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Physicochemical determinants of linear alkylbenzene sulfonate (LAS) disposition in skin exposed to aqueous cutting fluid mixtures

Ronald E Baynes, James D Brooks, Beth M Barlow and Jim E Riviere

Center for Chemical Toxicology Research and Pharmacokinetics (CCTRP), College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606, USA

Linear alkylbenzene sulfonate (LAS) is added to cutting fluid formulations to enhance the performance of metal machining operations, but this surfactant can cause contact dermatitis in workers involved in these operations. The purpose of this study was to determine how cutting fluid additives influence dermal disposition of ^{14}C-LAS in mineral oil- or polyethylene glycol 200 (PEG)-based mixtures when topically applied to silastic membranes and porcine skin in an *in vitro* flow-through diffusion cell system. ^{14}C-LAS mixtures were formulated with three commonly used cutting fluid additives; 0 or 2% triazine (TRI), 0 or 5% triethanolamine (TEA), and 0 or 5% sulfurized ricinoleic acid (SRA). LAS absorption was limited to less than a 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 mixture. TRI 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. In summary, physicochemical interactions in these mixtures influenced availability of LAS for absorption and distribution in skin, and could ultimately influence toxicological responses in skin. *Toxicology and Industrial Health* 2002; **18:** 237–248.

Key words: *cutting fluids; LAS; mixtures; physicochemical interactions; skin*

Introduction

Surfactants are frequently incorporated in agrochemical and industrial formulations to enhance delivery and/or performance of the active ingredients in the commercial formulation. They however have been associated with many cases of occupational contact irritant dermatitis (Mathias, 1986; Palmer *et al*., 1975; Mathur and Shanker, 2001), and various studies have demonstrated these irritant effects in various animal models (Wilhelm *et al*., 1991; Spoo *et al*., 1991). The model anionic surfactant, sodium lauryl sulfate (SLS), can be cytotoxic at its critical micelle concentration (CMC = 8 mM or 0.24%) (Wilhelm *et al*., 1991; Bloom *et al*., 1994), and it is not surprising to observe dermatitis where surfactants such as SLS are routinely used at concentrations much greater than the CMC. Earlier studies have demonstrated that 24-hour exposure to 5% SLS or 5% linear alkylbenzene sulfonate (LAS) can cause necrolysis of the epidermis (Gisslen and Magnusson, 1966). However, very little is known about surfactant

Address all correspondence to: Ronald E Baynes, College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606, USA
E-mail: Ronald_Baynes@ncsu.edu

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disposition in skin when simultaneously exposed to a chemical mixture or a complex formulation.

Although anionic surfactants are not readily absorbed into the blood stream following topical exposure, they can penetrate various skin layers (Patil *et al*., 1995) and can influence dermal absorption of other chemicals (Baynes and Riviere, 1998; Riviere *et al*., 2001). There is, however, limited information on whether one or more formulation additives can influence surfactant absorption and distribution in skin. In this study, we hypothesized that inert ingredients in cutting fluid formulations influence surfactant disposition in skin. Toxicologically, these interactions in the various compartments of the skin can ultimately modulate inflammation and skin sensitization in occupationally exposed individuals.

Cutting fluid has been incriminated as a major cause of occupational contact irritation dermatitis amongst workers in the metalworking industry (Koh *et al*., 2001), and their harmful effects on skin have been reported (Al-Humadi *et al*., 2000; Shvedova *et al*., 2001). As it is very difficult to assess all of these commercial formulations, our approach utilized biological and inert membranes of varying complexity to mimic worker exposure to various mixtures of LAS, and evaluate the effects of major formulation additives on LAS disposition in skin using a factorial experimental design. This approach allows one to identify which cutting fluid additive or group of additives has a significant effect on LAS disposition in skin.

The small number of cutting fluids additives selected for testing were representative of major constituents in most aqueous cutting fluids (Childers, 1994). These usually include a biocide (e.g., triazine, TRI), a fatty acid performance lubricant (e.g., sulfurized ricinoleic acid, SRA), and a corrosive inhibitor (e.g., triethanolamine, TEA). Many of the cutting fluids used in the metalworking 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 (5%) or polyethylene glycol 200 (PEG) (5%) to mimic this exposure.

LAS transport was evaluated in *in vitro* porcine skin sections as porcine skin is similar anatomically and biochemically to human skin. Absorption was also assessed in an inert silastic membrane to evaluate chemical interactions that can influence LAS transport processes from the skin surface. This inert membrane is analogous to the stratum corneum (SC) in skin as it is a homogenous lipid matrix, but not biologically reactive to irritant effects of the additives or LAS. Physicochemical studies were also performed to characterize surface chemical interactions that may be modulating LAS disposition. Finally, LAS disposition in simple (binary) and complete mixtures was also assessed in isolated perfused porcine skin flaps as this skin model has an intact microvasculature, is anatomically similar to human skin, and is predictive of *in vivo* human percutaneous absorption (Monteiro-Riviere, 1991; Wester *et al*., 1998). Interactions identified from this model system should closely reflect LAS disposition in skin in an *in vivo* occupational scenario.

Methods

Chemicals

Radiolabeled LAS (sodium 2-dodecylbenzene sulfonate-ring-UL-^{14}C) (specific activity = 50.77 mCi/mmol) was obtained from Wizard Laboratories (West Sacramento, CA, USA). Radiochemical purity was 99.12%. TRI was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). SRA, TEA and mineral oil (MO), were obtained from Sigma Chemical Company (St. Louis, MO, USA), and PEG, average M.W. 200, was obtained from Across Organics (New Jersey, USA). ^{14}C-LAS was dissolved in water, and was used to prepare the surrogate cutting fluid mixtures summarized in Table 1.

Physicochemical studies

Solubility and CMC determinations

LAS solubility was determined by preparing four standards and eight unknown (saturated and unsaturated) concentrations (Table 1) and tested using a Corning Conductivity Meter 441 (Corning Inc. – Corning, NY, USA). The concentration was determined by plotting a best-fit line ($R^2 > 0.99$) 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

Table 1. LAS mixtures prepared in water and either 5% mineral oil or 5% PEG for physiochemical and diffusion studies

1-Component mixture	2-Component mixtures	3-Component mixtures	4-Component mixture
LAS[1]	LAS+SRA	LAS+SRA+TRI	LAS+SRA+TRI+TEA[1]
	LAS+TRI	LAS+SRA+TEA	
	LAS+TEA	LAS+TRI+TEA	

PEG = polyethylene glycol 200; SRA = 5% sulfated ricinoleic acid, TRI = 2% triazine; TEA = 5% triethanolamine.
[1] Mixtures tested in porcine skin flap.

conductivity was determined to be the solubility point. The solubility value was determined using the slope of the standards. The CMC was also determined with the aid of a conductivity meter. Briefly, concentrations of LAS (mmol) 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 versus 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 four to five determinations.

Viscosity and pH determinations

LAS 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 five minutes at 25°C. Using an Excel spread sheet, the time points and viscosity (Pa) 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.

SClvehicle partition coefficient (PC) determination

SC/vehicle PC were determined according to methods previously described by Baynes *et al*. (2000). In brief, SC 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 mixtures (Table 1) with ^{14}C-LAS 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, 10 µL of the vehicle was removed for direct counts using Ecolume (ICN Costa Mesa, CA, USA). The SC sample was removed, gently blotted to remove excess solution and then analysed as described below.

Flow through diffusion cell experiments

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, USA). Silastic membranes (250 µm) were obtained from Dow Corning, Corporation (Midland, MI, USA). 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 µg/cm^2) 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, USA), and the pH was maintained between 7.3 and 7.5. Perfusate flow rate was 4.0 mL/hour, 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 one to two), taped-stripped six times (tape one to six) with cellophane tape to determine SC 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)

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% O_2 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 μg/cm^2 ^{14}C-LAS 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 two hours post-dosing and then at one hour intervals for a total of eight 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 eight-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 15 mL aliquots of soluene for 48 hours at 40°C before further analyses as described below.

Chemical analysis

For determination of ^{14}C-LAS, perfusate, swabs, dose skin, and SC samples were combusted in a Packard Model 306 Tissue Oxidizer (Packard Chemical Co., Downers Grove, IL, USA) and then analyzed by Packard Model 1900TR Liquid Scintillation Counter (Packard Chemical Co., Downers Grove, IL, USA) for total ^{14}C determination.

Calculations and statistics

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

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

LAS flux was determined from the apparent steady state slope derived from a plot of cumulative LAS versus time as depicted in Figure 1. *Tissue disposition* parameters such as surface, SC, and dosed skin were described above. For *PC* determinations, radioactivity content in the vehicle mixture and SC were normalized to 1000 mg vehicle (C_{vehicle}) and 1000 mg SC (C_{sc}), respectively. The log SC/vehicle PC was determined from the equation:

$$\text{Log PC} = \log C_{\text{SC}}/C_{\text{vehicle.}}$$

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

Results

Physicochemical interactions

Figure 2 depicts the physicochemical characteristics of the eight mixtures evaluated in the diffusion

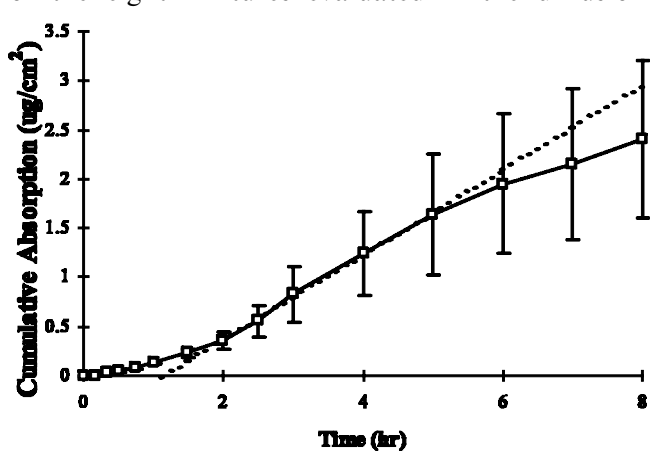

Figure 1. Plot of cumulative absorption of LAS (μg/cm^2) in complete mineral-oil based mixture. The open squares represent the average of 4–5 determinations, and the broken line represents the best straight line at an apparent steady state.

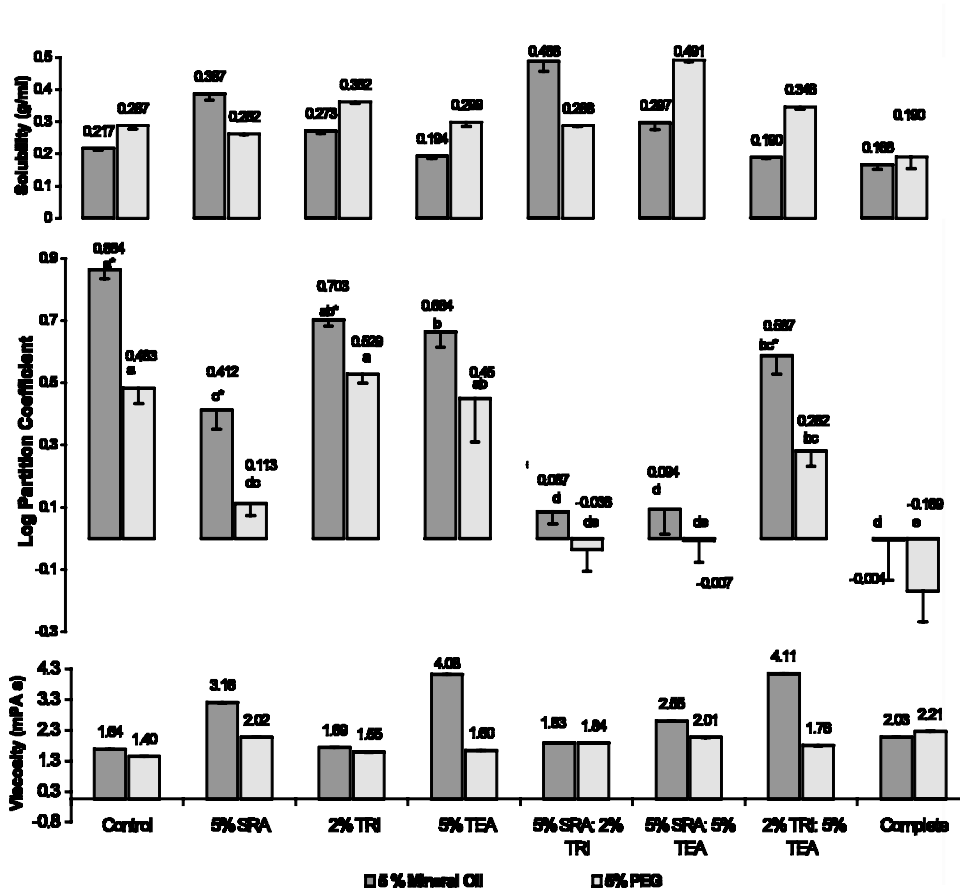

Figure 2. Influence of cutting fluid additives on the physicochemical characteristics of surrogate mineral oil-based and PEG-based cutting fluid formulations. Control refers to LAS only and no other additive present in the mixture. Means with different letters represent significant differences between treatments within a mineral oil- or PEG-based mixture ($P < 0.05$). * indicates significant differences between mineral oil- and PEG-based mixtures for each treatment.

studies. Viscosity ranged from 1.4×10^{-3} to 4.11×10^{-3} mPaS, 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 (Table 2). 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 was 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 to 11.23 for other cutting fluid mixtures. LAS solubility was greater in PEG than in mineral oil for six/eight mixtures, and solubility ranged from 27 to 49%. SRA or SRA+TRI increased LAS solubility about twofold in mineral oil, while SRA+TEA increased LAS solubility almost twofold in PEG. For most of the cutting fluid mixtures (seven/eight), LAS was more likely to partition into the SC with mineral oil mixtures,

Table 2. CMC for LAS in PEG-based cutting fluid surrogate mixtures ($n = 4–5$)

Mixtures	CMC ± SE (g/100 mL)
Water[1]	0.14 ± 0.01^{c}
Control	0.30 ± 0.01^{a}
5% SRA	0.02 ± 0.01^{e}
2% TRI	0.16 ± 0.01^{c}
5% TEA	0.13 ± 0.01^{c}
5% SRA+2% TRI	0.06 ± 0.01^{d}
5% TEA+2% TRI	0.32 ± 0.03^{a}
Complete	0.20 ± 0.01^{b}

[1]Water mixture did not contain PEG. TRI = triazine; TEA = triethanolamine; SRA = sulfated ricinoleic acid; complete = all additives present; control = only LAS present.
Means with different letters represent significant differences between treatments ($P < 0.05$).

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 (Figure 3) never exceeded 0.32% dose during these eight-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, and a similar trend was observed in the silastic membrane and skin flap exposure studies (Figure 3), although the differences were not significant in the skin flap (Figure 4). 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 twofold 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.

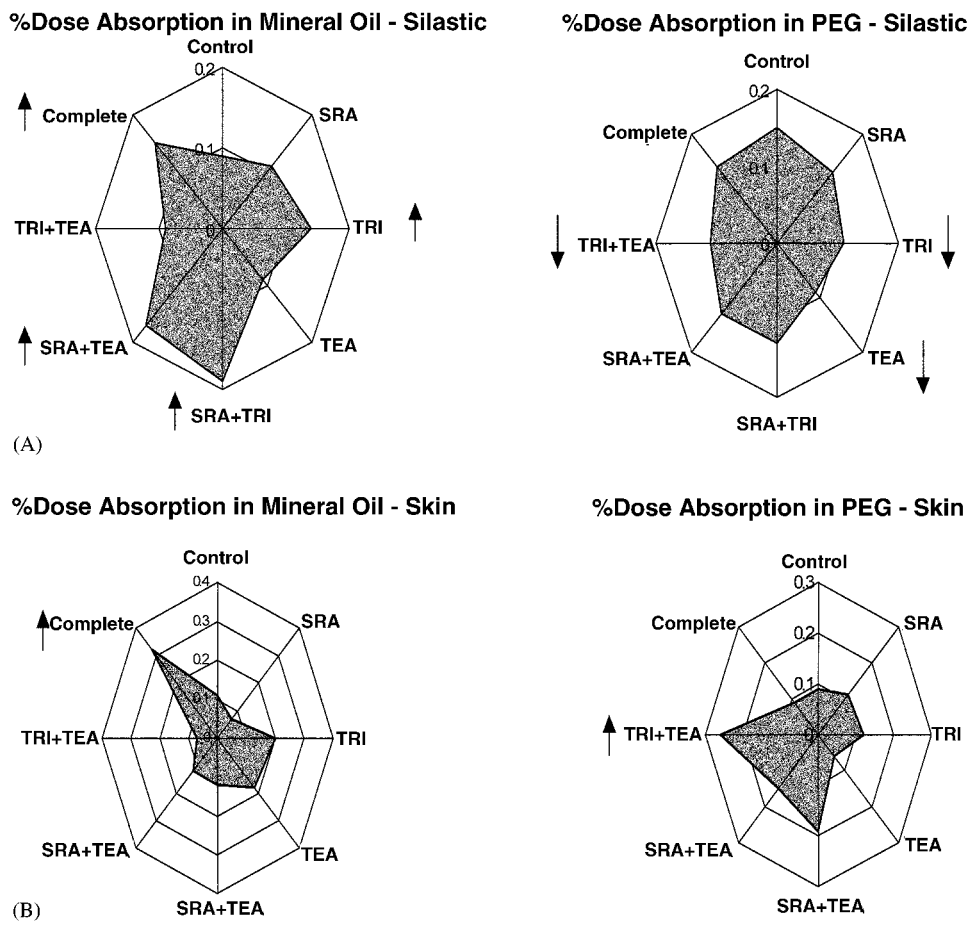

Figure 3. Compass plots depicting LAS absorption (% dose) in mineral oil-based and PEG-based mixtures in flow-through diffusion cell system using (A) silastic membranes and (B) porcine skin sections. Control refers to LAS only and no other additive present in the mixture. Arrows indicate significant increase or decrease in absorption when compared to the control mixture ($P < 0.05$).

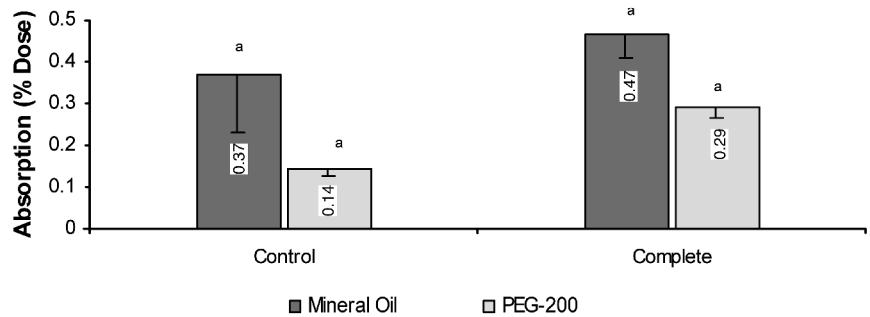

Figure 4. LAS absorption (% dose) in mineral oil-based and PEG-based mixtures in porcine skin flaps. Control refers to LAS only and no other additive present in the mixture. Means with the same letters represent no significant differences between treatments within a mineral oil- or PEG-based mixture ($P > 0.05$).

Table 3. Permeability of ^{14}C-LAS dosed in mineral oil- or PEG-200-based mixtures in silastic membranes and porcine skin sections ($n = 4$–5)

	Permeability ± SE (cm/hour × 10^{-5})	Permeability ± SE (cm/hour × 10^{-5})
Mixtures[1]	Mineral oil	PEG-200
Silastic membrane		
Control	1.40 ± 0.16^{b}	1.62 ± 0.33^{a}
TRI	1.24 ± 0.12^{bc2}	0.79 ± 0.09^{cd}
TEA	1.11 ± 0.15^{bcd2}	0.62 ± 0.07^{d}
SRA	0.69 ± 0.14^{d}	1.45 ± 0.15^{ab2}
TRI+TEA	0.77 ± 0.14^{cd}	1.02 ± 0.08^{bcd}
TRI+SRA	1.08 ± 0.01^{bcd}	0.88 ± 0.19^{cd}
TEA+SRA	1.90 ± 0.01^{a2}	1.16 ± 0.03^{abc}
Complete	1.90 ± 0.23^{a}	1.24 ± 0.15^{abc}
Porcine skin		
Control	0.59 ± 0.10^{cb}	1.44 ± 0.19^{a2}
TRI	1.52 ± 0.49^{a}	1.27 ± 0.46^{a}
TEA	0.66 ± 0.21^{cb}	0.47 ± 0.03^{b}
SRA	0.59 ± 0.13^{cb}	0.54 ± 0.07^{b}
TRI+TEA	0.30 ± 0.02^{c}	1.40 ± 0.32^{a2}
TRI+SRA	0.73 ± 0.14^{cb}	0.86 ± 0.06^{ab}
TEA+SRA	0.58 ± 0.06^{cb}	0.42 ± 0.08^{b}
Complete	0.87 ± 0.16^{b}	0.52 ± 0.05^{b}

[1] TRI = triazine; TEA = triethanolamine; SRA = sulfated ricinoleic acid; complete = all addtives present; contro 1 = only LAS present. Means with different letters represent significant differences between treatments ($P < 0.05$).
[2] Significant differences between mineral oil and PEG mixtures. Means with different letters represent significant differences between treatments ($P < 0.05$).

LAS deposition in skin layers

Figure 5A and B depicts distribution of LAS into the dose skin, SC, and retention on the skin surface. LAS deposition in these skin compartments decreased in the following order: surface > SC > dosed skin. In mineral oil mixtures, surface levels ranged from 70.6% dose for the complete mixture to 27.9% dose for the control mixture, however, LAS levels were significantly greater ($P < 0.05$) in the SC 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 SC 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 46.7% dose to 73.4% 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 (Figure 5C). The distribution pattern was different when compared to porcine skin sections; i.e., 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.

Discussion

LAS absorption

LAS absorption ($< 0.5\%$ dose) and permeability ($< 1.6 \times 10^{-6}$ cm/hour) in these diffusion experiments was relatively small. The data from our *in*

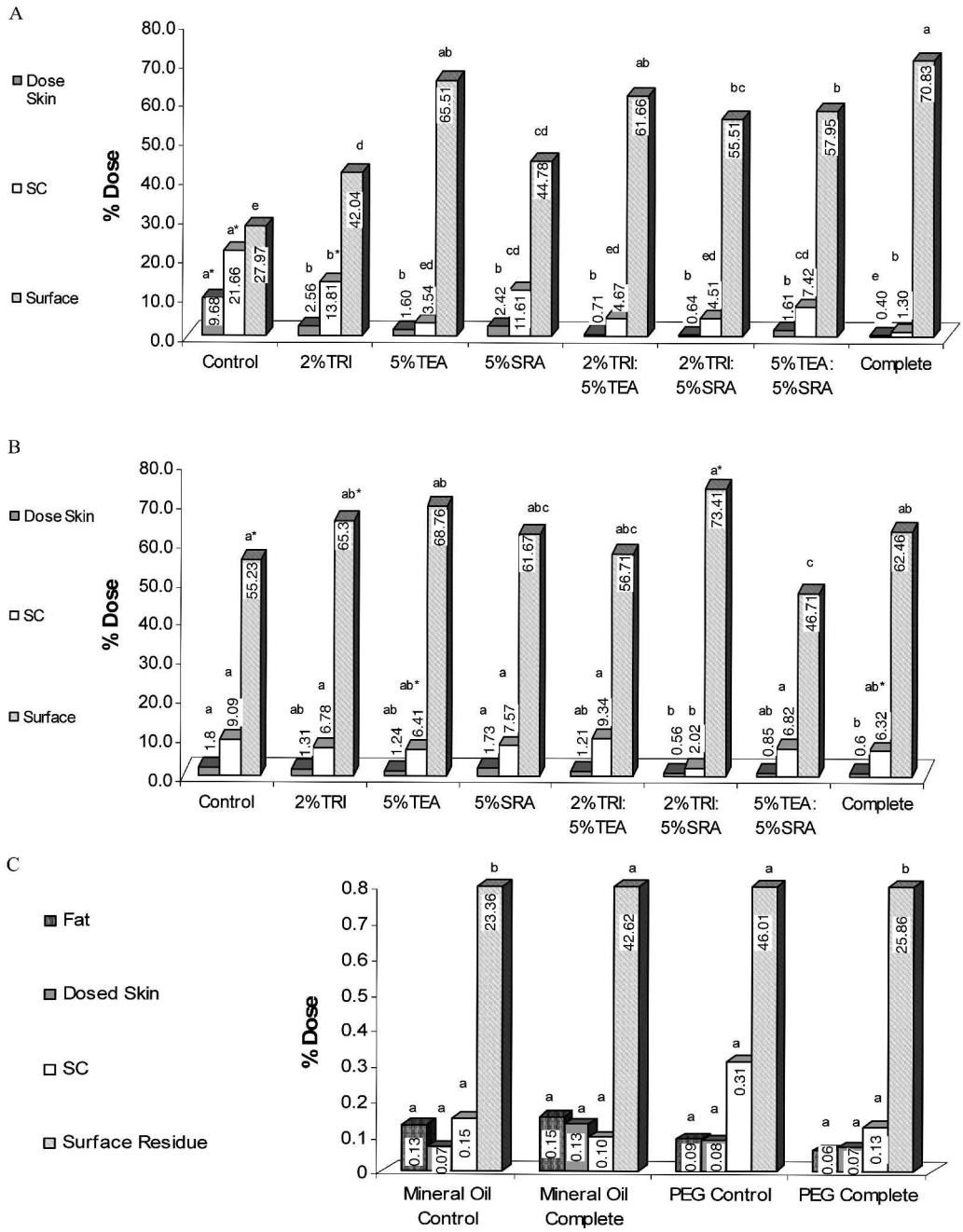

Figure 5. LAS distribution (% dose) on skin surface, SC, and dose skin (epidermis + dermis) in (A) porcine skin sections dosed with mineral oil-based mixtures, (B) porcine skin sections dosed with PEG-based mixtures, and (C) perfused porcine skin flap dosed with either mineral oil- or PEG-based mixtures. Control refers to LAS only and no other additive present in the mixture. Means with different letters represent significant differences between treatments within a mineral oil- or PEG-based mixture ($P < 0.05$). * indicates significant differences between mineral oil- and PEG-based mixtures for each treatment.

vitro porcine skin and *ex vivo* porcine skin flap studies are, however, similar to those from previous *in vivo* studies in rats, rabbits, guinea pigs, and human (Drotman, 1977; Hasegawa and Sato, 1978). Furthermore, LAS permeability in human epidermis was 6.0×10^{-6} cm/hour after a six or 24 hour exposure (Howes, 1975), a value comparable to that seen in the present porcine skin studies.

The limited LAS permeability in skin 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 SLS can form micelles or spherical aggregates above the 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. It is also worth noting that as alkyl chain length increases from 12 to 15, the CMC for LAS also decreases (Ohki and Tokiwa, 1970). The presence of a fatty acid chain in the form of SRA could have resulted in the 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. This decrease in CMC infers that fewer LAS molecules or monomers in the aqueous phase of the mixture are available for diffusion, resulting in less absorption. LAS and many other anionic surfactants are also known to have a high affinity for SC proteins (keratin) in the skin, and this chemical–biological interaction has also been blamed for the decrease in surfactant absorption in skin (Faucher and Goddard, 1978). There is evidence that these anionic surfactants enhance membrane permeability by interaction with membrane lipids, but only recent studies have demonstrated that it's the monomer and not the micelle that is responsible for this interaction (Xia and Onyuksel, 2000).

Compared to LAS, SLS, has a smaller molecular weight (288.38) than LAS (348.48), and therefore is more permeable than LAS. SLS permeability in excised rat skin (1.4×10^{-2} cm/hour) (Patil *et al*., 1995) was as expected, greater than that in human epidermis (2.89×10^{-3}–1.1×10^{-4} cm/hour) or rat SC (0.7×10^{-4} cm/hour) (Howes, 1975; Effendy *et al*., 1996), and SLS absorption in human skin *in vitro* after a 24-hour exposure was as much as 3.22% dose (Effendy *et al*., 1996). These longer *in vitro* permeability studies, however, run the risk of surfactant-induced skin barrier changes, which can result in greater permeability.

Our physicochemical studies demonstrated that LAS is more likely to partition from the topical mixture into the SC with mineral oil-based mixtures than with PEG-based mixtures. This is consistent with the fact that LAS was more soluble in PEG mixtures, and therefore more likely to remain in the more hydrophilic PEG formulation rather than partition into the SC. The water-soluble LAS monomers would rather partition from a more lipophilic formulation containing mineral oil into the SC and viable epidermis. Similar work in our laboratory demonstrated a comparable partitioning behavior for PCP in the SC for aqueous and nonaqueous mixtures (Baynes *et al*., 2002), and this inverse relationship between chemical solubility and permeability has also been described for several pharmaceuticals (Kikwai *et al*., 2002).

LAS absorption in mineral oil-based mixtures was significantly greater in complete cutting fluid mixtures (LAS+TRE+TRI+SRA) when compared to the control mixture (LAS only), and this mixture effect was the trend in the three membrane model systems used in this study. However, LAS was least likely to partition into the SC in these complete mixtures when compared to the control mixture. This may suggest that the complete mixture effect on absorption may not be driven entirely by chemical partitioning behavior and/or simply that the SC better retains LAS in simple aqueous systems thereby reducing dermal absorption.

Although LAS is negatively charged in an aqueous solution, the presence of other additives, such as triethylanolamine and/or TRI, could have contributed protons making these monomers neutral and more likely to diffuse across the membrane. The effect of formulation pH on the permeability of weak acids and bases has been reported in the literature (Gorukanti *et al*., 1999). The silastic membrane data suggests a possible chemical interaction between LAS and TRI 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. TRI is classified as a category I skin irritant, and obviously has a high corrosive activity with a pH of 11 at 5% solution (EPA, 1997). Although TRI has a low log PC (0.11), it is plausible to assume from its corrosive behavior that it would modulate LAS diffusion in several mixtures. However, this mechanism may not

be strictly chemical–biological in nature, and this may be modulated in the presence of SRA and TEA. 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 (Chidambaram and Burgess, 2000). This chemical interaction more likely promoted micelle formation which in turn reduced accessibility of the surfactant for binding sites to the SC (Paye and Pierard, 2000).

LAS distribution in skin

Our physicochemical analyses of these mixtures predicted that most of the applied LAS dose would not readily penetrate skin, but rather remain in the dosing solution as the mixtures became more complex. However, as described earlier, this did not necessarily result in decreased LAS permeability and absorption with complete mineral oil mixtures. However, it was not surprising that most of the applied dose remained on the skin surface after an eight-hour exposure and even less in the SC and dosed skin, which consist of viable epidermis and some dermis. Other studies, however, demonstrated the opposite trend with SLS, i.e., greater levels of SLS were observed in the dermis and viable epidermis than the SC or surface, although this was after a 24- or 48-hour exposure (Fullerton *et al*., 1994; Patil *et al*., 1995; Effendy *et al*., 1996). We were also able to demonstrate in both the *in vitro* model and *ex vivo* skin flap model that mineral oil was more effective than PEG to retain LAS on the surface for complete mixtures, while this was reversed for the control mixture. However, a greater percentage of LAS was distributed to the deeper tissues with the *in vitro* diffusion cell model than with the *ex vivo* skin flap model, which closely mimics the *in vivo* scenario. As has been previously proposed by Patil *et al*. (1995), the *in vitro* model does not have micro-capillaries like the *in vivo* model or in our case, the perfused skin flap model that can clear the surfactant, and therefore more of the surfactant will be retained in the deeper skin tissues of the *in vitro* model.

The distribution gradient between the surface, SC, and dose skin for the control mixture (LAS only) in mineral oil was not as significant as for other mineral oil or PEG mixtures. This suggests that the presence of these cutting fluid additives reduced the availability of LAS monomers for distribution to other skin layers. The presence of TEA, which was shown to significantly increase mixture viscosity, should be considered the principal contributor to this increased surface retention of LAS. It should also be noted that whereas as much as 9.7% remained in the dosed skin from control mixture, only 0.4–2.6% dose remained in the skin for all other mixtures. This suggests a greater absorption potential for the LAS control mixture beyond eight hours and/or that the presence of several of the other additives encouraged partitioning from these tissues into the perfusate as indicated from our absorption and permeability data for the complete mixture in mineral oil. Although TRI, like TEA, did not affect LAS partitioning into the SC when compared to control, this additive appears to have played a significant role in enhancing LAS levels in silastic membrane and porcine skin. TRI has a relatively small molecular weight, it is hydrophilic, and it could have acted as a co-solvent in these aqueous mixtures. As Wilhelm *et al*. (1994) demonstrated SLS-enhanced absorption for several hydrophilic drugs, it is possible that LAS enhanced TRI penetration of the SC could have triggered membrane changes that in turn also influenced LAS absorption.

In summary, the dermal absorption and distribution of LAS in these mixtures is influenced by the physicochemical characteristics of the mixture. Using various membrane diffusion models to probe for underlying chemical and biological interactions,

we were able to isolate and identify the potential influence of TRI, SRA, and TEA on LAS disposition in skin in a complex mixture system that may be of an occupational concern in the metalworking industry. 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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References

Al-Humadi, N.H., Shvedova, A.A., Batteli, L., Diotte, N., Castranova, V. and Kommineni, C. 2000: Dermal and systemic toxicity after application of semisynthetic metalworking fluids in B6C3F1 mice. *Journal of Toxicology and Environmental Health A* 61, 579–89.

Baynes, R.E. and Riviere, J.E. 1998: Influence of inert ingredients in pesticide formulations on dermal absorption of carbaryl. *American Journal of Veterinary Research* 59, 168–75.

Baynes, R.E., Brooks, J.D. and Riviere, J.E. 2000: Membrane transport of naphthalene and dodecane in jet fuel mixtures. *Toxicology and Industrial Health.* 16, 225–38.

Baynes, R.E., Brooks, J.D., Mumtaz, M. and Riviere, J.E. 2002: Effect of chemical interactions in pentachlorophenol mixtures on skin and membrane transport. *Toxicological Science* 69, 295–305.

Bloom, E., Sznitowska, M., Polansky, J., Ma, Z.D. and Maibach, H.I. 1994: Increased proliferation of skin cells by sublethal doses of sodium lauryl sulfate. *Dermatology* 188, 263–68.

Bronaugh, R.L. and Stewart, R.F. Methods for *in vitro* percutaneous absorption studies II. The flow-through diffusion cell. *Journal of Pharmaceutical Science* 74, 64–67.

Chidambaram, N. and Burgess, D.J. 2000: Effect of nonionic surfactant on transport of surface-active and non-surface-active model drugs and emulsion stability in triphasic systems. *AAPS Pharmscience* 2, 1–11.

Childers, J.C. 1994: The chemistry of metalworking fluids. In Beyers, J.E., editor, *Metalworking fluids*. New York, NY: Marcel Dekker, 165–89.

Cooper, E.R., Merritt, E.W. and Smith, R. 1985: Effect of fatty acids and alcohols on the penetration of acyclovir across human skin *in vitro*. *Journal of Pharmacological Science* 74, 688–89.

Drotman, R. 1977: Metabolism of cutaneously applied surfactants. In Drill, V.A. and Lazar P., editors, *Cutaneous toxicity*. New York, NY: Academic Press, 95–109.

Effendy, I., Weltfriend, S., Kwangsukstith, C., Singh, P. and Maibach, H.I. 1996: Effects of all-trans retinoic acid and sodium lauryl sulphate on the permeability of human skin *in vitro*. *British Journal of Dermatology* 135, 428–32.

EHC. 1996: *Linear alkylbenzene sulfonates and related compounds*, Environmental Health Criteria 169. Geneva: World Health Organization, 1996.

EPA. 1997: *Reregistration eligibility decision. 1,3,5-Triethylhexahydro-s-triazine*, United States Environmental Protection Agency. EPA-738-R-97-004.

Faucher, J.A. and Goddard, E.D. 1978: Interaction of keratinous substances with sodium lauryl sulfate: II permeation through stratum corneum. *Journal of the Society of Cosmetic Chemists* 29, 339–52.

Fisher Scientific Accumet AR10 pH meter, Vernon Hills, IL, USA.

Fullerton, A., Broby-Johansen, U. and Agner, T. 1994: Sodium lauryl sulfate penetration in an in vitro model using human skin. *Contact Dermatitis* 30, 222–25.

Gisslen, H. and Magnusson, B. 1966: Effects of detergents on guinea pig skin. *Acta Dermato-Venereologica* 46, 269–74.

Gorukanti, S.R., Li, L. and Kim, K. 1999: Transdermal delivery of antiparkinsonian agent, benztropine. I. Effects of vehicles on skin permeation. *International Journal of Pharmacology* 192, 159–72.

Hasegawa, H. and Sato, M.P. 1978: Permeation of LAS though rat skin. In *Tokyo, science and technology agency*. Tokyo: Research Coordination Bureau, 172–75.

Howes, D. 1975: The percutaneous absorption of some anionic surfactants. *Journal of the Society of Cosmetic Chemistry* 26, 47–63.

Kikwai, L, Kanikkannan, N, Babu, R and Singh, M. 2002: Effect of vehicles on the transdermal delivery of melatonin across porcine skin in vitro. *Journal of Controlled Release* 83, 307–11.

Koh, D., Leow, Y.H. and Goh, C.L. 2001: Occupational allergic contact dermatitis in Singapore. *The Science of the Total Environment* 270, 97–101.

Mathias, C.G.T. 1986: Contact dermatitis from use or misuse of soaps, detergents, and cleansers in the workplace. *Occupational Medicine: State of the Art Reviews* 1, 205–18.

Mathur, A.K. and Shanker, R. 2001: Dermal toxicity of linear alkylbenzene sulphonate and nickel in guinea pigs. *Journal of Toxicology – Cutaneous and Ocular Toxicology* 20, 23–27.

Monteiro-Riviere, N.A. 1991: Comparative anatomy, physiology, and biochemistry of mammalian skin. In Hobson, D.W., editor, *Dermal and ocular toxicology: fundamentals and methods*. Boca Raton, Fl: CRC Press, 3–71.

Ohki, K. and Tokiwa, F. 1970: Physicochemical properties of alpha-olefin-sulfonate sodium salts. *Journal of the Chemical Society of Japan* 91, 534–39.

Palmer, A.K., Readshaw, M.A. and Neuff A.M. 1975: Assessment of the teratogenic potential of surfactants. Part III – dermal application of LAS and soap. *Toxicology* 4, 171–81.

Patil, S., Sing, P., Sarasour, K. and Maibach, H.I. 1995: Quantification of sodium lauryl sulfate into the skin and underlying tissues after topical application-pharmacological and toxicological implications *Journal of Pharmceutical Science* 84, 1240–44.

Paye, M. and Pierard, G.E. 2000: Skin care/detergents. In Gabard, B., Elsner, P., Surber, C. and Treffel, P., editors, *Dermatopharmcology of topical preparations*. New York, NY: Springer, 297–315.

Riviere, J.E., Bowman, K.F., Monteiro-Riviere, N.A., Dix, L.P. and Carver, M.P. 1986: The isolated perfused porcine skin flap (IPPSF). I. A novel in vitro model for percutaneous absorption and cutaneous toxicology studies. *Fundamental and Applied Toxicology* 7, 444–53.

Riviere, J.E., Qiao, G., Baynes, R.E., Brooks, J.D. and Mumtaz, M. 2001: Mixture component effects on the in vitro dermal absorption of pentachlorophenol. *Archives of Toxicology* 75, 329–34.

Shvedova, A.A., Kisin, E., Kisin, J., Castranova, V. and Kommineni, C. 2001: Elevated oxidative stress in skin of B6C3F1 mice affects dermal exposure to metal working fluid. *Toxicoloy and Industrial Health* 16, 267–76.

Spoo, J.W., Rogers, R.A. and Monteiro-Riviere, N.A. 1992: Effects of formaldehyde, DMSO, benzoyl peroxide, and sodium lauryl sulfate on isolated perfused porcine skin. *In Vitro Toxicology* 5, 251–60.

Wester, R.C., Melendres, J., Sedik, L., Maibach, H. and Riviere, J.E. 1998: Percutaneous absorption of salicylic acid, theophylline, 2,4-dimethylamine, diethyl hexyl phthalic acid, and p-aminobenzoic acid in the isolated perfused porcine skin flap compared to man in vivo. *Toxicology and Applied Pharmacology* 151, 159–65.

Wilhelm, K.P., Surber, C. and Maibach, H.I. 1991: Effects of sodium lauryl sulfate-induced skin irritation on *in vitro* percutaneous penetration of four drugs. *Journal of Investigative Dermatology* 96, 963–67.

Xia, W.J. and Onyuksel, H. 2000: Mechanistic studies on surfactant-induced membrane permeability enhancement. *Pharmacology Research* 17, 612–18.