of the syringe suggesting loss due to MM's volatility. GA patch test reagents concentrations ranged from 27 to 45% of the labeled (1% in petrolatum) amount, independent of expiration date. No GA concentration pattern between tip and plunger was observed. These data suggest that false negative EPT results may be due to instability of volatile or self-polymerizing chemical allergens in the test reagents.

PS 1430 Adjuvant Effect of Dibutyl Phthalate (DBP) in an Animal Model of Contact Hypersensitivity.

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Recent studies have demonstrated that certain phthalates can have adjuvant effects in contact hypersensitivity models, exacerbating inflammatory responses. According to human exposure estimates, perfumes containing DBP can result in topic applications as high as 0.4 mg DBP/day. The aim of the present study was to investigate the adjuvant effect of DBP in the oxazolone-induced contact hypersensitivity model using human relevant doses. Adult male Balb/c mice were divided into 5 different groups (n=6/group). These animals received oxazolone (75 μg/animal) in hairless abdomen (induction). After five days, mice received oxazolone (75 μg/ear; positive control and DBP exposed groups) or vehicle (negative control group) in the right ear (sensitization). In addition, in the sensitization day and in the two subsequent days, DBP groups received three different doses (0.04, 0.4 and 4 mg DBP/ear) twice a day, while positive and negative controls received vehicle (acetone). All exposures were topical. For three subsequent days after sensitization ear thickness (edema) was measured with the use of a micrometer. After the last measurement, animals were decapitated and the ears were collected for the determination of N-acetyl-fl-D-glucosaminidase (NAG) and Myeloperoxidase (MPO) activity. The study was in accordance to the ethics committee of the Federal University of Paraná. Ear thickness was increased in positive control when compared to vehicle only (negative control) group. No difference was seen between positive control and the lowest DBP dose group. However, oxazolone-induced edema was increased in the groups treated with 0.4 and 4 mg DBP/ear when compared to positive control. Similar results were found in MPO and NAG activity. The groups treated with the two highest DBP doses displayed significantly higher enzymatic activity when compared to positive control group. These results indicate that human relevant doses of DBP can have adjuvant effects in the oxazolone-induced contact hypersensitivity in mice.

PS 1431 Characterization of the Mouse Allergy Model to Understand Mechanisms of Drug Allergy.

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Developed as a modification of the Lymph Node Proliferation Assay, the mouse allergy model (MAM) appears to be a promising tool for predicting the potential of drug development candidates to produce hypersensitivity reactions (HR). In this model, drugs associated with HR in the clinic produce a marked increase (compared to controls) in the cellularity of the draining lymph nodes (LN).The objective of this study was to characterize the phenotype of draining LN cells to identify new parameters that can be used to enhance the sensitivity and specificity of the MAM and to better understand the mechanism(s) for the response. Drugs that are associated with HR in the clinic (abacavir and amoxicillin) were selected as positive controls for this study. Negative control drugs (metformin and cimetidine) were selected based on the low number of reported HR for these compounds. Groups of 5 mice per group were injected subcutaneously with drug (100 mg/kg) or vehicle once daily for three consecutive days. After a two day rest, cells from the draining brachial LN were isolated and analyzed by flow cytometry. A significant increase in total LN cell number (compared to vehicle) was observed for mice treated with the positive control drugs. Compared to vehicle and negative control animals, an increase in CD4+ and CD8+ T cells and B cells was observed in the draining LN of abacavir and amoxicillin treated animals. Positive control drugs produced significant decreases (~25% compared to control) in the percentage of naïve T cells and increases (~27% compared to control) in the percentage of L-selectin (CD62L) and CD44 double-negative T cells. The negative control drugs induced slight, but statistically insignificant, changes in the expression of these markers. Drugs associated with HR in the clinic produced changes in draining LN cellularity and phenotype that are not observed for negative control drugs. Changes observed in adhesion molecule expression may suggest an effect of positive control drugs on lymphocyte trafficking.

PS 1432 Characterization and Comparison of Methylene Diphenyl Diisocyanate Haptenated Human Serum Albumin and Hemoglobin.

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Methylene diphenyl diisocyanate (MDI) is widely used as a cross-linking agent in the manufacture of polyurethane products. Exposure to diisocyanates (dNCO), such as MDI, is known to cause occupational asthma. MDI haptenation of proteins is central to dNCO immunological sensitization, however, the resultant protein conjugates are complex and difficult to characterize. The objective of the present study was to characterize hemoglobin (Hb) and human serum albumin (HSA) following conjugation to different molar concentrations of MDI. MDI-protein conjugates were acid digested to obtain free methylene dianiline (MDA). MDA was extracted, derivatized with fluorescamine and analyzed by HPLC-fluorescence. MDI-Hb was also digested with trypsin and specific amino acid conjugation sites determined by ultra-performance liquid chromatography-quadruple-tandem time-of-flight mass spectrometry. The trinitrobenzene sulfonic acid assay (TNBS) and denaturing gel electrophoresis were used to determine the extent of cross-linking. MDI conjugation was observed to be dependent on the MDI: protein ratio and the concentration of protein. Greater binding to HSA than Hb was observed and MDI bound to only eight binding sites on Hb compared to twenty for HSA (at 40:1 molar ratio of MDI: protein). Self-polymerization of MDI onto protein was observed on some amino acids at higher MDI concentrations. The TNBS assay was used to confirm cross linking in MDI-HSA with approximately 60% loss of amine reactivity at 40:1 MDI: HSA. More cross-linking was observed at 0.5 mg/ml HSA than at 5mg/ml at 40:1 MDI: HSA It is concluded that MDI has a greater reactivity toward HSA than Hb with respect to the number of residues haptenated and amount of MDI bound per mole of protein.
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PS 1433 Dimethylfumarate: Potency Prediction and Clinical Experience.

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Dimethylfumarate (DMF) was the cause of a major outbreak of allergic contact dermatitis as a consequence of its use as an antifungal agent in leather products, particularly when used in furniture. This became known, as "toxic sofa dermatitis". However, what has not previously been established is why the risks to human health had not been assessed adequately for this chemical. The purpose of these investigations was, therefore, to determine whether the frequency and severity of reactions to DMF arose as a function of its intrinsic skin sensitizing potency and/or the nature and extent of exposure. The intrinsic sensitizing potency of DMF was measured using the standard local lymph node assay (LLNA) with determination of an EC3 value; the threshold in the LLNA and which serves as an indicator of relative skin sensitising potency in humans. The EC3 value for DMF was 0.35% when tested in dimethylformamide as a vehicle, indicating that this chemical is a strong, but not an extreme, skin sensitiser in this mouse model. DMF was found therefore to have a relative sensitising potency that is comparable with formaldehyde, also a strong human skin sensitiser. The conclusion is drawn that the frequency and intensity of allergic contact dermatitis reactions to DMF suggest that it was the prolonged, repeated and occlusive exposure over large skin areas to this chemical, combined with strong sensitising potency, that together generated the "perfect storm" conditions that caused the DMF epidemic.

PS 1434 Characterization of the Allergenic Potential of Proteins: An Assessment of the Kiwifruit Allergen Actinidin.

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Assessment of the potential allergenicity (IgE-inducing properties) of novel proteins is an important component of the overall safety assessment of foods. Resistance to digestion with pepsin is commonly measured to characterize allergenicity, although the association is not absolute. We have shown previously that the measurement of specific IgE antibody production induced by systemic (intraperitoneal) exposure of BALB/c strain mice to a range of proteins correlates with

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