constituents on the dermal bioavailability, additional dermal studies were conducted using reformulated enamel paint with the titanium dioxide and xylene cosolvent replaced by toluene. PBPK model simulation of the exhaled breath data from these exposures required a Kp value roughly half the value from the intact paint (0.032 cm/hr) although the toluene concentration was more than 12 times greater. These data suggest the permeability of toluene is influenced by the exposure concentration and less so by the exposure matrix. (Supported by NIOSH grant 1-RO1-OH03658-01A2).

1852 SKIN PENETRATION AND EVAPORATION OF p-MENTHANE-3, 8-DIOL IN ETHANOL AND IN LOTION FORMULATION AFTER TOPICAL APPLICATION TO EXCISED PIG AND RAT SKIN: A MODEL FOR HUMAN DERMAL ABSORPTION.

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p-Menthane-3, 8-diol (3-8DIOL), a plant based product, was recently introduced as a topical insect repellent in the commercial product, "OFF! Botanicals" Lotion. The objective of this study was to provide an estimate of the potential for its systemic absorption in man. Carbon-14 labeled repellent formulated in the lotion or ethanol solution was applied to excised pig skin in an *in vitro* test system predictive of skin absorption in man. Twenty-four hours after application, radiolabel recovered from the dermis and receptor fluid was summed to determine percent absorption. At a dose of approximately 80 μg/cm^2 of 3-8DIOL in the lotion, a value of 3.5±0.8% was obtained with pig skin (N=6). The corresponding value for 3-8DIOL in ethanol was not significantly different (3.0±1.2%, N=6, $p>0.05$, ANOVA). For reference purposes, the pig skin absorption of piperonyl butoxide (PBO) at 100 μg/cm^2 and N, N-diethyl-m-toluamide (DEET) at 500 μg/cm^2 were significantly higher (15±6% and 23±3%, respectively, N=6, $p<0.05$, ANOVA). For additional reference, absorption of all compounds was found to be higher with excised rat skin ($p<0.05$, ANOVA) than with excised pig skin. Most of the applied dose of 3-8DIOL was found to evaporate from pig skin (77±8% for the lotion and 87±1% for ethanol solution), thus contributing to the relatively small percutaneous absorption values observed. Although methodological differences (such as contact time, etc.) need to be considered further, the absorption of DEET and PBO determined in the pig *in vitro* system is greater than what was determined previously in humans. This provides confidence that using the pig-derived dermal absorption value for 3-8DIOL does not underestimate systemic exposure and thus it would be appropriate for human exposure assessments.

1853 INDUCTION OF ADIPOSE DIFFERENTIATION RELATED PROTEIN AND NEUTRAL LIPID DROPLETS ACCUMULATION IN KERATINOCYTES BY SKIN IRRITANTS.

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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 identified by DD-PCR the upregulation by skin irritants of adipose differentiation related protein (ADRP) in reconstituted human epidermis. ADRP is a lipid storage droplet-associated protein, governing the deposition and release of lipids from droplets. The purpose of this study was to characterize in a human keratinocyte cells line (NCTC 2544) SDS-induced ADRP expression, to identify the biochemical events that lead to ADRP expression, and finally, to understand the function of ADRP in SDS cytotoxicity. SDS induced a dose and time related production of ADRP, which was associated with lipid droplets accumulation. Lipid accumulation following SDS treatment was likely to be due to intracellular redistribution rather that lipid neosynthesis, as indicated by equivalent 14C-oleate incorporation into di- and tri-acylglycerols. Other skin irritants, namely benzalkonium chloride, tributyltin, and phorbol 12-myristate 13 acetate, induce lipid droplets accumulation as well, indicating a common effect probably related to the essential role of lipid droplets in eukaryotic cells. SDS-induced ADRP expression and lipid droplets accumulation could be modulated by staurosporine, a broad spectrum protein kinases inhibitor, and by BAPTA, a calcium chelator, suggesting a role of calcium and protein phosphorylation in SDS-induced lipid accumulation. Modulation of SDS-induced ADRP expression by specific antisense oligonucleotide or by BAPTA resulted in increased cytotoxicity, indicating a protective role of ADRP and lipid accumulation in the process of cell damage induced by skin irritants.

1854 DERMAL ABSORPTION AND TOXICITY STUDY OF ACETONE-BASED SKIN COATINGS IN MINIATURE SWINE.

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Objective: This study was performed to evaluate both the systemic absorption of acetone and the potential for dermal toxicity from acute and chronic application of a skin coating material made from an acetone solution of polyvinylidene fluoride (PVDF) and acrylic polymers. Methods: Yucatan miniature pigs weighing 16-20 kg were topically administered a single dose of an acetone/PVDF/acrylic coating (3 animals per sex) or acetone alone (1 animal per sex) for acute evaluation. Acetone levels in blood were evaluated at regular intervals between 0 and 240 minutes. After a 3-4 day washout period, skin was abraded and a chronic 7-week study was completed with 2 daily applications (minimum of 6 hours between applications) of test material or acetone for 5 days per week. Trough blood acetone levels were taken before the first application of each week. Peak levels were taken after the second application on the last day of each week. Body weights and food consumption were recorded weekly. Clinical chemistry and hematologic parameters were evaluated. At necropsy, skin and major organs were removed for histopathological examination. Results: No evidence of toxicity was observed in any of the treatment groups. In the acute study, pigs either showed no perceptible elevation of acetone levels or slightly increased levels that would be considered non-toxic to humans. In addition, there was no evidence for elevated blood acetone levels after chronic treatment. There were no significant microscopic differences between any treatment or control groups. The most significant histopathological finding was minor disruption of the keratin layer, an observation that was also seen in untreated areas of skin. Conclusion: Repeated dosing of PVDF/acrylic coating formulations containing acetone are non-toxic and non-irritating.

1855 ACUTE TOXICITY ASSESSMENT OF BREAKFREE CLP®: A SMALL ARMS CLEANING COMPOUND.

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BreakFree CLP® ("BreakFree") is a weapons cleaning compound that is in use by the Armed Forces. BreakFree is a complex mixture made up of polyalphaolefin oil (65%), synthetic oils, esters and other synthetic proprietary ingredients (27%), isoparaffinic hydrocarbons (5%), and dibasic ester (3%). Like so many commercial mixtures, there is very little information available on the toxicity of BreakFree. Studies were conducted to characterize the dermal toxicity of BreakFree following single or repeat application. BreakFree was applied neat to the shaved backs of male and female CD-1 mice, 50 μL/application, 3 times/week for 2 weeks. Mice were then sacrificed 24 hours and 2 weeks after initiation of dermal applications. Final body, liver, and kidney weights, and blood chemistry and hematology profiles were compared with those of animals treated with deionized H_2O or acetone (negative controls) or 2.5% croton oil in acetone (positive control). Gross observations at 2 weeks included moderate dermal irritation (skin irritation) for BreakFree-treated animals and marked dermal irritation and scabbing in croton oil-treated animals. Final relative body and kidney weights were significantly lower for BreakFree-treated animals at 24 hours. There was evidence of epidermal acanthosis, and dermal inflammation in both 2 week BreakFree- and croton oil-treated animals, but differed in that serocellular crusts and multifocal ulceration was apparent for croton oil-treated skin. Blood concentrations of total protein, sodium, and alanine aminotransferase were significantly higher for BreakFree mice. Our findings indicate that repeat, unprotected handling of BreakFree could result in significant dermal irritation with possible histopatholgical damage to the epidermis and dermis. Blood chemistry profiles are suggestive of possible liver toxicity, but need to be confirmed.

1856 DERMAL PERMEATION OF THE SULFATED FATTY ACID, RICINOLEIC ACID, IS INHIBITED BY COMPLEX MIXTURE ADDITIVES.

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Performance of many cutting fluid formulations is dependent on its lubricant properties, which can often be improved by adding a sulfated fatty acid such as sulfated recinoleic acid (SRA). SRA like many of the other formulation ingredients are potential dermal irritants, yet little is known about its permeability in skin, and if other cutting fluid additives influence its dermal permeation. The purpose of this study was to assess H^3-SRA permeation when topically applied to inert silastic

membranes and porcine skin in *in vitro* flow-through diffusion cell system as aqueous mineral oil (MO) or aqueous polyethylene glycol (PEG) mixtures. H^3-SRA mixtures were formulated with 3 commonly used cutting fluid additives; namely, 0 or 2% triazine (TRI), 0 or 5% linear alkylbenzene sulfonate (LAS), and 0 or 5% triethanolamine (TEA). Formulation additives had little or no effect on SRA partitioning from the formulation into the stratum corneum (SC) in MO-based mixtures; However, in PEG-based mixtures the additives significantly decreased partitioning into the SC. The pH of SRA control and SRA+LAS mixture remained in physiological range (7.0 - 7.4), but all other mixtures were more basic pH (9.3 - 10.3). In silastic membranes, SRA absorption ranged from 1.22 to 12.84% dose and permeability and absorption were significantly reduced to one level by LAS and then another level by other additives or combination of additives in either MO- or PEG-based mixtures. In porcine skin, absorption ranged from 0.1 to 0.57% dose, and again formulation additives significantly decreased SRA absorption and permeability in both MO- and PEG-based mixtures. The observed decreasing trend of SRA permeation in both silastic and skin membranes is suggestive that this interaction is more physicochemical in nature than chemical-biological. The presence of other formulation additives that increased the pH of the mixture resulted in more charged SRA molecules that are not absorbed across the skin as readily. Supported by NIOSH Grant R01-OH-03669.

1857 PERCUTANEOUS ABSORPTION OF 2, 6-DI-*TERT*-BUTYL-4-NITROPHENOL (DBNP) IN ISOLATED PERFUSED PORCINE SKIN.

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DBNP (2, 6-di-*tert*-butyl-4-nitrophenol) has been reported as a potential contaminant in submarines. This yellow substance forms when lubrication oil mist containing the antioxidant additive 2, 6-di-*tert*-butylphenol passes through an electrostatic precipitator and is nitrated. Percutaneous absorption of ^{14}C-DBNP was assessed in the isolated perfused porcine skin flap (IPPSF). Four treatments were studied (n=4 flaps/treatment): 40.0mg/cm^2 in 100% ethanol; 40.0mg/cm^2 in 85% ethanol/15% water; 4.0mg/cm^2 in 100% ethanol; and 4.0mg/cm^2 in 85% ethanol/15% water. DBNP absorption was minimal across all treatment groups, with the highest absorption detected being only 1.08% applied dose in an aqueous ethanol group. The highest mass of ^{14}C-DBNP absorbed was only 0.5µg. The majority of the applied dose remained on the surface of the skin. This suggests that there is minimal dermal exposure of DBNP when exposed topically to skin. Supported by GEO-CENTERS, INC., Subcontract GC-3291-044-01-099 under SPAWAR SYSCEN NHRC Contract No. N66001-98-D-2600, D.O. 0044.

1858 ABSORPTION THROUGH PORCINE SKIN EXPOSED TO VARIOUS DOSES OF JET FUEL MARKER COMPONENTS DETERMINED WITH GC-FID USING HEAD SPACE SPME FIBER.

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Jet fuels (mixture of aliphatic and aromatic hydrocarbons) have been found to be potentially toxic to skin. In the past, we have studied the percutaneous absorption and mixture effects of selected individual hydrocarbons of jet fuels. Recently, dose related IL-8 release from human epidermal keratinocytes by jet fuel aromatic hydrocarbons has been studied. The present study is an ongoing approach to simultaneously observe the dose related percutaneous absorption of a number of aliphatic and aromatic hydrocarbons. Mixtures containing undecane (4.1%), dodecane (4.7%), tridecane (4.4%), tetradecane (3%), pentadecane (1.6%), naphthalene (1.1%) and dimethyl naphthalene (1.3% of jet fuels) in hexadecane was used to dose porcine skin diffusion cells. Treatments (n=4 cells) were 1X, 2X and 5X concentrations. Perfusate samples were analyzed with GC-FID using head space solid phase micro-extraction fiber technique. We have standardized the assay to have good linear correlation for all the tested components in media standards. Dosed components from perfusate were detected even with the lowest dose except for tetradecane which was detected with the highest dose only. Data indicates a dose dependent increase in absorption for naphthalene, dimethyl naphthalene and lower molecular weight aliphatic hydrocarbons. Absorption parameters including diffusivity, permeability and steady state flux were determined. This approach provides a baseline to access component interactions among themselves and with the diluent (solvents). Supported by USAFOSR F49620-01-1-0080.

1859 THE CYTOTOXICITY OF JET FUEL AROMATIC HYDROCARBONS AND DOSE-RELATED INTERLEUKIN-8 RELEASE FROM HUMAN EPIDERMAL KERATINOCYTES.

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Many aromatic hydrocarbons are known carcinogens with the ability to readily penetrate the skin with high absorption flux and cause skin irritation. In order to evaluate the *in vitro* cutaneous toxicity of individual aromatic hydrocarbons and their potential in inducing skin inflammation, we evaluated the LD_{50}, highest non-cytotoxic (5% mortality) dose (HNTD) and IL-8 release of 9 aromatic hydrocarbons in human epidermal keratinocytes (HEK). LD_{50} ranged from 1.8 mM (0.03%) for cyclohexylbenzene to 82.9 mM (0.74%) for benzene with a rank order potency of cyclohexylbenzene> trimethylbenzene≥xylene>dimethylnaphthalene>ethylbenzene>toluene>benzene. The HNTD value ranged from 0.1 mM (0.001%) for cyclohexylbenzene to 48.2 mM (0.43%) for benzene. There was a dose-related differential response in IL-8 release at 24 hr. Toluene, xylene, trimethylbenzene, cyclohexylbenzene and dimethylnaphthalene significantly decreased IL-8 release at the HNTD, while IL-8 release did not continue to decrease or significantly increase (cyclohexylbenzene and dimethylnaphthalene) at LD_{50}. IL-8 significantly increased with both doses of methylnaphthalene and naphthalene. The presence of hexadecane and mineral oil greatly attenuated the cytotoxicity to HEK cells elicited by individual aromatic hydrocarbons. This work was supported by the US Air Force Office of Scientific Research F49620-01-1-0080.

1860 CYTOTOXICITY OF THE JP-8 JET FUEL COMPONENTS m-XYLENE, 1-METHYLNAPHTHALENE, AND n-NONANE IN KERATINOCYTES.

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Cell culture methods are being developed to assess the dermal toxicity of volatile chemicals. Such tests are useful in the ranking of chemicals for irritancy, but they are not useful for quantitative risk assessment for two reasons. First, the amount of volatile chemical in the exposure media may decrease with time. Second, EC50 are reported as the concentrations in the media and not the cells. We have recently developed an *in vitro* approach for toxicity testing of volatile chemicals that avoids these problems. This system was used to expose keratinocytes grown on a collagen matrix to culture medium containing m-xylene, 1-methylnaphthalene (1-MN), or n-nonane. Partition coefficients were measured and used to estimate the chemical concentration in the keratinocytes. The EC50 for m-xylene at 1, 2, and 4 hours were 1248.46 ± 78.01, 1028.88 ± 11.12, and 860.8 ± 84.6 µg m-xylene per gram of keratinocytes, respectively. The EC50 for 1-MN in the keratinocytes at 1, 2, and 4 hours were 6494.3 ± 460.1, 4319.82 ± 372.61, 2201.06 ± 196.27 µg 1-MN per gram keratinocytes, respectively. Although marginal cytotoxicity was observed at 1 hr, the EC50 for n-nonane at 2 and 4 hours were 915.6 ± 155.5 and 980.7 ± 139.5 µg n-nonane per gram cells, respectively. These results suggest a time- and dose-dependent effect of m-xylene and 1-MN of keratinocyte viability, with little cytotoxic effect by n-nonane. This study supports the potential use of our exposure system for determining equivalent external doses for toxic endpoints.

1861 SIX MONTH SAFETY AND IMMUNOLOGY STUDY IN BABOONS OF ALLOGENEIC BABOON MESENCHYMAL STEM CELLS LABELED WITH FLUORESCENT DYE.

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The purpose of this study was to determine the safety and immunologic consequences of administering allogeneic baboon mesenchymal cells (MSCs) by routes of clinical significance. MSCs are rare cells found in bone marrow and other tissues that have the capacity to be expanded to large numbers for tissue repair. MSCs from male donors were labeled with fluorescent dye for tracking purposes and injected into female baboons as follows: group 1 control animals (n=3) received vehicle (95% plasmalyte:5% recipient plasma); group 2 (n=3) received DiI-labeled