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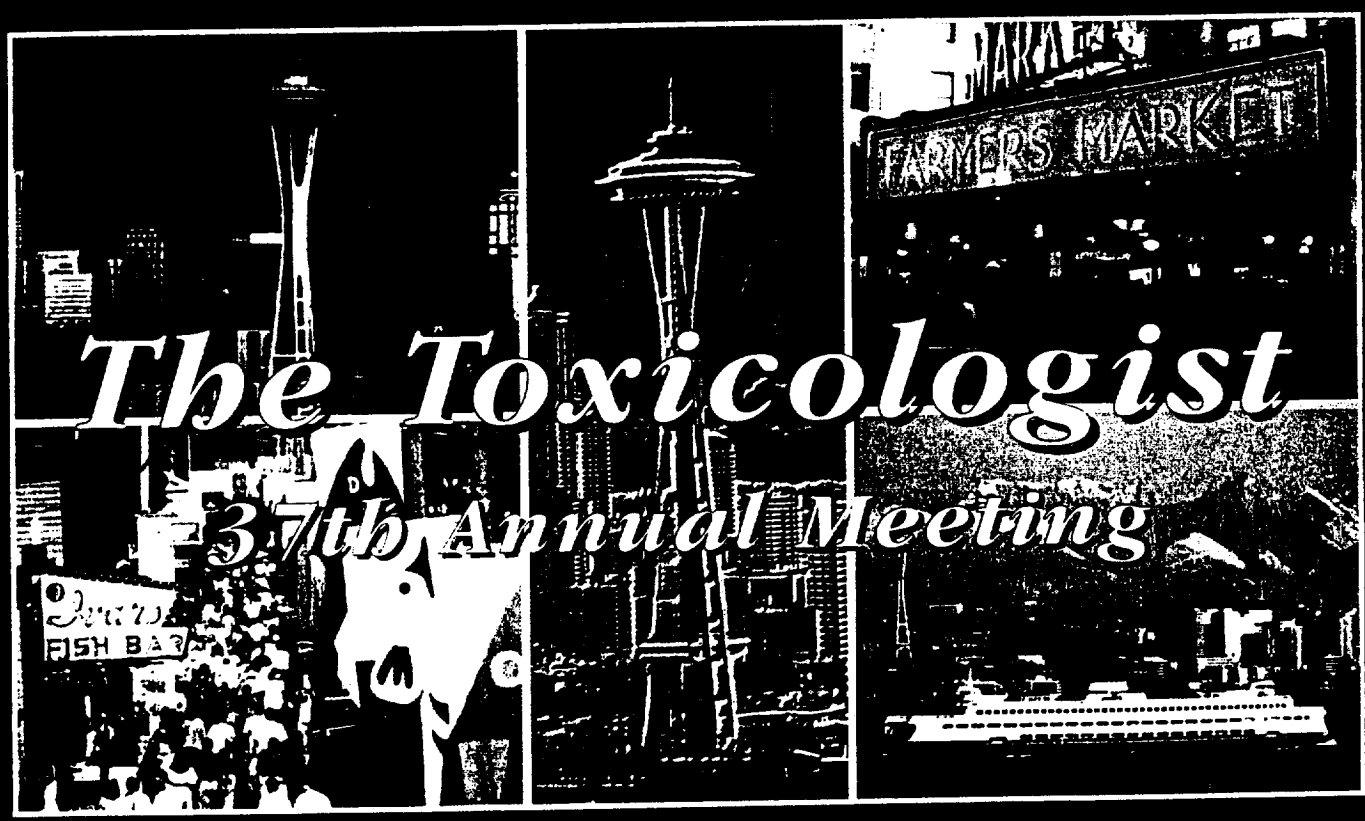

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high levels of particulate matter. The Environmental Quality Board of Puerto Rico has stated that Catafio (an industrialized area) has a history of exceeding National Ambient Air Quality Standards. The Health Department of Puerto Rico has reported the highest rates of respiratory illnesses and all types of cancers in this municipality. Using the EPA standard sampling methodology, total suspended particles (TSP) and particulate matter of less than 10 μm (PM10) were collected in Catafio and Fajardo (control area) for each season of the year. The particulate matter from the air filters and from reference standard material (SRM 1649, urban dust) were extracted sequentially with three solvents of different polarities using 24-hour Soxhlet extraction. The extracts obtained were tested for cytotoxicity on human keratinocytes using the Neutral Red Bioassay (NRB). The hexane extract from SRM 1649 was the most toxic with a NR_{50} of 5 μg/mL. Similar toxicity to the SRM1649 were found in the Catafio's hexane and dichloromethane extracts for winter and summer samples, respectively. Particle extracts showed NR_{50} values between 6 to 120 μg/mL for other seasons. None of the control extracts had demonstrated detectable levels of toxicity in the winter extracts, although in other seasons the NR_{50} fluctuated between 11 to 93 μg/mL Most of the dichloromethane extracts showed non-detectable toxicity levels. Fractionations and cytotoxicity tests to some hexane toxic extracts have been done and will be presented along with other fractions.

1945 ANTITUMOUR-ACTIVITIES OF COUMARIN AND 7-OH-COUMARIN IN HUMAN MALIGNANT CELL LINES.

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Coumarin is found in many medicinal plants and therefore also used in phytomedicine for the treatment of venous diseases. The metabolic pathways of coumarin in the human body lead to the intermediate 7-OH-coumarin and consequent glucuronidation in the intestine and liver. Clinical trials with coumarin or coumarin/cimetidine combinations in adjuvant tumour therapy revealed beneficial effects in patients with malignant melanomas (Thornes, D. et al., Eur. J. Oncol. 15, 431–435, 1989) and metastatic kidney carcinomas (Marshall, M.E. et al., J.Ir. Coll. Physicians. Surg. 22 (Suppl.I) 73, 1993). Coumarin was also active in the prevention of tumour growth in experimental models of carcinogen-induced tumours (Wattenberg, L.W. et al., Cancer Res. 39, 1651–1654, 1979) or onkogen-induced tumours in transgenic mice (Tseng, A. Jr., J. Ir. Coll. Physicians. Surg. 22 (Suppl.I), 49–50, 1993). Growth-inhibitory effects of C and 7-OH-C were also reported on human malignant cell lines in vitro (Marshall, M.E. et al., J. Cancer Res. Clin. Oncol. 120 (Suppl.I), S39-S412, 1994). We tested the antitumour activities of coumarin (C) and its known metabolites 7-OH-coumarin (7-OH-C) in several human tumour cell lines in culture. C as well as 7-OH-C inhibited cell proliferation of a gastric carcinoma cell line, a colon-carcinoma cell line (Caco-2), a hepatoma-derived cell line (HepG2) and a lymphoblastic cell line (CCRF CEM) in a concentration-dependent way. In equimolar concentrations the IC_{50}-values were 1.59–3.57 mM for C and 0.68–2.69 mM for 7-OH-C. The glucuronide of 7-OH-C was ineffective in this respect. Besides antiproliferative and cytotoxic effects coumarin exerts also immunomodulatory activities; all effects together characterize coumarin as a so-called "biological response modifier" and support the chance to use coumarin-containing phytotherapeutic preparations in the adjuvent treatment of tumour diseases; the recommended daily dosage is 100-1000 mg coumarin.

1946 PREDICTABILITY OF ASSESSING CATARACTOGENIC POTENTIAL OF DRUG CANDIDATES USING EXPLANT LENS CULTURE.

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The 5-lipoxygenase inhibitor CJ12918 (250 mg/kg/d) was associated with opacity formation in Sprague-Dawley rats after 1 month. The time course of toxicity and diminishing systemic exposure to CJ12918 suggested that opacity formation was linked to extensive metabolite formation. The incidence of opacity formation was reduced 33% with the less metabolically labile structural analog CJ13454, despite increased systemic exposure to the parent drug (AUC(0–8hr): 67 vs 2 μg•hr/ml for CJ12918) and similar drug levels in the lens (≤16 vs ≤17 ng/lens). The reference analog ZD2138 was without effect and was not detected in the lens. The present study utilized explanted lenses from Sprague-Dawley rats to determine the predictability and limitations of assessing cataractogenic potential of these drugs *in vitro*. Lenses were placed in organ culture and exposed daily to either vehicle (0.2% EtOH), CJ12918, CJ13454 or ZD2138 for up to 7 days. The role of metabolite formation was assessed using an arachlor-induced S9 microsomal fraction. In contrast to *in vivo* results, ZD2138 was a potent cataractogenic agent *in vitro*. After 2 days of exposure to 15 μg/ml, lens clarity and ATP content in the presence of S9 was as follows (appearance, % of control ATP): ZD2138 (53% cloudy, 32 ± 1%), CJ12918 (42% cloudy, 40 ± 3%), CJ13454 (100% clear, 101 ± 12%). Lens clarity and ATP content for all 3 drugs decreased proportionately with increased drug concentration (40 μg/ml) or length of exposure. Parent drug levels in the lens were <0.5 μg for ZD2138 and ranged between 0.6–1.3 μg for CJ12918 and CJ13454. Overall, these data suggest that with some limitations, explanted lenses can be used as an *in vitro* tool for discriminating compounds with potential to cause lenticular opacities. However, since the *in vitro* model bypasses potential differences in metabolism, pharmacokinetics and accumulation of various compounds into the target tissue *in vivo*, the model alone cannot be used as a definitive determinant of cataractogenic risk.

1947 DETERMINATION OF THE PARTITION COEFFICIENTS OF METHYL ETHYL KETOXIME IN WATER, OLIVE OIL AND RAT BLOOD.

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Methyl ethyl ketoxime (MEKO; 2-butanone oxime) is an industrial antioxidant and corrosion inhibitor. In long term inhalation studies with male rodents, MEKO induced liver tumours. Knowledge of tissue dose may be an important factor in elucidating the carcinogenic potency of MEKO. Tissue dose can be calculated for various exposure conditions by means of a physiologically based toxicokinetic model. Among other parameters, such a model requires knowledge of partition coefficients (PCs). A PC describes the ratio of the concentrations of MEKO in a liquid phase or a tissue to the concentration in the gas phase, at equilibrium.

The enrichment of MEKO in water, olive oil and blood of Fischer 344/N rats was measured according to Johanson and Filser (1993; Arch Toxicol 67:151–163). Defined amounts of liquids were incubated with defined amounts of gaseous MEKO at 37°C in closed all-glass systems. Concentration-time courses of MEKO in the gas phase were monitored using a gas chromatograph equipped with a FID and a stainless steel column (0.7 m) packed with Porapak Q (60–80 mesh). The oven temperature was 180°C. MEKO was stable in olive oil. However, in water and blood slow hydrolysis to 2-butanone occurred. PCs matrix:air (mean ± SD [n]) were for water 22300 ± 3400 [9], for olive oil 5000 ± 600 [6] and for blood 20400 ± 3700 [6]. Assuming a body composition of 70% water and 10% fat, the thermodynamic equilibrium constant whole body:air is calculated by means of the PCs water:air and olive oil:air to be 16100. The similar values of the PCs water:air and blood:air indicate an uniform distribution within the body, with the exception of adipose tissue. From the high value of the PC blood:air it has to be deduced that inhalative uptake is limited by the alveolar ventilation. *Sponsored by Allied Signal.*

1948 DISPLACEMENT OF A FLUORESCENTLY LABELED FATTY ACID ANALOGUE FROM FATTY ACID CARRIER PROTEINS BY WYETH-14,643, AMMONIUM PERFLUOROOCTANOATE, POTASSIUM PERFLUOROOCTANE SULFONATE AND OTHER KNOWN PEROXISOME PROLIFERATORS.

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It is hypothesized that fatty acid displacement from fatty acid-carrier proteins is an initial event leading to peroxisome proliferation and tumor formation observed in animal studies. To test this hypothesis, displacement of the fluorescently labeled fatty acid analogue 11-(5-dimethylaminonapthalenesulfphonyl)-undecanoic acid (DAUDA) from the fatty acid-carrier proteins, bovine serum albumin (BSA) and rat liver fatty acid-binding protein (L-FABP), by some known peroxisome proliferators (PP) was measured *in vitro* as a percent loss of initial fluorescence (excitation = 350 nm and emission = 500 nm). The known peroxisome proliferators, Wyeth-14,643 (WY), ammonium perfluorooctanoate (APFO) and potassium perfluorooctanesulfonoate (PFOS), when added to solutions containing 1 μM L-FABP and 1 μM DAUDA resulted in up to a 66% reduction (obtained with PFOS) in initial fluorescence at a concentration of 10 μM PP. Results for BSA were very similar to those seen with L-FABP, wherein PFOS yielded the maximum

decrease in initial fluorescence of 67%. The effect of additional known and suspect PP on these parameters is currently under investigation. These results support the hypothesis that fatty acids are displaced from carrier proteins by known PP. Future work will investigate the *in vivo* correlation between disruption of fatty acid processing and peroxisome proliferation.

1949 CHARACTERIZATION OF IN VITRO HEPATOCYTE SYSTEMS.

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There are a number of different in vitro hepatocyte systems available to use as potential models of in vivo hepatotoxicity. The ability of in vitro systems to be useful for understanding in vivo effects is based on a thorough understanding of cellular physiology and mechanisms of toxicity. In this study, the effects of cadmium (a model hepatotoxicant) on freshly isolated rat hepatocytes were characterized in culture and suspension/culture. Freshly isolated rat hepatocytes were either cultured for 24 or 48 hrs, or put into suspension for 4 hours and then transferred to culture for 24 hrs. Hepatocytes were exposed to cadmium chloride for 4 hours and cytolethality was measured by lactate dehydrogenase release. Hepatocytes in suspension/culture (LC_{50} = 3 nmol/million cells) were shown to be three times more sensitive to cadmium cytolethality than in culture for 24 hrs (LC_{50} = 9 nmol/million cells). Therefore, the first objective was to examine a mechanism which could differentiate cadmium cytolethality in these in vitro systems. Kukongviriyapan and Stacey (J. Cell Phys. **140**, 491, 1989) showed that cadmium transport was greater in freshly isolated and 48 hr cultured hepatocytes than in 24 hr cultures. We hypothesized that cytolethality would be modulated by cadmium transport. Our data showed that 24 and 48 hr cultures have similar LC_{50} values, therefore transport does not appear to be the mechanism which differentiates the sensitivity of hepatocyte systems to cadmium cytolethality. The second objective of our studies was to determine if isolated hepatocytes could be used to examine the sex difference in cadmium toxicity seen in vivo. Female rats have been shown to have an increased sensitivity to cadmium toxicity. Isolated hepatocytes were exposed to progesterone in conjunction with cadmium to study a possible mechanism for the sex difference. Progesterone (300 μg/ml) significantly increased cadmium (2 – 7 μM) cytolethality in male rat hepatocytes. However, hepatocytes isolated from female rats were not more sensitive than male hepatocytes to cadmium-induced cytolethality. These studies emphasize the importance of identifying specific endpoints of toxicity and the challenges of comparing in vitro with in vivo data.

1950 EVALUATION OF THE EFFECTS OF KUPFFER CELLS ON GALACTOSAMINE-INDUCED HEPATOTOXICITY USING THE PRECISION-CUT RAT LIVER SLICES.

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D-galactosamine (GalN) is one of the most extensively studied hepatotoxicants and known to induce liver damages via depletion of uridine triphosphate. In addition, recent *in vivo* studies have focused upon the involvement of Kupffer cells in the pathogenesis of GalN-induced liver injury, suggesting that Kupffer cells enhance the toxicity by releasing biologically active products such as superoxide anion or tumor necrosis factor α. To investigate the role of Kupffer cells in GalN-induced hepatotoxicity *in vitro*, we used the precision-cut liver slice system. Liver slices prepared from untreated or Kupffer cell-inactivated ($GdCl_3$-treated) rats were exposed to various concentrations of GalN (~20mM) and cultured for 24 hours. GalN at concentrations of more than 5mM caused dose-dependent loss of intracellular potassium and leakage of lactate dehydrogenase (LDH) from the slices of untreated rats. Histological examination revealed diffuse necrosis of hepatocyte. The toxicity of GalN, however, was markedly reduced in the slices from Kupffer cell-inactivated rats. Moreover, pre-treatment with dexamethasone, which inhibits the synthesis of inflammatory mediators, reduced the GalN-induced toxicity in slices from untreated rats. These results indicate the participation of Kupffer cells in GalN-induced hepatotoxicity *in vitro*, and suggest that inflammatory factors may play an important role in Kupffer cell-mediated hepatotoxicity of GalN. Liver slices may provide a useful model for studying roles of nonparenchymal cells in chemically induced hepatotoxicity.

1951 EFFECT OF COUMARIN ON UNSCHEDULED DNA SYNTHESIS IN PRECISION-CUT HUMAN LIVER SLI

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Coumarin (1,2-benzopyrone) occurs naturally in various plants and es oils and is known to exhibit marked species differences in both metal and hepatotoxicity. In this study we have examined the effect of cou on unscheduled DNA synthesis (UDS) in cultured human liver slices. sion-cut liver slices (200–300 μm) were prepared with a Krumdieck slicer. Liver slices were cultured for 24 hr in RPMI 1640 culture m containing 5% fetal calf serum, [^{3}H]thymidine and 0–5 mM coumarin, a dynamic organ culture system in an atmosphere of 95% O_2/5% C serve as positive controls, human liver slices were also cultured wit and 0.05 mM 2-acetylaminofluorene (2-AAF), 0.002 and 0.02 mM afla B_1 (AFB_1) and 0.005 and 0.05 mM 2-amino-1-methyl-6-phenyl-imidaz *b*]pyridine (PhIP, a heterocyclic cooked food mutagen). Liver slices fixed in formalin and processed for autoradiographic evaluation of Compared to control cultures, 0.05–5 mM coumarin had no effect on expressed as the net grain count or as the percentage of centrilobular he cyte nuclei with >5 and >10 net grains. In contrast, all three known muta produced a marked induction of UDS in human liver slices. Net grain c values (mean of 4 experiments) in control, 0.05 mM 2-AAF, 0.02 mM A and 0.05 mM PhIP treated cultures were 0.33, 4.4, 7.1 and 18.2, respecti These results suggest that coumarin is not a genotoxic agent in human l (Supported by RIFM and Rhône-Poulenc, Inc.).

1952 USE OF PRECISION-CUT RAT LIVER SLICES FOR STUDIES OF XENOBIOTIC METABOLISM AND TOXICI COMPARISON OF THE KRUMDIECK AND BRENDEL TISSUE SLICERS.

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In this study we have compared freshly cut and cultured liver slices produc from untreated male Sprague-Dawley rats by the Krumdieck (K) and Brend Vitron(BV) tissue slicers. No significant differences were observed in lev of protein, potassium, total glutathione (i.e. GSH + GSSG), reduced glutat one and cytochrome P-450 and activities of 7-ethoxyresorufin O-deethyla and 7-benzoxyresorufin O-debenzylase in freshly cut slices produced by t two tissue slicers. However, levels of oxidised glutathione were significant greater in liver slices produced by the BV tissue slicer. Precision-cut liv slices produced by both tissue slicers were cultured in RPMI 1640 mediu for O (i.e. a 1 hr preincubation period), 24 and 72 hr in a dynamic orga culture system in an atmosphere of either 95% O_2/5 % CO_2 or 95 % air/ % CO_2. Apart from small differences in glutathione levels in O and 24 h cultured liver slices, no significant differences in the above parameters wer observed between liver slices prepared with both tissue slicers and culture in both gas phases. With liver slices produced by both tissue slicers 50 μM sodium arsenite produced a greater induction of heat shock protein 70 i slices cultured for 24 hr in a high oxygen than in an air atmosphere. This data suggests that both the K and BV tissue slicers can readily produce precision-cut liver slices for studies of xenobiotic metabolism and toxicity However, for any given application of precision-cut liver slices it is desirable to establish optimal culture conditions. (Supported by Wyeth-Ayerst Research)

1953 INDUCTION OF CYTOCHROME P-450 (CYP) ISOENZYMES BY RIFAMPICIN IN CULTURED PRECISION-CUT HUMAN LIVER SLICES.

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In this study we have examined the effect of rifampicin (RIF) on CYP isoenzymes in cultured precision-cut human liver slices. Liver slices were prepared from samples of human liver (surplus to transplant requirements) with a Krumdieck tissue slicer and cultured in RPMI 1640 medium in an atmosphere of 95% O_2/5% CO_2 employing a dynamic organ culture system. Treatment of liver slices for 72 hr with 50 μM RIF induced testosterone 6β-hydroxylase activity, but had no effect on 7-ethoxyresorufin O-deethylase and 7-methoxyresorufin O-demethylase activities. A variability was observed