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## AGE AND SEX CONTRIBUTE TO ASTHMA HETEROGENEITY IN ANIMAL MODELS.

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Asthma is a lung disorder characterized by eosinophil infiltration and airway hyperresponsiveness (AHR). However, the predominance of airway obstruction vs inflammation differs in asthmatics, with numerous asthma phenotypes being described (eosinophilic, neutrophilic, childhood onset, steroid-resistant, etc). The purpose of the present study was to assess contributions of age and sex to the profile of asthma symptoms in guinea pig and mouse models of asthma. Female and male Hartley guinea pigs and Balb/c mice were sensitized and challenged with ovalbumin (OVA) before sexual maturity (Y/Y) or after sexual maturity (A/A) and eosinophil infiltration and AHR determined. For mice, Y/Y and A/A animals were sensitized 19 or 82 days postnatal, respectively. With guinea pigs, Y/Y and A/A animals were sensitized at 9 or 57-84 days postnatal, respectively. Eosinophil peroxidase in homogenized lung was used as an indicator of eosinophilia. In guinea pigs, AHR was assessed by measurement of pulmonary resistance to i.v. methacholine in anesthetized animals. In mice, AHR to aerosolized methacholine was determined using unrestrained barometric plethysmography to measure PenH. In the guinea pig, 24 hours after OVA challenge, eosinophils increased significantly in the lung in Y/Y females with minimal airway hyperresponsiveness detected. In contrast, A/A female guinea pigs accumulated very few eosinophils into the lung after OVA challenge, but the airway hyperresponsiveness was marked. In contrast to guinea pigs, OVA challenge of mice resulted in greater increases in eosinophils in A/A animals than Y/Y animals whether male or female. Marked AHR was evident in both Y/Y and A/A mice. These studies clearly indicate that age and sex contribute to asthma heterogeneity and need to be carefully considered when using animal models of asthma. (Support: Department of Defense CDMRP; DAMD 17-02-1-0191).

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## ZN/CU SUPEROXIDE DISMUTASE AUTOANTIBODY IN CEMENTED TUNGSTEN CARBIDE WORKERS.

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Asthma and hard metal pneumoconiosis have been associated with exposure to aerosols containing nickel, cobalt, and tungsten in the cemented tungsten carbide industry. Only a few studies reported metal-specific IgE in sera of hard metal aerosol exposed workers, but antibody prevalence was very low. In the present study, sera from 140 cemented tungsten carbide workers were screened for specific IgG against chromium ( $Cr^{3+}$ ), copper ( $Cu^{2+}$ ), cobalt ( $Co^{2+}$ ), zinc ( $Zn^{2+}$ ) and nickel ( $Ni^{2+}$ ), haptenized to albumin (HSA) and superoxide dismutase (SOD). SOD was used as a protein carrier for haptenization, because its physiologically active form is Zn/Cu-SOD and it is an important endogenous antioxidant enzyme. Antigens were characterized using MALDI-TOF mass spectrometry (MS). Mass spectra of haptenized SOD showed 0-3 Co, 0-3 Zn, and 0-1 Ni ions bound per SOD molecule. Quantification of specific binding of Cu was not possible due to the large numbers of copper ions bound to SOD, consistent with non-specific binding. Haptenization to HSA was evident by color change resulting from binding of the metals to HSA. Specific chromium and cobalt IgGs were not found using either of the haptenized proteins. Casein was not sufficient to block non-specific IgG binding from 19 sera. These sera were re-screened using bovine serum albumin blocked ELISA plates. Within this subgroup, specific-IgG was found for Ni-SOD in one worker, and IgG specific to both Cu- and Zn-SOD was found in the sera of 10 workers. Anti-apo-SOD IgG was not found. This is the first report of autoantibody against Zn/Cu-SOD. It is not known if the 7.1% prevalence rate of anti-Zn/Cu-SOD IgG in this group of cemented tungsten carbide workers is excessive. The relationship of Zn/Cu-SOD autoantibody to reported respiratory symptoms and respiratory disease has not yet been established in this workforce.

The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent agency determination or policy.

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## THE ROLE OF THIOLS AND DISULFIDE FORMATION IN MERCAPTOBENZOTHIAZOLE ALLERGENICITY.

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The rubber accelerator, 2-mercaptopbenzothiazole (MBT), can cause allergic contact dermatitis (ACD) from gloves and other rubber products, but the chemical mechanism leading to MBT's allergenicity is unknown. It was hypothesized that the thiol

group is critical to MBT's covalent binding/haptenation to nucleophilic protein residues. Oxidative transformation of MBT to the disulfide 2, 2'-dithiobis(benzothiazole) (MBTS) was observed within the glove matrix when hypochlorous acid, iodine or hydrogen peroxide were used as oxidants. Cysteine reduced MBTS to MBT with subsequent formation of the mixed disulfide 2-amino-3-(benzothiazol-2-yl disulfanyl)-propionic acid. Simultaneous reduction of MBTS and disulfide formation with Cys34 on bovine serum albumin was observed, suggesting a potential route of protein haptenation through covalent bonding between cysteinyl residues on proteins and the MBT/MBTS thiol moiety. MBT metabolites were not found following incubation with isoniazid and dexamethasone-induced rat liver microsomes and a NADPH regeneration system. Guinea pigs were sensitized to MBT using a modified guinea pig maximization assay (GPMT) and cross-reactivity towards MBTS and the free thiol lacking or blocked compounds benzothiazole (BT), 2-hydroxybenzothiazole (HBT) and 2-(methylthio)benzothiazole (MTBT) assessed. MBT and MBTS, but not BT, HBT or MTBT elicited ACD in MBT-sensitized animals. Both chemical and experimental animal studies demonstrate that the thiol group is required for MBT allergenicity and also suggest that MBT first undergoes non-enzyme mediated oxidation to MBTS with subsequent haptenation via the formation of mixed disulfides with sulphydryl groups on proteins. This work was supported by an interagency agreement with the NIEHS (Y1-ES0001-06). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent agency determination or policy.

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## ANTI-INFLAMMATORY ACTIVITY OF FISETIN IN HUMAN MAST CELLS (HMC-1).

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Mast cells play an important role in the pathogenesis of allergic diseases through the release of inflammatory mediators such as, histamine, cysteinyl leukotrienes, cytokines, and chemokines. Flavonoids, like fisetin are naturally occurring molecules with antioxidant, cytoprotective, and anti-inflammatory actions. The aim of our study was to examine whether fisetin modulates the inflammatory reaction in a stimulated human mast cell (HMC-1). HMC-1 cells were stimulated with 40 nM of phorbol-12-myristate 13-acetate plus 1  $\mu$ M of calcium ionophore A23187 (PMACI). The expression of pro-inflammatory cytokines was measured by reverse transcriptase-polymerase chain reaction. The nuclear transcription factor (NF)- $\kappa$ B promoter-mediated luciferase activity and the DNA-binding activity were detected by luciferase assay and electrophoretic mobility shift assay. Nuclear translocation of p65 NF- $\kappa$ B and phosphorylation of mitogen-activated protein kinases such as, p38, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) were detected by the Western blotting. Fisetin decreased the gene expression and production of PMACI-stimulated tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , IL-4, IL-6 and IL-8 in HMC-1. Fisetin suppressed NF- $\kappa$ B activation induced by PMACI, leading to expression of I $\kappa$ B- $\alpha$  phosphorylation and degradation. Fisetin also suppressed NF- $\kappa$ B promoter-mediated luciferase activity and DNA-binding activity. In addition, fisetin inhibited PMACI-induced phosphorylation of p38 MAPK, ERK, and JNK. We observed that the selective inhibitors of p38 MAPK, ERK, JNK and NF- $\kappa$ B reduced PMACI-induced cytokine expression in activated HMC-1 cells. These data display that the inhibitory effect of fisetin on the pro-inflammatory cytokines was p38 MAPK, JNK, ERK and NF- $\kappa$ B dependent. Pharmacological actions of fisetin produce new suggestion that fisetin is a potential medicine for treatment of inflammatory diseases through the down-regulation of mast cell activation.

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## DOSE METRICS IN THE ACQUISITION OF SKIN SENSITIZATION : THRESHOLDS AND DOSE PER UNIT AREA.

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Allergic contact dermatitis is a common occupational and environmental health problem. Skin sensitization is acquired following topical exposure of an inherently susceptible individual to an appropriate amount of a contact allergen; that required to induce a cutaneous immune response of the magnitude necessary for a degree of immunological priming (sensitization) that will result in a dermal inflammatory reaction being provoked following subsequent exposure. The amount of chemical necessary to result in the acquisition of skin sensitization will be determined primarily by two factors: (a) the inherent potency of the contact allergen, and (b) the con-

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## Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

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