examine the estrogenic and dioxin like activites using pERE-Luc and pCYP1A1-Luc reporter system. River water was extracted using combined solid-phase extraction in static adsorption mode with soxhlet extraction. Chemicals adsorbed to the XAD-4 resin were recovered 98.24± 5.90% by elution with ethyl acetate and methylene chloride (1:9). Kumho River of Korea showed 0.77 pM EEQ in upstrean and 7.7pM EEQ in downstream. Kum River of Korea showed 3.5pM and 1.7pM EEQ in upstream and downstream respectively. Mankyung River of Korea showed 61fM and 0.41 pM EEQ in upstream and downstream respectively. Miho Stream of Korea showed 0.2pM EEQ only in the upstream. All these samples were tested with pCYP1A1-Luc activity and results showed there were more dioxin like activities in sediments than water from the river.

[PA4-22] [ 10/19/2000 (Thr) 10:00 - 11:00 / [Hall B] ]

## No teratogenicity of Phthalates using in vitro battery system

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Phthalates have been used as plasticizers in polyvinyl chloride plastics such as cable coating, flooring, and blood bags. It was generally demonstrated that many phthalates is a developmental toxicant in rodents. However, in vitro teratogenic effects of phthalates are not clearly known. The aim of this study was to investigate the teratogenic potential of phthalates (DEHP, BBP, and DBP) using in vitro battery system. Short-term in vitro battery system (whole embryo culture and limbbud and midbrain cell micromass culture) has been proposed as a preliminary screening method of teratogens.In whole embryo culture, rat embryos at gestation day 9.5 were cultured in rat IC serum for 48 h. Micromass culture of embryonic limbbud and midbrain cells was performed based on the method of Flint. After 5 days of culture, cell proliferation was assessed by neutral red uptake and cell differentiation was determined by hematoxylin-stained foci area or alcin blue staining, respectively. In whole embryo culture, there were no morphological abnormalities of embryo at any concentration of phthalates. However, phthalates tested in our studies decreased growth and development of embryo only at higher concentration. Although in vitro battery system did not detect the embryotoxicity of phthalates, these results suggest that phthalates (DEHP, DBP, and BBP) itself are able to alter normal embryonic growth and development.

[PA4-23] [ 10/19/2000 (Thr) 10:00 - 11:00 / [Hall B] ]

## Comparative evaluation of a 20-day thyroid/pubertal male assay and Hershberger assay for the detection of androgenic/antiandrogenic activity

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Several different screening and testing methods for the detection of endocrine disruptors (EDCs) have been proposed recently. A rodent Hershberger assay is one of the screening methods recommended by EDSTAC and OECD. A rodent 20-day thyroid/pubertal male assay is also one of alternative methods to replace the Tier I Screening Battery. The purpose of our study is to evaluate comparatively short-term in vivo screening methods to detect substances with androgenic/antiandrogenic activity. Hershberger assay was performed utilizing immature Sprague-Dawley male rats castrated at 6 weeks of age. Testosterone (0.4 mg/kg/day) was subcutaneously (s.c.) injected for 10 days. Additionally, a pure androgen antagonist, flutamide (1, 5, and 10 mg/kg/day) was administered by oral gavage after testosterone treatment. In testosterone treatment group, glans penis (GP), seminal vesicles (SV), ventral prostate (VP), levator ani muscle

and bulbocavernous muscle (LABC), and cowper's glands (CpG) weights were significantly increased. Flutamide inhibited the testosterone-induced re-growth of accessory sex glands (SV, VP and Cp) and organs (GP and LABC) with dose-dependent manner. A rodent 20-day thyroid/pubertal male assay was performed utilizing immature age-matched intact male rats (33 days of age). All rats (10 rats/group) were treated with testosterone (1.0 mg/kg/day, by s.c.) and flutamide (1, 5, and 25 mg/kg/day, by oral) from PND 33 to PND 53, respectively. Testosterone increased significantly the SV and VP weights, with some effect on GP, LABC, and Cp. When flutamide was administered to immature male rats, the accessory sex organs (SV, VP, GP, LABC, and CpG) growth and development was blocked in dose-dependent manner. However, testes weights were significantly increased following flutamide administration (5 and 25 mg/kg/day). In conclusion, 20-day thyroid/pubertal male assay showed similar sensitivity to Hershberger assay for the screening of androgenic/antiandrogenic compounds.

[PA4-24] [ 10/19/2000 (Thr) 10:00 - 11:00 / [Hall B] ]

## 3MC and DPB effects on the expression of drug metabolising enzymes via RT-PCR.

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In order to understand the mechanism of the regulation of drug metabolizing enzyme gene expression, we have studies the induction of CYP1A1 and GSTα, μ, π enzymes in monkey that is treated with 3-methylcholanthrene (3MC) and dibutylphthalate (DBP). The mRNA levels were measured by RT-PCR and enzymatic activity was measured via EROD in brain, intestine and liver. And the copy number of CYP1A1 per 3/20㎍ was measured by competitive PCR. In the case of adult monkey, treatment with 3MC induced CYP1A1 mRNA in brain by 2-fold, in intestine by 11-fold and in liver by 10-fold respectively. And the treatment with DBP induced CYP1A1 mRNA. GST μ was not induced by the treatment with 3MC and DBP. GSTα was not induced by the treatment with 3MC and DBP in liver and brain, but it was induced in intestine (1.5-fold). GSTπ was slightly induced by the treatment with 3MC and DBP in brain, intestine and liver. In the case of fetus monkey, the basal levels of fetus CYP1A1 mRNA and GSTs mRNA were low in comparison to adult monkey and as the age of monkey increased, the basal levels of CYP1A1 mRNA and GSTs mRNA were also increased. The treatment with 3MC induced CYP1A1 mRNA in brain and liver, but it didn't significantly induce CYP1A1 mRNA in intestine. GSTμ and GSTα was not induced by the treatment with 3MC and DBP. GST π was slightly induced by the treatment with 3MC and DBP. The copy number of CYP1A1 per RT product of 3/20㎍ total RNA was about $1\times10^6$ in the 3MC-treated liver, $5\times10^4$ in control liver, $5\times10^3$ in 3MC-treated intestine and $1\times10^3$ in 3MC-treated brain.

[PA4-25] [ 10/19/2000 (Thr) 10:00 - 11:00 / [Hall B] ]

## De novo Synthesis Pathway of Ceramide Causes Hypoxia -induced Apoptosis of SH-SY5Y Human Neuroblastoma Cells

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Ceramide has been implicated to be a second messenger in the cell signaling pathway involved in a variety of cellular responses ranging from cell differentiation, cell cycle arrest, cellular senescence, apoptosis to cell survival and cell proliferation in a number of cells. However, there is little information of a role of ceramide in apoptosis in hypoxic injury known to induce necrotic cell death. Ceramide generation was measured in SH-SY5Y cells metabolically labeled with [3H]serine after exposure to chemical hypoxia induced by cobalt chloride. Chemical hypoxia resulted in a