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Skin absorption of six performance amines used in metalworking fluids

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ABSTRACT: Every year, 10 million workers are exposed to metalworking fluids (MWFs) that may be toxic. There are four types of MWFs: neat oils and three water-based MWFs (soluble oil, semisynthetic and synthetic), which are diluted with water and whose composition varies according to the mineral oils ratio. MWFs also contain various additives. To determine the absorption of six amines used as corrosion inhibitors and biocides in MWFs, porcine skin flow-through diffusion cell experiments were conducted with hydrophilic ethanolamines (mono-, di- and triethanolamine, MEA, DEA and TEA respectively) and a mixture of lipophilic amines (dibutylethanolamine, dicyclohexylamine and diphenylamine). The six amines were dosed in four vehicles (water and three generic water-based MWF formulations) and analyzed using a scintillation counter or gas chromatography/mass spectrometry. These 24 h studies showed that dermal absorption significantly ($P < 0.05$) increased from water for the six amines (e.g. 1.15 ± 0.29% dose; DEA in water) compared to other formulations (e.g. 0.13 ± 0.01% dose; DEA in semisynthetic MWF) and absorption was greatest for dibutylethanolamine in all the formulations. The soluble oil formulation tended to increase the dermal absorption of the hydrophilic amines. The permeability coefficient was significantly higher ($P < 0.05$) with TEA relative to the other hydrophilic amines (e.g. $4.22 \times 10^{-4} \pm 0.53 \times 10^{-4}$ cm h^{-1} [TEA in synthetic MWF] vs. $1.23 \times 10^{-4} \pm 0.10 \times 10^{-4}$ cm h^{-1} [MEA in synthetic MWF]), except for MEA in soluble oil formulation. Future research will confirm these findings in an *in vivo* pig model along with dermatotoxicity studies. These results should help MWF industries choose safer additives for their formulations to protect the health of metalworkers. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: skin absorption; metalworking fluids; flow-through diffusion cell; ethanolamines; biocide

Introduction

Metalworking fluids (MWFs) are used in various metalworking operations to cool, lubricate and remove debris from the work surfaces of metal parts, and to treat and protect machine tools. For this reason, they are applied to the various machines and inhalation and dermal contact by metalworkers is probable (Park *et al*., 2009b). There are two groups of MWFs: neat oils used directly by the metalworking industries and water-based MWFs (Wb-MWFs), which are diluted with water according to this ratio: 5% Wb-MWF and 95% water (Park *et al*., 2009b). MWF concentrates are diluted with water to produce "tankside" concentrations recommended by MWF manufacturers. These Wb-MWFs can be separated into three types: soluble oils (SO), semisynthetic (SS) fluids and synthetic (SYN) fluids. Soluble oil concentrates contain 60–90% mineral oil, semisynthetic oil concentrates contain 2–30% mineral oil and synthetic oil concentrates contain no mineral oil (ASTM, 2013), but contain various carboxylic acid salts, ethanolamines, ethylene glycols and plant seed oils. The use of these Wb-MWF types has increased in recent years at the expense of neat oils (Verma *et al*., 2006). Therefore, in North America, Asia and Europe, respectively 88%, 65% and 61% of MWF usage is water-based (Byers, 2011). The composition of each MWF is complex and varies in each industry according to the different mixtures added before or during their use. Nevertheless, generic formulations can be established to conduct permeability studies. The most common additives of the MWFs are surfactants (e.g., linear alkylbenzene sulfonate), biocides (e.g., triazine), lubricants (e.g., sulfurized ricinoleic acid) and corrosion inhibitors such as ethanolamines, and the local skin toxicity of several of these additives has been evaluated (Monteiro-Rivière *et al*., 2006).

The biocides used in MWFs can have different chemical properties. The Center for Chemical Toxicology Research and Pharmacokinetics (CCTRP) has recently tested phenolic biocides (Vijay *et al*., 2009) and is currently focused on amino-based biocides and/or corrosion inhibitors. It is important to note that the combination of all these compounds represent only 5% of the Wb-MWF tankside composition. However, certain component parts should be studied because they can cause various adverse effects in workers routinely exposed to MWFs.

About 10 million industrial workers are exposed to MWFs via inhalation of the aerosols generated in the machining process and via dermal contact. This daily contact with MWFs affects the health of employees. Much has been published about the microorganisms that grow and the biofilms that form in MWFs (e.g., Lucchesi *et al*., 2012; Perkins and Angenent, 2010; Saha and Donofrio, 2012; Sandin *et al*., 1990; Trafny, 2013). Much has been published regarding inhalation exposure and various diseases of the respiratory system such as asthma, upper respiratory tract infections, chronic bronchitis and hypersensitivity pneumonitis (e.g., Gordon, 2004; Henriks-Eckerman *et al*., 2007; Park *et al*., 2008, 2009a,2009b; Rosenman *et al*., 1997; Verma *et al*., 2006). Much has been published regarding dermal

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exposure and its effect of irritant or allergic contact dermatitis, eczema, skin eruptions, acne or bacterial or fungal infections (e.g., Cherrie and Semple, 2010; Geier *et al*., 2004; Henriks-Eckerman *et al*., 2007; Park *et al*., 2009a,2009b; Roff *et al*., 2004; Sprince *et al*., 1996; Verma *et al*., 2006; van Wendel de Joode *et al*., 2005). Less has been published regarding dermal absorption and distribution of MWF constituents (e.g., Al-Humadi *et al*., 2000; Baynes and Vijay, 2010; Baynes *et al*., 2002, 2003; Baynes and Riviere, 2004; Vijay *et al*., 2007, 2009; Xu *et al*., 2013).

Topical exposure is an important pathway for metalworking chemicals that can produce toxicological manifestations ranging from acute irritation to proliferation and tumor formation (Monteiro-Rivière *et al*., 2006; NTP, 1999, 2004). Studies also demonstrated that they could cross this biological barrier leading to erythema and intracellular epidermal edema (Monteiro-Rivière *et al*., 2006). Some studies have also shown that chronic exposure to MWFs is associated with increased risk of certain types of cancer, such as breast (Thompson *et al*., 2005) and prostate (Agalliu *et al*., 2005). These cancers could be due to the contamination of MWFs by some metals (e.g., nickel) and nitrosamines (e.g., *N*-nitrosodiethanolamine, NDELA). In 1956, it was demonstrated that NDELA was a potential carcinogen (Magee and Barnes, 1956). Now, NDELA is classified as a category 1B carcinogen ("presumed to have carcinogenic potential for humans") according to the Globally Harmonized System of Classification and Labelling of Chemicals. In 1977, it was determined that a reaction could occur between sodium nitrite, a corrosion inhibitor used at that time in MWFs, and diethanolamine (DEA) to form a potential carcinogen, i.e., NDELA (Zingmark and Rappe, 1977). Consequently, nitrites have not been used in metalworking since the 1980s (Byers, 2011) and metalworking industries tend to reduce the use of DEA for this reason. For example, the concentration of DEA in MWFs has been limited to 0.2% in Germany since 1993 (Geier *et al*., 2004).

For all these reasons, it is important to study the types of components present in MWFs to determine their toxicity and the differences between their permeability profiles. The permeability of some components of the MWFs (linear alkylbenzene sulfonate, triazine, ricinoleic acid) has previously been evaluated by our laboratory (Baynes *et al*., 2002, 2003; Baynes and Riviere, 2004). These studies indicated that biocides and corrosion inhibitors could influence skin absorption of the other additives. For example, it has been shown that triazine permeability is increased in mineral oil-based mixtures containing triethanolamine (TEA) and ricinoleic acid relative to mixtures without these two substances (Baynes *et al*., 2003). Lubricants and surfactants have only a minimal effect on skin absorption of the other additives.

The focus of the current study relates to the permeability of six amino-based additives that are often used as biocides and corrosion inhibitors in MWFs. Amines are widely used in MWFs because these molecules are able to solubilize water-insoluble materials, neutralize acids, buffer the MWF pH to a desired level (usually between 8.5 and 9.5) and prevent rust formation (Byers, 2011). The machines and metal parts are in contact with water so it is necessary to add corrosion inhibitors to protect them and neutralize the corrosive agents. Some amines can also be used as biocides to control the growth of bacteria or fungi. Therefore, the amines are a very interesting and beneficial group of chemicals that serve multiple important functions in MWFs. For this reason, the laboratory decided to focus its research on six of these amino-based corrosion inhibitors and biocides and more precisely on four ethanolamines and two cyclic amines. The ethanolamines are the most common amines used in MWFs (Byers, 2011). The simplest amine in this group is monoethanolamine (MEA), a primary amine with one ethanol group. Then, there are DEA and TEA, respectively a secondary and a tertiary amine substituted by two and three ethanol groups. Another interesting derivative of the ethanolamine group is dibutylethanolamine (DBEA). They are all used as corrosion inhibitors for rust prevention by the metalworking industries. DBEA also has biocidal properties. Dicyclohexylamine (DCHA) and diphenylamine (DPA) are also used in MWFs. They are cyclic secondary amines respectively characterized by the presence of two cyclohexyl (saturated rings) and two phenyl (unsaturated rings) groups. Although they are not classified as biocides by the United States Environmental Protection Agency (EPA), these two compounds are widely used by metalworking industries to control the growth of microorganisms in MWFs. Classified biocides are stringently regulated because studies have demonstrated that most are hazardous substances. For example, triazine was widely used by metalworking industries until 2008 when the EPA concluded that industries must significantly reduce its use because it leads to a release of formaldehyde, a highly toxic and potentially carcinogenic compound (EPA, 2008). Therefore, industries try to use "biocide-free" MWFs containing for example DCHA, which is not classified as a biocide but demonstrates some biocidal properties.

As these six corrosive and/or toxic amines are in daily contact with the metalworkers' skin, studies are necessary to determine, first, if they can cross the skin. Then, it is important to calculate their permeability in different vehicles (water and Wb-MWF formulations). Porcine skin flow-through diffusion cell studies (Bronaugh and Stewart, 1985) were used to determine the rate and extent of absorption of these MWF chemicals through skin *in vitro*. Porcine skin serves as the closest animal model of human skin that is available (Godin and Touitou, 2007) and there is a high correlation of dermal absorption between the two species (Reifenrath *et al*., 1984; Wester *et al*., 1998).

Materials and methods

Porcine Skin Flow-through Diffusion Cell Experiment

Four 24 h porcine skin flow-through experiments were conducted in Bronaugh two-compartment Teflon diffusion cell systems to evaluate the penetration of amino-based corrosion inhibitors and biocides in water and in three generic Wb-MWF formulation types. Fresh skin was obtained from four white, weanling Yorkshire/Landrace cross pigs (30–40 kg). The dorsum of the pigs was clipped and dermatomed to a thickness of 500 μm with a Padgett electric dermatome (Padgett Instruments Inc., Kansas City, MO, USA). Then, each piece of skin was punched into a circular disk and placed into the diffusion cell and secured in place by a screw cap dosing device to provide a dosing surface area of 0.64 cm^2 (diameter: 0.9 cm). The skin was dosed within 30 min of death of the porcine skin donor, so skin integrity testing was not necessary (OECD 428, 2004). For each experiment, a dosing solution of 1 ml (infinite dose) containing 0.25% of each studied compound in water, soluble oil, semisynthetic and synthetic MWF ($n = 7$ cells for each vehicle condition) were applied on to skin disks obtained from a single donor pig per experiment. The water doses acted as a baseline to which the other 95% water/5% MWF doses were compared. Ultrapure water was procured from the college laboratory facility and the three generic MWF concentrates were provided by

Cimcool Fluid Technology (Cincinnati, OH, USA). The generic soluble oil MWF concentrate contained 0% water, 60% naphthenic oil, 20% sulfonates, 5% ethanolamines, 0% ethylene glycols, 5% fatty acids and 10% fatty amides. The generic semisynthetic MWF concentrate contained 55% water, 12% naphthenic oil, 6% sulfonates, 3% ethanolamines, 3% ethylene glycols, 5% fatty acids and 8% fatty amides. The generic synthetic MWF concentrate contained 70% water, 0% naphthenic oil, 4% phosphates, 8% ethanolamines, 5% ethylene glycols, 4% fatty acids and 10% carboxylic acid salts. Each of these was mixed with water at a ratio of 5% MWF concentrate to 95% water.

The three hydrophilic ethanolamines could not be extracted from the aqueous perfusate for analysis. Therefore, these three compounds were tested using radiochemical analysis. The three ^{14}C-radiolabeled ethanolamines (purity: 99%) obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA), were dosed separately. Their specific activity (55 mCi mmol^{-1}) was adjusted with non-radiolabeled MEA, DEA or TEA (purity: 99%; Sigma-Aldrich, St. Louis, MO, USA) to obtain 0.5 μCi ml^{-1}. The three lipophilic amines (DBEA, DCHA and DPA), obtained from Sigma-Aldrich (purity: 99%), were dosed together using a mixture containing 0.25% of each compound. Then, the diffusion cells were occluded with Parafilm® M pieces (Pechiney Plastic Packaging, Chicago, IL, USA) to prevent evaporation of the volatile compounds. The dermal side of the skin disks was perfused using Krebs–Ringer bicarbonate buffer (pH maintained between 7.3 and 7.5) spiked with dextrose and bovine serum albumin (4.5%) to mimic the oncotic pressure found *in vivo*. The perfusate and the diffusion cells temperatures were maintained at 37 °C using a Brinkmann constant temperature circulator (Brinkmann Inc., Westbury, NY, USA). The flow rate was maintained at 4 ml h^{-1} using a peristaltic pump (Watson-Marlow PumpPro, Wilmington, MA, USA). Perfusate samples ($n = 7$ for each vehicle) were collected in borosilicate glass scintillation vials (Fisher Scientific, Pittsburgh, PA, USA) at 15 min intervals for the first 2 h after dosing, at 1 h intervals for the next 6 h and at 4 h intervals until the end of the 24 h experiments. Blank sample controls were collected before the dose application. The sample vials were occluded during sample collection to retard the evaporation of the volatile test compounds. Perfusate samples that were collected were kept frozen at −20 °C until analysis.

At the end of the experiments with the hydrophilic ethanolamine test compounds, the remaining dose was removed from the surface of the skin into a liquid scintillation vial before the skin disk was transferred to waxed paper. Each skin disk was then swabbed with a 1% soap solution (Ivory dishwashing liquid; Procter & Gamble, Cincinnati, OH, USA) with a cotton-tipped swab, and tape-stripped (Scotch Magic Tape; 3 M, St. Paul, MN, USA) six times. The skin disks were then dissolved in BioSol, a 3% KOH solution (National Diagnostics, Atlanta, GA, USA). The fingertips of the gloves that were used during the swabbing and tape stripping, as well as the forceps rinse, were extracted with ethanol. The dosing device was also extracted with ethanol and the tape strips were dissolved in ethyl acetate. The extracted dosing device, glove fingertips and forceps rinse were analyzed for mass balance purposes.

No mass balance was attempted for the non-radiolabeled lipophilic amines because of the difficulty of extracting and purifying the lipophilic amines from skin and tape strip samples to the extent necessary for injection on to a gas chromatograph.

Perfusate Samples Analysis

Liquid scintillation counter. One milliliter of each perfusate, surface swab, tape strip, fingertip and rinse sample from the experiments with the hydrophilic ethanolamines (MEA, DEA and TEA) was combined with 15 ml of Bioscint scintillation cocktail (National Diagnostics, Atlanta, GA, USA). The entire skin disk and remaining dose samples, rather than an aliquot, were also combined with scintillation cocktail. These samples were then analyzed on a Tri-Carb 2910 TR liquid scintillation counter (Perkin Elmer, Waltham, MA, USA) for total ^{14}C determination.

Gas chromatography mass spectrometry. A liquid–liquid extraction of the DBEA, DCHA and DPA was performed by diluting 500 μl of each perfusate sample and standards into 750 μl of dichloromethane (methylene chloride; Fisher Scientific, Pittsburgh, PA, USA). After 12 h of gentle agitation on an Adams Nutator (Clay Adams, Parsippany, NJ, USA), these samples were centrifuged at 6797 × g for 10 min at 20 °C (Sorvall ST16R Centrifuge, Thermo Scientific, Waltham, MA, USA) to separate the water phase and the dichloromethane phase. A 250 μl aliquot of the lower dichloromethane phase of each sample was transferred into a total recovery vial (Waters Corporation, Milford, MA, USA). Then, an Agilent Technologies 7890A GC System coupled to an Agilent Technologies 7000 GC-MS Triple Quad equipped with Combi-PAL autosampler was used to analyze the three lipophilic amines. Two microliters of each sample and standard were injected and the pulsed splitless mode was used with an injection pulse pressure of 40 psi until 0.75 min. The chromatographic separations were performed on a DB5 MS ultra-inert capillary column (30 m × 0.25 mm ID × 0.25 μm thickness) and the helium flow was 1.2 ml min^{-1}. The inlet temperature was 220 °C and the temperature of the column oven was initially 60 °C, then ramped up to 270 °C at 30 °C min^{-1} and held post-run for 2 min at 280 °C. The calibration standards were analyzed daily along with the samples and the chemical concentration in the samples was calculated from the linear standard curve ($R^2 = 0.99$). The limit of detection was 0.001 μg ml^{-1} and the limit of quantification (LOQ) was 0.005 μg ml^{-1} for each of the lipophilic amines. A 12-point standard curve (range 0.001–10 μg ml^{-1}) was made fresh on each day of the analysis and run along with the perfusate samples. Twelve samples of blank perfusate were spiked with each amine and treated in the same manner as the samples; therefore, no correction for recovery was necessary.

Calculations and Statistics

Absorption was the summation of the total mass (μg) in all of the fraction-collected perfusate samples over the entire 24 h dosing period. For the radiolabeled experiments, the remaining dose, dosing device, surface swabs, tape strips, skin and rinses were the total amount of each test compound detected in each sample vial. Remaining dose + swabs indicated the dose that was on the surface of the skin at the end of the experiment, and absorption + skin indicated the penetration of test compound into or through the skin. The mass (μg), as well as the percentage dose, were calculated for all the samples. Permeability coefficient (Kp) (cm h^{-1}) was calculated from the ratio of steady-state flux (μg cm^{-2} h^{-1}) to the concentration (μg cm^{-3}) of the initial topical dose. Apparent steady-state flux was

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obtained from the slope of the cumulative absorption versus time curve (0–24 h). Statistical analysis was performed using SAS 9.2 for Windows software (Statistical Analysis Software, SAS Institute Inc., Cary, NC, USA, 2008). The resulting means of all the calculated values of the test compounds in the four vehicles (water, soluble oil, synthetic and semisynthetic MWF) ($n = 7$) were compared to one another using one-way ANOVA and multiple comparison methods such as Fisher's least significant difference and Duncan's methods. Because water alone is not used as a MWF by the metalworking industry, an ANOVA was also run on just the three Wb-MWFs without the 100% water doses. The level of significance, between each value (within each vehicle and within each test compound), used was $\alpha = 0.05$ ($P < 0.05$) and is indicated respectively by lower and upper case letters in the figures and tables.

Multiple linear regression analysis was conducted using the Abraham model (Abraham and Martins, 2004). This linear free-energy relationship model is a particular type of QSAR model that is widely used in skin permeability studies to quantify the relationship between skin permeability and molecular descriptors of the solutes:

$$\log Kp = c + eE + sS + aA + bB + vV$$

where logKp is the logarithm of the permeability coefficient obtained for each molecule in each vehicle, E is the solute excess molar refractivity, S is the solute dipolarity/polarizability, A is the solute hydrogen bond donor acidity, B is the solute hydrogen-bond acceptor basicity, V is the solute McGowan characteristic volume, c is the intercept, and e, s, a, b, and v are weighting coefficients. While S, A and B are somewhat related to the molecular polarity of the test compound, E and V are related to the molecular size of the solute. The five Abraham solute descriptors for each solute were estimated using ACD/Percepta (ACD/Labs, 2012 release, Advanced Chemistry Development, Inc., Toronto, Canada). In addition to the five Abraham solute descriptors of the basic model, we also added a vehicle indicator variable to determine the influence of the vehicle on the predicted value of logKp.

Results

Absorption and Permeability Coefficient

The mean absorption (% dose) of each compound in each vehicle is shown in Fig. 1. The absorption was significantly higher ($P < 0.05$) in water relative to the other vehicles (e.g., 28.84 ± 7.13 μg [DEA in water] vs. 3.35 ± 0.2 μg [DEA in SS]; Table 1) for each hydrophilic ethanolamine (upper case letters in Fig. 1). The absorption of MEA in soluble oil was significantly higher than the absorption in synthetic and semisynthetic types of MWFs (Fig. 1 and Table 1). TEA was significantly the most absorbed hydrophilic ethanolamine in all the vehicles relative to the others (e.g., 57.49 ± 9.35 μg [TEA in water]; vs. 28.84 ± 7.13 μg [DEA in water]; Table 1). However, TEA absorption was not significantly different ($P > 0.05$) relative to MEA in the soluble oil type of MWFs (25.32 ± 5.69 μg [TEA in SO] vs. 16.90 ± 3.61 μg [MEA in SO]; Table 1). The same results were obtained for the permeability coefficient that is significantly higher in water (relative to the other vehicles) and with TEA (relative to the other test compounds), except for MEA in generic soluble oil formulation. As these two parameters are correlated, similar results seem logical.

The amount of hydrophilic ethanolamine remaining on the skin surface was significantly lower in water than in the other vehicles (e.g., 2392 ± 10 μg [DEA in SYN] vs. 1915 ± 256 μg [DEA in water]; Table 1), which is consistent with the greater absorption from the water doses for all the ethanolamines, and in SO for MEA (2171 ± 97 μg [MEA in water] vs. 2088 ± 100 μg [MEA in SO]; Table 1). For the same reason, the penetration (absorption + skin) and the amount in the stratum corneum were also significantly higher using water as a vehicle. However, there was no significant difference between the amount of ethanolamines retained in the stratum corneum in water and soluble oil formulations (e.g., 0.46 ± 0.11 μg [MEA in water] vs. 0.46 ± 0.08 μg [MEA in SO]; Table 1). Therefore, these results showed that as there was a greater amount of molecules that crossed the skin using water (and secondly soluble oil), and there was more hydrophilic compound in the most external layer of the skin, the stratum corneum. For the dose remaining on the skin surface, there were few significant differences seen between the ethanolamines among the vehicles: MEA in water (2171 ± 97 μg) was greater than TEA in water (1267 ± 328 μg), and DEA in soluble oil (2344 ± 27 μg) was greater than MEA in the same vehicle (2088 ± 100 μg). No other differences were noted between the ethanolamines among the vehicles for this parameter.

The amount of molecules retained in the stratum corneum was significantly higher with TEA in all vehicles relative to MEA, while no differences were observed between TEA in all vehicles relative to DEA. There were no differences between MEA and DEA in soluble oil or synthetic, while DEA in the stratum corneum was significantly higher than MEA in water and semisynthetic MWFs. As the amount of compound that crossed the skin was generally low relative to the amount that was retained in the skin disks, it is logical that the skin and the penetration (skin + absorption) calculations showed approximately the same results. For these two skin disposition parameters, no consistent pattern was discernible comparing the three ethanolamines for each vehicle. For example, there was significantly more TEA retained in the skin at 24 h in water relative to MEA, and in soluble oil and semisynthetic relative to DEA. However, there was less TEA in the skin relative to MEA when the synthetic formulation was used. Therefore, we can assume that the mechanisms that determined the amount of these three ethanolamines that penetrated into and through the skin were multifactorial and not necessarily dependent entirely on the test compound.

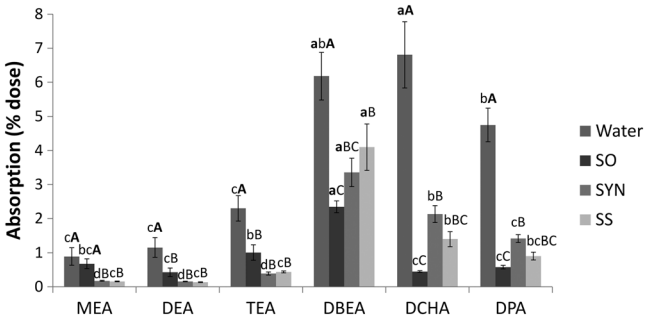

Figure 1. Mean absorption (% initial dose) of MEA, DEA, TEA, DBEA, DCHA and DPA in water, and 5% SO, 5% SYN and 5% SS type of metalworking fluids. The dose in each diffusion cell was 2500 μg. Means with the same letter are not significantly different ($P > 0.05$). Lower case letters indicate comparison of the means between test compounds within vehicles. Upper case letters indicate comparison of the means between vehicles within test compound. DBEA, dibutylethanolamine; DCHA, dicyclohexylamine; DEA, diethanolamine; DPA, diphenylamine; MEA, monoethanolamine; SO, soluble oil; SS, semisynthetic; SYN, synthetic; TEA, triethanolamine.

Table 1. Mean experimental (±SEM) absorptions (μg), permeability coefficients ($cm\,h^{-1}$) and amounts of hydrophilic molecule (μg) in the remaining dose plus the swabs, in the tape strips, in the skin disks, and the absorption plus the skin amount (μg) of MEA, DEA and TEA in water and SO, SYN and SS type of metalworking fluids. The dose in each diffusion cell was 2500 μg

Compound	Vehicle	Absorption (μg)			Permeability coefficient ($cm\,h^{-1}$)			Remaining dose + swabs (μg)			Tape (μg)			Skin (μg)			Skin + absorption (μg)		
		Mean	SEM		Mean × 10^{-3}	SEM × 10^{-3}		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM	
MEA	Water	22.27	(6.46)	bA	0.77	(0.22)	bA	2171	(97)	aB	0.46	(0.11)	bA	41.00	(15.23)	bAB	63.271	(21.69)	bA
	SO	16.90	(3.61)	abA	0.53	(0.11)	abA	2088	(100)	bB	0.46	(0.08)	bA	58.18	(16.52)	abA	75.078	(20.12)	abA
	SYN	4.34	(0.38)	bB	0.12	(0.01)	bB	2378	(7)	aA	0.30	(0.03)	bAB	11.72	(1.11)	aBC	16.063	(1.48)	aB
	SS	3.88	(0.29)	bB	0.10	(0.01)	bB	2366	(9)	aA	0.20	(0.04)	bB	10.16	(0.51)	abC	14.044	(0.80)	bB
DEA	Water	28.84	(7.13)	bA	1.17	(0.29)	bA	1915	(256)	abB	4.99	(1.89)	aA	89.24	(25.97)	abA	118.09	(33.09)	abA
	SO	10.67	(3.16)	bB	0.35	(0.10)	bB	2344	(27)	aA	2.45	(0.86)	abAB	22.54	(5.00)	bB	33.213	(8.16)	bB
	SYN	3.89	(0.24)	bB	0.10	(0.01)	bB	2392	(10)	aA	1.18	(0.22)	abB	6.57	(0.73)	bB	10.463	(0.97)	bB
	SS	3.35	(0.26)	bB	0.09	(0.01)	bB	2367	(11)	aA	1.53	(0.38)	aB	8.10	(0.90)	bB	11.449	(1.16)	bB
TEA	Water	57.56	(9.35)	aA	2.42	(0.36)	aA	1267	(328)	bB	8.32	(1.53)	aA	148.61	(35.17)	aA	206.17	(44.52)	aA
	SO	25.32	(5.69)	aB	0.88	(0.20)	aB	2244	(67)	abA	5.08	(1.66)	aAB	73.59	(18.32)	aB	98.902	(24.01)	aB
	SYN	9.71	(1.13)	aB	0.42	(0.05)	aB	2311	(60)	aA	2.88	(1.09)	aB	7.40	(0.73)	bC	17.11	(1.86)	aC
	SS	10.93	(0.73)	aB	0.49	(0.04)	aB	2346	(20)	aA	1.83	(0.35)	aB	11.03	(0.66)	aC	21.955	(1.39)	aC

DEA, diethanolamine; MEA, monoethanolamine; SO, soluble oil; SS, semisynthetic; SYN, synthetic; TEA, triethanolamine.
Means with the same letter are not significantly different ($P > 0.05$). Lower case letters indicate comparison of the means between test compounds within vehicles. Upper case letters indicate comparison of the means between vehicles within test compound.

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The absorption (μg) and the permeability coefficient (cm h^{-1}) of the three lipophilic amines were significantly ($P < 0.05$) higher using water as a vehicle (e.g., 170.21 ± 24.23 μg [DCHA in water] vs 34.91 ± 5.41 μg [DCHA in SS] for the absorption; Table 2). The DBEA was significantly the most absorbed molecule and it had the significantly higher permeability coefficient relative to DCHA and DPA in the three Wb-MWF vehicles, as well as relative to all the other test compounds in all the vehicles (Fig. 1 for the absorption).

A separate *t*-test was run between DCHA (saturated ring compound) and DPA (unsaturated ring compound) alone. No significant difference was observed between their Kp values in the different vehicles, except in the synthetic vehicle where the Kp of DCHA was significantly higher ($1.74 \times 10^{-3} \pm 0.24 \times 10^{-3}$ cm h^{-1} [DCHA in SYN] vs. $1.07 \times 10^{-3} \pm 0.10 \times 10^{-3}$ cm h^{-1} [DPA in SYN]; Table 2). Significant differences were obtained for their absorption, except in water and the semisynthetic type of MWFs, and the pattern obtained for their Kp values were different (SO = SYN = SS for DPA vs. SYN ≥ SS ≥ SO for DCHA; Table 2). Conversely, DCHA and DPA had the same rank order for their absorption in the different vehicles (water > SYN ≥ SS ≥ SO; Table 2).

Recoveries were calculated for the hydrophilic ethanolamines only. The mean recoveries ranged from 82.4% to 96.5%, with a median of 95.2% and a mean of 93.1%. This indicates that the occlusion of the sample vials was reasonably effective at retarding evaporation. No mass balance was attempted for the non-radiolabeled lipophilic amines because of the difficulty of extracting and purifying the lipophilic amines from skin and tape strip samples to the extent necessary for injection on to a gas chromatograph.

Absorption Profiles

The cumulative concentrations (μg cm^{-2}) of each test compound collected in the perfusate samples were also calculated to determine the steady-state absorption profile of each molecule in all the vehicles. Two distinct profiles have been established: one for the hydrophilic ethanolamines and one for the lipophilic amines. TEA (Fig. 2a) and DCHA (Fig. 2b) cumulative absorption profiles are presented here as examples. Approximately 50% of the final amount of each absorbed molecule crossed the skin 16 h after dosing. The flux increased slowly and reached steady state at approximately 3–4 h for the lipophilic amines and 5–9 h for the hydrophilic ethanolamines on average (data not shown).

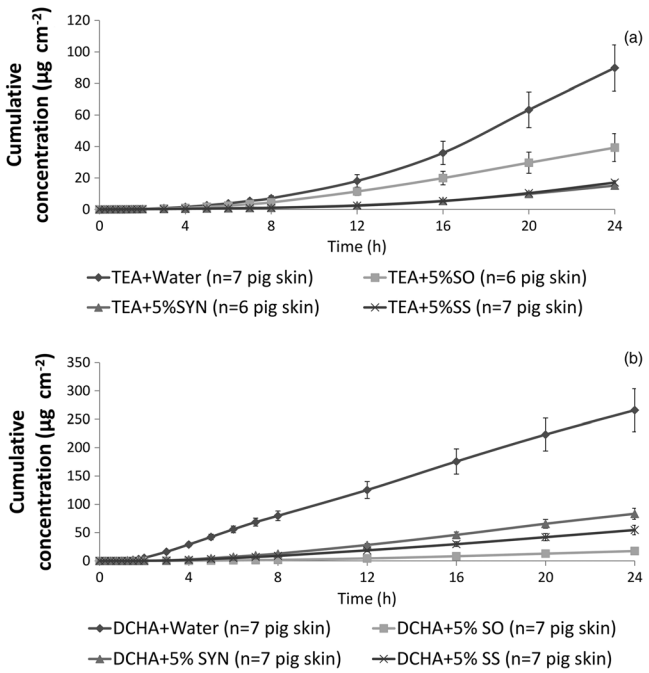

Figure 2. Absorption profiles of representative hydrophilic ethanolamines (a) and representative lipophilic amines (b). (a) Cumulative concentration ± SEM (μg cm^{-2}) of TEA in water and SO, SYN and SS types of metalworking fluids during a 24 h flow-through diffusion cell experiment. (b) Cumulative concentration ± SEM (μg cm^{-2}) of DCHA in water and SO, SYN and SS type of metalworking fluids during a 24 h flow-through diffusion cell experiment. DCHA, dicyclohexylamine; SO, soluble oil; SS, semisynthetic; SYN, synthetic; TEA, triethanolamine.

Table 2. Mean experimental (±SEM) absorption (μg) and permeability coefficient (cm h^{-1}) values of DBEA, DCHA and DPA in water and SO, SYN and SS type of metalworking fluids. The dose in each diffusion cell was 2500 μg

Compound	Vehicle	Absorption (μg)			Permeability coefficient (cm h^{-1})		
		Mean	SEM		Mean × 10^{-3}	SEM × 10^{-3}	
DBEA	Water	154.51	(17.41)	aA	4.38	(0.50)	aA
	SO	58.57	(4.34)	aC	2.00	(0.15)	aB
	SYN	83.89	(10.43)	aBC	2.57	(0.37)	aB
	SS	102.45	(17.05)	aB	3.19	(0.64)	aAB
DCHA	Water	170.21	(24.23)	aA	4.83	(0.61)	aA
	SO	11.14	(0.66)	bC	0.44	(0.02)	bC
	SYN	53.24	(6.23)	bB	1.74	(0.24)	bB
	SS	34.91	(5.41)	bBC	1.14	(0.21)	bBC
DPA	Water	118.77	(12.36)	aA	3.72	(0.39)	aA
	SO	14.46	(1.33)	bC	0.47	(0.05)	bB
	SYN	35.33	(2.75)	bB	1.07	(0.10)	bB
	SS	22.56	(2.90)	bBC	0.65	(0.09)	bB

DBEA, dibutylethanolamine; DCHA, dicyclohexylamine; DPA, diphenylamine; SO, soluble oil; SS, semisynthetic; SYN, synthetic. Means with the same letter are not significantly different ($P > 0.05$). Lower case letters indicate comparison of the means between test compounds within vehicles. Upper case letters indicate comparison of the means between vehicles within test compound.

As previously stated, the cumulative absorption profiles obtained for both the hydrophilic ethanolamines and the lipophilic amines (e.g., Fig. 2a,b) also showed the significantly highest absorption in water compared to other formulations.

The three lipophilic amines were all significantly less absorbed in soluble oil (relative to all the other vehicles) but no consistent pattern was obtained for the two other vehicles between these three compounds. Unlike the hydrophilic compounds, 50% of the final amount of the absorbed molecule crossed the skin approximately 13 h after dosing. The flux was very high for the first 4 h when it reached steady state (especially for DBEA and DCHA). Figure 3 demonstrates the distinct pattern differences seen in the concentration profiles between the lipophilic DBEA and the hydrophilic DEA when dosed in water.

Multiple Linear Regression

Multiple linear regression analyses were conducted to determine if the vehicle indicator, a coefficient attributed to each vehicle according to the absorption obtained with all the test compounds, could explain the variations obtained for the logKp. These regression analyses demonstrated that the physicochemical parameters used in the Abraham model (Abraham and Martins, 2004) may influence the permeability of these six test compounds in the various MWF formulations. Figure 4 shows the importance of the vehicle indicator influence on logKp. Indeed, the coefficient of determination increased from 0.54 (Fig. 4a) to 0.71 (Fig. 4b) when a vehicle indicator was incorporated into the model.

Discussion

Absorption and permeability coefficient data indicate that the largest hydrophilic ethanolamine (TEA) crossed the skin more readily than the smaller molecules (DEA and MEA), which was surprising. It has been demonstrated that MEA can be metabolized in the skin and incorporated into skin phospholipids (Klain *et al.*, 1985). However, if this were the driving force of the differences seen, the amount of radiolabel remaining in the skin following the MEA doses would be greater than the other two ethanolamines, which was not the case. The thermodynamic activity of these amines in the various formulations could explain the permeability differences between these formulations. In other words, the chemical partitioning and diffusion in skin can be affected by the chemical having a tendency to escape from the formulation site to a site of different activity (Ishii *et al.*, 2010; Megrab *et al.*, 1995).

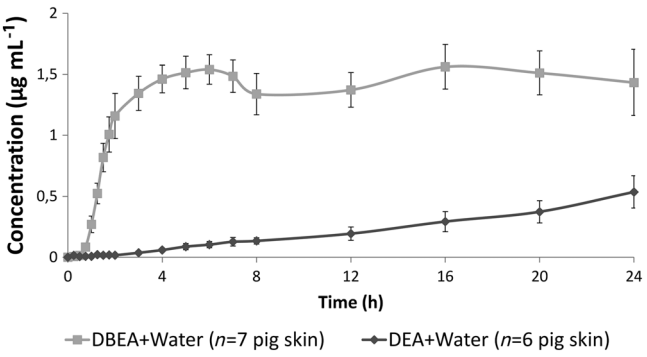

Figure 3. Concentration (µg ml^{-1}) of DEA and DBEA in water. Distinct patterns may be explained by the different logKow values. DBEA, dibutylethanolamine; DEA, diethanolamine.

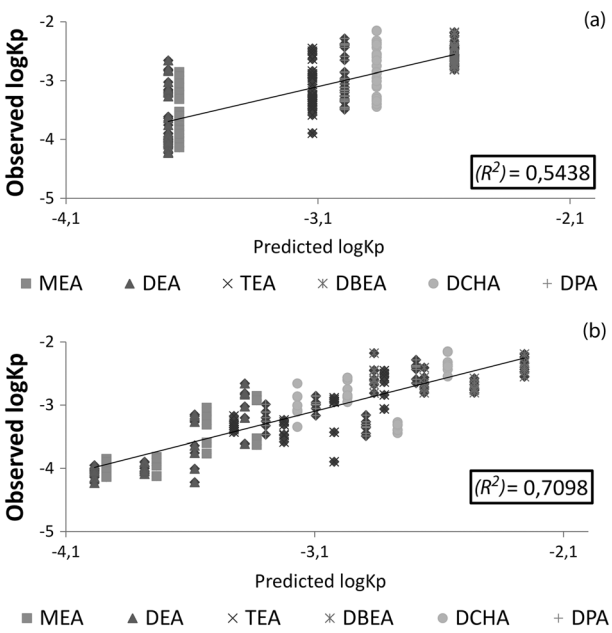

Figure 4. Test compound permeability in water and metalworking fluids using a simple Abraham multiple linear regression model (a) and adding a vehicle indicator to this model (b). Adding a coefficient for each vehicle leads to splitting the six lines (one for each tested molecule) into 24 lines (one for each vehicle for the six test compounds). The coefficient of determination (R^2) increases from 0.54 to 0.71, which indicates that predictions made using the model used to obtain (b) would be more robust. DBEA, dibutylethanolamine; DCHA, dicyclohexylamine; DEA, diethanolamine; DPA, diphenylamine; MEA, monoethanolamine; TEA, triethanolamine.

The obtained results showed no significant difference between the amount of MEA and TEA in the skin disks in soluble oil and semisynthetic types of MWFs, and the significant differences obtained with the two other vehicles showed, relative to TEA, a greater amount of MEA in the skin with water but a lower amount in skin with synthetic vehicle. Another explanation could be the speed of absorption: the percutaneous penetration of MEA appears to be relatively slow (Klain *et al.*, 1985), which could explain the greater amount of TEA, relative to the MEA, in the collected perfusate samples after 24 h.

There was no significant difference ($P > 0.05$) in absorption and permeability coefficient between the three lipophilic amines in water (Table 2), but they all showed a significantly greater absorption in water relative to the three hydrophilic ethanolamines (Fig. 1), which was surprising. Indeed, we might expect similar absorption and Kp for the three ethanolamines as they have similar chemical structures, notwithstanding their different hydrogen-bonding potentials. However, the significant differences seen between the two groups might be explained by the logarithm of the octanol–water partition coefficient (logKow or logP). Indeed, the logKow of MEA, DEA and TEA are all close to −1 (respectively −1.06, −1.08, −1.05) whereas DBEA, DCHA and DPA have logKow of 2.86, 3.69 and 2.97, respectively (logKow estimation by ACD/Percepta, ACD/Labs 2012 release, Advanced Chemistry Development, Inc.). These latter three compounds are less soluble in water and can more readily cross the skin compared to the three hydrophilic molecules that are more likely to stay in the water vehicle.

This study demonstrated that water is the vehicle that allows the greatest skin absorption for all the tested molecules, which was also demonstrated in the literature (Vijay *et al.*, 2007).

However, other studies demonstrated that the permeation is higher in synthetic, semisynthetic and finally in soluble oil type of MWFs (Vijay *et al*., 2009). We found this absorption pattern with the three lipophilic amines but not with the three hydrophilic ethanolamines. This difference is shown more clearly in Fig. 3, which represents the concentration ($\mu g\,ml^{-1}$) of DBEA and DEA in water. The logKow explained the lipophilic amines greater absorption in water and we could suppose that the hydrophilic ethanolamines are less absorbed because they remain in the water dose rather than partitioning into the stratum corneum. Thus, it would be interesting to conduct *in vivo* studies to validate these interactions between the skin and MEA, DEA and TEA. It would also be useful to conduct phospholipidomic studies to determine if there are different levels of incorporation of each ethanolamine, relative to the others, into the skin phospholipids in the presence of these MWF formulations.

Results showing the influence of physicochemical parameters used in the Abraham model on the permeability of the amines are consistent with our previous work (Vijay *et al*., 2007) where we demonstrated these quantitative structure–permeability relationships in similar MWF formulations. Moreover, the model used to obtain Fig. 4(b), i.e., the model incorporating a vehicle indicator variable, would be more robust in predicting the logKp of unknown molecules albeit, in a narrow chemical space as defined by the six amines used in this study. Data obtained in this study could be pooled with previous results obtained by our laboratory (Monteiro-Rivière *et al*., 2006; Vijay *et al*., 2009), and a predictive model could be designed to estimate logKp of unknown molecules in different vehicles. With this model, industries could rapidly evaluate if molecules can easily cross the skin in different types of MWF formulations and choose the safest additives, that is, the compounds having the lowest Kp, to protect the health of metalworking employees.

In summary, these flow-through diffusion cell experiments demonstrated that the six tested amines were significantly more absorbed when applied in water relative to the three generic Wb-MWF formulations, except for MEA in soluble oil (Fig. 1). DBEA absorption was most impacted (more than four-fold) by the semisynthetic formulation when compared to the other five amines. DCHA and DPA crossed the skin more readily with the synthetic MWF formulation relative to the other MWF formulations, particularly to the soluble oil MWF (Table 2). In general, the absorption of the three hydrophilic ethanolamines was most impacted by the soluble oil formulation (Table 1). Moreover, DBEA was the most significantly absorbed compound across all vehicles relative to all the other amines, except for DCHA in water (Fig. 1).

Two distinct absorption profiles were established, one for the hydrophilic ethanolamines and the other for the lipophilic amines (Fig. 2a,b). Indeed, their respective concentration vs. time profile patterns were very different, particularly in water (Fig. 3), which might be explained by their different logKow values (close to 3 for DBEA, DCHA and DPA; close to −1 for MEA, DEA and TEA).

As anticipated, absorption and skin retention of all of the amines were highest from the water doses. Of more interest to this discussion was the ANOVA examining the means of the three Wb-MWFs alone, without the water doses at 100%. The water doses acted as a baseline to which the other 95% water/5% MWF doses were compared, but water alone is not used in the metalworking industry. This ANOVA demonstrated significantly higher absorption, permeability, skin retention and penetration (skin + absorption) of the hydrophilic ethanolamines from the topical soluble oil formulation than from the synthetic and semisynthetic formulations; that is, for MEA, DEA and TEA: $SO > SS = SYN$. When an ANOVA was run on just the three Wb-MWFs with the lipophilic amines, without the water doses, the differences seen in the absorption and permeability of the lipophilic amines followed a converse pattern to the hydrophilic ethanolamines, but were not always significant: (DCHA: $SYN > SS$ SO); (DPA: $SYN > SS \geq SO$); (DBEA: $SS \geq SYN \geq SO$). These ANOVA results among the Wb-MFWs, without the water doses, are not included in Table 1 or Fig. 1.

As formation of a reservoir of molecules in the skin can lead to local skin toxicity, metalworking industries should avoid the use of soluble oil formulations when simple hydrophilic ethanolamines are used in the formulations as corrosion inhibitors and instead use synthetic or semisynthetic Wb-MWFs. If soluble oil formulations are used, these studies suggest that the lipophilic amines may be a safer choice.

However, dermatotoxicity experiments should be conducted on these six amines to test these hypotheses. According to the results obtained from toxicity tests (Monteiro-Rivière *et al*., 2006) and with validation *in vivo* studies, recommendations could be given to the metalworking industries to determine which compounds and MWF formulations could be safe and thus alter the formulation of MWFs to protect the health of metalworkers.

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Conflict of Interest

The Authors did not report any conflict of interest.

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