parameters, such as serum hormone concentrations, testicular and epididymal histology and sperm morphology and motility, in peripubertal and post-pubertal boars. Nine, 7-month-old and eight, 12 to 14-month-old boars were each divided into two groups which were orally exposed to either 0 or 100 mg VCZ/kg body weight for 14 days. Serum samples collected prior to and during the study were analyzed for concentrations of testosterone and estradiol. Following euthanasia, semen was collected from the cauda epididymis for computer-assisted analysis, and tissues were selected and prepared for histologic examination. Statistical analyses were performed using ANOVA and the general linear model (GLM) procedure. Testosterone concentrations increased initially and then decreased in VCZ-treated post-pubertal boars. Estradiol levels were higher in boars of both age groups dosed with VCZ. Histologic changes in the testes and epididymides, as well as abnormal sperm morphology and decreased sperm motility, were observed with administration of VCZ. Exposure to VCZ adversely affected various reproductive parameters in peripubertal and, in particular, post-pubertal boars. VCZ-associated histologic changes were more severe in swine than those reported in rodents administered the same dosage of VCZ. The boar shows promise as a sensitive comparative model for studying the effects of EDCs on male reproductive morphology and function.

547 DELAYED PREPUTIAL SEPARATION (PPS) AND SP22 MEASUREMENT IN RATS ADMINISTERED BROMOCHLOROACETIC ACID (BCA) IN DRINKING WATER.

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Reproductive effects of BCA were determined in a dose range finding study (DRFS) and definitive two-generational study. Adult male and female CD®(SD) rats were administered BCA in drinking water for two weeks in the DRFS (10/sex/group) and ten weeks in the definitive study (25/sex/group) before mating. Females were dosed until the weaning of their litters. The F1 post-weanlings received BCA until acquiring puberty [PPS in males, vaginal patency (VP) in females] in the DRFS, and through mating to generate F2 offspring in the definitive study. The concentrations were 0, 50, 200, 400, 600, 800, and 1000 ppm BCA *ad libitum* in the DRFS and 0, 30, 300, and 600 ppm BCA in the definitive study. Age of male PPS was used to assess reproductive development. The males at ≧ 600 ppm in both studies, showed significant delay of PPS vs. the control males; At 600 ppm mean PPS delays were 2.2 days in the definitive study and 2.7 days in the DRFS; At 800 ppm, males had mean delays of 4.7 days and those males at 1000 ppm had delays of 5.2 days. Ages at acquisition were adjusted using body weight (BW) at acquisition as a covariant. In the DRFS, F1 females at ≧ 600 ppm had VP delays of 6.2 days; comparable delays were not seen in the definitive study. In the DRFS, the F0 females, males and F1 offspring at both weaning and puberty had dose-related decreases in BW. In the definitive study, BW was unaltered in F0 males, but was decreased in 600 ppm F0 females. The F1 males and females had decreased BW at 600 ppm at pnd 21 and as adults; the males also had decreased BW at 300 ppm. The sperm membrane protein SP 22 was determined in the caudal sperm of adult F0 and F1 males as a measure of adult reproductive competence and an important biomarker of fertility. Significant decreases in SP22 were seen with increasing BCA concentrations in both F0 and F1 males in the definitive study. This work was funded by a cooperative agreement from USEPA, RTP.

548 USE OF 5α-DIHYDROTESTOSTERONE (DHT)-EXPOSED UROGENITAL SINUSES (UGS) FROM FEMALE MICE TO INVESTIGATE INHIBITION OF PROSTATIC BUDDING CAUSED BY 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD).

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Mouse prostate development starts as projections (buds) of UGS basal epithelial cells into the surrounding mesenchyme on Gestation Day (GD) 16. *In utero* TCDD exposure blocks ventral bud formation and reduces dorsolateral bud number in male UGS. Prostatic budding is an androgen-dependent process, and only rudimentary UGS buds are evident in normal female UGS. TCDD appears to inhibit budding without affecting androgen signaling but the inhibitory mechanism remains unknown. Experiments were conducted to determine if DHT-exposed female UGSs can be a model system to investigate prostatic bud formation. This system allows androgen exposure times to be regulated. Pregnant mice were implanted with DHT-releasing pellets on GD 13, 14, 15, or 16. Each dam was then treated with single dose of 5 μg/kg TCDD or vehicle 24, 48, 72, or 96 hours later, depending on how early pellet implantation occurred, such that treatment occurred no later than GD 17. UGSs were collected from female fetuses on GD 19 and observed by light microscopy. All DHT- and vehicle-exposed female UGSs displayed characteristic male UGS development, including UGS enlargement, dorsal sulcus formation, and region-specific bud formation patterns. A male pattern UGS-urethral angle (~100°) was also present when DHT exposure began before GD 16. These results demonstrate that exposure of female UGSs to DHT can closely replicate normal male UGS development. TCDD exposure on GD 16 or earlier blocked formation of all ventral and almost all dorsolateral buds regardless of when DHT exposure began. In males a similar time-course occurs and ventral budding is completely blocked by TCDD, but dorsal, lateral, and anterior budding is less vulnerable. The use of DHT-exposed female UGSs therefore provides a useful tool to investigate effects of TCDD on prostatic budding. The sex difference in region-specific sensitivity to budding inhibition by TCDD may facilitate elucidation of the mechanism by which TCDD inhibits prostatic budding. (Supported by NIH ES01332)

549 *IN VIVO* EXPOSURE OF PREPUBERTAL RATS TO METHOXYCHLOR (M) INHIBITS *EX VIVO* LEYDIG CELL (LC) BASAL AND HUMAN CHORIONIC GONADOTROPIN (HCG)- STIMULATED TESTOSTERONE (T) FORMATION.

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M is pesticide which has been used as a replacement for DDT. Its active metabolite is reported to be 2, 2-bis(p-hydroxyphenyl)-1, 1, 1-trichloroethane (HPTE), and both M and HPTE exhibit weak estrogenic and/or antiandrogenic activities and have adverse reproductive effects on animals. In the current studies, prepubertal male rats (12 animals per treatment group) were gavaged once daily between 24-30 days of age with 0, 5, 40 or 200 mg/kg body weight of M in corn oil to evaluate whether LC from immature rats are sensitive to M. Animals were sacrificed ~24 h after the last exposure, and LC were isolated by density-gradient centrifugation of dispersed testes. *Ex vivo* LC T formation was measured after 4 h of incubation under basal conditions and following exposure to 10 mIU/ml hCG. In addition, because immature LC express high 5α-reductase (5α-R) activity, which causes the majority of synthesized T to be metabolized to dihydrotestosterone and its hydroxylated metabolites, a 5α-R inhibitor was added to assess total T biosynthetic capacity. Final body weights of M-treated animals were no different than control; however, testes and seminal vesicle weights declined significantly to 81 and 69% of control, respectively, in animals exposed to 200 mg/kg M. *Ex vivo* LC T formation over 4 h under basal conditions declined significantly to 29% of control at the highest dose of M. A similar pattern of decline in T was observed when LC were incubated with a 5α-R inhibitor, but T levels were >30-fold higher. LC T formation following exposure to hCG for 4 h declined significantly to 34% of control in animals exposed to 200 mg/kg M, and a similar pattern of T decline was observed when LC were incubated with a 5α-R inhibitor, except T levels were ~60-fold higher. Of interest, serum T, LH and FSH levels were unaffected by M. These results suggest that LC from immature rats are highly sensitive to the inhibitive effects of M.

550 *IN UTERO* EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN ALTERS SONIC HEDGEHOG AND BONE MORPHOGENIC PROTEIN 4 EXPRESSION IN THE DEVELOPING MOUSE UROGENITAL SINUS.

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2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits mouse ventral prostate development through inhibition of epithelial bud formation in the fetal urogenital sinus (UGS). Prostate bud formation occurs as proliferating epithelial buds penetrate into the surrounding mesenchyme. Sonic hedgehog (Shh) in the UGS epithelium activates mesenchyme-mediated paracrine signaling to stimulate epithelial proliferation in emerging buds. Bone morphogenetic protein (Bmp4) expressed in the mesenchyme inhibits epithelial proliferation and budding is accompanied by clearing of BMP4 expression at the sites of bud emergence. To determine if impaired ventral prostate bud formation following TCDD exposure is associated with disruption of mesenchymal-epithelial interactions involving these pathways, expression of *Shh* and *Bmp4* in male UGS of vehicle- and TCDD-exposed fetuses were compared by whole mount *in situ* hybridization. Pregnant dams were exposed to TCDD (5 μg/kg, po) or vehicle on gestation day 13 (GD13), and male fetuses removed daily from GD14-GD19 for comparison of gene expression. *Shh* expression was uniformly distributed in UGS epithelium lining the urethra of both vehicle- and TCDD-exposed UGS up to GD17. Starting at GD18, concentrated expression in the nascent buds was evident in the dorsolateral region in both groups but was absent from the ventral region of TCDD-exposed UGS. Lack of Shh staining in this area was likely due to the absence of ventral bud formation in TCDD-ex-

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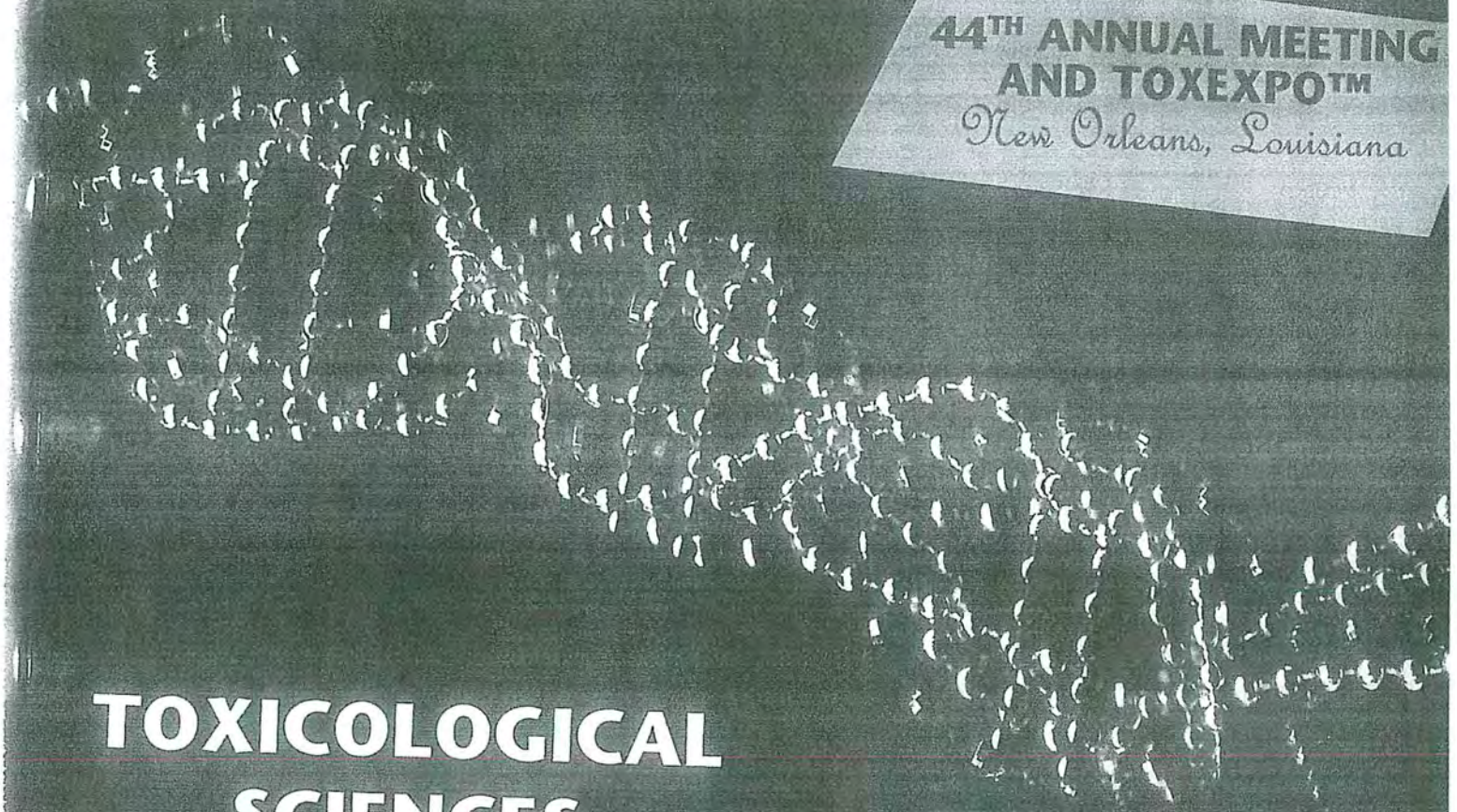

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