PS 82 COMPARISON OF 3H-THYMIDINE INCORPORATION AND NON-RADIOACTIVE CELL COUNTEND AS ENDPOINTS OF THE LOCAL LYMPH NODE ASSAY (LLNA) IN ROUTINE TESTING.

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The local lymph node assay as described in the OECD guideline 429 is based on measuring lymph node cell proliferation by 3H-Thymidine incorporation into the lymph node. Alternative endpoints were evaluated recently (Basketter et al. 2008). The evaluation of cell proliferation by the cell counts in the single cell suspensions produced from the ear lymph nodes (LLNC) as described by Vohr et al. (2000) proved to be useful for evaluation of LLNA if the cut off stimulation index (SI) for positive tests was adjusted to 1.5, reflecting the overall range of cell count increase (Ehling et al. 2005). This has been demonstrated in a multi-center study and a study on 13 epoxy resin constituents (epoxides and amines) (Gamer et al. 2008). We present data from routine studies with a 170 industrial chemicals and agrochemical formulations (54% were identified as sensitizers, 74% as irritants, and 46% as both sensitizers and irritants) and additionally the 24 substnces of the performance standard list obtained during routine testing using both the LLNC and the radioactive endpoints (dpm). Results indicate that there is a correlation between LNCC and dpm values. Furthermore equivalent estimated concentrations (ECs) for the prediction of skin sensitizing potency are obtained in the majority of cases with both measurements. The results indicate that if an adjusted reference SI is used for LNCC, equivalent designation of test substances as sensitizers were obtained compared with 3H-thymidine incorporation. Overall, 3H-thymidine incorporation identified more substances as weak and LNCC identified more as moderate or strong.

PS 83 VEHICLE-DEPENDENT EFFECTS ON HEXYLCINNAMALDEHYDE RESPONSES IN THE LLNA.

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Hexylcinnamaldehyde (HCA) is the default, preferred positive control substance in the Local Lymph Node Assay (LLNA), due to its moderately potent dermal sensitizing properties. We have tested the effects of use of varying vehicle solvents for HCA on the endpoints of Stimulation Index (SI), lymphotype subsets (immunophenotyping; IP), and irritation as measured by ear swelling. Herein, we show that the choice of vehicle does biologically and statistically significantly ($P>0.05$) impacts the HCA endpoint values, most importantly, the SI. HCA at 25% was evaluated in all vehicles (AOO, DMSO, Acetone, Ethanol and DAE433, petrolatum, PG, etc.), and compared to naïve or untreated controls, as appropriate. Most importantly, some vehicles (besides the default AOO) that are commonly used were more prone to causing or enhancing irritation induced by vehicle-alone treatment or either decreased or increased SI values and variability when compared to AOO. Generally, DAE433 was a 'better' vehicle than DMSO alone, resulting in lower background proliferation and less irritation (ear swelling day1 through Day 6). Petrolatum gave good results as a vehicle for 25% HCA with an SI=10.3, comparable to AOO SI=9.8 and DMF SI=9.9. Acetone and PEG had significantly higher SI values and B:T cell ratios than DMSO and AOO. DMSO and DMA had the lowest SI values for 25%HCA, at 7.8 and 5.8, respectively. In addition, DMSO was significantly irritating to the ears of mice as a vehicle, and caused very pronounced ear swelling when used as a vehicle for HCA (>15% increase). In conclusion, the vehicle chosen for the LLNA can significantly affect multiple endpoints of interest in the assay, especially the Stimulation Index, and that this variability should be taken into account when testing similiar or borderline test substances in vehicles other than AOO.

PS 84 RESPIRATORY TRACT RESPONSES IN WISTAR AND BN RATS, SENSITIZED AND CHALLENGED BY INHALATION WITH THE CONTACT ALLERGEN DINITROCHLOROBENZENE (DNCB).

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All chemical respiratory allergens studied sofar can also induce skin sensitization/allergy in test animals. The question is if in turn contact (skin) allergens can induce allergy in the respiratory tract. The contact allergen dinitrochlorobenzene (DNCB) was tested first in Th2-prone Brown Norway (BN) rats, using a protocol that successfully identified chemical respiratory allergens like trimellitic anhydride. Dermal sensitization induced DNCB-specific IgG in serum. A subsequent single inhalation challenge with DNCB did not provoke apnoeic breathing or allergic inflammation in the respiratory tissues (signs of respiratory allergy), but the allergy-associated genes for Ccl2 (MCP-1), Ccl4 (MIP-1beta), Ccl7 and Ccl17 were upregulated in lung tissue. Next, DNCB was tested in Th1-prone Wistar rats. Again, a single inhalation challenge in sensitized rats did not provoke apnoeic breathing, but induced a minimal lymphocytic infiltrate in the nasal tissues and larynx. Repeated inhalation challenges (twice a week for 4 weeks) in Wistar rats induced DNCB-specific IgG antibodies in serum and a pronounced, predominantly lymphocytic, inflammation in the nasal tissues and larynx. The inflammation may be the upper respiratory tract analogue of hypersensitivity pneumonitis/allergic alveolitis. The relevance of these findings to man, and possible progression of the airway inflammation should be investigated to support or dismiss discrimination between contact and respiratory allergens in relation to respiratory allergy.

PS 85 STEPS TOWARDS THE DEVELOPMENT OF AN INTEGRATED APPROACH FOR THE PREDICTION OF SKIN SENSITIZATION POTENTIAL USING DATA FROM SEVERAL ALTERNATIVE TEST SYSTEMS.

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A number of different in vitro, in chemico and in silico methods have been developed to examine the skin sensitization potential of chemicals. However, it is the general view that a single test method cannot replace the current animal tests for such a complex endpoint. Therefore, development of an integrated approach is needed which incorporates data from several test systems for the prediction of the skin sensitization effects of chemicals. Two approaches are being taken to develop a prediction model that would quantify in some way data for epidermal bioavailability generated in silico, peptide reactivity from an in chemico assay and dendritic cell activation from an in vitro U937 cell line-based test system. The first approach uses recursive partitioning methodology in order to build a classification tree. The algorithm starts with the complete data set and seeks to partition it into separate subgroups of more homogeneous subsamples. An optimal split variable and a corresponding threshold value are selected. Then the sample is split into two subgroups: (1) observations with split variable values less than the threshold, and (2) observations with split variable values greater than the threshold. This same binary partitioning is then applied separately to the two subsets. The process is repeated until the increase in subset homogeneity and/or the resulting sample sizes are too small to continue. The second approach explores use of a Bayesian network which in addition to integrating data, can serve as a decision tool for guiding testing strategies based on identification of the most informative tests. Both approaches hold promise as a step forward towards making efficient use of alternative data.

PS 86 DIFFERENTIATION OF PROHAPTENS FROM DIRECT ACTING CONTACT CHEMICAL ALLERGENS USING A CYTOCHROME P450 REDUCTASE DEFICIENT MOUSE MODEL.

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The murine local lymph node assay (LLNA) is a well accepted, validated method for identification of chemical contact allergens. Both direct acting haptens and prohaptens (requiring metabolic activation) can be identified, but not differentiated by this assay. The objective of this study was to assess the utility of a pan microsomal metabolic deficient mouse (CPR low/low) to distinguish between direct acting haptens and prohaptens in the LLNA. LLNA cell proliferation was compared in C57BL/6J (B6) wild type vs. homozygous CPR low/low mouse, congenic with the B6 strain, having a hypomorphic NADPH-cytochrome P450 reductase (CPR) gene resulting in low microsomal enzyme activity. The known prohaptens, benzo(a)pyrene (BaP), carvone oxime (CVO) and paracetamol (PCM) and direct binding haptens, oxazolone (OX), 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (EtOX), and N-acetylbenzoquinoneimine (NABQI) employed in the present study. Skin microsomes from the wild type (WT) and CPR low/low homozygous (HM) and heterozygous (HT) knock-out (KO) mice were assayed and compared for CPR activity. Lymphocyte proliferative responses to BaP, CVO and PCM were significantly abrogated by 36.4%, 45.2% and 50.8%, respectively; in CPR low/low KO mice versus WT mice; while the lymphocyte proliferative responses to the direct

acting haptens OX, EtOX and NABQI were comparable. CPR activity, determined as Units/mg protein was determined to be significantly lower in the CPR low/low KO mice compared to the WT. Results of the present study suggest potential utility employing the LLNA in the CRP low/low mouse in conjunction with WT to differentiate pro- vs. direct acting haptens.

PS 87 PLASMACYTOID DENDRITIC CELL-BASED ASSAY AS AN *IN VITRO* ALTERNATIVE FOR CHEMICAL ALLERGENICITY SCREENING.

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Sponsor: P. J.

Human dendritic cells (DC) have been used as an alternative to existing animal models utilized throughout industry to monitor products for contact sensitization. Such methods are necessary to comply with the ban on animal testing imposed by the Cosmetics Directive in the EU. In this study, we investigated whether CD86 expression in plasmacytoid DC (pDC) can be used to identify contact allergens. Human DC were generated from CD34+ progenitor cells and the pDC fraction (CD123+/CD11c-) was harvested using FACS sorting. The pDC were exposed to an expanded list of chemical allergens (n=49) or irritants (n=42). Concentrations of each chemical that resulted in >50% viability as determined by FACS analysis of propidium iodide stained cells were used. Allergens were identified based on stimulation index (SI) calculated by the fold increase in CD86 expression levels. A material that had an SI ≥1.5 in at least 50% of the pDC donors (n=2-5 donors) was considered an allergen. For 71 of the 91 materials tested, historical mouse local lymph node assay (LLNA) and human clinical data were available. Using the in vitro pDC assay, CD86 expression increased ≥1.5 fold for 37 of 39 allergens but not for 26 of 32 non-allergens. Based on these results, a prediction model was developed to classify chemicals as allergens or non-allergens. The in vitro assay performance was sensitivity=95%, specificity=81%, and accuracy=89%; these results were slightly better than those obtained using the LLNA assay: sensitivity=85%, specificity=84%, and accuracy=85%. Transferability of the test method was evaluated using 7 test articles in 3 laboratories. The results showed that all samples were correctly identified in the 3 labs. In conclusion, the CD86 expression level in pDC appears to be a sensitive and specific predictor of allergenicity. The pDC assay is advantageous because high throughput screening of chemicals is possible, donor-to-donor variation can be monitored, the cells are of human origin, and the assay is cost effective.

PS 88 CELL LINE-BASED PREDICTION OF SENSITIZATION BY COUPLING KERATINOCYTE METABOLISM AND DENDRITIC CELL ACTIVATION.

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Frequency of allergic contact dermatitis is constantly increasing and extended knowledge about the sensitizing process is essential for the development of preventive strategies. Many compounds are not immunogenic themselves but are activated outside or inside the skin by non-enzymatic oxidation (prehaptens) or enzymatic transformation (prohaptens, e.g. cytochrome P450) prior induction of an immune response. This necessary bioactivation step has not yet been actively integrated in a cell line-based prediction approach. We cocultured HaCaT keratinocytes with THP-1 as dendritic cell-like cells (ratio 1:0.75). The sensitizing potential can be determined by analyzing the maturation markers CD86 and CD54 on cocultured THP-1 cells. Coculturing augmented cytochrome P450 1 activity in HaCaT cells after treatment with chemicals. The assay allowed the differentiation between the irritant sodium dodecyl sulfate and allergens without influencing cell viability. We achieved the sensitivity to testing prohaptens and their differentiation from prehaptens and haptens using lipopolysaccharide, 2,4-dinitrochlorobenzene, eugenol and isoeugenol. For the prohapten eugenol the upregulation of the CD86 expression was 4-fold higher in the presence of HaCaT. In concordance with the hapten concept, responses to 2,4-dinitrochlorobenzene and to the prehapten isoeugenol were not modified or even slightly reduced under these terms. A coculture assay with HaCaT and THP-1 cells is easy to perform, reproducible, avoids donor variance, and allows the detection of prohaptens under rather physiological conditions. Thus, this assay is a reasonable approach to search for the sensitizing potential of compounds, and as one part of an integrated testing strategy it may replace the local lymph node assay.

PS 89 DEVELOPMENT OF A MOUSE MODEL TO ASSESS THE ALLERGENICITY OF HYDROLYSED COW'S MILK BASED INFANT FORMULAE.

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The EC-directive 2006/141/E requires objective and scientifically verified data to claim hypoallergenicity of hydrolysed formulae. However, no validated animal models are currently available to assess the potential residual sensitizing capacity, although guinea pig assays are frequently used. This study aims to validate a recently developed mouse model for cow's milk allergy to assess the potential allergenicity of hydrolysed cow's milk-based infant formulae. To that end, a multicenter transferability study was performed to introduce the model in 4 independent research centers. C3H/HeOuJ mice (3-4 weeks, Charles River) were sensitized by oral administration of whey (2 and 20 mg) at weekly intervals for 5 weeks. One week after the last sensitization the acute allergic skin response (ear swelling at 1 hr) and anaphylactic symptoms were determined upon intradermal ear injection of whey. Subsequently, mice were challenged orally with 50 mg whey and blood samples were taken after 30 minutes. Serum was analyzed for whey-specific immunoglobulins and mMCP-1. All protocols, test substances, and procedures were standardized. All participating research laboratories detected elevated levels of whey-specific IgE/IgG1/IgG2a and serum mMCP-1 as a reflection of mast cell degranulation. Acute allergic skin responses were observed in 3 out of 4 research centers and anaphylactic symptoms were present at all 4 research centers. These results are indicative of good interlaboratory transferability. In the next phase of the validation process, whey hydrolysates will be included to assess whether the proposed mouse oral sensitization model is suitable for evaluation of hypoallergenic cow's milk formulae.

PS 90 PREFERENTIAL REACTIVITY OF CONTACT AND RESPIRATORY LOW MOLECULAR WEIGHT CHEMICAL ALLERGENS UNDER COMPETITIVE CONDITIONS.

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Low molecular weight chemicals are capable of causing allergic diseases of the skin and respiratory tract. Individual materials are typically, though not always, associated with one form of disease or the other – generating a Th1 or Th2 type response. The reason for this divergence is unclear; however chemical interaction with proteins is an important common step. Using a peptide reactivity model, the reactivity of reference skin (dinitrochlorobenzene [DNCB], dinitrofluorobenzene [DNFB]) and respiratory allergens (toluene diisocyanate [TDI], methylene diphenyl diisocyanate [MDI]) was investigated. Of particular interest was determining if there exist preferences for binding to either cysteine (Cys) or lysine (Lys). One set of assay was conducted by reacting synthetic peptides containing either Cys or Lys alone with an excess of test chemical. In order to evaluate the effect of competition, assays were conducted by preparing reaction mixtures of these same peptides in 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. The samples were analyzed for depletion of peptide by HPLC/UV after 24 h incubations. When incubated with single peptides, DNCB and DNFB were observed to have increased reactivity to Cys when compared to both TDI and MDI. Although DNCB lacked significant reactivity, DNFB and the isocyanates had comparable reactivity with the Lys peptide. Under competitive conditions, however, both DNCB and DNFB (contact allergens) showed preferential binding to Cys; while TDI and MDI (respiratory allergens) exhibited preferential binding to Lys. The preferences were most evident at the 6:1 and 9:1 reaction ratios; pairwise comparisons showed that this was not due to concentration dependence. The observed differences may be relevant for the ability of these chemicals to induce divergent allergic responses.

PS 91 ALLERGEN EXPOSURE RESULTS IN CHANGES IN THE LEVEL OF EXPRESSION AND FREQUENCY OF B220^{+} LYMPHOCYTES.

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The murine local lymph node assay (LLNA) is a validated alternative for the predictive identification of skin sensitizing chemicals. As with other predictive test methods, false-positives are known to occur, particularly to a small minority of skin

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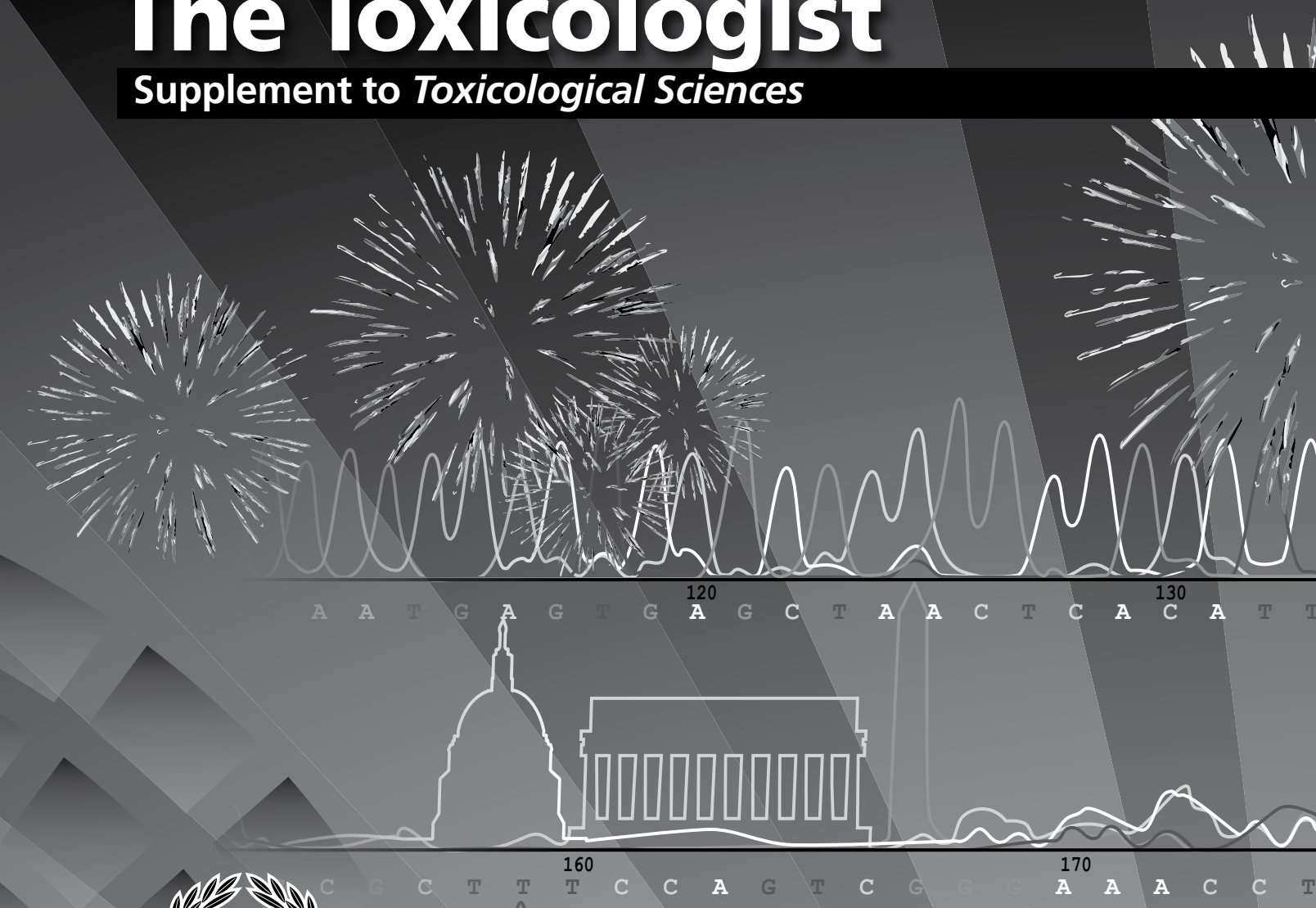

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

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