to determine the extent to which acetaminophen alters expression of specific ER-regulated genes in different cells. The ability of acetaminophen to compete with binding of 3H-estradiol to purified ERα or ERβ was determined. Acetaminophen (106-fold molar excess) did not compete with 3H-estradiol binding ERα or ERβ, indicating this drug does not bind ER in the same manner as E2. The effect of acetaminophen on expression of specific genes was determined in ER + breast cancer cells (MCF-7) and endometrial carcinoma cells (Ishikawa). In MCF-7 cells, c-myc is induced by estradiol. Ribonuclease protection assays were used to examine c-myc gene expression in MCF-7 cells; c-myc expression was normalized to expression of 18S rRNA. Estradiol induced an ~3-fold increase in c-myc expression 1 hr after addition to cells, and acetaminophen induced c-myc RNA ~2-fold 2 hr after addition to cells. In Ishikawa cells, estradiol induces alkaline phosphatase activity, which is measured spectrophotometrically. Acetaminophen did not induce alkaline phosphatase activity; rather, acetaminophen inhibited estradiol-induced alkaline phosphatase activity in a dose dependent manner. The acetaminophen-mediated reduction of estradiol-induced alkaline phosphatase activity was not due to direct inhibition of this enzyme activity or to general toxicity. These findings demonstrate that acetaminophen can exhibit estrogenic or anti-estrogenic activity on different ER-regulated processes in different cells in a manner that appears to be independent of ligand binding to ER.

1120 INVESTIGATING THE MECHANISM OF METHOXYCHLOR TOXICITY THROUGH ESTROGEN RECEPTORS ALPHA AND BETA USING CDNA ARRAY TECHNOLOGY.

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The reproductive effects of the pesticide methoxychlor during utero exposure is similar to, but not identical to that observed for the sex steriod estrogen. The dissimilarities may be due to distinct patterns of estrogen receptor α (ERα and estrogen receptor β (ERβ expression in selected targer tissues. We have shown previously that the *in vivo* metabolite of methoxychlor, 2,2-bis (p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), has selective agonist activity through ERα and antagonist activity through ERβ and androgen receptor. To investigate the mechanism of methoxychlor-induced toxicity, we subcutaneously dosed C57BL/6 male and female weanling mice daily with either vehicle (corn oil), 17β-estradiol (E2) (50μg/kg), HPTE (500mg/kg), E2+HPTE, or flutamide (45mg/kg) for three days. On day 4, several target organs were harvested. We observed an increase in uterine wet weight of 3.8-fold with E2, 2.6-fold with HPTE, and 8.9-fold with E2+HPTE. Flutamide treatment had no effect on uterine wet weight, and neither E2, HPTE, nor flutamide significantly altered ovarian or testicular wet weights. RNA from prostates, testes, uteri and ovaries was isolated and used to probe Clontech Atlas Mouse cDNA expression arrays. HPTE altered the expression of several of the same genes as E2, such as glutathione peroxidase, insulin-like growth factor binding protein-6, and cathespin D, while other genes were differentially regulated, such as interleukin-11, insulin-like growth factor binding protein-4, and integrin beta. Quantitation of gene changes will be required using standard molecular techniques. These data will provide a better understanding of the roles of ERα and ERβ in both normal endocrine function and its alteration by environmental chemicals. (ES09106 & ES04917)

1121 EFFECTS OF OCTYLPHENOL ON TESTOSTERONE BIOSYNTHESIS BY CULTURED PRECURSOR CELLS (PC) AND IMMATURE LEYDIG CELLS (ILC) FROM RAT TESTES.

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4-Tert-octylphenol (octylphenol, OP) is a degradation product of alkylphenol ethoxylates, widely used as a surfactant additive in the manufacture of detergents, plastics and pesticides. OP has been reported to mimic estrogen in many cellular systems and, thus, to potentially alter normal sexual growth/maturation. In the present studies, the direct effects of OP on 10 mIU/ml human chorionic gonadotropin (hCG)- stimulated testosterone (T) biosynthesis by cultured PC and ILC were examined. PC are mesenchymal cells that differentiate into ILC, mainly through the actions of luteinizing hormone, between ~ 14 to 28 d after birth, and ILC are converted to adult Leydig cells during the next 4 to 5 weeks of maturation. PC and ILC were isolated by elutriation and Percoll gradient centrifugation of collagenase-dispersed testes from 23-day-old Sprague-Dawley rats. Increasing concentrations of OP (1-2000 nM) progressively decreased hCG-stimulated T starting at 100 or 500 nM, to maximal declines of 30 to 70% below control at the highest concentration in both PC and ILC following exposure for 24 h. OP, similarly, decreased 1 mM 8-Br-cAMP-stimulated T in both PC and ILC, suggesting that the main site(s) of action occur after cAMP formation. To further localize the site(s) of action of OP, the conversion of 1 μM 22 (R) hydroxycholesterol, pregnenolone, progesterone or androstenedione to T after exposure to increasing OP concentrations and hCG was evaluated. Progressive declines in 22 (R) hydroxycholesterol, pregnenolone and progesterone conversion to T were observed, but not androstenedione, suggesting that P450c17 activity, which converts progesterone to androstenedione, is inhibited by OP but not 17β-hydroxysteroid dehydrogenase activity, which converts androstenedione to T. OP also may inhibit cholesterol side-chain cleavage and 3β-hydroxysteroid dehydrogenase-isomerase activities, but additional studies are necessary to confirm this possibility. Interestingly, 17β-estradiol (1-1000 nM) had no effect on hCG-stimulated T formation in both PC and ILC, and concomitant exposure to 100 nM ICI 182,780, a pure estrogen antagonist, did not alter the inhibitive effects of OP. These results suggest that OP effects on both PC and ILC do not mimic the actions of estrogen and are not mediated through the classic estrogen receptor -α or -β pathway.

1122 MONO-(2-ETHYLHEXYL) PHTHALATE SUPPRESSES ESTRADIOL BY DECREASING AROMATASE MRNA EXPRESSION LEVEL AS SHOWN BY REAL TIME RT-PCR.

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Di-(2-ethylhexyl) phthalate (DEHP) is a commercially important plasticizer that is ubiquitous in the environment. DEHP and its active metabolite mono-(2-ethylhexyl) phthalate (MEHP) are reproductive and endocrine toxicants in animal models. MEHP has a direct effect on the granulosa cells of the rat ovary to suppress estradiol production by affecting aromatase, the rate limiting enzyme for the conversion of testosterone to estradiol. To better understand the mechanism of action of MEHP, we tested the hypothesis that MEHP decreases transcription of the aromatase gene. Granulosa cells were obtained from 28 day old Fisher 344 rats that had been treated with PMSG either 24 or 48 hours earlier to induce follicle development. Cells were cultured for 48 hours with FSH (2.5 ng/mL) and testosterone (500nM) and 0mM, 0.001mM, or 100mM MEHP (in DMSO). As previously found, 100mM MEHP significantly decreased mean estradiol levels in media compared to control and low dose with $p<0.0001$. Moreover, MEHP had a greater effect on estradiol production from cells obtained 24 hours after PMSG treatment compared to cells obtained 48 hours after PMSG treatment. This is consistent with the understanding that MEHP affects the differentiating granulosa cell. RNA was analyzed using the TaqMan real-time PCR assay to measure aromatase message. In 4 separate experiments, samples treated with 100mM MEHP had half ($p<0.005$) as much aromatase message compared to control samples when normalized to GAPDH. The 0.001mM treatment group was not statistically different from controls. This was the case for both the 24 hour and 48 hour post-PMSG treatment. These results indicate that the suppression of estradiol by MEHP may occur at a transcriptional level. Future experiments will examine the effects of MEHP on the aromatase protein. We anticipate that these studies will uncover the molecular mechanisms by which MEHP inhibits granulosa cell differentiation and ovulation.

1123 OXIDATIVE STRESS BY SODIUM ARSENITE INHIBITS ADRENAL CHOLESTEROL METABOLISM WHILE SHOWING BOTH STIMULATION AND SUPPRESSION STEROIDOGENIC ACUTE REGULATORY OF PROTEIN.

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The steroidogenic acute regulatory protein (StAR) is a key participant in the hormonally activated transfer of cholesterol to cytochrome P450scc. Here we show that two activators of stress activated protein kinase pathways (SAPK), arsenite and anisomycin, each potentiate the maximum stimulation of StAR mRNA (3.5 kb) by Br-cAMP at low concentrations (respectively, 20 μM, 2.5 fold and 0.2 μM, 5 fold). Hydrogen peroxide (100 μM), a strong activator of NFκB, was ineffective in this stimulation. At higher concentrations (>40 μM) of each activator StAR mRNA and protein expression was reduced sharply, reaching almost complete suppression by arsenite. None of these agents changed basal StAR expression indicating potentiation and suppression of the protein kinase A stimulation as opposed to a direct effect on transcription. Time courses for StAR stimulation by Br-cAMP indicated that at low concentrations of arsenite rates of transcription were elevated after a 1 hr lag. At higher concentrations an early increase was followed after 2 hr by a rapid decrease indicating onset of increased degradation of StAR mRNA. Hydrogen

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