PS 2181 Timecourse of Recovery following UV-Induced Damage Using a Full-Thickness Human Skin Equivalent

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Solar ultraviolet (UV) radiation is known to have deleterious effects on human skin. The UVB (280-320 nm) and UVA (320-400 nm) spectrum of solar radiation have been shown to affect keratinocytes, the major cellular constituent of the epidermis, by causing direct DNA damage and/or indirect DNA damage and cytotoxicity through the formation of reactive oxygen species. In the present study, a commercially available human skin equivalent (HSE) (EpiDerm-FT™) and excised human skin was exposed to solar-simulated light to gain insight into the temporal UV-induced response of human epidermal tissue. In vitro HSEs were irradiated with a single UVR dose approximately equivalent to either 6 or 9.5 minimal erythemal doses (MEDs) for an individual of EPA skin phototype 2. Cutaneous damage and recovery were then monitored for a period of seven days. Histological analysis showed a dose dependent formation of apoptotic sunburn cells in epidermal KCs at 24 hrs post-irradiation. By day 3 post-irradiation, a thinning of the viable epidermal cell layers was evident with maximum epidermal degeneration observed at day 4. Resumption of epidermal proliferation and differentiation was evident in both 6 and 9.5 MED tissues by days 5-7, leading to regeneration of viable epidermal layers. Excised human skin tissues irradiated with the same UV doses displayed responses very similar to those observed in the in vitro HSEs. DNA damage indicated by cyclopyrimidine dimer (CPD) formation was assessed by immunohistochemistry. CPD positive basal KCs decreased steadily in number each day, and were almost completely undetectable seven days post-irradiation. CPD formation could be completely blocked through topical application of OTC sunscreens. Finally, elevated levels of IL-8 and MMP-1 were induced following UV-irradiation demonstrating an early inflammatory response followed by an extended period of matrix remodeling activity. These results demonstrate that HSEs are useful for UV-induced photocarcinogenesis studies and evaluation of sunscreens.

PS 2182 Effect of Exposure Area on Nerve Agent Absorption through Skin *In Vitro*

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Static diffusion cells are used to determine the penetration of chemicals through skin *in vitro*. These chemicals may be constituent parts of topically applied pharmaceutical formulations, being evaluated for hazard assessment purposes or for medical countermeasure development. Static diffusion cells traditionally have a limited surface area defined by the edge of the donor chamber over which a penetrant may spread during a diffusion cell study. Should the penetrant spread rapidly to this containment limit the penetration rate can be determined. Where chemicals are applied within vehicles, creams and ointments and are applied over the entire skin surface area within the diffusion cell, spread to the containment limit occurs at study commencement. However, for the hazard assessment of small droplets of toxic chemicals, such as organophosphate nerve agents, limiting skin surface spread *in vitro* could lead to underestimation of percutaneous penetration and hence underestimation of systemic toxicity *in vivo*. This study considered the dependency of the percutaneous penetration of the nerve agents ^{14}C-VX and ^{14}C-GD on skin surface spread on pig and guinea pig skin. Two types of static diffusion cell were used with areas of 2.54 cm^2 and 14 cm^2 available for diffusion. For both VX and GD lateral diffusion (either on the skin surface or within the superficial layers) was measured by autoradiography. Chemical spread to the edge of the 2.54 cm^2 cell but not the 14 cm^2 cell. Amounts of nerve agent penetrating in the 2.54 cm^2 cell were less than in the 14 cm^2 cell, but penetration rates expressed per unit area were similar. The results support the conclusion that the smaller diffusion cell artificially limited the skin surface spread of both ^{14}C-VX and ^{14}C-GD resulting in less chemical penetrating at the same rate. This study has shown that it is important account for surface area spread when reporting nerve agent absorption through skin.

PS 2182a *In Vitro* Dermal Permeation of Nicotine from Surrogate E-Cigarette Liquids

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Rationale and Scope: Nicotine (NIC) in propylene glycol (PG) plus flavorings form the main delivery components of electronic cigarettes (e-cigarettes). Some flavoring constituents, e.g. limonene, are known to enhance dermal penetration. Workers mix these liquids and fill e-cigarette cartridges in the manufacturing process, creating the potential for dermal exposure. No information currently exists on the dermal absorption of nicotine from propylene glycol vehicle. Therefore, *in vitro* human epidermal permeation of NIC from 24 mg/mL ("high strength") solutions in PG and from neat NIC were measured. Preliminary studies from 24 mg/mL NIC in PG with the addition of 5% (w/w) limonene were also undertaken. Procedures: Both steady-state fluxes (Jss, μg/cm^2/h) and lag times (τ, h) were determined from infinite dose exposures of 8 h (neat NIC, 9 samples from 3 donors) or 26 h (NIC + PG, 9 samples from 3 donors; NIC + PG + limonene, 3 samples from 1 donor) using static diffusion cells. NIC was quantified by HPLC. Results: Jss from NIC + PG was 4.0 ± 2.3 (mean ± SD). Addition of 5% limonene substantially enhanced flux to 84 ± 23. Jss from neat NIC was 175 ± 57. Measured τ's were: NIC + PG: 10.1 ± 3.3; NIC + PG + limonene: 5.2 ± 2.6; neat NIC: 1.9 ± 0.7. Application of a risk assessment paradigm predicts, for an 8 h exposure to the volar surface of fingertips (73 cm^2) followed by washing, the following average total NIC uptakes (mg): NIC + PG: 7.3; NIC + PG + limonene: 110; neat NIC: 152. Because the typical NIC intake from a smoked cigarette ≈ 1 mg, these numbers may also be thought of as "cigarette equivalents" consumed over an 8 h work shift. Conclusions: These data suggest the potential for substantial systemic nicotine uptake from occupational exposures in the manufacture of e-cigarettes. The addition of flavorings may significantly enhance systemic uptake.

PS 2182b Using Mode of Action in Predicting Skin Toxicity

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The aim of this study was to develop and test different skin toxicity Modes of Action (MOA). MOA describes the key events and processes starting with interaction of an agent with the cell leading to different biological response. MOA can be looked at as a representation of existing knowledge concerning the linkage(s) between initial chemical binding, defined as the molecular initiating event (MIE), intermediate events on cell, tissue and organ level and biological outcome. MOA can be used to identify key events for which non-animal tests can be developed, thereby facilitating mechanism-based, predictive toxicological assessments with low uncertainty and high human relevance.
We focused on 5 well studied skin toxicants and based on the present knowledge, created their biological network. In addition we created a computational model of biological pathways describing cellular processes activated by these compounds by manually annotating and processing molecular information from the literature from the public domain (PubMed articles and FDA reports) and made the data computable. Moreover, we created bioinformatics analysis tools for assessing chemical structure similarity, allowing us to group the compounds based on their chemical structure in addition to their MOA.
We tested this database for other known skin toxicants that are not covered by the database. Based on their structure similarity to other skin toxicant and/or know biological interaction partners we were able to predict skin toxicity of these compounds and suggest possible MOA.

PS 2182c Identification of Putative Skin Sensitizers Using QSAR Models Enriched by *In Vitro* Data

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Repetitive exposure to a chemical agent can induce an immune reaction in susceptible individuals leading to skin sensitization. We have developed computational models capable of accurately assessing the skin sensitization potential of environmental chemicals. To this end, we have (i) compiled, curated, and integrated the largest publicly-available database of skin-sensitizing chemicals; (ii) used this data to generate and validate QSAR models for skin sensitization; and (iii) employed these models to identify putative sensitizers among chemicals in the Scorecard and Tox21 databases. A random forest method was employed for QSAR modeling of compounds characterized by SiRMS and Dragon descriptors, and the OECD-compliant model validation workflow was followed. The overall classification accuracies of QSAR models discriminating sensitizers from non-sensitizers were 68-88% when evaluated on several external validation sets. When compared to the OECD QSAR toolbox skin sensitization module, our models afforded significantly higher Positive and Negative Predictive Rates. When applied to chemicals within the applicability domains, the models could reliably identify positive and negative sensitizers with 94% and 71% certainty, respectively. Statistically significant descriptors from high-accuracy models yielded SAR rules that could guide structural optimization of chemicals of interest. Using these models, we have identified putative skin sensitizers in the ScoreCard and Tox21 databases as primary hits for

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