793 EFFECTS OF OCCUPATIONAL LEVELS OF TOLUENE DIISOCYANATE ON GLUTATHIONE-DEPENDENT ENZYMES AND GLUTATHIONE IN HUMAN BRONCHOEPITHELIAL CELLS.

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Toluene diisocyanate (TDI) is a recognized asthmogen, yet the mechanism of its toxicity remain unclear. We have previously shown a rapid reaction of TDI with glutathione (GSH), a key cellular antioxidant. Glutamyl transpeptidase (γ-GT) plays a key role in the synthesis and degradation of GSH. Our earlier *in vitro* studies using the fluorescent dye, 5-chloromethyl-fluorescein diacetate (CMFDA), have shown a decrease in intensity of the thiol staining as a function of the length of TDI exposure in differentiated and undifferentiated human bronchial epithelium (HBE) cells. Because CMFDA activation requires functional intracellular esterases and glutathione-S-transferases (GST), the present study was undertaken to evaluate the activity of these two enzymes as well γ-GT activity. Differentiated air/liquid cultures of HBE cells were exposed to 20 ppb TDI vapor for 5 or 30 minutes. Following exposure, cell viability was analyzed, cellular GSH levels were titrated using the thiol specific fluorophore ThioGlo, and GST and γ-GT enzymatic activities were spectrophotometrically determined. Cellular esterase activity was assayed by incubating cells with 5-(and-6-)-carboxyfluorescein succidinimil ester followed by confocal microscopy imaging. Exposure of cultures to 20 ppb TDI was not cytotoxic. Esterase and GST activities were not affected. Therefore, the reduction in CMFD fluorescence detected following TDI exposure was not due to cytotoxicity, decreased esterase activity, nor reduced GST activity. TDI exposure of HBE cells resulted in a 30 % decrease in GSH levels and 35% decrease in γ-GT activity. This reduction of intracellular GSH and inhibition of a GSH-dependent enzyme can alter cellular redox status. The resulting oxidative stress may be a significant factor in determining human susceptibility to TDI-induced lung diseases. Supported by NIEHS 05651 and NIEHS ES06694.

794 ACETAMINOPHEN DECREASES GLUTATHIONE IN RAT BRONCHOALVEOLAR LAVAGE CELLS IN VIVO.

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We have previously shown *in vitro*, that acetaminophen (APAP) leads to a biotransformation-dependent decrease in cellular reduced glutathione (GSH) in rat and human alveolar macrophages, as well as type II pneumocytes. In order to verify whether this also occurs in vivo, we studied, in rats, the effect of APAP administration on GSH levels in liver, lung and bronchoalveolar lavage (BAL) cells. After an overnight fast, male Wistar rats (244-294 g) were treated with APAP, given as a single oral dose of 100 (n=6) or 1000 (n=6) mg/kg bw. The controls (n=6) received the same volume (12.5 ml/kg bw) of the vehicle (50% propylene glycol/water). Three hours after APAP administration, GSH levels decreased significantly in the liver from 3.14±0.24 μmol/g in controls, to 2.79±0.15 μmol/g (100 mg/kg) and 2.16±0.26 μmol/g (1000 mg/kg), but not in the lung. In BAL cells, GSH content decreased from 15.6±3.1 nmol/mg protein in controls, to 12.7±1.4 nmol/mg protein ($p=0.077$) and 11.0±2.5 nmol/mg protein ($p=0.018$), after 100 and 1000 mg/kg, respectively. There were no changes in BAL cell viability (trypan blue exclusion), and protein content. In all groups, alveolar macrophages represented more than 90% of recovered BAL cells. A significant increase in the percentage of BAL polymorphonuclear granulocytes was observed at 1000 mg/kg APAP (5.1±2.1%) compared with controls (1.3±0.5%). At the APAP doses and time point studied, no changes were observed in epithelial lining fluid volume (urea dilution technique), protein content, and gamma-glutamyltranspeptidase activity. The results have demonstrated that high doses of APAP decrease GSH in rat BAL cells in vivo. Although there was no evidence of overt pulmonary toxicity, the decrease in GSH content may diminish the anti-oxidant defence of pulmonary macrophages. (This work was supported by the European Respiratory Society and partly by INCO/Copernicus (EU) (IC15-CT96-0314).

795 DIESEL EXHAUST PARTICULATE (DEP) AFFECTED THIOL LEVELS IN BOTH ALVEOLAR MACROPHAGES (AM) AND LYMPH NODE CELLS (LNC).

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Glutathione (GSH) and cysteine (CYS) are major intracellular antioxidants and play an important role in protecting against electrophilic xenobiotics. The purpose of this research was to study the thiol regulation by AM and LNC, and the effect of DEP on this process. AM, and thymic and tracheal lymph node lymphocytes were obtained from rats 3 days after intratracheal instillation of DEP/saline or saline. Cells were cultured ± cystine for 16 hour's at 37C° and 5% CO2, or used immediately to determine GSH-reductase activity (GSH-R). Determination of cysteine and GSH levels in MBB-stained cells and derivitized culture supernatant was achieved *via* reverse phase high performance liquid chromatography (HPLC). Both AM and LNC showed enhanced levels of cysteine when incubated with cystine. This enhancement was significantly augmented in cells obtained from DEP exposed rats. The DEP exposure was also shown to enhance GSH production in AM, but not in LNC when cystine was added to the cell culture. Intracellular GSH levels in LNC were decreased in the DEP exposed cells cultured with or without cystine. DEP was found to interact directly with cystine, cysteine, and to a lesser extent with GSH. DEP did not react with oxidized GSH or directly react with GSH-R; however, GSH-R activity was greatly induced in LNC from animals exposed to DEP. DEP caused increased uptake and reduction of disulfides in these immune effector cells. The mechanism of this stimulus is not known, but does not appear to be in response to significant intracellular thiol oxidation/depletion by DEP. (Supported in part by NIH1RO1-HL62630).

796 EFFECT OF ETHACRYNIC ACID PRETREATMENT ON SULFUR MUSTARD CYTOTOXICITY TO HUMAN EPIDERMAL KERATINOCYTES *IN VITRO*.

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Sulfur mustard (HD) is a powerful alkylating agent that is not only a potent vesicant, but also profoundly cytotoxic. The biochemical mechanism(s) for these actions is not well understood, but a number of working hypotheses have been developed to study ways to interfere with cytotoxicity. *in vitro* models are used to study relevant biochemical markers that are modified as a function of HD exposure. HD reacts spontaneously with glutathione creating glutathione conjugates, which are excreted through the mercapturic acid pathway in mammals. It is unknown whether any enzymes such as the glutathione-S-transferases are involved in the detoxification of HD through formation of conjugates. An inhibitor of glutathione-S-transferase, ethacrynic acid (EAA), has been used as a pretreatment compound in human epidermal keratinocytes grown in culture. Cells grown in both 24 - and 96-well tissue culture plates were pretreated with 1- 5 ug EAA/well for various periods of time and then challenged with HD. The cells were incubated for an additional 24 hours before viabilities were measured by the MTS-PMS colorometric assay (Promega). The 24-hour viabilities were determined directly by adding MTS reagent to the tissue culture plate, which were then incubated for 4 hrs at 37C and read at 490 nm in a microplate reader. Dose response curves were generated that showed HD cytotoxicity beginning at 50 uM and reaching a maximum at 300 uM. Preliminary results suggest that pretreatment of HEK for 24 hours with EAA appears to give some protection against HD challenge, but the mechanism involved is unclear. Glutathione-S-transferase levels are currently under analysis. Other compounds that stimulate synthesis of the glutathione-S-transferase are under investigation.

797 ASSESSING THE ROLE OF OVARIAN GSH LEVELS IN OVOTOXICITY IN RATS.

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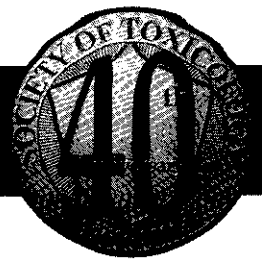
The diepoxide metabolite (VCD) of the occupational chemical 4-vinylyclohexene is ovotoxic in rats following repeated exposures. Daily dosing of VCD for 15d destroys 50% of the smallest ovarian follicles. Since VCD acutely reduced hepatic levels of the antioxidant, glutathione (GSH), these studies addressed if reduced GSH levels are involved in the destruction of ovarian follicles. Thus, immature female Fischer 344 rats (n=3-12 per group) were given a single dose or dosed once daily for 15d with VCD (0.57mmol/kg, ip) or the GSH synthesis inhibitor buthionine sulfoximine (BSO, 2mmol/kg). Animals were killed 2, 6, or 26h following a single dose, and 2 or 26h following 15d of dosing. Liver and ovarian tissues were collected for determination of GSH levels by HPLC. Also, ovaries were collected following repeated dosing and histologically processed for counting follicles. Following a single dose, both VCD (51±5% of control) and BSO (42±9% of control) reduced hepatic GSH within 2h ($p<0.05$), but only BSO reduced ovarian GSH (64±5% of control at 6h, $p=0.05$). Within 26h, liver and ovarian GSH levels had returned to control levels with either treatment. Two h after the 15th dose,



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Preface

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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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