stimulation of peripheral-blood mononuclear cells with KLH but no responses were elicited with human samples. In conclusion, KLH-specific antibodies and an apparent DTH response were induced in NHPs following a successful clinical protocol. The differential ability of prednisolone to modulate the DTH response in NHPs and humans implies that the responses may not have been mediated by the same cell types; however, this could not be further examined due to the lack of skin biopsy samples from the clinical study. Commercially available reagents to detect Ig isotypes in NHP are highly desirable to further our understanding of the TDAR response in NHPs.

## W 2619 GOOD PRACTICES IN THE STUDY DESIGN, DATA ANALYSIS AND REPORTING OF T-DEPENDENT ANTIBODY RESPONSE STUDIES.

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The T-Cell Dependent Antibody Response (TDAR) assay is commonly used to evaluate effects of investigational small molecule drugs or biologics on the immune system, including in nonhuman primates (NHPs). While this functional assay is recommended in the guidance document ICH S8 Immunotoxicity Studies for Human Pharmaceuticals, the assay is not standardized. An interlaboratory retrospective analysis indicated that immunization of NHPs with either keyhole limpet hemocyanin (KLH), sheep red blood cells or tetanus toxoid led to measurable primary and secondary antibody responses. Also, it was demonstrated that antibody responses can be obtained in response to immunization with bacteriophage. Interanimal variability in peak response values is significant with all antigens evaluated and studies need to be powered accordingly (8 animals per group [if all animals can be evaluated without interference from pre-existing cross-reactive antibodies] allows detection of 3-fold statistically significant differences between groups). Interanimal variability and peak antibody response values are similar in male and female NHPs and data from both genders may be combined for statistical analysis purposes (if appropriate based on pharmacokinetics and target biology) to increase the power to detect differences between treatment groups. In order to account for the kinetics of the antibody response, the area under the curve (AUC) defined by antibody titers across study days can be calculated and analyzed in addition to pairwise comparisons at each given time point after immunization. Points to consider when interpreting TDAR studies include: the exclusion of data from animals with pre-existing cross-reactive antibodies against the immunogen, the ability of animals to mount appropriate primary IgM and IgG and secondary IgG responses, how group mean antibody titers compare at each time point and as AUC, the overlap between titers in control and treated animals, the evaluation of treatment effect by combining its statistical significance (p-value) and the magnitude of the effect (the treated/control ratio and its confidence interval).

## PL 2620 KEY EVENTS OF THE INNATE IMMUNE RESPONSE AS TOOLS FOR IDENTIFICATION OF CHEMICAL SENSITIZERS *IN VITRO*: DO WE NEED MORE?

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Abstract: Sensitization is the toxicological endpoint associated with compounds having the intrinsic ability to adversely trigger the adaptive immune system. Martin et al. (2011) demonstrated that innate responses in keratinocytes (KC) and Langerhans cells (LC) are crucial. The presented study compared existing *in vitro* tests based upon innate key mechanisms for their capacity to properly label and classify chemicals. Methods: The performance of selected tests when exposed to well characterized sufficiently water-soluble skin sensitizers (N=35), respiratory sensitizers (N=9), and non-sensitizers (N=21) (including 5 irritants) (www.sens-it-iv.eu) was analyzed retrospectively. The tests included the IL-18 test (Corsini et al., 2009), the KeratinoSens (Natsch et al., 2010)), 2 reconstituted human epidermis (RHE) models (SkinEthic, CellSystems), the MUSST (Python et al., 2007), h-CLAT (Sakaguchi et al., 2009), VitoSens (Lambrechts et al, 2010), and GARD (Johansson et al., 2011). Results were expressed in terms of concordance (%) with the available *in vivo* data. Results: The KeratinoSens and the IL-18 test identified the chemicals correctly as skin sensitizers in 87 and 95% of the cases. The MUSST and h-CLAT tests (overall: 78%) scored high with extreme and strong sensitizers (86-100%), but poorly with moderate and weak sensitizers (53-60%). Stronger irritants were found positive in these tests. The genomic marker profile of the VitoSens and GARD tests correctly labled 89 and 98% of the chemicals. They performed equally well across the chemical potency classess and did not score the irritants on the list. The RHE models did not discriminate sensitizers from irritants, but irritative responses were found useful for classification of the chemicals (92%). Conclusion: Tests addressing multiple pathways (IL-18, VitoSens, GARD) performed best. The chemicals on the Sens-it-iv list with sufficient solubility in water were properly identified by one test or a combination of 2 tests (96-98%). In 92% of the cases a proper classification was obtained.

## PL 2621 GARD—A GENOMIC BIOMARKER ASSAY FOR PREDICTION OF SENSITIZERS USING A CELL-BASED *IN VITRO* ALTERNATIVE TO ANIMAL TESTS.

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Background: Allergic contact dermatitis and respiratory conditions such as occupational asthma are both caused by an adverse immune response towards chemical haptens. These diseases affect a significant proportion of the population, with increasing incidences, which leads to a substantial economic burden for society. Current tests for skin sensitizing chemicals rely on animal experimentation, whereas no such assays exist for respiratory sensitizers. New legislations on the registration and use of chemicals within chemical and cosmetic industries imply a need of alternative, human cell-based assays for the prediction of sensitization. The aim is to replace animal experiments with *in vitro* tests displaying a higher predictive power. Results: We have developed a novel cell-based assay for the prediction of sensitizing chemicals, called Genomic Allergen Rapid Detection, GARD. By analyzing the transcriptome of the human cell line MUTZ-3 after 24 h stimulation, using well characterized skin sensitizing chemicals (N=20), respiratory sensitizing chemicals (N=9) and nonsensitizing chemicals (N=20), we have identified genomic biomarker signatures with potent discriminatory ability. Using a Support Vector Machine for cross-validation, the prediction accuracy of the assay is estimated to 98%. The identified transcriptional biomarkers are involved in biological pathways with immunologically relevant functions, which can shed light on the process of human sensitization. Conclusions: Gene signatures have been identified and demonstrated to have the power to predict sensitization. This simple and robust cell-based assay has the potential to completely replace or drastically reduce the utilization of test systems based on experimental animals. Being based on human biology, the assay is proposed to be more accurate for predicting sensitization in humans, than the traditional animal-based tests.

## PL 2622 ESTABLISHMENT OF AN *IN VITRO* PHOTOASSAY TEST USING NCTC2544 CELLS AND IL-18 TO DISCRIMINATE PHOTOIRRITANTS FROM PHOTOALLERGENS.

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PURPOSE: Differentiation between photoallergenic and phototoxic reactions induced by low molecular weight compounds represents a current problem. The use of keratinocytes as a potential tool for the detection of photoallergens as opposed to photoirritants is considered an interesting strategy for developing *in vitro* methods. We have previously shown that the human keratinocyte cell line NCTC2455 and the production of IL-18 ia a good model for screening sensitizers. The main purpose of this work was to explore the NCTC2544 model as an *in vitro* model to identify photoallergenic and discriminate from phototoxic chemicals. METHODS: The effect of UVA radiation (3 J/cm2) over NCTC2544 cells irradiated and non irradiated, and treated with increasing concentrations of various compounds including negative compounds (irritants and allergens), ibuprofen and acridine (photoirritants); ketoprofen and chlorpromazine (photoirritant and photoallergen); benzophenone, 4-ter-butyl-4-methoxy-dibenzoylmethane, 2-ethylexyl-p-methoxycinnamate and 6-methylcumarin (photoallergens) was investigated. Twenty four hours after exposure, cytotoxicity was evaluated by the MTT assay, while the production of IL-18 was measured by a commercially available ELISA kit. RESULTS: At the maximal concentration assayed with non cytotoxic effects, allergens and photoallergens induce the production of IL-18, whereas irritants and photoirritants failed. Indicating that the NCTC2544 assay can also be used to identify *in vitro* photoallergens. This project was funded by Ministero dell'Istruzione, dell'Università e della Ricerca (IT10B3A3AA) and by Ministerio de Ciencia e Innovación (IT2009-0014).

## PL 2623 PYRIDOXYLAMINE REACTIVITY KINETICS AS AN AMINE-BASED PROBE FOR SCREENING ELECTROPHILIC CONTACT ALLERGENS.

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Covalent protein binding is an initial step in dermal contact sensitization. We previously reported the utility of a nitrobenzenethiol (NBT) binding assay for screening thiol reactive haptens (Chipinda et al Chem. Res. Tox. 2010). The present

study describes an amine (pyridoxylamine (PDA)) absorbance and fluorescence-based reactivity kinetic probe to complement the NBT assay for identification of amine-selective haptens. Rate constants for 32 chemicals including 5 anhydrides, 6 aldehydes, 2 quinones, among other classes, were determined where reaction times to completion ranged from 20 s to > 2 h. Preliminary assessment suggests labile reactive intermediates are formed during PDA reaction to quinones and benzyl bromide. No reactivity was observed with some thiol-selective sensitizers such as propiolactone or nonsensitizers such as sulfanilamide and benzocaine. Strong correlation (R2 = 0.81) was obtained between PDA reactivity constant (pseudo-first order; k) and the local lymph node assay threshold (EC3) values. Reaction completion time of the sensitizers also correlated strongly (R2 = 0.74) with EC3 values suggesting utility of transforming this chemical reactivity method into a simple, inexpensive end-point assay. The results from the PDA method also highlight the utility of combining this amine based method with the previous NBT based model to be able to identify thiol selective, amine selective and non-selective electrophilic contact allergens.

## PL 2624 EVALUATION OF *IN VITRO* REACTIVITY ASSAYS FOR ASSESSING THE SENSITIZATION POTENTIAL OF CHEMICALS.

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Assessment of skin sensitization potential of chemicals is an important aspect of the safety evaluation process. Recent regulations, as well as responsible stewardship programs, have advocated for the development of non-animal approaches that can reliably predict skin sensitization potential for new chemicals. As the majority of chemical sensitizers are electrophiles that can react with nucleophilic sites on proteins, several assays have focused on characterizing this property *in vitro* for prediction of sensitization potential. Herein, we describe our evaluation of two such *in vitro* approaches, the KeratinoSens assay and the Direct Peptide Reactivity Assay (DPRA). The KeratinoSens assay uses a human keratinocyte cell line (HaCaT) in which activation of the Nrf2-ARE pathway is quantified via a luciferase reporter gene to assess chemical reactivity. In the DPRA, reactivity of the test material to cysteine and lysine containing peptides is assessed through quantification of peptide depletion using HPLC-UV-MS. To explore the utility of these assays for identifying sensitizers, 28 (17 sensitizers and 11 non-sensitizers) and 9 (6 sensitizers and 3 non-sensitizers) chemicals were tested using the KeratinoSens assay and DPRA, respectively. The KeratinoSens assay exhibited 88% sensitivity, 91% specificity and 89% overall accuracy relative to available *in vivo* data. The DPRA exhibited 83% sensitivity, 100% specificity and 89% overall accuracy. However, integrating the results of the two assays improved the sensitivity, specificity and accuracy levels to 100% for the nine common chemicals. The data sets also identified several characteristics for the conduct of these assays that need to be recognized to avoid false positives and negatives. Overall, the results provide support for the use of these *in vitro* assays to identify chemicals with skin sensitization potential and that integrating their results enhances the overall predictive capability.

## PL 2625 *IN VITRO* SKIN IRRITATION AND CORROSION TESTING SERVING DIFFERENT REGULATORY CLASSIFICATION SCHEMES.

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Skin corrosion or irritation refers to irreversible or reversible tissue damage to the skin following the application of a test substance. Traditionally, this was tested using the *in vivo* Draize skin irritation test. Due to animal welfare considerations and regulatory provisions such as the European Cosmetics Regulation, *in vitro* tests using different reconstructed human skin models were developed and have now gained regulatory acceptance (OECD TG 431 and OECD 439). Depending on the up-front prediction of the severity of the effects (skin corrosive, skin irritant, or nonirritant) by expert judgments or structure activity relationship, different testing schemes can be applied, e.g. the top-down or bottom-up strategies proposed for skin irritation testing by COLIPA (MacFarlane et al., 2009). We have used the EpiDerm™ (Mattek, USA) and compared it to the results of Draize skin irritation test *in vivo* which had been performed due to regulatory requirements. Over 100 substances have been tested including a wide range of different chemical classes. The test results were assessed according to the UN-GHS, EU-CLP, Brazilian and US EPA guidelines. We could confirm a good correlation of the *in vitro* results with the *in vivo* data for corrosive and non-irritating substances. Irritating substances were occasionally incorrectly classified by not more than one class off the *in vivo*-based classifcation. Moreover, the *in vitro data* could serve all four different regulatory classification schemes.

## PL 2626 REFINEMENT OF THE APPLICABILITY DOMAIN AND PREDICTIVITY OF THE KERATINOSENS ASSAY, A NOVEL *IN VITRO* SKIN SENSITIZATION ASSAY.

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Determination of skin sensitization potential is a critical toxicological endpoint in the development of novel ingredients used in consumer and industrial products. With the European Union regulatory deadline to ban animal testing of cosmetic ingredients for skin sensitization quickly approaching, and many companies proactively choosing to eliminate animal testing due to ethical considerations, alternative methods are urgently needed. The KeratinoSens assay is a cell-based reporter gene assay which may be used to assess the potential of chemicals to induce skin sensitization in humans. A feature all chemical allergens have in common is their intrinsic electrophilicity, or their potential to be transformed to electrophilic chemicals. The Nrf-2-electrophile sensing pathway comprised of the repressor protein Keap1, the transcription factor Nrf2, and the antioxidant response element (ARE), is capable of detecting skin sensitizers. In the KeratinoSens assay, the induction of a luciferase gene under the control of the antioxidant response element (ARE) derived from the human gene AKR1C2 gene is quantified. Thus far, over 100 chemicals have been evaluated using the KeratinoSens assay and the results indicate a good predictive value (~ 85%). In this study we sought to further refine the applicability domain and predictivity of the assay by testing neat chemicals and mixtures of fragrances, preservatives, industrial solvents, and surfactants with a broad range of potencies for their ability to induce the luciferase gene. In parallel, cytotoxicity was assessed by both Neutral Red Uptake (NRU) and MTT assays. The results from the KeratinoSens assay and parallel cytotoxicity assays were compared to the available correlative *in vivo* and human clinical data. The results indicate that the KeratinoSens assay may be used to evaluate a broad range of materials with reproducibility and a high predictive value.

## PL 2627 AN *IN VITRO* TEST BATTERY FOR THE PREDICTION OF SKIN SENSITIZERS BASED ON KEY EVENTS OF THE TOXICITY PATHWAY.

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Skin sensitization is a common health problem caused by repeated contact with an allergen (or hapten or pro-hapten). Currently, skin sensitization relies on animal testing (OECD 406, 429), and so far no validated and regulatory accepted *in vitro* methods are available. The purpose of this study was to establish an *in vitro* test strategy for the prediction of skin sensitizers based on the key events leading from the hapten contact to skin sensitization. For such an integrated approach several *in vitro* assays are required. We tested a total of 10 assays and – based on their performance – we selected three assays adressing chemical reactivity towards proteins and dendritic cell activation: (1) direct peptide reactivity assay (DPRA) to measure chemical reactivity towards proteins with model peptides with nucleophilic amino acid residues (2) LuSens, an antioxidant response element (ARE) dependent luciferase gene activity in a recombinant cell line (3) myeloid U937 skin sensitization test (MUSST) or human cell line acitvation test (h-CLAT) for measurement of cell surface markers (CD54 and/or CD86) on cells with dendritic cell like characteristics. The in-house validation of the *in vitro* assays was performed with a panel of 60 substances of known sensitizing potential including the 22 performance standard substances of the murine local lymph node assay. Out of the 60 substances 54 were applicable to the test methods; 6 were insoluble. A combination of DPRA and LuSens adresses chemical reactivity and offered a sensitivity of 100% (enabling the exclusion of a sensitizing potential). The MUSST adressed dendritic cell activation and offered a specificity of 100% (proofing a sensitizing potential). The combination of the two elements resulted in an accuracy of 94% for the 54 substances.

## PL 2628 BUILDING HYPOTHESES OF PATHWAY PERTURBATION USING 'OMICS DATA AND MACHINE LEARNING TOOLS.

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Assays that measure perturbations to biochemical pathways linked to adverse effects have been proposed as a way to move toxicology testing from high-dose *in vivo* trials to quicker assays (*in vitro* or *in vivo* using in model systems) at doses relevant for

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