binding in the liver, which involves direct oxidation to a quinone methide, the reactive metabolite that covalently binds in the skin is a sulfate. The sulfate responsible for the rash is formed in the skin of rats and in human skin incubations but not in mice which develop no rash. Further work is being done to confirm the role of IL-1β in NVP-induced skin rash. Funding: Canadian Institutes of Health Research.

PS 1051 *In Vitro* Discrimination of Skin Sensitizing Haptens and Prohaptens in a Modified Keratinosens Assay with an Added Metabolic Activation Step.

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Prohaptens are chemicals which may cause skin sensitization after being converted into electrophilic molecules by skin enzymes. In vitro sensitization assays ideally should detect the potential of molecules to act as prohaptens. The metabolic activation system most commonly used in in vitro toxicology is Aroclor-induced rat liver S9 fraction. Even if this system contains higher enzyme activites as compared to those reported in skin, it may serve as a surrogate system to study the potential of chemicals to form reactive, skin sensitizing metabolites. To test this concept, the luciferase induction in KeratinoSens reporter cells treated with chemicals in presence and absence of S9 fractions was measured. Suspected prohaptens such as methylisoeugenol, eugenol, trans-anethol or benzo(a)pyrene gave no, or weak, gene induction in absence of S9 fractions, and a strongly enhanced luciferase induction in presence of S9 proving their prohapten status. Haptens like DNCB or cinnamic aldehyde gave a reduced response in presence of S9. We then evaluated whether this metabolic activation assay might be implemented in a tiered screening strategy to screen negatives in the classical KeratinoSensTM assay to enhance sensitivity. To this aim all chemicals classified negative in the classical KeratinoSensTM assay were retested with this activation step. Among the 77 chemicals found as correct-negatives, 74 were also negative in presence of metabolic activation, thus this counterscreen does only slightly reduce specificity. However, based on this comprehensive screening, we found that only a small fraction of the known skin sensitizers need activation by the S9/P450 system, and thus the KeratinoSensTM -S9 assay may be useful for the in vitro evaluation of specific classes of potential prohaptens, rather than as a general screening approach. These results will be presented along with results on the predictivity and reproducibility of the KeratinoSensTM assay as accumulated during the prevalidation studies conducted for ECVAM.

PS 1052 Toxicogenomic Characterization of Sensitizer and False-Positive Responses in the Local Lymph Node Assay (LLNA).

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Recent publications have highlighted chemistries which yield false positive responses in the LLNA when compared with guinea pig and human data. A toxicogenomic approach was applied to provide insight into the molecular and cellular mechanisms that may explain these differential responses. Auricular lymph node gene expression responses were evaluated in female CBA mice exposed to equipotent doses of 9 chemical sensitizers and 7 false positives per the standard LLNA dosing regimen. Lymph nodes were analyzed for 3HTdR incorporation on day 6 and gene expression responses on study days 4 and 6. Statistical analyses identified 779 and 473 differentially expressed genes (DEGs) between sensitizers and false positives on days 4 and 6. Class-based comparison of DEGs showed that the most enriched functional categories in the sensitizer-specific subsets were consistent with mechanisms involved in the acquisition of antigen-mediated skin sensitization. Key immune responses at the 4 day time point were restricted to genes involved in early T-cell development including pathways involved in Il2 regulation (Il2 and Egr4), Tbata and the pre T-cell receptor alpha (Ptcra). Day 6 responses were more consistent with a mature T-cell response and included genes involved in the DC/T-cell maturation process such as Il21, Lag3 and Fxyd4. In contrast, false positives exhibited a strong pro-inflammatory expression profile including markers for activated macrophages and neutrophils such as Cd5l, Il12b, Mpo, Defa4 and class I stefins. Expression of these genes in the absence of dermal irritation suggested these responses were not solely driven by skin irritation. These gene expression profiles suggest a differential cellular recruitment to the lymph nodes following skin exposure to true sensitizers and false positives and provide a potential new endpoint that could be applied to address false positives and enhance the predictive value of the LLNA.

PS 1053 Inductive Effects on Reactivity of the Contact Allergen Benzoquinone and Its Derivatives to Proteins.

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Benzoquinone (BQ) and substituted benzoquinones (SB) are used for dye and cosmetics production. BQ is an electrophile known to covalently modify proteins via Michael Addition (MA) but the reactivity, reaction mechanistic domains and allergenicity of SB are unknown. Electron withdrawing and electron donating substituents on BQ were assessed for effects on BQ reactivity and allergenicity. Alternative potential protein binding mechanisms were explored. BQ binding to Cys34 on human serum albumin (HSA) was studied and for BQ and SB reactivity studies, nitrobenzenethiol (NBT) was used as a model nucleophile. Hammett and Taft (HT) constants were used to evaluate the influence of these substituents on chemical reactivity. Both NBT binding studies and HT values demonstrated chlorine SB to be more reactive than methyl and t-butyl SB. Production of semiquinone radicals from SB and characterization of SB-NBT adducts demonstrated that haptenation may also occur via free radical mechanism which is pH dependent, and vinylic substitution mechanisms, in addition to the predominant MA. BQ and SB dermal allergenicity as evaluated in the murine local lymph node assay (LLNA) was consistent with that predicted by reactivity and HT data. These results demonstrate the effect of substituents on BQ reactivity and allergenicity while suggesting potential utility of chemical reactivity data and HT values for electrophilic allergen identification and potency ranking.

PS 1054 Interaction of Para-Phenylenediamine with Human N-Acetyltransferases.

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The contact allergen para-phenylenediamine (PPD) is known as a good substrate for N-acetyltransferase 1 (NAT1) but we also found that concentrations above 50 μM are accompanied by inhibition of NAT1 activity in human keratinocytes. Here, we investigated the substrate and inhibition characteristics of PPD on NAT enzymes. First we measured whether next to PPD and mono-acetylated PPD (MAPPD) the PPD oxidation product Bandrowskis Base (BB) can also be acetylated. We therefore incubated PPD, MAPPD and BB with human recombinant NAT1 and NAT2 and found them to be good substrates for both enzymes.
NAT1 inhibition characteristic of PPD was further studied using the THP-1 cell line which served as model for antigen-presenting cells. Both PPD and MAPPD are N-acetylated by THP-1 and the acetylation is accompanied by NAT1 inhibition. Concentrations above 1 μM PPD clearly reduced enzyme activity already after 8h while 47% reduction was measured after 24h (200 μM). Independent of the substrate-based enzyme inhibitions, certain compounds are known to oxidize the catalytic cysteine or form adducts with NAT protein. Therefore we studied whether PPD, MAPPD and/or oxidized PPD including BB also interact with recombinant NAT protein itself in the absence of acetyl coenzyme A. All but MAPPD interact with the protein after 2h and the greatest inhibition was found for oxidized PPD (up to 50%). From these results we can conclude that the observed NAT inhibition may be caused by both substrate dependent and independent effects. NAT1 activity in PPD-treated THP-1 cells was completely restored after incubation in fresh culture medium for 24h, whereas inhibition caused by 24h treatment with MAPPD could be restored for only 10%.
In sum our data indicate that PPD and its oxidation products can inhibit NAT in two different ways. In addition we demonstrated that PPD and BB are acetylated by NAT1 and 2, suggesting that certain amounts of PPD and eventually formed BB inside the body may be detoxified by NAT1 in skin and additionally by NAT2 expressing organs.

PS 1055 Pharmacodynamic Profiling of EGFR Inhibitors in HaCaT Cells.

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Skin Rash is a serious adverse effect of EGFR inhibitors observed during anticancer therapy in the clinic and appears to be linked to inhibition of the target pathway. The EGFR inhibitors erlotinib and afatinib were investigated at increasing concentrations (0, 0.001, 0.01, 0.1, 1 and 10 μM) in the human keratinocyte cell line

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