idative stress-mediated toxicity in dopaminergic SH-SY5Y cells. Thus, these results suggest that paraquat may cause oxidative stress in dopaminergic neurons of human brain, and support the hypothesis that paraquat is an environmental risk factor implicated in the incidence of PD.

2171 DIFFERENTIAL INDUCTION OF CYCLOOXYGENASE-2 (COX-2) AND HEME OXYGENASE-1 (HO-1) BY UVB LIGHT IN GROWING AND CALCIUM-DIFFERENTIATED PRIMARY CULTURES OF MOUSE KERATINOCYTES.

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Exposure to ultraviolet light as a component of sunlight has been demonstrated to be a major factor in the development of skin cancer. The high energy wavelengths of the ultraviolet B (UVB) spectra (290-320 nm) can cause damage to DNA and also initiate an inflammatory response as well as intracellular oxidative stress. Numerous studies have shown that the presence of inflammatory mediators and reactive oxygen species contribute to the development of cancer. COX-2, responsible for the formation of prostaglandins, is known to be induced by UVB in human skin. HO-1, known to be induced by reactive oxygen species, is an effective cellular antioxidant. In the present studies, we compared the effect of UVB on the expression of COX-2 and HO-1 in undifferentiated and calcium-differentiated primary mouse keratinocytes. Both cell types constitutively express small amounts of COX-2 and HO-1 mRNA as measured by real-time PCR. Differentiated cells, however, express 2-fold more HO-1 than undifferentiated cells. UVB light (2.5 to 25 mJ/cm^2) caused a dose-dependent increase in expression of both COX-2 and HO-1 mRNA and protein as measured western blotting. Undifferentiated cells showed a greater response to UVB light than differentiated cells in expression of both COX-2 and HO-1, with approximately twice the induction at the maximal UVB dose. In contrast, the differentiated cells exhibited higher absolute induction at every UVB dose. Taken together, these data indicate that not only the dose of UVB but also the differentiation state of the keratinocytes is critical to expression of either a pro-inflammatory enzyme, COX-2, or an antioxidant and protective enzyme, HO-1. Supported by NIH grants ES05022, CA100994, CA093798 and ES011932

2172 DECREASING MALATHION APPLICATION TIME FOR LICE TREATMENT REDUCES TRANSDERMAL ABSORPTION.

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Background: Head lice are the most common parasitic infestation in the United States and Europe. Children are commonly infected with head lice requiring topical treatment with pediculicides. The instructions for the Ovide® 0.5% malathion formulation include placing formulation on dry hair and leaving it on for eight to twelve hours. Ovide®, however is effective at killing 100% of both lice and nits in ten minutes. Our concern of over exposing children to malathion has led us to examine whether significantly more malathion will penetrate transdermally when applied for the recommended eight hours than for a shorter but apparently equally effective period. Methods: *In vitro* absorption studies were performed to determine if reducing malathion application time decreases skin absorption. Ovide® was placed on either full thickness haired rat skin or human abdominal skin for 0.5, 2, 4, or 8 hours before removal, skin was thoroughly washed with shampoo and penetration was allowed to continue for either 24 (rat and human), 48 (rat) or 72 (rat) hours. Results: A 0.5 hour exposure caused 0.36 + 0.14 % of the donor malathion to penetrate through human skin after 24 hours and 2.1 + 0.6% remained in the skin after washing with shampoo. After 8 hours of topical applications penetration was approximately 3 fold greater (1.02 + 0.41) and 3.4 + 0.5% remained in the skin ($p<0.05$ vs 0.5 hr). The relationship between absorption and exposure time also occurred for haired rat skin ($p<0.05$) although the magnitude was smaller than for human skin. This differential continued for the full 72 hours studied. Conclusions: Reducing Ovide® application from the suggested 8-12 hours to 30 minutes can significantly reduce transdermal absorption of malathion, without decreasing the product's efficacy. Further clinical studies in children are warranted to confirm the efficacy of this shortened application time.

2173 DERMAL PENETRATION OF SODIUM LAURYL SULPHATE AND ITS EFFECT UPON THE ABSORPTION OF OTHER CHEMICALS *IN VITRO*.

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Sodium Lauryl Sulphate (SLS) (an anionic surfactant) is a known skin irritant. The irritation caused by SLS produces reversible changes in the barrier function of the skin. The objective was to investigate the dermal penetration and localisation of SLS through full-thickness and dermatomed human skin *in vitro* and its effect upon the dermal penetration of testosterone and water. Human breast skin was mounted in flow-through diffusion cells. Receptor fluid (MEM + 2%w/v BSA) was pumped at 1.5ml/hr (pH 7.4, 32°C). 2%w/v ^{14}C-SLS in water (25µl) was applied to the surface (0.64cm^2) of full thickness (~1mm) and dermatomed (280µm) skin for 1hr, removed, and the distribution determined at 0, 3, 6, 12 and 24hrs after removal of the SLS. SLS (0, 1, 2, 4 and 10%w/v [100µl]) was applied to full-thickness skin for 1hr then rinsed off. ^{3}H-water (100µl) was applied to the treated surface for 11hrs. In a further study, ^{14}C-testosterone (10µl) was applied for 24hrs to full-thickness and dermatomed skin pre-treated with SLS. Following a 1hr application of SLS (2%w/v), 4-5% of the applied dose was absorbed into full-thickness skin, and 1-1.5% into dermatomed skin, with no significant change in distribution over time. SLS did not readily pass into the receptor fluid, remaining within the stratum corneum and dermis after 24hrs. The total amount of water penetrating to the receptor fluid increased with concentration of SLS pre-treatment. However, with increasing concentration of SLS, testosterone penetration into the receptor fluid, decreased through both full-thickness and dermatomed skin, remaining within the skin instead. It appears that SLS localisation affected the skin such, that the passage of a hydrophilic compound (water) into the receptor fluid was increased, whilst the penetration of a lipophilic compound (testosterone) was reduced. It is believed that SLS forms micelles within the stratum corneum and dermis, preventing lipophilic chemicals from passing into the receptor fluid *in vitro*.

2174 INVESTIGATION OF SKIN BARRIER CREAMS FOR LOWERING PENETRATION OF JP-8 JET FUEL THROUGH *IN VITRO* PIG SKIN.

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Skin irritation remains a concern for Air Force personnel exposed to JP-8 jet fuel. One manner to lower or eliminate skin and systemic JP-8 exposure is to use a skin-enhancement barrier cream. To determine inhibition of JP-8 penetration for a number of existing marketed products, we applied an even coverage of each barrier cream on a 10 cm2 section of harvested dorsal pig skin (dermatome avg. 0.6mm). After application of even coverage of a barrier cream at room temp onto pig skin, and the skin was weighed, placed in static penetration cell at 37C for 45 min, and exposed to JP-8 (2mL) for 4 hr. JP-8 chemical component penetration was captured in VOLPO-saline (20 mL) into the lower chamber. Samples (20µl) were withdrawn from the lower chamber sidearm and heated (140C) to stable vapor phase using a headspace sampler for component separation on a non-polar SPB-1 column with FID detection. Total average area of eluted hydrocarbon vapor from samples (n=3) was compared between the coated and non-coated pig skin after the 4hr penetration study. Generally, products tested ranged from no barrier properties, to a 35% rate of the control. Also, after completion of the 4hr penetration experiment, the pig skin was wiped with water and paper towels, and skin punch samples (4mm diameter) were taken with a dermal biopsy punch for vapor headspace analysis. These skin punch results showed the best barrier creams for both barrier maintenance as well as those aiding surface removal of JP-8 from skin. The results varied from no benefit to 15% of the JP-8 amount in unprotected skin. The best barriers creams to JP-8 were pr88, Ply No. 9, Chimal, SERPACWA and Prolin skin guard. These results show that not all creams promoting non-polar barrier qualities would create a barrier to JP-8 penetration in this model. Further work will test the degree of protection against JP-8 irritation each cream offers with regards to skin exposure of JP-8 to New Zealand white rabbits.

2175 EFFECT OF ACUTE AND CHRONIC EXPOSURE TO THE CLEANSER, TRICHLOROETHYLENE, ON THE DERMAL ABSORPTION OF THE BIOCIDE TRIAZINE.

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Triazine is often added to cutting-fluid formulations in the metal machining industry as a preservative that inhibits the growth of biological material. Unfortunately, triazine has been shown to cause contact dermatitis in exposed workers.

Trichloroethylene, TCE, is a solvent used for cleaning and rinsing. The purpose of this study was to examine the effect of TCE on the dermal absorption of the biocide triazine. A porcine skin *in vitro* flow-through diffusion cell system was used for these studies. In one set of experiments, the diffusion cells were dosed topically with 14C-Triazine mixtures containing TCE and the 3 most common cutting-fluid components as aqueous mineral oil (MO) or polyethylene glycol (PEG) mixtures. The cutting-fluid components were 5% linear alkylbenzene sulfonate (LAS), 5% triethanolamine (TEA), and 5% sulfated ricinoleic acid (SRA). In another set of experiments, the diffusion cells were dosed with 14C-Triazine mixtures containing the cutting-fluid components in aqueous MO or PEG-based mixtures after having been pre-exposed in situ to TCE for 96 hours. In the MO-based formulations, the absorption of 14C-Triazine ranged from 3.05 to 3.65% dose. In the PEG-based formulations, the absorption of 14C-Triazine ranged from 2.70 to 4.06% dose. In both sets of experiments, the porcine skin that was pre-exposed in situ to TCE showed greater absorption of 14C-Triazine. The chronic effects of TCE appear to be more important in the PEG-based mixtures than in the MO-based mixtures. Supported by NIOSH Grant R01-OH-03669

2176 EFFECT OF *IN VIVO* JET FUEL EXPOSURE ON SUBSEQUENT *IN VITRO* DERMAL ABSORPTION OF AROMATIC AND ALIPHATIC HYDROCARBONS.

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The percutaneous absorption of topically applied jet fuel hydrocarbons (HC) through skin previously exposed to fuel has not been studied, although this exposure is the occupational norm. Pigs were exposed to JP-8 fuel soaked cotton for 1 and 4 days with repeated daily exposures. Pre-exposed and unexposed skin was dermatomed and placed in in-vitro diffusion cells. Five cells with exposed and four cells with unexposed skin were dosed with a mixture of 14 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. Five cells containing JP-8 exposed skin were dosed solely with diluent to determine skin HC retention. Flux, diffusivity and permeability were calculated. There was a 2- and 4-fold increase in absorption of specific aromatic HC like ethyl benzene, o-xylene and TMB through 1 and 4-day JP-8 pre-exposed skin, respectively. Similarly, dodecane and tridecane were absorbed more in 4-day than 1-day JP-8 pre-exposed skin. Absorption of naphthalene and DMN was 1.5 times greater than controls in 1 and 4-day pre-exposures. CHB, naphthalene and DMN had significant skin retention in 4-day pre-exposures as compared to 1-day exposures that might be capable of further absorption days post exposure. The possible mechanism of an increase in HC absorption in fuel pre-exposed skin may be via lipid extraction from the stratum corneum as indicated by Fourier Transform Infrared (FTIR) spectroscopy. This study suggests that pre-exposure of skin to jet fuel enhances the subsequent *in vitro* absorption of HC. Single dose absorption data from naive skin may not be optimal to predict the toxic potential for repeated exposures. For certain compounds, persistent absorption may occur days after the initial exposure. Supported by USAFOSR F49620-01-1-0080

2177 ABSORPTION OF LAWSONE THROUGH HUMAN SKIN.

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Lawsone (2- hydroxy-1, 4-naphthoquinone) is the principal color ingredient in henna, a color additive approved with limitations for coloring hair by the Food and Drug Administration under 21 CFR 73.2190. The safety of lawsone as a coloring agent in hair dye products has recently been evaluated by the Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) of the EU. The SCCNFP concluded that lawsone was mutagenic and not suitable for use as a hair coloring agent. Studies were conducted to measure the extent of lawsone absorption through human skin. Lawsone skin absorption was determined from two hair coloring products and two shampoo products, all containing henna. [^{14}C]-Lawsone (Sp. Ac. 22.9 mCi/mmol) was added to each commercial product and applied to excised, non-viable human skin (approx. 240 microns thick) mounted in flow-through diffusion cells perfused with a physiological buffer (HEPES-buffered Hanks' balanced salt solution, pH 7.4). Products remained on the skin for 5 min (shampoos) and 1 h (hair color paste). For the henna hair paste products, 0.29 and 1.4% of the applied dose was absorbed into the receptor fluid in 24 h while 2.2 and 3.7% remained in the skin. For the henna shampoo products, 0.32 and 0.34% of the applied dose was absorbed into the receptor fluid at 24 h while 3.6 and 6.8% remained in the skin. For all products, most of the lawsone applied was washed from the surface of the skin (83 - 102%) at the end of the exposure period. Extended absorption studies were conducted for 72 h to determine if skin levels of lawsone in the 24 h studies might eventually be percutaneously absorbed. These studies determined that the majority of the lawsone remained in the skin with little diffusing out into the receptor fluid. For example, the 72 h receptor fluid values following administration of henna paste products were only 0.48 and 1.61%. Since most of the receptor fluid values did not increase significantly during the extended studies, the receptor fluid values at 24 h should be used in a subsequent exposure assessment.

2178 SKIN PENETRATION OF BREAK-FREE CLP IN THREE SPECIES; SPRAGUE DAWLEY RAT, CD-1 MOUSE, AND YORKSHIRE PIG.

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Break-Free CLP (a complex mixture of polyalphaolephin oil, proprietary ingredients and other hydrocarbons) is used by the US and some European military, police departments and private citizens to clean, lubricate and protect firearms. Skin exposures from this widespread use are likely, although it is unknown whether systemic toxicity can result from skin contact. The purpose of this study was to determine the penetration of Break-Free in laboratory animal skin so that the hazards to humans can be estimated. We used static diffusion cells and GC/MS headspace analysis of receptor and skin samples to estimate the flux of liquid Break-Free across the skin for up to six hours. The sum of the concentrations of six major constituents of Break-Free was used as a surrogate for the concentration of the mixture. The constituents were decane (RT = 2.86 min), butanedioic acid dimethyl ester (RT= 3.2 min), undecane (RT= 4.25 min), dodecane (RT= 6.9 min), hexanedioic acid dimethyl ester (RT= 8.8min), and tridecane (RT= 11.4 min). Average total flux values were 9.78 ug/cm2/h for pig, 43.2 ug/cm2/h for rat, and 131 ug/cm2/h for mouse. Concentration of chemical in the skin following a 6 hour exposure was 0.949 ug/mg skin in pig, 9.78 ug/mg skin in rat, and 31.6 ug/mg skin in the mouse. As expected, different components of Break-Free were found in the receptor solution than were found in the skin following exposure. The major Break Free components found in the receptor solution of all three species were decane and undecane, while the skin had highest concentrations of butanedioic acid dimethyl ester and dodecane. All six measured components were present to some extent in both receptor solution and skin of all species examined. We conclude that penetration of Break-Free is measurable but low in these species. The potential for systemic toxicity from this mixture can be evaluated based on toxicity of the individual components. (Funded by Naval Health Research Center Detachment Environmental Effects Laboratory)

2179 CHANGES IN GENE EXPRESSION IN RAT EPIDERMIS AFTER JET FUEL (JP-8) EXPOSURE TO THE SKIN.

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An estimated 13 million US workers are potentially exposed to chemicals and as a result irritant contact dermatitis is a common occupational disease. Understanding the molecular mechanisms behind skin irritation may establish prophylactic and therapeutic interventions for jet fuel-induced skin disease. No previous studies have shown how JP-8 fuel affects gene expression of skin cells located in the epidermal layer. The purpose of our study was to identify genomic changes in rat epidermis resulting from jet fuel-induced skin irritation. Five hundred microliters of JP-8 was placed in a Hill Top Chamber and secured with a Lomir rodent jacket onto the shaved mid-scapular region of male Fisher 344 rats. Exposure time was one hour, and the skin was collected and observed at 1, 4, and 8 hours after the beginning of the exposure. Skin samples were compared with samples from sham-treated rats. At the conclusion of the experiment, skin was excised, and the epidermis was removed in 5 micron sections using a cryotome. Total RNA was isolated from the epidermal layer. We measured changes in gene expression using the Affymetrix gene array (Rat Genome U34A chip). This experiment showed that changes in gene transcripts occur as early as one hour and become progressively greater after initiation of treatment with JP-8. At one hour, 116 genes were up regulated and 14 were down regulated. At four hours, 175 gene transcripts were up regulated and 100 were down regulated. At eight hours, 327 transcripts were up regulated and 473 were down regulated. When transcripts showing the greatest changes were cross-referenced within the Simplified Gene Ontology database (GeneSpring), the molecular function categories associated with these transcripts differed over time. After one hour, the nucleic acid binding genes were up-regulated the most. Enzymes were the most changed category of genes at the two later time points. These studies illustrate the rapid response of gene expression and the sequence of gene induction immediately after JP-8 exposures to the skin. (Funded by Air Force Office of Scientific Research)

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