DERMAL ABSORPTION OF CUTTING FLUID MIXTURES

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TABLE OF CONTENTS:

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CMC	Critical micelle concentration
IPPSF	Isolated perfused porcine skin flap
Log $K_{O/W}$	Log octanol/water partition coefficient
MO	Mineral oil
PEG	Propylene glycol 200
SC	Stratum corneum
LAS	Linear alkylbenzene sulfonate
RA	ricinoleic acid
TCE	Trichloroethylene
TRI	Triazine
TRE	triethanolamine
NDELA	Nitrodiethanloamine
SMFT	Silastic membrane flow through diffusion cell
PSFT	Porcine skin flow through diffusion cell

Abstract:

This work has focused on understanding how cutting fluid additives, contaminants, and metal-working cleanser can influence the dermal disposition of potential skin irritants. Representatives of three commonly used classes of additives (emulsifier, lubricant, and biocide) were investigated in this study. The three representative additives, linear alkylbenzene sulfonate (LAS), sulfate ricinoleic acid (RA), and triazine, were used in the initial phases of this research to answer these questions. Diffusion of these additives across skin and inert membranes was determined experimentally to help identify physicochemical and chemical-biological interactions when workers are exposed to similar complex cutting fluid formulations. Because triazine was shown to be more readily absorbed across skin, it was used as a chemical marker to further assess contaminant and cleanser effects on dermal absorption.

LAS absorption in skin was limited to less than 0.5% dose and the additives in various combinations influenced the physicochemical characteristics of the dosing mixture. LAS was more likely to partition into the stratum corneum (SC) in mineral oil mixtures, and LAS absorption was significantly greater in the complete cutting fluid mixture. Triazine enhanced LAS transport, and SRA decreased LAS critical micelle concentration (CMC) which reduced LAS monomers available for membrane transport. TEA increased mixture viscosity, and this may have negated the apparent enhancing properties of TRI in several mixtures

Physicochemical interactions which influenced ricinoleic acid partitioning into the stratum corneum modulated ***ricinoleic acid*** diffusion as evidenced by reduced permeability as the mixture became more complex. Although *in vitro* diffusion may differ from *in vivo* diffusion in human skin, physicochemical interactions between ricinoleic acid and cutting additives appear to play a significant role in membrane diffusion. The human health implications here are that the more complex the mixture, the less able ricinoleic acid is to partition and diffuse across skin. This can result in greater retention of these potentially irritant fatty acids in the upper epidermis which will eventually penetrate into the viable epidermis and absorbed into the blood stream.

Several of the additives did not enhance ***triazine*** partitioning into the stratum corneum, but significantly enhanced the apparent permeability of triazine in both skin and silatsic membrane systems. The significant enhancer effects in silastic membrane as well as increased deposition in the stratum corneum and skin, especially with PEG mixtures, is suggestive of a significant chemical mechanism associated with these apparent triazine-additive interactions. The subsequent promotion of apparent triazine permeability in skin may be related to these interactions as well as bio-membrane alterations that are often manifested as acute irritant dermatitis in metal machine workers. Because of the significant additive effects on triazine permeability in these cutting fluid formulations, the effect of chemical contaminant and solvent effects involved triazine as the marker.

Contaminants Effects: Dermal absorption of the irritant biocide, triazine appears to be greater in soluble oil cutting fluids than in synthetic cutting fluids. This effect can be more pronounced when cutting fluids are contaminated with nitrosamines and leached metal ions. These contaminants by themselves did not significantly increase triazine absorption, but can significantly increase its deposition into the skin surface, stratum corneum and viable epidermis. More long-term exposure studies are required to determine if these mixture interactions are consistent beyond an 8-hour exposure, and whether this is related to an enhanced irritant response in workers simultaneously exposed to cutting fluids containing biocides and contaminants.

Solvent Cleanser Effects: This study was the first to demonstrate that chronic exposure to solvents such as TCE could have a more significant effect on the permeation of water-soluble irritants (e.g., triazine) than the simultaneous topical exposure to the solvent. Our study further demonstrated little to no TCE effects on triazine diffusivity, although there was enhanced permeability in TCE pre-treated skin, which suggest that TCE altered partitioning behavior of triazine in these cutting fluid mixtures. This study demonstrated in our 8-hour perfusion studies that an almost 2-fold increase in permeability is possible. This does not account for significant increase in deposition of these and other irritants into the viable epidermis to illicit an irritant or contact dermatitis. This therefore may be sufficient to be an occupational concern as the worker has already compromised the epidermal barrier of the skin to not only biocided such as triazine but also other water soluble toxicants (e.g., NDELA) that may be result in more harmful systemic effects.

In ***summary***, these cutting fluid mixture studies demonstrated that cutting fluid additives, contaminants, and solvent cleansers can have a significant effect on solute permeability in skin. This study demonstrated this possibility using a water-soluble biocide, triazine, that is sometimes added to cutting fluid formulations as a preservative. The inert membrane studies strongly suggested that physicochemical interactions contribute significantly to solute permeability in skin.

Future Physicochemical Research: Preliminary membrane coated fiber (MCF) studies have demonstrated that the ***inert*** MCF can discriminate between different solutes and solute clusters. For this reason, future work will utilize a diverse series of MCFs with varying membrane properties to characterize physicochemical interactions associated with the presence of cutting fluid additives in cutting fluid formulations/mixtures. The MCF technique has the added advantage of probing these interactions within a linear solvation energy relationship (LSER) framework without confounding interactions present in the biological membrane system. Finally, this proposed LSER framework allows for comparison of solute diffusion as well as mixture effects on solute diffusion in the MCF and the skin membrane systems. The differences in ***interaction coefficients*** between these membrane systems will ***identify*** and ***quantify*** unique physicochemical properties that influence dermal absorption of solutes in a defined cutting fluid formulation.

Finally, further work is needed to better characterize the mechanisms influencing these interactions, as these interactions can be modified at the formulation step if it is determined that it predisposes dermal deposition of one or more of these occupational irritants.

Significant Findings:

The primary objective of this project **(RO1-OH-03669, 2000-2004)** was to identify significant physicochemical and chemico-biological interactions that influence the dermal disposition of three cutting fluid additives that have been identified as causing ***occupational irritant dermatitis*** amongst workers who are exposed to formulations containing these additives. A complete factorial experimental design was employed to dissect out these interactions utilizing inert silastic membranes as well as porcine skin in *in vitro* flow-through diffusion cell systems. The scientific findings are related to the following 3 Specific Aims of the Project:
1) ***Determine statistically significant chemical-chemical and chemical-biological interactions for five component mixtures using three skin model systems possessing increasing levels of biological complexity. Physical chemistry studies will first be conducted to identify the chemical interactions.***
2) ***Determine interactions with common cutting oil contaminants, N-nitrosodiethanolamine and nickel, and a cleansing solvent, trichloroethylene, using a reduced factorial design.***
3) ***Using biomarkers of irritation, determine significant changes in epidermal barrier structure and function after exposure to chemical mixtures that significantly alter component disposition in skin.***

- We have thus far identified ***significant mixture interactions*** that are unique for each of the three cutting fluid additives in soluble oil and synthetic cutting fluid surrogate mixtures, and which clearly influenced additive disposition in skin. The details of these unique observations were recently published (Baynes et al., 2002a, Baynes et al.2003; Baynes and Riviere, 2004) or have been recently submitted for publication (Baynes et al., 2005a; Baynes et al., 2005b).

- In companion *in vivo* and *in vitro* studies, the biological effects of these additive mixtures provided tentative evidence of a link between biological irritant response and additive disposition in skin (Monteiro-Riviere et al., 2005c).

- The biocide component in these mixtures, triazine, was more permeable in skin than the surfactant or the fatty acid additives, and is therefore one of the main reasons why this and other classes of biocides warrant further investigation. Triazine permeability in both silastic and porcine skin was significantly increased in both mineral oil- and synthetic (PEG)-based aqueous mixtures when the ***complete additives package*** was added to the formulation (Baynes et al., 2003).

- We identified synergistic interactions between ***triethanolamine*** (TEA) and ***sulfated ricinoleic acid*** (SRA) that enhanced triazine permeability, and we also recently observed significant enhancer effects with the contaminant, NDELA, but not nickel (Baynes et al., 2005a). It is also conceivable that the alkanolamine and fatty acid interaction led to formation of lipophilic ion pairs that resulted in statistically significant enhanced permeability of the biocide. This clearly was a physicochemical interaction as it was ***also*** observed in the inert silastic membranes. However, this was not predicted from the triazine SC/formulation partition coefficient studies, although the PEG-based formulations significantly enhanced triazine partitioning into the SC as well as its permeability in skin.

- Our laboratory has recently demonstrated enhanced permeability of triazine following a 4-day pre-treatment with TCE (Baynes et al., 2005b). This was observed with PEG and MO mixtures, and strongly supports the argument that workers who have had prior exposure to degreasers and cleansers are more likely to absorb these additives than the general population who have not had prior exposure to these chemical mixtures. Surprisingly, simultaneous exposure to the solvent did not appear to influence triazine disposition in skin.

- Our research also identified ***solute-micelle interactions*** in these cutting fluid mixtures. Linear alkylbenzene sulfonate (LAS) is an anionic surfactant which did not readily diffuse across either membrane system in either solvent system (Baynes et al., 2002a), however, this surfactant reduced triazine partitioning into the SC but had little effect on its dermal permeability. This was also observed with the cutting fluid lubricant, sulfated ricinoleic acid (SRA). While it is hypothesized that micellar interactions reduced additive partitioning into the stratum corneum, we also discovered that several of the cutting fluid additives, especially the fatty acid additive, significantly reduced the critical micellar concentration (CMC) for LAS (Baynes et al., 2002a). This interaction was also supported by the inhibitory effect of SRA+LAS on triazine partitioning and permeability in skin. In addition to these interactions, SRA partitioning into the stratum corneum and diffusion in skin was significantly inhibited by additives other than LAS. ***The fact that this occurred in both inert and biological membrane systems and because these additives affected formulation pH provided strong evidence of dominant physicochemical interactions modulating solute permeability in skin.***

Translation of Findings:

Metal-machining fluids and oils are known to cause skin irritation and toxicity, yet very little is known about how the various additives in these formulations influence irritant diffusion and distribution in workers' skin. Under the auspices of this **grant (RO1-OH-03669)**, we demonstrated that the frequently used biocide, triazine, was more likely than other performance additives such as surfactants and lubricants to penetrate porcine skin if a worker was exposed to cutting fluids via the skin. This work also demonstrated that triazine more so than other cutting fluid additives was primarily responsible for the inflammatory response in skin. As previously hypothesized in the original grant application, the cutting fluid additives and/or contaminants that were simultaneously present in these formulations significantly influenced the dermal absorption disposition of triazine. The nickel contaminant was not as important as the nitrosamine contaminant in influencing the skin absorption of triazine. We also demonstrated that if workers were repeatedly exposed to the grease cleanser/remover, TCE, that this will significantly enhance the skin absorption of triazine. Comparative analysis of additive permeability in inert silastic membranes and porcine skin membranes allowed for determining whether mixture effects were physicochemical or chemical-biological in nature. Our studies to date provided evidence that physicochemical interactions between the irritant biocide and the cutting fluid formulation may be more important than chemico-biological interactions in determining how much of these industrial toxicants penetrate and distribute in skin.

SCIENTIFIC REPORT:

Background for the Project:

Background to Dermal Risk Assessment: Topical exposure to chemicals in the workplace is widely accepted as a primary route by which xenobiotics gain access to the systemic circulation. In addition, the skin is a direct target for metalworking and other industrial chemicals which may produce toxicological manifestations ranging from acute irritation to proliferation and tumor formation. Most investigations have only dealt with exposure to a single chemical, yet *multiple chemical exposure is the actual scenario in both the workplace and the environment*. In contrast, studies primarily conducted for topical and transdermal pharmaceuticals teach us that significant interactions may occur within members of a chemical mixture (e.g. principal components, vehicles, additives) which would alter their *rate* and *extent* of *penetration* into and *absorption* from the skin. This would have effects both on assessing the systemic exposure to topical applied chemicals as well as direct chemical-induced irritant contact dermatitis where the skin itself is the target organ.

Workers in the metal machining industry have a high incidence of occupational irritant dermatitis that is related to exposure to hundreds of cutting fluid formulations. It would be impossible to assess the effect of these many formulations on the dermal absorption of key cutting fluid additives that are thought to cause occupational irritant dermatitis as well as be absorbed and have more serious systemic effects. It would be more prudent to utilize robust and mechanistically-defined skin models to predict what additive or additives will influence important dermatopharmacokinetic parameters rather than performing extensive *in vivo* and *in vitro* toxicokinetic studies to estimate the dermal disposition of these industrial irritants.

Current dermal risk assessments of hazardous chemicals are plagued by the many conflicting experimental protocols and internal dose calculations that limit characterization of solute absorption in skin. Calculating solute absorption has been based on (1) percent dosed absorbed into the systemic circulation and/or (2) steady state flux which can be used to calculate solute permeability. The latter is more useful primarily because *solute permeability is concentration independent*, whereas expressing penetration as a fraction of applied dose may cause large errors associated with variations in external dosing and exposure times. Permeability is therefore ***preferred for extrapolating across dose*** in dermal risk assessment and also better suited for assessing and ultimately extrapolating across formulation and mixture effects which is the purpose of this grant application. Assuming that solutes obey Fick's first law of diffusion as they diffuse across the human epidermal membrane, **skin permeability** can be defined by the equation,

$$\textbf{Permeability (cm/hr), kp} = J_{ss}/C_v$$

where J_{ss} represents solute steady state flux and C_v the solute dosing concentration. Solute permeability is dependent on solute diffusivity, D, (cm^2/hr) in the membrane and its ability to ***partition*** from the dosing solution to the stratum corneum layer of skin. The latter is referred to as the stratum corneum-vehicle partition coefficient, K_{sv}, and is often correlated to octanol-water partition coefficients, Ko/w. Permeability can therefore be re-defined by equation 2, where l = membrane thickness.

$$Kp = \frac{D * K_{s/v}}{l}$$

It is quite conceivable that in some chemical mixtures scenarios that not only will the physicochemical properties of the targeted solute be altered, but also the biological membrane. *Therefore solute diffusivity and/or partitioning behavior can be influenced by chemical mixtures.* These interactions have **not** been adequately addressed in many of the current risk assessment models. This project derived these parameters in a chemical mixture scenario; namely, cutting fluids formulations used in the metal machining industry.

Background to Additive Effects: Commercial cutting fluids are really mixture formulations of performance additives. These additives may have various and specific performance functions during metal machining, but workers have developed irritant dermatitis following occupational exposure to many of these formulations, and prior to this project, the dermal disposition of these offending additives in skin has not been characterized in the presence of these mixtures. It therefore seemed feasible that many of these performance additives can be grouped into various chemical categories based on well known toxic effects to skin, and factorial experimental design can be used to identify what chemical or group of chemicals can influence additive dispostion in the skin of workers exposed to these cutting fluids. The 4 chemical categories or groups investigated in this project were (1) Surfactants (e.g., linear alkylbenzene sulfonate, LAS), (2) Biocides (e.g., triazine, TRI), (3) Fatty acid performance lubricant (e.g., sulfurized ricinoleic acid, SRA), and a (4) Corrosive inhibitor (e.g., triethanolamine, TEA). Many of the cutting fluids used in the metal-working industry are either oil-water mixtures or strictly synthetic aqueous formulations. In order to assess skin disposition in these two broad classes of cutting fluids, the above additives were formulated in either mineral oil (MO) or polyethylene glycol (PEG) 200 to mimic this exposure.

(1) The influence of surfactants on solute permeability has been extensively reported in the literature, however the mechanism of this interaction has not been quantitatively assessed. Our laboratory has demonstrated that surfactants themselves are not readily absorbed across skin or inert membranes irrespective of the solvents or other additives present in the formulation (Patil *et al.*, 1995; Baynes *et al.*, 2002b). However, several of our mixture studies have demonstrated that surfactants can increase or decrease solute absorption (Baynes *et al.*, 1996; Baynes and Riviere, 1998; Baynes *et al*, 2002b). Other recent studies have demonstrated that surfactants *by themselves* can have little or no effect on triazine permeability (Baynes *et al.*, 2003), however, many of the other classes of biocides have significantly greater logKo/w values. These studies strongly suggest that permeability is dependent on physicochemical properties of the solute (e.g., log Ko/w) and of the surfactant mixture (e.g., critical micelle conc., CMC). It is therefore no surprise that solute diffusion across a membrane can be limited by *solute solubilization in surfactant micelles* (Perez-Buendia *et al.*, 1989). This solute-micelle interaction is a kinetic process and these intermolecular forces modulate the availability of solute molecules for diffusion across skin. Getting a better handle on these interactions becomes more complicated when an organic solvent is added to the surfactant mixture. Our studies with cutting fluid additives have also demonstrated significant changes in CMC (Baynes et al., 2002) in the presence of various solvents.

(2) Biocides and prescrvatives are used in many industrial applications to improve the shelf-life of aqueous industrial and cosmetic formulations. There are many classes of industrial biocides and preservatives, that have been associated with ***occupational irritant dermatitis*** in

workers involved in metal machining operations. Its worth noting that while many of these biocides are potential irritants, ***very little is known about their dermal disposition*** in occupational exposure conditions where the worker is exposed to these chemical mixtures over a prolonged period. Performing an ***occupational dermal risk assessment*** therefore requires data and information that is not only relevant to these occupational conditions but also amenable to meaningful extrapolation provided there is some physicochemical understanding of what *physicochemical* and *chemico-biological* interactions dictate partitioning and diffusion behavior of the biocide under any given defined conditions.

(3) Lubricants used in these formulations are usually fatty acids (e.g., Ricinoleic acid, RA), and although very little is known about their enhancer properties, several investigators have demonstrated that a related fatty acid, oleic acid, is a very effective transdermal enhancer, with significantly greater enhancement with hydrophilic than hydrophobic drugs. One recent study has demonstrated, however, that ricinoleic acid is a less effective permeation promoter than oleic acid for polar drugs. Fatty acids are often added to topical drugs and cosmetic products to improve skin condition, lubricity, and as a formulation aid in many skin pharmaceutics. Fatty acids such as oleic acid and to a lesser extent its related cis-12-monohydroxylated derivative, ricinoleic acid, are known to significantly enhance skin permeation of drugs. However, fatty acids also have industrial uses such as in metal machining industry where they and/or their derivatives are formulated with cutting fluids to enhance lubricity and thus reduce friction between metal parts. It is through such occupational activities that workers' skin can be exposed to various formulations containing fatty acids.

(4) Triethanolamine (TEA) is an alkanolamine, and is used as a corrosive inhibitor in cutting fluids and cosmetic products. TEA is a mild primary irritant and influences dermal absorption of additives since it is readily absorbed in neat or aqueous solutions. There is evidence that alkanolamines are effective penetration enhancers of drugs.

Specific Aims

The following 3 Specific Aims of the Project were:

1) Determine statistically significant chemical-chemical and chemical-biological interactions for five component mixtures using three skin model systems possessing increasing levels of biological complexity. Physical chemistry studies will first be conducted to identify the chemical interactions.

2) Determine interactions with common cutting oil contaminants, N-nitrosodiethanolamine and nickel, and a cleansing solvent, trichloroethylene, using a reduced factorial design.

3) Using biomarkers of irritation, determine significant changes in epidermal barrier structure and function after exposure to chemical mixtures that significantly alter component disposition in skin.

Procedures & Methodology

***Preparation of Chemical and Contaminant Mixtures** (For Specific Aims #1, #2, and #3):*

In the in vitro dermal perfusion studies, radiolabeled isotopes for LAS, TRI, and RA were used as markers. Radiolabeled linear alkylbenzene sulfonate (sodium 2-dodecylbenzene sulfonate-ring-UL-^{14}C, LAS) (specific activity = 50.77 mCi/mmol) was obtained from Wizard Laboratories, West Sacramento, CA. Radiochemical purity was 99.12%. Radiolabeled ^{14}C-triazine (hexahydro-1,3,5-triethyl-s-triazine -ring-UL-^{14}C; specific activity = 10.00 mCi/mmol) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). Radiochemical purity was 99.12%. Radiolabeled ^{3}H-ricinoleic acid (^{3}H-RA, [12-^{3}H-hydroxy-cis-9-octadecenoic acid] with specific activity = 20.00 mCi/mmol) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). Radiochemical purity was 99%. ^{14}C-LAS was dissolved in water, and was used to prepare the surrogate cutting fluid mixtures summarized in **Table 1**. In separate experiments, ^{14}C-triazine was dissolved in 50/50 (v/v) water: ethanol, and was used to prepare the surrogate cutting fluid mixtures summarized in **Table 1**. ^{3}H-RA was dissolved in ethanol, and then used to spike castor oil which consisted of predominantly (> 90%) unlabeled ricinoleic acid. This radiolabeled fatty acid mixture was used to prepare all surrogate cutting fluid mixtures summarized in **Table 1**.

Linear alkylbenzene sulfonate (LAS) was obtained from Aldrich Chemical Company, Milwaukee, WI. Sulfated ricinoleic acid (SRA), triethanolamine, (TEA) and Mineral oil (MO), were obtained from Sigma Chemical Company, St. Louis, MO, and poly(ethylene glycol), average M.W. 200, (PEG) was obtained from Acros Organics, Morris Plains, NJ.

Non radiolabeled additives LAS and TRI were obtained from Aldrich Chemical Company, Milwaukee, WI. Non-labelled sulfated ricinoleic acid (SRA), triethanolamine, (TEA) and Mineral oil (MO), were obtained from Sigma Chemical Company, St. Louis; and poly(ethylene glycol), average M.W. 200, (PEG) was obtained from Across Organics, New Jersey.

1-Component Mixture	2-Component Mixtures	3-Component Mixtures	4-Component Mixture
RA,	RA+TRI	RA+TRI+LAS	RA+TRI+LAS+TEA
	RA+LAS	RA+TRI+TEA	
	RA+TEA	RA+LAS+TEA	

Table 1. Factorial design for cutting fluid mixtures spiked with a radioloabelled marker. In this example, ^{3}H-RA was the chemical marker of interest. This was repeated for radiolabelled for LAS and TRI mixtures prepared in water and either 5% mineral oil or 5% PEG for physiochemical and diffusion studies. PEG = polyethylene glycol 200; RA = 5% ricinoleic acid, TRI = 2% triazine; TEA = 5% triethanolamine; LAS = 5% Linear alkylbenzene sulfate .

Comtaminants. (For Specific Aims #2) Cutting fluids can become contaminated with metals (e.g., nickel, Ni) and nitrosamines (e.g., N-nitrosodiethanolamine, NDELA), and there is concern that these classes of contaminants will modulate dermal disposition and ultimately the

toxicity of cutting fluid additives such as biocides (e.g., triazine, TRI. The dermal absorption and deposition of ^{14}C-TRI when topically applied to porcine skin in an *in vitro* flow-through diffusion cell system as mineral oil (MO) or polyethylene glycol (PEG) mixtures. ^{14}C-TRI mixtures were formulated with NDELA and/or Ni or with 3 other cutting fluid additives (5% linear alkylbenzene sulfonate, 5% triethanolamine, and 5% sulfurized ricinoleic acid) and one or both of these contaminants.

Degreasers & Cleansers: (For Specific Aimis #2). 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.

Physicochemical Studies: (For Specific Aims #1)

Solubility and CMC Determinations: LAS solubility was determined by preparing 4 standards and 8 unknown (saturated and unsaturated) concentrations (Table 1) and testing them using a Corning Conductivity Meter 441 (Corning Inc. – Corning NY, USA). The concentration was determined by plotting a best-fit line (R^2>. 99) and slope of the standards using an Excel spreadsheet. The unknowns were plotted against the conductivity and the point at which there was no change in conductivity was determined to be the solubility point. The solubility value was determined using the slope of the standards. The *Critical Micelle Concentration* (CMC) was also determined with the aid of a conductivity meter. Briefly, concentrations of LAS (mmolar) and mixtures without LAS were prepared separately. Known amounts of LAS were titrated into the various mixtures and the conductivity was then determined with the conductivity meter. A conductivity vs. concentration plot was generated, and the CMC was determined as the last LAS concentration in solution before the slope of the line ($r^2 > 0.99$) changed. The CMC was reported as an average of 4-5 determinations.

Viscosity and pH Determinations: LAS and TRI solutions were formulated as described in Table 1. and then tested in a Stresstech Rheometer (Reologica Instruments AB, Lund, Sweden/ATS Rheosystems, Bordentown, NJ) for 5 minutes at 25°C. Using an Excel spread sheet, the time points and viscosity (Pa s) were plotted on a graph to determine the plateau, which is the viscosity. The pH was tested using a Fisher Scientific Accumet AR10 pH meter. The temperature was 30°C and the meter was calibrated to two points.

Stratum Corneum (SC)/Vehicle Partition Coefficient Determination: SC/Vehicle partition coefficients were determined according to methods previously described by Baynes et al. (2000). In brief, stratum corneum and epidermis layers were removed from abdominal skin of a female weanling Yorkshire pig by heat treatment and then treated with 0.25% trypsin (Sigma Chemical Co., St. Louis, MO) to dissolve the epidermis. The remaining SC was dried and weighed (5-8 mg sample) and placed in vials. About 3 ml of the LAS, TRI, or RA mixtures (Table 1) with ^{14}C-LAS, ^{14}C-TRI, or ^{3}H-RA, was added to the SC sample vial (n = 4), capped, sealed and allowed to

remain undisturbed at room temperature for 24 hours. At 24 hours, 10µl of the vehicle was removed for direct counts using Ecolume (ICN Costa Mesa, CA). The SC sample was removed, gently blotted to remove excess solution and then analyzed as described below.

Flow through Diffusion Cell Experiments *(For Specific Aims #1 and #2)*

The flow-through diffusion cell system as previously described by Bronaugh and Stewart (1985) was used to perfuse porcine skin and silastic (polydimethylsiloxane) membranes. Porcine skin was obtained from the dorsal area of weanling female Yorkshire pigs. The skin was dermatomed to a thickness of 500 µm with a Padgett Dermatome, (Padgett Instruments Inc, Kansas City, MO). Silastic membranes (250 µm) were obtained from Dow Corning, Corporation, Midland, MI. Each circular skin and silastic section was punched to provide a dosing surface area of 0.64 cm^2 and then placed into a two-compartment Teflon flow-through diffusion cell. Skin and silastic discs were perfused using Krebs-Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin and dosed with 20 µl of LAS (1577 $\mu g/cm^2$), 20 µl of 2% triazine (625 $\mu g/cm^2$), or 20 µl of 5% RA in various mixtures described in **Table 1**. The temperature of the perfusate and flow-through cell was maintained at 37°C using a Brinkmann constant-temperature circulator (Brinkmann Inc., Westbury, NY), and the pH was maintained between 7.3 and 7.5. Perfusate flow rate was 4.0 mL/hr, and perfusate samples were collected at 0, 10, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 240, 300, 360, 420, and 480 minutes post dosing. At the end of the perfusion, the dose area was swabbed twice with soapy solution to determine surface content (Swab 1-2), taped-stripped six times (Tape 1-6) with cellophane tape to determine stratum corneum content, and removed from the skin disc with a 0.64 cm^2 punch biopsy to determine dose area skin deposition. These tissue samples were saved for radiochemical analysis described below.

Isolated Perfused Porcine Skin Flaps (IPPSFs). *(For Specific Aims #1, #2, and #3)*

Porcine skin flaps were surgically prepared according to procedures previously reported in the literature (Riviere *et al.*, 1986). Skin flaps were perfused in a non-recirculating system with oxygenated (95% O2 and 5% CO_2) Krebs-Ringer bicarbonate buffer spiked with glucose and bovine serum albumin. The perfusion chambers were maintained at 37°C and a relative humidity of 50-60%. Fifty microliter doses of 502 $\mu g/cm^2$ ^{14}C-LAS or other radiolabelled marker chemicals in simple and complete mixtures (**Table 1**) were applied to each dose site of 5.0 cm^2. Venous perfusate samples were collected at 30 minute intervals for the first 2 hours post-dosing and then at 1-hour intervals for a total of 8 hours. Monitoring vascular resistance (VR) (perfusate pressure/flow) and cumulative glucose utilization assessed skin flap viability. Arterial perfusate samples were collected hourly and compared with venous samples to determine CGU, while VR was defined as the ratio of arterial pressure to perfusate flow rate. At the end of the 8-hour perfusion, the dose area was swabbed with cotton swabs containing soapy solution and skin and fat tissues were obtained from the dose site and surrounding area and allowed to digest in soluene before further analyses as described below.

Chemical Analysis *(For Specific Aims #1 and #2)*

For determination of ^{14}C-LAS, ^{14}C-TRI, or ^{3}H-RA, perfusate, swabs, dose skin, and stratum corneum samples were combusted in a Packard Model 306 Tissue Oxidizer (Packard

Chemical Co., Downers Grove, IL) and then analyzed by Packard Model 1900TR Liquid Scintillation Counter (Packard Chemical Co., Downers Grove, IL) for total ^{14}C determination.

HPLC Analysis of NDELA:

Perfusate samples (200 µl) were precipitated with 1ml of acetonitrile, vortexed for 10 seconds, and filtered through 0.45 µm PVDF acrodisc syringe filter into new clean 16x125mm glass tubes. The filtrate was evaporated to dryness (about 15 minutes) at 55°C under nitrogen (16 inches Hg). This was then reconstitute with 200 µl of HPLC grade water, and injected directly onto a HPLC system (Waters Model 616 with 717 plus autosampler, and 996 photodiode array detector, Waters Corp., Milford, Mass.). The HPLC conditions were as follows: Column: YMC ODS-AQ S3 120 Å 4.6 x 100mm Guard Column: YMC ODS AQ; Column temperature: 30°C; Mobile phase: 100% pH 3.0 20mM NaH_2PO_4; Flow rate: 0.75 ml/min; Injection volume: 10.0 µl; Detection: 240nm UV.

The limit of quantitation for NDELA in perfusate samples was 0.1µg/mL. The NDELA assay was validated for inter- and intra-day precision and accuracy, and over a concentration range of 0.1 µg/mL to 5.0 µg/mL, the recoveries ranged from 87% to 101% and the coefficient of variation ranged from 0.5% to 8.3%.

Nickel Analysis using ICP emission spectroscopy:

The sample was placed in cleaned pyrex glass test tube, dried at 105°C for 24 hours, and then ashed in Muffle furnace at 500°C. Tubes were removed from muffle furnace and allowed to cool and 2.5 mL of 6N HCl (Ultra-hi purity grade) was added, and this was then heated until boiling on a sand bath for 5 minutes. Approximately 5 mL of deionized-distilled water was then added. Ni content was determined using ICP emission spectroscopy where all readings were based on peak area. All readings were initially corrected by standard blank and acid blanks used to correct samples with < 2.5 mLs media unknown. Media blanks were used to correct the remainder of samples with sample size = 2.5 mL. The two wavelengths used for analysis were Ni_231.604 & Ni_221.648. All unknowns and acid blanks spiked with Sc to test signal suppression due to high salt matrix. Final average Ni concentration in digests were corrected for signal suppression if the ratio < 0.98. Acid blanks were used to account for mismatch between standards acid background and low sample volumes for first unknowns in each set. Acid blanks were "subtracted" to account for possible over correction using standard blank value to calculate the net intensity.

For 0.050 µgNi/mL in receptor media, the %CV was 1.0 (n=15) at both wavelengths. The average %CV for zero readings was 4.0 for Ni_231 and 7.5 for Ni_221. The limit of quantitation for nickel in perfusate samples was estimated to be 0.004 µgNi/mL for Ni_231 and 0.005 µgNi/mL for Ni_221. The working LOQ was defined as 0.007µgNi/mL.

Analysis of perfused media for Trichloroethylene:

The method used for analyzing the perfused media for trichloroethylene, TCE, in the porcine skin *in vitro* flow-through diffusion cell experiments involved liquid-liquid extraction with hexane followed by gas chromatography-electron capture detection. Briefly, 1 mL of sample and 1 mL of hexane were placed into 16x125mm borosilicate glass screw-top tubes and capped. The samples were then gently shaken for 20minutes using an Adams Nutator. If necessary, the samples were then centrifuged for 10 minutes at 4000 RPM and 20 degrees C in order to clarify any emulsion that had formed. A 200 µL aliquot was removed from the top

hexane layer and placed into GC vials containing small volume inserts. The gas chromatograph was a Hewlett-Packard 5890 series II gas chromatograph with electron capture detection. The data acquisition system consisted of a Toshiba Equium computer with Hewlett-Packard Chemstation Software for Windows 95 version G1701AA rev. A.03.02. A DB-5 megabore (30m L; 0.53mm I.D.; 3 μm film thickness) column was used for all analysis. The column oven temperature was programmed to begin at 40°C for 4 minutes then increase to 100°C at 25°C/minute and hold for 2 minutes. The Nitrogen carrier gas and make-up gas flow rates were 2.0 mL/minute and 60 mL/minute, respectively. The detector temperature was 310°C. The inlet temperature was 240 °C. The split/splitless inlet vent flow was 50 mL/min. The septum purge vent flow was 2-3 mL/minute. Injection volume was 2 μL. A 4mm splitless, single taper liner was used. Upon injection, the purge gas was off for one minute then on for the remainder of the run. The retention time of TCE under these conditions was 2.8 minutes.

An external standard calibration curve, recoveries and blanks were run with every set of samples as part of quality control. The limit of quantitation was determined experimentally to be 0.05 μg/mL TCE based on the lowest recovery that had an overall coefficient of variation, CV, less than 15%. In other words, the IPPSF media spiked at 0.05 μg/mL of TCE gave an average recovery of 116% with CV of 12% (n=42). The slope of the external standard calibration plot was linear in the range of 0.005μg/mL TCE to 1.0μg/mL TCE. The average slope was 65026 ± 4712 with a CV of 7.2% and an average R^2 of 0.991 ± 0.001 with a CV of 0.1%. The overall extraction efficiencies, %recovery (CV%, n), for TCE spiked media were as follows: 116% (43% n=24), 148% (20% n=42), 116% (12% n=42), and 117% (12% n=42), for TCE concentrations of 0.005, 0.01, 0.05, and 0.1 μg/mL, respectively.

***Calculations and Statistics** (For Specific Aims 1#, #2, and #3)*

Absorption in all three model systems was defined as the total percentage of initial dose detected in the perfusate for the entire 8-hr perfusion period. The *apparent permeability* (cm/hr) of LAS in the diffusion cell system was determined from the following equation:

$$\text{Permeability (cm/hr)} = \text{Flux } (\mu g/cm^2/hr) \;/\; \text{Dose } ((\mu g/cm^3)$$

LAS, TRI, and RA flux were determined from the apparent steady state slope derived from a plot of cumulative toxicant vs time. *Tissue disposition* parameters such as surface, stratum corneum (SC), and dosed skin were described above. For *partition coefficient* (PC) determinations, radioactivity content in the vehicle mixture and stratum corneum (SC) were normalized to 1000 mg vehicle ($C_{vehicle}$) and 1000 mg SC (C_{sc}), respectively. The log SC/Vehicle partition coefficient was determined from the equation:

$$\text{Log PC} = \text{Log } C_{SC} / C_{Vehicle}$$

Standard errors were determined for all data sets. For analysis of total absorption, flux, permeability, diffusivity, skin surface, SC, and dosed area data, multiple comparison tests were performed using ANOVA with significance level at 0.05. Paired comparisons were also made to assess silastic vs. skin effects and mineral oil vs. PEG effects. All analyses were carried out using SAS 6.12 for Windows software (SAS Institute Inc., Cary, NC). A least significant difference (LSD) procedure was used for multiple comparisons on all parameters assessed.

***Dermatotoxicity Studies** (For Specific Aims #3)*

In vivo: Female weanling Yorkshire pigs weighing 20-30 kg were acclimated for one week prior to the study. The pigs were housed in an AALAC accredited facility on elevated floors and provided water and 15% protein pig and sow pellets ad libitum. Pigs (n=4/treatment) were exposed to 5% mineral oil (MO) or 5% polyethylene glycol (PEG) aqueous mixtures containing the additives TRI (2%), TEA (5%), LAS (5%), SRA (5%), or additive mixtures. Approximately 24 hrs prior to the topical application of the compound, the pig was sedated with a telazol-ketamine-xylazine cocktail (TKX) and the excess hair on the back carefully clipped. On the day of the experiment, each pig was sedated and placed in a sling. Sixteen sites, 8 on each side of the midline, were randomized on the back of the pig. Eight sites were immediately treated with 200µl of the appropriate cutting fluid (single or in combination) in a Hill Top Chamber. Four hrs later, the pig was again sedated with TKX and the remaining 8 sites treated as above. Eight hrs after the initial treatment, the pig was sedated with TKX, erythema and edema were evaluated, and 6mm biopsies were harvested and fixed in 10% neutral buffered formalin. The biopsies were processed through graded ethanols, cleared in Clear-Rite 3, infiltrated, and embedded in Paraplast®. The skin was sectioned at 6µm, stained with hematoxylin and eosin, and analyzed for intracellular and intercellular epidermal edema, dermal edema, and dermal inflammation on an Olympus BH-2 microscope.

Isolated perfused porcine skin flap (IPPSF): The IPPSF was created as previously described for the dermal absorption studies. The flaps were perfused for 1 hr prior to dosing with a modified Krebs/Ringer bicarbonate buffer (pH 7.4) containing bovine serum albumin, glucose, penicillin G, amikacin, and heparin, during which 1.0ml arterial and 3.0ml venous samples were collected to assess glucose utilization. Once flap viability was confirmed, the perfusion was interrupted and each flap was removed from the chamber. A Stomahesive® (ConvaTec, Princeton NJ) template with a 1.0cm x 5.0cm (5.0cm^2) dose area was secured to the flap with Skin-Bond® (Smith & Nephew, Inc., Largo FL). Each flap was returned to the chamber and treated with 50µl of the vehicle, additive, or additive mixture. Flap perfusion was resumed, with the venous perfusate sampled at 0, 0.5, 1, 2, 4, and 8 hrs for IL-8 assay. IPPSF's (n=4/treatment) were exposed to MO or PEG controls, single additive in vehicle, and complete additive mixtures in vehicle.

The IL-8 assay was performed using a Swine IL-8 Enzyme Linked-Immuno-Sorbent Assay kit (Biosource International, Inc., Camarillo, CA). The timed samples were thawed, diluted 1:1 in a standard diluent buffer, and thoroughly mixed. Samples (triplicate) and Standards (duplicate) were pipetted (100µl) into the appropriate wells of the antibody-coated ELISA plate. The plate was sealed and incubated for 2 hrs, rinsed, and incubated for 1 hr with 100µl of biotinylated anti-IL-8 biotin conjugate. Following a rinse, 100µl of Streptavidin-HRP (1:1000) was pipetted into each well and incubated for 30 min. The plate was incubated in the dark with Stabilized Chromagen (100µl) for 30 min, and the absorbance of each well read at 450nm using the Labsystems Multiskan RC plate reader (Fisher Scientific). The absorbance of each sample was converted to concentration (pg/ml) by fitting to the standard curve using Genesis-Lite Windows based microplate software version 3.0 (Life Sciences International UK Ltd.). The values were normalized by subtracting the mean of the time 0 value.

Cell Culture: Cryopreserved HEKs were purchased from Cambrex Bioproducts (Walkersville, MD) and stored under liquid nitrogen until passage. Cells (approximately 263,000) were diluted in 15ml of KGM-2 (serum-free keratinocyte basal media supplemented with human epidermal growth factor, insulin, bovine pituitary extract, gentamicin, and amphotericin-B) in each $75cm^2$ culture flask and grown in a humidified 5% CO_2 incubator at 37°C. The keratinocytes were harvested once they reached 80% confluency, stored under liquid nitrogen until plated, or plated immediately in 96-well culture plates at a density of approximately 8,000 cells per well. Once the monolayer reached approximately 80%, the HEKs were treated with the additives LAS (range 0.5-500μM), TRI (range 0.001-1000μM), SRA (range 0.034-34mM), and TEA (range 0.4-400mM) and the vehicles MO (0.01-10%) and PEG (0.01-10%) diluted in KGM-2. Plates containing the TRI and MO were covered with a membrane that allowed the exchange of gases but not the evaporation of these volatile compounds. At 24 hrs post-treatment, the media was replaced with KGM-2 containing MTT (0.5mg/ml; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and the plates incubated for 3 hrs. The HEKs were rinsed briefly in Hank's Balanced Salt Solution and destained with isopropanol for 2 hrs. The absorbance, read at 540nm using the Labsystems Multiskan RC plate reader, was converted to percent viability relative to the untreated controls.

Statistics: The means from the above dermal toxicity studies were generated and the significant difference ($p<0.05$) determined using a Student's t-test in the ANOVA procedure of PC SAS (version 8.01).

Results

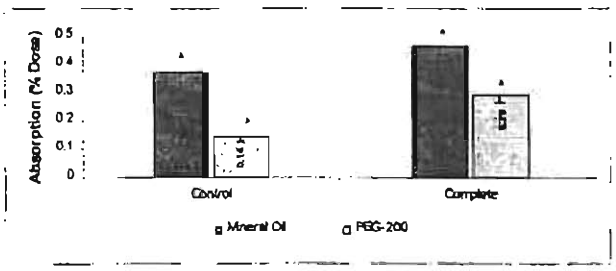
LAS Physicochemical and Permeation Studies: *(Specific Aims 1)*

LAS physicochemical studies

Viscosity ranged from 1.4×10^{-3} to 4.11×10^{-3} mPa.S., and it was always greater in mineral oil than PEG. However, the presence of SRA, TEA, or TRI+TEA, appeared to increase viscosity among mineral oil mixtures. Compared to other additives, SRA was most effective at significantly depressing LAS CMC in PEG-based mixtures. Consistent CMC values were difficult to obtain for mineral oil mixtures, therefore only CMC values for PEG were obtained. The pH for corresponding mineral oil and PEG mixtures were similar (data not presented). While the pH for the control mixture (LAS alone) or LAS+SRA mixtures were within physiological range (6.52 – 7.24), the pH values ranged from 9.4 – 11.23 for other cutting fluid mixtures. LAS solubility was greater in PEG than in mineral oil for 6/8 mixtures, and solubility ranged from 27 – 49%. SRA or SRA+TRI increased LAS solubility about 2-fold in mineral oil, while SRA+TEA increased LAS solubility almost 2-fold in PEG. For most of the cutting fluid mixtures (7/8), LAS was more likely to partition into the SC with mineral oil mixtures than with PEG mixtures. LAS partitioning into SC in mineral oil was greatest for the control, which was statistically similar to TRI alone The presence of SRA alone or combinations of additives significantly ($p < 0.05$) *decreased* LAS partitioning into SC in mineral oil and PEG mixtures.

LAS Absorption and Permeability:

LAS absorption across both silastic membranes and porcine skin never exceeded 0.32% dose during these 8-hour exposures. In porcine skin exposed to mineral oil mixtures, the complete mixture significantly increased LAS absorption ($p < 0.05$) when compared to the control mixture (see figure), and a similar trend was observed in the silastic membrane and skin flap exposure studies although the differences were not significant in the skin flap. The complete mineral oil mixture significantly increased LAS permeability in silastic membranes, but only slightly in porcine skin (Table 3).



In the mineral oil mixture, TRI alone significantly increased LAS absorption in silastic membrane and LAS permeability in porcine skin. With PEG mixtures, there was no significant influence of the complete mixture on LAS absorption in skin or silastic membranes, although the complete PEG mixture significantly reduce LAS permeability in porcine skin while increasing absorption two-fold in the skin flaps. LAS permeability in both skin and silastic membranes in PEG mixtures was greatest when no additives (i.e., control mixture) were present. TEA significantly reduced LAS absorption in silastic membranes and permeability in both membranes with PEG-based mixtures, yet when combined with TRI, this mixture significantly enhanced LAS absorption in skin.

LAS Deposition in Skin Layers

LAS deposition in these skin compartments decreased in the following order: surface > stratum corneum > dosed skin. In mineral oil mixtures, surface levels ranged from 70.63% dose

for the complete mixture to 27.97% dose for the control mixture, however LAS levels were significantly greater ($p < 0.05$) in the stratum corneum and dosed skin for the control mixture than when additives or combinations of additives were present in the dosing mixture. This trend was also observed in silastic membrane, although the data is not presented in this manuscript.

In PEG mixtures, deposition in the stratum corneum and dosed skin was also greater with the control mixture than the complete mixture. In silastic membranes this effect was statistically significant ($p < 0.05$). Surface levels on the skin ranged from 46.71% dose to 73.41% dose for PEG mixtures, and there were not as many differences between PEG mixtures as compared with mineral oil mixtures. LAS levels in the silastic membrane were greatest with TRI dosed in either mineral oil or PEG, and for both mineral oil and PEG mixtures, the complete mixture resulted in the greatest levels on the silastic surface.

The porcine skin flap model allowed one to determine deposition into the subcutaneous fat layer as well as other skin layers described for the porcine skin sections. The distribution pattern was different when compared to porcine skin sections; that is, LAS levels in deeper skin layers were similar or comparable to those in the SC for the mineral oil and PEG mixtures. In essence the gradient in LAS distribution in the skin layers of skin flap was not as great as that in the porcine skin sections.

Triazine Phyiscochemical and Permeation Studies. *(For Specific Aims 1#)*

Triazine Physicochemical Properties

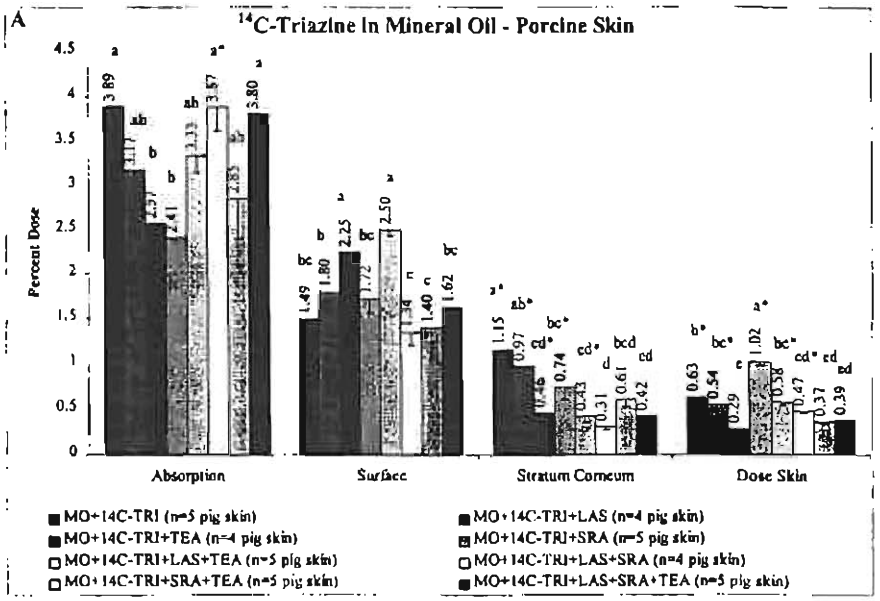
The pH of triazine control mixtures (triazine+water+diluent) was within range of the literature values of pH = 11. However, SRA or the presence of SRA+LAS, SRA+TEA, or SRA+LAS+TEA decreased mixture pH by at least one pH unit, with SRA alone having the greatest effect on mixture pH. The LAS+TEA mixture appeared to have the greatest effect on mixture viscosity in mineral oil but not synthetic mixtures. The presence of LAS significantly decreased triazine partitioning into the stratum corneum ($p < 0.05$), and this was most significant with complete mixtures and more likely associated with mineral oil than PEG mixtures. SRA had a similar but not such a significant effect as LAS, while TEA by itself had no effect on triazine partitioning into the stratum corneum.

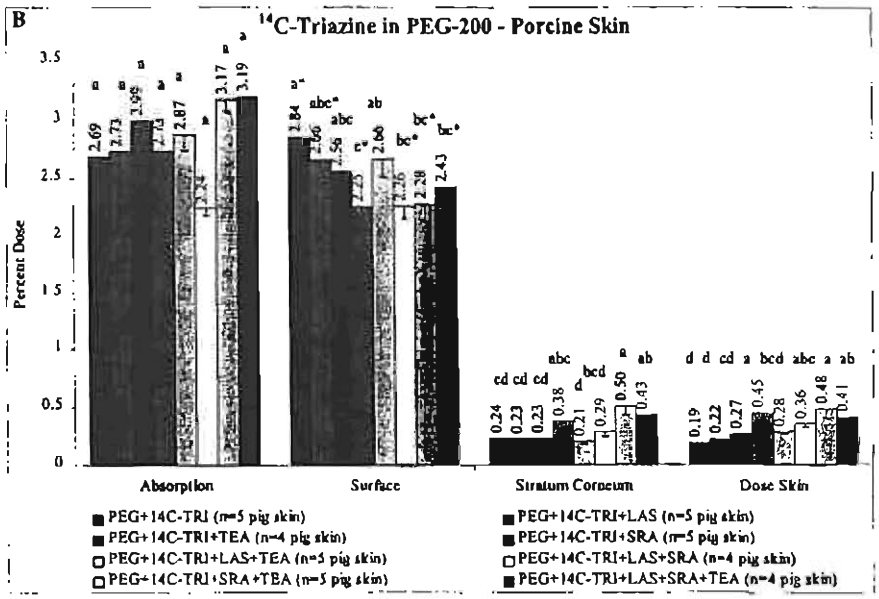
Triazine Dermal Absorption and Permeability

Triazine absorption peaked rapidly in both silastic membranes and porcine skin and rapidly declined to low levels for the remaining 8-hr exposure. Triazine absorption ranged from 12.61 – 18.63% dose in silastic membranes and 2.41 – 3.89 % dose in porcine skin sections (see Figure). In silastic membranes exposed to PEG mixtures, LAS alone had no effect on triazine absorption, TEA alone decreased absorption, and SRA alone and all other mixtures significantly increased triazine absorption ($p < 0.05$). SRA+TEA and complete mixtures significantly increased triazine permeability. For mineral oil mixtures, there was a reverse trend as additives were formulated into these surrogate mixture; that is, the presence of one or more additives significantly reduced triazine absorption with the complete mixture having the most significant effect. Of interest, only SRA and SRA+TEA appeared to increase triazine permeability, if not significantly, for all mixtures involved. The silastic membrane/skin permeability ratio for triazine ranged from 7.09 to 17.01 in mineral oil mixtures and 6.00 to 14.14 in PEG mixtures.

The greatest silastic/skin permeability ratios were associated with SRA in mineral oil mixtures (17.01) and with SRA+LAS (14.14) in PEG mixtures.

In porcine skin (se Figure below), a trend similar to triazine absorption in silastic membranes was observed with the PEG mixtures, although the differences were not statistically significant. Interestingly, SRA+TEA and the complete mixture significantly increased the





apparent permeability of triazine in PEG mixtures. In mineral oil mixtures, the complete mixture also significantly increased triazine permeability and although SRA or TEA alone significantly reduced triazine absorption, they increased triazine permeability when combined.

Triazine Membrane and Skin Deposition

In silastic membranes, triazine deposition in the membrane at 8 hrs significantly decreased with the presence of one or more additives in PEG mixtures and although not statistically significant in mineral oil mixtures, a consistent trend was observed. In the porcine dosed skin, triazine deposition followed a similar trend with mineral oil mixtures, but deposition was significantly reversed in PEG mixtures. It should be noted that porcine skin retained more of the triazine (0.19 – 1.02% dose) than did the silastic membranes (0.05 – 0.2% dose) and that triazine was more likely to be retained in the skin with mineral oil mixtures than PEG after an 8-hr exposure.

Finally, triazine deposition in stratum corneum at 8 hrs was significantly reduced by the presence of additives in mineral oil, but the trend was reversed in PEG mixtures. This trend in mineral oil mixtures is consistent with the partitioning studies. As with dosed skin, triazine was more likely to be retained in the stratum corneum with mineral oil mixtures than with PEG after an 8-hr exposure, and triazine levels ranged from 0.31 – 1.15% dose with mineral oil mixtures to 0.21 – 0.50% dose with PEG mixtures.

Ricinoleic Acid (RA) Physicochemical and Permeation Studies (For Specific Aims 1#)

RA Physicochemical Studies.

The pH of the control mixtures (ricinoleic acid only) or mixtures containing ricinoleic acid and LAS only were within physiological range (6.52 – 7.53). However, the presence of other additives increased the pH by at least 1 - 3 pH units to a very basic pH ranging from 9.31 – 10.28 (Figure 2). Ricinoleic acid partitioning into the stratum corneum was significantly reduced in the more complex PEG mixtures ($p < 0.05$), while this trend was reversed with mineral oil mixtures. In fact, the presence of one or more additives in mineral oil mixtures resulted in significantly greater ricinoleic acid partitioning into the stratum corneum compared with PEG-200 mixtures ($p < 0.05$).

RA Dermal Permeation and Deposition.

Ricinoleic acid absorption peaked within 3 hours for most mixtures with the greatest ricinoleic acid peak concentrations being associated with the control mixtures containing PEG in both membranes (Figure 3 and 4). At 8 hours after topical exposure, ricinoleic acid absorption ranged from 1 – 13% dose in silastic membranes, but only 0.1 – 0.3% dose in porcine skin membranes (Figure 5 and 6). Compared to other mixtures, ricinoleic acid permeability and absorption in either membrane system was significantly greater when dosed by itself than when other additives were present in the mixture ($p < 0.05$). That is, the presence of cutting fluid additives in these mixtures had a negative effect on ricinoleic acid absorption and permeability. The only exception was in porcine skin exposed to mineral oil mixtures where TEA significantly enhanced absorption compared to control mixture. It should also be noted that LAS alone had one level of effect, while other additives or combination of additives had a more significant effect on reducing ricinoleic acid absorption and permeability ($p < 0.05$).

At the end of the 8-hour perfusion experiments, as much as 5% dose of ricinoleic acid was detected in the dosed skin and as much as 16% dose was detected in the stratum corneum with mineral oil formulations. Considerably less ricinoleic acid remained in these skin tissues with PEG mixtures and this was statistically significant for 5/8 of the mixtures ($p < 0.05$).

Significantly greater levels of ricinoleic acid were detected in the dosed skin and stratum corneum with the control mixtures (ricinoleic acid only) or with LAS mixtures ($p < 0.05$), and reflected the same general trend observed with the absorption data. That is, the presence of more additives in these mixtures reduced ricinoleic acid deposition in skin tissues. It should also be noted that ricinoleic acid deposition in these tissues was always greater in mineral oil than PEG mixtures, and statistically significant for 6/8 of these mixtures ($p < 0.05$).

Contaminant mixture effects on TRI permeation (For Specific Aims #2)

Triazine Absorption in Skin

In these 8-hour *in vitro* perfusions, triazine absorption peaked within 45 minutes after topical application, and then began to decline near background levels within 3-4 hours. In mineral oil-based mixtures lacking cutting fluid additives, the presence of both Ni and NDELA *significantly increase* triazine peak flux when compared to controls ($p < 0.05$). These trends were not observed in PEG-based mixtures, but peak fluxes tended to be greater in mineral oil-based mixtures than in PEG-based mixtures and this was statistically significant when NDELA was present.

In all mixtures tested, the *percentage dose absorbed and permeability of triazine was greater in mineral oil-based mixtures* (2.87 - 3.64% dose) than when dosed in PEG-based mixtures (2.29 – 3.04% dose). This was statistically significant ($p < 0.05$) for cutting fluid mixtures containing NDELA or NDELA+Ni. However, NDELA and/or Ni had no significant effect on percent dose absorption of triazine or its permeability after 8 hours in mineral oil mixtures, but their presence significantly ($p < 0.05$) reduced triazine absorption in PEG-based mixtures.

Triazine Deposition in Skin, SC, and Surface

Triazine depostion in the viable skin tissue below the stratum corneum ranged from 0.46 – 1.20% dose in mineral oil mixtures and 0.28 – 0.53 % dose in PEG mixtures. *Once again the differences between mineral oil and PEG observed in the perfusate were reflected in the viable skin.* Interestingly, this was statistically significant ($p < 0.05$) for mixtures containing just nickel and no NDELA. Nickel also significantly increased triazine deposition in skin when compared to other control mixtures.

The same trends occurred in the stratum corneum when one compares mineral oil and PEG mixtures for the additives-free mixtures. However, this is reversed when the cutting fluid additives are present. Nickel also seems to play a significant role in increasing triazine deposition in the SC. On the skin surface, triazine deposition was greater in PEG mixtures than with mineral oil mixtures. This was statistically significant for three-quarters of the control mixtures. Finally, NDELA caused the greatest retention of triazine on the skin surface for both PEG-based and mineral oil-based mixtures, and this was statistically significant for PEG-based mixtures with cutting fluid additives.

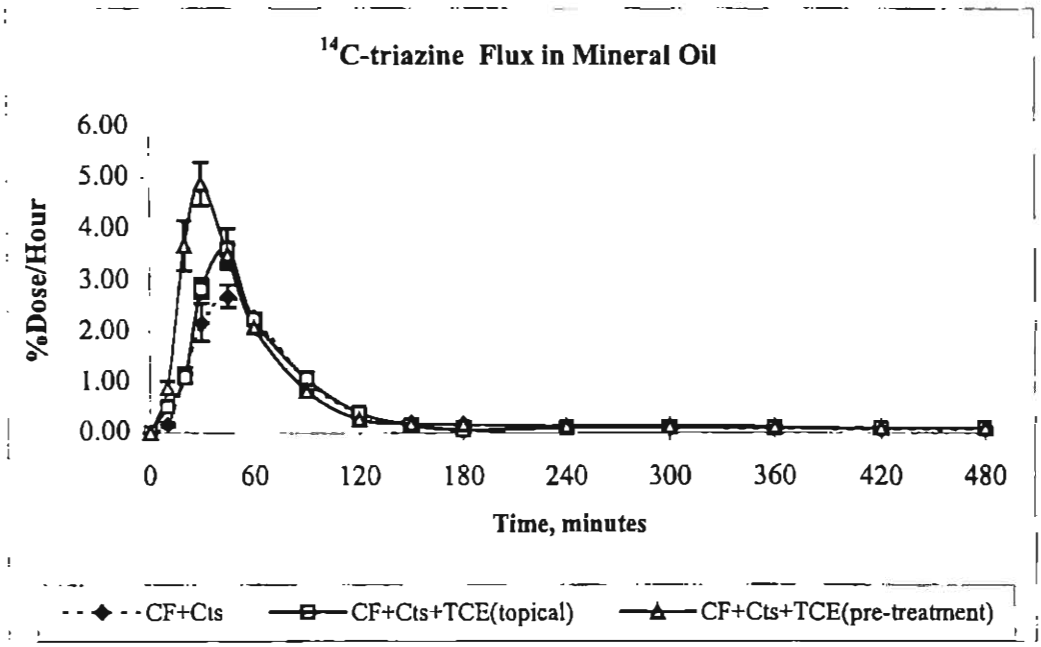
NDELA and Nickle Disposition

Within limits of our assay previously described, little or no traces of nickel or NDELA were detected in perfusate samples from porcine skin sections dosed with nickel or NDELA mixtures.

Cleanser (TCE) effects on TRI permeation (For Specific Aims #2,)

Triazine Absorption

The mineral oil (MO) base cutting fluid resulted in greater absorption in comparison to the polyethylene glycol-200 (PEG) base. The 96-hour TCE pretreatment of skin significantly ($p < 0.05$) enhanced triazine absorption when dosed with cutting fluids containing NDELA and nickel contaminants. **(see figure below: CF = cutting fluids; Cts = contaminants).** TCE pretreatment also delayed triazine time to peak in both MO- and PEG-based cutting fluids with statistically significant effects in MO-base cutting fluids. Simultaneous topical application of TCE with cutting fluid mixtures did not significantly affect triazine absorption. However, the data trend also suggests that contaminants may similarly influence triazine absorption with topical TCE application.



Dosed Skin Deposition

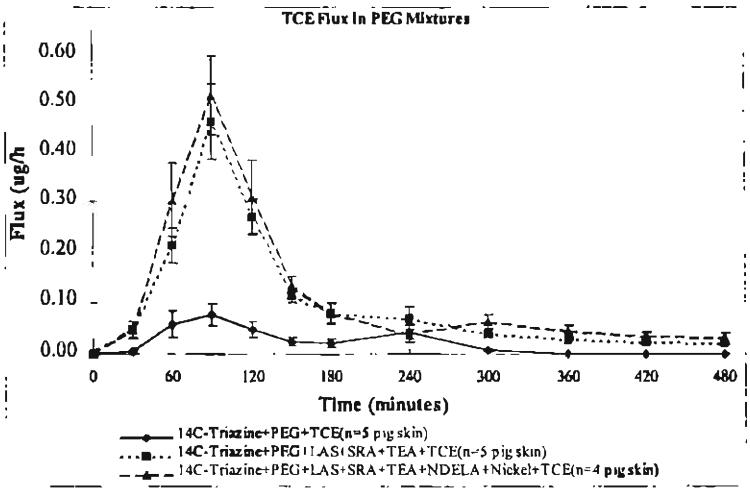
With only one exception, TCE consistently increased triazine deposition in the dosed skin area **(Figure 2)**. This was statistically significant in TCE pretreated skin dosed with cutting fluids in either MO- or PEG-based cutting fluids. This was also statistically significant for the control mixtures in both the topically treated and TCE pretreated skin dosed with either MO- and PEG-based mixtures.

Stratum Corneum Deposition

Topically treated skin or TCE-pretreated skin caused a statistically significant increase in triazine deposition the stratum corneum with PEG-based control mixtures but was associated with a significant decrease with PEG-based cutting fluid mixtures containing contaminants. Triazine deposition in the stratum corneum was not significantly affected by topical TCE or TCE pretreated skin in the MO base.

TCE absorption

Cutting fluids increased topical TCE absorption in both MO and PEG with PEG being more significant (**see Fig below**). The contaminants in the cutting fluids increase topical TCE absorption even more in the PEG based vehicle. With MO based cutting fluids and contaminants TCE absorption is not affected by TCE-pretreated skin, however, when compared to MO-based mixtures, the data suggests that PEG based cutting fluids decrease TCE absorption in TCE pretreated skin.



In vivo and in vitro effects of cutting fluid mixtures (For Specific Aims #3)

In vivo:

With the single additive, slight erythema was present in the 2% TRI and 5% LAS (with PEG, 8 hrs; with MO, 4 and 8 hrs) and the 5% LAS/2% TRI (with MO, 8 hrs). Slight erythema was also seen with other 2-component mixtures. The 3-component mixture 5% TEA/5% SRA/2% TRI exhibited the greatest overall erythema in both vehicles (Table 1). The only statistically significant ($p<0.05$) vehicle effect was found in the 5% LAS/2% TRI mixture at 8 hrs. No macroscopic edema was present in any of the cutting fluid treatments (Table 2). Mixtures containing 2% TRI exhibit the greatest microscopic intracellular epidermal edema, followed by mixtures containing SRA (Table 3). A significant ($p<0.05$) vehicle effect was present in the 4-component mixture. Individual cutting fluids exhibited no intracellular epidermal edema above that of controls. Intercellular epidermal edema was minimal in most of the individual and combination cutting fluids (Table 4), with a minimal effect noted with 5% TEA and 2% TRI. Dermal edema (Table 5) and dermal inflammation (Table 6) were the greatest in mixtures containing TRI or SRA (Figures 1 and 2). Individual cutting fluids exhibited about the same dermal edema as the controls, with dermal inflammation with 2% TRI greater than the controls.

IPPSF:

IL-8 concentration (pg/ml) increased from 0 hrs to 8 hrs in all additive treatments. ^{14}C-LAS in PEG caused a significant ($p<0.05$) release of IL-8 at 1 hr, while the controls were significantly higher ($p<0.05$) than ^{14}C-LAS+MO+SRA+TEA+TRI at 2 and 4 hrs (Figure 3). No significant differences were noted at 8 hrs post-treatment. The 4-component mixture containing ^{14}C-TRI+PEG caused significantly higher ($p<0.05$) levels of IL-8 release at 1 and 2 hrs. By 4 hrs, the control was significantly higher ($p<0.06$) than the remaining treatments (Figure 4). ^{3}H-

SRA in MO caused the release of significantly higher ($p<0.05$) concentrations of IL-8 at 0.5, 1, 2, and 4 hrs post-treatment (Figure 5). By 8 hrs, ^{3}H-SRA+MO+LAS+TRI+TEA had significantly higher ($p<0.05$) concentrations of IL-8 relative to the other SRA treatments.

Cell Culture:

HEKs were treated with each additive and vehicle to determine the concentration of their toxic effect. Cell viability threshold in LAS was 20.0μM (0.0007%; Figure 6), in TRI at 10.0μM (0.0002%; Figure 7), in SRA at 34.0μM (0.001%; Figure 8), and in TEA at 10000μM (0.15%; Figure 9). In the vehicles, MO showed no toxic effect up to 10.0% (Figure 10) while PEG viability plateaued at 1.0% (Figure 11).

Discussion:

LAS Absorption LAS is used as an emulsifier in many cutting fluid formulations, and there is limited knowledge about its dermal disposition. This research demonstrated that LAS permeability in skin is limited and it can be attributed to its large molecular weight and charge that limit its diffusion across the lipid matrix of the SC. This is reflected in almost similar permeability values in both silastic membrane and procine skin. Anionic surfactants such as LAS and sodium lauryl sulfate can form micelles or spherical aggregates above the critical micelle concentration (CMC) which are effectively too large to diffuse across a membrane. Our experimentally derived CMC value (0.14%) for LAS in only water was very similar to the literature value of 0.11% (EHC, 1996), making it fair to assume that our observed additive effects on the CMC of these LAS mixtures are valid. The presence of a fatty acid chain in the form of SRA could have resulted in formation of a longer alkyl chain length or more LAS aggregates as evidenced from the significantly decreased CMC values when SRA was added to these LAS mixtures.

Although LAS is negatively charged in an aqueous solution, the presence of other additives such as triethylanolamine and/or triazine could have contributed protons making these monomers neutral and more likely to diffuse across the membrane. The silastic membrane data suggests a possible chemical interaction between LAS and triazine that may have contributed to the significant increase in LAS absorption in mineral oil-based mixtures. In the case of PEG mixtures, the presence of TRI+TEA had a significant effect on LAS absorption in skin, but not silastic membrane suggesting that the former mixture effect is probably a chemical-biological interaction. TEA does not appear to enhance LAS absorption in either solvent system, but it increased the viscosity of these mixtures. This is a plausible explanation for its ability to reduce LAS absorption in silastic membranes for several PEG mixtures.

Fatty acids are known to enhance dermal permeability (Cooper et al., 1985), however, the effects of fatty acids on the permeability of surfactants have not been fully characterized. In this study, SRA did not appear to enhance LAS absorption. Interestingly, LAS solubility was the greatest with SRA+TRI in mineral oil and SRA+TEA in PEG, and the presence of SRA consistently lowered the log PC in mineral oil and PEG mixtures and thus significantly reduced LAS permeability in porcine skin in PEG. SRA significantly reduced LAS CMC, thereby reducing LAS monomers available for diffusion across the skin. It is also conceivable that the fatty acid formed mixed micelles with the surfactant and more likely promoted micelle formation which in turn reduced accessibility of the surfactant for binding sites to the stratum corneum.

Triazine Absorption: This study demonstrated that more than one of these cutting fluid additives modified the physicochemical properties of a simple triazine mixture, and simultaneously altered triazine permeability across skin. This was most evident with complete PEG mixtures in inert and biological membranes.

Triazine is a very water soluble biocide, and therefore does not readily diffuse or partition into skin or a related lipophilic membrane. In spite of this, it was still surprising that many of the cutting fluid additives or combinations of additives, which are also dermal irritants, significantly reduced triazine partitioning into the stratum corneum. It is plausible to assume that these additives increased polarity and solubility of the mixture making it less favorable for triazine to partition from the mixture into the stratum corneum. As SRA significantly reduced mixture pH by 1-2 pH units, it is safe to assume that this influenced the polarity of triazine which is a weak base. According to the Henderson-Hasselbalch pH-partitioning hypothesis, this interaction can result in more ionized molecules that are less likely to diffuse across or into a lipophilic membrane such as the SC (Swarbrick, et al., 1984, Smith et al., 2000). However, this does not support our observations with LAS that had no effect on mixture pH, but consistently and significantly reduced triazine partitioning more so than SRA. A more plausible explanation may be related to the interaction between triazine and LAS micelles especially as these dosing mixtures contained supra-micellar concentrations at 5% LAS (EHC, 1986). The influence of micelles on membrane diffusion of various drugs and toxicants has been well documented (Xia, W.J.; Onyuksel, 2000).

The present *in vitro* study demonstrated that while absorption in silastic membrane can be considerable (12-18% dose), absorption is significantly less in porcine skin (2 – 4% dose). This difference can be attributed to the more heterogenous composition of skin that contains aqueous domains that are more likely to retain polar triazine molecules than the homogenous lipophilic silastic membrane where triazine will readily partition from the membrane into the aqueous perfusate (Bronaugh and Stewart, 1985). Also, this study demonstrated that triazine absorption in porcine skin was comparable to literature values for other triazines that are less toxic to skin

Mixture effects on triazine absorption and permeability in porcine skin appeared to be related to cutting fluid additives as well as the diluent effects of mineral oil and PEG. The SC partitioning data as well as the silastic membrane data suggest that triazine diffusion should be limited by the presence of several of these additives in either diluent. Surprisingly, triazine permeability was increased significantly with several complex mixtures in either diluent, which is not predictable from the partitioning data. A plausible explanation for this observation may be related to the fact that these additives increased diffusivity by altering polar transport pathways in biological membranes even though partitioning into the SC and silastic membrane was compromised by these additives. Dermal diffusion of hydrophilic solutes such as triazine are more likely to occur via so-called "polar" or "pore" routes which are conceptually imperfections in the lipid bilayers (Flynn et al., 1981; Anderson et al., 1988; Mitragotri, et al., 2003). There is convincing evidence that hydration can lead to induction of new pores and reduced tortuosity/path length of existing pores in porcine skin sections especially for hydrophilic solutes in aqueous environments utilized in this study (Tang et al., 2002)

It is worth noting that the trend for increased triazine absorption in PEG mixtures was observed in both silastic as well as porcine skin membranes, and supports the fact that PEG was more effective than mineral oil at solubilizing mixture additives and probably behaved as a co-solvent for triazine and other cutting fluid additives. The SC partitioning data supports this

argument as PEG was more likely to enhance triazine partitioning into the SC than mineral oil especially in complete mixtures.

The permeability data also demonstrated that SRA+TEA significantly enhanced triazine diffusion in complex cutting fluid mixtures in either PEG- or mineral oil-based mixtures and in both membrane systems. SRA is a fatty acid, and although very little is known about its enhancer properties, several investigators have demonstrated that a related fatty acid, oleic acid, is a very effective transdermal enhancer (Cooper et al., 1985; Green et al., 1988) with significantly greater enhancement with hydrophilic than hydrophobic drugs (Kim et al., 1996). One recent study has however demonstrated that ricinoleic acid is a less effective permeation enhancer than oleic acid for polar drugs (Song et al., 2001). Our study also demonstrated that SRA or SRA+LAS had the greater effect on triazine permeability in silastic membranes than in skin. It is plausible to infer from this membrane comparisons that SRA may have enhanced triazine permeability in silastic membranes by increasing the availability of triazine as well as behaving as a permeation enhancer in the lipophilic silastic membranes. This physicochemical interaction may be characteristic of polar solutes similar to triazine in similar membranes, but may not be applicable to nonpolar solutes as observed with other fatty acids.

TEA is an alkanolamine, and the enhancer properties of this class of chemicals have also been well documented. It is no surprise that this pair of additives (TEA+SRA) had the most significant effect on triazine permeability across both membrane systems. Other dermal absorption studies with chemically related compositions have proposed formation of a lipophilic ion pair as a possible mechanism for skin penetration enhancement (Aungst et al., 1990). However, our SC partitioning experiments do not support this hypothesis as SRA or SRA-containing mixtures did not enhance triazine partitioning into the SC. Finally, the physicochemical and diffusion data demonstrated that LAS did not enhance triazine diffusion in these mixtures, which is not entirely unusual for anionic surfactants.]

It should also be noted that the addition of additives to mineral oil-based mixtures significantly decreased stratum corneum and skin depostion of triazine, while this was reversed with PEG. The latter reflects the penetrating enhancer effect of PEG over mineral oil in biologically intact membranes (i.e., skin sections) in the presence of several of these additives. The finding that these additives significantly reduced triazine deposition into the silastic membrane, but increased deposition in viable skin, supports the argument that these additives in the presence of PEG promotes triazine diffusion via a chemico-biological mechanism as reflected in the permeability data previously described.

Although tissue levels are significantly greater with many of the mineral oil mixtures than with PEG, tissue levels for complete mixtures in both diluents were similar. This has toxicological implications because if this trend continues with addition of more cutting fluid additives to PEG-based cutting fluids, then these mixtures maybe more of an occupational concern than mineral oil where the increasing presence of additives has the opposite effect on tissue deposition. The latter will influence local irritation and the former may enhance absorption which influences systemic toxicity. This research was focused on interactions with 4 other cutting fluid additives, but in the real world there is simultaneous dermal exposure to numerous other additives as well as cutting fluid contaminants. In fact, triazine has been implicated with the increased presence of nitrosodiethanolamine in several cutting fluid formulations (Loeppky et al., 1983) which may not only be a carcinogenic concern, but may also modulate dermal absorption of triazine.

Ricinoleic Acid Absorption. Ricinoleic acid is used to enhance lubricity of metal-machining operations, but in spite of its known dermal irritant effects in workers, its dermal disposition has not been well characterized. Our study demonstrated that although dermal absorption of ricinoleic acid into the perfusate can be minimal (< 0.4%), as much as 16% of the dose can be retained in the stratum corneum for up to 8 hours. From an occupational health perspective, this may be significant enough to cause dermal irritation in metal-machining workers.

The most surprising observation from this study was that the presence of cutting fluid additives significantly reduced dermal absorption and skin tissue deposition of ricinoleic acid. These cutting fluid additives are themselves dermal irritants and should theoretically enhance solute permeability as recently demonstrated with related chemical mixtures (Baynes et al., 2003). However, because the opposite effect was observed, this suggests that chemical-chemical interactions more so than chemical-biological interactions Cutting fluid additives clearly had a significant effect on mixture pH and ricinoleic acid partitioning into the stratum corneum which further demonstrates that several physicochemical mechanisms modulating ricinoleic acid diffusion in skin were operational. Firstly, ricinoleic acid is a weak acid (pKa = 6), and because the presence of the additives increased the mixture pH by as much as 3 pH units, it is plausible to assume that this will probably result in formation of more of the anionic than the free fatty acid form..

The stratum corneum partitioning experiments further demonstrated that ricinoleic acid partitioning in the stratum cornuem was significantly inhibited by the presence of cutting fluid additives with PEG-200 but not mineral oil mixtures. This trend may be related to the fact that cutting fluid additives enhanced ricinoleic acid solubility in PEG-200 and thereby reduced partitioning from the dosing mixture into the membrane. At the end of the 8-hr exposure in the diffusion cells, this trend was also observed in the stratum corneum and dosed skin layers. These differences between mineral oil and PEG were however not reflected in ricinoleic permeability and absorption in silastic or skin membranes.

One or more cutting fluid additives significantly decreased ricinoleic acid absorption and permeability in both skin and silastic membrane. This interaction was more significant in silastic membrane than skin because of potential interactions between the diffusing fatty acid and other fatty acids in skin. The deeper epidermis contains a greater proportion of free fatty acids while silastic membranes contain no free fatty acids.

Although LAS significantly reduced ricinoleic acid absorption, the other additives triethanolamine and triazine either individually or as a mixture with LAS had a more significant effect on ricinoleic acid absorption. The fact that this was observed in both membranes reinforces the argument that these two additives had a significant effect on mixture pH and possibly the proportion of free acid form available for dermal transport. As an anionic surfactant, LAS is expected to enhance dermal absorption of most hydrophilic solutes (Wilhelm et al., 1994; Xia and Onyuksel, 2000). However, in this case it may have negatively altered fatty acid diffusion by direct chemical complexation with LAS micelles as previously reported with other solute-surfactant mixtures (Chidambaram and Burgess, 2000) or possibly altered lipid pathway in the epidermis. The latter mechanism is not likely as this surfactant-membrane interaction does not occur in silastic membranes, and yet we observed similar LAS effects on ricinoleic acid absorption in these inert membranes.

Effect of cutting fluid contaminants: This study demonstrated that the contaminants, nickel and NDELA, can influence the dermal disposition of the cutting fluid biocide, triazine. Modulation of the dermal absorption of triazine in the presence of these contaminants was more likely associated with mineral oil-based mixtures than with PEG-based mixtures. The use of mineral oil-based mixtures and PEG-based mixtures in this study was our attempt to prepare surrogate mixtures of the two major types of industrial cutting fluid formulations typically classified as soluble oil and synthetic fluids, respectively. It is very likely that other industrial diluents could have resulted in differential biocide disposition in skin.

Triazine is a water soluble biocide and one would expect this solute to more readily partition from the mineral oil formulation into skin than from a PEG-based formulation as predicted from our earlier studies (Baynes *et al.*, 2003). However, what was unexpected was that the contaminants, NDELA and/or nickel further enhanced this effect. It is plausible to assume that these contaminants altered triazine diffusion in skin by changing the physicochemical properties of these surrogate formulations. NDELA is miscible in water in all proportions, soluble in polar organic solvents, but insoluble in non-polar organic solvents. These solubility properties suggest that NDELA was behaving as a better co-solvent or vehicle for the polar triazine in mineral oil than in PEG. Put simply, NDELA is more likely to partition from a less miscible formulation (e.g., mineral oil) than from a miscible formulation (e.g., PEG), and in so doing enhance triazine diffusion in skin. Our data strongly supports this hypothesis, as we observed a significant decrease in triazine absorption when NDELA or Ni was added to PEG mixtures.

Our previous skin permeation work with this biocide (Baynes *et al.*, 2003) demonstrated that cutting fluid additives can significantly increase triazine permeability in mineral oil-based and PEG-based mixtures. Nickel salts are relatively water soluble, and there are no reports in the literature to suggest that metal salts modulate solute absorption in skin. The sparse literature reports that nickel absorption in skin is limited, variable, and it has very long lag times (Fullerton *et al.*, 1988). Its plausible to assume that nickel-induced irritation could have contributed to the limited mixture effects observed with PEG mixtures in this study.

NDELA is a water-soluble contaminant and previous percutaneous studies have demonstrated that dermal absorption can vary considerably depending on dosing vehicle and topically applied dose (Lethco *et al.*, 1982; Airoldi *et al.*, 1983; Bronaugh *et al.*, 1981; Franz *et al.*, 1993). There are no previous studies to demonstrate whether this or other nitrososamines are penetration enhancers, yet dihydroxy groups are part of its molecular structure and this suggests that it is a potential dermal enhancer. Although our studies are not conclusive, it is safe to assume that these contaminants may not be effective dermal enhancers for polar solutes such as triazine, but could be so for less polar or nonpolar cutting fluid irritants. Further studies will determine whether specific mixture interactions will more likely occur for irritants with different physicochemical characteristics. Lipoidal formulations appear to increase permeability of NDELA in human skin (Bronaugh *et al.*, 1981), however, we were unable to observe NDELA absorption during these 8-hour studies in mineral oil mixtures. It is probable that NDELA would have been detected at latter time points as demonstrated in other studies, and this may lead to increased triazine absorption as originally hypothesized.

Closer examination of our skin and surface deposition data demonstrated that NDELA caused the greatest retention of triazine on the skin surface. Again, it is safe to assume that NDELA made the formulation more polar, which encouraged triazine to remain in the formulation and not partition into the skin from the surface. This was clearly evident for PEG-

based mixtures and consistent with our absorption data with PEG as a diluent. Nickel may have had a slightly different physicochemical mechanism, although the results were similar. Histochemical studies have demonstrated that nickel can be selectively taken up by Langerhans cells in human epidermis and nickel has a high affinity for keratin which accounts for reservoir formation that contributes to its sensitization. Our studies demonstrated that nickel enhanced retention of triazine in the stratum corneum, which in itself suggests a possible ion-pairing interaction between this metal ion and the diffusing triazine solute which led to retention.

Effects of the Cleanser, trichloroethylene (TCE): Workers involved in metal-machining will at some stage of the machining operation use a cleanser to remove the cutting fluids from the finished metal product. While the use of TCE in this regard is being reduced, it does serve as a useful agent to assess acute and chronic effects on dermal disposition of irritants present in cutting fluid formulations.

This study demonstrated that simultaneous topical exposure to TCE or prior topical exposure to TCE can influence the dermal disposition of the biocide, triazine. These observations were with few exceptions evident in the three mineral oil-based or three PEG-based mixtures evaluated in this study. These findings are not entirely surprising as TCE and related solvents are known to impair skin barrier function by modifying epidermal lipid composition and structure and thus modulate chemical absorption (Abrams et al., 1993; Tsai et al., 2001). The latter study further demonstrated that some solvents such as acetone may enhance permeability not by altering solute partitioning behavior, but by altering diffusion kinetics in the stratum corneum. More recent studies with ampiphilic enhancers have countered that enhanced partitioning may be more important than diffusivity in permeation enhancement (He et al., 2004). Our study demonstrated no TCE effects on triazine diffusivity, although there was enhanced permeability in TCE pre-treated skin , which suggest that TCE altered partitioning behavior of triazine in these cutting fluid mixtures. Irrespective of the molecular mechanism involved, organic solvents are generally thought to be good permeation enhancers (Marjukka et al., 1999).

One unexpected observation from our study was that simultaneous topical exposure to TCE had little or no significant effect on triazine absorption into the perfusate. Furthermore, TCE did not modulate triazine deposition into the viable epidermis in the presence of cutting fluid additives, while TCE appears to reduce triazine partitioning into the stratum coneum (Figure 2 & 3). These tissue distribution data suggest that its very unlikely that TCE-enhanced effects on triazine absorption will be realized beyond the 8-hour experimental period.

Our laboratory and others have reported solvent system (e.g., ethanol, acetone) enhancement on dermal absorption of numerous toxicants with widely variable physcochemical properties (Baynes et al., 2002b; Baynes and Riviere, 1998; van der Merwe and Riviere, 2005). In the latter study, an aqueous ethanol system almost doubled triazine absorption, but aqueous propylene glycol had minimal effect. There is limited data in the literature describing the dermal enhancement effects of TCE or related halogenated volatile organics. In one study, methylene chloride like acetone only changed the absorption profile of the very lipophilic dioxin (3,3',4,4'-tetrachlorobiphenyl, TCB) but not the extent of TCB absorption (Qiao and Riviere, 2000). Compared to many other solvent systems, its important to note that TCE and related halogenated chemicals tend to be more water-soluble (0.11%) than many other longer chain aliphatic solvents (Morgan et al., 1991), but they are less water-soluble than ethanol or acetone. This may explain why TCE as a topical vehicle in a predominantly aqueous system had little influence on the dermal absorption of triazine which is a very polar substance. The latter is important to this study

as aqueous systems were evaluated to mimic the work-place exposure scenario and not neat solvent systems as often is the case in many experimental studies reported in the literature.

Skin pre-treatment with TCE significantly increased triazine permeability and absorption in mineral oil-based and PEG-based cutting fluids containing contaminants. As TCE pre-treatment significantly influenced triazine permeability in these mixtures, it is plausible to assume a TCE-induced modulation of triazine partitioning behavior in skin. Triazine ditribution into the dosed skin area showed the same trend (Figure 2), which supports the partitioning theory described above. The stratum corneum data suggest similar trend with cutting fluids but an almost opposite trend when the contaminants were present in the cutting fluids. The latter is more likely a reflection of what remained in the stratum corneum after most of the available traizine had permeated into the viable epidermis and into the perfusate. In this case, TCE pre-treatment enhanced triazine diffusion in the presence pf cutting fluids contaminated with nickel and NDELA, with the latter components playing a major role in triazine diffusion as previously reported in our laboratory (Baynes et al., 2005a).

A further surprising observation was that these contaminants significantly enhanced TCE absorption (3-10-fold) in TCE topical mixtures but not in TCE pre-treated skin. Cutting fluids with or without contaminants significantly enhanced TCE absorption by as much as (3-10 fold) suggesting that the cutting fluid additives by themselves are potent enhancers of not only triazine absorption as previously reported (Baynes et al., 2003) but could be so for more lipophilic substances such as TCE.

In comparing across triazine and TCE flux profiles (Figures 1 and 4), triazine absorption was greater in mineral oil-based mixtures than PEG, while TCE absorption was greater in PEG than in mineral oil mixtures. It is very likely that aqueous base solutions influenced partitioning behavior of these two physicochemically diverse diffusing solutes. For example, TCE is more lipophilic than triazine, and therefore will more readily partition from a PEG-based mixture than from a mineral oil-based mixture, and vice-versa for triazine. Comparison of flux profiles of both solutes indicate that the triazine flux peak was about 50 minutes before TCE Peak flux irrespective of the aqueous base mixture. Although triazine is more polar than TCE, the simultaneous presence of this lipophilic chemical (TCE) in the dosing solution was not sufficient to significantly influence the flux profile of triazine.

Conclusions:

This work focused on understanding how cutting fluid additives, contaminants, and metal-working cleanser can influence the dermal disposition of potential skin irritants. Three additives (LAS, RA, and triazine) were used in the initial phases of this research to answer these questions and because triazine was shown to be more readily absorbed across skin, it was used as a chemical marker to assess cutting fluid contaminant and cuuting fluid cleanser effects on dermal absorption.

LAS absorption was limited to less than 0.5% dose and the additives in various combinations influenced the physicochemical characteristics of the dosing mixture. LAS was more likely to partition into the stratum corneum (SC) in mineral oil mixtures, and LAS absorption was significantly greater in the complete cutting fluid mixture. Triazine enhanced LAS transport, and the presence of SRA decreased LAS critical micelle concentration (CMC) which reduced LAS monomers available for transport. TEA increased mixture viscosity, and this may have negated the apparent enhancing properties of TRI in several mixtures

Physicochemical interactions which influenced ricinoleic acid partitioning into the stratum corneum modulated ***ricinoleic acid*** diffusion as evidenced by reduced permeability as the mixture became more complex. Although *in vitro* diffusion may differ from *in vivo* diffusion in human skin, physicochemical interactions between ricinoleic acid and cutting additives appear to play a significant role in membrane diffusion. The human health implications here are that the more complex the mixture, the less able ricinoleic acid is to partition and diffuse across skin. This can result in greater retention of these potentially irritant fatty acids in the upper epidermis which will eventually penetrate into the viable epidermis and absorbed into the blood stream.

Several of the additives did not enhance ***triazine*** partitioning into the stratum corneum, but significantly enhanced the apparent permeability of triazine in both membrane systems especially in PEG. The significant enhancer effects in silastic membrane as well as increased deposition in the stratum corneum and skin, especially with PEG mixtures, is suggestive of a significant chemical mechanism associated with these apparent triazine-additive interactions. The subsequent promotion of apparent triazine permeability in skin may be related to these interactions as well as bio-membrane alterations that are often manifested as acute irritant dermatitis in metal machine workers.

Contaminants Effects: Dermal absorption of the irritant biocide, triazine appears to be greater in soluble oil cutting fluids than in synthetic cutting fluids. This effect can be more pronounced when cutting fluids are contaminated with nitrosamines and leached metal ions. These contaminants by themselves did not significantly increase triazine absorption, but can significantly increase its deposition into the skin surface, stratum corneum and viable epidermis. More long-term exposure studies are required to determine if these mixture interactions are consistent beyond an 8-hour exposure, and whether this is related to an enhanced irritant response in workers simultaneously exposed to cutting fluids containing biocides and contaminants.

TCE Cleanser effects: This study demonstrated that potential chronic exposure to solvents such as TCE could have a more significant effect on the permeation of water-soluble irritants (e.g., triazine) than the simultaneous topical exposure to the solvent. This study only evaluated a 4-day exposure to the cleanser, but workers could be repeatedly exposed to this or related solvents during the cleaning of the finished metal products and this could result in a more severely impaired epidermal barrier. This study demonstrated in our 8-hour perfusion studies that an almost 2-fold increase in permeability is possible. This does not account for significant increase in deposition of these and other irritants into the viable epidermis to illicit an irritant or contact dermatitis. This therefore may be sufficient to be an occupational concern as the worker has already compromised the epidermal barrier of the skin to not only biocided such as triazine but also other water soluble toxicants (e.g., NDELA) that may be result in more harmful systemic effects.

In ***summary***, these cutting fluid mixture studies demonstrated that cutting fluid additives and contaminants had a significant effect on solute permeability in skin. The inert membrane studies strongly suggested that physicochemical interactions contribute significantly to solute permeability in skin.

Future Physicochemical Research: Preliminary membrane coated fiber (MCF) studies have demonstrated that the ***inert*** MCF can discriminate between different solutes and solute clusters. For this reason, future work will utilize a diverse series of MCFs with varying membrane properties to characterize physicochemical interactions associated with the presence of cutting fluid additives in cutting fluid formulations/mixtures. The MCF technique has the

added advantage of probing these interactions within a linear solvation energy relationship (LSER) framework without confounding interactions present in the biological membrane system. Finally, this proposed LSER framework allows for comparison of solute diffusion as well as mixture effects on solute diffusion in the MCF and the skin membrane systems. The differences in ***interaction coefficients*** between these membrane systems will ***identify*** and ***quantify*** unique physicochemical properties that influence dermal absorption of solutes in a defined cutting formulation.

Finally, further work is needed to better characterize the mechanisms influencing these interactions, as these interactions can be modified at the formulation step if it is determined that it predisposes dermal deposition of one or more of these occupational irritants.

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