immunosuppressive agents with harsh side effects. Cannabinoids, which are a group of compounds derived from the marijuana plant (Cannabis sativa), are known to mediate their signals through the cannabionoid receptors, CB1 and CB2, and have been effective as treatment for cancer associated pain, nausea and appetite loss. Recently, their anti-inflammatory properties have been studied. Moreover, the use of cannabinoid therapy for MS has also been exploited. However, the proposed mechanism of action needs to be explored further. We used experimental autoimmune encephalomyelitis (EAE), a murine model of MS, to explore the anti-inflammatory role of cannabidiol (CBD) and its effects on myeloid-derived suppressor cells (MDSCs). EAE disease paradigms were consistently reduced with CBD treatment, as a significant reduction in clinical scores of paralysis and decrease in cellular infiltration, marked improvement of CNS tissue integrity, and reduced demyelination via histopathological analysis were observed. In addition, CBD treatment led to a reduction in the percentage and absolute number of T cells particularly the CD4+ T cells infiltrating the CNS (spinal cord and brain), which were significantly increased in the untreated EAE mice. However, there was a profound increase in MDSC induction in the spleens, CNS, and peritoneal wash of CBD treated EAE mice as compared to the untreated EAE controls. Both granulocytic and monocytic MDSCs were increased in CBD treated EAE mice. Together, these studies demonstrate that CBD treatment may ameliorate EAE via the induction of MDSCs which suppress the aberrant autoimmune response. (Supported by NIH grants R01 AT006888, R01 ES019313, R01 MH094755, P01 AT003961, P20 RR032684 and VA Merit Award BX001357).

PS 1426 Attenuation of Trichloroethene-Mediated Autoimmune Response in iNOS-null MRL+/+ Mice.

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Exposure to trichloroethene (TCE), a ubiquitous environmental contaminant, is associated with an autoimmune response both in humans and animal models. However, mechanisms underlying TCE-mediated autoimmunity remain largely unknown. Previous studies from our laboratory in MRL+/+ mice suggest that reactive oxygen and nitrogen species (RONS) may contribute to TCE-induced autoimmunity. The current study was undertaken to further assess the role of oxidative and nitrosative stress in TCE-induced autoimmunity by using iNOS-null MRL+/+ mice. iNOS-null mice were backcrossed to MRL +/+ mice for 10 generations and then N10 heterozygous mutants were intercrossed to obtain homozygous mutants. Female MRL+/+ and iNOS-null MRL+/+ mice were given TCE (10 mmol/kg, i.p., every 4th day) for 6 weeks; their respective controls received corn oil only. TCE treatment led to significant induction of anti-malondiadelhyde (MDA)- and anti-4-hydroxynonenal (HNE)-protein adduct antibodies along with increased iNOS in sera, and increased nitrotyrosine (NT) in sera, livers and kidneys in MRL+/+ mice, suggesting an overall increase in oxidative and nitrosative stress. The TCE-induced oxidative stress was also associated with significant increases in serum anti-nuclear antibodies (ANA) and anti-double stranded DNA antibodies (anti-dsDNA) levels. Interestingly, iNOS and NT levels were negligible in both controls and TCE-treated iNOS-null MRL+/+ mice. However, TCE treatment in iNOS-null mice still led to significant increases in serum anti-MDA-/HNE-antibodies along with increases in serum ANA and anti-dsDNA compared to controls. Remarkably, the increases in serum ANA and anti-dsDNA induced by TCE in the iNOS-null mice were significantly less pronounced compared to that in MRL+/+ mice. Our results provide further evidence for a role of oxidative/nitrosative stress in TCE-induced autoimmune response, and iNOS elimination attenuates this autoimmune response. Supported by NIH ES016302.

PS 1427 Mouse Model of Halogenated Platinum Salt Hypersensitivity.

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Occupational exposure to halogenated platinum salts can trigger the development of asthma. Concern for increased asthma risk exists for the general population due to the use of platinum (Pt) in catalytic converters and its emerging use as a diesel fuel additive. To investigate airway responses to Pt, we developed a mouse model of Pt hypersensitivity. Previously, we confirmed the dermal sensitizing potency of ammonium hexachloroplatinate (AHCP) using an ex vivo [3H]methyl thymidine labeling version of the local lymph node assay in BALB/c mice. Here, we investigated the ability of AHCP to induce airway responses in mice sensitized by the dermal route. Mice were sensitized through application of 100 µL 1% AHCP in DMSO to the shaved back on days 0, 5 and 19, and 25 µl to each ear on days 10, 11 and 12. Unsensitized mice received vehicle. On day 24, mice were challenged by oropharyngeal aspiration (OPA) with 0 or 100 µg AHCP in saline. Before and immediately after dosing, airway responses were assessed using whole body plethysmography (WBP). A dose dependent increase in immediate airway responses (IAR) was observed in AHCP sensitized and challenged mice. On day 26, changes in ventilatory responses to methacholine (Mch) aerosol were assessed by WBP; dose-dependent increases in Mch responsiveness occurred in sensitized mice. Bronchoalveolar lavage fluid harvested from sensitized mice contained an average of 7.5% eosinophils compared to less than 0.5% in control mice ($p < 0.05$); significant increases in total serum IgE was observed for all sensitized mice. This model will be useful for assessing both relative sensitizing potency and cross-reactivity between different halogenated Pt salts and for investigating the possible adjuvant effects of diesel exhaust particles. This abstract does not represent EPA policy.

PS 1428 Effect of Analgesic Administration on the Guinea Pig Maximization Test.

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Guinea pig maximization tests have been associated with inflammation at cutaneous induction sites due to the use of 1-chloro-2, 4-dinitrobenzene (DNCB) as a positive control and Complete Freund's Adjuvant (CFA). CFA enhances the sensitization potential of the test substances and its use is required by ISO 10993-10. To alleviate the potential for pain and distress, we evaluated the use of the analgesic, buprenorphine hydrochloride (HCl). Analgesics can modulate the inflammatory response and may interfere with the detection of contact sensitization. The purpose of this study was to determine if the administration of buprenorphine HCl, as a refinement to reduce the potential for pain and distress, would affect the results of the guinea pig maximization test. DNCB and Rubbercare® Gloves were used as the test articles. The experimental design was consistent with the procedures described in ISO 10993- 10 and the guinea pig maximization test (Magnusson and Kligman), with additional parameters evaluated. The experimental design consisted of 10 groups with each group receiving different concentrations of the test articles with or without analgesic treatment. Twenty-four to 30 hours after topical induction, the groups treated with buprenorphine HCl were given 0.006 mg/kg every 12 hours for a total of three doses. Three animals per group were terminated on study day 10 for hematology, coagulation and histologic evaluation of treatment sites. The remaining animals were terminated after completion of the sensitization test. Body weight gain, clinical observations, pain assessment, and sensitization potential were evaluated. Clinical observations, hematology, coagulation or histopathology of treatment sites were similar in groups that received analgesics compared to groups that did not. At least 60% to 100% of animals in each group were sensitized with no difference between corresponding groups with or without analgesic treatment. Based upon the results of this study, the use of the analgesic, buprenorphine HCl, did not interfere with the evaluation of the results of the guinea pig maximization test.

PS 1429 Chemical Assessment of "In-Use" Allergic Contact Dermatitis Patch Test Reagents from Dermatology Clinics.

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Epicutaneous patch tests (EPT) are commonly used to identify chemical agents of allergic contact dermatitis in dermatology patients. Test validity and assessment of allergic reaction severity are highly dependent on the use of reliable chemical allergen test reagents. The purpose of the present study was to measure the actual concentration of nickel sulfate (NiSO4), methyl methacrylate (MM), formaldehyde (FA) and glutaraldehyde (GA) compared to the labeled concentrations of commercial reagents found in dermatology clinics where patch testing is routinely performed. The commercial reagents, NiSO4, MM and GA are supplied either dissolved or suspended in petrolatum (usually in syringe, multiuse containers) while FA is diluted in water. Participating clinics submitted in-date and out-dated reagents to the laboratory for analyses. Both NiSO4 and FA levels were at or above the labeled concentration. NiSO4 particulate was uniformly distributed throughout the petrolatum. In contrast, MM was low and variable in commercial allergen reagents. "In-use" MM reagent syringes were all ≤56% of the 2% label concentration with no observable relationship to expiration date. One MM syringe purchased directly from the manufactured was 70% of the labeled concentration. Lower MM levels in syringes were consistently measured at the tip vs. plunger end

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.
This work was supported by an Interagency agreement with the NIEHS (Y1-ES-0001).

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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