1588 TOPICAL HYDROCARBON ABSORPTION IN PORCINE SKIN PREVIOUSLY EXPOSED TO JP-8 JET FUEL.

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The percutaneous absorption of topically applied jet fuel hydrocarbons (HC) through skin previously exposed to JP-8 has not been investigated, although this exposure scenario is the occupational norm. Pigs were exposed to JP-8 soaked cotton fabrics for 1 and 4 days with repeated daily exposures. Pre-treated and untreated skin was then dermatomed and placed in in-vitro diffusion cells. Five cells with exposed skin and four cells with unexposed skin were dosed with a mixture of 14 different HC consisting of nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, ethyl benzene, o-xylene, trimethyl benzene (TMB), cyclohexyl benzene (CHB), naphthalene, and dimethyl naphthalene (DMN) in water + ethanol (50:50) as diluent. Another five cells containing JP-8 exposed skin were dosed with diluent only in order to determine the residue potential of jet fuel HC in skin. The data indicated that there was 2-3-fold and 3-4-fold increase in absorption of nonane, undecane, dodecane and tridecane through 1 and 4 days JP-8 pre-exposed skin respectively. Similarly, ethyl benzene, o-xylene and TMB were absorbed 2-3 times more than in controls in 1 day while > 4 times in 4 days JP-8 pre-exposed skin experiments. The absorption of naphthalene and DMN was 1.5 times higher than controls in both 1 and 4 days pre-exposures. The CHB, naphthalene and DMN were found to have significant persistent skin residues capable of considerable further absorption in 4 days pre-exposures as compared to 1-day exposures. The absorption parameters of flux, diffusivity and permeability were calculated for the studied HC. The possible mechanism of increased HC absorption in fuel pre-exposed skin may be *via* lipid extraction from stratum corneum as indicated by electron microscopy. This study suggests that single dose application data for jet fuel HC cannot be used to predict the toxic potential for repeated exposures and that for certain compounds, persistent absorption may occur days post-exposure. Supported by USAFOSR F49620-01-1-0080.

1589 A MURINE MODEL FOR CUTANEOUS PHOTOAGING: OBSERVATIONAL, HISTOPATHOLOGIC AND MOLECULAR ENDPOINTS.

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Photoaging connotes changes in aging skin chronically exposed to sunlight. Clinically, the changes involve skin texture and appearance (wrinkling, sagging, discoloration), microarchitecture (e.g., epidermal thickness, elastin and collagen fiber content and arrangement, cell type and number,) and functional capacity (e.g., repair). Identifying compounds that affect (e.g., increase, halt or reduce) these deleterious responses to solar exposure begins with employing a reliable experimental model to define those responses. The (HRS/Skh-1) hairless albino mouse undergoes photoaging changes when chronically exposed to UVR. Groups of female hairless mice were exposed to 0, 3, 3.75, 4.5, 5.25 or 6 minimal erythema doses (MED) of solar-simulated, environmentally relevant UVR per week, for 4 or 8 weeks. Clinical observations were performed weekly and skinfold thickness (SFT) measured at weeks 0, 2, 4, 6 and 8 weeks. Skin samples harvested at 4 or 8 weeks were frozen or formalin-fixed, sectioned, stained and evaluated histopathologically using visual and image analysis techniques. Mean SFT increased in a UVR dose-dependent manner up to 5.25 MED/week. Incidence and severity of erythema, edema, flaking, thickening (assessed visually) and wrinkling increased in a UVR dose-dependent manner up to 4.5 MED/week. Mast cell density (MSD) and average epidermal thickness (AET) was greatest at 4 weeks at UVR exposures of 5.25 MED and 6.0 MED, respectively. Elastin content (EC) was increased at 4 weeks but the increase was not UVR dose-dependent. MSD, AET and EC was reduced at 8 weeks for all groups, as compared with 4 weeks. The hairless mouse along with the use of environmentally relevant UVR is a predictable and repeatable photoaging model for the evaluation of both safety (i.e., enhancement) and efficacy (i.e., prevention and/or amelioration) by novel compounds using these observational and histopathological endpoints.

1590 DIFFERENTIAL REGULATION OF COX-2 EXPRESSION BY ULTRAVIOLET LIGHT IN KERATINOCYTES AND MACROPHAGES.

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Chronic exposure to sunlight is a significant causative factor in the development of skin cancer. Modifications of DNA and other critical cellular macromolecules by the higher energy shorter solar wavelengths comprising the UVB spectra (290 to 320 nm) are the most damaging to the skin. Recent studies have demonstrated a link between expression of cyclooxygenase-2 (COX-2), formation of prostaglandins and the development of skin cancer. UVB is also known to induce COX-2 in human skin cells. In the present studies, we compared the effects of UVB on COX-2 expression in JB-6 and PAM 212 cells, two murine keratinocyte cell lines, and RAW264.7, a murine macrophage cell line. In macrophages, bacterially derived lipopolysaccharide (LPS, 1 μg/ml) was found to rapidly induce COX-2 protein expression as determined by western blotting. The keratinocyte cell lines constitutively expressed COX-2. LPS caused a 3-4 fold increase in expression of this protein. Whereas in PAM 212 cells, UVB light (10-25 mJ/cm^2) caused a 3-4 fold increase in COX-2 expression, it had no effect on expression of the protein in JB6 keratinocytes or RAW264.7 macrophages. In PAM 212 cells, the combination of UVB light and LPS did not further increase COX-2 expression while in JB6 cells, UVB light had no effect on LPS-induced COX-2 expression. In contrast, in macrophages, UVB light was found to suppress LPS-induced expression of COX-2. In macrophages, UVB light-induced the JNK and p38 MAP kinases. Inhibition of these kinases with JNK inhibitor II (1, 9-pyrazoloanthrone) or the p38 kinase inhibitor SB-203580 was found to reverse the inhibitory effects of UVB light on LPS-induced expression of COX-2. Taken together these data indicate that the effects of UVB light on COX-2 expression is dependent on cell type. In macrophages, MAP kinases appear to mediate the inhibitory effects of UVB light on LPS-induced COX-2 expression. Supported by NIH grants CA100994, ES005022 and ES006897.

1591 EPIDERMAL CYTOKINE SECRETION INDUCED BY CHEMICAL CONTACT AND RESPIRATORY ALLERGENS.

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Repeated topical exposure of BALB/c strain mice to chemical contact and respiratory allergens results in preferential T helper (Th)1- and Th2-cell activation, respectively. In addition, it has been shown that respiratory allergens, such as trimellitic anhydride (TMA), stimulate epidermal Langerhans cell (LC) migration with delayed kinetics compared with contact allergens, such as 2, 4-dinitrochlorobenzene (DNCB). Experiments using anti-interleukin (IL)-10 antibodies *in vivo* suggest that cutaneous IL-10 may contribute to the differential regulation of LC migration by these chemicals. To investigate further the mechanistic basis for the development of polarised immune responses, we have examined the production of epidermal cytokines provoked following a single topical application to BALB/c strain mice of DNCB (1%), TMA (25%) or vehicle (acetone:olive oil, 4:1; AOO). Skin explants were excised from mice exposed on the dorsum of both ears for various periods (30min-6hr) to chemical and were cultured on medium prior to analysis of supernatants for the presence of tumour necrosis factor α (TNF-α), IL-1β, IL-1α, IL-6, IL-10, IL-12p40, IL-12p70 and IL-17 using the Bio-PlexTM cytokine array system. Enhanced production of IL-1β, a cytokine involved in the initiation of LC migration, was detected only following exposure to DNCB, with 15-fold increases induced by 6hr of exposure. In addition, only exposure to DNCB was associated with early (2hr) secretion of IL-17. In contrast, up-regulation of IL-10, a cytokine that inhibits LC mobilization, was evident only for TMA during the first 3hr of exposure, with 2 to 3-fold increases in IL-10 release being induced. Small increases in IL-1α levels were apparent for both chemicals. No alterations in either IL-6, IL-12p40 or IL-12p70 secretion were recorded and TNF-α remained undetectable throughout. These data suggest that discrete epidermal cytokine secretion profiles induced following exposure to chemical contact and respiratory allergens might contribute to the polarisation of immune responses, possibly through effects on LC function.

1592 *IN VIVO* AND *IN VITRO* DERMATOTOXICITY OF CUTTING FLUID MIXTURES.

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Cutting fluids are widely used in the metal-machining industry to lubricate and reduce heat generation when metals are cut by a metal-cutting tool. Unfortunately, these cutting fluids have caused occupational contact irritant dermatitis (OCID), and many of the additives used in these cutting fluid mixtures are thought to be responsible for OCID in workers. The purpose of this study was to assess single or various combinations of these additives in initiating the OCID response following an acute 8 hr exposure in porcine skin *in vivo* and *in vitro* using the isolated perfused porcine skin flap (IPPSF). Pigs (n=4) were exposed to 5% mineral oil (MO) or 5% polyethylene glycol (PEG) aqueous mixtures containing various combinations of 2% triazine (TRI), 5% triethanolamine (TEA), 5% linear alkylbenzene sulfonate (LAS), or 5% sulfurized ricinoleic acid (SRA). Erythema and edema were evaluated and skin biopsies for histology were obtained at 4 and 8 hrs. IPPSF's

(n=4) were exposed to control MO or PEG mixtures and complete MO or PEG mixtures, and perfusate samples were collected hourly to determine IL-8 release. The only significant mixture effects were SRA+MO+LAS+TRI+TEA causing an increase in IL-8 release. Exposure to TRI alone appeared to increase erythema, edema, and dermal inflammation compared to the other additives, while SRA alone was least likely to initiate a dermal inflammatory response. In 2-component mixture exposures, the presence of TRI appeared to increase the dermal inflammatory response at 4 and 8 hrs especially with the PEG mixtures. In the 3- and 4-component mixtures, MO mixtures are more likely to incite an inflammatory response than PEG mixtures. In summary, these preliminary studies suggest that the biocide, TRI, is the more potent of the 4 performance additives in causing dermal irritation, and this may vary depending on whether the worker is exposed to a synthetic- or MO-based fluid.(Supported by NIOSH Grant R01-OH-03669)

1593 INVESTIGATION OF THE SENSITIZATION POTENTIAL OF FRAGRANCE INGREDIENT 3 AND 4-(4-HYDROXY-4-METHYLPENTYL)-3-CYCLOHEXENE-1-CARBOXALDEHYDE (HMPCC).

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3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC, CAS Nos. 31906-04-4 and 51414-25-6) is a fragrance ingredient with a sweet, light and floral odor used in all types of cosmetic and household products. Three guinea pig studies (a modified Draize and two Magnusson and Kligman maximization tests) and a murine local lymph node assay indicate that the material has a weak potential to induce skin sensitization. The results from human predictive tests (numerous repeated insult patch tests and one human maximization test) conducted at various concentrations, also predict that the material is a weak skin sensitizer. The diagnostic patch test data indicate that the material is a moderate to strong skin sensitizer. The skin sensitization potential of this material is summarized and a dermal sensitization risk assessment is presented.

1594 ASSESSMENT OF SKIN ABSORPTION AND METABOLISM OF ARACHIDONIC ACID & GLYCERYL ARACHIDONATE USING *IN VITRO* DIFFUSION CELL TECHNIQUES.

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Arachidonic acid (AA), has been used in cosmetics as a surfactant-cleansing and emulsifying agent. Glyceryl arachidonate (GA), a skin conditioning agent and emollient, may be partially metabolized by ester hydrolysis in skin to AA. Based on the Cosmetic Ingredient Review (CIR) Panel's concern that there was a lack of dermal absorption data for AA, *in vitro* percutaneous absorption and metabolism studies were initiated. To simulate normal consumer use, AA and GA were applied in an oil in water emulsion (2mg/cm^2) to skin samples 200um thick in flow-through diffusion cells perfused with a physiological buffer. To assay for permeation, *via*ble fuzzy rat and human skin were dosed with [^{14}C]AA containing ~0.5uCi (0.01mg) AA /cell while [^{3}H]GA was applied to *via*ble and cadaver human skin at ~0.5uCi (0.003mg) GA/cell. For metabolism analysis, the skin equivalent Epiderm was dosed with [^{3}H]GA under similar conditions. Receptor fluid fractions were collected at 6h intervals over a 24h dosing period. Skin penetration was determined by liquid scintillation counting and expressed as a percent of the applied dose. High performance liquid chromatography was used to assess metabolism to AA. Absorption of AA through rat skin was 19.8 ± 5.3 % (mean ± SEM) compared to only 1.4 ± 0.3 % through human skin. Total AA penetration (receptor fluid plus skin levels) in rat and human skin was 52.3 ± 7.3 and 20.1 ± 5.4 %, respectively. Total GA penetration of *via*ble skin was found to be 11.3 ± 2.1% with only 3.2 ± 0.5% absorbed through the skin. In cadaver skin, 4.8 ± 0.8% GA was absorbed through skin with a total penetration of 6.7 ± 1.2%. Assay of the Epiderm receptor fluid found ~50% absorption of radioactivity and 3.0 ± 2.1% GA conversion to AA. In conclusion, percutaneous absorption of AA was less through human than rat skin, while GA absorbed through Epiderm was metabolized to AA.

1595 MIXTURE EFFECTS OF JET FUEL ALIPHATIC AND AROMATIC HYDROCARBONS ON HUMAN EPIDERMAL KERATINOCYTES.

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Jet fuels are complex mixtures of aliphatic (ALI) and aromatic (ARO) hydrocarbons that vary significantly in individual cytotoxicity and proinflammatory activities in human epidermal keratinocytes (HEK). In order to elucidate the dermatotoxicity of a complex mixture like jet fuels, structural differences, exposure time and dosage were investigated on HEK toxicity assessed by mortality and IL-8 release. ALI and ARO hydrocarbons were grouped into 4 categories: highly cytotoxic (octane, nonane, decane for aliphatics and cyclohexalbenzene, trimethylbenzene, xylene for aromatics), low cytotoxic (tetradecane, pentadecane, hexadecane for aliphatics and benzene for aromatics), high IL-8 release (decane, undecane, dodecane for aliphatics and dimethylnapthalene, cyclohexylbenzene, ethylbenzene for aromatics) and low IL-8 release (tetradecane, pentadecane, hexadecane for aliphatics and benzene, toluene, xylene for aromatics). The 4 categories of ALI hydrocarbons were mixed with each other, or cross-mixed with each of the 4 categories of ARO hydrocarbons. The resulting cytotoxicity and IL-8 production from HEK were evaluated at 24 hrs. The results showed an antagonistic cytotoxic effect between ALI and ARO hydrocarbons in which ALI attenuated the degree of HEK mortality caused by the ARO hydrocarbons. On the other hand, the ARO hydrocarbons reduced the significant increase of IL-8 induced by ALI hydrocarbons. Synergistic effects between low IL-8 inductive and low cytotoxic hydrocarbons were found and the highest cytotoxic and IL-8 inductive responses did not completely correspond to the mixture of highly cytotoxic and highly IL-8 inductive hydrocarbons. This study supports the concept that the ARO dictate the degree of HEK mortality, while the ALI are the major contributor to inciting the proinflammatory response. Mixture effects must be considered when evaluating cytotoxicity to HEK. Support: NSC Taiwan 92-2313-B-005-117

1596 DIPHOTERINE:SKIN SENSITIZATION STUDY IN THE GUINEA PIG.

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Diphoterine is an eye/skin decontamination solution for chemical splashes, produced by the PREVOR laboratory, France. Its chemical and physical properties allow a quasi polyvalent rinsing of chemical splashes with a quick return to a physiological state. Diphoterine has been used since several years by workers in industries on European Market. No sensitization cases have been reported. Here we present the results obtained on a possible delayed sensitizing capacity of Diphoterine. It was evaluated in the Guinea pig, in accordance with the general requirements of OECD Guideline and Directive 67/548/EEC. The experimental technique is based on those of Magnusson-Kligman and Guillot and coll. The sensitivity and the reliability of the experimental method are verified at least six months, by use of a positive control group in which animals are treated with DNCB used as a 1% alcoholic solution. The application of the test substance Diphoterine did not induce coloration of the application site. Grading of any skin lesions was therefore possible. Primary induction was performed at day 1 by intradermal injection, the sensitization phase by topical application at day 9 and the challenge at day 22 by topical application with 100% v/v concentration of the substance. Determination of the degree of allergenicity at times 24 and 48 hours was based upon the percentage of animals in the group showing a reaction, rather than on the severity on the latter. Under the experimental conditions adopted, results obtained were as follows:no irritation reaction was noted at times 24 and 48 hours in animals of the negative control group and in animals treated during the challenge phase with Diphoterine at the Maximum Non-Irritant Concentration. The test substance showed no allergenicity at 24 and 48 hours. The test substance showed no allergenicity of Class at 24 hours and 48 hours. According to the terminology employed, it is considered that Diphoterine is free of sensitizing capacity in the Guinea pig. Keywords:Diphoterine, Skin, Allergy

1597 ROLE OF SP-1 IN SDS-INDUCED ADIPOSE DIFFERENTIATION RELATED PROTEIN SYNTHESIS IN HUMAN KERATINOCYTES.

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Skin irritation is a complex phenomenon, and keratinocytes, owing of their anatomical location and production of inflammatory mediators, play an important role in it. We have recently characterized the expression and protective role of adipose differentiation related protein (ADRP) in skin irritation (J. Invest. Dermatol. 121: 337, 2003). In particular, ADRP expression is induced to recover functional cell membrane following the cell damage induced by skin irritants. The purpose of this study was to characterize in a human keratinocyte cells line (NCTC 2544) the biochemical events that lead to ADRP expression following SDS treatment, and, in particular, to investigate the role of transcription factor SP-1. Analysis of ADRP promoter region revealed indeed a potential binding site for the transcription factor SP-1 together with AP-2, NF-1, c-myc and Pax-1. We found that SDS induces a dose and time related SP-1 activation as evaluated by measuring the DNA binding activity, which is correlated with SDS-induced ADRP mRNA expression. Furthermore, SDS-induced SP-1 activation, ADRP mRNA expression and lipid droplets accumulation could be modulated by mithramycin A, an antibiotic that selectively binds to the GC box preventing SP-1 binding and gene expression,

Society of Toxicology
43rd Annual Meeting
Baltimore, Maryland

THE TOXICOLOGIST

a supplement to
TOXICOLOGICAL SCIENCES

An Official Journal of the Society of Toxicology

Volume 78, Number S-1, March 2004

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, workshop, roundtable, platform and poster sessions of the 43rd Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 21-25, 2004.

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