Developmental and Reproductive Toxicology

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Endocrine disruption has been documented in recent years as a major route by which environmental chemicals can produce developmental and reproductive toxicity. This session is comprised or eight oral presentations that examine the endocrine disrupting activity of a variety of persistent organic pollutants and will include aspects of exposure, analytics and effects of various compounds (alkylphenols, pesticides, PCBs and their metabolites, PBDEs and dioxins) in biological systems in vitro and in vivo.

While estrogens and xenoestrogens are detectable in waste water of sewage treatment plants, there is little information on their effects on aquatic plants. A study by Schultis and coworkers (Germany) analyzed such waste water samples using a modified yeast estrogen screening assay (LYES), the in vitro E-Screen assay using human MCF-7 cells and instrumental analysis methods. Not only do their results demonstrate the presence of phenolic xenoestrogens and estrogen equivalents in these samples, but their data support the assumption that waste water's estrogenic substances can indeed accumulate in the biofilms and plants from sewage treatment plants. Their results not only indicate that further studies on the effects of xenoestrogens on aquatic plants are needed, but they suggest that the ability of these plants to accumulate xenoestrogens may provide an avenue in which to facilitate elimination of estrogenic substances from waste water.

In the subsequent presentation, Gordon et al. (USA) have developed and utilized a recombinant human ovarian cell line that contains an estrogen- and estrogen receptor (ER)-responsive firefly luciferase reporter gene for high throughput screening analysis of the xenoestrogenic chemicals. This "LUMI-CELL-ER" bioassay was used to screen thirteen organochlorine pesticides for xenoestrogenic activity. The authors report the ability of ten of these compounds to activate the ER-responsive bioassay system, albeit the compounds were about five orders of magnitude less potent than that of 17-beta-estradiol (17BE2). This ER bioassay is reported to have an EC50 of about 2×10^{-11} M for 17BE2, a level of detection far lower than any limit likely to be imposed by regulatory agencies. The LUMI-CELL-ER bioassay provides a rapid, sensitive and relatively inexpensive method for detection of estrogen agonists and/or antagonists.

It is well established that polychlorinated biphenyls (PCBs) as well as their metabolites are endocrine disruptors. While the several PCBs are know to exert antiestrogenic activities in vitro and in vivo, numerous PCB metabolites have been reported to exhibit estrogenic activity. This latter aspect is especially relevant to lower chlorinated PCBs since they are more easily hydroxylated by intracellular enzymes. The studies of Ptak and coworkers (USA and Poland) report that PCB3 and its hydroxylated metabolites are able to strongly stimulate estrogen secretion, from ovarian follicular cells *in vitro*. The relative rank order potency of the tested chemicals was determined to be PCB3 < 4'-OH-PCB3 < 3',4'-OH-PCB3. Their results show that the stimulatory action of (PCB3) and/or its metabolites on estrogen-release into the medium in 24/48h exposure studies is not due to membrane damage, but likely due to effects on follicular steroidogenesis. In contrast, long-term exposure (96h) resulted in cytotoxicity and membrane damage, effects likely due to the pro-apoptotic activity of PCB3 and its metabolites. Interestingly, oxidation of PCB3 decreases the pro-apoptotic activity and increase estrogen secretion. These studies open new questions about the mechanism of action of these compounds.

Methylsulfonyl PCB metabolites ($MeSO_2$-PCBs) belong to persistent contaminants that are ubiquitously present in humans and the environment and many have been observed to interact with and regulate the activity of steroid hormone receptors. 4-$MeSO_2$-PCB-149 is the most abundant PCB metabolite in human adipose tissue and milk, residing there at a level of 1.5 ng/g lipids. In addition to those compounds which directly interact with the estrogen receptor, it is important to also identify exogenous compounds which can alter aromatase activity (CYP19). Studies by Heneweer and coworkers (The Netherlands, Sweden) examined and compared the ability of various $MeSO_2$-PCBs to affect aromatase activity in primary human fibroblasts and in a human adrenocortiocarcinoma cell (H295R) cell line. Their results revealed the ability of $MeSO_2$-PCBs to inhibit aromatase activity and suggest that the decrease in aromatase activity was found to be due mainly to catalytic inhibition of CYP19 rather than modulation of CYP19 gene expression via the glucocorticoid receptor.

Polybrominated flame retardants (i.e. brominated diphenyl ethers (BDEs)) are found in commercial and household products and are have been found widely distributed in the environment and in biological samples. Recent evidence suggests that some BDEs and/or their metabolites can exert endocrine disrupting activities, including that of aromatase (CYP17), a key enzyme in the synthesis of estrogen. Studies by Canton et al (The Netherlands, Sweden) have examined the effect of ten BDEs on aromatase (CYP17) activity in a human adrenocortical carcinoma cell (H295R) line. Their studies revealed that 10 uM hexabromocyclododecane-D, BDE99 and BDE100 inhibited CYP17 aromatase activity to 50% of control, while tribromophenol and tetrabromobisphenol A induced activity 2-fold above control. While it remains to be established whether concentrations of these compounds in humans and wildlife are high enough to exert interactions with CYP17 and CYP19, they show a possible new mechanistic pathway by which certain brominated flame retardants may interfere with the endocrine system.

The results of animal experiments presented over the last years have demonstrated that bone tissue can be negatively affected by exposure to persistent organochlorines (POCs). Studies by Lind and coworkers (Sweden) have examined the mechanisms by which POCs exert their action on bone in rats in vivo. In addition to examining the effects of POCs (particularly PCB126) on bone, they have also examined the effects of this model compound on serum vitamin D and thyroxine levels as well as the effect of estrogen on this response. Their in vivo studies in rats dosed i.p. for three months with 384 ug PCB126/kg bw (total dose) demonstrate that estrogen modulates PCB126 induced effects on vertebral bone tissue as well as on serum levels of thyroxine. Although the dose of PCB126 used in this study was rather high, the results do support the view that resident estrogen status can plays an important role on modulating the bone toxicity of PCB126.

Environmental pollution in Arctic regions is a public concern especially given the high consumption of contaminated fish and wildlife by Arctic inhabitants. Studies by Stern et al (Canada, Sweden) examined the effects of perinatal exposure to a mixture of PCBs, organochlorines and methylmercury on skeletal development in rat pups at levels to which Canadian Arctic Indians are documented to be exposed. Exposure to the maternal dose of 5 mg of “Arctic mixture” /kg/day caused decreased body weight, bone length and cortical geometrical parameters in both male and female pups. These data are in agreement with previously reported effects of TCDD on bone growth in rat pups. Interestingly, in contrast, low-dose maternal exposure to the Arctic mixture (0.05 mg/kg) actually resulted in significantly increased bone length and cortical thickness in male and female pups. The mechanism responsible for this novel observation of a dose-dependent opposing effect of a complex mixture on bone growth remains to be elucidated.

Effects of chemicals on aromatase activity can modulate the level of sex hormones in an organism, with inhibition of estrogen synthesis. While the effects of xenobiotic aromatase inhibiting/inducing chemicals has been well documented in cells in culture, the developmental effects in vivo have been less studied, particularly in nonmammalian species. Studies by Yamashita and colleagues (Japan) have examined the effects of in ovo exposure of Imazalil (an aromatase-inhibiting chemical) and Atrazine (aromatase-activating chemical) on sexual differentiation in chick gonads. The authors report that in *ovo* exposure to Imazalil inhibits sexual differentiation of the ovary via it ability to inhibit aromatase activity; no effects on aromatase mRNA or protein levels were observed. In contrast, in *ovo* exposure to atrazine influences sexual differentiation of the ovary but by different mechanisms, possibly by the induction of aromatase in the right gonad. Aromatase activity in the left gonad from female chicks was unaffected by atrazine, in contrast to that reported in human cell lines (i.e. H295R) where induction is observed. Interestingly, no effects of either compound on differentiation of male gonads were observed.