236 CROSS-REACTIVITY OF ACID ANHYDRIDES ASSESSED BY AIRWAY CHALLENGE IN RATS SENSITIZED WITH TRIMELLITIC ANHYDRIDE (TMA).

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Previous studies using inhaled organic acid anhydride (OAA)-protein conjugates in studies of airway hyperreactivity have revealed immunologic cross-reactivity among OAAs. The present investigation evaluated cross reactivity between phthalic anhydride, (PA), tetrachlorophthalic anhydride (TCPA) and TMA with respect to their ability to induce asthma-like airway responses in TMA-hapten-sensitized Brown Norway rats. Four to 40 mg of dry TMA powder was applied to anesthetized rats' backs (hair removed with scissors) on days 0, 7, 14 and 21 and washed off with water 4 hours after each application. Inhalation challenge with TMA aerosol (40 mg/m^3;10 min) was performed on day 35 and Penh, an index of airway resistance, was measured. The sensitized rats developed early(EAR)- and late(LAR)-phase obstructive responses. One week after TMA inhalation exposure, rats were challenged with TMA, PA or TCPA aerosol (40mg/m^3, 10 min; n = 8 in each group). EAR developed in all rats challenged with TMA, 3 rats challenged with PA, and 7 rats challenged with TCPA. The LAR developed in all TMA-challenged, sensitized rats, but none of the TMA sensitized rats challenged with PA or TCPA developed the LAR. We have previously reported that low dose TMA airway challenge results in EAR-only similar to the airway responses observed in the present cross-reactivity studies. It is concluded that cross-reactivity exists between TMA and both PA and TCPA. These airway responses are most likely immune mediated and the reason why this cross-reactivity was manifested only by EAR may be related to lower relative antigenic potency of PA and TCPA to that of TMA in TMA-sensitized rats.

237 INHALATION EXPOSURE OF TRIMELLITIC ANHYDRIDE (TMA) AEROSOL IN A BROWN NORWAY RAT MODEL.

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Inhalation is one of the TMA exposure routes for sensitization and subsequent asthma. The present work reported some characteristics after inhalation exposure of TMA aerosol. Brown Norway rats (n=8/group) were exposed, once a week from day 0 to day 63 with 40 mg/m^3 TMA or to day 70 with 4 mg/m^3 TMA in a nose-only system for 10 min. Enhanced pause (Penh, an index of airway resistance) was recorded in Buxco chambers overnight (⟩12 hours). Sera were collected weekly for antibody analyses. For the group with 40mg/m^3 of TMA, specific IgE level was significantly increased after day 7 and reached the peak by day 49. By day 14 these rats developed early- (EAR) and late-phase airway responses (LAR) following exposure. For the group exposed to 4mg/m^3 of TMA, specific IgE level was significantly increased by day 21 and reached the peak by day 56; however, no obviously airway responses were noted. Two weeks after the final exposure the 4 mg/m^3 TMA exposed rats were challenged with 40 mg/m^3 TMA resulting in both an EAR and LAR response. The specific IgE levels for rats with high dose of TMA were greater than that with low dose of TMA. We conclude that high dose and short term inhalation exposure may induce specific IgE and airway responses; low dose and short term exposure can induce specific IgE production to establish sensitization, while the development of airway responses needs high dose of TMA for challenge.

238 INTERSPECIES VARIATION AND LINEAGE SPECIFICITY IN HEMATOPOIETIC TOXICITY TESTING.

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In the search for efficient and cost effective ways to screen lead compounds for hematotoxicity, the use of Colony Forming Cell (CFC) assays has received a great deal of attention. These standardized assays allow the detection of toxicity on hematopoietic progenitor subsets (erythroid, myeloid, megakaryocytic) to evaluate potential cytopenic conditions, as well as mesenchymal progenitors to evaluate potential damage to the bone and connective tissue. As these assays require the growth of primary cells in culture for up to 14 days, they offer a high level of sensitivity and allow the quantitative and qualitative evaluation of colony growth and morphology, providing high functional content. Using CFC assays, the toxic effects of three antineoplastic compounds were tested on erythroid (CFU-E, BFU-E), myeloid (CFU-GM) and mesenchymal (CFU-F) progenitor growth. 5-Fluorouracil (5-FU), Hydroxyurea (HU) and Paclitaxel were incubated in culture with both human and murine bone marrow progenitor cells for 10-14 days, after which colonies were scored and assessed. Results indicate that each compound displays a unique spectrum of toxicity on each progenitor lineage; erythroid, myeloid and mesenchymal lineages showing different relative susceptibility to toxicity depending on the compound tested. In addition, up to 10-fold differences were seen between human and murine progenitor sensitivity to each compound. Our data highlights the multifaceted nature of bone marrow and unique specificity of action of individual compounds. The results also illustrate the importance of using the most appropriate species model in preclinical studies to improve predictivity and clinical success.

239 A HIGH THROUGHPUT CELL-BASED ASSAY FOR ASSESSMENT OF HEPATOTOXICITY USING CRYOPRESERVED HUMAN HEPATOCYTES.

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Screening for hepatotoxicity during early stage of drug discovery may add another important tool, among others, to improve the quality of drug candidates advancing into lead optimization. We report here a high throughput cell-based assay (384-well plate) capable of detecting acute hepatotoxicity. Test compounds were exposed to cryopresevered primary human hepatocytes for 18 hours and cytotoxicity was measured using a luciferin/luciferase assay that quantifies cellular ATP levels. In a proof of concept study, we evaluated 30 reference compounds with known hepatotoxicity along with 8 non-hepatotoxicants using human hepatocytes obtained from three donors. Dose-dependent cytotoxicity was observed for 23 of the potential hepatotoxicants, such as aflatoxin B, tamoxifen, troglitazone, ketoconazole and flutamide, with measurable TC50 values (<115 uM) within the test concentration range. No apparent cytotoxicity was observed for the other 6 compounds (TC50 >500 uM) including phenytoin, Carbamazapine and crotaline. However, all of these six except carbon tetrachloride were reported hepatotoxic as a result of combination therapy with other drugs. For the 8 non-hepatotoxicants tested (Buspirone, Theophyline and Ibuprofen etc), none of them showed cytotoxicity (TC50 >500 uM). This data indicates that our assay is reasonably predictive in detecting acute hepatotoxicity of drug candidates and can be employed in the high throughput mode during lead optimization process. It should also be of interest for retrospective mechanistic studies of heptotoxicity observed *in vivo*.

240 PHASE I AND II RESULTS OF A VALIDATION STUDY TO EVALUATE *IN VITRO* CYTOTOXICITY ASSAYS FOR ESTIMATING RODENT AND HUMAN ACUTE SYSTEMIC TOXICITY.

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Studies have identified a correlation between *in vitro* cytotoxicity and acute oral toxicity. NICEATM and ECVAM initiated a three-phase multi-laboratory validation study to evaluate the usefulness of two standardized *in vitro* basal cytotoxicity assays for estimating rodent and human acute toxicity and the extent that they may reduce animal use. Seventy-two coded chemicals (12 from each of five acute oral hazard categories; 12 unclassified/nontoxic chemicals) will be tested in mouse 3T3 fibroblasts and in normal human epidermal keratinocytes (NHK) using neutral red (NR) uptake assays. Protocols were optimized after each of the first two phases to minimize intra- and inter-laboratory variation prior to testing 60 chemicals in Phase III. Phase Ia established the historical databases for the positive control chemical, sodium laurel sulfate (SLS), for each of three laboratories. Three chemicals were tested in Phase Ib and nine in Phase II. Technical challenges arose in Phases Ia/Ib (i.e., formation of NR dye crystals; uneven growth of NHKs; slow growth of 3T3 cells) that were resolved with Phase II protocols. Significant variation in NHK growth occurred in Phase II with various lots of media and supplements. The optimized final protocols are being tested in Phase III. Rodent oral LD50 values were estimated using prediction models based on the Registry of Cytotoxicity data and Phase I/II results. Human toxicity will be estimated using a prediction model based on data from human poisoning reports and the Multicentre Evaluation of *In Vitro* Cytotoxicity (MEIC). Supported by: N01-ES-35504, N01-ES-75408; EPA IAG DW-75-93893601-0; European Commission 19416-2002-04 F2ED ISP GB.

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