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IN VITRO DERMAL PENETRATION OF 4-CHLORO-3-METHYLPHENOL FROM COMMERCIAL METAL WORKING FLUID AND AQUEOUS VEHICLES

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The biocide 4-chloro-3-methylphenol (CMP, CAS number 59-50-7) is a common additive to metal-working fluids (MWF) and building materials. National Institute for Occupational Safety and Health (NIOSH) researchers previously identified and quantified CMP in a commercial water-soluble MWF, TRIM VX, and demonstrated irritancy and sensitization potential of both TRIM VX and CMP alone after dermal exposure in a murine model. In the current study, the in vitro human epidermal permeability of CMP contained in a working dilution of TRIM VX (20% in water) was evaluated and, for comparison, permeability from an aqueous buffer was also assessed. CMP penetration was also measured from transient exposures to 20% TRIM VX. To address differences in penetration rates from 20% TRIM VX and from buffer, the role of thermodynamic activity of CMP in the 2 vehicles on dermal penetration was investigated. Static headspace gas chromatography was used to measure vapor pressures and infer fractional thermodynamic activities of CMP in the mixtures. Permeability coefficient (k_p) of CMP from 20% TRIM VX was $(4.1 \pm 0.8) \times 10^{-3}$ cm/h (mean ± SD, $n = 5$), and CMP was found at a concentration of 3555 ± 191 μg/ml in this donor. In contrast, k_p was 0.18 ± 0.03 cm/h ($n = 5$) at a similar concentration (3919 ± 240) from buffer donor. Steady-state fluxes from 20% TRIM VX and buffer were comparable when expressed as functions of thermodynamic activity of CMP in the donor, rather than as concentrations. Transient (20 or 40 min) exposures of epidermal membranes to 20% TRIM VX ($n = 4$) resulted in total penetration of 4.2 ± 1.2 and 7.3 ± 0.8 μg/cm^2, respectively; these amounts are comparable to amounts predicted using a simple algebraic equation.

4-Chloro-3-methylphenol (CMP; Table 1) is a biocide and preservative used in a large number of commercial products and preparations such as metal-working fluids (MWF), construction materials, medicines, and adhesives (Dooms-Goossens et al., 1981; Archer & MacDonald, 1984; Lewis & Emmett, 1987; van Faassen et al., 1990; Rajpar et al., 2006). About 100 million gallons of MWF is produced annually in the United States and more than 1 million workers are exposed to MWF (NIOSH, 1998; ILMA, 2000). Research has demonstrated that some MWF exposures cause both allergic and irritant contact dermatitis (de Boer et al., 1989a; 1989b; Sprince et al., 1996). Because CMP was shown to be a sensitizer (Archer & MacDonald, 1984; Lewis & Emmett, 1987; Rajpar et al., 2006), it may be the crucial chemical species responsible for MWF-induced contact dermatitis. However, no information is available about CMP dermal penetration resulting from exposure to commercial MWF.

TRIM VX is a soluble oil MWF concentrate manufactured by Master Chemical Corporation, Perrysburg, OH. The Material Safety Data Sheet accompanying our purchase

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The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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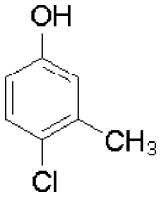
TABLE 1. Properties of 4-Chloro-3-methylphenol (CMP)

CAS number	Structure	MW	log K_{ow}	MP (°C)	BP (°C)	Sol (mg/ml)
59-50-7		142.6	3.10	67	235	5.45

Note. CAS number: Chemical Abstracts Service Registry Number; MW, molecular weight; log K_{ow}, base 10 logarithm of octanol–water partition coefficient; MP, melting point; BP, boiling point; Sol, solubility in buffer, 32°C (measured here). Information from Hazardous Substances Data Bank (http://toxnet.nlm.nih.gov/index.html).

of this product listed two components: petroleum oil 30–40% and triethanolamine 1–10%. TRIM VX is claimed to be nonirritating and not a sensitizer. Recently, NIOSH researchers identified, quantified and fractionated CMP in TRIM VX (Brown & Robinson, 2008). In an in vivo murine model, TRIM VX was identified as both a contact sensitizer and an irritant when tested in a combined local lymph node assay (Anderson et al., 2009). TRIM VX was determined to be the most potent sensitizer of the nine MWF tested in that study based on lymphocyte proliferation. Anderson et al., (2009) also found significant lymphocyte proliferation following dermal exposure to CMP alone. It is therefore important to better characterize the dermal penetration potential of this compound from MWF.

Previously, Vijay et al. (2009) measured penetration of CMP through pig skin from generic metal working fluid formulations and from two commercial fluids in which CMP was added (Vijay et al., 2007). The main purpose of the present studies was to evaluate human epidermal penetration of CMP following exposure to an in-use formulation of a commercial MWF. The presence and quantity of CMP in TRIM VX were determined. The steady-state in vitro human epidermal permeability of CMP was assessed from a recommended working dilution of TRIM VX and, for comparison, from an aqueous buffer vehicle. Results of CMP penetration from transient exposures of skin to TRIM VX are also presented. A secondary goal was to investigate the role of thermodynamic activity of CMP in the respective vehicles on penetration rates. In order to address differences in penetration rates from TRIM VX and from buffer vehicles, static headspace gas chromatographic analysis of CMP/buffer and CMP/TRIM VX mixtures was performed to assess fractional thermodynamic activity of CMP as a function of concentration in these two vehicles, and the skin permeability was also measured from TRIM VX fortified with additional CMP.

METHODS

Chemicals

Acetonitrile, phosphoric acid, sodium bicarbonate, HEPES, 4-chloro-3-methylphenol (99%), and 2-methyl-4,6-dinotrophenol were all analytical grade and purchased from Sigma-Aldrich (St. Louis, MO). Hanks balanced salt solution (HBSS) was purchased from Gibo-Invitrogen Corporation (Carlsbad, CA). TRIM VX (batch number 071607H) was purchased from Rite Way Tool Company (Pittsburgh, PA).

Buffer

Buffer consisted of HEPES-buffered HBSS (pH 7.4 at 37°C): 5.96 g HEPES was stirred into 1000 ml HBSS. Then 0.32 g $NaHCO_3$ and 0.05 g gentamicin sulfate were added. Buffer was filtered (Nalgene, 0.2 μm pore size) and degassed prior to use by warming to 40°C and stirring under lab vacuum.

Skin

Caucasian female (age range 16–46 yr) skin samples were obtained fresh from breast reduction (8 donors) or panniculectomy (1 donor) surgrical procedures from the West Virginia University Skin Bank. The Skin Bank maintains Human Subjects Review Board approval for all collections, but the specific use of this tissue in our experiments was deemed "not human subject research" and therefore not subject to approval. Skin was frozen (–85°C) on the same day as surgery and used within 154 d of storage.

Heat-separated epidermal membranes were used. The thawed skin was submersed in 60°C buffer for 45–60 s, and epidermis was teased from the dermis using cotton swabs. Skin disks were cut using a 5/8-inch-diameter stainless-steel punch. Visual inspection of each disk under dissecting microscope (20× power) was performed to eliminate any samples with obvious defects (holes).

Donor Solutions

TRIM VX was mixed with water to a 20% dilution (v/v). This is the highest concentration for in-use formulation recommended by the manufacturer. This dilution, herein referred to as 20% TRIM VX, was used for all studies described here. For comparisons, penetration studies were performed with CMP dissolved in buffer, and with 20% TRIM VX that was supplemented with additional CMP. The CMP donor concentrations are given in Table 2.

Static Headspace Gas Chromatography of Donor Solutions

It was postulated that differences in CMP penetration at similar concentrations from the two vehicles, buffer and 20% TRIM VX (see Results section), could be explained by differences in solution thermodynamics. A brief presentation of the theory appears in the Appendix.

CMP solutions in both buffer and 20% TRIM VX solvents were studied. Saturated CMP–buffer solutions were made by mixing excess CMP in buffer for 48 h at 32°C. (This temperature was selected to duplicate the skin surface temperature in our diffusion cell experiments.) In addition, a known concentration (3.90 mg/ml) was mixed to serve as a reference point. Creation of saturated CMP in 20% TRIM VX was problematic, as high levels of CMP led to instability in the TRIM VX/water emulsion. Greater than approximately 150 mg CMP added per ml 20% TRIM VX led to visible separation of the emulsion; therefore, this level is referred to here as the "saturated" concentration, with the understanding that true saturation could not be achieved in this solvent. Saturated solutions were prepared in triplicate. These were diluted with solvent (v/v) to achieve 20, 40, 60, and 80% saturated solutions. Pure buffer (0% saturation) and 20% TRIM VX with no added CMP were also studied. For the latter, this corresponds to 2.3% saturation (3.6 mg/ml CMP found in 20% TRIM VX, divided by 153.6 mg/ml). It should be noted that fortification of TRIM VX with additional CMP would not be performed in the

TABLE 2. CMP Fractional Thermodynamic Activity in Donor Vehicle (a/a_s), Skin Permeability (k_p), Lag Time (τ), Steady-State Flux, and Electrical Impedance (Z)

Donor vehicle	n	Donor [CMP] (μg/ml)	a/a_s	k_p (cm/h)	τ (h)	SS flux (μg cm^{-2} h^{-1})	Z_{pre} (kΩ)	Z_{post} (kΩ)
Buffer	5	384 ± 19	0.07	0.10 ± 0.03	0.09 ± 0.07	38.1 ± 9.4	25 ± 22	41 ± 40
Buffer	5	3919 ± 240	0.71	0.18 ± 0.03	0.17 ± 0.09	690 ± 109	27 ± 22	44 ± 37
20% TRIM VX	5	3555 ± 191	0.02	(4.1 ± 0.8)e-3	0.38 ± 0.14	14.6 ± 3.0	20 ± 7	30 ± 9
20% TRIM VX + *	4	17,922 ± 3345	0.11	(4.4 ± 1.2)e-3	0.26 ± 0.23	78.3 ± 16.9	—	—
20% TRIM VX + *	4	142,876 ± 29,556	0.84	(1.8 ± 0.1)e-2	0.22 ± 0.18	2589 ± 395	—	—

Note. Data presented as means ± SD. Asterisk indicates 20% TRIM VX fortified with additional CMP. Z_{pre}, Z_{post}: 10-Hz impedance measured before and after experimental exposure.

industrial setting; it was done here solely to investigate the role of thermodynamic activity in skin flux measurements.

Aliquots (0.5 ml) of the 32°C solutions were added to prewarmed 10-ml headspace vials. In addition, 0.5 ml of solid CMP (685 mg) was added to each of 3 headspace vials to obtain A_0 [Eq. (9) in Appendix]. Sample incubation and injections were automated using a CombiPal autosampler (CTC Analytics) and Varian 3800 gas chromatograph (GC) equipped with a flame ionization detector. Samples were incubated at 32°C for 2 h with intermittent agitation. Preliminary studies confirmed headspace equilibration with this incubation procedure. A 100-μl sample of headspace vapor was injected into the GC in splitless mode. The capillary column phase was trifluoropropylmethyl polysiloxane (RTX 200MS, Restek). The initial column temperature of 60°C was held for 1 min and then ramped to 280°C at 25°C/min, followed by a 4-min hold. The retention time for CMP was 7.6 min.

Diffusion Cell Studies

Vertical, static, "Franz-type" diffusion cells (PermeGear) were used. Receptor compartments (5 ml) were filled with warmed, degassed buffer. The diameter of exposed skin was 9 mm. The water-jacketed cells were maintained at 37°C via a recirculating water bath, which maintained 32°C at the skin surface. At time 0, approximately 0.5 ml of donor solution ("infinite" dose) was added to the diffusion cells, and donor compartments were covered with parafilm. Receptor fluid samples (0.5 ml) were taken at time 0 and at set times throughout the duration of the experiment, and this volume was replaced with fresh buffer.

Steady-State Exposures Donor solutions were applied to multiple (two or three) replicates from individual skin donors and were replaced each hour to maintain infinite dose conditions. Samples were taken at time 0, 0.5, and 1 h and at each subsequent hour for the 7-h duration of the experiment for CMP analysis. For buffer and 20% TRIM VX donors, 5 individual skin donors were used. For 20% TRIM VX supplemented with additional CMP, 4 individual donors were used.

The total amount of penetrated CMP as a function of time of exposure was calculated from the measured receptor concentrations for each skin disk. The average of the permeabilities and lag times for skin disks from each skin donor was taken as the value for that individual. Permeability coefficient (k_p) and lag time (τ) were calculated as described previously (Frasch & Barbero, 2005). Steady-state fluxes were calculated as the product of k_p and donor CMP concentration.

Transient Exposures In order to more realistically simulate occupational or environmental exposures and to test the predictions of a simple algebraic equation, epidermal membranes were transiently exposed to 20% TRIM VX donor. From each of $n = 4$ individual skin donors, 2 replicates were exposed ("infinite" dose) for either 20 or 40 min. At the end of the exposure period the donor was removed; skin samples were rapidly rinsed 4× with fresh buffer and then patted dry with cotton swabs. Receptor samples were taken at 10-min intervals for the first hour, then at 1.5, 2, 3, and 4 h, and analyzed for CMP. Thus, the CMP accumulation measured over the course of the experiment included that which penetrated during the exposure period, plus additional penetration from the skin reservoir remaining at the end of the exposure period.

Total mass accumulations measured at 4 h were compared with amounts predicted using a simple equation previously validated by us (Frasch & Barbero, 2008):

$$m = k_p A C_v t_{\text{exp}} \tag{1}$$

Here A is the area of skin exposed to chemical of concentration C_v and t_{exp} is the exposure period, here either 20 or 40 min. This equation predicts the asymptotic total mass accumulation for transient exposures to volatile compounds, and to nonvolatile compounds when the exposure time exceeds the lag time. The k_p values used for this calculation were derived from the steady-state exposures.

Skin Impedance The low-frequency impedances of epidermal membranes mounted in diffusion cells were measured before and after steady-state exposures of buffer–CMP and 20% TRIM VX donors in order to evaluate membrane integrity. Before the exposures, donor cells were filled with buffer. Ag–AgCl electrodes (World Precision Instruments) were placed in the donor and receptor compartments, and the membrane was placed in series with a 10.37-kΩ resistor and sine-wave generator (Sanford Research Systems DS345) set at a frequency of 10 Hz, 1000 mV peak-to-peak voltage. Voltages were measured between the donor and receptor compartments with a Tektronix TDS 360 oscilloscope. Buffer was removed from donor cells and membranes were patted dry with cotton swabs prior to donor exposure. After the exposures, skin and donor compartments were rinsed with fresh buffer, and the measurements were repeated.

CMP Analytical Method

A high-performance liquid chromatography (HPLC) method was developed for CMP quantification in receptor fluid and is described elsewhere (Zang et al., 2010). Briefly, the HPLC system was an Agilent 1100 series instrument with diode array detector and autosampler. The column was an Onyx C_{18}, 3.5 μm particle size, 100 mm × 4.6 mm (Phenomenex, Inc.). The mobile phase was 50% acetonitrile and 50% H_2O containing 0.1% H_3PO_4. 2-Methyl-4, 6-dinitrophenol was used as an internal standard. Quantification of CMP in diffusion-cell samples was done using internal standard (50 μl of 25 μg/ml), added to 450 μl of appropriately diluted sample. The responses of CMP and internal standard were measured in duplicate at their individual peak retention times, 5.56 and 6.67 min, respectively. Peak ratios were calculated and the amount of CMP was determined using calibration curves generated for each experiment at five different concentrations that spanned the range of measured concentrations.

RESULTS

Figure 1 shows measured fractional thermodynamic activity of CMP in both buffer and 20% TRIM VX ($n = 3$ each). Data are shown as functions of percent saturation in the respective vehicles. For CMP in buffer, the data are linear and signify that Henry's law holds for this solvent; that is, the partial pressure of CMP in buffer is directly proportional to its concentration in buffer. The activity measured from the known concentration of 3.90 mg/ml CMP in buffer corresponds, via linear interpolation, to a 71.5% saturated solution. Extrapolation of this to 100% saturation leads to a calculated solubility of CMP in buffer at 32°C of 5.45 mg/ml. For CMP in 20% TRIM VX, there is deviation from linearity at higher than approximately 60% saturation. This can be explained by the fact that true saturation was not achieved for CMP in 20% TRIM VX. As discussed in the Methods section, phase separation occurred at high CMP concentrations. For the CMP + 20% TRIM VX formulation labeled 100% saturation, this represents the point where visible separation of the emulsion occurred. The formulation phase separates before attaining maximum thermodynamic activity of CMP.

Estimation of fractional thermodynamic activities of donor CMP in the skin penetration experiments was performed by interpolation

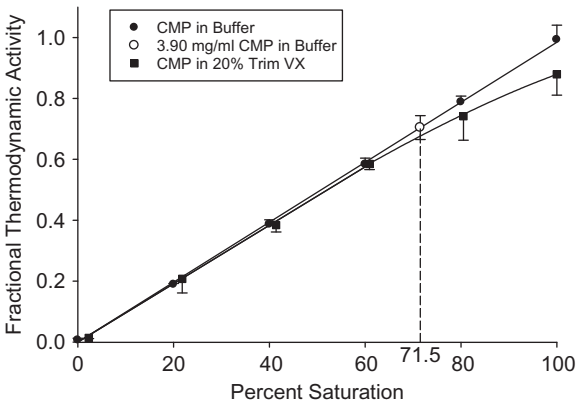


FIGURE 1. Fractional thermodynamic activity of CMP in buffer and in 20% TRIM VX. Known concentration of 3.90 mg/ml CMP in buffer provides a reference point and corresponds to 71.5% saturation. Data represent means and standard deviations of three independent measurements.

between measured values, and these are reported in Table 2.

Table 2 summarizes CMP penetration data from both buffer and 20% TRIM VX donor vehicles. Comparing the results from row 2 versus row 3, large differences in flux and hence k_p were observed despite similar CMP concentrations in the two vehicles (3.9 vs. 3.6 mg/ml). Flux from buffer was 47× that from 20% TRIM VX at these concentrations.

CMP in buffer at about 0.1× the higher concentration (0.38 vs. 3.9 mg/ml; row 1 vs. row 2) resulted in permeability of about 0.6 that from the higher concentration (0.10 vs. 0.18 cm/h), indicating the possibility of permeation enhancement or barrier degradation resulting from the high concentration of CMP in buffer.

In order to address differences in penetration rates at similar concentrations from 20% TRIM VX compared with buffer vehicle, studies were performed in which 20% TRIM VX was fortified with additional CMP. Comparing rows 3 and 4, similar k_p values were measured with a 5× increase in CMP donor concentration (17.9 vs. 3.6 mg/ml). Thus, flux increased directly with donor (CMP), but flux was still substantially less than from aqueous buffer with only approximately 20% of the donor concentration (row 4 vs. 2). When CMP was added to approach the saturation value for 20% TRIM VX vehicle (row 5), flux increased disproportionately, with k_p approximately 4× greater. Flux from this donor was nearly 4× greater than that from the highest concentration of CMP in buffer donor.

Time courses of in vitro epidermal penetration of CMP from 20% TRIM VX are shown in Figure 2. Figure 2A displays mass accumulation from unadulterated 20% TRIM VX, i.e., with no added CMP. Figures 2B and 2C show accumulation from 20% TRIM VX with added CMP.

Figure 3 shows the penetration time courses of CMP from aqueous buffer at two different donor concentrations.

The steady-state flux data are displayed in Figure 4. Figure 4A displays flux from both vehicles as a function of donor CMP concentration.

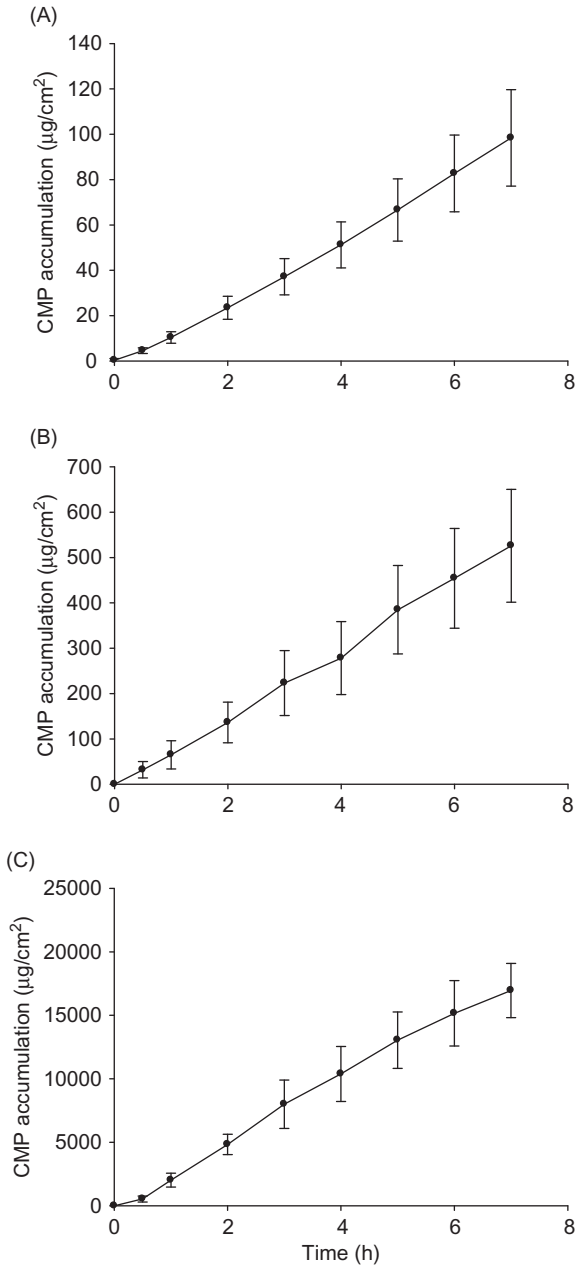


FIGURE 2. CMP mass accumulation from 20% TRIM VX donor. Displayed are means ± SDs, $n = 5$ (A) or 4 (B and C). (A) Unadulterated donor, mean [CMP] = 3555 μg/ml. (B) Donor + added CMP, mean [CMP] = 17,922 μg/ml. (C) Donor + added CMP, mean [CMP] = 142,876 μg/ml.

Figure 4B shows the same results, but expressed as a function of fractional thermodynamic activity of CMP in the two vehicles. The large disparity in penetration rates in Figure 4A is substantially reduced when activity, rather than concentration, is the metric for chemical

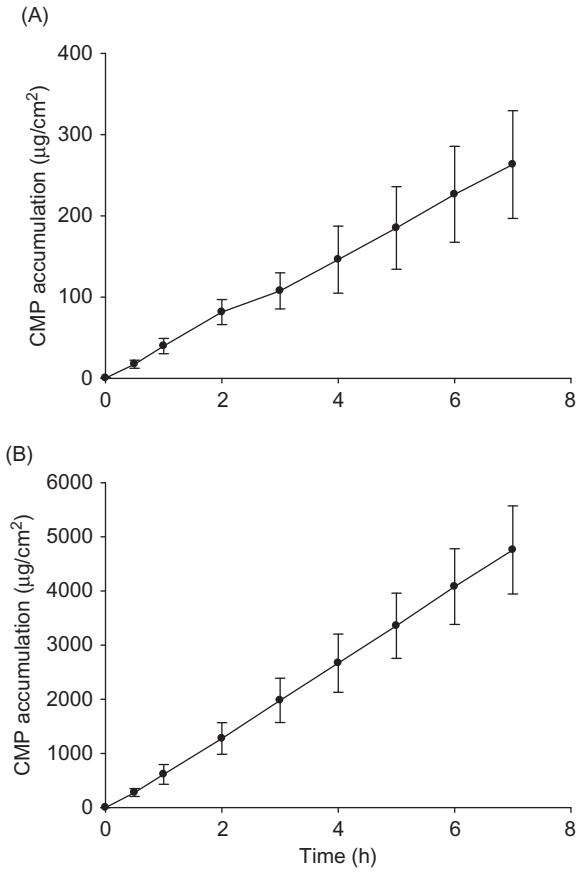


FIGURE 3. CMP mass accumulation from aqueous (buffer) donor. Displayed are means ± SDs, $n = 5$. (A) Mean donor [CMP] = 384 μg/ml. (B) Mean donor [CMP] = 3919 μg/ml.

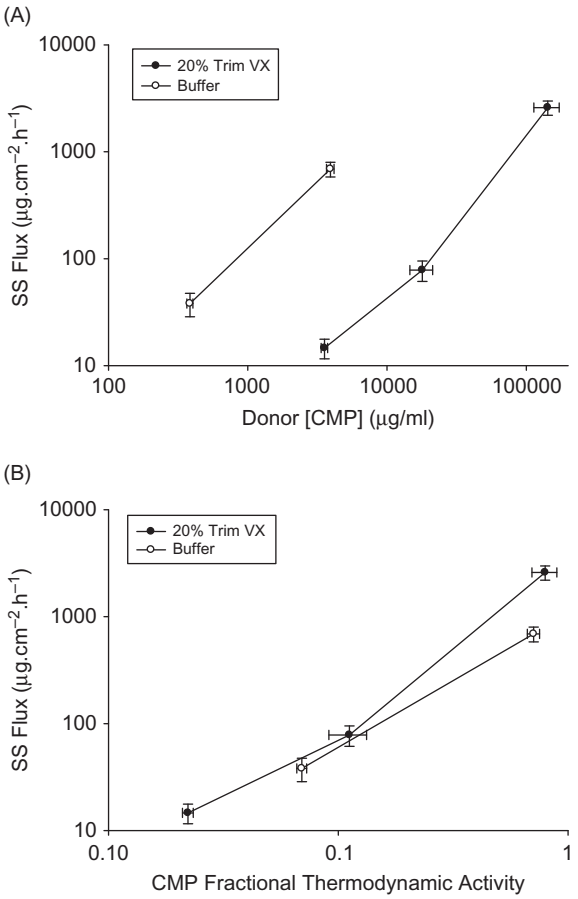


FIGURE 4. Steady-state CMP fluxes from two vehicles, buffer and 20% TRIM VX. (A) Flux as function of donor CMP concentration. (B) Flux as function of fractional thermodynamic activity of CMP in the respective donor vehicles.

driving force. This result is considered further in the Discussion section.

Data on CMP penetration from transient exposures to 20% TRIM VX are presented in Table 3 and Figure 5. Figure 5 demonstrates that penetration of CMP continues to occur well after the exposure period, even though skin surfaces were thoroughly rinsed after the exposures. This is understood on the basis that CMP in the skin at the end of the exposure period is still available for penetration; this "skin reservoir" supplies additional CMP after the exposure ceases, and needs to be considered in dermal risk assessments of occupational and environmental exposures to this chemical. The measured values of total penetration from transient exposures were not statistically significantly different from the amounts predicted using Eq. (1) (Table 3).

DISCUSSION

In these investigations the compound 4-chloro-3-methylphenol was identified and quantified in a commercial metal-working fluid, TRIM VX. A 20% mixture of TRIM VX

TABLE 3. Mass Accumulation From Transient Exposure to 20% TRIM VX

t_{exp} (min)	Measured (μg/cm²)	Predicted (μg/cm²)
20	4.2 ± 1.2	4.9 ± 1.2
40	7.3 ± 0.8	9.7 ± 2.4

Note. Data presented are means ± SD, $n = 4$; $p > .1$ (not significant) for measured vs. predicted comparisons (unpaired t-test). t_{exp}: Exposure time. Measured: Total CMP accumulation at 4 h. Predicted: Predicted total mass accumulation using Eq. (1), with k_p and C_v obtained from the steady-state experiments (Table 2).

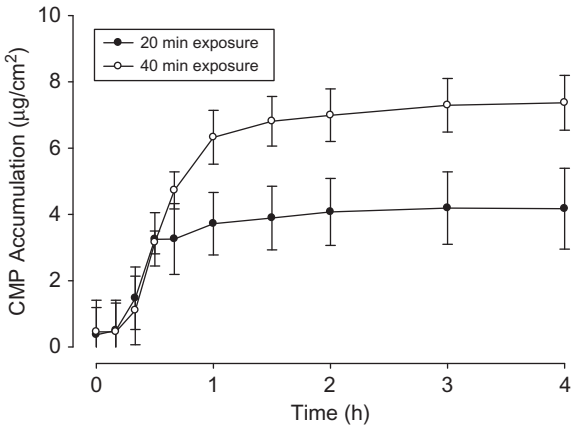


FIGURE 5. CMP mass accumulation from 20% TRIM VX applied as transient exposures; $n = 4$ each.

in water, the highest working concentration recommended by the manufacturer, contains CMP at a concentration of 3.6 mg/ml. At this dilution, penetration of CMP through human epidermal membranes was observed with a permeability coefficient of 4.1×10^{-3} cm/h and a lag time of 0.38 h.

An equivalent concentration of CMP in buffer yielded a substantially greater k_p of 0.18 cm/h. Stated differently, steady-state flux from buffer was much greater than that from 20% TRIM VX for a given concentration (Figure 4A). These results are consistent with those of Vijay et al. (2007, 2009), who also found lower penetration rates for biocides (including CMP) in generic MWF formulations compared with water. This may seem odd at first, especially considering that MWF typically contain solvents, detergents, surfactants, and emulsifiers that could potentially serve as penetration enhancers. Specifically, TRIM VX was shown to contain oleic acid and α-terpineol (Anderson et al., 2009), both of which are potent penetration enhancers (Green et al., 1988; Williams & Barry, 1992; Sapra et al., 2008).

To understand these results, it is useful to consider the driving force for diffusion. Generally this is thought of as the chemical concentration gradient, but when comparing different vehicles, it is more appropriate to consider instead the gradient in chemical potential (Higuchi, 1960; Barry et al., 1985a, 1985b). For the steady state, it can be shown (Appendix) that flux is proportional to thermodynamic activity. Steady-state flux of a chemical from different solvents will be equal at equivalent activities, assuming there are no interactions of either solvent or solute with the membrane. Although the data (Figure 4) here are somewhat sparse, they support the use of thermodynamic activity as the metric for comparison of flux data from different vehicles. In Figure 4B, it is apparent that thermodynamic activity does not entirely explain the flux data: Flux from 20% TRIM VX is significantly greater than that from buffer at CMP activity approaching 1. It appears that 20% TRIM VX plus CMP leads to an increase in permeability under this extreme tested condition.

These data serve as a reminder that permeability coefficients are vehicle specific (Theeuwes et al., 1976). It is incorrect to use the k_p of a compound measured from one vehicle to estimate penetration of that compound from a different vehicle. Instead, the two k_p values can be related through the relative solubility of the compound. Because maximal activity occurs at the solubility limit of the solute in its respective solvent, the following relationship may be useful as a first approximation in estimating relative k_p values:

$$k_{p,2} = k_{p,1}\frac{S_1}{S_2} \tag{2}$$

Penetration rates are frequently reported from aqueous donor, and numerous models exist that can predict permeability coefficients from aqueous donor (NIOSH, 2010). The present data suggests that penetration of CMP from a complex mixture such as metal-working fluid can be predicted from these simple systems. As an example of the use of Eq. (2), taking our k_p measured from an aqueous vehicle (~0.14 cm/h), along with solubility of 5.45 mg/ml, leads to a predicted k_p from 20% TRIM VX of 5×10^{-3} cm/h, reasonably close to our measured k_p of 4.1×10^{-3} cm/h.

This equation holds in cases where the skin membrane is not modified by the presence of the vehicle or by the solute–vehicle

system. The higher permeability coefficient measured from 20% TRIM VX fortified with high concentration of CMP (Table 2) indicates permeation enhancement or barrier disruption; consequently, use of Eq. (2) would not be accurate without the inclusion of some sort of "damage factor." If Eq. (2) were valid over the entire concentration ranges studied here, the two curves in Figure 4B would directly overlap.

Others reported on the dermal penetration of CMP. Vijay et al. (2009) measured k_p of 0.030 cm/h for CMP in water in dermatomed pig skin, and much lower values, comparable to ours, for CMP added to generic MWF donor vehicles (range 2.9×10^{-3}–5.8×10^{-3} cm/h, depending on the formulation). The aqueous permeability results reported here are two- to threefold greater than the value 0.055 cm/h found by Roberts et al. (1977), also using human epidermis. Their measured lag time of 0.28 h compares favorably with those measured here (Table 2). While these authors reported no damage to skin at any concentration up to saturation, our data showed some evidence for damage, with k_p increasing 1.8× from low to high concentration. Membrane degradation, however, is not supported by the impedance measurements reported here. There are clear trends toward increasing impedance following exposures for both buffer and 20% TRIM VX vehicles; however, the differences listed in Table 2 are not significant and therefore no conclusive statements can be drawn from these data. Overall, the differences in skin penetration here compared with other studies and other species are in accord with what one typically finds in studies in vitro (Barbero & Frasch, 2009).

In this study, the time course following transient exposures to 20% TRIM VX was measured. The total asymptotic mass accumulation resulting from these exposures can be predicted using a simple algebraic equation [Eq. (1)]. This equation is not a function of the membrane lag time; instead, it requires knowledge only of permeability coefficient and exposure duration. Data presented here demonstrate that Eq. (1) may be useful in predicting total skin penetration resulting from transient or short-term exposures. A thorough theoretical analysis of the transient dermal exposure has been presented elsewhere (Frasch & Barbero, 2008).

To summarize, the biocide 4-chloro-3-methylphenol was quantified in a commercial metal-working fluid, TRIM VX. The in vitro human epidermal absorption of this compound was measured from a normal working dilution (20% in water) of TRIM VX, and for comparison from an aqueous vehicle. Data demonstrated that steady-state fluxes from the two donor vehicles are comparable at similar activities in the solvent but not at similar concentrations. Finally, CMP accumulation was measured from short-term or transient exposures to 20% TRIM VX and it was found that predictions of a simple algebraic equation compare favorably with the total penetrated amounts.

REFERENCES

Anderson, S. E., Brown, K. K., Butterworth, L. F., Fedorowicz, A., Jackson, L. G., Frasch, H. F., Beezhold, D., Munson, A. E., and Meade, B. J. 2009. Evaluation of irritancy and sensitization potential of metalworking fluids and components. *J. Immunotoxicol*. 6: 19–29.

Archer, C. B., and MacDonald, D. M. 1984. Chlorocresol sensitivity induced by treatment of allergic contact dermatitis with steroid creams. *Contact Dermatitis* 11: 144–145.

Barbero, A. M., and Frasch, H. F. 2009. Pig and guinea pig skin as surrogates for human in vitro penetration studies: A quantitative review. *Toxicol. In Vitro* 23:1–13.

Barry, B. W., Harrison, S. M., and Dugard, P. H. 1985a. Correlation of thermodynamic activity and vapor diffusion through human skin for the model compound, benzyl alcohol. *J. Pharm. Pharmacol*. 37:84–90.

Barry, B. W., Harrison, S. M., and Dugard, P. H. 1985b. Vapor and liquid diffusion of model penetrants through human skin—Correlation with thermodynamic activity. *J. Pharm. Pharmacol*. 37:226–235.

Brown, K. K., and Robinson, K. 2008. High-performance liquid chromatography identification, quantification, and fractionation of a suspect allergen, 4-chloro-3-methylphenol, in an LLNA-positive metalworking fluid. *Tribol. Trans*. 51:101–106.

Cussler, E. L. 1984. *Diffusion: Mass transfer in fluid systems*, pp. 180–181. Cambridge: Cambridge University Press.

de Boer, E. M., van Ketel, W. G., and Bruynzeel, D. P. 1989a. Dermatoses in metal workers. (I). Irritant contact dermatitis. *Contact Dermatitis* 20:212–218.

de Boer, E. M., van Ketel, W. G., Bruynzeel, D. P., 1989b. Dermatoses in metal workers. (II). Allergic contact dermatitis. *Contact Dermatitis* 20:280–286.

Dooms-Goossens, A., Degreef, H., Vanhee, J., Kerkhofs, L., and Chrispeels, M. T. 1981. Chlorocresol and chloracetamide: Allergens in medications, glues, and cosmetics. *Contact Dermatitis* 7:51–52.

Frasch, H. F., and Barbero, A. M. 2005. Application of solid-phase microextraction to in vitro skin permeation experiments: Example using diethyl phthalate. *Toxicol. In Vitro* 19:253–259.

Frasch, H. F., and Barbero, A. M. 2008. The transient dermal exposure: theory and experimental examples using skin and silicone membranes. *J. Pharm. Sci*. 97:1578–1592.

Green, P. G., Guy, R. H., and Hadgraft, J. 1988. In vitro and in vivo enhancement of skin permeation with oleic and lauric acids. *Int. J. Pharm*. 48:103–111.

Higuchi, T. 1960. Physical chemical analysis of percutaneous absorption process with creams and ointments. *J. Soc. Cosmet. Chem*. 2:85–97.

Independent Lubricant Manufacturers Association. 2000. 1999 Volume survey: Report on the volume of lubricants manufactured in the United States and Canada by independent lubricant manufacturers in 1999. Alexandria, VA: Independent Lubricant Manufacturers Association.

Kolb, B. 1980. Physiochemical applications for headspace gas chromatography. In *Applied headspace gas chromatography*, ed. B. Kolb, pp. 1–11. London: Heyden & Son Ltd.

Lewis, P. G., and Emmett, E. A. 1987. Irritant dermatitis from tri-butyl tin oxide and contact allergy from chlorocresol. *Contact Dermatitis* 17:129–132.

National Institute for Occupational Safety and Health. 1998. *Criteria for a recommended standard: Occupational exposure to metalworking fluids*. NIOSH Publication #98-102. Cincinnati: NIOSH.

National Institute for Occupational Safety and Health. 2010. *Skin permeation calculator*. http://www.cdc.gov/niosh/topics/skin/skinPermCalc.html.

Rajpar, S. F., Foulds, I. S., Abdullah, A., and Maheshwari, M. 2006. Severe adverse cutaneous reaction to insulin due to cresol sensitivity. *Contact Dermatitis* 55:119–120.

Roberts, M. S., Anderson, R. A., and Swarbrick, J. 1977. Permeability of human epidermis to phenolic compounds. *J. Pharm. Pharmacol*. 29:677–683.

Sapra, B., Jain, S., and Tiwary, A. 2008. Percutaneous permeation enhancement by terpenes: mechanistic view. *The AAPS Journal*. 10:120–132.

Sprince, N. L., Palmer, J.A., Popendorf, W., Thorne, P. S., Selim, M. I., Zwerling, C., and Miller, E. R. 1996. Dermatitis among automobile production machine operators exposed to metal-working fluids. *Am. J. Ind. Med*. 30:421–429.

Theeuwes, F., Gale, R. M., and Baker, R. W. 1976. Transference: A comprehensive parameter governing permeation of solutes through membranes. *J. Membr. Sci*. 1: 3–16.

van Faassen, I., Verweij-van Vught, A. M., Lomecky-Janousek, M. Z., Razenberg, P. P., and van der Veen, E. A. 1990. Preservatives in insulin preparations impair leukocyte function. In vitro study. *Diabetes Care* 13:71–74.

Vijay, V., White, E. M., Kaminski, M. D., Jr., Riviere, J. E., and Baynes, R. E. 2009. Dermal permeation of biocides and aromatic chemicals in three generic formulations of metalworking fluids. *J. Toxicol. Environ. Health A* 72:832–841.

Vijay, V., Yeatts, J. L., Jr., Riviere, J. E., and Baynes, R. E. 2007. Predicting dermal permeability of biocides in commercial cutting fluids using a LSER approach. *Toxicol. Lett*. 175:34–43.

Welty, J. R., Wicks, C. E., and Wilson, R. E. 1984. *Fundamentals of momentum, heat, and mass transfer*, 3rd ed., pp. 480–481. New York: John Wiley & Sons.

Williams, A. C., and Barry, B. W. 1992. Skin absorption enhancers. *Crit Rev. Ther. Drug Carrier Syst*. 9:305–353.

Zang, L.-Y., Barbero, A. M., and Frasch, H. F. 2010. A rapid HPLC analysis for dermal penetration: The case study of 4-chloro-3-methylphenol (CMP) from metal working fluid Trim VX. *Open Anal. Chem. J*. 4:10–17.

APPENDIX: THERMODYNAMIC ACTIVITY AND SKIN PERMEATION

This analysis assumes that there are no effects of vehicle or solute on the dermal membrane barrier. Under ideal sink conditions, the steady-state flux (J_{ss}) of a chemical species through a homogeneous membrane of thickness h is proportional to its concentration in the donor vehicle (C_v):

$$J_{ss} = K_{mv} C_v D / h \tag{3}$$

where D is effective diffusivity and K_{mv} is membrane–vehicle partition coefficient. Fluxes from different vehicles at the same concentration will in general differ, owing to different membrane–vehicle partition coefficients. When comparing fluxes from different vehicles, or when using nonideal donor solutions, it is instructive to keep in mind that the generalized driving force for diffusion is the chemical potential gradient rather than the concentration gradient (Cussler, 1984; Welty et al., 1984). For this case, the thermodynamic activity (a) of the chemical can be related to its concentration C:

$$a = \gamma C \tag{4}$$

where γ is the activity coefficient, assumed here to be independent of C. The thermodynamic equilibrium condition requires that a in the donor solution be equal to a just within the membrane surface in contact with the donor. Then

$$(\gamma C)|_{membrane} = (\gamma C)|_{vehicle} \tag{5}$$

or

$$\frac{C_m}{C_v} = \frac{\gamma_v}{\gamma_m} = K_{mv} \tag{6}$$

Equation (6) and Eq. (4) in Eq. (3) lead to:

$$\begin{aligned} J_{ss} &= K_{mv} C_v D / h = \gamma_v C_v D / (\gamma_m h) \\ &= a_v D / (\gamma_m h) \end{aligned} \tag{7}$$

If we assume γ_m is constant for a given chemical species (once the molecules enter the membrane, their behavior is not dependent on the donor vehicle), it can be seen that steady-state flux of a chemical is directly proportional to its thermodynamic activity in the vehicle. This theory can be tested by comparing fluxes of a chemical species from different vehicles at similar thermodynamic activities.

Static headspace gas chromatography (SHSGC) can be used to measure thermodynamic activities of volatile chemicals in solutions (Kolb, 1980), and this approach has been employed by Barry et al. (1985a, 1985b) in dermal penetration studies. Under equilibrium conditions, the thermodynamic activity of a chemical species in solution is identical to the activity of the vapor phase above the solution. Thus, the following relationship holds:

$$\frac{P}{P_0} = \frac{a}{a_s} \tag{8}$$

Here P is the partial pressure of the species in the vapor phase, and P_0 and a_s are the vapor pressure and activity of the pure component. In SHSGC, the chromatographic peak area (A) produced by a volatile solute is proportional

to its partial pressure in the head space vapor. Thus,

$$\frac{A}{A_0} = \frac{P}{P_0} = \frac{a}{a_s} \tag{9}$$

where A_0 is the area produced by the pure component. Note that neither a nor a_s is directly determined here; instead, the ratio of the two is measured. This ratio corresponds here to the activity of the CMP solution relative to that of pure solid CMP at 32°C.

The analysis requires identical headspace and GC conditions for all measurements and also requires that the injected amount be far less than the column's capacity.