1847 THE INFLUENCE OF STORAGE TIME AND ARTIFICIAL SWEAT ON THE ERCUTANEOUS ABSORPTION OF EXPLOSIVES FROM SOILS.

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We determined the influence of sample storage time on the percutaneous absorption of C-14 labeled hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX), 2, 6-dinitrotoluene (26DNT) and 2, 4, 6-trinitrotoluene (TNT) from two soil types, Yolo having 1.9% carbon and Tinker having 9.5% carbon content. RDX soil samples stored at -20C for 27 months and 62 months were compared to freshly spiked soil samples. Similarly, 26DNT samples stored 35-36 months and TNT samples stored 18 months were compared to freshly spiked samples. Approximately 10 ug/cm2 of radiolabeled compound was applied in 10 mg/cm2 of soil to freshly excised pig skin pretreated with artifical sweat (5 ul) and mounted in skin penetration-evaporation chambers. Radiolabel recovered from the dermis and tissue culture media (receptor fluid) was summed to determine percent absorption from the soils. For each compound, percent absorptions of label were highest from Yolo soil. Storage did not significantly alter percutaneous absorption values for RDX, as values were all less than 1%, regardless of soil type or age. Similarly, 26DNT absorption was 1-2% for Tinker soil and 16-18% for Yolo soil, regardless of soil age. TNT absorption was approximately 0.5% from Tinker soil and 3-4% from Yolo soil for fresh and stored samples. HPLC analysis of 26DNT in receptor fluid at maximum flux indicated no metabolism or breakdown. For TNT, extensive conversion to monoamino derivaties and other metabolites was observed. The absorption of 26DNT from low carbon soil was reduced from 16-18% to near zero without sweat pretreatment, indicating that skin surface moisture was a critical variable in determining topical bioavailability.

1848 THE INFLUENCE OF SWEAT ON THE PERCUTANEOUS ABSORPTION OF CHLORPYRIFOS FROM NYLON CARPET FIBERS.

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Enhanced absorption of chlorpyrifos following exercise and a correlation between moisture and pesticide transfer (Williams et al., 2002) have been observed. Chlorpyrifos was chosen as a surrogate for semi-volatile chemicals used indoors. The percutaneous absorption of 14C-ring-chlorpyrifos from nylon carpet fibers was measured in porcine skin penetration-evaporation cells from nylon carpet fibers. Prior to application, synthetic sweat was applied to the skin surface in half of the cells. Radioactivity was measured in receptor fluid, dermis, epidermis, tape stripping samples, and vapor trap samples from a 24-hour period. Chlorpyrifos was successfully measured from nylon carpet fibers in the penetration-evaporation cells. The sum of radiolabel recovered from the dermis and receptor fluid was considered to represent the absorbed dose. There was no significant difference ($p > 0.05$) in percutaneous absorption or evaporative loss between cells that received the synthetic sweat application and cells that were run "dry" (1.5 ± 0.45 and 28.1 ± 4.96 percent for percutaneous absorption and evaporative loss, respectively). There was significantly more ($p < 0.05$) radiolabel recovered from tape stripping (5.4 ± 2.12 vs. 2.8 ± 0.59 percent) and in the epidermis (4.5 ± 0.78 vs. 3.1 ± 0.34 percent) from cells that received the synthetic sweat application. The percutaneous absorption of chlorpyrifos was found to correlate with an empirical model previously developed with nitro-compounds from soil. The synthetic sweat treatment facilitated transfer of chlorpyrifos from a treated substrate to the skin surface, but did not effect the rate or magnitude of percutaneous absorption in this study. This work has been supported in part through the Colgate-Palmolive/SOT Award for Student Research Training in Alternative Methods

1849 DERMAL DISPOSITION OF TRIAZINE IN CUTTING FLUID MIXTURES.

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Triazine is often added as a biocide/preservative to cutting fluids formulations used in the metal machine industry. Workers involved in metal machining are not only exposed to components in these cutting fluids, but biocides such as triazine which have been implicated in occupational contact irritant dermatitis (OCID). Little is known about how these cutting fluids and their ingredients influence the dermal disposition of triazine. The purpose of this study was to assess C^{14}-triazine membrane transport 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. C^{14}-triazine mixtures were formulated with 3 commonly used cutting fluid additives; namely, 0 or 5% linear alkylbenzene sulfonate (LAS), 0 or 5% triethanolamine (TEA), and 0 or 5% sulfurized ricinoleic acid (SRA). Triazine partitioning from the formulation into the stratum corneum (SC) was significantly reduced by LAS, while SRA significantly reduced the pH of the formulation. Triazine absorption ranged from 2.24 to 3.9% dose in porcine skin and 12.61 to 18.63% dose in silastic membranes. In silastic membranes, the complete mixture significantly reduced triazine absorption in MO-based mixtures, while in PEG-based mixtures triazine absorption and apparent permeability were significantly increased. In porcine skin, triazine permeability was significantly increased for both MO- and PEG-based complete mixtures and the trend was for greater triazine absorption in more complex PEG-based mixtures. Interestingly, SRA or TEA alone significantly reduced triazine absorption in MO-based mixtures, and this interaction appears to be more additive than synergistic. Although the physiochemical experiments suggest otherwise, triazine readily permeates a homogenous lipid membrane such as the SC, while triazine permeability and absorption was significantly enhanced by the complete mixture especially in PEG-based mixtures. Supported by NIOSH Grant R01-OH-03669.

1850 ABSORPTION OF 14C- RDX FROM SOILS THROUGH HUMAN SKIN.

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Cyclotrimethylenetrinitramine (RDX), a munition compound has been detected in water and soil as an environmental contaminant at production waste disposal sites and at certain military installations. The bioavailability of a chemical from soils depends on soil composition. We studied dermal absorption of 14C- RDX from two types of soils, Yolo (low carbon, 1.9%) and Tinker (high carbon, 9.5%), in human skin *in vitro* in flow-through diffusion cells. Soils (10 mg/cm2) containing a dose (10 mg/ cm2 / 0.05 m Ci) was applied to the skin and collected as diffused receptor fluid for every 6 hr up to 24 hrs. The soil content on the skin was washed with soap water and water with cotton swabs, and radioactivity present in washings was determined. The RDX absorbed in the skin (stratum corneum, epidermis and dermis) was also determined. Our results show that a total of approximately 2 .71 % (Yolo) and 2.24% (Tinker) of applied dose from soils was absorbed in the skin (receptor fluid and skin) in 24 hr. The absorption of RDX in the receptor fluid was about 1. 4 % (Yolo) and 0.66% (Tinker) soils in 24 hrs. The total recovery of applied dose (receptor fluid, skin and washings) was about 87 % (Yolo) and 94% (Tinker). The RDX absorption from soils in the skin was low when compared to RDX in acetone (6%) (Reddy et al., 2002). This shows that the bioavailability of RDX from soils is reduced considerably. The estimated levels of RDX absorption from soils can be used to evaluate health risks associated with dermal exposure (Supported by COE, abstract does not reflect US Army policy).

1851 DERMAL ABSORPTION OF TOLUENE FROM ENAMEL PAINT IN F344 RATS.

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Toluene is a component of many paint products and there is potential for both occupational and non-occupational dermal exposure to toluene in various matrices. To understand the significance of these exposures, the dermal bioavailability of toluene was assessed in F344 male rats using a combination of real-time exhaled breath analysis and physiologically based pharmacokinetic (PBPK) modeling. Animals were exposed to toluene present in a commercial enamel paint using a 1.7-cm diameter occluded glass patch system attached to a clipper-shaved area on the back of the rat. Immediately following exposure, individual animals were placed in glass off-gassing chambers and exhaled breath was monitored as chamber concentration using an ion trap mass spectrometer (MS/MS). The exhaled breath profiles from treated animals clearly demonstrated the rapid absorption of toluene. Peak chamber concentrations, representing exhaled breath, were observed within 1 hr from the start of exposure. The PBPK model describing the exposure and off-gassing chamber was used to model the exhaled breath data. A dermal permeability coefficient (Kp) of 0.073 cm/hr was found to describe each set of exhaled breath data. In comparison, the Kp value determined for enamel paint was identical to the Kp value for aqueous toluene (0.074 cm/hr) although toluene concentrations differed significantly (25 mg/ml versus 0.5 mg/ml). To evaluate the impact of paint

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