Cutaneous and Ocular Toxicology, 25: 235–247, 2006

ISSN: 1556-9527 print/1556-9535 online
DOI: 10.1080/15569520601013137

informa
healthcare

DERMATOTOXICITY OF CUTTING FLUID MIXTURES: *IN VITRO* AND *IN VIVO* STUDIES

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Cutting fluids are widely used in the metal-machining industry to lubricate and reduce heat generation when metals are cut by a metal-cutting tool. These cutting fluids have caused occupational irritant contact dermatitis (OICD), and many of the additives used in these cutting fluid mixtures are thought to be responsible for OICD in workers. The purpose of this study was to assess single or various combinations of these additives in initiating the OICD response following an acute 8-hour exposure in porcine skin in vivo and in vitro using the isolated perfused porcine skin flap (IPPSF) and human epidermal keratinocytes (HEK). Pigs ($n = 4$) were exposed to 5% mineral oil (MO) or 5% polyethylene glycol (PEG) aqueous mixtures containing various combinations of 2% triazine (TRI), 5% triethanolamine (TEA), 5% linear alkylbenzene sulfonate (LAS), or 5% sulfurized ricinoleic acid (SRA). Erythema and edema were evaluated and skin biopsies for histopathology were obtained at 4 and 8 hours. IPPSFs ($n = 4$) were exposed to control MO or PEG mixtures and complete MO or PEG mixtures, and perfusate samples were collected hourly to determine interleukin- (IL-) 8 release. The only significant ($p < 0.05$) mixture effects observed in IPPSFs were with SRA + MO that caused an increase in IL-8 release after 1 or 2 hours' exposure. In vivo exposure to TRI alone appeared to increase erythema, edema, and dermal inflammation compared to the other additives, while SRA alone was least likely to initiate a dermal inflammatory response. In 2-component mixture exposures, the presence of TRI appeared to increase the dermal inflammatory response at 4 and 8 hours especially with the PEG mixtures. In the 3- and 4-component mixtures, MO mixtures are more likely to incite an inflammatory response than PEG mixtures. TRI exhibited the highest toxicity toward HEK, which correlates well to the in vivo irritation and morphology results. In summary, these preliminary studies suggest that the biocide, TRI, is the more potent of the 4 performance additives in causing dermal irritation, and this may vary depending on whether the worker is exposed to a synthetic (PEG)- or MO-based fluid. These findings will however require further clinical studies to validate these acute dermal effects as well as human cumulative irritation following exposure to similar cutting fluid formulations in the workplace.

Keywords: Alkylbenzene sulfonate; Cutting fluids; HEK; Mixtures; Ricinoleic acid; Triazine; Triethanolamine

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INTRODUCTION

Topical exposure to environmental chemicals is the primary route that xenobiotics use to gain access to the systemic circulation. Skin is a major organ and direct target for metal-working and industrial chemicals that can produce toxicological manifestations ranging from acute irritation to proliferation and tumor formation (1,2). Most studies have dealt with single chemical exposures, yet it is multiple chemical exposures that is the actual scenario in both the workplace and in the environment. Studies have shown that single chemical toxicity data are not predictive of the toxicity of complex mixtures (3). Cutting fluids are widely used in the metal-machining industry to lubricate and reduce heat generation when metals are cut by a metal-cutting tool. During the machinery process, the cutting fluid becomes contaminated with by-products that can act as irritants or may be potential carcinogens if systemically absorbed. Until very recent efforts (4–6), little was known about the dermal absorption and disposition of cutting fluid components and contaminants individually or as a mixture. Cutting fluids have caused occupational irritant contact dermatitis (OICD), and many of the additives used in these cutting fluid mixtures are thought to be responsible for OICD in workers (7–9). OICD can result from exposure to a single large stimulus, repetitive stimuli, or a combination of several different stimuli that may surpass a critical level. Cutting fluids tend to be alkaline and are often soap-like. As such they denature keratin, defat the skin, and remove water from the skin to cause dryness, fissures, and eczema.

The small number of cutting fluids additives selected for testing was representative of major constituents in most aqueous cutting fluids (10). These usually include a surfactant (e.g., linear alkylbenzene sulfonate, LAS), 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 metal-working industry are either oil–water mixtures or strictly synthetic aqueous formulations. 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.

Surfactants are frequently associated with OICD and irritant effects in various animal models (11,12). The classical anionic surfactant sodium lauryl sulfate (SLS) can be cytotoxic at or greater than its critical micelle concentration (CMC = 8 mM or 0.24%). For example, guinea pig skin exposed to 5% SLS or 5% linear alkyl benzene sulfonate (LAS) for 24 hours developed necrolysis of the epidermis (13). LAS is often formulated in industrial cutting fluids. Triazine (TRI) is often added to cutting fluids and other industrial formulations as a biocide or preservative to extend the shelf-life of the formulation. However, many of these biocides are known irritants, and OICD dermatitis at the macroscopic and cellular level has been widely reported (8,9,14). Fatty acids such as ricinoleic acid are often formulated with cutting fluids to enhance lubricity. While these fatty acids are known more for their dermal penetration enhancement (15), dermal irritation as a single chemical or part of a mixture has not been reported. Corrosive inhibitors such as the alkanolamine (e.g., triethanolamine, TEA) are thought to be mild primary irritants and could influence dermal absorption of additives since they are readily absorbed in neat or aqueous solutions.

The purpose of this study was to assess in *in vivo* porcine skin single or various combinations of these additives in initiating the OICD response following an acute 4-hour and 8-hour exposure, and *in vitro* using the isolated perfused porcine skin flap (IPPSF). In addition, individual additives and vehicles were exposed to human epidermal keratinocytes (HEK) to determine their relative cytotoxicity.

MATERIALS AND METHODS

In Vivo

Female weanling Yorkshire pigs weighing 20–30 kg were acclimated for 1 week before 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 additives, individually or in mixtures, 2% triazine (TRI), 5% triethanolamine (TEA), 5% linear alkylbenzene sulfonate (LAS), and 5% sulfurized ricinoleic acid (SRA). Approximately 24 hours before 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 200 μL of the appropriate cutting fluid (single or in combination) in a Hill Top Chamber. Four hours later, the pig was again sedated with TKX and the remaining 8 sites treated as above. Eight hours after the initial treatment, the pig was sedated with TKX, and erythema and edema evaluated using a Draize scoring system (erythema: 0, no change; 1, very slight change; 2, pale red in defined area; 3, definite red in well-defined area; 4, crimson red; edema: 0, no change; 1, very slight change; 2, slight change with edges barely defined; 3, moderate change, with area raised 1 mm; 4, severe change, with area raised more than 1 mm and extending beyond the exposure area). After this, 6-mm biopsies were harvested and fixed in 10% neutral buffered formalin for histopathological evaluation. Biopsies were processed through graded ethanols, cleared in Clear-Rite 3 (Richard-Allan Scientific, Kalamazoo, MI, USA), infiltrated, and embedded in Paraplast® (Tyco Health Care Group, Mansfield, MA, USA). The skin was sectioned at 6 μm, stained with hematoxylin and eosin (H&E), and analyzed for intercellular epidermal edema, intracellular epidermal edema, dermal edema, and dermal inflammation on an Olympus BH-2 microscope. Scoring for edema and inflammation was assessed on the criteria: 0, no change; 1, mild alterations; 2, moderate alterations; and 3, severe alterations. The mean values ± SEM were calculated for each treatment.

Isolated Perfused Porcine Skin Flap (IPPSF)

The IPPSF, created on the abdomen of the pig, has been utilized to study the toxicity and percutaneous absorption of numerous compounds (16). Two single-pedicle axial pattern skin flaps, each lateral to the ventral midline, were created during stage I and harvested 48 hours later during stage II surgery. The flaps were cannulated, flushed with heparinized saline to clear the vasculature of blood, and each transferred to a perfusion chamber. The flaps were perfused for 1 hour before

dosing with a modified Krebs/Ringer bicarbonate buffer (pH 7.4) containing bovine serum albumin, glucose, penicillin G, amikacin, and heparin, during which 1.0-mL arterial and 3.0-mL 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 1.0 cm × 5.0 cm (5.0 cm^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 50 μL of the vehicle, additive, or additive mixture. Flap perfusion was resumed, with the venous perfusate harvested at 0, 0.5, 1, 2, 4, and 8 hours and frozen at −80°C for later interleukin- (IL-) 8 assay. IPPSFs (n = 4/treatment) were exposed to MO or PEG mixtures with single additive, complete additive mixtures in vehicle, or no treatment (control).

The IL-8 assay was performed using a swine IL-8 enzyme-linked immunosorbent assay kit (ELISA; Biosource International, Inc., Camarillo, CA). The timed samples were thawed, diluted 1:1 in a standard diluent buffer, and thoroughly mixed. Samples in triplicate and standards in duplicate were pipetted (100 μL) into the appropriate wells of the antibody-coated ELISA plate. The plate was sealed and incubated for 2 hours, rinsed, and incubated for 1 hour with 100 μL of biotinylated anti-IL-8 biotin conjugate. Following a rinse, 100 μL of Streptavidin-HRP (1:1000) was pipetted into each well and incubated for 30 minutes. The plate was incubated in the dark with stabilized chromagen (100 μL) for 30 minutes, and the absorbance of each well read at 450 nm 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 neonatal HEK were purchased from Cambrex Bioproducts (Walkersville, MD) and stored under liquid nitrogen until passage. Cells (approximately 263,000) were diluted in 15 mL of KGM-2 (serum-free keratinocyte basal media supplemented with human epidermal growth factor, insulin, bovine pituitary extract, gentamicin, and amphotericin-B) in each 75-cm^2 culture flask and grown in a humidified 5% CO_2 incubator at 37°C. The keratinocytes were harvested once they reached 60% confluency, stored under liquid nitrogen until plated, or plated immediately in 96-well culture plates at a density of approximately 7000 cells per well. Once the monolayer reached approximately 70%, the HEKs were treated with the additives LAS (range 0.5–500 μM; 0.00002–0.02%), TRI (range 0.001–1000 μM; 0.00000002–0.02%), SRA (range 0.034–34 mM; 0.001–1%), and TEA (range 0.4–400 mM; 0.006–6%) 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 (Diversified Biotech; Boston, MA) that allowed the exchange of gases but not the evaporation of these volatile compounds. At 24 hours' post treatment, the media were replaced with KGM-2 containing MTT (0.5 mg/mL; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and the plates incubated for 3 hours. The HEK were rinsed briefly in Hanks' balanced salt solution and destained with agitation in isopropanol for 2 hours. The

absorbance, read at 540 nm using the Labsystems Multiskan RC plate reader, was converted to percent viability relative to the untreated controls.

Statistics

The means were generated and treatments statistically compared using analysis of variance (ANOVA) (SAS 8.01 for Windows; SAS Institute, Cary, NC). A least significant difference (LSD) procedure was used for multiple comparisons within MO- or PEG-based treatment groups and paired Student's *t*-test was used for comparisons between MO and PEG treatments. Means with different (superscripted) letters within the same group represent significant differences ($p < 0.05$) between treatments.

RESULTS

In Vivo Studies

With the single additive, slight erythema was observed with TRI and LAS (with PEG, 8 hours; with MO, 4 and 8 hours) and the TRI + LAS (with MO, 8 hours). Slight erythema was also seen with other 2-component mixtures in both PEG and MO. The 3-component mixture TRI + TEA + SRA exhibited the greatest overall erythema in both vehicles at 4 hours (Table 1). The only statistically significant ($p < 0.05$) vehicle effect was found in the LAS + TRI mixture at 8 hours.

Table 1 Mean erythema following treatment with linear alkylbenzene sulfonate (LAS), triazine (TRI), sulfurized ricinoleic acid (SRA), and/or triethanolamine (TEA) in polyethylene glycol (PEG) or mineral oil (MO) vehicle

5% Vehicle >> treatment	PEG, 4 hours (±SEM)	PEG, 8 hours (±SEM)	MO, 4 hours (±SEM)	MO, 8 hours (±SEM)
No treatment control	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}
5% vehicle control	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}
5% SRA	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}
5% LAS	0.00 ± 0.00^{b}	0.75 ± 0.48	0.25 ± 0.25	0.25 ± 0.25
2% TRI	0.25 ± 0.25^{b}	1.00 ± 0.71	0.25 ± 0.25	0.00 ± 0.00^{f}
5% TEA	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}
5% SRA + 5% LAS	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}
5% SRA + 2% TRI	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}
5% SRA + 5% TEA	0.25 ± 0.25^{b}	0.00 ± 0.00	0.25 ± 0.25	0.50 ± 0.29
5% LAS + 2% TRI	$0.00 \pm 0.00^{b,B}$	0.00 ± 0.00^{B}	$0.00 \pm 0.00^{d,B}$	0.75 ± 0.25^{A}
5% LAS + 5% TEA	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}
5% TEA + 2% TRI	0.25 ± 0.25^{b}	0.50 ± 0.50	0.50 ± 0.50	0.25 ± 0.25
5% SRA + 5% LAS + 2% TRI	0.00 ± 0.00^{b}	1.00 ± 0.58	0.25 ± 0.25	1.00 ± 0.58^{e}
5% LAS + 5% TEA + 5% SRA	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}
5% TEA + 5% SRA + /2% TRI	0.75 ± 0.48^{a}	0.75 ± 0.75	1.00 ± 0.41^{c}	0.50 ± 0.50
5% LAS + 5% TEA + 2% TRI	0.00 ± 0.00^{b}	0.50 ± 0.50	1.00 ± 0.41^{c}	0.50 ± 0.50
5% LAS + 5% TEA + 5% SRA + 2%TRI	0.00 ± 0.00^{b}	0.75 ± 0.75	0.75 ± 0.48	0.25 ± 0.25

Different letters within each treatment (a and b, c and d, e and f) and across each treatment (A and B) denote significant differences ($p < 0.05$).

Table 2 Mean intracellular epidermal edema following treatment with the linear alkylbenzene sulfonate (LAS), triazine (TRI), sulfurized ricinoleic acid (SRA), and/or triethanolamine (TEA) in polyethylene glycol (PEG) or mineral oil (MO) vehicle

5% Vehicle >> treatment	PEG, 4 hours (±SEM)	PEG, 8 hours (±SEM)	MO, 4 hours (±SEM)	MO, 8 hours (±SEM)
No treatment control	0.38 ± 0.08	0.33 ± 0.07	0.47 ± 0.10	0.34 ± 0.10^{f}
5% vehicle control	0.42 ± 0.10	0.37 ± 0.10	0.53 ± 0.13	0.55 ± 0.09
5% SRA	0.38 ± 0.24	0.06 ± 0.06^{d}	0.13 ± 0.13	0.19 ± 0.12^{f}
5% LAS	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00	0.19 ± 0.12^{f}
2% TRI	0.13 ± 0.13^{b}	0.25 ± 0.14	0.00 ± 0.00	0.50 ± 0.29
5% TEA	0.00 ± 0.00^{b}	0.06 ± 0.06^{d}	0.25 ± 0.25	0.38 ± 0.24^{f}
5% SRA + 5% LAS	0.69 ± 0.19	0.81 ± 0.45	0.44 ± 0.06	0.38 ± 0.13^{f}
5% SRA + 2% TRI	0.88 ± 0.13	0.44 ± 0.21	0.44 ± 0.21	0.38 ± 0.13^{f}
5% SRA + 5% TEA	0.50 ± 0.29	0.88 ± 0.43	0.94 ± 0.39	0.75 ± 0.14
5% LAS + 2% TRI	0.31 ± 0.12	1.25 ± 0.43	0.38 ± 0.22	1.38 ± 0.38
5% LAS + 5% TEA	0.25 ± 0.14	0.25 ± 0.14	0.38 ± 0.24	0.38 ± 0.13^{f}
5% TEA + 2% TRI	0.38 ± 0.13	0.75 ± 0.43	0.25 ± 0.14	1.00 ± 0.35
5% SRA + 5% LAS + 2% TRI	0.25 ± 0.14	0.75 ± 0.14	0.63 ± 0.24	1.13 ± 0.52
5% LAS + 5% TEA + 5% SRA	0.50 ± 0.20	0.63 ± 0.13	0.75 ± 0.25	0.75 ± 0.43
5% TEA + 5% SRA + 2% TRI	0.88 ± 0.13	0.88 ± 0.13	0.38 ± 0.24	1.63 ± 0.55^{e}
5% LAS + 5% TEA + 2% TRI	1.00 ± 0.41^{a}	1.25 ± 0.25	0.50 ± 0.20	1.25 ± 0.25
5% LAS + 5% TEA + 5% SRA + 2%TRI	0.88 ± 0.13^{B}	$1.50 \pm 0.29^{c,A}$	0.50 ± 0.20^{B}	0.75 ± 0.14^{B}

Different letters within each treatment (a and b, c and d, e and f) and across each treatment (A and B) denote significant differences ($p < 0.05$).

No macroscopic edema was present in any of the cutting fluid treatments. Mixtures containing TRI exhibited the greatest intracellular epidermal edema, followed by mixtures containing SRA (Table 2). A significant ($p < 0.05$) vehicle effect was present in the 4-component mixture. Individual cutting fluids exhibited no intracellular epidermal edema compared to controls. Intercellular epidermal edema was minimal in most of the individual and combination cutting fluids, with a minimal effect noted with TEA and TRI. Dermal edema and dermal inflammation (Figs. 1 and 2) were the greatest in mixtures containing TRI or SRA. Individual cutting fluid components exhibited similar responses for dermal edema as the controls, with dermal inflammation with TRI greater than the controls.

IPPSF Studies

IL-8 concentration (pg/mL) increased from 0 hours to 8 hours in all additive treatments. SRA + MO caused significantly ($p < 0.05$) greater release of IL-8 at 1 and 2 hours post treatment; no treatments caused a significant increase of IL-8 over controls at 4 and 8 hours (Fig. 3). In mixtures containing a PEG vehicle, no treatments caused significant release of IL-8 above controls.

Cell Culture Studies

HEK were treated with each of the four additives and the two vehicles to determine their effect on cell viability. LAS caused significant ($p < 0.05$) cell death from 0.002% to 0.02% concentration (Fig. 4), TRI at 0.02% (Fig. 5), SRA from 0.01% to

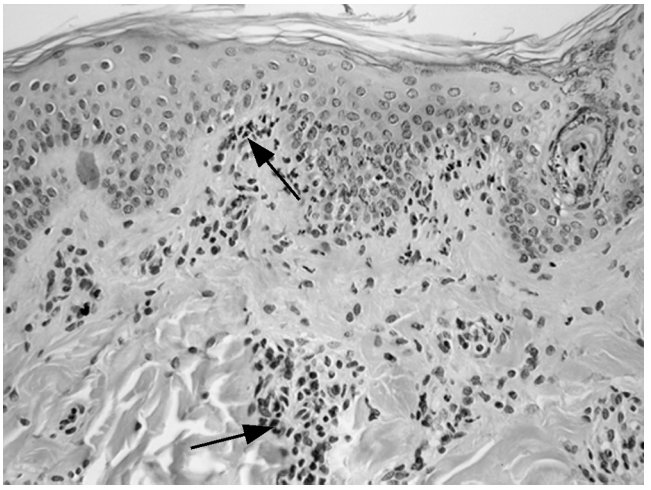

Figure 1 Light micrograph of porcine skin treated with 5% LAS, 5% TEA, and 2% TRI for 8 hours depicting dermal inflammation (arrow). H&E. ×175.

1.0% (Fig. 6), and TEA from 0.3% to 6.0% (Fig. 7). In the vehicles, MO caused no significant ($p > 0.05$) cell death, while PEG caused significant ($p < 0.05$) cell death from 5% to 10.0%.

DISCUSSION

The selected cutting fluid additives, LAS, TRI, SRA, and TEA individually and in a mixture, with PEG or MO vehicle appear to have initiated the OICD

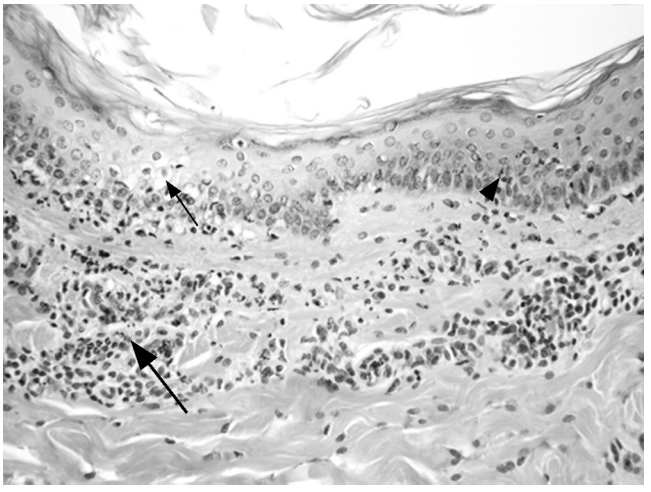

Figure 2 Light micrograph of porcine skin treated with 5% LAS, 5% TEA, and 2% TRI for 8 hours exhibiting intracellular epidermal edema (small arrow), epidermal infiltrates (arrow head), and dermal inflammation (large arrow). H&E. ×175.

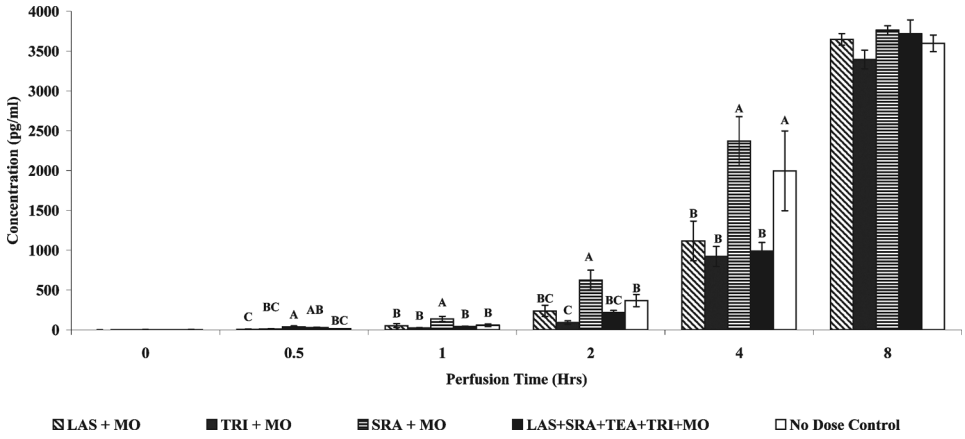

Figure 3 Mean normalized IL-8 released from IPPSF treated with mixtures in MO vehicle. Different letters within each treatment denote significant differences ($p < 0.05$).

response following an acute exposure in porcine skin *in vivo* and in the IPPSF. In addition, the keratinocyte cell culture studies have shown that the individual compounds can cause cell mortality.

TRI is an indicator of potential cutaneous toxicity for individuals handling these mixtures. TRI is very soluble in water, but less soluble in organic solvents and very alkaline in water (pH of 11 with 5% TRI in water [17]). Previous work in our laboratory has confirmed the effect of selected cutting fluid additives on TRI absorption in skin (5). Not surprisingly, cutting fluid mixtures containing the additive TRI most often caused erythema and intracellular epidermal edema within 4 to 8 hours following exposure. Irrespective of whether occupational dermatitis is

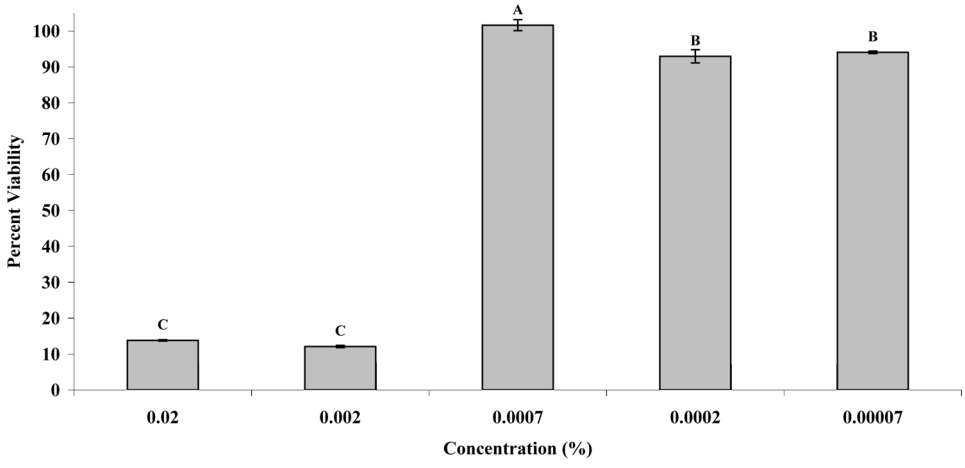

Figure 4 Viability of HEK treated with different concentrations of LAS for 24 hours. Different letters within each treatment denote significant differences ($p < 0.05$).

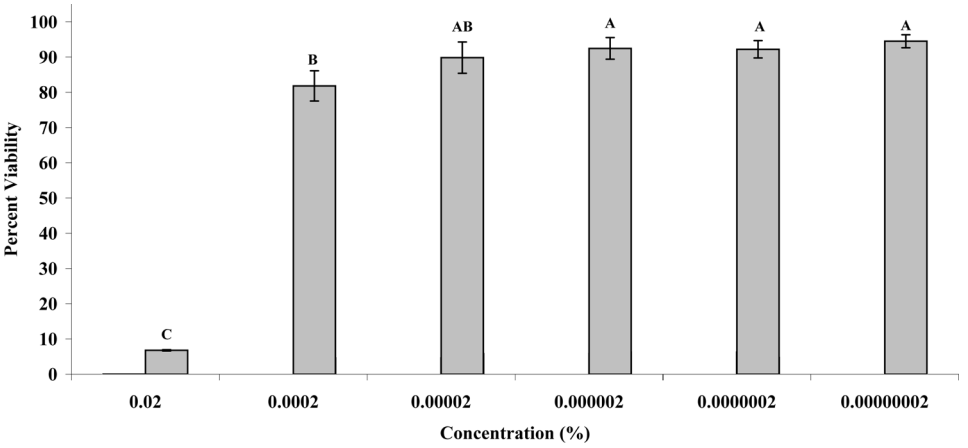

Figure 5 Viability of HEK treated with different concentrations of TRI for 24 hours. Different letters within each treatment denote significant differences ($p < 0.05$).

due to irritation or sensitization, TRI was able to penetrate the skin surface to illicit the response. In occupational scenarios where the skin is consistently hydrated, these water-soluble molecules could readily diffuse across the stratum corneum barrier. This event can become more of a health concern when one recognizes that cutting fluids may also contain other potential irritants that could modulate the dermal disposition of TRI. The presence of two other additives, TEA and SRA, appear to enhance the erythemic response. The IL-8 response data were not conclusive regarding TRI effects.

SRA alone did not cause any macroscopic effects (edema or erythema) in *in vivo* skin. However, several of the noted responses were associated with TRI + SRA mixtures. Fatty acids and their derivatives are nontoxic to the skin since they

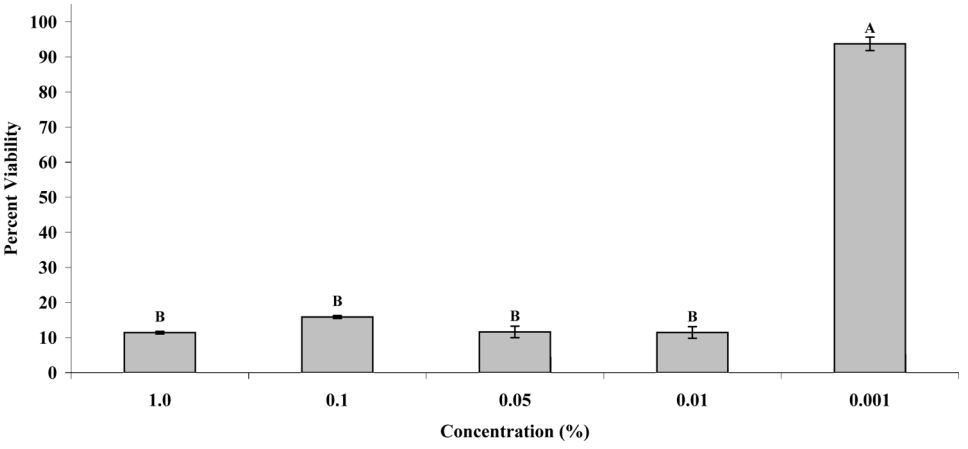

Figure 6 Viability of HEK treated with different concentrations of SRA for 24 hours. Different letters within each treatment denote significant differences ($p < 0.05$).

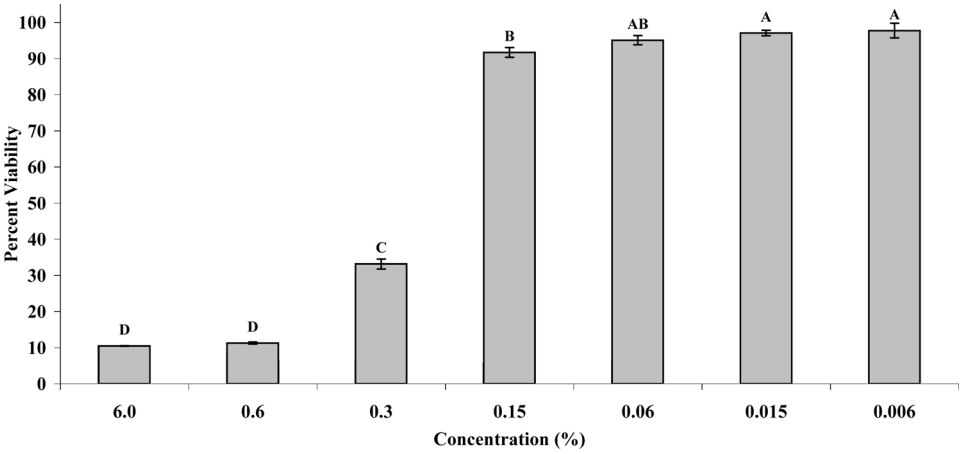

Figure 7 Viability of HEK treated with different concentrations of TEA for 24 hours. Different letters within each treatment denote significant differences ($p < 0.05$).

are widely used as emollients in many registered cosmetic formulations. However, numerous reports in recent years have noted that the presence of ricinoleic acid in these products may cause severe dermal reactions (18,19). Contact allergy has been attributed to a fatty acid ester EM-550 in cutting fluids after a thorough investigation, which included patch testing with components present in cutting fluids. Castor oil, which consists mostly of ricinoleic acid (90%), oleic acid (7%), and linoleic acid (3%), is often included in cutting fluid formulations to enhance lubricity. Recent *in vivo* studies have demonstrated that ricinoleic acid can produce both a pro-inflammatory and an anti-inflammatory response in guinea pig skin following topical exposure (20). The alkanolamine TEA, added to the cutting fluid mixture to inhibit corrosion, is a mild primary irritant. Studies have found skin ulcerations and epidermal erosion in rats at the site of TEA application (21).

In the present study, the pig was treated with acute doses of the cutting fluid additives LAS, TRI, SRA, and TEA, individually or in combination. Slight skin erythema was present in some areas treated with 5% LAS and 5% SRA + 5% TEA. Sites that exhibited irritation were treated with 2% TRI alone, or with 2-, 3-, and 4-component mixtures. The 3-component mixture TEA + SRA + TRI caused the greatest erythema at 4 hours with both PEG and MO vehicles, while LAS + SRA + TRI caused erythema in both vehicles at 8 hours.

Previous work in our laboratory with jet fuel (22,23), hydrocarbon components of jet fuel (24,25), and the pesticide permethrin (26) have found the inflammatory mediator IL-8 to be a sensitive biomarker to cutaneous irritation in both the IPPSF and HEK cell culture. IL-8 concentrations above controls were found only in the perfusate of flaps treated with the full mixture in MO; no significant concentrations above controls were found in mixtures with PEG. The full mixture was also responsible for a significant increase ($p < 0.05$) in TRI permeability by as much as 2-fold in porcine skin in both the MO and the PEG vehicles (5). The cutting fluid additives also increased TRI deposition in the epidermal cell layers, while this was generally reversed for LAS and SRA (4,6).

Toxicity studies of the four additives in HEK are lacking, so each target toxic concentration was based on other cell culture systems and determined within a concentration range. An increase of arachidonic acid release was shown in C3H-10T1/2 cells after treatment with 5.0–50 μM concentrations of LAS (27) and in HPKII cells after a single treatment of 100 mM TEA (28). Treatment with 340 μM ricinoleic acid in rat isolated intestine showed stimulation of cyclooxygenase and lipoxygenase products (29), while TRI was shown to lower the EC50 at 0.097 μM in the microalga Chlorella vulgaris after a 96-hour exposure (30). LAS, SRA, and TEA caused a toxic effect on HEK in the projected range. TRI, however, showed no toxicity up to the concentration of 0.0002% (10.0 μM). This may be due to the volatile nature of TRI, allowing little time for this compound to come in contact with the HEKs. The cell culture plate membrane may have prevented some TRI from evaporating, but some may have volatilized into the head space of the wells and therefore did not reach the HEK in the required dose. While PEG caused slight toxicity to the HEK at concentration up to 1%, MO-induced toxicity was low at all concentrations. The primary reason was that MO was not miscible in the aqueous medium and therefore had little direct contact with the cells. The cell viability threshold, the concentration of compound that provides HEK viability between 90% and 100%, indicates the relative toxicity of each compound. With this in mind, TEA was least toxic to HEK with a 0.15% (10,000 μM) dose concentration, followed by SRA at 0.001% (34.0 μM), LAS at 0.0007% (20.0 μM), and TRI at 0.00002% (1.0 μM). MO showed a nontoxic effect as high as 10%, while PEG exhibited a cell viability threshold at 1%. TRI toxicity in HEK may be underestimated due to its volatility. The response seen in the *in vivo* data (erythema, intracellular epidermal edema, dermal edema, and dermal inflammation) showed an irritation effect.

In summary, 2% TRI, alone or with 2-, 3-, and 4-component mixtures *in vivo* porcine skin, caused more consistent macroscopic (erythema) and microscopic (intracellular epidermal edema, dermal edema, and dermal inflammation) responses. The IPPSF studies show no correlation to absorption of the additives and IL-8 release. While the cell culture studies investigated the toxicity of individual components at different concentrations, the results did show that the additive TRI was more toxic to HEK than the vehicles or other additives. These results taken together strongly suggest that TRI may play a more important role in OICD when several of the tested additives are included in similar cutting fluid formulations. These observations would need to be further complemented by a clinical study to validate these findings and to also determine cumulative irritation in the workplace.

ACKNOWLEDGMENTS

The authors would like to thank the staff of the Center for Chemical Toxicology Research and Pharmacokinetics (CCTRP). This research was supported by NIOSH grant R01-OH-03669. In conducting research using animals, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, revised 1985).

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Cutaneous and Ocular Toxicology

ISSN: 1556-9527 (Print) 1556-9535 (Online) Journal homepage: http://www.tandfonline.com/loi/icot20

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To cite this article: Nancy A. Monteiro-Riviere, Alfred O. Inman, Beth M. Barlow & Ronald E. Baynes (2006) Dermatotoxicity of Cutting Fluid Mixtures:*In Vitro* and *In Vivo* Studies, Cutaneous and Ocular Toxicology, 25:4, 235-247, DOI: 10.1080/15569520601013137

To link to this article: https://doi.org/10.1080/15569520601013137