## 1819 ABBREVIATED ONE-GENERATION STUDY OF DIETARY BISPHENOL A (BPA) IN CD-1® MICE.

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This study duplicated the species/strain, route, and top 2 dietary BPA concentrations (5000 and 10, 000 ppm) employed in a previous RACB study (Reel et al., 1985), but with shorter duration, one breeding, and enhanced evaluation of parental systemic/reproductive toxicity to provide the context for previously reported F1 offspring effects at these doses. F0 mice (20/sex/group) were exposed ad lib to BPA for 2-wk prebreed; 1-wk mating (F0 males necropsied after mating; 3-wk total exposure); and ca. 3-wk gestation. F0 dams and F1 litters were necropsied at parturition (pnd 0). Parental systemic toxicity was present at both BPA doses: increased liver and kidney weights (WT) in both sexes; for F0 dams: reduced body weights (BW), weight gain, and feed efficiency during pregnancy; prolonged gestation (no effects on precoital interval or ovarian or uterine WT); histopathology in liver (dose-related hepatocellular hypertrophy) and kidneys (dose-related tubular epithelial degeneration, necrosis and regeneration); elevated serum BUN at 10, 000 ppm; and reduced serum Na, K, and Cl at 5000 ppm (consistent with renal toxicity). F1 offspring toxicity was observed only at 10, 000 ppm: slightly reduced total and live pups/litter, with no effects on pre-/postimplantation loss or pup BW. This study resulted in comparable daily BPA intakes (for both sexes and doses) and confirmed litter size effects at 10, 000 ppm by Reel. It also demonstrated parental systemic toxicity at both doses. Our data support the interpretation of litter size effects at 10, 000 ppm as caused by the compromised status of dams (after 5-6 wks total exposure), while the lesser systemic toxicity at 5000 ppm did not cause litter size effects. F1 litter size effects at 5000 ppm were observed by Reel only for 4th-5th litters after much longer exposure duration (>90 days).

## 1820 ALTERATIONS IN THE RAT ESTROUS CYCLE BY DIBROMOACETIC ACID (DBA): RELATIONSHIP TO A SUPPRESSION IN ESTRADIOL (E2) METABOLISM?

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Of the large variety of chemicals formed by chlorination of public drinking water, a number are suspected to cause reproductive alterations in humans and test animals. Members of one class, the haloacetic acids (of which DBA is a member) have been reported to alter rat sperm production & gonadal hormonal activity at elevated doses. In females, DBA was found to induce a persistent alteration in estrous cyclicity, something found in the present study using 14d exposures (0-270 mg/kg, po). Body wts were unaffected. While a fall in progesterone (seen *in vitro*) could impact cyclicity, it may be that E2 levels are also altered. *in vitro* data did not show shifts in ovarian E2 release, but an impact on E2 metabolism could affect blood levels. Consequently, cycling female rats were treated (po) daily for 2 wks with DBA (0, 30, 90, 270 mg/kg in water, pH adj. to 6.8). After 8d (during which cycles continued), females were ovariectomized & 3d thereafter implanted with estradiol capsules (OVX-E2). Daily tail nick blood was then taken for 3d. Non-E2 capsules were also implanted & blood taken (0 & 270 mg/kg DBA). Additional OVX-E2 rats (270 mg/kg) received phenobarbital (PB, 0.1% in drinking $H_2O$) for 4d before sampling to stimulate E2 metabolism. After capsule implantation, an initial serum E2 rise then fell over the next 2d. By 72h, DBA caused a dose-related increase in E2 that at the highest dose was approx. 2 1/2-fold above controls. PB reduced DBA-elevated E2 levels. Data suggest that alterations in cyclicity seen following DBA exposures could be at least partially due to a suppression in hepatic E2 metabolism. The rise in E2 is reversible & speaks against suicide inhibition of the relevant P450 activity. The data indicate that DBA given at these doses over 2 wks acts as an endocrine disrupting chemical. While it is conceivable that lower doses over more extended periods could have similar effects on reproductive functioning, such studies remain to be conducted. (This abstract does not reflect EPA policy.)

## 1821 EFFECTS OF METHOXYCHLOR (M) OR ITS ACTIVE METABOLITE, 2, 2-BIS (P-HYDROXYPHENYL)-1, 1, 1-TRICHLOROETHANE (HPTE), ON TESTOSTERONE (T) FORMATION BY CULTURED ADULT RAT LEYDIG CELLS (LC).

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M is a pesticide developed as a replacement for dichlorodiphenyltrichloroethane (DDT). Its active metabolite is reported to be HPTE. Both M and HPTE have been shown to exhibit weak estrogenic or antiandrogenic activities in various *in vivo* or *in vitro* testing protocols. In the present studies, we examined the direct effects of M or HPTE on T biosynthesis by cultured LC from young adult rats. Increasing concentrations of M or HPTE (1-1000 nM) caused a progressive decline in both basal and 10 mIU/ml human chorionic gonadotropin (hCG)- or 1 mM 8-Br-cAMP-stimulated T following exposure for 4 or 24 h, beginning at 100 or 500 nM, although the declines with HPTE generally were greater. To localize the site(s) of action of HPTE, LC were exposed to HPTE (1-1000 nM) alone for 24 h, then fresh media containing steroid precursors of T were added to follow the ability of exposed cells to convert these substrates to T over 4 h. The conversion of 0.01 mM pregnenolone, progesterone, or androstenedione to T was unaffected by prior exposure to HPTE; however, there was a progressive decline in the conversion of 0.01 mM 22(R)-hydroxycholesterol to T, suggesting that among the enzymes involved in the conversion of cholesterol to T, P450 cholesterol side-cleavage activity is inhibited by HPTE. The concomitant inclusion of the pure estrogen antagonist, ICI 182, 780, did not alter the inhibitive effects of HPTE, suggesting that the actions of HPTE are not mediated by binding to estrogen receptor alpha or beta. Also of interest, the addition of the native estrogen, 17beta-estradiol, or the antiandrogens, cyproterone acetate (1-1000 nM) or hydroxyflutamide (1-1000 nM), had no effect on basal or hCG-stimulated T following exposure for 24 h, suggesting that the actions of HPTE are not due to its estrogenic or antiandrogenic properties.

## 1822 EXPRESSION OF BIOTRANSFORMATION ENZYMES IN OVARIAN INTERSTITIAL CELLS: EFFECT OF DOSING WITH 4-VINYLCYCLOHEXENE AND ITS DIEPOXIDE METABOLITE IN MICE.

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Our laboratory is investigating the role of ovarian metabolism in 4-vinylcyclohexene-induced ovotoxicity in mice. This study was designed to evaluate the effect of repeated dosing with the ovotoxicants 4-vinylcyclohexene (VCH) and its diepoxide metabolite (VCD) on expression of several biotransformation enzymes in the ovarian interstitium, whose function is poorly understood. Female B6C3F$_1$ mice (d28) were dosed daily (15d) with vehicle (Con), VCH (800 mg/kg) or VCD (80 mg/kg). Ovaries were removed and fixed for immunohistochemistry, or isolated into interstitial cells. We evaluated the effects of VCH/VCD dosing on mRNA expression and protein distribution of cyp 2e1, cyp 2a4/5, and microsomal epoxide hydrolase (mEH) using realtime PCR or confocal microscopy, respectively. Luteinizing hormone receptor was used as a marker for confocal visualization of interstitial cells. mRNA encoding *cyp* 2e1, *cyp* 2a4/5, and *mEH* was present in interstitial cells from control mice. However, after repeated dosing, expression was significantly decreased only for *mEH* (60±37% of Con, VCH, $p<0.05$), compared to control. Intense immunostaining for cyp 2e1, cyp 2a4/5, and mEH proteins was seen in interstitial cells from control mice. VCH dosing decreased staining intensity for cyp 2e1 (19±2.4% of Con VCH, $p<0.05$), while increasing staining intensity (39±5.1% above Con VCH, $p<0.05$) for cyp 2a4/5, compared to control. Although not significant, there was a downward trend in staining intensity for mEH, (20±10.3% of Con VCH, 32±19.3% of Con VCD) compared to control. Taken together, mRNA and protein for several biotransformation enzymes were investigated in ovarian interstitial cells. Their expression was affected by repeated dosing with VCH/VCD. Thus, these cells may play an important role in the ovarian metabolism of xenobiotics. (ES08979, ES06694, ES07019)

## 1823 EFFECT OF BLOCKADE OF GLUTATHIONE (GSH) SYNTHESIS ON OVARIAN FOLLICULAR ATRESIA.

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The mammalian ovary contains a finite complement of oocytes, most of which are destined to undergo the apoptotic process of degeneration termed follicular atresia. Our previous work has shown that healthy, growing follicles, but not atretic follicles, express high levels of the modulatory subunit of glutamate cysteine ligase (GCL), the rate limiting enzyme in the synthesis of the antioxidant tripeptide GSH (Luderer et al, 2000, Toxicol Sciences:54:257). Depletion of cellular GSH causes apoptosis in many cell types; however, the effect of GSH depletion on ovarian follicular atresia has not been investigated. To test the hypothesis that GSH depletion causes increased follicular atresia, cycling adult rats were treated with i.p. injections of 5mmol/kg buthionine sulfoximine (BSO), a specific inhibitor of GCL, in saline or with saline alone at 0700 and 1900h on proestrus (n=2, saline; n=3, BSO) or estrus (n=3 saline; n=4, BSO), and sacrificed at 0700h the next day. One ovary was homogenized for total GSH assay using the Tietze method. The other ovary was cryosectioned and subjected to terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) using the Cell Death Detection Kit (Roche

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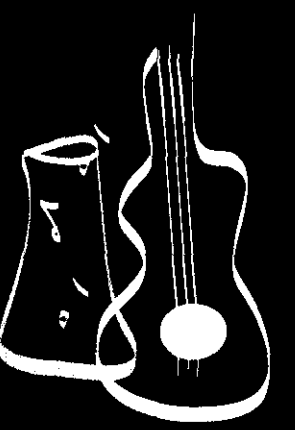

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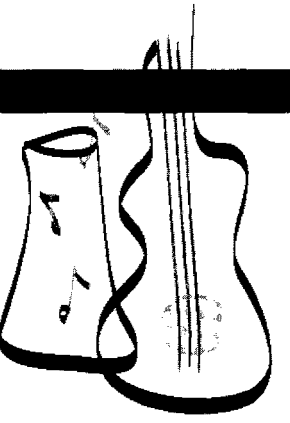

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# *Preface*

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**An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 385.**

**The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 411.**

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