

and cell proliferation of bisphenol A in the presence of a rat liver S9 mix containing cytochrome P 450 enzymes and Cu(II). In the present study, we found that BPA in combination with Cu(II) exhibited an enhancement in cytotoxicity, which was inhibited by reactive oxygen species scavenger. For cell proliferation assay MCF-7 cells were seeded on a 96-well multi-well-plate at  $1.5 \times 10^3$  cells per well. After 24hr cultivation, the S9 mix and Cu(II) was added to the wells as an S9 mix group (+S9), and medium was added to the other wells as a none-S9 mix group (-S9), then 5 different concentrations of various BPA were added to each well. After 5 days, a sulforhodamine B (SRB) assay was conducted to measure cell proliferation. +S9 mix group enhanced the proliferation of MCF-7 cells at much lower concentrations than -S9 mix group which was inhibited by the ROS scavenger. These results suggest that reactive oxygen species reacts with Cu(I) leading oxidative stress. Also the formation of reactive oxygen species induced by BPA was dose-dependently by inhibited by tamoxifen, which suggests that the effect of BPA was estrogenic action via estrogen receptors.

[PA3-16] [ 10/18/2001 (Thr) 14:00 - 17:00 / Hall D ]

### **Toxicity Identification Evaluation of Water Pollution using in vitro bioassay**

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So far, investigation of environmental pollution has been achieved in field study. This remains the most exhaustive approach, current dimensions of environmental researches and their inherent complexity require that relatively inexpensive and simple laboratory procedures are developed to make possible the screening of large numbers of sites and samples. At this point, micro-bioassay has been highlighted. The purpose of this study is to evaluate the water pollution using micro-bioassay. Micro-bioassay methods were optimized and validated for the sensitive and quantitative determination of total toxic effects of the water samples. EROD bioassay was focused to detect PAHs, PCBs and dioxinlike components in the water. The EROD bioassay was executed in rat hepatoma cell line, H4II E cell lines. 50L of river water was adsorbed using XAD-2 resin column. Pollutants adsorbed to the XAD-2 resin were extracted by elution with methanol (sample I), and with ethyl acetate (sample II). Toxic effects of extracts were determined by micro-bioassay methods.

[PA3-17] [ 10/18/2001 (Thr) 14:00 - 17:00 / Hall D ]

### **Oxidative damage by bisphenol A induced lipid peroxidation and apoptosis**

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It is very important to understand the ROS generation of estrogen-like chemicals. Of such chemicals, we were especially interested in bisphenol A for its wide spreadness in environmental and structural similarity with aneuploidogenic stilbene estrogen diethylstilbestrol. The purpose of the this study was to evaluate the lipid peroxidation and DNA fragmentation by bisphenol A in the presence of a rat liver S9 mix containing cytochrome P 450 enzymes and Cu(II) in HaCaT cell lines. The specific content of malondialdehyde, an end product of lipid peroxidation, was also found to increase with concentration. The fragmentation of intact DNA, a parameter of apoptotic cell death, was evaluated qualitatively by agarose gel electrophoresis analysis and quantitatively by diphenylamine reation method. BPA induced apoptotic cell death in a dose-dependent manner. When HaCaT cells were exposed to 50uM BPA for 48h, the DNA fragmentaion was significantly increased to 54%. The effect of radical scavenger on the apoptotic cell death induced by BPA was investigated. The DNA fragmentation induced by BPA was significantly inhibited by addition of ROS scavenger to the culture medium. Also we examined the enzyme activities of Cu,Zn-SOD, Mn-SOD, catalase, and GPx in the cells. The activities of Cu,Zn-SOD, glutathione peroxidase, Catalase were found to decrease with concentration. However, the activity of

Mn-SOD were unchanged. This indicated that elevated oxidative stress caused by an imbalance between the production and removal of ROS and free radicals occurred in cells.

[PA3-18] [ 10/18/2001 (Thr) 14:00 - 17:00 / Hall D ]

### Determination of PCBs in Korean Adipose tissues and Endocrine Disrupting Effects

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Polychlorinated biphenyls(PCBs) are members of the halogenated aromatic group of environmental pollutants. Because of their unique physical and chemical properties, notably their stability and widespread use, PCBs are sidely distributed and transported throughout the global environment. In fact, residues of PCBs have been identified in air, water, aquatic and marine sediments, and human tissue samples. Although the mechanism of the effects of these PCBs on estrogenic function are still not entirely understood, the toxicities of the PCBs have been studied intensively. Some PCBs exert dioxin-like activities mediated through the aryl hydrocarbon receptor and some congeners are hypothesized to possess endocrine disruptive potential and to induce CYP1A. We examined antiestrogenic potentials of some PCB congeners(PCB 52, 118, 138, 153, 180)in vitro which detected in Korean adipose tissues. As a result, PCB 118, 153 inhibited aromatase activities using tritiated water release assay in JEG-3 cell line. PCB 118, 138, 153 induced CYP1A activities using ethoxyresorufin o-deethylase bioassay in H4IIE cell line. And PCB 118, 138, 153, 180 showed antiestrogenic activities by E-Screen assay in MCF-BUS cell line. This study demonstrated that PCB congeners could have and antiestrogenic activities and affect estrogen biosynthesis depend on their structure.

[PA3-19] [ 10/18/2001 (Thr) 14:00 - 17:00 / Hall D ]

### Solvent Microextraction of Methamphetamine and its metabolite, amphetmaine, in urine with simultaneous back-extraction

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Solvent microextraction was developed as a sample preconcentration for the determination of methamphetamine and its metabolite, amphetamine, in urine. Although liquid-liquid extraction(LLE) and solid-phase extraction(SPE) have been used most commonly for the preconcentration and cleanup of samples prior to the GC or GC/Mass analysis, their relative poor selectivity to the target drugs, analogues, or impurities produced poor resolution between the target drugs and impurities and resulted the low detection limit in GC or GC/Mass analysis. Furthermore the use of the relatively large amount of extracting organic solvent for the extraction of the target drugs from urine in LLE might be harmful to thamphetamine testers. Solvent microextraction employs a microliter size liquid membrane and receiving phase. The small apparatus of the solvent microextraction was composed of 2.0 mL reaction vessel, stirrer, Teflon ring, 1.0 mL syringe, stirrer bar, and Teflon stopper. The n-octane liquid membrane was confined inside a small Teflon ring and layered over 1.0 mL urine or aqueous sample which was already adjusted to alkali with 6N-NaOH. The receiving droplet of 0.05M-NaH<sub>2</sub>PO<sub>4</sub>(pH = 2.3) was suspended in the n-octane liquid membrane from tip of a microsyringe needle. When the sample was stirred the basic drugs like methamphetamine and amphetamine in urine diffused into the n-octanof phase because the basic drugs were in the form of molecular state, not ionized state, under alkaline conditions. Successively the molecular basic drugs in organic phase diffused into the acidic aqueous microdroplet suspended on the needle because the basic drugs turned into the ionized form in the acidic aqueous media. After extraction of the basic drugs in the aqueous media for ten minutes, the microdrop was taken back into the syringe and transferred into the 2.0 mL reaction vial. The microsyringe was rinsed out with ethanol 2 times then added in the above solution. The solution was evaporated under nitrogen stream. The residue was derivatized with trifluoroacetic anhydride and then injected into the GC/MS. The MS spectra by microextraction and by common LLE were compared each