cryogenic grinding, for hair analysis. The hair is milled to fine powder using the Cryogenic Mill. The fine powder is directly extracted with acidic methanol. The residue evaporated under N_2 stream was derivatized with pentafluoroacetic anhydride and injected into GC/MS with SIM mode. The standard calibration curves for methamphetamine and amphetamine were obtained from the hair powder blanks spiked with methamphetmaine and amphetamine standards. The recoveries of amphetamine and methamphetamine from the spiked hair powder blanks were over 96 %. The correlation coefficients of the standard calibration curves for amphetamine and methamphetamine were over 0.997. Ten hair samples which were already analysed with hair cutting preparations were analyzed with cryogenic grinding preparations and the results were compared with each other. Amphetamine and methamphetamine extraction were much improved by cryogenic grinding. These results showed that extraction of amphetamine and methamphetamine in hair was dependent on the hair sample preparations. Furthermore, the time consumed for sample preparation decreased when the hair sample preparation was done by cryogenic grinding. These all results suggested that this cryogenic grinding could be utilized as one of the useful sample preparations for hair analysis.

[PA4-2] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Gas chromatographic/Mass spectrometric Determination of 2-Chlorobenzylidene malononitrile (CS gas) metabolites, 2-Chlorohippuric acid and 2-Chloromercapturic acid, in Postmortem Specimen, Liver

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There were several kinds of lacrimators, commonly called tear gases or riot control agents, used for incapacitating or dispersing of rioters in the contaminated environment or chemical warfare. The representative and popular lacrimatory agents used are 2-chlorobenzylidine malononitrile (CS gas) and chloroacetophenone (CN gas). The major urinary metabolites of CS in rats were reported to be 2-chlorohippuric acid and 2-chloromercapturic acid. Our main goals are to develop GC/MS analysis methodology of these two metabolites using new derivatizing reagent, trimethylsilyldiazomethane, in postmortem specimen, liver. The liver samples was taken from the postmortems of which the cause of was due to the intoxication of CS. The samples were homogenated and the metabolites under acidic condition were extracted with Isolute C_{18} column. The residues were derivatized with trimethylsilyldiazomethane ($TMSCHN_2$) to methylate the hydroxy grops. These solutions were injected into the GC/MS. To quantitate the concentration of 2-chlorohippuric acid and 2-chloromercapturic acid in samples 168 m/z and 125 m/z were selected, respectively. The concentrations of 2-chlorohippuric acid and 2-chloromercapturic acid in different postmortem specimen were calculated from the standard calibration curve and blank blood and the results were shown in Table 1.
The concentrations of 2-chlorohippuric acid and 2-chloromercapturic acid in the control blood from hospital were 46.3 ng/mL and 7.2 ng/mL, respectively. However, the concentrations of 2-chlorohippuric acid and 2-chloromercapturic acid in postmortem specimen, in m073, m074, and m077 were over 130 ng/mL and over 628 ng/mL, respectively. In case of 2-chloromercapturic acid, the concentrations in postmortem specimen were about 100 times higher than that in normal blood. This results suggested that the dead persons would be intoxicated with C.S. Generally almost all the papers pertaining to 2-chlorohippuric acid and 2-chloromercapturic acid analysis with GC or GC/MS reported that first the hydroxyl groups of 2-chlorohippuric acid and 2-chloromercapturic acid were derivatized with diazomethane. U. langenbeck, et al reported that hippuric acid was most efficiently derivatized with diazomethane among bis (trimethylsilyl)acetamide, bis(trimethylsilyl)trifluoroacetamide, and trimethylphenylammonium hydroxide derivatizing reagents. However, this diazomethane is highly toxic, in situ prepared and should be treated with care and safe. Newly applied methylating reagent, trimethylsilyldiazomethane, was relatively not toxic and very quantitavely reacted with the hydroxyl group of carboxylic acid at room temperature but with same reactivity as diazomethane. Our results suggested that this trimethylsilyldiazomethane was very usfull substituent for diazomethane for methylation of 2-chlorohippuric acid and 2-chloromercapturic acid.

[PA4-3] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Down-regulation of Cytochrome P450 1A1 expression by o,p'-DDT

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The effects of o,p'-DDT on cytochrome P450 1A1 expression was investigated in cultured mouse hepatoma Hepa-1c1c7 cells. o,p'-DDT alone did not affect P450 1A1-specific 7-ethoxyresorufin O-deethylase (EROD) activity. In contrast, the TCDD-inducible EROD activities were markedly reduced upon concomitant treatment with TCDD and o,p'-DDT in a dose dependent manner. Treatment with ICI 182.780, an estrogen-receptor antagonist, did not affect the suppressive effects of o,p'-DDT on TCDD-inducible EROD activity. TCDD-inducible P450 1A1 mRNA levels were markedly suppressed upon treatment with TCDD and o,p'-DDT, and this consistent with their effects on EROD activity. A transient transfection assay using dioxin-response element (DRE)-linked luciferase and an electrophoretic mobility shift assay revealed that o,p'-DDT reduced the transformation of the aryl hydrocarbons receptor to a form capable of specifically binding to the DRE sequence in the promoter region of the P450 1A1 gene. These results suggest that the down regulation of P450 1A1 gene expression by o,p'-DDT in Hepa-1c1c7 cells might be an antagonism of the DRE binding potential of the nuclear aryl hydrocarbon receptor but is not mediated through the estradiol receptor.

[PA4-4] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Sphinganine 1-phosphate , New biomarker of fumonisin B1 exposure

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Fumonisins are produced by fusarium verticilloides and other fusarium that grow on corn worldwide. They cause liver cancer promotion and subchronic liver and kidney effects in rats, mice, rabbits, horses and swine. The impact of fumonisins on human health remains unclear, but epidemiologic evidence suggests that consumption of fumonisin-contaminated corn contributes to human esophageal cancer and neural tube defects in southern africa and china. On molecular level, fumonisins inhibit ceramide synthase and disrupt sphingolipid metabolism including the accumulation of sphinganine(Sa).
Recently, we studied the effect of fumonisin B1 on sphinganine 1-phosphate(Sa1P) by using HPLC method in vitro and in vivo.
In vitro, HaCaT cells and LLC-PK1 celle were sensitive to fumonisin B1. In HaCaT cells and LLC-PK1 cells treated with fumonisin B1 50uM, Sa1P was elevated by 23-fold and 300-fold at 72 hr, respectively. But, CHO cells and Chang cells did not go through cell death even 50uM fumonisin B1 exposure. Interestingly, although there was a big increase in sphinganine, no increase in Sa1P was observed in CHO and Chang cells.
In vivo study, we compared the effects of fumonisin B1 on Sa1P elevation with Sa.
Mice (n=8) were cut the abdomen open after 1hr (acute) and 5 days (chronic) of given fumonisin B1 (0.5 and 5 mg/kg body weight) by i.p injection. The increase in sphinganine 1-phosphate were significantly observed than sphinganine in all tissues except liver. In brain, sphinganine 1-phosphate was increased although the elevation of sphinganine did not observed.
This study may settle limitation of using Sa elevation as a biomarker of fumonisin B1 exposure and may be suggested as a new biomarker of fumonisin B1 exposure.

[PA4-5] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

In vitro effects of polychlorinated biphenyls on the AhR and ER activity.

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Polychlorinated biphenyls (PCBs) are persistent environmental contaminants that elicit a broad spectrum of toxic effects in mammals and other vertebrate species. Because of their lipophilicity, chemical stability and resistance to biodegradation, PCBs tend to accumulate in the food chain and environmental matrices including human adipose tissues, blood and milk.
Certain congeners of PBCs exert dioxin-like activities such as immuno-, reproductive-, neuro-, dermal-, and hepatotoxicity and carcinogenesis through interacting with aryl hydrocarbon receptor (AhR). In vivo and in vitro studies have shown that some PCB mixtures, individual congeners and their metabolites exhibit