

## Effects of Aryl Hydrocarbon Receptor Null Mutation and *in Utero* and Lactational 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Exposure on Prostate and Seminal Vesicle Development in C57BL/6 Mice

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Experiments were conducted to determine the effects of aryl hydrocarbon receptor (AhR) null mutation and *in utero* and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure, alone and in combination, on prostate and seminal vesicle development in C57BL/6 mice. AhR heterozygous (*Ahr*<sup>+/-</sup>) mice were mated, and pregnant females were dosed orally on gestation day 13 with TCDD (5 µg/kg) or vehicle. Pups underwent necropsy on postnatal days (PNDs) 35 and 90. Comparison of vehicle-exposed AhR knockout (AhRKO; *Ahr*<sup>-/-</sup>) with wild-type (*Ahr*<sup>+/+</sup>) pups revealed that the AhR is necessary for normal dorsolateral prostate, anterior prostate, and seminal vesicle development but apparently not for ventral prostate development. In wild-type mice, *in utero* and lactational TCDD exposure reduced ventral prostate weight by 79–87% and mRNA expression for its major androgen-dependent secretory protein (MP25) by 99%. Yet high levels of mRNA for a secretory protein normally produced primarily by the lateral prostate (PSP94) were expressed. These effects were predominantly AhR dependent because TCDD had little if any effect in AhRKO mice. TCDD reduced dorsolateral prostate weight in wild-type but not AhRKO mice and had no significant effect on expression of mRNA for PSP94 or for probasin, a major androgen-dependent secretory protein. The PSP94 results suggest that TCDD may have caused a respecification of prostatic gene expression. TCDD reduced anterior prostate weight by more than half, and expression of mRNA for its major androgen-dependent secretory protein (renin-1) was greatly reduced. These effects were AhR dependent. Seminal vesicle weight was reduced by TCDD in wild-type mice but was increased in AhRKO mice on PND 35 and decreased on PND 90 (relative weight only). Androgen receptor mRNA levels were not significantly altered in any prostate lobe, and all organs appeared histologically normal in all groups. Serum testosterone concentrations were unchanged, and modest reductions in serum 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol concentrations could

not account for the effects on sex organs. Collectively, these results indicate that the AhR signaling pathway plays a role in normal accessory sex organ development and that *in utero* and lactational TCDD exposure disrupts development of these organs via spatially and perhaps temporally specific mechanisms.

**Key Words:** 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; *in utero*; lactational exposure; TCDD; aryl hydrocarbon receptor null mutation; AhRKO mice; prostate; seminal vesicles; gene expression; development; mice.

Initial reports that male reproductive system development is exceptionally sensitive to *in utero* and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure (Mably *et al.*, 1992a,b,c), and most subsequent publications that further characterize these effects and elucidate mechanisms responsible for causing them, were based on experiments conducted in the rat. With the advent of mutant mice lacking the aryl hydrocarbon receptor (AhR) and other proteins, the development of conditional mutations in mice, and other technologies (e.g., DNA microarrays) that are more advanced for studying mice than rats, we have begun to use mouse models to investigate effects of *in utero* and lactational TCDD exposure on development of the male reproductive system.

In earlier work on the mouse, we found that cumulative maternal doses of 3 and 9 µg TCDD/kg on gestation days (GDs) 12–14 caused modest reductions in ventral prostate weight on postnatal day (PND) 50 and anterior prostate weight on PNDs 65 and 95. Male mating ability (with control females) was also reduced by the high maternal dose. In contrast, testis, epididymis, vas deferens, dorsolateral prostate, and seminal vesicle weights were unaffected, as were cauda epididymal and ejaculated sperm numbers (Sommer and Peterson, 1997). In a separate experiment, *in utero* and lactational exposure to 15, 30, or 60 µg TCDD/kg on GD 14 reduced ventral prostate and anterior prostate weight and cauda epididymal sperm numbers. However, no significant effects were seen on testis, epididymis, dorsolateral prostate, or seminal vesicle weight, anogenital distance, time to testis descent or preputial separation,

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serum testosterone concentrations, or daily sperm production (Theobald and Peterson, 1997). Both experiments were conducted with ICR mice, a wild-type outbred strain derived from the CD-1 strain. No other laboratories have published reports of possible effects of *in utero* and lactational TCDD exposure on male reproductive system development in the mouse.

Although the studies summarized previously demonstrate that male reproductive organ development in the mouse can be vulnerable to TCDD, each of the 3 types of AhR knockout (AhRKO; *Ahr*<sup>-/-</sup>) mice that has been developed (Fernandez-Salguero *et al.*, 1995; Mimura *et al.*, 1997; Schmidt *et al.*, 1996) was backcrossed to a C57BL/6 background. To take advantage of AhRKO mice, it was necessary to determine effects of *in utero* and lactational TCDD exposure on C57BL/6 mice.

Our initial objectives were to use AhRKO mice on a C57BL/6J background, and their wild-type (*Ahr*<sup>+/+</sup>) littermates, to determine (1) the possible role of the AhR in normal development; (2) the effects of *in utero* and lactational TCDD exposure on development, and (3) the extent to which effects of *in utero* and lactational TCDD exposure on development are mediated by the AhR. Effects on survival, hydronephrosis, body weight, absolute and relative weights of the liver, heart, spleen, thymus, kidney, lung, submandibular gland, testis, and epididymis at 3 stages of development, and daily sperm production and cauda epididymal sperm numbers have been described elsewhere (Lin *et al.*, 2001a). We now report effects of AhR null mutation and *in utero* and lactational TCDD exposure, alone and in combination, on ventral prostate, dorsolateral prostate, anterior prostate, and seminal vesicle development, including effects on gene expression in each prostate lobe.

## MATERIALS AND METHODS

**Animals, genotyping, and treatments.** Details have previously been described by Lin *et al.* (2001a). Briefly, AhRKO mice developed in the Bradfield laboratory (Schmidt *et al.*, 1996) were backcrossed to C57BL/6J mice for 9 to 11 generations. Mice were housed in temperature-, humidity-, and light-controlled rooms in plastic cages with heat-treated chipped aspen bedding. Feed and tap water were available *ad libitum*. All procedures were approved by the University of Wisconsin Animal Care and Use Committee.

Heterozygous (*Ahr*<sup>+/-</sup>) females were paired overnight with *Ahr*<sup>+/-</sup> males. Pregnant mice were given a single oral dose of TCDD (5 µg/kg) or vehicle (95% corn oil/5% acetone, 5 ml/kg) on GD 13 (GD 0 = plug positive). This dose was chosen because pilot experiments found it to be the highest that could be given without causing mortality in offspring. Dosing was on GD 13 to coincide with the onset of testosterone synthesis in fetal mouse testes (Pointis *et al.*, 1979). Genotyping was done by polymerase chain reaction (PCR) analysis of ear punch tissue taken at 10 to 16 days of age, as previously described (Benedict *et al.*, 2000). Pups were weaned on PND 21.

**Necropsies and sample preparation.** Mice were euthanized by CO<sub>2</sub> overdose on PNDs 35 and 90. Accessory sex organs were identified as described by Sugimura *et al.* (1986), removed, weighed, and frozen in polypropylene vials with liquid nitrogen or placed overnight in Z-5 fixative (Anatech Ltd., Battle Creek, MI). Fixed samples were stored in 70% ethanol, dehydrated in graded ethanol, embedded in paraffin, sectioned at 5 µm, and stained with hematox-

ilin and eosin. Five sections per organ (every 10th section) from each of 3 mice from each age, genotype, and treatment group were examined microscopically by an experienced pathologist (T.D.O.).

**Real-time reverse-transcription PCR mRNA quantification.** Total RNA was isolated using RNeasy Mini Kits (Qiagen, Valencia, CA) according to the manufacturer's protocol. Oligo-dT primed first-strand cDNA was synthesized using Omniscript reverse transcriptase (Qiagen) in 20 µl reactions containing 500 ng total RNA following manufacturer's instructions. Final reverse transcription (RT) reactions were diluted to 100 µl for storage and real-time LightCycler (Roche Molecular Biochemicals, Indianapolis, IN) quantitative PCR analysis. PCR was performed in a 20 µl reaction volume containing 5 µl of diluted RT reaction mixture, 1 × Qiagen PCR buffer, 4 mM MgCl<sub>2</sub>, 250 ng/µl bovine serum albumin (New England Biolabs, Beverly, MA), 0.5 µM of each primer, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.05 U/µl *Taq* DNA polymerase (Qiagen), and SYBR Green I (1:40,000; Molecular Probes, Eugene, OR). Primer sequences, product size, and annealing temperatures are shown in Table 1. Amplified RT-PCR products were cloned into pCRII plasmid vectors (Invitrogen, Carlsbad, CA). Clones containing cDNA fragments of the proper size were selected and confirmed as being correct inserts by matching restriction endonuclease digestion profiles to those predicted from published cDNA sequences. A single clone of purified plasmid was selected as the copy number standard for each gene. Serially diluted copy number standard plasmids and unknown RT samples were simultaneously amplified using the same reaction master mixture. After an initial 94°C 30-s melting step, the cDNA of interest was amplified for 40 three-step cycles (94°C 0-s hold melting, 5-s hold annealing, and 72°C 10-s hold extension). Fluorescent product (double-stranded DNA) was detected at the end of each cycle. The LightCycler software integrated fluorescence intensity versus cycle number for each tube and provided every reaction with a crossing point, defined as the fractional cycle number at which the second derivative maximum occurred. A crossing point versus initial copy number of cDNA standard was plotted as the standard curve, and initial copy numbers for unknown RT samples were estimated. After amplification, a melting curve was acquired by heating to 94°C, cooling to 5°C above the annealing temperature, and slowly heating to 94°C, with continuous fluorescence data collection. Identical melting curves between cDNA standards and unknown samples provided a reliability check that the fluorescence signal reflected only the specific RT-PCR product and assurance of the integrity of mRNA quantification results.

**Androgen assays.** Serum testosterone and 5α-androstane-3α,17β-diol concentrations were determined by radioimmunoassays as previously described (Loeffler and Peterson, 1999).

**Statistical analysis.** Analyses were conducted with the litter as the experimental unit (i.e., if results were obtained from any litter from 2 or more males of the same genotype, analyses were done on the average results). Parametric analyses were conducted on untransformed data and on log, square root, and inverse transforms as well as on ranked data. For data that passed Levene's test for homogeneity of variance and which appeared to be normally distributed, analysis of variance (ANOVA) was conducted. If a significant effect was found, the least significant difference test was used to determine which groups differed from the appropriate control group. All data were also analyzed by the Kruskal-Wallis nonparametric ANOVA and by the median test. The distribution-free multiple comparison test was used as the *post hoc* test for nonparametric analyses. Significance was set at  $p < 0.05$ . Results are presented as means ± standard error.

## RESULTS

As previously reported (Lin *et al.*, 2001a), neither AhR genotype nor TCDD exposure affected litter size or survival. Vehicle-exposed AhRKO mice were similar in weight to their wild-type counterparts on PND 35 and 7% heavier on PND 90,

TABLE 1  
LightCycler Real-Time RT-PCR Primer Sequences, Annealing Temperatures, and Product Sizes

Gene (GenBank accession no.)	Primer pairs	Temp. (°C)	Size
Androgen receptor (emb X59590)	AATCTGGATGTGGAGAGAGC AGAGAACAGAACACTAGCGC	60	166
Cytokeratin 8 (emb X12789)	ACCAGGAGCTTATGAACGTC AGGAGCTCATTCCGTAGCTG	65	204
Cyclophilin (emb X52803)	ATCACGGCCGATGACGAGCC TCTCTCCGTAGATGGACCTGC	65	217
MP25 (emb X06246)	AGAGCCCAGAATGTCCTGGG TTATCACGTGCTCTCCGTCC	65	214
PSP94 (emb X16642)	TGCCACCATGGAAGCTTGGC TAGCGTTGGTACAGCAGGTG	65	224
Renin 1 (gb U89840)	ACTCGGTGACTGTGGGTGG AGGTGGGAACCCCTGTTGTAG	65	220
SVS II (dbj AK020661)	AAACAGAGGAAGACTTATCCC AGGCGAGTCCTTGATATTGATC	60	237

Note. MP25, mouse prostatic secretory glycoprotein. PSP94, prostate secretory protein of 94 amino acids. SVS II, seminal vesicle secretion II protein. Size, product size (base pairs).

but TCDD exposure had no significant effect on body weight in either wild-type or AhRKO mice on PND 35 or 90.

Absolute and relative ventral prostate weights were not significantly affected in vehicle-exposed mice by AhR null mutation on either PND 35 or 90 (Fig. 1). *In utero* and lactational TCDD exposure reduced absolute and relative ventral prostate weights in wild-type mice at both times tested by 79–87% but had no significant effect on ventral prostate weight in AhRKO mice.

On PND 90, gene expression in mouse prostate lobes was quantitated at the level of mRNA. Results are shown relative to cyclophilin mRNA to normalize expression on a per cell basis (Weisinger *et al.*, 1999).

Cytokeratin 8 mRNA expression was used as a marker for structurally cytodifferentiated luminal epithelial cells (Fuchs, 1988). AhR null mutation had no significant effect on cytokeratin 8 mRNA expression in the ventral prostate, but *in utero* and lactational TCDD exposure caused a 21% reduction in wild-type mice (Fig. 2A). TCDD did not significantly affect cytokeratin 8 mRNA expression in AhRKO mice, however. Functional cytodifferentiation of the ventral prostate was analyzed by quantitating mRNA for MP25, its major androgen-dependent secretory glycoprotein in mice (Mills *et al.*, 1987). AhR null mutation had no significant effect on MP25 mRNA expression in vehicle-exposed mice, but expression in TCDD-exposed wild-type mice was reduced to less than 1% of the control value (Fig. 2B). In contrast, TCDD had no significant effect on MP25 mRNA expression in AhRKO mice. However, MP25 mRNA expression relative to cytokeratin 8 mRNA expression was significantly reduced (by 25%) by TCDD in AhRKO mice (not shown). Expression of mRNA for PSP94, a prostatic secretory protein produced primarily by the lateral prostate (Xuan *et al.*, 1999), was increased 4-fold in TCDD-exposed wild-type mice but was unchanged in the other 2

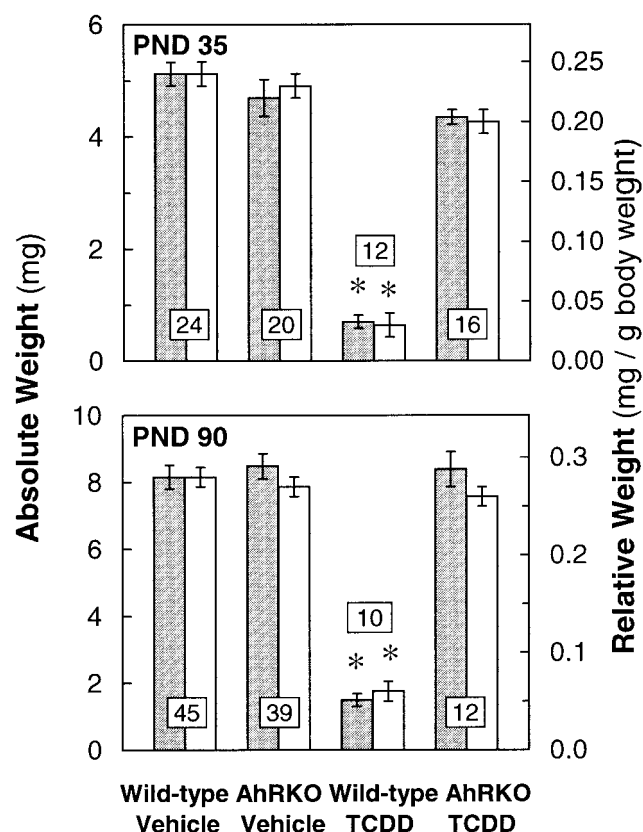


FIG. 1. Effects of AhR genotype and *in utero* and lactational TCDD exposure on absolute (shaded bars) and relative (open bars) ventral prostate weight on PND 35 and PND 90. Dams were orally dosed with TCDD (5  $\mu$ g/kg) or vehicle (5 ml/kg) on GD 13. Values represent means  $\pm$  SE; the number of replicates (litters) is shown in each bar. \*Significantly different from vehicle-exposed wild type at  $p < 0.05$ .

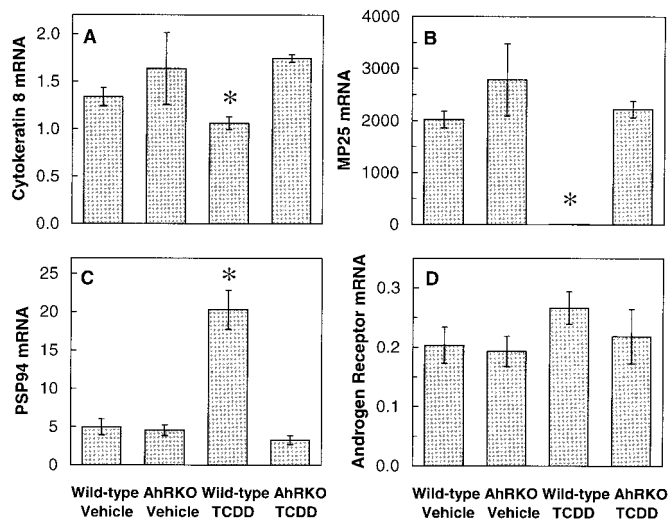


FIG. 2. Effects of AhR genotype and *in utero* and lactational TCDD exposure on (A) cytokeratin 8, (B) MP25, (C) PSP94, and (D) androgen receptor mRNA (relative to cyclophilin mRNA) in the ventral prostate on PND 90. Dams were orally dosed with TCDD (5  $\mu\text{g}/\text{kg}$ ) or vehicle (5 ml/kg) on GD 13. Values represent means  $\pm$  SE;  $n = 5\text{--}7$  litters. \*Significantly different from vehicle-exposed wild type at  $p < 0.05$ .

groups (Fig. 2C). Androgen receptor mRNA expression was not significantly affected by AhR null mutation or by *in utero* and lactational TCDD exposure (Fig. 2D).

Absolute and relative dorsolateral prostate weights in vehicle-exposed mice were reduced by 19 and 16%, respectively, on PND 35, and relative dorsolateral prostate weight was reduced by 10% on PND 90 by AhR null mutation (Fig. 3). *In utero* and lactational TCDD exposure reduced absolute and relative dorsolateral prostate weights in wild-type mice much more on PND 35 (by 56 and 53%, respectively) than on PND 90 (by 25 and 17%, respectively). TCDD exposure had no significant effect on dorsolateral prostate weight in AhRKO mice.

In the dorsolateral prostate, cytokeratin 8 mRNA expression was not significantly affected by either AhR null mutation or *in utero* and lactational TCDD exposure (Fig. 4A). Probasin is a major androgen-dependent secretory protein in mice that is produced by the dorsolateral and anterior prostate (Johnson *et al.*, 2000). Probasin mRNA levels were not significantly affected relative to cyclophilin mRNA (Fig. 4B) but were significantly reduced by TCDD in wild-type mice (by 29%) and in AhRKO mice (by 39%) relative to cytokeratin 8 mRNA (not shown). There were no significant effects on PSP94 mRNA levels, a lateral prostate-specific gene product (Fig. 4C). MP25 mRNA expression was 2 orders of magnitude smaller in the dorsolateral prostate than in the ventral prostate, and neither genotype nor treatment affected its expression (results not shown). Finally, there were no significant effects of AhR null mutation or *in utero* and lactational TCDD exposure on androgen receptor mRNA expression (Fig. 4D).

Absolute and relative anterior prostate weights were reduced

in vehicle-exposed mice by AhR null mutation by 19 and 16%, respectively, on PND 35 but were unchanged on PND 90 (Fig. 5). TCDD exposure reduced absolute and relative anterior prostate weights in wild-type mice at both times tested, by 53–67%, but had no significant effects in AhRKO mice at either time.

Cytokeratin 8 mRNA expression was not affected in the anterior prostate by AhR null mutation or TCDD exposure (Fig. 6A). Functional cytodifferentiation was assessed by measuring mRNA for renin-1, an androgen-dependent protein highly expressed in mouse anterior prostate (Fabian *et al.*, 1993). Renin-1 mRNA expression was reduced nearly 60% by *in utero* and lactational TCDD exposure in wild-type mice (Fig. 6B) but was not otherwise affected. Expression of mRNA for a seminal vesicle-specific gene, SVS II (Lundwall, 1996), suggested that AhR null mutation and TCDD exposure caused the anterior prostate in some mice to synthesize high levels of mRNA for this protein, but no statistically significant effects were observed (Fig. 6C). Androgen receptor mRNA levels did not differ significantly among any of the groups (Fig. 6D).

Absolute and relative seminal vesicle weights in vehicle-

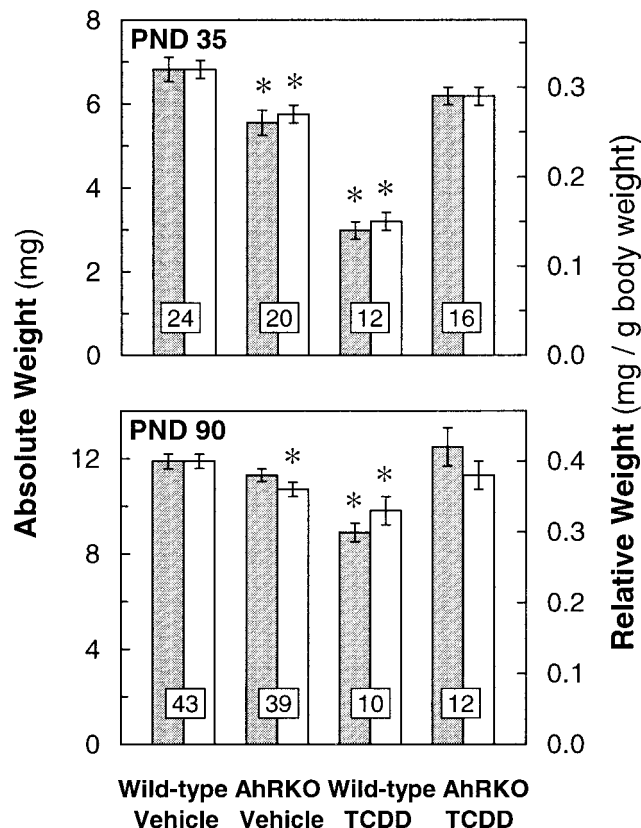


FIG. 3. Effects of AhR genotype and *in utero* and lactational TCDD exposure on absolute (shaded bars) and relative (open bars) dorsolateral prostate weight on PND 35 and PND 90. Dams were orally dosed with TCDD (5  $\mu\text{g}/\text{kg}$ ) or vehicle (5 ml/kg) on GD 13. Values represent means  $\pm$  SE; the number of replicates (litters) is shown in each bar. \*Significantly different from vehicle-exposed wild type at  $p < 0.05$ .

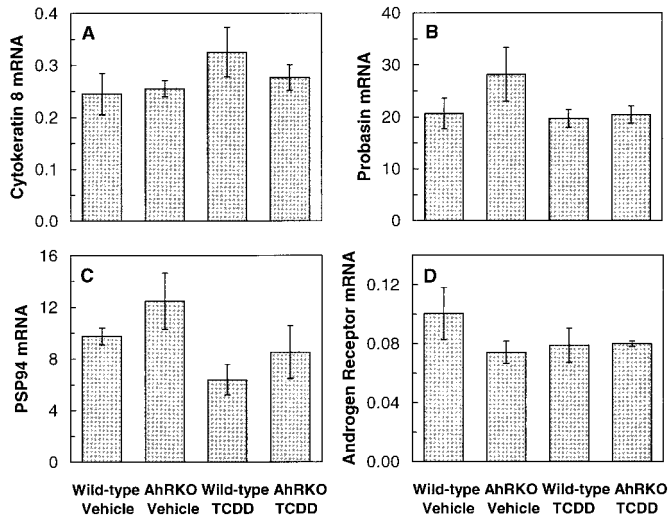


FIG. 4. Effects of AhR genotype and *in utero* and lactational TCDD exposure on (A) cytokeratin 8, (B) probasin, (C) PSP94, and (D) androgen receptor mRNA (relative to cyclophilin mRNA) in the dorsolateral prostate on PND 90. Dams were orally dosed with TCDD (5  $\mu$ g/kg) or vehicle (5 ml/kg) on GD 13. Values represent means  $\pm$  SE;  $n = 5-7$  litters.

exposed mice were reduced by 19 and 18%, respectively, on PND 35 and by 8 and 14%, respectively, on PND 90 by absence of the AhR (Fig. 7). TCDD exposure reduced absolute and relative seminal vesicle weights in wild-type mice by 42 and 39%, respectively, on PND 35 and by 25 and 20%, respectively, on PND 90. In AhRKO mice, TCDD exposure increased absolute and relative seminal vesicle weights by 25 and 23%, respectively, on PND 35, whereas relative seminal vesicle weight was decreased by 9% on PND 90.

Seminal vesicles and prostate lobes were examined histologically on PNDs 35 and 90. Although weight and gene expression in each organ often differed greatly from one group to another, ventral prostates, dorsolateral prostates, anterior prostates, and seminal vesicles from each genotype and treatment group appeared to be histologically normal at both times studied (not shown).

Serum concentrations of  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol, the primary circulating androgen prior to puberty, were reduced on PND 21 in vehicle-exposed mice by 37% by AhR null mutation (Fig. 8). The reduction in serum  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol concentrations in wild-type mice caused by TCDD exposure at this time was almost identical. However, concentrations of this androgen were not significantly altered on PND 21 by TCDD in AhRKO mice. There were no significant differences in serum  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol concentrations among any of the groups on PND 35.

Serum testosterone concentrations were not significantly affected by AhR null mutation in vehicle-exposed mice or by TCDD exposure in either wild-type or AhRKO mice on either PND 35 or PND 90 (Fig. 8).

Male fertility was not affected by AhR null mutation or by

*in utero* and lactational TCDD exposure at any time tested (not shown).

## DISCUSSION

### Role of the AhR in Prostate and Seminal Vesicle Development

AhR null mutation caused small but statistically significant reductions in absolute and relative testis and epididymis weights in C57BL/6 mice (Lin *et al.*, 2001a). However, whether the AhR is involved in the development of other male reproductive organs had not been reported. AhR null mutation had no detectable effect on the ventral prostate, suggesting that the AhR plays little if any role in the development of this organ in mice. In contrast, AhR null mutation inhibited dorsolateral prostate development, as shown by reductions in absolute and relative weight on PND 35 and relative weight on PND 90. No significant effects on gene expression or histology were found, however, suggesting that this organ is somewhat small in adult AhRKO mice but otherwise completely normal. AhR null

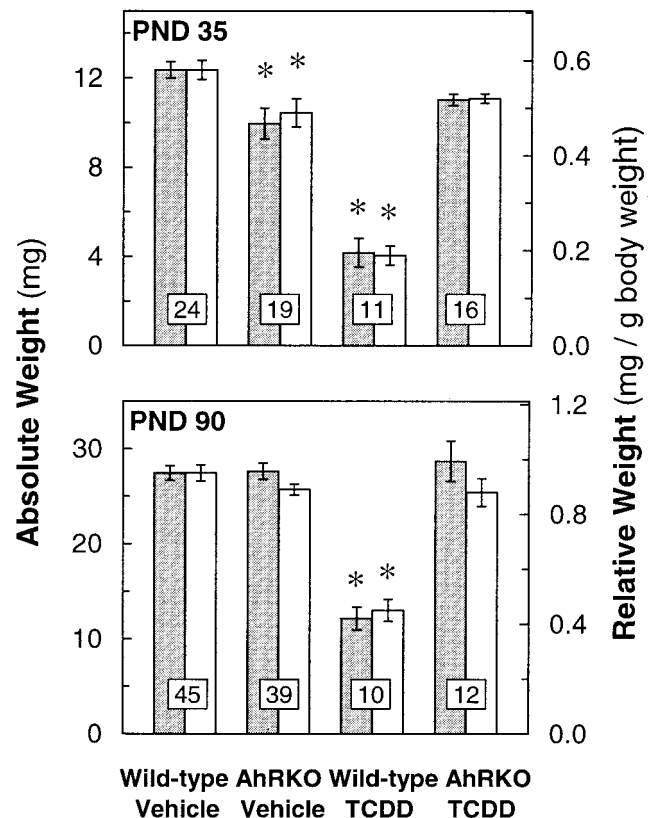


FIG. 5. Effects of AhR genotype and *in utero* and lactational TCDD exposure on absolute (shaded bars) and relative (open bars) anterior prostate weight on PND 35 and PND 90. Dams were orally dosed with TCDD (5  $\mu$ g/kg) or vehicle (5 ml/kg) on GD 13. Values represent means  $\pm$  SE; the number of replicates (litters) is shown in each bar. \*Significantly different from vehicle-exposed wild type at  $p < 0.05$ . Weights are for paired organs.

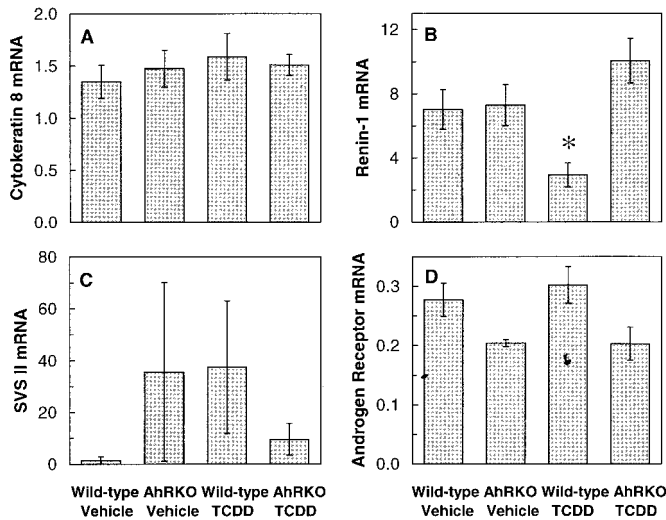


FIG. 6. Effects of AhR genotype and *in utero* and lactational TCDD exposure on (A) cytochrome 8, (B) renin-1, (C) SVS II, and (D) androgen receptor mRNA (relative to cyclophilin mRNA) in the anterior prostate on PND 90. Dams were orally dosed with TCDD (5  $\mu\text{g}/\text{kg}$ ) or vehicle (5 ml/kg) on GD 13. Values represent means  $\pm$  SE;  $n = 4\text{--}6$  litters. \*Significantly different from vehicle-exposed wild type at  $p < 0.05$ .

mutation also impaired anterior prostate development, as shown by reductions in absolute and relative weight on PND 35. By PND 90, however, there were no significant effects on weight or gene expression, and histology appeared normal at both times. These results suggest that the anterior prostate, although slow to develop when the AhR is absent, is completely developed in adulthood. The seminal vesicles appear to be the accessory sex organ most vulnerable to AhR null mutation. Although the magnitude of the effect decreased with time, absolute and relative seminal vesicle weights were reduced at both times tested. Histological appearance remained normal, however.

In summary, the AhR plays a heretofore unrecognized role in normal dorsolateral prostate, anterior prostate, and seminal vesicle development, albeit one that apparently does not involve structural or functional cytodifferentiation of the prostate. Whether delayed and/or incomplete development of these organs seen in the absence of the AhR is due to interactions of the AhR with one or more endogenous ligands or to actions of unliganded AhR remains to be determined.

#### Effects of *in Utero* and Lactational TCDD Exposure on Prostate and Seminal Vesicle Development, and the Role of the AhR in Mediating the Effects of TCDD

We had previously reported that *in utero* and lactational TCDD exposure reduces ventral prostate and anterior prostate weight in ICR mice, whereas dorsolateral prostate and seminal vesicle weights were unaffected (Sommer and Peterson, 1997; Theobald and Peterson, 1997). No other publications described the effects of TCDD on accessory sex organ development in

the mouse, and effects on histology and gene expression were unknown.

The magnitude of the effects of TCDD on ventral and anterior prostate development seen in the present study are substantially greater than those previously reported. In addition, dorsolateral prostate and seminal vesicle development have now been shown to be affected by *in utero* and lactational TCDD exposure. Accessory sex organs in the C57BL/6 mouse are, therefore, substantially more sensitive to *in utero* and lactational TCDD exposure than are those of ICR mice.

**Ventral prostate.** *In utero* and lactational TCDD exposure caused a profound inhibition of ventral prostate development in wild-type mice, far greater than had previously been shown in any species. Effects on the ventral prostate were substantially greater than those on other sex organs. Although extremely small, the ventral prostate appeared to be reasonably well cytodifferentiated structurally, as evidenced by a small (though significant) reduction in the ratio of cytochrome 8 mRNA to

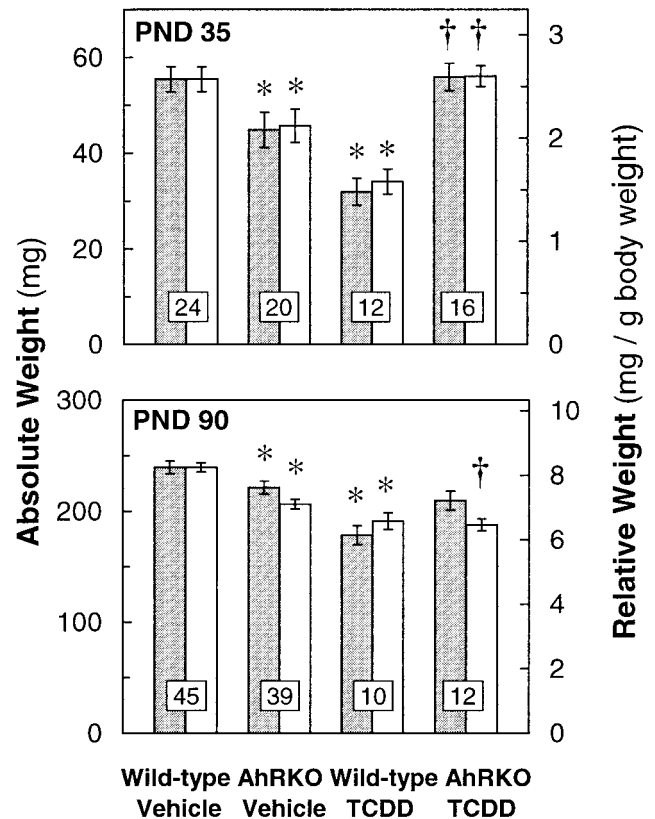


FIG. 7. Effects of AhR genotype and *in utero* and lactational TCDD exposure on absolute (shaded bars) and relative (open bars) seminal vesicle weight on PND 35 and PND 90. Dams were orally dosed with TCDD (5  $\mu\text{g}/\text{kg}$ ) or vehicle (5 ml/kg) on GD 13. Values represent means  $\pm$  SE; the number of replicates (litters) is shown in each bar. \*Significantly different from vehicle-exposed wild type at  $p < 0.05$ . †Significantly different at  $p < 0.05$  (data from TCDD-exposed AhRKO mice were compared only with the corresponding data from vehicle-exposed AhRKO mice). Weights are for paired organs.

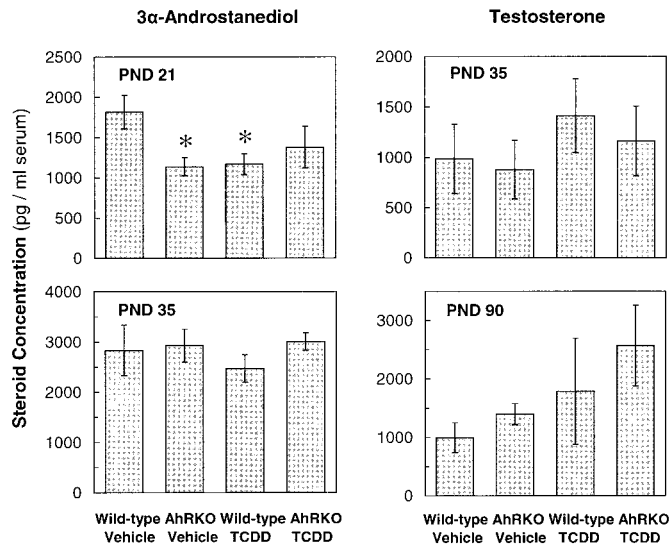


FIG. 8. Effects of AhR genotype and *in utero* and lactational TCDD exposure on serum 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol and testosterone concentrations on PND 21, 35, and/or 90, as shown. Dams were orally dosed with TCDD (5  $\mu$ g/kg) or vehicle (5 ml/kg) on GD 13. Values represent means  $\pm$  SE;  $n = 5$ –10 litters. \*Significantly different from vehicle-exposed wild type at  $p < 0.05$ .

cyclophilin mRNA, and by the observation that its histological appearance was normal.

Effects of TCDD on ventral prostate growth and gene expression were clearly AhR dependent, with the exception that MP25 mRNA expression per structurally cytodifferentiated luminal epithelial cell (i.e., relative to cytokeratin 8 mRNA expression) was slightly reduced in AhRKO mice. The severe effects of *in utero* and lactational TCDD exposure on wild-type mice appeared to be permanent in that organ weight and MP25 mRNA expression were greatly reduced in mature adulthood (PND 90).

The finding that ventral prostate epithelial cells in TCDD-exposed wild-type mice looked normal histologically normal suggests that the severe inhibitory effect of TCDD on MP25 mRNA expression is not representative of its effects on secretory protein gene expression in general. If expression of mRNA for all secretory proteins had been reduced as severely as it was for MP25 (by 99%), epithelial cells in the ventral prostate should have resembled (visually) those typical of castrated mice. The observation that these cells looked normal indicates that they were secreting proteins at rates roughly comparable to that of epithelial cells in control ventral prostates. Consequently, although the mRNA expression data indicate that secretion of MP25 (and possibly other proteins) was severely inhibited by TCDD, the histology results indicate that expression of other secretory proteins (on a per cell basis) would have been unchanged or even increased.

The most interesting effect of TCDD on ventral prostate development, other than the severe reductions in organ weight

and mRNA expression for a ventral-specific secretory protein, was the high expression of mRNA for a protein normally secreted primarily by the lateral prostate. PSP94 mRNA expression per ventral prostate cell was 4 times greater in TCDD-exposed than in vehicle-exposed mice, and expression per structurally cytodifferentiated luminal epithelial cell was 6.5-fold greater. Because gene expression analyses for the ventral and dorsolateral prostate were not run concurrently, PSP94 mRNA expression in these 2 organs cannot be quantitatively compared with each other. Consequently, the observation that PSP94 mRNA expression per cell appeared to be twice as great in the ventral prostate of TCDD-exposed mice as in the dorsolateral prostate of vehicle-exposed mice provides only a rough approximation of relative gene expression in the 2 organs. Nevertheless, it is safe to conclude that TCDD caused cells in the ventral prostate to express PSP94 mRNA at a level roughly comparable to that of cells in control dorsolateral prostate. This increase is not simply due to a generalized increase in expression of this gene, because PSP94 mRNA expression in the dorsolateral prostate of TCDD-exposed mice was clearly not increased and, in fact, tended to be decreased. Collectively, these results suggest that *in utero* and lactational TCDD exposure may have caused a respecification of gene expression in the ventral prostate toward that characteristic of the lateral prostate. The molecular basis for this apparent alteration in lobe-specific gene expression remains to be determined. Although numerous reports exist that TCDD alters gene expression in various tissues and organs (including alterations in the differentiation of various cell types), to the best of our knowledge this is the first report that any AhR ligand may be capable of causing one organ to display gene expression characteristic of another.

**Dorsolateral prostate.** Effects of *in utero* and lactational TCDD exposure on dorsolateral prostate development in wild-type mice were not nearly as great as effects on the ventral prostate. In addition, unlike the ventral prostate, the inhibition of dorsolateral prostate growth lessened substantially with time. The reductions in dorsolateral prostate weight were AhR dependent. Gene expression analysis found no significant effects of TCDD on either structural or functional cytodifferentiation per cell, although the reduction in organ weight implies that mRNA expression per dorsolateral prostate was probably reduced. Probasin mRNA expression per structurally cytodifferentiated luminal epithelial cell was slightly decreased in both wild-type and AhRKO mice, suggesting that this effect was AhR independent. Histologically, TCDD had no effect on either PND 35 or PND 90.

Although *in utero* and lactational TCDD exposure caused substantial lateral prostate-like gene expression in the ventral prostate of wild-type mice, no evidence was found that it caused ventral prostate-like gene expression in the dorsolateral prostate.

**Anterior prostate.** Effects of TCDD on anterior prostate weight and on androgen-dependent, lobe-specific gene expression were AhR dependent and were intermediate in severity between its effects on the other 2 prostate lobes. Similarly, the apparent recovery in organ weight between PND 35 and PND 90 was far less than that observed in the dorsolateral prostate but greater than that seen in the ventral prostate.

The lack of an effect on cytokeratin 8 mRNA expression and the observation that histological appearance was unchanged indicate that *in utero* and lactational TCDD exposure did not affect structural cytodifferentiation of the anterior prostate. Yet its luminal epithelial cells were not fully cytodifferentiated functionally, as shown by the major reduction in renin-1 mRNA expression. In addition, TCDD caused the anterior prostate in some wild-type mice to produce mRNA characteristic of the seminal vesicles: 2 of 6 mice had SVS II mRNA levels at least an order of magnitude greater than the highest level seen in any wild-type mouse exposed to vehicle. Further research is needed to determine whether this possible respecification of anterior prostate gene expression is real, and if so, how widespread it may be.

**Seminal vesicles.** *In utero* and lactational TCDD exposure significantly reduced absolute and relative seminal vesicle weight in wild-type mice at both times tested. The magnitude of these reductions decreased with time. A surprising finding is that TCDD significantly increased absolute and relative seminal vesicle weight on PND 35 in AhRKO mice. The effect on absolute weight disappeared by PND 90, whereas the increase in relative weight seen on PND 35 became a small but significant decrease by PND 90. Despite these changes in weight, no changes in seminal vesicle histology were observed. Effects of TCDD on seminal vesicles were far smaller than those on the ventral prostate, in contrast to the effects of AhR null mutation, in which the ventral prostate appeared to be unaffected while the seminal vesicles were the organ most affected.

#### *Observations Common to Accessory Sex Organs*

Serum  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol concentrations were reduced 36–37% by AhR null mutation and by TCDD (in wild-type mice) on PND 21, but otherwise no significant differences in  $5\alpha$ -androstane- $3\alpha,17\beta$ -diol, or testosterone concentrations were observed. These reductions were relatively small, and the effects of TCDD and AhR null mutation differed greatly from one organ to another, suggesting that possible reductions in serum androgen concentrations are not a primary factor in abnormal prostate and seminal vesicle development.

Impaired ventral, dorsolateral, and anterior prostate development do not appear to be due to insufficient androgen receptor mRNA expression. There were no statistically significant effects on androgen receptor mRNA expression in any prostate lobe.

Despite a number of severe changes in organ weight and gene expression, neither AhR null mutation nor *in utero* and

lactational TCDD exposure, alone or in combination, had any detectable effect on the histological appearance of any accessory sex organ. These observations, in combination with the almost complete lack of effect on cytokeratin 8 mRNA expression, demonstrate that AhR null mutation and TCDD had little if any effect on structural cytodifferentiation of these organs. We conclude that these organs weighed less than normal primarily because fewer cells were present rather than because their cells were too small. Whether these organs are small because cell division is inhibited or apoptosis is increased, or some combination of these, remains to be determined.

Each accessory sex organ was affected differently by AhR null mutation and by *in utero* and lactational TCDD exposure. Differences were seen in the magnitude of the effects on organ weight and on expression of mRNA for a major secretory product characteristic of that organ, the extent to which effects appeared to be developmental delays or permanent effects, and whether evidence of altered regional specificity in gene expression was found. The organ most sensitive to TCDD (the ventral prostate) was the only organ not affected by AhR null mutation. There were also differences in which effects of TCDD appeared to be AhR dependent or AhR independent.

We previously reported that TCDD can cause one effect in wild-type mice but cause the opposite effect in AhRKO mice, produce similar effects in wild-type and AhRKO mice, and significantly affect AhRKO mice without significantly altering the same endpoint in wild-type mice (Lin *et al.*, 2001a). Results of the present study provide additional examples of effects of TCDD that may not be fully AhR dependent: specifically, reduced MP25 mRNA expression (relative to cytokeratin 8) in the ventral prostate, reduced probasin mRNA expression (relative to cytokeratin 8) in the dorsolateral prostate, and alterations in seminal vesicle weight. Although most effects of TCDD seen in our lab and elsewhere required the presence of the AhR, these results provide additional evidence for either multiple forms of the AhR in mice (one or more of which are still present in AhRKO mice) or for AhR-independent effects of low-level TCDD exposure.

In summary, *in utero* and lactational TCDD exposure (and to a lesser extent AhR null mutation) can substantially alter prostate and seminal vesicle development in the mouse. Effects on the prostate are clearly lobe specific and may be due to regional differences in the effects of TCDD on the urogenital sinus, the organ from which prostate lobes develop. We are currently examining the effects of TCDD on region-specific urogenital sinus bud formation (Lin *et al.*, 2001b) and region-specific urogenital sinus gene expression (Lin *et al.*, 2002b), as well as ductal branching morphogenesis in each prostate lobe (Ko *et al.*, 2001). The developmental stage at which prostate lobes and seminal vesicles are most vulnerable to TCDD exposure varies from 1 organ to another, as will be reported separately (Lin *et al.*, in press).

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