the lungs in male and female rats starting at an age of 2 days. Furthermore, the practicability of nose-only inhalation treatment in juvenile rats as young as day 4 pp has been proven.
ASR data show the practicability of this test as early as day 10 pp with a coordinated animal response showing a significant pre-pulse inhibition around day 15 pp. The development of a gender-specific response starts around day 40 pp in coincidence with sexual maturation. Adult-like patterns of habituation can be assessed by day 60 pp.
The described techniques permit new insights into human neonatal risk assessment and therefore these animal models are suitable for regulatory studies. The presented data are important for correct planning and interpretation of juvenile animal studies.

PS 741 SKIN-SENSITIZING POTENCY OF HALOGENATED PLATINUM SALTS.

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The relationship between occupational exposure to halogenated platinum (Pt) salts and Pt-specific allergic sensitization is well-established. Although human case reports and clinical studies demonstrate that Pt salts are potent skin sensitizers, no studies have been published that investigate whether there are differences in potencies of halogenated Pt salts. In this study, we evaluated ammonium hexachloroplatinate (AHCP), ammonium tetrachloroplatinate (ATCP) and cis-dichlorodiamine (CDDP) using the local lymph node assay. For 3 consecutive days, BALB/c mice were dosed topically on the dorsum of both ears with vehicle, 25% trimellitic anhydride (TMA, positive control) or one of 3 concentrations of Pt salt. On day 5, lymph nodes were harvested and single-cell suspensions were labeled ex vivo with [3H]methyl thymidine. Lymphocyte proliferation was determined by scintillation counting. Concentration-dependent increases in ear thickness and lymphocyte proliferation were observed for all 3 Pt salts. None of the doses tested resulted in a skin irritant response since erythema was minimal and the maximum increase in ear thickness was less than 25% (n = 12). The EC3 values for AHCP, ATCP and CDDP were determined to be 0.58, 0.23 and 0.33%, respectively (n = 12). In complementary studies, lymph node cells were labeled ex vivo with bromodeoxyuridine (BrdU) to investigate an alternative to radioisotopic labeling. Incorporation of BrdU was determined by ELISA. In this case, the EC2 values for AHCP, ATCP and CDDP were determined to be 0.12, 0.95 and 0.17%, respectively (n = 6). The stimulation index of TMA was 4.6% (+/-1.2) and 2.9% (+/-0.3) in the radioisotope- and BrdU-labeling procedures, respectively. We conclude that AHCP, ATCP and CDDP are categorized as strong sensitizers according to the Globally Harmonized System for Classification and Labeling of Chemicals. Furthermore, these data suggest that BrdU labeling may not be as sensitive a procedure as labeling with radioisotope. This abstract does not reflect EPA policy.

PS 742 THE EFFECT OF ACTIVATING AND DEACTIVATING SUBSTITUENTS ON THE ALLERGENICITY OF BENZOQUINONE AND BENZOQUINONE DERIVATIVES.

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Benzoquinone (BQ) is an electrophilic contact allergen whose extreme skin sensitization (SS) potency is known to be mediated through Michael Addition (MA). It is also hypothesized that BQ will haptenate proteins via free radical formation. The reactivity and subsequent SS potency of BQ derivatives (BQD) is, however, unknown. The objective of this study was to assess the negative inductive effects (activating) and positive inductive effects (deactivating) of substituents on BQ reactivity to a nucleophilic thiol and utilize this in chemico assay to predict the allergenicity of BQD. Reactivity of BQ and BQD to nitrobenzenethiol (NBT) was used as a surrogate for protein binding of the electrophiles. Pseudo-first order rate constants (k) of chlorine, methyl and t-butyl substituted BQD reactions to NBT were determined at pH 7.4 and 5.5. Electron paramagnetic resonance (EPR) studies to probe a potential free radical mediated binding mechanism of BQD to nucleophiles were also performed. The k values demonstrated the chlorine substituted (activated) BQD to be more reactive than the methyl and t-butyl substituted (deactivated) BQD, correlating with the respective EPR intensities. The results suggest that binding of BQD to proteins may also occur via free radical mechanism which is pH dependent, in addition to the predominant MA mechanism. Local lymph node assays (LLNA) for BQ and BQD were conducted in BALB/c mice. The LLNA EC3 values for the BQD were of the order; chlorine < methyl < t-butyl substituted BQD, consistent with that predicted by reactivity data. Results of the present study suggest potential utility of employing chemical reactivity data for electrophilic allergen identification and potency ranking.

PS 743 REACTIVITY PROFILE OF CONTACT AND RESPIRATORY LOW MOLECULAR WEIGHT ALLERGENS IN A COMPETITIVE PEPTIDE REACTIVITY ASSAY.

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It is well established that certain low molecular weight chemicals cause allergic diseases of the skin and respiratory tract. Individual chemicals are typically associated primarily with one or other form of disease, generating selective Th1 or Th2 type responses. The reasons for this divergence are unclear; however selective modification of specific amino acids may play a role. The reactivity of chemical allergens to single nucleophile peptides is increasingly well-described with standardized assays for use in hazard assessment; adapting these methodologies to evaluate competitive binding to multiple nucleophiles may assist in defining preferential modifications. Using a peptide reactivity model, the reactivity of reference respiratory (phthalic anhydride [PA], maleic anhydride [MA]) and skin allergens (dinitrochlorobenzene [DNCB], dinitrofluorobenzene [DNFB]) were investigated. One set of assays was conducted by reacting peptides containing either cysteine (Cys) or lysine (Lys) alone with an excess concentration of test chemical. To evaluate the effect of competition, assays were conducted by preparing reaction mixtures of these same peptides at various concentrations relative to the other. The ratios utilized were 1:1, 3:1, 6:1 and 9:1; in each case the total peptide content was constant. When incubated with single peptides PA and MA (respiratory allergens) were observed to have increased reactivity to Lys, while DNCB and DNFB (contact allergens) preferentially reacted to Cys. These preferences were conserved or enhanced under competitive conditions. Under such conditions PA and DNCB were observed to deplete exclusively Lys and Cys, respectively. MA and DNFB both maintained the preferences observed with individual peptides. The selective reactivity demonstrated here may have important mechanistic relevance for the ability of these chemicals to induce divergent immune responses.

PS 744 CHANGES IN THE FREQUENCY OF B220$^+$ LYMPHOCYTES FOLLOWING TOPICAL EXPOSURE TO LINALOOL.

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Linalool is a terpene alcohol that is known to undergo autoxidation forming a variety of hydroperoxides and other degradation products. Linalool is not considered to be a skin sensitizer; however, the products formed during autoxidation are known to be contact allergens. In the murine local lymph node assay (LLNA), high purity samples of linalool have been reported to have a weak sensitization potential. As with other predictive test methods, false-positives are known to occur in the LLNA, particularly to subsets of certain classes of skin irritants. While these have not typically presented interpretive difficulties, strategies have been developed to assist in eliminating false positives in the LLNA. One approach is to align the LLNA with monitoring the frequency of B220$^+$ lymphocytes in skin draining lymph nodes (LN). In the present study, assays were conducted by topically treating CBA/Ca mice with high purity linalool (50% or 100%; both with and without an antioxidant), dinitrochlorobenzene (DNCB; 0.25%; contact allergen control), benzalkonium chloride (BZC; 2%; irritant control) or with vehicle (acetone) alone using the standard LLNA dosing regimen. Draining LN were isolated and the frequency of B220$^+$ cells measured by flow cytometry and expressed as a ratio of the test to vehicle control groups. Treatment with DNCB induced a marked increase in B220$^+$ cell frequency, with ratios ranging from 2.5 to 4.4 fold. Exposure to BZC resulted in low level increases in B220$^+$ cell frequency, with ratios ranging from 1.2 to 1.4 fold. Linalool was not observed to cause significant increases in B220$^+$ cells; ratios of ≤ 1.2 were obtained for all treatment groups. These low levels were very consistent with those recorded for the concurrent irritant control BZC. These data suggest that the positive responses reported for high purity linalool in the standard LLNA maybe due to the irritant properties of the material.

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