PS 487 CULTURE OF ORGANOTYPIC, 3-DIMENSIONAL TISSUE MODELS – IMPORTANCE OF SUBSTRATE PROPERTIES.

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Highly differentiated, organotypic tissue models are being used increasingly in lieu of animals to meet regulatory testing requirements. The reproducibility of these tissue models is of prime importance so that US and EU regulators and industry can be assured that the toxicological system is reproducible both during the validation process and afterwards. The purpose of this study was to investigate the effects of various tissue culture inserts (TCI) on the tissue morphology and reproducibility. Tissue culture inserts (TCI) from 5 commercial manufacturers were obtained and standardized culture conditions were used to produce the skin (EpiDerm™) and ocular (EpiOcular™) organotypic tissue models. These tissue models were then subjected to quality control (QC) tests which include histological evaluation and determination of the exposure time of a common surfactant (Triton X-100) that reduces the tissue viability to 50% (ET-50). Of the 5 TCI tested, tissue histology for tissues cultured on 2 TCI was distinctly different and inferior to the standard tissues while the histology for tissues produced on the remaining TCI were structurally equivalent to the control tissues. The average ET-50 for EpiDerm produced with the best substitute TCI was 7.64 +/- 0.95 hr (n=6) and was not statistically different than that of tissues cultured on the control TCI, 7.73 +/- 0.56 hr (p = 0.79, paired student t-test); similarly, the ET-50 for EpiOcular cultured on the best alternative TCI was 22.6 +/- 5.0 min (n=5), which was not statistically different than that of the control tissue, 27.2 +/- 3.0 min (p = 0.18, paired student t-test). In summary, the TCI is one of the crucial parameters in producing high quality, reproducible, organotypic tissue models but multiple commercially available TCI appear to have appropriate properties.

PS 488 *IN VITRO* EYE IRRITATION ASSESSMENT OF COLORED SUBSTANCES BY USING THE SKINETHICTM HCE OCULAR TEST METHOD.

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The in vitro SkinEthicTM reconstructed Human Corneal Epithelium (HCE) is part of an ongoing ECVAM formal in vitro eye irritation validation study aiming a possible incorporation of the test method in a tiered test strategy to replace the Draize eye test (OECD TG 405). The test method, based on tissue viability measurement by using the MTT assay, was specifically designed to allow the discrimination between irritant and non irritant substances. The reduced MTT (purple formazan) is quantified by a standard colorimetric method. During formazan extraction, any unspecific color remaining in the tissue or developed in situ may be extracted and consequently induce a possible final viability overestimation. In this study specific controls were introduced allowing the use of the test method for the irritancy prediction of colored/coloring substances. The protocol consisted mainly in a short 10 minutes topical treatment or a long 1h +16 hrs post-treatment incubation period. After rinsing, some colored substances retained in the epithelium can induce a residual staining. Unspecific color, was quantified by using treated tissue controls following the standard protocol course but not exposed to MTT. These controls enabled the quantification of Non Specific Optical Density (NSOD) and the correction of final measurements (true OD due to mitochondrial activity). Histological analysis can be conducted in order to document strong coloring substances (> 30% relative to negative controls). In this study, 9 irritants and 10 NC colorants have been evaluated following this strategy.
We showed that the SkinEthicTM-HCE assay is a suitable method for the in vitro eye irritation prediction of coloring substances. The applicability domain of this assay can therefore be extended to these substance families

PS 489 AN EVALUATION OF THE EPIDERM™ CORROSIVITY AND CORROSITEX® ASSAYS FOR PREDICTING SKIN CORROSIVITY OF CHEMICAL PRODUCTS WITH EXTREME ALKALINE PH.

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The purpose of this study was to evaluate the EpiDerm™ Corrosivity (OECD 431) and Corrositex® Time Monitor (OECD 435) as in vitro methods to predict skin corrosivity for extreme pH (≤2 or ≥ 11.5) products. Extreme pH can be a useful predictor of irritation but may lead to over classification in weakly buffered systems. Hazard classification guidelines such as the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) recommend testing with a validated in vitro method to confirm a non-corrosive classification for an extreme pH product. Our objective was to identify a method that could accurately identify corrosive and non-corrosive alkaline products. 10/15 products tested on the Epiderm assay predicted the same skin classification when compared with the in vivo data. The remaining five formulas over-predicted the skin classification when compared with the in vivo data. There were no products in which the Epiderm under-predicted the skin classification when compared to the in vivo results. The Corrositex assay was able to accurately predict 3/7 formulas, four products were over predicted as corrosive by Corrositex; none were under predicted. Epiderm results were also compared to classifications made under JDI's internal hazard assessment process which bridges new formulas to classification guidelines based on an extensive database of historical in vivo data on specific chemical and formulation categories. This weight of evidence approach is consistent with GHS guidance on use of professional judgment to classify a product. JDI's process accurately predicted the in vivo data 14/18 times. Two products were under predicted and two over predicted. The EpiDerm assay is a promising alternative to the use of animals to confirm non-corrosive classifications; however limitations were seen with higher solvent levels. The Corrositex assay did not reliably identify non-corrosive formulations.

PS 490 A SIMPLE AND FAST KINETICS SCREENING ASSAY FOR ELECTROPHILIC DERMAL SENSITIZERS USING NITROBENZENETHIOL.

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The need for alternatives to animal based skin sensitization testing has spurred research on the use of in-vitro, in silico and in chemico methods. Glutathione and other select peptides have been used to determine the reactivity of electrophilic allergens to nucleophiles but available peptide-based methods are inadequate to accurately measure the rapid kinetics observed with many chemical sensitizers. A kinetic spectrophotometric chemometric assay involving the reactivity of electrophilic sensitizers to nitrobenzenethiol was evaluated. Stopped flow techniques and conventional UV spectrophotometric measurements enabled determination of reaction rate constants ranging from 6 x10-3 M-1s-1 to 2.7 x 104 M-1s-1. Rate constants were measured for 3 extreme, 5 strong, 3 moderate and 2 non-sensitizers. Nine of 13 and 2/13 tested skin sensitizers exhibited pseudo-first order and second order kinetics, respectively. In 2/13 chemicals deviations from first and second order were apparent where the chemicals exhibited complex, likely mixed order kinetics. The reaction rates of the electrophiles correlated positively with their EC3 values within the same mechanistic domain. Detailed specific chemical knowledge of the test chemical is of paramount importance, since false negatives were observed with sensitizers like trimellitic anhydride and oxazolone. Findings from this model show that for the same mechanistic domain, skin sensitization is driven mainly by electrophilic reactivity. This simple and rapid absorbance based method can be incorporated into the battery of alternative non-animal assays for use in skin sensitization prediction.

PS 491 INTEGRATED *IN VITRO* VAGINAL SAFETY SCREENING APPROACH FOR BATH AND BODY WASH PRODUCTS UTILIZING SKINETHIC HUMAN VAGINAL EPITHELIUM (HVE) MODEL.

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A common goal of many personal care companies is to assure the safety of their products without animal testing, due to concerns about ethical and animal welfare issues as well as the relevancy of the animal model to humans. To address these issues, we have developed an *in vitro* testing program to support the safety evaluation of potential vaginal irritation in a number of bath and shower cleanser products. A series of surfactant-containing formulations, diluted to 10% in water to mimic the maximum concentration expected in bath water, were tested. The formulations were applied topically onto the surface of commercially-available SkinEthic HVE three-dimensional human vaginal epithelium tissues over various exposure times. The ET_{50} (i.e. the exposure time expected to reduce relative viability of the tissues to 50% of controls) for each candidate was determined. The test results were compared to reference formulations and available human clinical data. The vaginal irri-

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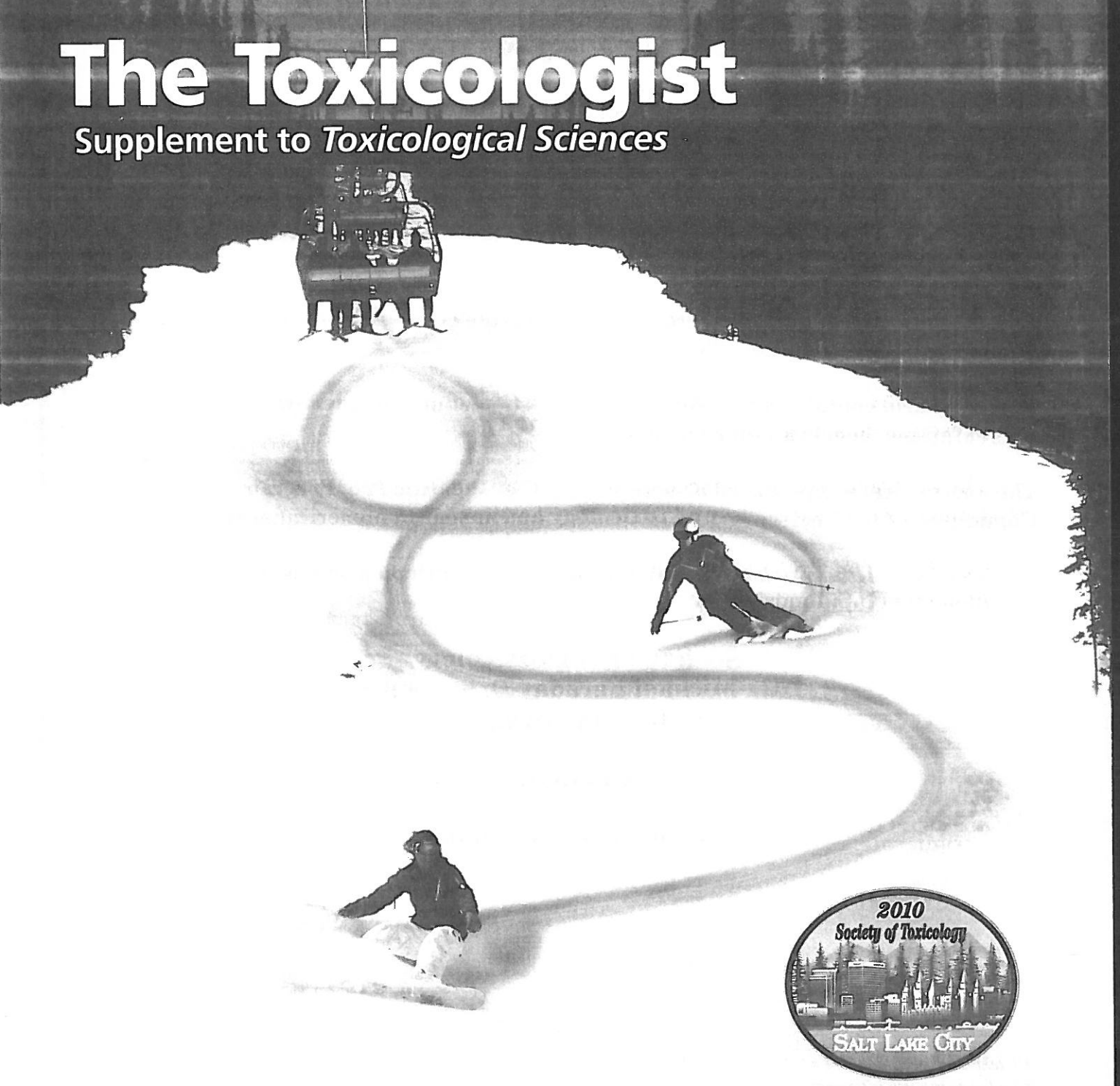

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 49th Annual Meeting of the Society of Toxicology, held at the Salt Palace Convention Center, March 7–11, 2010.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 473.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 496.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

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