PS 631 NOVEL *DUOX* INDUCTION IN EPIDERMAL KERATINOCYTES BY IFN-γ AND IL-4.

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Dual oxidase (DUOX) 1 and 2 are peroxide-generating members of the NOX family of membrane proteins. DUOX proteins have been implicated as peroxide donors during wound healing in mammalian mucosal epithelia and in hydroxylation events that lead to host defense barrier formation in plant tissues. Keratinocyte differentiation in mammals leads to formation of a moisture retaining, protective barrier to the environment. Our studies found DUOX was upregulated during keratinocyte differentiation, suggesting it may be an essential part of this process as well. We have previously shown that IL-4 and IFN-γ cytokine balance regulates DUOX mRNA expression in human airway epithelium *in vitro* in a dose-dependent manner, and that the IFN-γ signaling mechanism is independent of the canonical pathways. We consider this novel role for DUOX in keratinocyte differentiation in the context of response to Th1 and Th2 cytokine agonists. To determine how *DUOX* gene induction in keratinocytes is affected by Th1/Th2 cytokines, the responses of *DUOX1* and *DUOX2* to IL-4 and IFN-γ were examined in epidermal keratinocyte cultures using real time QPCR. Specific dose-response relationships were observed. Exposure to IFN-γ caused a 3 to 5-fold induction of *DUOX2* (EC50 50 ng/mL); no induction of *DUOX1* was observed. However, in contrast to our studies in HBE1 cells, exposure to IL-4 generated both a 3-fold induction of *DUOX1* and a 4 to 6-fold induction of *DUOX2* (EC50 of 10 ng/mL). These inductions were dependent on *de novo* protein synthesis, and no receptor crosstalk was observed between IL-4 and IFN-γ in these cells. Our data are biologically consistent with historically proposed DUOX functions and suggest that imbalanced Th1/Th2 cytokine expression might disrupt differentiation. These findings may help explicate anomalies in wound healing under physiologic, pharmacologic or environmental conditions that also alter cytokine profile. These and future studies to explicate the role of DUOX in keratinocytes may provide a novel mechanistic perspective to assess environmental and industrial toxicant exposure in damaged or healthy skin.

PS 632 *IN VIVO* DERMAL UPTAKE PATTERN FOR 20 DILUTE AQUEOUS ORGANIC CHEMICALS IS POORLY PREDICTED BY MODELS BASED ON *IN VITRO* DATA.

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Updating a similar analysis done in 1994 that addressed only 9 chemicals, previously published data on *in vivo* dermal uptakes from dilute aqueous solution were expressed as permeability coefficients (Kp, in cm/h) for 22 organic chemicals (including 5 phthalates) containing C, H, O, N, S, and/or Cl, and for the organophosphate malathion. Multiple regression on physico-chemical properties yielded a highly predictive model, log Kp = 1.942 – 2.297/√(log Kow) – 0.007551 MW (r = 0.967), for 20 of the 22 considered chemicals (log Kow ≥ 0.8), where log = base-10 logarithm, Kow = octanol:water partition coefficient, and MW = molecular weight. Butylparaben (a cytotoxic antimicrobial) and malathion (the sole organophosphate considered) were excluded as outliers, with Kp values ~10-fold less and ~100-fold greater than predicted, respectively. While *in vivo* Kp measures are all predicted within 2-fold for 20 chemicals, geometric mean errors >10-fold are predicted by five previous models fit only or primarily to *in vitro* diffusion-cell data. Those earlier models include two (one ~20 years old still used by USEPA) intended to adjust estimates of steady-state diffusion measured *in vitro* to better predict total uptake from dilute aqueous solution when non-steady-state exposure conditions may also occur. Models fit solely or primarily to measures of aqueous organic chemical penetration through dermal tissue *in vitro* thus cannot yet reliably predict corresponding dermal uptake measured *in vivo*. Consequently, regulatory approaches to dermal exposure assessment that rely primarily or exclusively on *in vitro* data remain seriously flawed. Instead, models fit only to *in vivo* data should be preferred for non-hydrophilic chemicals, and more *in vivo* data should be generated to support improved, accurate dermal exposure assessment for such aqueous organics, until wide discrepancies between dermal uptakes measured *in vivo* and those measured *in vitro* can be explained and predicted reliably.

PS 633 PREDICTING SKIN PERMEABILITY FROM COMPLEX CHEMICAL MIXTURES: THE IMPACT OF BIOLOGICAL SKIN MODEL SYSTEMS ON QUANTITATIVE STRUCTURE PERMEATION RELATIONSHIPS (QSPR).

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Dermal absorption of topically applied chemicals usually occurs from complex chemical mixtures, yet most attempts to predict dermal permeability use data collected from aqueous binary mixtures. The focus of this research was to develop quantitative structure permeation relationships (QSPR) for predicting chemical absorption from mixtures through skin using two different *in vitro* porcine skin biological systems. A total of 16 diverse chemicals were applied in 384 treatment combinations in pig skin flow-through diffusion cells (PSFT) and 20 chemicals in 119 treatment combinations in the isolated perfused porcine skin flap (IPPSF). Apparent permeability coefficient (k_p*) was calculated using chemical flux into perfusate in the PSFT, while area under the curve (AUC) was calculated for the IPPSF. These data were then fit with a modified Abraham and Martin dermal QSPR model including a sixth term called a mixture factor (MF) to account for mixture interactions based on physico-chemical properties of the mixture components. Goodness of fit was assessed using correlation coefficients (r^2), internal and external validation metrics (q^2_{LOO}, $q^2_{L25\%}$, q^2_{EXT}), and applicable chemical domain. Different mixture factors were needed for each model system. Based on standard deviation and p-values of the model parameters greater than α=0.05, the Abraham and Martin model could be reduced to four terms plus the MF for each biological system. The term which could be eliminated from each model differed in the PSFT (hydrogen-bond acceptor basicity) and in the IPPSF (dipolarity/polarizability). These findings suggest that a QSPR model for estimating apparent k_p* as a function of chemical mixture composition is possible and that the nature of the QSPR model selected is dependent upon the biological level of the *in vitro* test system used. Both of these findings having implications when dermal absorption data is used for *in vivo* risk assessments. (Supported by NIOSH OH-07555)

PS 634 *IN VITRO* EVALUATION OF THE EFFECT OF DOSE, RECEPTOR FLUID, AND VEHICLE ON THE ABSORPTION OF FIVE PHYSICOCHEMICALLY DIFFERENT COMPOUNDS IN PORCINE SKIN.

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Understanding the interactions that control the extent and rate of passive drug absorption through skin is of great interest in pharmaceutical and toxicological research. These interactions involve the physicochemical properties of the test compound, the vehicle, and the anatomy of the skin. This research aimed to identify the rate limiting factors which determine the extent and rate of compound absorption through porcine skin from three vehicles (propylene glycol, water and ethanol), by testing the effect of common experimental variables. Two traditional *in vitro* diffusion cell systems (static and flow-through) were employed to evaluate the effect of finite (20 μL) and infinite (1000 μL) doses and the presence/absence of bovine serum albumin (BSA, 4.5%) in the receptor. Five compounds (caffeine, diclofenac sodium, mannitol, salicylic acid and testosterone) selected on the basis of lipophilicity, molecular weight and commercial 14-C availability were applied at a concentration of 1.28 μg/μL. Flux of each compound into the receptor phase was monitored over 24 hours. Levels of radioactivity were also determined in the stratum corneum (assessed by tape stripping) and the remaining epidermis. Apparent permeability coefficients and percentage of the applied dose absorbed were then calculated and compared. Each compound had its own unique absorption profile. A slight variation in the percent dose absorbed was observed when comparing the presence/absence of BSA, being more evident with the finite doses. The correlation between absorption data from both diffusion cell systems was stronger when BSA was present. For the majority of the compounds, absorption was greatest with a water vehicle. For the infinite dose, the dominant absorption pattern was water>ethanol>propylene glycol. This suggests the influence of these variables should be considered when designing predictive absorption models. (Supported by Novartis Animal Health, Inc.)

PS 635 CHARACTERIZATION OF NORMAL SKIN THICKNESS FOR VARIOUS BODY REGIONS, AGES, AND GENDERS OF YUCATAN MINIATURE SWINE.

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This poster presents the results of a histologic skin characterization study in the Yucatan minipig. Four skin samples [neck, back (lumbar), flank, abdomen] were collected by 8-mm punch biopsy from various age animals and immediately fixed in 10% neutral buffered formalin. The number of hairs per surface area were enumerated then samples were processed, embedded in paraffin, sectioned, and stained with H & E for histologic evaluation. The strateum corneum, cellular epidermis, and dermis layers were measured microscopically using an image analyzing system. Each component measurement was taken at random 5 times and averaged for each sample. Gender, age and body region specific means (μM), ± SDs, and observed ranges are presented for 5 inter-follicular skin components (full thickness, stratum

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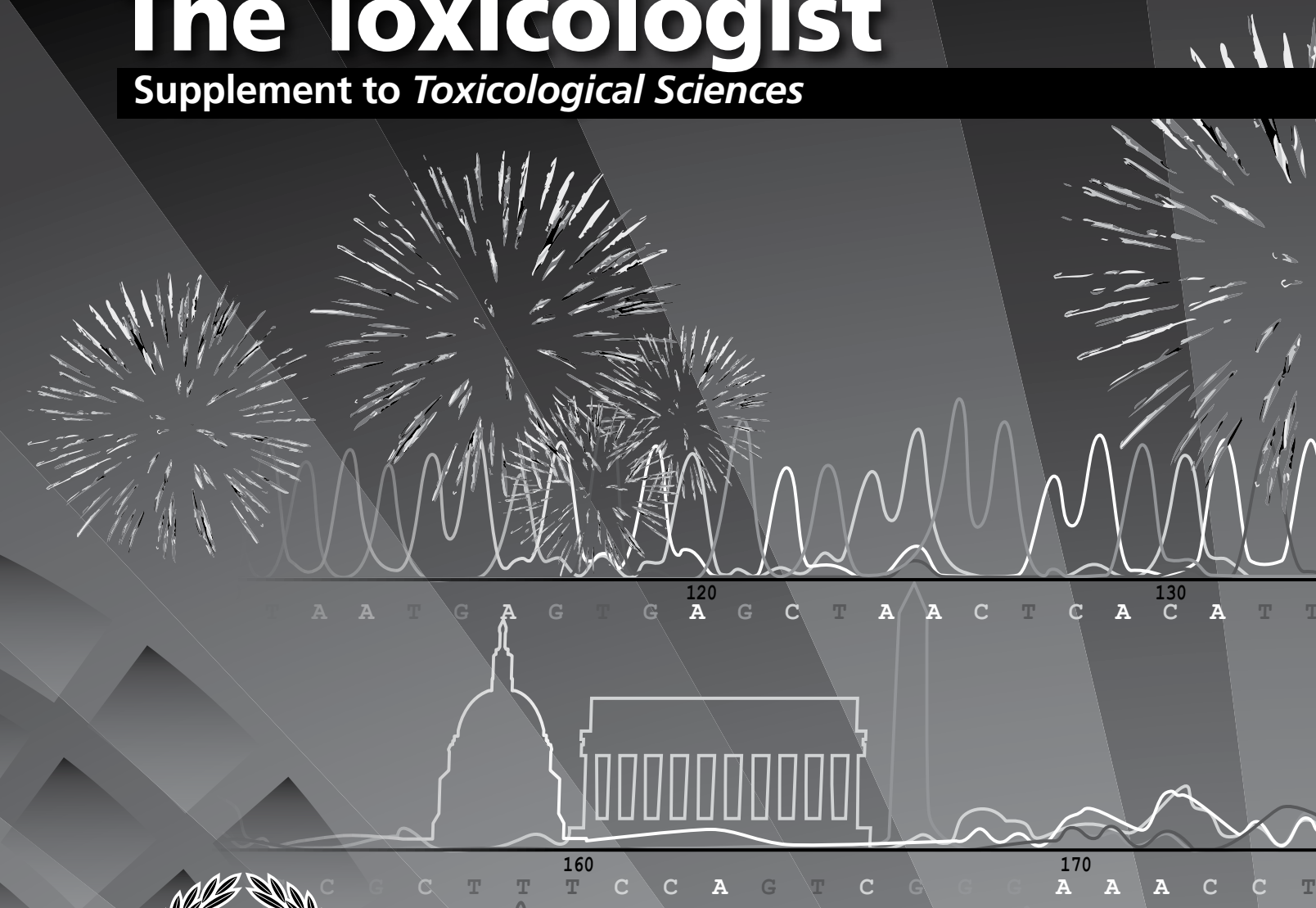

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50th Anniversary Annual Meeting and ToxExpo™ Washington, D.C.

An Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 120, Supplement 2
March 2011

www.toxsci.oxfordjournals.org

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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.

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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