed to DDE, PB, or PCN compared with the levels in the control animals (Fig. 2A). Quantitative reverse transcriptase-PCR demonstrated that the amount of 3A1 mRNA was greater in treated animals than in controls (Fig. 2C). Levels of mRNA in the livers of rats exposed to DDE (100 mg/kg) were 11-fold greater than in controls, compared with 4.4- and 6.8-fold increases for PB (40 mg/kg) and PCN (40 mg/kg), respectively. Hepatic expression of 2B1 protein was higher in both DDE- and PB-treated rats compared with controls (Fig. 2B). The level of 2B1 mRNA was greater in the livers of DDE- and PB-treated animals compared with controls (Fig. 2D). The level of 2B1 mRNA was increased 130-fold in DDE-treated and 40-fold in PB-treated rats. PCN treatment did not affect either 2B1 protein or mRNA in rat liver (Fig. 2, B and D).



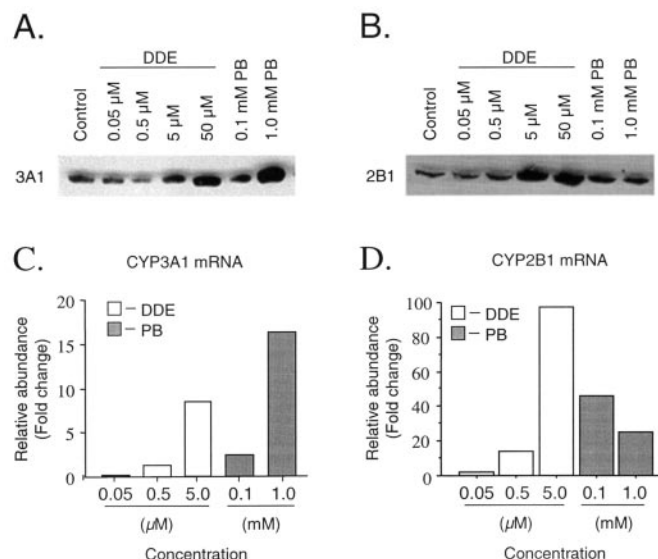
In cultured primary hepatocytes from male rats, the level of CYP 3A1 protein was increased in cells exposed to PB and to DDE at concentrations of 5  $\mu$ M and 50  $\mu$ M (Fig. 3A). The level of 3A1 mRNA was 8-fold higher at 5  $\mu$ M DDE compared with controls (Fig. 3C). The 3A1 mRNA was increased 2.5- and 16-fold at 0.1 and 1.0 mM PB, respectively. However, the level of 3A1 protein appeared to be consistently lower in hepatocytes treated with 0.05 or 0.5  $\mu$ M DDE than in the DMSO-treated cells (Fig. 3).

The level of CYP 2B1 protein and mRNA was increased in the primary hepatocytes treated with DDE at 5  $\mu$ M and 50  $\mu$ M (Fig. 3B). The level of 2B1 mRNA was increased 14-fold at 0.5  $\mu$ M and 95-fold at the 5  $\mu$ M DDE (Fig. 3D). The levels of 2B1 protein and mRNA were higher in PB-treated cells compared with the control. The level of 2B1 mRNA was increased 45- and 25-fold in cells treated with 0.1 mM and 1.0 mM PB, respectively.

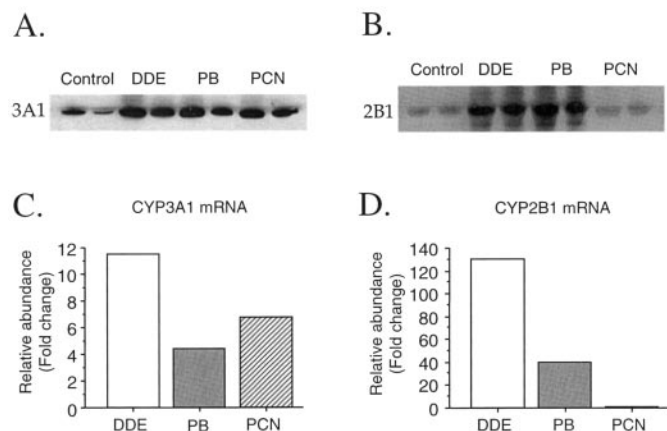
To examine the effect of in utero and lactational exposure to DDE on perinatal hepatic enzyme expression, female Sprague-Dawley rats were exposed to dietary concentrations of 0, 2.5, 25, or 250 ppm DDE throughout pregnancy and lactation until pup weaning on PND 21. Pup exposure was largely limited to transplacental transfer during gestation and lactational exposure until postnatal day 14. In rats euthanized on postnatal day 14, hepatic expression of CYP 3A1 was significantly higher in pups from dams exposed to 25 or 250 ppm DDE as compared with pups from untreated dams (Fig. 4A). Hepatic expression of 2B1 was significantly increased in pups from the 2.5, 25, or 250 ppm DDE groups compared with pups from untreated dams (Fig. 4B). Additional  $F_1$  pups from each litter were shifted to regular diet at weaning on postnatal day 21 and maintained treatment free from postnatal day 21 to 90. Following the 69-day untreated recovery period, the expression of 3A1 remained elevated in the livers of rats exposed during gestation and lactation to maternally administered dietary DDE at concentrations of

2.5, 25, or 250 ppm compared with rats from untreated dams (Fig. 5A). No differences were observed in 2B1 expression between pups from treated and untreated dams following the same recovery period (Fig. 5B).

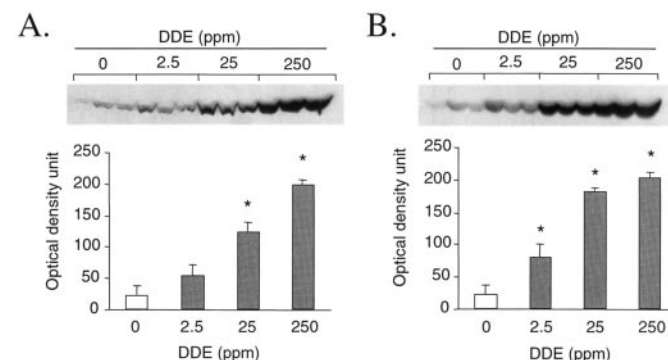
Immunoblot analysis showed that the expression of PXR in the livers of young adult male Sprague-Dawley rats was not altered after 7 days of treatment with DDE, PB, or PCN (Fig.



**Fig. 3.** Effect of DDE and PB treatment on the expression of P450 protein and mRNA in cultured primary hepatocytes from adult male Sprague-Dawley rats. Expression of CYP3A1 (A) and CYP2B1 (B) was determined in hepatocytes that were incubated with DMSO vehicle, DDE ranging from 0.05  $\mu$ M to 50  $\mu$ M, or PB at concentrations of 0.1 mM or 1.0 mM. Aliquots of 20  $\mu$ g of protein from cultured hepatocyte cell lysate were separated by SDS-PAGE, and immunoblot analysis was performed for cytochrome P450 isozymes 3A1 and 2B1. The amounts of CYP3A1 (C) and CYP2B1 (D) mRNA were quantified by real-time quantitative PCR. Samples were run in triplicate for each treatment group of hepatocytes. Fold change values were determined by normalizing to GAPDH and expressed as fold change relative to the control. Data presented are from one typical experiment of a minimum of two.



**Fig. 2.** Effect of DDE, PB, and PCN treatment on the expression of cytochrome P450 protein and mRNA in the livers of Sprague-Dawley rats. Expression of CYP3A1 protein (A) and CYP2B1 protein (B), and of CYP3A1 mRNA (C) and CYP2B1 mRNA (D) was determined in the livers of male Sprague-Dawley rats receiving daily gavage doses of corn oil vehicle, 100 mg of DDE, 40 mg of PB, or 40 mg of PCN/kg body weight for 7 days. Aliquots of 20  $\mu$ g of microsomal hepatic protein were separated by SDS-PAGE. Immunoblot analysis was performed for CYP3A1 and 2B1 as described under *Materials and Methods*. Real-time quantitative PCR was performed with samples run in triplicate for each animal. Fold change values were determined by normalizing to GAPDH and expressed relative to the control.

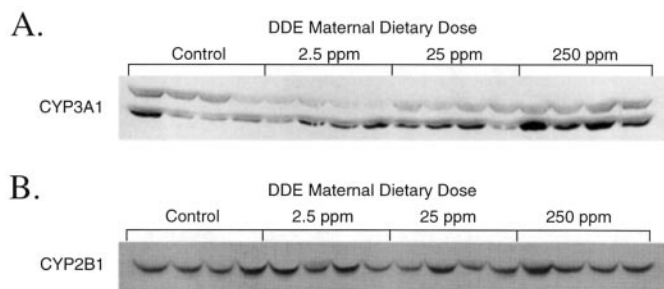


**Fig. 4.** Effect of maternally administered dietary DDE exposure during gestation and lactation on hepatic P450 protein expression in juvenile male Sprague-Dawley rats. Expression of CYP3A1 (A) and CYP2B1 (B) protein was determined in liver samples of 14-day-old male Sprague-Dawley rat pups from dams that were exposed to 0, 2.5, 25, or 250 ppm DDE in the feed from 8 weeks before pregnancy through gestation and lactation. Pups were exposed in utero through transplacental transfer and postnatally via lactation until postnatal day 14. Aliquots of 15  $\mu$ g of microsomal protein from rat liver were separated by SDS-PAGE and immunoblotted for CYP3A1 and 2B1. Band densities were measured and normalized against background. Differences in band densities between groups were evaluated by ANOVA and pairwise comparisons by Fisher's least significant difference. \*, significantly higher than controls ( $p < 0.05$ ).

6A). However, the CAR protein level was lower in the liver tissue of rats treated with DDE, PB, or PCN compared with controls (Fig. 6B). In cultured hepatocytes, the level of PXR protein was not affected by DDE or PB treatment (Fig. 6C). No remarkable change in the level of CAR protein was seen in the primary hepatocytes treated with DDE, although its level appeared to be lower in the cells treated with 0.1 mM PB (Fig. 6D).

The ability of DDE to transactivate PXR and CAR was investigated in cells transfected with CAR and PXR expression plasmids. Transactivation of PXR was studied in COS7 cells transiently transfected with rat PXR and measured by luciferase reporter gene expression. In DDE-treated cells, there was a dose-dependent increase in PXR transactivation in the dose range of 1 to 50  $\mu$ M ( $p < 0.05$ ) (Fig. 7). PXR transactivation in cells exposed to 10  $\mu$ M PCN, a known PXR activator and CYP3A inducer (Kocarek et al., 1995; Moore and Klierer, 2000), was far greater than that in DDE-treated cells. The effect of DDE treatment on CAR activation was measured in the expression of luciferase reporter gene in HepG2 cells transiently transfected with rat CAR and an NR-1 reporter plasmid. In cells treated with DDE at 25 or 50  $\mu$ M, the transcriptional activity of CAR was significantly enhanced compared with DMSO-treated cells ( $p < 0.05$ ) (Fig. 8). CAR transcriptional activity was lower in cells treated with 20 or 50  $\mu$ M PCN compared with DMSO-treated controls ( $p < 0.05$ ) (Fig. 9), with 85 to 90% transcriptional activity observed in cells treated with PCN in this dose range.

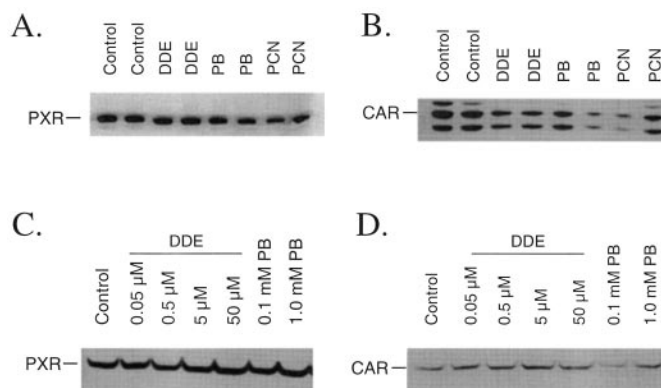
A time-dependent change in nuclear translocation of CAR was observed through immunoblot analysis of nuclear fractions from the livers of DDE-treated male Sprague-Dawley rats. CAR protein was undetectable in liver nuclear extracts from untreated rats or 3 h after a single i.p. injection of 40 mg of DDE/kg (Fig. 10A). Nuclear presence of CAR became detectable at 6 h and, to a lesser extent at 24 h, after the injection of DDE. Higher content of CAR in the nucleus was also observed in rats at 6 h after an injection of PB at 100 mg/kg. No differences were observed in the level of PXR protein detected in nuclear fraction of liver tissue from DDE-treated rats of the different treatment groups (Fig. 10B).



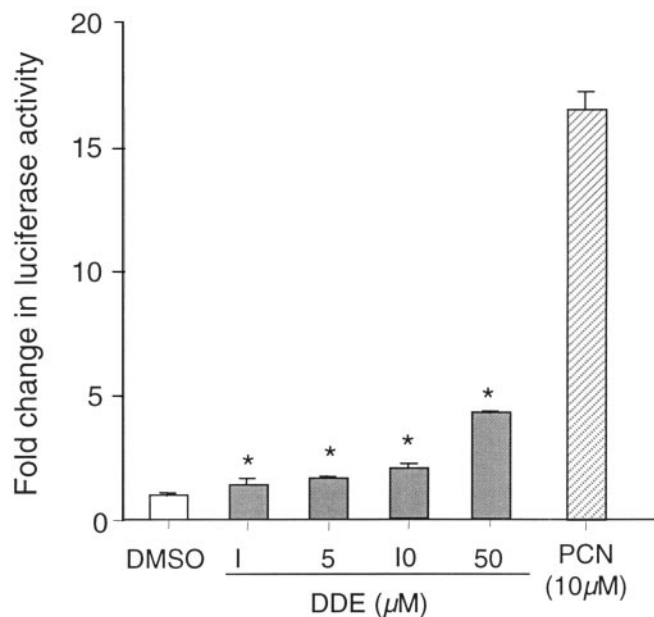
**Fig. 5.** Effect of maternally administered dietary DDE exposure during gestation and lactation until postnatal day 21 on hepatic P450 protein expression in 90-day-old male Sprague-Dawley offspring rat. Expression of CYP3A1 (A) and CYP2B1 (B) protein was determined in the livers of 90-day-old male Sprague-Dawley rat pups from dams that were exposed to 0, 2.5, 25, or 250 ppm DDE in the feed from 8 weeks before pregnancy through gestation and lactation. Pups were exposed in utero through transplacental transfer and postnatally via lactation until weaning on postnatal day 21, at which point pups were shifted to a regular diet without DDE. Aliquots of 15  $\mu$ g of microsomal protein from rat liver were separated by SDS-PAGE, and immunoblot analysis was performed for CYP3A1 and 2B1.

## Discussion

The effect of the environmental pollutant DDE on CYP 3A1 and 2B1 enzyme expression in young adult and developing male Sprague-Dawley rats and the ability of DDE to interact with the nuclear receptors CAR and PXR were investigated. The induction of hepatic 3A1 and 2B1 by both DDE and PB was demonstrated in vivo as well as in primary hepatocytes in vitro, indicating that DDE, similar to PB, effects the up-regulation of 2B1 and 3A1 through cellular machinery intrinsic to the hepatocytes. DDE also activates both CAR and PXR, which are known to function as transcription regula-

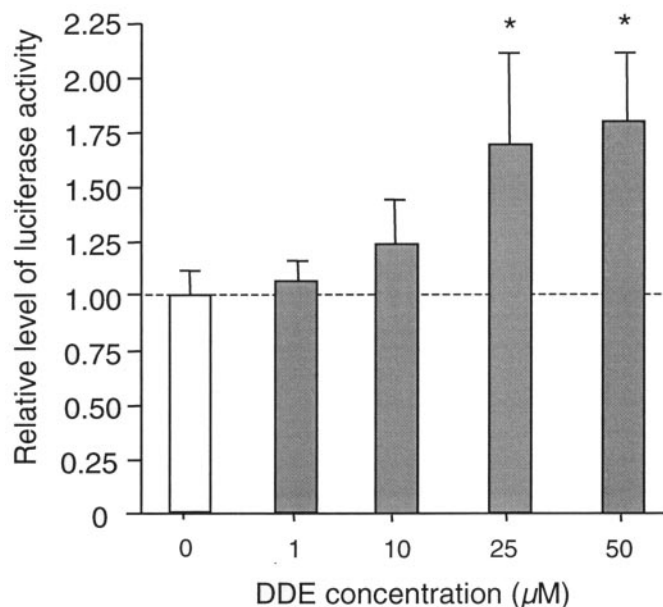


**Fig. 6.** Immunoblot analysis of PXR and CAR expression in Sprague-Dawley rat liver and primary hepatocytes. Hepatic expression of PXR (A) and CAR (B) was determined in male Sprague-Dawley rats receiving daily gavage doses of corn oil vehicle, 100 mg of DDE, 40 mg of PB, or 40 mg of PCN/kg body weight for 7 days. The expression of PXR (C) and CAR (D) was determined in isolated and cultured hepatocytes that were incubated with DMSO vehicle, concentrations of DDE ranging from 0.05  $\mu$ M to 50  $\mu$ M, or PB at concentrations of 0.1 mM or 1.0 mM. Aliquots of 20  $\mu$ g of tissue homogenate protein from rat liver or primary hepatocytes were separated by SDS-PAGE, and immunoblot analysis was carried out for PXR and CAR protein expression.

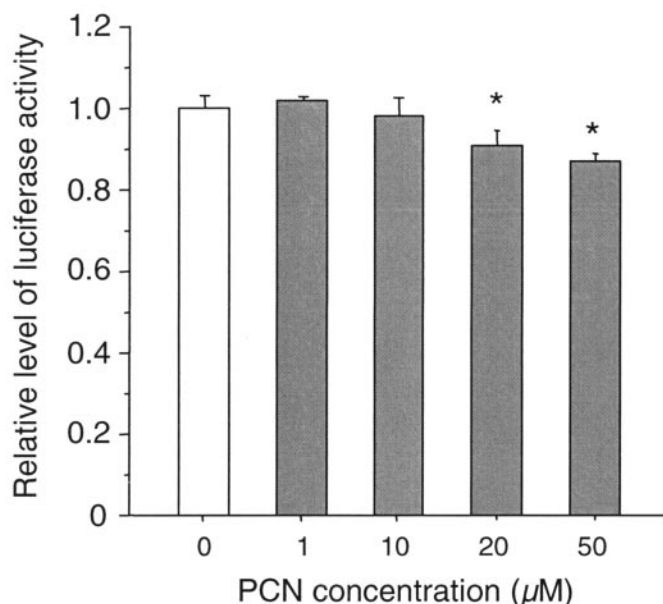


**Fig. 7.** Effect of DDE and PCN treatment on PXR transactivation. COS7 cells were cotransfected with the rat PXR expression plasmids and a luciferase reporter gene plasmid. Cells were treated with 1, 5, 10, or 50  $\mu$ M DDE. \*, significantly different from DMSO control as determined by ANOVA and Dunnett's test ( $p < 0.05$ ).

tors for CYP 2B1 and 3A1. DDE-induced CAR and PXR transactivation was consistent with DDE-caused induction of CYP 3A1 and 2B1. DDE treatment was shown to result in translocation of CAR into the nuclei. These data indicate that DDE induces rat hepatic CYP 3A1 and 2B1 via transactivation of the nuclear receptors CAR and PXR.



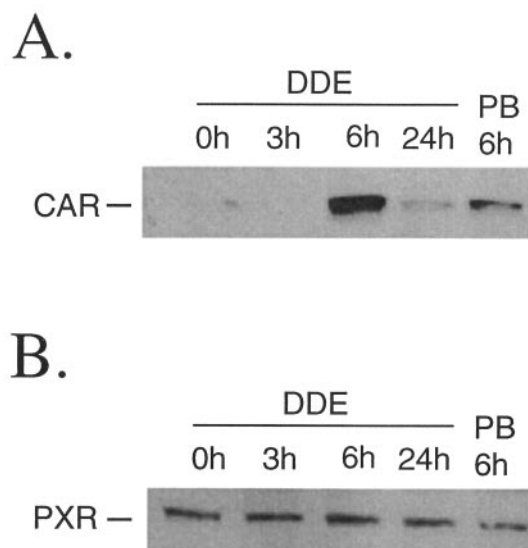
**Fig. 8.** Effect of DDE treatment on rat CAR transactivation. HepG2 cells were cotransfected with plasmids for rat CAR and pRL-SV40 and a (NR1)<sub>5</sub>-tk-luciferase reporter plasmid. Cells were incubated with different concentrations of DDE for 24 h, lysed, and measured for luciferase activity. Relative transcriptional activities were expressed by normalizing values to DMSO-treated results. \*, significantly different from DMSO control as determined by ANOVA and Dunnett's test ( $p < 0.05$ ).



**Fig. 9.** Effect of PCN treatment on rat CAR transactivation. HepG2 cells were cotransfected with plasmids for rat CAR and pRL-SV40 and a (NR1)<sub>5</sub>-tk-luciferase reporter plasmid. Cells were incubated with different concentrations of PCN for 24 h, lysed, and measured for luciferase activity. Relative activities were expressed by normalizing values to DMSO-treated results. \*, significantly different from DMSO control as determined by ANOVA and Dunnett's test ( $p < 0.05$ ).

The expression of PXR protein in primary hepatocytes or in the livers of male rats was not markedly affected by exposure to DDE, PB, or PCN. A down-regulation of PXR expression was detectable in the livers of PB- and PCN-treated rats. Similarly, the expression of hepatic CAR was decreased in DDE-, PB-, and PCN-treated rats. Although the effects of xenobiotics on the expression of CAR and PXR have not been extensively studied, it has been demonstrated that the cytokine interleukin-6 down-regulates CAR and PXR expression in primary human hepatocytes and decreases ligand-mediated induction of CYP 2B and 3A enzyme expression (Pascucci et al., 2000a,b). In contrast, a different relationship was seen in the current study: the expression of nuclear receptors was repressed, whereas the expression of their target genes was enhanced. This relationship was seen in the cases of PXR with CYP 3A1 in the PB- and PCN-treated rats and CAR with CYP 2B1 in the DDE-, PB-, and PCN-treated rats (Fig. 6). One possible explanation is that the levels of PXR and CAR might be affected by interactions between the test compounds and glucocorticoid signals. Glucocorticoids are known to change the expression of both CAR and PXR (Pascucci et al., 2000a,b). Glucocorticoids also transactivate PXR, probably through recruiting a transcriptional enhancer, CCAAT/enhancer binding protein  $\alpha$  (Rodrigues et al., 2003). In vivo, DDE may have the potential to reduce the production of glucocorticoids by inhibiting steroidogenesis in the adrenal (Johansson et al., 2002), possibly resulting in repression of CAR and PXR expression. Phenobarbital is also known to inhibit steroidogenesis. Repression of CAR and PXR, however, was not observed in vitro in the current study.

The reduced CYP 3A1 in primary hepatocyte at low (sub-micromolar) DDE doses (Fig. 3A) is similar to the effect caused by another PXR agonist, mifepristone (RU486). The mechanism for the reduction of 3A expression by PXR ago-



**Fig. 10.** Nuclear accumulation of CAR and PXR proteins in liver cells following treatment with DDE and PB. Nuclear accumulation of CAR (A) or PXR (B) was determined in liver samples of male Sprague-Dawley rats that received a single i.p. injection of 40 mg/kg DDE or 100 mg/kg PB and were sacrificed 3, 6, or 24 h after DDE treatment or 6 h after PB treatment. Nuclear extracts were also prepared from the livers of untreated male Sprague-Dawley rats as 0-h controls. Aliquots of 30  $\mu$ g of hepatic nuclear extracts were separated by SDS-PAGE, and immunoblot analysis was performed.



nists at submicromolar dose range is unclear (Pascucci et al., 2003). In our primary hepatocyte experiment, DEX was used in the culture media at a concentration of approximately 25 nM. Since DEX is an activator of PXR (Schuetz et al., 2000), a low dose of DDE may competitively inhibit DEX effects on PXR, resulting in a decreased glucocorticoid effect on CYP 3A1 expression. This inhibition could then be reversed when DDE reaches higher doses. Such considerations regarding the potential role of glucocorticoid, however, are not expected to affect the conclusion that DDE transactivates PXR and CAR and induce CYP 2B1 and 3A1.

The characteristics of DDE transactivation of PXR and CAR are consistent with other structurally diverse receptor activators that are regulators of CYP 2B and 3A families (Honkakoski et al., 1998a,b; Blizard et al., 2001). CAR is transcriptionally active in the absence of a ligand in cell-based assays (Baes et al., 1994; Choi et al., 1997; Kawamoto et al., 1999). In the liver, CAR is sequestered in the cytoplasm of hepatocytes. Treatment with CAR activators, such as phenobarbital or 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, results in nuclear accumulation of CAR in the hepatocytes (Kawamoto et al., 1999, 2000; Yoshinari et al., 2001). Disruption of nuclear translocation of CAR inhibits CAR-mediated regulation of gene expression (Kawamoto et al., 1999). Therefore, nuclear translocation is an obligatory step for CAR transactivation and CAR-mediated gene expression. Similar to previous reports with PB, DDE caused nuclear accumulation of CAR within 6 h of dosing that persisted for longer than 24 h (Kawamoto et al., 1999; Yoshinari et al., 2001). Greater nuclear accumulation at 6 h than at 24 h after the DDE treatment is consistent with the previously reported time course of nuclear accumulation of CAR in PB-treated Wistar Kyoto rats (Yoshinari et al., 2001). DDE-responsive nuclear accumulation of CAR, together with DDE-mediated CAR transcriptional activation, suggests an interaction of DDE with CAR that is similar to that of PB and other known CAR activators. DDE and PB treatment did not result in detectable nuclear accumulation of PXR. Currently little is known regarding the subcellular localization of PXR, which may differ from that of CAR.

The manner in which DDE interacted with CAR was similar to that of PB, whereas the interaction between DDE and PXR in inducing 3A1 was comparable to that of PCN. The effects of DDE on CAR and PXR transactivation and CYP 3A1 and 2B1 expression indicate that DDE interacts with both CAR and PXR pathways. Therefore, DDE belongs to the category of compounds that are common activators of both CAR and PXR (Moore et al., 2000, 2002).

Although DDE is not as potent as PCN in transactivating PXR, hepatic 3A1 mRNA levels were higher in DDE-treated rats than in PCN-treated rats. PXR activity is known to correlate well with CYP 3A1 expression (Moore and Klierer, 2000). However, CAR and PXR can cross-regulate both 3A1 and 2B1 expression (Honkakoski et al., 1998a,b; Xie et al., 2000b; Goodwin et al., 2001). Our data demonstrate that DDE activates both CAR and PXR and induces both CYP 3A1 and 2B1, whereas PCN activates PXR but not CAR and induces 3A1 but not 2B1. Thus, the greater effects of DDE than of PCN on CYP 3A1 induction may be related to cross-regulation of 3A1 through DDE-mediated activation of CAR.

Maternally administered DDE during gestation and lactation induced CYP 3A1 and 2B1 expression in the liver tissue

of the offspring on postnatal day 14. Induction of CYP 2B1 was observed at a lower dose of DDE (2.5 ppm) than 3A1 (25 ppm). Exposure to 2.5 ppm DDE is roughly equivalent to the World Health Organization Daily Advisory Intake, which is 20 µg/kg/day of DDT, the parent compound of DDE. This estimate is based on an assumption of an 80-kg person consuming 800 g of food per day. Induction of 3A1 protein persisted following cessation of lactational exposure on PND 21 and an untreated recovery period of 69 days, whereas 2B1 protein expression returned to control levels following the recovery period. The fact that DDE is slowly metabolized and has a long half-life does not explain the more transient response in 2B1 expression. The current study indicated that CAR and PXR are likely targets for environmental chemicals and that enzyme modulation may occur at dose levels relevant to environmental exposure. CAR and PXR may function as xenosensors with a broad range of ligands and regulate P450 enzymes and other cellular modulators relevant to the metabolism of exogenous substrates (Xie et al., 2000a; Moore et al., 2002). In addition to protecting the body against xenobiotics, there may be other consequences associated with activating these receptors, however. Significant induction of P450 enzymes by environmental chemicals may lead to activation of protoxicants and alteration of the metabolism of drugs and endogenous substances. In humans, the CYP3A family is responsible for the metabolism of approximately 50% of all drugs (Thummel and Wilkinson, 1998), and chemical-drug interactions via induction of metabolic enzymes may cause serious health consequences. Additionally, the metabolism of endogenous substances, such as steroid hormones that are substrates for CYP 3A1 and 2B1 and other hepatic enzymes, may affect normal endocrine function via altered hormone biotransformation. PB, in this regard, increases hepatic metabolism of androgens (Elias and Gwinup, 1980) while inducing developmental abnormalities that reflect androgen deficiency (Gupta et al., 1980; Dessens et al., 2001). A possible linkage between DDE effects on enzyme induction and its reproductive effects deserves examination.

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#### References

- Arlotto MP, Trant JM, and Estabrook RW (1991) Measurement of steroid hydroxylation reactions by high-performance liquid chromatography as indicator of P450 identity and function. *Methods Enzymol* **206**:454–462.
- Baes M, Gulick T, Choi HS, Martinoli MG, Simha D, and Moore DD (1994) A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. *Mol Cell Biol* **14**:1544–1551.
- Blizard DT, Sueyoshi T, Negishi M, Dehal SS, and Kupfer D (2001) Mechanism of induction of cytochrome p450 enzymes by the proestrogenic endocrine disruptor pesticide-methoxychlor: interactions of methoxychlor metabolites with the constitutive androstane receptor system. *Drug Metab Dispos* **29**:781–785.
- Choi HS, Chung M, Tzameli I, Simha D, Lee YK, Seol W, and Moore DD (1997) Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. *J Biol Chem* **272**:23565–23571.
- Dessens AB, Cohen-Kettenis PT, Mellenbergh GJ, Koppe JG, Poll NE, and Boer K (2001) Association of prenatal phenobarbital and phenytoin exposure with genital anomalies and menstrual disorders. *Teratology* **64**:181–188.
- Elias AN and Gwinup G (1980) Effects of some clinically encountered drugs on steroid synthesis and degradation. *Metabolism* **29**:582–595.
- Goodwin B, Moore LB, Stoltz CM, McKee DD, and Klierer SA (2001) Regulation of the human CYP2B6 gene by the nuclear pregnane X receptor. *Mol Pharmacol* **60**:427–431.
- Gupta C, Shapiro BH, and Yaffe SJ (1980) Reproductive dysfunction in male rats following prenatal exposure to phenobarbital. *Pediatr Pharmacol* **1**:55–62.

- Gupta C, Yaffe SJ, and Shapiro BH (1982) Prenatal exposure to phenobarbital permanently decreases testosterone and causes reproductive dysfunction. *Science (Wash DC)* **216**:640–642.
- Honkakoski P, Moore R, Washburn KA, and Negishi M (1998a) Activation by diverse xenochemicals of the 51-base pair phenobarbital-responsive enhancer module in the CYP2B10 gene. *Mol Pharmacol* **53**:597–601.
- Honkakoski P, Zelko I, Sueyoshi T, and Negishi M (1998b) The nuclear orphan receptor CAR-retinoid X receptor heterodimer activates the phenobarbital-responsive enhancer module of the CYP2B gene. *Mol Cell Biol* **18**:5652–5658.
- Johansson MK, Sanderson JT, and Lund BO (2002) Effects of 3-MeSO<sub>2</sub>-DDE and some CYP inhibitors on glucocorticoid steroidogenesis in the H295R human adrenocortical carcinoma cell line. *Toxicol In Vitro* **16**:113–121.
- Kawamoto T, Kakizaki S, Yoshinari K, and Negishi M (2000) Estrogen activation of the nuclear orphan receptor CAR (constitutive active receptor) in induction of the mouse Cyp2b10 gene. *Mol Endocrinol* **14**:1897–1905.
- Kawamoto T, Sueyoshi T, Zelko I, Moore R, Washburn K, and Negishi M (1999) Phenobarbital-responsive nuclear translocation of the receptor CAR in induction of the CYP2B gene. *Mol Cell Biol* **19**:6318–6322.
- Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, and Wilson EM (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature (Lond)* **375**:581–585.
- Kliwer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, et al. (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92**:73–82.
- Kocarek TA, Schuetz EG, Strom SC, Fisher RA, and Guzelian PS (1995) Comparative analysis of cytochrome P450A induction in primary cultures of rat, rabbit, and human hepatocytes. *Drug Metab Dispos* **23**:415–421.
- Moore JT and Kliwer SA (2000) Use of the nuclear receptor PXR to predict drug interactions. *Toxicology* **153**:1–10.
- Moore LB, Maglich JM, McKee DD, Wisely B, Willson TM, Kliwer SA, Lambert MH, and Moore JT (2002) Pregnane X receptor (PXR), constitutive androstane receptor (CAR), and benzoate X receptor (BXR) define three pharmacologically distinct classes of nuclear receptors. *Mol Endocrinol* **16**:977–986.
- Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, Liddle C, Blanchard SG, Willson TM, et al. (2000) Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J Biol Chem* **275**:15122–15127.
- Nims RW and Lubet RA (1995) Induction of cytochrome P-450 in the Norway rat, *Rattus norvegicus*, following exposure to potential environmental contaminants. *J Toxicol Environ Health* **46**:271–292.
- Pascussi JM, Gerbal-Chaloin S, Drocourt L, Maurel P, and Vilarem MJ (2003) The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. *Biochim Biophys Acta* **1619**:143–153.
- Pascussi J, Gerbal-Chaloin S, Pichard-Garcia L, Fabre J, Maurel P, and Vilarem M (2000a) Dexamethasone induces pregnane X receptor and retinoid X receptor- $\alpha$  expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol Pharmacol* **58**:361–372.
- Pascussi J, Gerbal-Chaloin S, Pichard-Garcia L, Fabre J, Maurel P, and Vilarem M (2000b) Dexamethasone enhances constitutive androstane receptor expression in human hepatocytes: consequences on cytochrome P450 gene regulation. *Mol Pharmacol* **58**:1441–1450.
- Rodrigues E, Vilarem MJ, Ribeiro V, Maurel P, and Lechner MC (2003) Two CCAAT/enhancer binding protein sites in the cytochrome P450A1 locus. Potential role in the glucocorticoid response. *Eur J Biochem* **270**:556–564.
- Schuetz EG, Schmid W, Schutz G, Brimer C, Yasuda K, Kamataki T, Bornheim L, Myles K, and Cole TJ (2000) The glucocorticoid receptor is essential for induction of cytochrome P-450B by steroids but not for drug or steroid induction of CYP3A or P-450 reductase in mouse liver. *Drug Metab Dispos* **28**:268–278.
- Thummel KE and Wilkinson GR (1998) In vitro and in vivo drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol* **38**:389–430.
- Waxman DJ and Azaroff L (1992) Phenobarbital induction of cytochrome P-450 gene expression. *Biochem J* **281**:577–592.
- Wei P, Zhang J, Dowhan DH, Han Y, and Moore DD (2002) Specific and overlapping functions of the nuclear hormone receptors CAR and PXR in xenobiotic response. *Pharmacogenomics J* **2**:117–126.
- Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander-Tetri BA, Brunt EM, Guzelian PS, and Evans RM (2000a) Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature (Lond)* **406**:435–439.
- Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, Guzelian PS, and Evans RM (2000b) Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev* **14**:3014–3023.
- Yoshinari K, Sueyoshi T, Moore R, and Negishi M (2001) Nuclear receptor CAR as a regulatory factor for the sexually dimorphic induction of CYB2B1 gene by phenobarbital in rat livers. *Mol Pharmacol* **59**:278–284.
- You L, Casanova M, Aarchibeque-Engle S, Sar M, Fan LQ, and Heck HA (1998) Impaired male sexual development in perinatal Sprague-Dawley and Long-Evans hooded rats exposed in utero and lactationally to p,p'-DDE. *Toxicol Sci* **45**:162–173.
- You L, Chan SK, Bruce JM, Archibeque-Engle S, Casanova M, Corton JC, and Heck H (1999) Modulation of testosterone-metabolizing hepatic cytochrome P-450 enzymes in developing Sprague-Dawley rats following in utero exposure to p,p'-DDE. *Toxicol Appl Pharmacol* **158**:197–205.
- You L, Sar M, Bartolucci E, Ploch S, and Whitt M (2001) Induction of hepatic aromatase by p,p'-DDE in adult male rats. *Mol Cell Endocrinol* **178**:207–214.
- Zhang H, LeCluyse E, Liu L, Hu M, Matoney L, Zhu W, and Yan B (1999) Rat pregnane X receptor: molecular cloning, tissue distribution, and xenobiotic regulation. *Arch Biochem Biophys* **368**:14–22.
- Zhu W, Song L, Zhang H, Matoney L, LeCluyse E, and Yan B (2000) Dexamethasone differentially regulates expression of carboxylesterase genes in humans and rats. *Drug Metab Dispos* **28**:186–191.

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