protein) of several GSH-dependent enzymes, including γ-glutamylcysteine synthetase (81.1), GSH peroxidase (29.0), glutathione disulfide reductase (24.5), and GSH *S*-transferase (32.8). In contrast, very low activity (in mU/mg protein) was observed for γ-glutamyltransferase (2.03) and alkaline phosphatase (2.63), consistent with a low content of brush-border microvilli. The former contrasts markedly with γ-glutamyltransferase activity in freshly isolated rat proximal tubular cells (~70 mU/mg protein). NRK-52E cells also exhibited high activities (in mU/mg protein) of (Na^++K^+)-ATPase (139) and malic dehydrogenase (219), suggesting that these cells contain a high density of mitochondria; this was confirmed with the MitoTracker® Orange fluorescent dye and confocal microscopy. Uptake and total cellular accumulation of [^{14}C]-α-methylglucose was significantly higher when cells were exposed at the basolateral surface as compared to exposure at the brush-border surface (4 *vs.* 2.5 μmol/mg protein). Similarly, uptake of GSH (measured with either [L-^{3}H-glycyl]-GSH or by HPLC) was nearly 2-fold higher across the basolateral membrane than across the brush-border membrane. Susceptibility to chemically-induced apoptosis was probed using *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC; 10 μM to 1 mM) and 1 μM staurosporine as a positive control. Morphology, flow cytometry analysis for cell cycle and annexin V, and caspase-3 activation showed time- and concentration-dependent induction of apoptosis by both DCVC and staurosporine. Thus, with the exception of GSH degradation and oxidation activities, the NRK-52E cells exhibit functions and susceptibilities that are similar to those in freshly isolated rat proximal tubular cells.

803 IMPROVED DETECTION OF ACUTE DRUG-INDUCED LIVER TOXICITY BY MEASUREMENT OF SERUM ALPHA GLUTATHIONE S-TRANSFERASE IN CYNOMOLGUS MONKEYS.

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Alpha Gluthathione S - Transferase (alpha GST) is an enzyme that detoxifies many harmful substances by coupling them to reduced glutathione. The high concentration and uniform distribution of alpha GST in hepatocytes and its small molecular size suggest that alpha GST could serve as an early indicator for liver damage. In our present study, we focused on the (1) validation of the Biotrin 'human alpha GST assay' (Biotrin HEPKIT-Alpha, Biotrin International, Dublin, Ireland) for assaying alpha GST in macaque serum samples according to national and international standards including the Good Laboratory Practice Regulations, (2) the generation of normal background data on serum alpha GST levels in cynomolgus monkeys; (3) the definition of appropriate dose levels of positive control compounds to induce liver toxicity and (4) the investigation of the nature and degree of hepatic responses to common vehicles used in routine primate toxicity studies. Results from our experiments in cynomolgus monkeys (Macaca fascicularis) are presented which show that, as in man, monitoring alpha GST is clinically more useful than conventional biochemical liver function tests, since it is an earlier and more sensitive biomarker of acute hepatotoxicity than eg Alanine-Aminotransferase. This renders alpha GST a valuable marker for acute liver injury in routine toxicity studies with non-human primates.

804 CHARACTERIZATION OF THE RAT α-GLUTATHIONE S-TRANSFERASE (GST) ELISA™ (BIOTRIN) USING RAT HEPATOMA (H4IIE) CELLS.

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The release of intracellular proteins, such as GST, into the culture medium can be used as an indicator of cell death. The GST assay has been used *in vitro*, however some important aspects of the assay have not been evaluated. The present study characterizes the GST ELISA for use as an assay to effectively measure cell death in H4IIE cells. The primary objectives of the present study were to determine if the assay could accurately measure GST in cell culture medium containing 20% serum, assess metabolic stability of GST in media at 37°C, investigate freezer stability of GST at −80°C, and evaluate the importance of plate mixing. To determine if the assay could accurately measure GST in cells that had become detached from plates, but remained intact after compound exposure, H4IIE cells were seeded into 96-well plates (10,000 cells/200μL/well). Cells were lysed immediately with digitonin (DIG, 1 mM) or frozen at −80°C. Total GST was determined in both DIG-treated and frozen suspensions of cells. No significant difference was found between the two treatments. GST stability in a cell matrix was evaluated at 37°C by treating cells with 1 mM DIG to obtain maximum GST release. Aliquots were collected at 0, 24, 48, and 72 hr for GST determination. GST recovered from media relative to GST at time 0 was 79% at 24 hr, 74% at 48 hr, and 63% at 72 hr. Stability of GST at −80°C was determined by adding known amounts of purified GST standard (0, 2, 10, 20, and 40 μg/L) into media. GST was determined on day 0 and used as a reference. After 2 weeks GST was 95% of day 0, followed by 81% after one month, 76% after two months, and 65% after 6 months. To evaluate the importance of plate mixing during the ELISA assay, GST standards were assayed with and without mixing. Absorbance values were increased 15% as a result of plate mixing. In conclusion, the GST ELISA is a reliable assay for assessing cell death *in vitro*. It is reproducible, highly specific, stable, and because of its 96-well format, lends itself to high throughput analyses.

805 PHENOLIC COMPOUNDS CAUSE DEPLETION OF GSH, OXIDATIVE STRESS AND CYTOTOXICITY IN NORMAL HUMAN DERMAL CELLS.

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Phenolic compounds are known to cause skin rashes, burns and ulceration, dermal inflammation and necrosis, irritant and allergic contact dermatitis, eczematous "black-spot" dermatitis, leukoderma and cancer promotion. In many cases, the mechanisms underlying these toxic effects are unknown. Enzymatic oxidation of phenols is an effective metabolic pathway leading to their bio-activation. We hypothesized that cytotoxic effects of phenolic compounds are due, at least in part, to generation of phenoxyl radicals, reactive intermediates of their one-electron oxidation by different intracellular peroxidase activities (*e.g.*, PGHS, myeloperoxidase). Phenoxyl radicals are capable of depleting the intracellular pool of GSH and oxidizing protein sulfhydryls. To experimentally test this hypothesis, we measured cell viability, peroxidation, intracellular levels of GSH and protein SH-groups in six human cell lines: keratinocytes, melanocytes, fibroblasts, astrocytes, dendritic and Jurkat cells exposed to twelve phenolic compounds. Western blot analysis of PGHS-2 in cultured keratinocytes after a single application of 2-methoxy-4-propenylphenol for 6 or 18 h showed that the protein level was elevated about 2-fold over control. We found that 18 h incubation of cultured cells in the presence of some phenolic compounds (100-500 μM) caused pronounced cytotoxicity and significant depletion of GSH. Incubation of ascorbate-preloaded keratinocytes with phenols produced an EPR-detectable signal of ascorbate radicals, indicating that redox-cycling of phenoxyl radical is likely involved in oxidative effects. In summary, while phenolics are known to act as radical scavengers, their enzymatic (PGHS-catalyzed) reactive intermediates, phenoxyl radicals, interact with vital intracellular thiol reductants and cause cytotoxic effects.

806 DIVERGENT CYTOKINE PROFILES PROVOKED IN MURINE LYMPH NODE CELLS BY CHEMICAL ALLERGENS: A STATISTICAL ANALYSIS.

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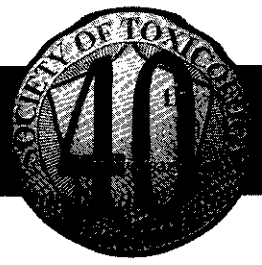
We have demonstrated previously that topical exposure of BALB/c strain mice to chemical contact and respiratory allergens provokes divergent cytokine secretion profiles consistent with the preferential activation of T helper (Th) 1 and Th2-type cells, respectively. Thus, draining auricular lymph node cells (LNC) pooled from animals treated with the contact allergen 2,4-dinitrochlorobenzene (DNCB) express high levels of interferon γ(IFN-γ) and interleukin (IL)-12, but little of the type 2 cytokines IL-4, IL-5, IL-10 or IL-13. The converse, Th2-type, phenotype is observed following topical exposure to the respiratory allergen trimellitic anhydride (TMA). There was, however, considerable inter-experimental variation in the absolute amount of cytokine secreted by allergen-activated LNC; although in every experiment the polarization of expression was similar. We have therefore analyzed statistically the differences in cytokine secretion profiles stimulated by DNCB and TMA. Data from 5 independent experiments were evaluated by Student's t test based on two-way analysis of variance of the log transformed data, allowing for the replicate structure of the experimental design. DNCB-stimulated LNC produced approximately twice as much IFN-γ and IL-12 than did TMA-activated LNC ($p<0.05$), whereas exposure to TMA resulted in between 3- and 10-fold higher levels of IL-4, IL-5 or IL-13 ($p<0.01$) and IL-10 ($p<0.05$). These data confirm that topical exposure of BALB/c strain mice to the reference contact and respiratory allergens DNCB and TMA induces the selective activation of Th1- and Th2-type cells, respectively.



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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