and each average was submitted to two-sided statistical analysis. Differences in the thickness of the epidermis, dermis and hypodermis (the skin layer in which the compound was deposited) were observed craniocaudally in rats of both sexes, while differences in mice were limited to the dermis in males and the hypodermis in both sexes. In male rats, the epidermis and dermis were thinnest and the hypodermis was thickest at the scapular region. In female rats, greater epidermal thickness at each region compared to the lumbar region was deemed biologically insignificant due to its small magnitude (<2 microns). As was found in males, the dermis was thinnest and the hypodermis thickest at the scapular region. In mice, similar differences were observed – the dermis was thinnest at the scapular region in male mice and the hypodermis was thickest at this region in mice of both sexes. In conclusion, sites used for dorsal subcutaneous injection of xenobiotics in rodents are not equivalent. In particular, the thickness of the hypodermis varies significantly. This, along with the volume and formulation of the compound injected, may influence the occurrence of injection site lesions and make their interpretation difficult regarding relevance for human safety.

2086 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN INDUCED MATRIX METALLOPROTEINASE-1 EXPRESSION IN A2058 MELANOMA CELLS REQUIRES THE AHR AND ERK PATHAWAYS.

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There has been a 34% increase in melanoma-related mortality in the United States from 1973 to 1992. Although excessive UV exposure is considered the main etiological factor in melanoma initiation, epidemiological and experimental evidence suggests that exposure to environmental carcinogens contributes to melanoma. Our data show that activation of the aryl hydrocarbon receptor (AhR) pathway activates expression and activity of the matrix metalloproteinases (MMPs). Matrix metalloproteinases are a family of zinc- and calcium-dependent proteinases classified by extracellular matrix substrate specificity and are essential for tissue remodeling events and cancer progression. We examined the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the expression of MMP-1, a proteolytic enzyme that degrades type I collagen, in A2058 melanoma cells. We have shown that TCDD increased MMP-1 expression is AhR dependent and that it requires sequences in the distal portion of the promoter region. Recently it has been discovered that approximately 60% of all melanomas contain an activating mutation, V599EBRAF, in the Ras/Raf MAP kinase pathway. To investigate the contribution of the Ras/RAf signaling pathway on TCDD/AhR-induced MMP expression, we used specific chemical inhibitors for several signaling pathways and examined the effect of TCDD and AhR-activation on phosphorylation events in these cascades. TCDD treatment caused alterations in ERK phosphorylation, and co-treatment with the MEK inhibitor, U0126, abolished phosphorylation of ERK. Further, a minimal MMP-1 promoter construct is induced 4 fold by TCDD, but is not inducible upon co-treatment with U0126. These data demonstrate that TCDD-induced expression of MMP-1 in A2058 cells requires Ras/Raf signaling as well as the AhR pathway.

2087 GLUTATHIONE-MEDIATED PROTECTION AGAINST CHROMIUM-INDUCED DERMAL TOXICITY.

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Occupational exposure to compounds containing chromium has been identified as a risk factor for developing irritant and contact dermatitis among workers. Induction of oxidative stress has been implicated as one of the major mechanisms responsible for the toxicity and the adverse health effects due to exposure to chromium. Presently, we have studied the role of glutathione in the toxicity of chromium following its dermal exposure in mice. The tissue glutathione levels of C57BL/6 mice were depleted by providing them with drinking water containing 20 mM buthionine sulfoximine (BSO). Hexavalent chromium (K2Cr2O7) was applied on the skin of control and BSO-fed mice on alternate days at doses of 0, 1, 5 and 10 mg/kg b.w. either for 2 or 4 weeks. The chromium-induced lesions noticed on the skin of the mice correlated with the dose and duration of the chemical application. In general, the animals with depleted tissue levels of glutathione exhibited more severe skin lesions compared with the control animals indicating the enhanced susceptibility of the GSH-depleted mice to chromium-induced dermal toxicity. Similarly, the amounts of 8-hydroxydeoxy guanosine (8-OHdG) and malondialdehyde (MDA) – indicators of oxidative damage to tissue DNA and lipids, respectively, were higher in the skin of the GSH-depleted mice treated with chromium compared with the controls. The enhanced dermal toxicity of chromium noticed among the GSH-depleted mice compared with the corresponding controls was further supported by the results of gene expression and histopathology studies. In conclusion, these results suggest that the tissue level of glutathione is an important determining factor in the response of mice to the dermal toxicity induced by hexavalent chromium.

2088 DEVELOPMENT OF A METHOD FOR PHARMACOLOGICAL MANIPULATION OF EPIDERMIS USING INTRADERMAL INJECTIONS IN THE RAT.

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Our goal is to deliver signaling molecules or their antagonists directly to epidermal cells of intact rat skin in an attempt to understand chemical-induced changes in signaling pathways. The Stratum corneum limits penetration of most molecules including signaling proteins or their antagonists. We used an intradermal injection technique that avoids disrupting the skin at the site of experimental chemical exposures, by penetrating the skin with a needle about 20 mm away from the exposure site and tunneling subcutaneously to beneath the future site of exposure. In an attempt to determine if injected molecules would diffuse through the basal layer of the epidermis from below, we injected 50 µL of a 0.27 mg/mL solution of recombinant Enhanced Green Fluorescent Protein (EGFP) (27 KDa) in phosphate buffered saline. After 1.5 hours, injected skin was sectioned and studied under a fluorescent microscope. A high concentration of EGFP was observed in the injected region of the dermis. In addition, we were able to visualize some EGFP that moved from the injection site into the epidermal region. To investigate the efficacy of delivering signaling molecule antagonists, we also intradermally injected 100 µL of interleukin 1 receptor antagonist (Il-1ra) (17 KDa) or phosphate buffered saline as a control. We used antibody conjugated with Cy3 to detect Il-1ra, and a FITC-conjugated antibody to keratin 14 to locate the position of the basal membrane of epidermis. Preliminary results from the fluorescent microscopic study show the presence of Il-1ra in the epidermal region. The epidermis was stretched compared to control skin. Although the successful intradermal injection caused a protrusion on the surface like a blister, there was no separation of dermal layers in the region of the skin where the bleb is formed. We conclude that this intradermal injection technique could deliver signaling molecules and their antagonists, which are smaller that 27 KDa, to the rat epidermis. (Supported by the AFOSR)

2089 HUMAN SKIN IN ORGAN CULTURE AND HUMAN SKIN CELLS (KERATINOCYTES AND FIBROBLASTS) IN MONOLAYER CULTURE FOR ASSESSMENT OF CHEMICALLY-INDUCED SKIN DAMAGE.

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Human skin cells (epidermal keratinocytes and dermal fibroblasts) in monolayer culture and human skin in organ culture were exposed to agents that are known to produce irritation (redness, dryness, edema and scaly crusts) when applied topically to skin. Among the agents used were three well-accepted contact irritants (i.e., all-trans retinoic acid [RA], sodium lauryl sulfate [SLS] and benzalkonium chloride) and five contact allergens (oxazolone, nickel sulfate, eugenol, isoeugenol and ethylene glycol dimethacrylate [EGDM]). The corrosive organic mercury compound, aminophenyl mercuric acetate (APMA), was used as an addition test agent. Based on dose-dependent differences in i) cytotoxicity in a 4-hour assay with keratinocytes and fibroblasts, ii) growth suppression in a 2-day assay with the same cells, iii) histological changes (necrosis, altered proliferation and differentiation) in organ-cultured skin, and iv) the profile of secreted molecules from organ-cultured skin, the corrosive agent could be distinguished from the other contact irritants, which, in turn, could be distinguished from the agents with allergenic potential. The use of organ-cultured skin in conjunction with cells derived from the skin in monolayer culture may provide an initial approach to screening agents for deleterious changes in skin.

2090 SUSPECTED INFLUENCE OF P-GLYCOPROTEIN ON THE PENETRATION OF IVERMECTIN IN PORCINE SKIN.

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The purpose of this study was to determine the contribution of P-glycoprotein (P-gp), a transmembrane energy-dependent drug efflux pump, on the dermal absorption of ivermectin, as a possible P-gp substrate. An 8 hour flow-through diffusion cell experiment was performed on porcine skin with perfusate samples collected hourly. The skin was dosed with Ivomec Pour-On for cattle (Ivomec®) spiked with BODIPY-ivermectin (Molecular Probes, Eugene, OR). Verapamil was used as a P-gp inhibitor, while DMSO was used with the BODIPY-ivermectin as a positive control. The skin samples were frozen and viewed on a Nikon C-1 confocal system

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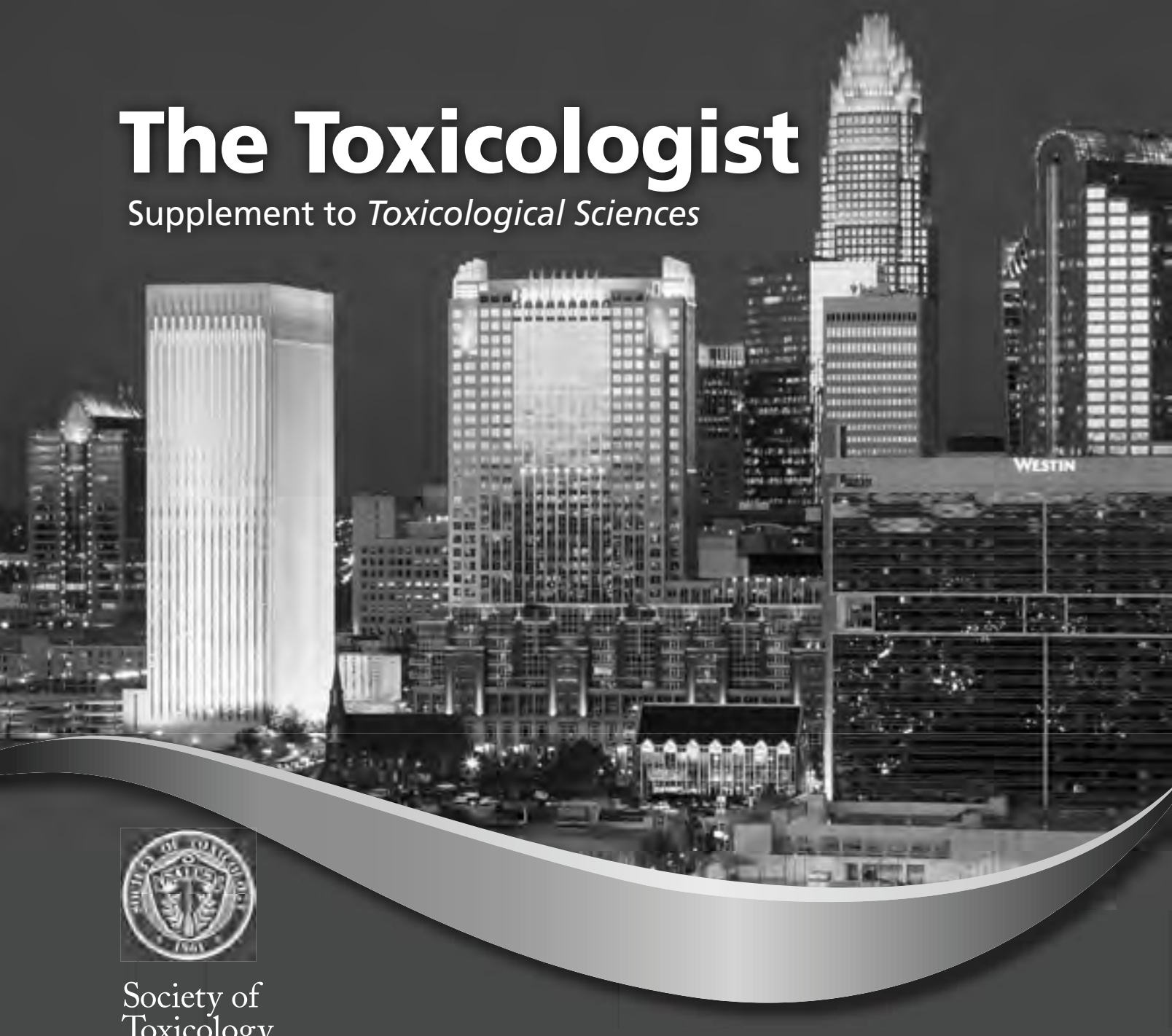

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 46th Annual Meeting of the Society of Toxicology, held at the Charlotte Convention Center, Charlotte, March 25–29, 2007.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

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

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