353 ADVERSE RESPONSES TO SEMISYNTHETIC METAL WORKING FLUIDS IN B6C3F1 MICE.

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About 10 million industrial workers of both sexes are exposed to Metal Working Fluids (MWFs) via inhalation or skin or both. Our preliminary results, following dermal application of 200ul of 50 % unused (neat) semi-synthetic MWF (pH 7 or pH 9.7) to the unshaved backs of 6 week old B6C3F1 mice, twice a week for 6 weeks , show significant increase in weights of the liver of both sexes. The present study is to determine if this weight change is related to oxidative stress consequent to MWF exposure. Therefore we exposed 6 month old mice of both sexes to MWFs following the above protocol except that the topical application was with 5% MWFs, 5 days a week for 13 weeks. Chemical analyses of tissues from these mice indicate significant reduction in ascorbic acid and glutathione levels in the liver of both sexes and in the gonads of the male. Malonaldehyde levels in the male liver and gonads show significant increase. The skin histamine levels are significantly increased in the female. These results suggest that MWFs are absorbed through the skin, exhibit liver toxicity, and may represent an important health risk to industrial workers exposed to these fluids.

354 RESTRAINT STRESS DIFFERENTIALLY AFFECTS CYTOKINE PRODUCTION AND EAR SWELLING IN ALLERGIC CONTACT DERMATITIS (ACD) AND IRRITANT CONTACT DERMATITIS (ICD).

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Previous studies have demonstrated that glucocorticoids suppress the immune response to antigen and that restraint stress increases serum corticosterone levels. Additional research using rats has shown that mild, acute restraint stress increases ear swelling and lymphocytic infiltration in allergic contact dermatitis (ACD), but not in irritant contact dermatitis (ICD). We hypothesized that mild restraint stress alters the murine cutaneous response to chemicals and that these changes would be reflected in the cytokine profile and be gender-specific. For ACD studies, male and female B6,129 mice were sensitized on the flank with 0.5% DNFB (Days 1 & 2) and challenged with 0.25% DNFB on the ear immediately following mild restraint stress (Day 6). For irritant studies, 5% croton oil was applied to the ear immediately after restraint. We measured ear swelling, cytokines TNF-a, IL-1b, IFN-g and IL-4 and serum corticosterone levels as an indicator of activation of the hypothalamic-pituitary axis. Restraint increased serum corticosterone levels in all mice. In the ICD studies, we measured irritant-induced increases in ear thickness and TNF-a and IL-1b production. Ear thickness and TNF-a levels were not altered by restraint stress, however, the concentration of IL-1b was suppressed at 8 and 24 hours. IFN g and IL-4 were minimally detectable in all treatment conditions. There were no gender differences in the irritant response. For ACD, in both males and females, DNFB induced ear swelling and increased TNF-a, IL-1b and IFN-g. Restraint stress coupled with DNFB treatment resulted in an additional, but transient, increase in ear thickness and, at 24 hours, increased IFN-g but decreased IL-1b. TNF-a was more elevated at 8 and 24 hours in females only, and IL-4 was not detected for any ACD treatment conditions. These data suggest a complex interaction between restraint stress-induced changes in the dermal response to chemicals that may be mediated by elevations in serum glucocorticoids in B6,129 mice.

355 USE OF GREEN FLUORESCENT PROTEIN IN A HUMAN ORGANOTYPIC STRATIFYING SQUAMOUS EPITHELIUM FOR MOLECULAR AND PHARMACOLOGICAL STUDIES OF NORMAL AND MALIGNANT EPITHELIAL GROWTH.

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We have developed a novel organotypic *in vitro* model for the study of normal and malignant human squamous epithelial cell proliferation and pharmacological response. This model utilizes expression of green fluorescent protein (GFP) in transfected epithelial cells to identify and isolate cells within an organotypic stratifying squamous epithelium. Prototypic non-malignant and malignant cell lines, BC-1-Ep/SL and SCC13y, respectively, have been developed which stably express GFP. GFP-labeled SCC13y cells (GFP13y) grown in mixed cultures with unlabeled BC-1-Ep/SL cells, do not appear to effect the normal stratification of BC-1-Ep/SL cells in organotypic cultures. Individual and groups of GFP13y cells are visualized in frozen sections using fluorescent microscopy. This report characterizes a number of standard skin differentiation and proliferation markers such as involucrin and PCNA. Data observed in studies using confocal microscopy and flow cytometry are also presented which demonstrate the growth of GFP13y cells over a period of 5-15 days in organotypic culture. This model has potential broad use with all types of stratified epithelia and associated tumors (oral cavity, cervix, trachea, cornea) and melanoma. We believe it is particularly valuable to screen for efficacy of pharmacological agents on normal and malignant epithelia cultured in a context which maintains the cell interactions present in functioning human epithelial tissue. By entirely reconstructing human epithelial cell growth within a tissue-like environment, epithelial cell growth characteristics can be monitored in a physiologically relevant context. Preliminary data characterizing the radiation response and antiproliferative response of a set of standard chemotherapeutic agents are described herein. We believe this approach may provide a more accurate assessment of epithelial cell proliferation kinetics and prove to be a valuable tool for the pharmacologic studies.

356 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCCD) CAUSES ALTERATIONS IN DIFFERENTIATION OF KERATINOCYTES IN ORGANOTYPIC CULTURE.

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Human exposure to the environmental toxin TCDD produces a severe skin pathology known as chloracne. Although several laboratories have previously reported on the aryl hydrocarbon receptor (AhR)-mediated cellular responses of normal human or rodent keratinocytes to TCDD *in vitro*, little evidence has been available to link these observations to the specific pathology observed in skin. To approximate the three dimensional microenvironment of human skin, we have established organotypic cultures utilizing BC-1-Ep/SL cells, a novel human keratinocyte cell line. The BC-1-Ep/SL cell line was isolated in our laboratory and is identical to normal keratinocytes in its growth and differentiation characteristics. These cells differentiate normally *in vitro* and are morphologically indistinguishable from normal human keratinocytes at both the light and electron microscope level. The BC-1-Ep/SL keratinocytes are immortal and have been shown to be non-tumorogenic in nu/nu mice. Upon treatment of the organotypic model system with TCDD, we have observed an altered morphology as compared to controls. In treated samples we observed a thickening of the granular and cornified superbasal layers of keratinocytes in organotypic cultures. This phenomenon was observed in organotypic culture seeded with normal human keratinocytes and subsequently with BC-1-Ep/SL keratinocytes. We present new data on the differentiation characteristics of TCDD-treated keratinocytes in organotypic culture and examine the effect of TCDD on epithelial-mesenchymal interactions *in vitro*.

357 EFFECT OF CACL2 CONCENTRATION ON THE RATE OF PROLIFERATION OF CULTURED HUMAN KERATINOCYTES.

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Topical formulations of drugs may contain $CaCl_2$ and citrate as excipients. In order to analyze the influence of calcium on the epidermal cells' behavior, the effects of $CaCl_2$ on the proliferation and cell cycle distribution of cultured normal human epidermal keratinocytes (NHEK) and HaCaT keratinocytes were investigated. The cells were continuously (24 h) or transiently (1, 2, 4 h) exposed to $CaCl_2$ (0.09, 0.2, 1, 2 and 5 mM), or to combinations of $CaCl_2$ and citrate (5 / 5 mM, and 40 / 40 mM). BrdU incorporation determined by ELISA was used as a marker for cell proliferation. Flow cytometric evaluation of the propidium iodide staining of fixed cells served for cell cycle distribution analysis. $CaCl_2$ induced a concentration-dependent decrease in keratinocyte cell growth with respect to non-treated cells. This effect was more pronounced for NHEK than for HaCaT cells. The combination of $CaCl_2$ and citrate (40 / 40 mM) strongly decreased this parameter in both cell types, and induced cytotoxicity as measured by LDH release. After 24 h treatment, NHEK and HaCaT cells incubated with 40 mM $CaCl_2$ + 40 mM citrate showed changes in the cell cycle distribution (increase in the percentage of cells in G1 phase and decrease in S phase) which were indicative

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