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Poster Abstracts
(numbers represent poster location)

1 **Absorption and Biotransformation of the Model Xeno-Estrogen Bisphenol A by the Skin**

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The hypothesis that skin contact could contribute to human exposure to bisphenol A (4,4'-dihydroxydiphenyl dimethylmethane, CAS # 80-05-7; BPA) has been repeatedly raised and debated at recent international conferences on endocrine disruptors. Among other things, it is based on the fact that BPA residues are known to be present in many manufactured goods and certain types of paper in daily use (thermal paper). We previously published several articles regarding the metabolic fate of BPA and other bisphenols[1,2]. Recently, we completed a study focusing on the use of short-term cultures of pig ear skin as an alternative method to study the absorption and fate of xenobiotics by the skin. This model was validated using model compounds such as 7-ethoxycoumarin [7-EC], benzo(a)pyrene [B(a)P] and testosterone, addressing in detail the metabolic capabilities of short-term cultures of pig ear skin. Both oxidative (phase I) and conjugative (phase II) activities were shown to be expressed by this *ex vivo* system[3,4]. We investigated the diffusion and biotransformation of BPA using ring labeled ^{14}C-BPA [50-800 nmol] applied on the surface of pig skin ear short term cultures. Next, we compared the results with experiments carried out using commercial human skin explants. Radioactivity distribution was measured in all skin compartments and in the diffusion cells of static cells diffusion systems. BPA and metabolites were further quantified by radio-HPLC. Results demonstrate extensive absorption of this model xenoestrogen [viable pig ear skin: 65%; viable human explants: 46%; non viable (previously frozen) pig skin: 58%], as well as biotransformation (in viable systems only). Major BPA metabolites produced by the skin were conjugates, which were identified as BPA glucuronide and BPA sulfate, respectively. Experiments with viable skin models unequivocally demonstrate that BPA is readily absorbed and metabolized by the skin. The trans-dermal route is expected to contribute to BPA exposure in human, when direct contact with BPA (free monomer) occurs. This is the first report documenting the fate of BPA in viable skin models.

(1) Jaeg, J.P., Perdu, E., Dolo, L., Debrauwer, L., Cravedi, J.P., Zalko, D. (2004). Characterization of new bisphenol A metabolites produced by CD1 mice liver microsomes and S9 fractions. J Agric Food Chem. **52**(15):4935-4942.

(2) Zalko D, Prouillac C, Riu A, Perdu E, Dolo L, Jouanin I, Canlet C, Debrauwer L, & Cravedi JP (2006) Biotransformation of the flame retardant tetrabromo-bisphenol A by human and rat sub-cellular liver fractions. *Chemosphere* 64(2):318-327.

(3) Jacques, C., Jamin, E.L., Perdu, E., Duplan, H., Mavon, A., Zalko, D., Debrauwer, L. In Press (2010). Characterisation of B(a)P metabolites formed in an ex vivo pig skin model using three complementary analytical methods. *Anal Bioanal Chem*. doi:10.1007/s00216-009-3389-1

(4) Jacques, C., Perdu, E., Dorio, C., Bacqueville, D., Mavon, A., Zalko, D. In press (2010). Percutaneous absorption and metabolism of ^{14}C-ethoxycoumarin in a pig ear skin model. *Toxicology In Vitro*. doi:10.1016/j.tiv.2010.04.006

Bisphenol A's Effects on Uterine Leiomyoma Growth

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Uterine fibroids are benign smooth muscle tumors that affect nearly 13.6 million women in the United States, resulting in 150,000-175,000 hysterectomies each year, but have no known cause. Studies from our laboratory and others have shown there are differences in cell signaling and gene expression between fibroids and adjacent myometrial cells, but the link between these differences and fibroid formation is not clear. In the environment, endocrine disrupting compounds, such as bisphenol A, exhibit estrogenic effects and have been linked to fibroids, yet little experimental data exists in the literature. Our study investigates the role of bisphenol A in growth of uterine fibroids. When exposed to bisphenol A in cell culture, uterine fibroid cells increased proliferation in a dose dependent manner, but normal smooth muscle cells did not exhibit the same dose dependence. Additionally, the cell cycle of uterine fibroids and uterine smooth muscle cells shifted in a dose dependent manner.

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3 *In Vitro* Biotransformations of Chlorinated Bisphenol A by Rat Sub-Cellular Liver Fractions

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Bisphenol A (BPA) is widely used in manufacture plastics, food can linings, dentistry sealants and thermal paper. Chlorinated BPA analogues are an emerging group of environmental contaminants and are closely related to BPA. Tetrachlorobisphenol-A (TCBPA) has been reported to be used as a flame retardant. Its presence, as well as the presence of lower chlorinated analogues (mono-, di- and tri-chloroBPA) has been unequivocally demonstrated in environmental samples. Recent results have demonstrated the presence of several chlorinated BPA analogues in human fat. BPA is readily chlorinated by reacting with sodium hypochlorite, which is commonly applied as a bleaching agent in paper factories. Moreover, BPA easily chlorinates in aqueous media, and chlorinated BPAs have been found in sea water. Contrary to BPA and to the flame retardant tetrabromobisphenol-A (TBBPA), nothing is known about the metabolic fate of chlorinated BPAs either *in vitro* or *in vivo*. We investigated the *in vitro* biotransformation of several chlorinated analogues of BPA using rat liver sub-cellular fractions. ^{14}C-labeled chlorinated BPAs were synthesized and analytical systems enabling the radio-HPLC separation of parent compound and their main metabolites were developed. The structural characterization of metabolites produced through oxidative biotransformation was carried out by LC-ESI-ITMS. Chlorinated BPAs undergo P450-dependant oxidation, which results in a cleavage next to the central carbon leading to the formation of hydroxylated chloro-phenols.

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4 **TBBPA Metabolites and Thyroid Hormone Signaling Disruption in *X. laevis*.**

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TBBPA disrupts thyroid hormone (TH) signalling after three days exposure in embryonic *Xenopus leavis* (Fini et al. Envi Sci Tech. 2007). We investigated whether disruption of TH signalling was due to the parent compound or its metabolites. Radio-labelled ^{14}C-TBBPA was used to follow its uptake. After 2h of exposure 80% of the ^{14}C-TBBPA was absorbed by the tadpoles. In less than 8h, 98% of the TBBPA was metabolized , with TBBPA and metabolites released into water. We identified four different metabolites as TBBPA conjugates: glucuronide-TBBPA, TBBPA-glucuronide-sulphate, sulphate-TBBPA and bisulphate-TBBPA. We then tested the effects of metabolites on transgenic amphibian model. We followed TH target gene expression using a reporter tadpole and expression of phase II enzymes implicated in metabolism of both TBBPA and TH. Only TBBPA, and not its metabolites, disrupted TH signalling. Thus, we conclude that perturbation of thyroid axis is due to rapid action of TBBPA itself.

5 **Gene Expression Analysis of Daphnia magna Exposed to Juvenile Hormone Agonists**

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Insect growth regulators (IGRs) possessing juvenile hormone (JH) activity disrupt the life functions of insects, resulting in two major adverse effects: suppression of fecundity or lethal morphogenetic effects. Over 4000 analogs (agonists) of JH, which are very diverse in chemical structure and JH activity, have been synthesized and widely used. However, comparison of molecular impacts among JH agonists has never been performed. In this study, we focus on two actions of JH agonists for Daphnia: suppression of fecundity and induction of parthenogenetic males, which seems to be specific for cladoceran crustaceans. To examine signaling pathways of JH agonists, we compared gene expression profiles in D. magna neonates exposed to fenoxycarb, methoprene, epofenonane using DNA microarray. We suggest hypothesis that induction of parthenogenetic males in cladoceran crustaceans is regulated through JH signaling.

6 **The Organochlorine o,p'-DDT Upregulates Vascular Endothelial Growth Factor A Expression in MCF-7 Breast Cancer Cells**

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DDT is a known estrogen mimic and endocrine disruptor; however, the mechanism(s) by which DDT affects cellular physiology remain elusive. DDT activates cell signaling cascades, culminating in activation of the estrogen receptor. Here, our objective is to identify novel mechanisms by which DDT alters cellular pathways independently of estradiol. We performed a superarray gene analysis in MCF-7 cells using estradiol or o,p'-DDT and found that DDT differentially upregulates vascular endothelial growth factor A (VEGF-A) expression. We further show a DDT-mediated increase in activation of the Hif-1 response element (HRE), a component of the VEGF promoter. The coactivator CBP augments this DDT-mediated increase in HRE activity and is dependent on the p38 MAPK pathway. Based on our data, we propose that DDT activates p38 which in turn phosphorylates and activates CBP. CBP then binds to and activates Hif-1, which binds the HRE within the VEGF promoter, thereby increasing expression of VEGF.

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7 **Distribution and Function of Phytoestrogens in Plants.**

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To understand why plant products are widely estrogenic in animals, we are investigating the distribution and function of estrogenic activity in plants, using yeast-based reporter assays for human estrogen receptors alpha and beta (ESR1 and ESR2). Data presented here were generated during assay optimization to show proof of principle. To comparatively quantify estrogenic activity from multiple species, we tested 50%-ethanol extractions of 12 plants used as herbs, spices, or essential oils. Samples represented several plant organs. As reported in the literature, plant extracts tended to have greater activity associated with ER beta, compared with ER alpha. Higher estrogenic activity with both receptors was found for celery seed, clover, curry, fenugreek, parsley, and sassafras. Cardamom, chipotle, and cinnamon were active with ER beta only. Ginger had almost no activity with either receptor. Nutmeg and clove oil were toxic to the yeast. Future studies will look at distribution of hormonal activity among plant organs and at different stages of development.

8 **Morphometric Study on External Genitalia Development in Male Rats Given Flutamide during Pregnancy**

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Hypospadias is the most common malformation in males, which is thought to be related to abnormalities of androgen production and timing or receptor function during male sexual differentiation at this early period of pregnancy. Fludamide, a potent nonsteroidal androgen receptor antagonist, has been used therapeutically to treat androgen-dependent prostate cancer and as a tool to study male reproductive development. Since fludamide is known to induce hypospadias when given pregnant rats, external genitalia was examined by morphometry in male and female rats given oral administrations of flutamide (45 mg/kg/day) from pregnant days 12 to 22. External genitalia dissected out from males and females (embryos, neonates, and adults) were fixed in Bouin's solution. Tissues were cut in paraffin for histological examination. Each part of the genitalia in serial sections was measured with a micrometer. Measurement of a major component of phallus revealed that males given flutamide mimicked the control females in fetal development of the genitalia, the urethra development involving urethral openings in males given flutamide being similar to that of the control females. Although adult males aged 60 days given flutamide *in utero* showed severe hypospadias, male neonates showed no sign of hypospadias, as well as females given flutamide *in utero*. These results indicate that flutamide exposure *in utero* induces female type genitalia in male neonates. Hypospadias seems to develop after puberty in males given flutamide *in utero*. (Supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.)

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9 **Modeling Mixtures of Environmental Estrogens Detected in U.S. Surface Waters with an *In Vitro* Estrogen Mediated Transcriptional Activation Assay (T47D-KBluc).**

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There is growing concern of exposure to fish, wildlife, and humans to water sources contaminated with estrogens and the potential impact on reproductive health. Environmental estrogens can come from various sources including concentrated animal feedlot operations (CAFO), municipal waste, agricultural and industrial effluents. Furthermore, U.S. EPA's drinking water contaminant candidate list 3 (CCL3) includes several estrogenic compounds which may require future regulation under the Safe Drinking Water Act. Using an in vitro transcriptional activation assay, this study evaluated estrogens from CCL3 both individually and as a seven estrogen mixture. In addition, mixtures that mirror primary estrogens found in swine, poultry and dairy CAFO effluent, and a mixture of estrogens found in hormone replacement therapy and/or oral contraceptives were tested. Mixtures were evaluated for additivity using both the concentration addition model (CA) and estrogen equivalence model (EEQ). In all cases, both the CA and EEQ models predicted the observed responses accurately. There was no evidence that any of the mixtures acted synergistically or in an antagonistic manner. Results indicate both additive models are appropriate for modeling estrogen mixtures. This study augments the understanding of estrogen mixture interactions. Abstract does not necessarily reflect EPA policy. NIEHS/EPA Interagency RW75-92285501-1; NCSU/EPA Cooperative Training Agreement CT833235-01-0.

10 **Molecular Evolution of the Inhibin α Subunit in Actinopterygian Fishes**

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A whole genome duplication (known as 3R or Fish Specific Genome Duplication) occurred in the stem lineage of ray-finned (actinopterygian) fishes approximately 350 mya. In some teleost fishes, 3R gene duplication has altered activin signaling mechanisms by way of duplication and functional retention of signaling components. The inhibin α subunit (INHA) dimerizes with activin subunits to produce inhibin, an activin receptor antagonists. We investigated the molecular evolution of INHA in fishes which speciated before and after 3R genome duplication, explored differences in amino acid sequences of functional motifs that putatively effect activin signaling in these fish species, and compared these results to human, mouse, and chicken INHA polypeptide sequences. We present evidence of conserved homology of hydrophobic residues associated with protein folding and secretion, phylogenetic alterations of glycosylation sites, a deletion event specific to cypriniformes, and an N-terminus extension in the mature protein region putatively analogous to a similar extension observed in mammals which increased the antagonistic activity of the inhibin ligand. These results suggest functional differences in inhibin signaling across clades of fish.

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11 **Effects of Estrogen and Phytoestrogens on Lung Matrix Proteins**

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Previous work has shown that the predominant estrogen receptor (ER) subtype in the lung is ERβ. Phytoestrogens, plant-produced ER agonists, have higher affinity at ERβ compared to ERα. To date, there are no published studies on E2 and phytoestrogen effects on lung extracellular matrix proteins in aging. Lungs from 4 groups (soy-, soy+, E2, SHAM) of aging rats (n=2/group) were used to examine effects of estrogen and dietary phytoestrogens on matrix metalloproteinases and their inhibitors (MMP-9, TIMP-1, TIMP-2), iNOS, and ERα mRNA via RT-PCR. Significant differences were found in gene expression of MMP-9 ($p=0.015$, soy+ > E2 or soy-) and TIMP-1 ($p=0.030$, sham > soy-, others were intermediate), both of which can affect lung collagen/elastin ratios, and in ERα mRNA ($p=0.026$, E2> soy-, with soy+ and sham intermediate). Gene expression of neither TIMP-2 nor iNOS differed significantly ($p=0.107$ and 0.466, respectively). Estrogenic compounds might affect lung structure/function at menopause.

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12 **Activation of Estrogen Signaling by Soy-Based Infant Formula and BPA**

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Soy-based formulas (SFs) account for nearly 25% of the formula market in the United States. Even though there is general agreement that the consumption of soy-rich diets can be beneficial to adults, the adequacy and safety of SF for infant use is still controversial. This study investigated possible additive estrogenic effects of SF and the plasticizer BPA, often present in baby feeding bottles. SF extracts potently activated both ERα and ERβ-mediated transcription in HeLa cells expressing an estrogen responsive luciferase reporter and either ERα or ERβ (HeLN). Addition of 100nM and 10nM BPA to HeLN-ERα and ERβ cells, respectively, resulted in increased transcription activation relative to extract alone. Simultaneous treatment of cells with BPA and the SF phytoestrogens genistein and daidzein at 10nM resulted in transcription activation comparable to that of 10nM estradiol. These results indicate that dietary components and environmental pollutants may act in concert on activation of ERs.

14 **Effects of Estrogen and Dietary Phytoestrogens on Lung Compliance**

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Some symptomatic menopausal women seek an HRT alternative found in soy-based foods and supplements, phytoestrogens, which have higher affinity for ERβ than ERα. The predominant ER subtype in the lung is ERβ. Previous studies found that ovariectomy (ovx) modifies rat lung morphology. We investigated the effects of estrogen and dietary phytoestrogens on lung compliance in aging rats. Female retired breeder Sprague-Dawley rats were in 4 groups: estrogen, soy +, soy- (all ovx), and sham ovx. Here we report that neither estrogen nor dietary phytoestrogens gave the protective effects that we predicted in a measure of static lung compliance or in an assay of lung elastin content. The presence of dietary phytoestrogens did, however, slow the rate at which rats gained weight after ovx when estrogen was not replaced. Taken together, estrogenic action may not be potent enough to elicit gross morphological and physiological changes in lung.

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15 **Over-Expression of miR-155 Disrupts Estrogen Receptor Signaling in MCF-7 Breast Cancer Cells.**

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microRNAs (miRNA) are short non-coding RNAs that regulate gene function by binding the 3'UTR of target mRNA. Changes in miRNA expression patterns have been linked to breast cancer and miRNA expression profiles correlate with breast cancer receptor status. miR-155 expression is deregulated in breast carcinomas and its ectopic expression is linked with an ER-negative phenotype. miR-155 confers drug resistance and increases proliferation in triple-negative breast cancer cell lines. Here we demonstrate that the ectopic expression of miR-155 in the ER-positive MCF-7 breast cancer cell line results in a dysregulation of ER signaling. Overexpression of miR-155 leads to alterations in ER regulated genes. Inhibition of ER activity through the use of the pure anti-estrogen ICI 182,780 results in restoration of gene expression. Western blot analysis of MCF-7-miR-155 cells reveals alterations in the ERK signaling pathway. Overexpression of miR-155 drives MCF-7 cells towards a PgR-negative and ER-positive phenotype which is suggestive of a hormone-independent phenotype observed in clinical tumor samples. Taken together our data demonstrates a role for miR-155 in the regulation of ER signaling in the ER-positive MCF-7 breast cancer cell line.

16 **ES 101: Curriculum and Pedagogy for Teaching**

Environmental Signaling

Robert Wallace, Thea Edwards, Syreeta Tilghman, Barbara Beckman, and John McLachlan

We have developed a set of case-based lessons to teach concepts of environmental signaling. They are problem-based modules, which use a student-centered constructivist approach to development of complex concepts. Background material is covered using a Just-In-Time methodology, and students are assessed by participation in group discussions, their writing on class topics, and final project. There are, so far, a dozen modules, and one additional one for younger students. The primary target of the class is upper-level undergraduate or beginning graduate students. Student surveys report that they feel more engaged in this class than in others, that they enjoy the chance to discuss and write about their ideas, and appreciate the different perspective they get from the class.