sitization and intranasal challenge compared to corresponding non-sensitized mice. Only TMI or HMDI sensitized and challenged mice, however, had increases in methacholine-induced airflow obstruction. While the cytokine response is predictive of the airway response to HMDI, TMXDI or DNCB, it is not predictive of the airway response to MDI, TMI, IPDI, or TDI. The data suggest that cytokine responses at the site of dermal exposure to a chemical respiratory sensitizer may not be predictive of the airway response after airway challenge (This abstract does not reflect EPA policy).

375 INITIAL INTERACTIONS OF ALLERGENS WITH AIRWAY EPITHELIAL CELLS: ANY ROLE IN THE INDUCTION OF TYPE I ALLERGY?

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Type I respiratory allergy is a Th2 dominated immune response to a protein allergen with development of allergen-specific IgE. One of the first cell types to come in contact with the allergen is the airway epithelial cell. The initial interaction of allergen with host cells may be crucial in determining whether a Th2-dominated (vs Th1-dominated) immune response will occur. In this study we examined the interactions of known type 1 allergens with the human airway type II-like bronchial epithelial cell line A549. A549 cells are grown on membrane filters such that the apical surface is exposed to air and the basal side is exposed to liquid medium (Novozymes design). The air/liquid interface system allows for better dosing of protein to the cells as compared to cells grown submerged in liquid medium. Culturing A549 cells with detergent enzyme protease allergens (Alcalase®, Savinase®, BPN'Y217L), amylase allergens (Fungamyl®, Termamyl®) or house dust mite rDer P 1 induced secretion of the pro-inflammatory cytokines IL-8 and MCP-1 while a non/poor-allergenic protein (bovine serum albumin, BSA) did not. IL-6 and GM-CSF proteins were not detected in the supernatants of allergen-stimulated A549 cells but this may be due to sensitivity to degradation by the detergent enzymes. Initial experiments using Luminex cytokine bead analysis of Alcalase-exposed A549 cells showed specific secretion of IL-1ra, IL-7, and IL-15 that was not induced by BSA. Incubation of A549 cells with the allergens was not cytotoxic as measured by Alamar Blue®. Reflective microscopy showed no change to the cell layer following exposure to enzyme. These allergens also consistently induced the secretion of M-CSF which has not been reported to be involved in type I allergy but may provide additional information on the mechanism of induction of the Th2 response. Additional cytokines/chemokines and other proteins are being tested. Whether the response of A549 cells can be used to screen proteins as potential allergens remains to be determined.

376 *IN VITRO* CHARACTERIZATION OF DENDRITIC CELL (DC) RESPONSES TO A CHEMICAL ALLERGEN AND A SKIN IRRITANT

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DC are key cells in the initiation of immune and allergic responses. Methods have been developed recently for the generation of these cells in culture from precursors. In the present series of experiments, mouse bone marrow derived DC were cultured for 24h with the chemical allergen dinitrobenzene sulfonic acid (DNBS; 0.1, 0.5 and 1mM), or with the non-sensitizing skin irritant benzene sulfonic acid (BS; 10, 50 and 100mM). Treatment with both chemicals was associated with a dose-dependent increase in cell death, determined by dual staining for annexin V/propidium iodide (PI), with a 90% loss of cell viability observed at 1mM DNBS and 100mM BS. However, whereas 1mM DNBS induced 5-fold more late (annexin V+/PI+) apoptotic cells than early (annexin V+/PI-) apoptotic cells, the converse was observed for 100mM BS with a 2-fold increase in early:late apoptotic cells. Flow cytometric analyses of DC for the maturation markers MHC class II (Ia), CD80, CD86, CD40 and CD54 revealed that exposure of DC only to DNBS at sub-toxic doses (0.5mM; 25% decline in cell viability) caused increased expression of Ia, CD80 and CD86. BS failed to cause DC maturation at any dose tested despite similar decreases in cell viability. Analysis of culture supernatants by cytokine array for interleukin (IL)-1α, 1β, 2, 6, 10, 12p40, 12p70, 17 and tumor necrosis factor α (TNF-α) revealed only modest changes in cytokine secretion associated with chemical treatment. However, co-culture with suboptimal doses of the known DC activators PAM3CSK4 (toll-like receptor [TLR] ligand 1/2) and macrophage-activating lipopeptide 2 (TLR 6/2), together with lower doses of BS, resulted in enhanced secretion of the proinflammatory cytokines IL-1α, IL-6 and TNF-α. No such synergy was observed for DNBS. These experiments suggest that chemical allergens and skin irritants interact differently with DC and raise the possibility that such differences may be exploited for the development of in vitro skin sensitization assays.

377 THE REDUCED LLNA AS A HAZARD IDENTIFICATION SCREEN FOR SKIN SENSITISATION

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For the identification of potential skin sensitising substances, the local lymph node assay (LLNA) has, for many years, been accepted as a full replacement for guinea pig methods. In part this was due to the scientific advantages provided by the LLNA, but also due to the significant animal welfare benefits this method offers. However, the desire to reduce further animal usage is in conflict with the need to complete toxicological evaluations of approximately 30,000 chemicals under new European legislation (REACH: Registration, Evaluation and Authorisation of Chemicals). Consequently, we have conducted a retrospective evaluation of a recently published LLNA dataset comprising 211 chemicals. The work addressed whether it is possible to obtain a satisfactory identification of skin sensitisation hazard using only a comparison of the top dose group with the concurrent vehicle treated control. In these analyses, negative results (ie non-sensitisers) were not accepted if the top dose group was treated with <10% of the test chemical. The results indicated that this reduced LLNA would have provided about 99% accuracy of identification of sensitisers and non-sensitisers: 166 of 168 sensitisers were correctly identified, as were all non-sensitizers (42/42). Only one chemical was tested below 10% and, being negative, had a result which was regarded for these purposes as invalid. Thus, overall, the reduced LLNA gave 208/210 predictions that were identical to the full assay; in only instance was the interpretation inconclusive due to the use of a low test concentration. In our view, it would be possible, for certain specific purposes, and in some circumstances, to consider the utility of a reduced LLNA as a contribution to a reduced requirement for animal numbers.

378 EVALUATION OF THE PERFORMANCE OF CANDIDATE GENES FOR PREDICTING SKIN SENSITIZATION POTENTIAL

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Previously we analyzed by real-time PCR analysis 60 candidate genes identified from microarray transcript profiling studies for their potential to serve as markers for the prediction of contact allergy. Twenty-nine of the 60 genes were found to display sensitivity and selectivity when analyzed with 3 strong allergens, 1 irritant, and 1 non-senstizer and thus were analyzed comprehensively using a training set of chemicals (n=20) that included multiple allergens, irritants, and non-sensitizers. Therefore, to prioritize the 29 genes of interest with respect to their robustness and selectivity the genes were scored according to their expression level induced by additional allergens and irritants. Scoring of fold-changes was made by determining a positive response for a gene in all chemicals tested and the number of chemicals tested per gene. A positive response was defined as being greater than a 2.0 fold change in the direction identified originally in the initial DNBS microarray experiment at one of the doses tested. In addition, a gene was scored positive regardless of the number of times it registered a positive in any experiment tested with that particular chemical and the number of times that a particular gene was tested and registered as positive or negative for a chemical was also included. Several of the analyzed genes have emerged as better predictors of DC activation and we have denoted the top 10 genes that display the most promise based on our selection criteria of fold-change levels induced in individual experiments and the scoring results and thus show potential for use in the development of an in vitro assay for skin sensitization testing. Validation of these genes with an expanded test set of chemicals is required and will be conducted with a fluorescent microsphere-based high throughput screening (HTS) method.

379 USE OF A MULTIDISCIPLINARY APPROACH THAT IDENTIFIED PHENOLIC DERMAL SENSITIZERS IN A WOUND CLOSURE TAPE

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A latex allergic patient exhibited a severe local reaction to a non-latex wound closure tape following surgery. A tape sample was extracted in acetonitrile (10 ml/g) and analyzed by GC-EI-MS (Gas Chromatograph-Electron Impact-Mass

Spectrometry) in the total ion monitoring mode. Components were identified by their ion mass fingerprint and elution at the same time as a corresponding standard from the GC column. The chemicals identified were 5-tert-butyl-4-hydroxy-2-methylphenyl sulfide (TBHMS), 2-tert-butyl-5-methyl-phenol (TBMP), 2,4-di-tert-butylphenol (DTBP), and erucamide (EA). Potential for sensitization for these chemicals was evaluated using two quantitative structure-activity relationship (QSAR) programs. The potential for TBHMS (the most potent sensitizer) to be a sensitizer was predicted by both DEREK for Windows and TOPKAT 6.1. The potential for TBMP and DTBP to induce sensitization was predicted by DEREK for Windows but not TOPKAT. These three phenolic chemicals were then tested for their sensitization potential using a modified local lymph node assay (LLNA). With the exception of TBMP (highest concentration 12.5% due to toxicity) test articles were evaluated at 50%, 25% and 12.5%. None of the animals exhibited body weight loss or skin irritation. LLNA analysis of the chemicals identified TBHMS>TBMP>DTBP as potential sensitizers with EC3 values of 0.2, 4.5, and 6.8 respectively. Following a clinical observation, these studies used a multidisciplinary approach combining GC-EI-MS to identify chemical components, QSAR to prioritize chemicals for animal testing, and the LLNA to determine the sensitization potential of chemicals in the product. Human patch testing will be conducted to confirm these findings. These studies were supported in part by IAG# NIEHS Y1-ES0001-06.

380 MODE OF ACTION AND THE INHALATION CARCINOGENICITY OF NAPHTHALENE

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The inhalation carcinogenicity of naphthalene is under evaluation by U.S. EPA's Integrated Risk Information System (IRIS) Program. Naphthalene is used as a moth repellant and toilet bowl deodorizer and is a constituent of polycyclic aromatic hydrocarbon mixtures, particulate organic matter, jet fuels and fossil fuels. In a two-year inhalation rat bioassay conducted by the National Toxicology Program (NTP, 2000), statistically significant increases in the incidences of two rare nasal tumors, olfactory neuroblastomas in male and female rats and adenomas of the respiratory epithelium in male rats, were observed. Increased incidences of lung adenomas and carcinomas were observed in female mice (NTP, 1992). The mode of action (MOA) of naphthalene carcinogenicity may involve metabolic intermediates, including naphthalene-1,2-oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone, which may damage tissue macromolecules either directly by their inherent electrophilicity or by the generation of reactive oxygen species. Naphthalene has produced negative results in most genotoxicity and mutagenicity tests; however, the role of genotoxicity in the MOA has not been characterized in target tissues. In addition, an understanding of the MOA is inadequate for determining why rats, but not mice, develop tumors originating in nasal tissues even though both species show nonneoplastic lesions in nasal epithelial tissues. According to EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), data on the MOA of naphthalene carcinogenicity are lacking. U.S. EPA held a peer consultation workshop in April 2005 to discuss further research to characterize naphthalene's carcinogenic MOA. (The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA).

381 AGE RELATED DIFFERENCES IN SUSCEPTIBILITY TO CARCINOGENESIS—A PRELIMINARY CLASSIFICATION OF NON-MUTAGENIC MODES OF ACTION TO STRUCTURE QUANTITATIVE ANALYSIS OF BIOASSAY DATA

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Previous work analyzed age-related differences in carcinogenic susceptibility for chemicals with a mutagenic mode of action. This work sets the stage for similar analyses for non-mutagenic modes of action. These are diverse, with potentially different implications for age-related differences in sensitivity. We therefore believe it is important to have a biologically-based classification to reveal such differences if they are present in the data. Our emerging system is structured around the classic initiation-promotion-progression framework for carcinogenesis:
(1) Pre-initiator or co-initiator modes of actions [e.g. induction of enzymes that activate parent chemicals to more DNA reactive forms (e.g. ethanol induction of CYP2E1, increasing susceptibility to vinyl chloride), inhibition of DNA repair attributed to a number of metallic ions, and expansion of the numbers of potential target stem cells that are susceptible to transformation].
(2) Promoter modes of action (e.g. increases in the replication rate or decreases in the death/differentiation rate of initiated cells).
(3) Progressor modes of action, (e.g. facilitation of the growth/spread of fully transformed cells; inhibition of growth repression; or impairment of immune surveillance).
Theory suggests early-life relative susceptibility for the first of these groups, and late-life relative susceptibility for the third. The discussion will cover details of the modes of action within each group, and experimental approaches to practically distinguish among the groups for specific non-mutagenic agents. We recognize that the limited number of agents with good early-life data, and chemical to chemical differences, may limit the applicability of grouped analyses.
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382 APPROACHES FOR DERIVING RELATIVE CARCINOGENIC POTENCY FACTORS FOR POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

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U.S. EPA's Integrated Risk Information System (IRIS) Program is undertaking a health assessment for PAH mixtures that considers three approaches (i.e., comparative potency, surrogate mixture, and relative potency factor) for estimating cancer risk. The relative potency factor (RPF) approach provides a cancer risk estimate for the PAH mixture by summing the carcinogenic potential of the individual PAHs relative to an index compound (e.g., benzo[a]pyrene [BaP]). This approach has been developed for seven individual PAHs in the Provisional Guidance for Quantitative Risk Assessment of PAHs (U.S. EPA, 1993) and is utilized extensively within U.S. EPA program offices and other regulatory agencies; however, this guidance does not reflect the most recent research, nor does it consider additional PAHs with carcinogenic potential (e.g., fjord-region PAHs). Approaches to RPF development are being evaluated based on current data. A thorough literature search identified in vivo cancer bioassays and both in vivo and in vitro assays of cancer-related endpoints (e.g., mutagenicity, clastogenicity, malignant transformation, and DNA adduct formation) for 74 PAHs. Dose-response information was extracted from studies in which at least one PAH was studied in addition to BaP, resulting in data for one or more endpoints for 49 PAHs. Several options for deriving a single RPF value for individual PAHs with multiple-endpoint datasets are being considered. One approach uses a framework in which RPFs from studies of greatest relevance and quality are preferred over RPFs from studies with quality concerns or those from endpoints with less certain links to tumor formation. Other approaches utilizing statistical based observations are also under consideration. (The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA).

383 DETERMINATION OF THE MAGNITUDE OF INTRASPECIES DIFFERENCES IN RED BLOOD CELL CHOLINESTERASE INHIBITION IN RESPONSE TO DICHLORVOS EXPOSURE

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Uncertainty factors are used in non-cancer risk assessments to account for potential inter- and intra-species sensitivity differences in response to chemical exposure, with a default 10-fold value for each. In the case of pesticides, an additional child-specific factor may be added to account for the potentially increased sensitivity of children. In the case of one pesticide, dichlorvos (DDVP), there is a large body of relevant human data, much of it from clinical studies examining potential pharmaceutical applications, that can be used to address these extrapolation issues. Published medical and toxicological literature and unpublished data generated in support of product registrations were reviewed, appropriate studies with useful dose-response information were identified, and selected data were analyzed to assess dichlorvos-specific intraspecies sensitivity differences. The critical effect identified as the response indicator was red blood cell (RBC) cholinesterase inhibition. Levels of inhibition (expressed as percent of normal RBC cholinesterase activity) were identified and plotted versus the dose and duration of exposure producing that effect. The data demonstrated that the DDVP doses producing specific levels of inhibition in various subpopulations of humans are virtually identical to those that elicit the same inhibition levels in healthy adults. There is thus no apparent difference in the extent of RBC cholinesterase inhibition following DDVP exposure that can be attributed to gender, age or certain disease states. The data indicate that the intraspecies uncertainty factor should be reduced to below 10 when RBC cholinesterase is the endpoint of concern.

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 45th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, March 5–9, 2006.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 500.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

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