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SURFACTANT EFFECTS ON SKIN ABSORPTION OF MODEL ORGANIC CHEMICALS: IMPLICATIONS FOR DERMAL RISK ASSESSMENT STUDIES

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Occupational and environmental exposures to chemicals are major potential routes of exposure for direct skin toxicity and for systemic absorption. The majority of these exposures are to complex mixtures, yet most experimental studies to assess topical chemical absorption are conducted neat or in simple aqueous vehicles. A component of many industrial mixtures is surfactants that solubilize ingredients and stabilize mixtures of oily components when present in aqueous vehicles. The purpose of this series of experiments was to use two well-developed experimental techniques to assess how solution interactions present in a pure nonbiological in vitro system (membrane coated fibers, MCF) compare to those seen in a viable ex vivo biological preparation (isolated perfused porcine skin flap, IPPSF). Two widely encountered anionic surfactants, sodium lauryl sulfate (SLS) and linear alkylbenzene sulfonate (LAS), were studied in 10% solutions. The rank orders of absorption were: water: pentachlorophenol (PCP) > 4-nitrophenol (PNP) > parathion > fenthion > simazine > propazine; SLS: PNP > PCP > parathion > simazine > fenthion > propazine; and LAS: PNP > PCP > simazine > parathion > fenthion > propazine. For all penetrants, absorption was greater in SLS compared to LAS mixtures, a finding consistent with smaller micelle sizes seen with SLS. For these low-water-solubility compounds, absorption was greater from aqueous solutions in nearly every case. The inert three-fiber MCF array predicted absorptive fluxes seen in the ex vivo IPPSF, suggesting lack of any biological effects of the surfactants on skin.

Topical exposure to chemicals in occupational and environmental scenarios continues to be a major potential route of exposure both for direct skin toxicity as well as for their systemic absorption. The majority of these exposures are to complex mixtures. In contrast, most experimental studies designed to assess topical chemical absorption are conducted in simple aqueous vehicles or with neat test chemicals. Numerous studies demonstrated vehicle effects on chemical absorption and have probed potential mechanisms for these effects (Bliss, 1939; Idson, 1983; Sloan et al., 1986; Qiao et al., 1996; Cross et al., 2001; Riviere et al., 2002, 2007; Rosado et al., 2003). Other studies quantified the effect of mixture interactions in quantitative structure–permeability relationships (QSPeR) (Riviere & Brooks, 2005, 2007; Baynes et al., 2008; Gregoire et al., 2008). There is little doubt that vehicles and formulation components modulate transdermal delivery of topically applied solutes.

A component of many industrial mixtures is surfactants that solubilize ingredients and stabilize mixtures of oily components when present in aqueous vehicles. In addition to being employed in detergents and in many industrial processes, and hence present in occupational and environmental chemical exposures, they also are specifically used as formulation ingredients in cosmetics and topical

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drugs (Hargreaves, 2003; Lavoue et al., 2003; Somasundaran et al., 2004; Khan et al., 2006; Augustijns & Brewster, 2007). The physical chemical actions of such compounds are well described and can be experimentally assessed using in vitro approaches. However, surfactants were also implicated as being cutaneous irritants (Wilhelm et al., 1991; deJong et al., 2007) that may alter a chemical's dermal absorption after topical application through biological mechanisms within the skin. A number of studies assessed the potential impact of surfactants on the dermal absorption of specific chemicals but did not attempt to tease out the different potential mechanisms of action that would directly impact the nature of experimental models required to quantify these effects.

The purpose of this series of experiments was to use two well-developed experimental techniques, sensitive to different aspects of surfactant action, to assess how solution interactions present in a pure nonbiological in vitro system compare to those seen in a viable ex vivo biological preparation. Two anionic surfactants widely used in commerce with a high potential for occupational exposure were studied. The relationship between a surfactant's activity in nonbiological and that in biological systems needs to be probed and understood before such data are incorporated into risk assessment models. This information is also important in developing QSPeR models since the nature of a surfactant's interaction with chemical penetrants must be defined to assess how they can be applied in mixture-interaction models.

MATERIAL AND METHODS

Study Chemicals

Penetrants Radiolabeled [^{14}C]-fenthion-ring-UL (specific activity = 15 mCi/mmol; purity 99%) and [^{14}C]-propazine-ring-UL (specific activity = 15 mCi/mmol; purity 99%) were obtained from American Radiolabeled Chemical (St. Louis, MO). Radiolabeled [^{14}C]-parathion-ring-UL (specific activity = 9.2 mCi/mmol; purity 96%), [^{14}C]-4-nitrophenol-UL (specific activity = 6.4 mCi/mmol; purity 99%), [^{14}C]-pentachlorophenol-UL (specific activity = 11.9 mCi/mmol; purity 96%), and [^{14}C]-simazine-ring-UL (specific activity = 15.5 mCi/mmol; purity 99%) were obtained from Sigma Chemical (St. Louis, MO). The analytical-grade reagents 4-nitrophenol (PNP), pentachlorophenol (PCP), propazine, simazine, fenthion, and parathion were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water was obtained from the in-house laboratory water purification system (Pure Water Solutions, Hillsborough, NC).

Surfactants Sodium lauryl sulfate (SLS; sodium dodecyl sulfate; CAS number 151-21-3; purity 99%) was obtained from Sigma Chemical (St. Louis, MO). Linear alkylbenzene sulfonate (LAS; dodecylbenzenesulfonic acid, sodium salt; CAS number 25155-30-0; technical grade) was obtained from Aldrich Chemical (Milwaukee, WI).

Experimental Design

These studies involved two separate series of experiments: determining dermal absorption of six chemicals topically applied in water and two surfactant solutions on an isolated perfused tissue model, and determining partitioning of the same six chemicals and mixtures into an array of inert membrane coated fibers. Table 1 lists the six penetrants and their physical chemical properties studied in these experiments. Figure 1 depicts their structures. All penetrants were applied in topical solutions consisting of either water, water + 10% SLS (sodium lauryl sulfate), or water + 10% LAS (linear alkylbenzene sulfonate), reflecting functional surfactant levels potentially encountered in occupational and consumer exposure scenarios. Table 2 confirms that the surfactant concentrations employed in these exposures greatly exceeded the critical micelle concentrations for these surfactants, indicating a level where clear surfactant actions are observed.

Isolated Perfused Porcine Skin Flap (IPPSF) Studies

The isolated perfused porcine skin flap (IPPSF) is a single-pedicle, axial-pattern, tubed skin flap obtained from the abdomen of female

TABLE 1. Identity and Physicochemical Properties of Penetrants Investigated in the IPPSF

Marker	Molecular mass (g/mol)	Melting point (°C)	Literature $LogK_{ow}$	log D ($pH_{6.0}$)	log D ($pH_{7.4}$)	Water solubility (mg/L)	Number of H-bond donors	Number of H-bond Acceptors
PNP	139.11	114	1.91	1.65	1.27	11600	1	4
PCP	266.34	174	5.12	3.66	2.43	14	1	1
Parathion	291.26	6.1	3.83	3.62	3.62	11	0	6
Fenthion	278.33	7.5	4.09	3.95	3.95	7.5	0	3
Propazine	229.71	213	2.93	2.93	2.93	8.6	2	5
Simazine	201.66	226	2.18	2.23	2.23	6.2	2	5

Note. PNP = *p*-nitrophenol; PCP = pentachlorophenol.

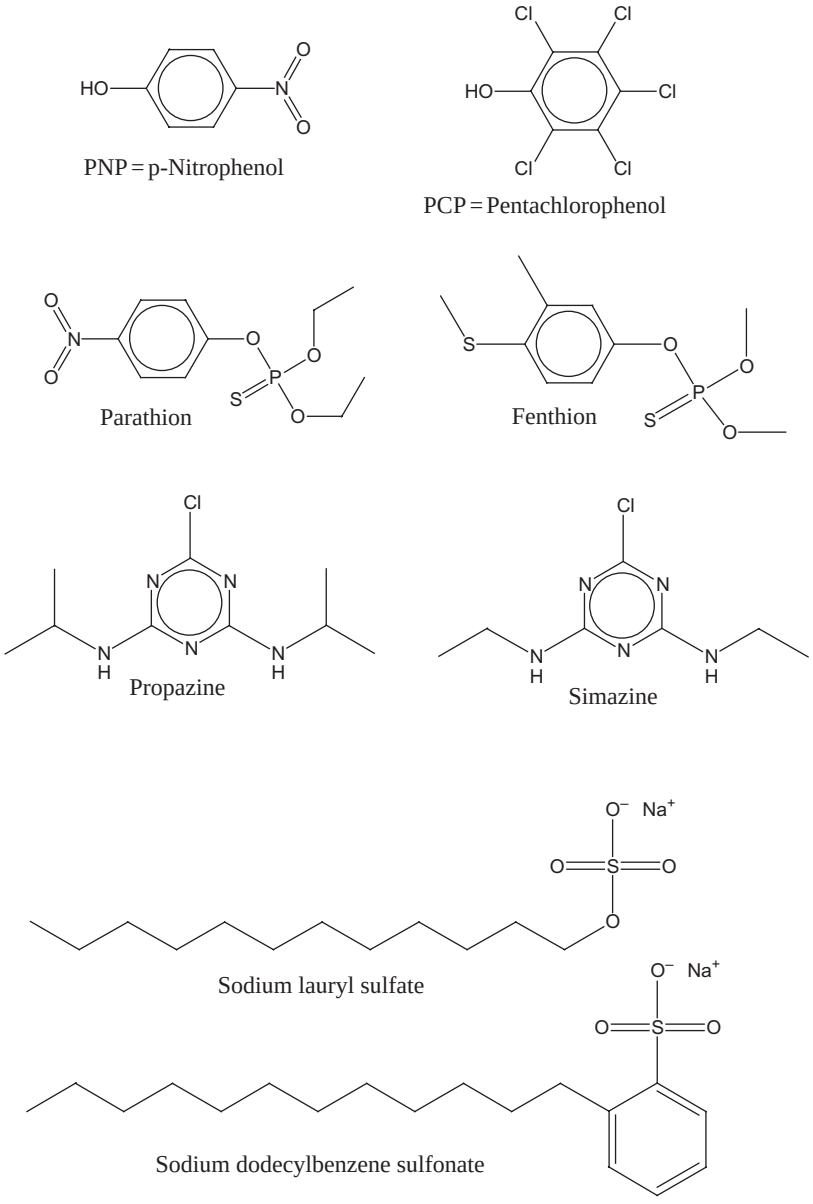

FIGURE 1. Chemical structures of the six marker compounds and two surfactants.

TABLE 2. Literature Critical Micelle Concentrations and Studied Mixture Concentrations

Surfactant	Literature CMC (mg/L)	Literature CMC (m*M*)	Our mixture conc. (mg/L)	Our mixture conc. (m*M*)
SLS	2300–2500	8.2–8.6	100,000	350
LAS	400–1400	1.2–4.0	100,000	290

Note. SLS = sodium lauryl sulfate = sodium dodecyl sulfate; LAS = linear alkylbenzene sulfonate = sodium 2-dodecylbenzene sulfonate; CMC = critical micelle concentration.

weanling Yorkshire pigs (*Sus scrofa*) that was previously shown to be predictive of in vivo human absorption (Riviere & Monteiro-Riviere, 1991; Wester et al., 1998). Two flaps per animal, each lateral to the ventral midline, were created in a single surgical procedure. The procedure involved surgical creation of the flap (measuring 4 cm × 12 cm) perfused primarily by the caudal superficial epigastric artery and its associated paired venae comitantes, followed by arterial cannulation and harvest in 48 h (Bowman et al., 1991). The IPPSF was then transferred to a perfusion apparatus that is a custom-designed temperature- and humidity-regulated chamber. Perfusion medium consisted of a modified Krebs Ringer buffer with bovine serum albumin. Normal perfusate flow was maintained at 1 ml/min/flap (3–7 ml/min/100 g) with a mean arterial pressure ranging from 30 to 70 mm Hg, settings consistent with in vivo values reported in the literature. Viability for up to 24 h was confirmed through biochemical studies and extensive light and transmission electron microscopy studies (Monteiro-Riviere et al., 1987). These techniques are fully described in the literature (Riviere et al., 1986; Riviere & Monteiro-Riviere, 1991).

Following arterial cannulation, and a 1-h predose perfusion period to determine skin flap viability, a flexible dosing template measuring 1 cm × 5 cm (Stomahesive, ConvaTec-Squibb, Princeton, NJ) was affixed to the skin surface with Skin Bond (Smith and Nephew, Inc., Largo, FL) to provide a dosing surface area of 5 cm^2. Doses of 100 µl of each mixture were applied to each dose site providing a surface concentration of approximately 10 µg/cm^2 of each ^{14}C-labeled marker compound. Perfusate samples (3 ml) were collected every 15 min for the first 2 h, and then every 30 min until termination at 8 h. Arterial perfusate samples were collected hourly and compared with venous samples to determine glucose utilization to assess continued skin flap viability. At the end of the 8-h perfusion, the dosing template was removed, and the dose area was swabbed with cotton swabs containing a soap solution (1% Ivory Liquid, Procter & Gamble, Cincinnati, OH), then tape-stripped 12 times (Scotch Magic Tape, 3M Corporation, St. Paul, MN). The dosed skin, the skin around the dose area, and the fat tissues were separated and digested in Biosol (National Diagnostics, Atlanta, GA) before further analysis as described later. The dosing template, surface swabs, glove fingertips, and leaks during skin flap dissection were extracted with ethanol for mass balance purposes. The tape strips were dissolved in ethyl acetate. The measurements analyzed were skin surface, stratum corneum, dosed skin, total skin, fat, total absorption, penetration, and area under the curve (AUC). Stratum corneum was the percentage of ^{14}C activity detected in the tape strips. Representative samples of 1 ml for the perfusate samples, dosing template, surface swabs, stratum corneum tape strips, skin, fat, and mass balance samples were pipetted into Bioscint liquid scintillation cocktail (National Diagnostics, Atlanta, GA) and counted on a Packard model 1900TR liquid scintillation counter (Packard Chemical Co., Downers Grove, IL).

Membrane-Coated Fiber (MCF) Partition Coefficients

The membrane-coated fiber (MCF) system was developed as an in vitro approach to experimentally determine partition coefficients from common vehicles or mixtures. Determination of partition coefficients using membrane-coated fibers is described in detail elsewhere (Xia et al. 2003, 2004; Yeatts et al. 2008). Results showed that a linear combination of three MCF partition coefficients is predictive of dermal absorption through skin from a chemical mixture (Baynes et al., 2008; Riviere et al., 2007). Basically, 6 fibers coated

with one of 3 membranes, polydimethylsiloxane (PDMS), polyacrylate (PA), or carbowax (CW), were immersed in one of the aforementioned mixtures for up to 24 h. Starting at approximately 18 h, the MCF were removed one at a time over 6 time points and transferred into the injector of a gas chromatograph (GC) for analysis, thus eliminating the need for an additional extraction step. The marker compounds that were partitioned into the membrane were thermally desorbed into the GC injector and a GC/mass spectroscopy (MS) spectrum was acquired. A chemical desorbed from the membrane was detected as a peak in the GC/MS spectrum and quantified by its peak area in the spectra against the calibration standard acquired under the same GC/MS conditions.

The partition coefficient (K) was calculated by using the following equation:

$$K = C_{me} / C_{de} = n^0 V_d / V_m (V_d C_0 - n^0) \quad (1)$$

where n^o was the amount of marker permeated into the membrane, C_o was the initial concentration of the given marker in the donor solution, V_d was the volume of the donor solution, V_m was the volume of the membrane, C_{me} was the equilibrium concentration in the membrane ($C_{me} = n°/V_m$), and C_{de} was the equilibrium concentration in the donor solution ($C_{de} = C_o V_d - n°/V_d$).

The volume of the membrane was calculated using the membrane diameter, the fiber core diameter, and the length of the membrane. The membrane length of all the membranes was 10 mm. The PDMS fiber core diameter was 0.11 mm, membrane diameter 0.3 mm, and membrane volume 0.652 µl. The PA fiber core diameter was 0.11 mm, membrane diameter 0.28 mm, and membrane volume 0.56 µl. The CW fiber core diameter was 0.16 mm, membrane diameter 0.26 mm, and membrane volume 0.451 µl.

The initial donor volume was 20 ml in all cases. The initial donor concentrations (ng/µl) varied depending upon solubilities of the markers in the water or water plus surfactant, as well as the individual membrane's ability to absorb the marker.

TABLE 3. Penetrant Concentrations at 100% Water Solubility Used for Micelle Size Determinations

Compound name	Concentration in 10%SDS (µg/ml):	Concentration in 10%LAS (µg/ml)
PNP	11,308	11,446
PCP	14	14
Parathion	11	11
Fenthion	7.5	7.5
Propazine	8.6	8.6
Simazine	6.2	6.2

Note. PNP = *p*-nitrophenol; PCP = pentachlorophenol.

Micelle Size Measurements

Surfactant micelles were assessed to confirm surfactant activity and assess micelle size in the exposure solutions. Stock solutions were gravimetrically prepared and diluted to approximately 100% of the water solubility value in 10% SLS or 10% LAS. The final concentrations used are shown in Table 3. Micelle sizes were measured on a Zetasizer Nano ZS system (Malvern Instruments Ltd., Malvern, Worcestershire, UK) equipped with a 633-nm laser. The Zetasizer Nano ZS system reports micelle sizes present in the mixture and the percentage of each micelle size. The most dominant size of micelle based on percent of total was reported.

Calculations and Statistics

Standard errors were determined for all data sets. IPPSF absorbtion was defined as the total percent detected in the perfusate for the entire 8-h perfusion period. Penetration was defined as the total amounts detected in the perfusate and all the skin and fat tissues, but not the stratum corneum. AUC (%D-h/ml) was calculated using the trapezoidal rule, wherein the mean of the concentration of two adjacent time points was multiplied by the elapsed time of those two samples and added to the remaining means. Analysis of variance with significance level at .05 was carried out using SAS 9.1 for Windows (SAS Institute, Cary, NC).

Multiple linear regression analysis (SAS 9.1) was used to define the relationship between

MCF log K versus the observed IPPSF AUC. Each mixture was tested separately with the three types of membrane-coated fibers (PDMS, PA, and CW) that yielded a partition coefficient for each mixture. These partition coefficients were used as the independent parameters to predict the observed log AUC using the model:

$$\log \mathrm{AUC} = i + a(\log K_{\mathrm{PDMS}}) + b(\log K_{\mathrm{PA}}) + c(\log K_{\mathrm{CW}}) \quad (2)$$

where log AUC is the area under the curve (percent dose-h/ml) of the observed IPPSF flux profiles; the log K terms are the partition coefficients for the PDMS-, PA-, and CW-coated fibers, respectively; i is the intercept; and a, b, and c are regression coefficients that reflect the relative contribution of each fiber partition coefficient to log AUC. Goodness of fit was assessed by r^2 and adjusted r^2. It was previously shown that a linear regression of the three log K_{MCF} partition coefficients is predictive of skin absorption from a mixture (Baynes et al., 2008; Riviere et al., 2007).

RESULTS

Figure 2 depicts the IPPSF perfusate flux profiles for the six penetrants in the three application mixtures. These data can be more easily compared by the histograms in Figure 3 and tabulations in Table 4. Recoveries ranged from 45 to 82% with most loss expected to be due to volatility. The rank orders of actual absorption across solvents were: water: PCP > PNP > parathion > fenthion > simazine > propazine; SLS: PNP > PCP > parathion > simazine > fenthion > propazine; and LAS: PNP > PCP > simazine > parathion > fenthion > propazine. Table 5 lists the ratios of the 10% SLS and 10% LAS values normalized by the water values.

For all penetrants in the IPPSF, absorption, peak flux, and penetration were greater in SLS mixtures than in LAS mixtures, although this was not always a statistically significant difference. Except for simazine, absorption, peak flux, and penetration were greater in water than in either surfactant mixture. Because simazine did not follow this basic pattern of water > SLS > LAS, 5 IPPSF replicates of each simazine dose were run, as compared to 3 replicates of the other 15 doses, in order to eliminate the possibility of experimental error contributing to this anomaly. These replicates of the simazine doses confirmed the absorption and penetration pattern SLS > water > LAS, while the peak flux followed the same water > SLS > LAS pattern seen in the other five test compounds. In examining the absorption profiles in Figure 2, the difference in simazine flux seems to be related to an earlier and enhanced peak concentration for LAS and a prolonged flux in SLS exposure not seen with the other compounds.

Table 6 lists the log K_{PDMS}, the log K_{PA}, and the log K_{CW} of the various membranes with respect to the markers in the three mixtures. Note that the same anomaly of SLS versus LAS absorption seen with the IPPSFs is also seen in the PDMS and PA partition coefficient data, suggesting that this effect is related to solution chemistry and not biological interactions in the skin. In the case of CW, both propazine and simazine had a reverse pattern from the other four penetrants relevant to surfactant effects. Table 7 lists the statistics and regression coefficients resulting from multiple linear regression analysis using single, double, and triple log K_{MCF} values. The MCF-predicted versus IPPSF-observed AUC, using the three log K_{MCF} partition coefficients, is shown in Figure 4. Table 8 lists the size of the SLS and LAS micelles measured at 22ºC and at the IPPSF perfusion conditions of 32ºC. In both cases, LAS micelles were twice the mean diameter of those seen in SLS.

DISCUSSION

Surfactants are common additives often encountered in topical environmental and occupational exposure scenarios. When making risk assessments, the U.S. Environmental Protection Agency often bases its dermal

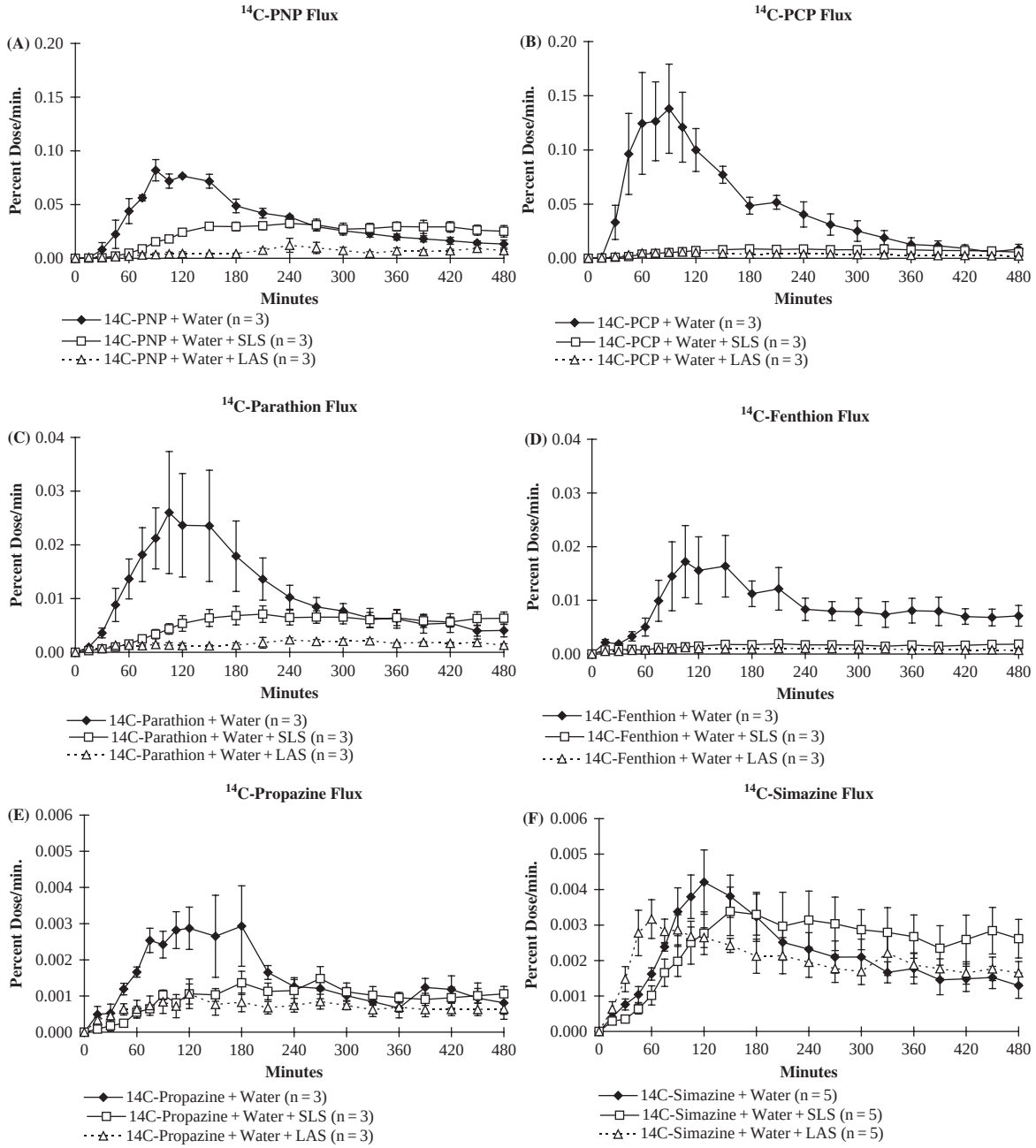

FIGURE 2. Mean (SEM) IPPSF flux profiles (percent dose/min) following topical doses of (A) *p*-nitrophenol, (B) pentachlorophenol, (C) parathion, (D) fenthion, (E) propazine, and (F) simazine in water, 10% SLS, and 10% LAS.

absorption estimates on data obtained from aqueous exposures without surfactants present (U.S. EPA, 2004). For the relatively lipophilic compounds studied, absorption from a vehicle containing a surfactant was less than that seen from an aqueous vehicle, supporting the U.S. EPA's risk assessment being a worst-case scenario for lipophilic compounds such as the pesticides studied here. Environmental exposures to surfactants are likely at much lower rates than the 10% surfactant scenarios used in this study.

These studies also demonstrate that an inert system such as the MCF three-fiber array

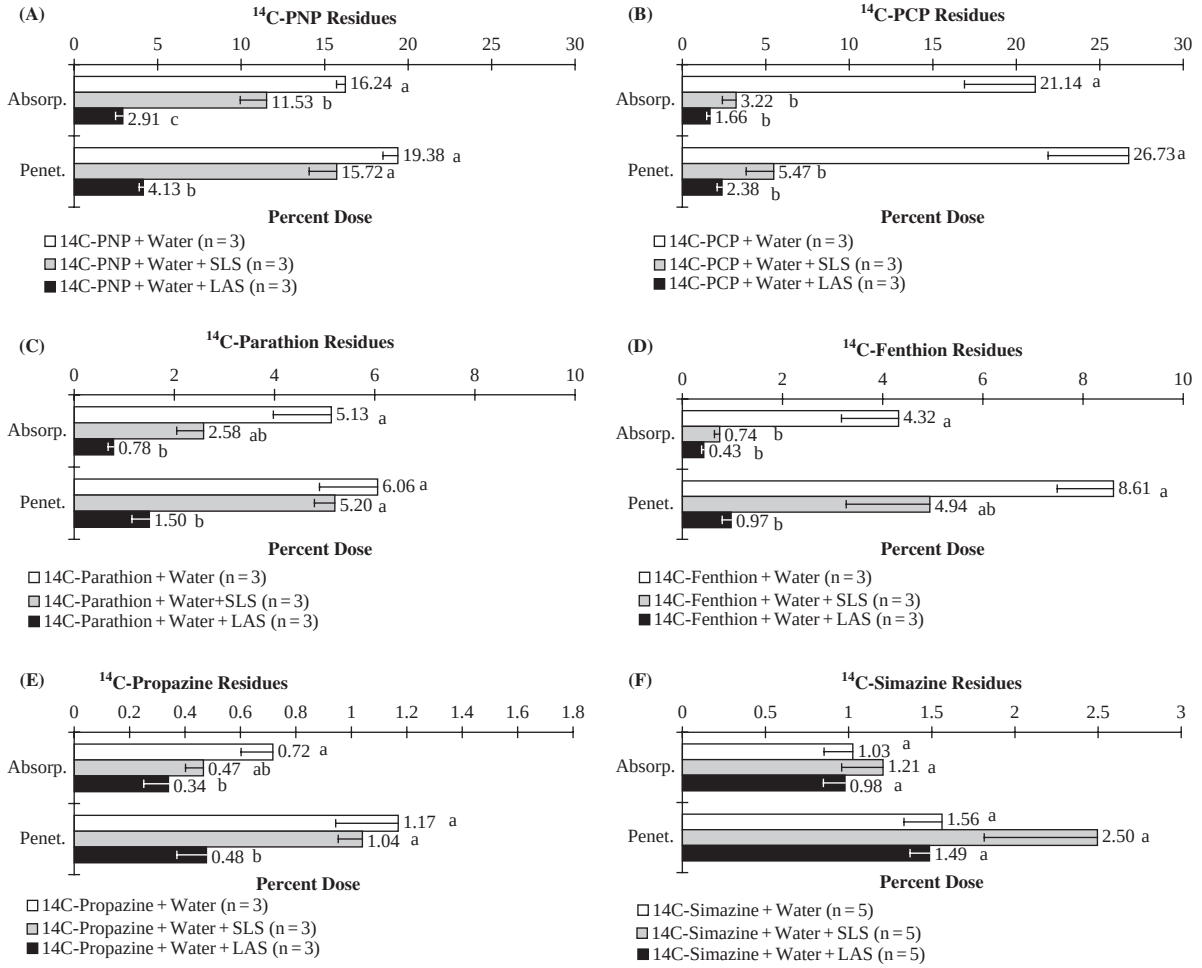

FIGURE 3. Mean (SEM) IPPSF percent dose absorption and penetration residues following topical doses of (A) *p*-nitrophenol, (B) pentachlorophenol, (C) parathion, (D) fenthion, (E) propazine, and (F) simazine in water, 10% SLS, and 10% LAS.

is capable of predicting surfactant effects on dermal absorption of the study compounds, suggesting that physicochemical interactions in the dosing mixture determine ultimate absorption (Figure 4). The present study suggested that any biological interaction between surfactant and skin tissue did not alter absorption of these compounds. The ability of the MCF system to predict mixture interactions was previously shown with other mixtures containing vehicles in addition to surfactants (Riviere et al., 2007; Baynes et al., 2008). This system may be conceptually viewed as representing a three-dimensional partition coefficient. However, for such predictions to be optimal, three physicochemically distinct membranes were required to reflect the diversity of chemical interactions seen between vehicles and skin.

Relative to water, absorption from SLS was greater than LAS in both systems for all compounds studied except for simazine (Table 5). Both of these anionic surfactants have a 12-carbon chain length, which was suggested to be optimal for surfactant enhancement of some compounds in skin after topical application (Cooper and Berner, 1984). As seen in Figure 1, the only difference in structure between SLS and LAS is the aromatic group in the head region of LAS. As seen in Table 8, SLS micelles are uniformly half the diameter of LAS micelles with penetrants having no further effect on their size. The most likely explanation

TABLE 4. Mean (SEM) IPPSF Residues Following Topical Doses of Mixtures in Water, 10% SLS, and 10% LAS

Mixture	Absorption (percent dose)		Peak flux (%D/min)		Time to peak (h)		Surface (percent dose)		Stratum corneum (percent dose)		Penetration (percent dose)		AUC (%D-h/ml)	
PNP + water ($n = 3$)	16.24	(0.53)^{a}	0.0855	(0.0073)^{a}	1.67	(0.08)^{b}	24.9	(4.05)^{a}	0.40	(0.09)^{ab}	19.38	(0.89)^{a}	0.277	(0.008)^{a}
PNP + water + SLS ($n = 3$)	11.53	(1.58)^{b}	0.0352	(0.0033)^{b}	4.50	(1.04)^{ab}	16.98	(4.19)^{a}	0.57	(0.15)^{a}	15.72	(1.64)^{a}	0.189	(0.025)^{b}
PNP + water + LAS ($n = 3$)	2.91	(0.43)^{c}	0.0151	(0.0050)^{c}	5.00	(1.26)^{a}	14.00	(3.82)^{a}	0.13	(0.04)^{b}	4.13	(0.24)^{b}	0.047	(0.007)^{c}
PCP + water ($n = 3$)	21.14	(4.24)^{a}	0.1475	(0.0424)^{a}	1.67	(0.44)^{b}	26.11	(10.60)^{a}	0.91	(0.18)^{a}	26.73	(4.83)^{a}	0.366	(0.071)^{a}
PCP + water + SLS ($n = 3$)	3.22	(0.82)^{b}	0.0103	(0.0018)^{b}	4.00	(1.04)^{a}	14.97	(1.47)^{a}	0.45	(0.25)^{a}	5.47	(1.65)^{b}	0.053	(0.013)^{b}
PCP + water + LAS ($n = 3$)	1.66	(0.21)^{b}	0.0059	(0.0014)^{b}	1.67	(0.08)^{b}	21.89	(2.54)^{a}	0.39	(0.19)^{a}	2.38	(0.29)^{b}	0.028	(0.004)^{b}
Parathion + water ($n = 3$)	5.13	(1.16)^{a}	0.0283	(0.0102)^{a}	1.83	(0.36)^{b}	17.48	(3.66)^{a}	0.19	(0.09)^{ab}	6.06	(1.16)^{a}	0.087	(0.020)^{a}
Parathion + water + SLS ($n = 3$)	2.58	(0.54)^{ab}	0.0074	(0.0016)^{b}	4.33	(0.83)^{ab}	17.07	(3.85)^{a}	0.38	(0.04)^{a}	5.20	(0.41)^{a}	0.042	(0.009)^{b}
Parathion + water + LAS ($n = 3$)	0.78	(0.11)^{b}	0.0027	(0.0006)^{b}	4.67	(0.93)^{a}	17.63	(3.20)^{a}	0.04	(0.02)^{b}	1.50	(0.35)^{b}	0.013	(0.002)^{b}
Fenthion + water ($n = 3$)	4.32	(1.14)^{a}	0.0194	(0.0062)^{a}	2.50	(0.58)^{a}	8.47	(1.93)^{b}	0.30	(0.05)^{a}	8.61	(1.12)^{a}	0.072	(0.019)^{a}
Fenthion + water + SLS ($n = 3$)	0.74	(0.10)^{b}	0.0022	(0.0002)^{b}	5.00	(1.50)^{a}	15.39	(6.21)^{b}	0.18	(0.07)^{ab}	4.94	(1.67)^{ab}	0.012	(0.002)^{b}
Fenthion + water + LAS ($n = 3$)	0.43	(0.04)^{b}	0.0013	(0.0001)^{b}	2.42	(0.79)^{a}	49.03	(6.73)^{a}	0.09	(0.03)^{b}	0.97	(0.18)^{b}	0.007	(0.001)^{b}
Propazine + water ($n = 3$)	0.72	(0.11)^{a}	0.0034	(0.0009)^{a}	2.00	(0.52)^{a}	40.93	(9.95)^{a}	0.13	(0.01)^{a}	1.17	(0.23)^{a}	0.012	(0.002)^{a}
Propazine + water + SLS ($n = 3$)	0.47	(0.06)^{ab}	0.0016	(0.0002)^{ab}	3.50	(0.50)^{a}	21.36	(3.78)^{b}	0.13	(0.10)^{a}	1.04	(0.09)^{a}	0.008	(0.001)^{ab}
Propazine + water + LAS ($n = 3$)	0.34	(0.09)^{b}	0.0011	(0.0003)^{b}	2.67	(0.67)^{a}	49.86	(4.35)^{ab}	0.04	(0.01)^{a}	0.48	(0.11)^{b}	0.006	(0.001)^{b}
Simazine + water ($n = 5$)	1.03	(0.17)^{a}	0.0046	(0.0008)^{a}	1.65	(0.10)^{b}	58.50	(9.27)^{a}	0.32	(0.08)^{a}	1.56	(0.23)^{a}	0.017	(0.003)^{a}
Simazine + water + SLS ($n = 5$)	1.21	(0.25)^{a}	0.0039	(0.0006)^{a}	3.60	(0.40)^{a}	36.46	(4.37)^{b}	0.12	(0.05)^{b}	2.50	(0.68)^{a}	0.020	(0.004)^{a}
Simazine + water + LAS ($n = 5$)	0.98	(0.13)^{a}	0.0037	(0.0005)^{a}	2.20	(0.88)^{ab}	24.74	(5.70)^{b}	0.05	(0.01)^{b}	1.49	(0.12)^{a}	0.016	(0.002)^{a}

Note. PNP = *p*-nitrophenol; PCP = pentachlorophenol. Means with the same superscript letter are not significantly different.

TABLE 5. Ratios of Mean IPPSF Residues Normalized by Water Values Following Topical Doses of Mixtures in Water, 10% SLS, and 10% LAS

Mixture	Absorption (percent dose)	Peakflux (%D/min)	Time to peak (h)	Surface (percent dose)	Stratum corneum (percent dose)	Penetration (percent dose)	AUC (%D-h/ml)
PNP + water + SLS ($n = 3$)	0.71	0.41	2.70	0.68	1.43	0.81	0.68
PNP + water + LAS ($n = 3$)	0.18	0.18	3.00	0.56	0.34	0.21	0.17
PCP + water + SLS ($n = 3$)	0.15	0.07	2.40	0.57	0.50	0.20	0.15
PCP + water + LAS ($n = 3$)	0.08	0.04	1.00	0.84	0.43	0.09	0.08
Parathion + water + SLS ($n = 3$)	0.50	0.26	2.36	0.98	2.01	0.86	0.48
Parathion + water + LAS ($n = 3$)	0.15	0.10	2.55	1.01	0.22	0.25	0.15
Fenthion + water + SLS ($n = 3$)	0.17	0.11	2.00	1.82	0.60	0.57	0.17
Fenthion + water + LAS ($n = 3$)	0.10	0.06	0.97	5.79	0.29	0.11	0.10
Propazine + water + SLS ($n = 3$)	0.65	0.47	1.75	0.52	1.00	0.89	0.63
Propazine + water + LAS ($n = 3$)	0.47	0.32	1.33	1.22	0.30	0.41	0.46
Simazine + water + SLS ($n = 5$)	1.18	0.86	2.18	0.62	0.39	1.60	1.15
Simazine + water + LAS ($n = 5$)	0.95	0.81	1.33	0.42	0.15	0.95	0.94

Note. PNP = *p*-nitrophenol; PCP = pentachlorophenol.

TABLE 6. Mean (SEM) log K_{PDMS}, log K_{PA}, and log K_{CW} of the Various Membranes With Respect to the Markers in the Three Mixtures

	log K_{PDMS}			log K_{PA}			log K_{CW}		
PNP + water	0.17	$(0.10)^a$	$n = 6$	2.04	$(0.06)^a$	$n = 6$	2.01	$(0.02)^a$	$n = 6$
PNP + water + SLS	–0.47	$(0.04)^b$	$n = 6$	1.14	$(0.02)^b$	$n = 4$	0.27	$(0.04)^b$	$n = 5$
PNP + water + LAS	–0.49	$(0.07)^b$	$n = 6$	1.28	$(0.06)^b$	$n = 6$	0.16	$(0.03)^c$	$n = 5$
PCP + water	1.07	$(0.04)^a$	$n = 5$	3.72	$(0.04)^a$	$n = 6$	2.80	$(0.02)^a$	$n = 6$
PCP + water + SLS	–0.25	$(0.02)^b$	$n = 6$	1.38	$(0.04)^b$	$n = 6$	1.46	$(0.02)^b$	$n = 6$
PCP + water + LAS	–0.17	$(0.02)^b$	$n = 6$	1.37	$(0.01)^b$	$n = 6$	1.28	$(0.03)^c$	$n = 6$
Parathion + water	3.12	$(0.03)^a$	$n = 5$	4.06	$(0.06)^a$	$n = 6$	3.74	$(0.03)^a$	$n = 6$
Parathion + water + SLS	0.55	$(0.04)^b$	$n = 6$	1.50	$(0.02)^b$	$n = 7$	1.34	$(0.03)^b$	$n = 6$
Parathion + water + LAS	0.30	$(0.02)^c$	$n = 6$	1.24	$(0.08)^c$	$n = 6$	0.96	$(0.03)^c$	$n = 6$
Fenthion + water	3.53	$(0.02)^a$	$n = 5$	4.29	$(0.04)^a$	$n = 6$	4.30	$(0.03)^a$	$n = 6$
Fenthion + water + SLS	0.63	$(0.04)^b$	$n = 6$	1.59	$(0.03)^b$	$n = 7$	1.52	$(0.02)^b$	$n = 6$
Fenthion + water + LAS	–0.22	$(0.05)^c$	$n = 5$	0.62	$(0.07)^c$	$n = 6$	0.14	$(0.05)^c$	$n = 6$
Propazine + water	1.92	$(0.05)^a$	$n = 5$	2.66	$(0.04)^a$	$n = 6$	2.31	$(0.02)^a$	$n = 6$
Propazine + water + SLS	0.16	$(0.06)^b$	$n = 4$	0.53	$(0.06)^b$	$n = 3$	–0.50	$(0.15)^c$	$n = 5$
Propazine + water + LAS	0.11	$(0.03)^b$	$n = 6$	0.61	$(0.09)^b$	$n = 6$	0.05	$(0.05)^b$	$n = 6$
Simazine + water	0.48	$(0.05)^a$	$n = 6$	1.80	$(0.02)^a$	$n = 6$	1.26	$(0.02)^a$	$n = 6$
Simazine + water + SLS	–0.84	$(0.11)^c$	$n = 6$	–0.07	$(0.06)^c$	$n = 6$	–0.33	$(0.01)^c$	$n = 6$
Simazine + water + LAS	0.06	$(0.06)^b$	$n = 6$	0.50	$(0.03)^b$	$n = 6$	0.66	$(0.03)^b$	$n = 6$

Note. PNP = *p*-nitrophenol; PCP = pentachlorophenol. Means with the same superscript letter are not significantly different.

TABLE 7. Statistics and Regression Coefficients (SEM) Values for IPPSF logAUC (n=18)

	Statistics				Regression coefficients		
r^2	Adjusted r^2	s	F	i	PDMS-logKp	PA-logKp	CW-logKp
0.042	–0.018	0.555	0.700	–1.550 (0.144)	0.094 (0.113)		
0.320	0.277	0.468	7.516	–1.917 (0.188)		0.248 (0.091)	
0.264	0.218	0.486	5.738	–1.774 (0.162)			0.211 (0.088)
0.709	0.670	0.316	18.245	–2.453 (0.175)	–0.609 (0.136)	0.761 (0.130)	
0.550	0.490	0.393	9.157	–2.033 (0.155)	–0.527 (0.171)		0.627 (0.152)
0.328	0.239	0.480	3.666	–1.973 (0.230)		0.383 (0.320)	–0.132 (0.299)
0.718	0.657	0.322	11.862	–2.419 (0.185)	–0.638 (0.145)	0.642 (0.223)	0.140 (0.210)

TABLE 8. Mean (SEM) SLS and LAS Micelle Diameter (nm) as Measured by Dynamic Light Scattering at 22°C and 32°C

	SLS micelle diameter (nm) (22°C)	LAS micelle diameter (nm) (22°C)	SLS micelle diameter (nm) (32°C)	SLS micelle diameter (nm) (32°C)
No marker	1.12 (0.01)^{bA}	2.08 (0.02)^{aA}	1.13 (0.01)^{bA}	2.05 (0.03)^{aA}
PNP	0.94 (0.01)^{bA}	1.98 (0.02)^{aA}	0.95 (0.01)^{bA}	1.90 (0.03)^{aA}
PCP	1.07 (0.01)^{bA}	2.09 (0.03)^{aA}	1.09 (0.02)^{bA}	2.00 (0.01)^{aB}
Parathion	1.11 (0.01)^{bA}	2.11 (0.03)^{aA}	1.11 (0.01)^{bA}	2.01 (0.02)^{aB}
Fenthion	1.10 (0.01)^{bA}	2.05 (0.01)^{aA}	1.10 (0.01)^{bA}	2.01 (0.01)^{aB}
Propazine	1.08 (0.01)^{bB}	2.07 (0.01)^{aA}	1.16 (0.01)^{bA}	2.03 (0.01)^{aA}
Simazine	1.09 (0.01)^{bB}	2.06 (0.02)^{aA}	1.12 (0.01)^{bA}	2.04 (0.03)^{aA}
Grand mean	1.10 (0.01)^{bA}	2.08 (0.01)^{aA}	1.12 (0.01)^{bA}	2.03 (0.01)^{aB}

Note. PNP = *p*-nitrophenol; PCP = pentachlorophenol. Means with the same superscript letter are not significantly different. Lower case letters compare the means of different surfactants within the same temperature. Upper case letters compare the means of the same surfactants between different temperatures.

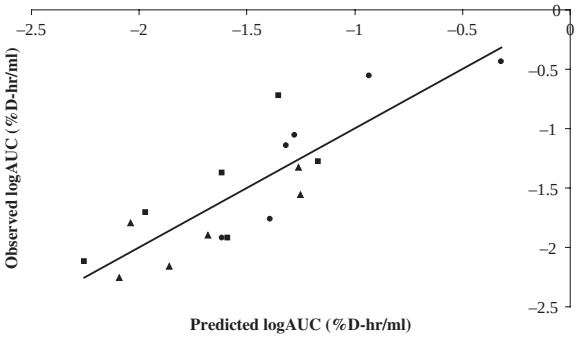

FIGURE 4. Predicted versus observed IPPSF log AUC (%D-h/ml) following topical doses of 6 markers in water (circle), 10% SLS (square), and 10% LAS (triangle); $r^2 = .7177$, adjusted $r^2 = .6572$. Regression equation: $y = -2.42(0.19) - 0.64(0.15)$*PDMS + $0.64(0.22)$*PA + $0.14(0.21)$*CW.

for the greater absorption in SLS versus LAS is the size of the micelles formed by the respective surfactants. One could postulate that in the case of LAS versus SLS, the presence of a benzene ring on the LAS micelle could potentially increase skin irritation. However, this is ruled out since the same pattern of absorption modulation was seen in both the MCF and IPPSF studies, indicating a physicochemical rather than physiological effect.

It should be noted that, unlike some studies which showed surfactant induced increased dermal absorption, our markers were co-dosed with the surfactants, rather than inducing irritation with SLS first as Wilhelm et al. (1991) did. This group induced irritation with SLS in dogs, then applied topical doses of hydrocortisone (HC), indomethacin (IM), ibuprofen (IB), and acitretin (AC) to see if there was an increase in absorption and skin distribution. Systemic absorption of topically applied drugs (as evaluated by urinary and fecal excretion) in SLS-irritated skin was significantly increased for HC (factor 2.6) followed by IB (1.9 times) and IM (1.6 times) but not increased for AC. However, drug concentrations in the viable epidermis and dermis were 70% lower in SLS-irritated than in normal skin for HC, but not different for IB, IM, and AC.

An interesting observation was that simazine did not follow the pattern seen with the other penetrants in both systems. The physicochemical properties of simazine are closely related to propazine. Indeed, the structures are similar (Figure 1). The effect is seen in the MCFs, also ruling out any potential effect due to irritation. The different response in simazine versus propazine was not seen in stratum corneum residues, as penetration and absorption were different but not SC amounts.

Within surfactant-containing mixtures, the highest surfactant enhancement was seen in compounds with the highest melting points. For these relatively low-water-solubility compounds, absorption was generally greater from aqueous solutions, reflecting the greater vehicle to skin partition coefficient. It needs to be stressed that more hydrophilic penetrants with

high water solubility may not show this pattern since their vehicle to stratum corneum partition coefficient would be less in the aqueous dosing solution (Riviere & Brooks, 2005).

REFERENCES

Augustijns, P., and Brewster, M. E., eds. 2007. *Solvent systems and their selection in pharmaceutics and biopharmaceutics*. New York: Springer.

Baynes, R. E., Xia, X. R., Imram, M., and Riviere, J. E. 2008. Quantification of chemical mixture interactions that modulate dermal absorption using a multiple membrane coated fiber array. *Chem. Res. Toxicol*. 21:591–599.

Bliss, C. I. 1939. The toxicity of poisons applied jointly. *Ann. Appl. Biol*. 26:585–615.

Bowman, K. F., Monteiro-Riviere, N. A., and Riviere, J. E. 1991. Development of surgical techniques for preparation of in vitro isolated perfused porcine skin flaps for percutaneous absorption studies. *Am. J. Vet. Res*. 52:75–82.

Cooper, E. R., and Berner, B. 1984. Interaction of surfactants with epidermal tissues. In *Surfactants in cosmetics*, ed. M. M. Rieger, p. 85. New York: Marcel Dekker.

Cross, S. E., Pugh, W. J., Hadgraft, J., and Roberts, M. S. 2001. Probing the effect of vehicles on topical delivery. *Pharm. Res*. 18:999–1005.

DeJong, C. M., Lutter, R., Verberk, M. M., and Kezic, S. 2007. Differential cytokine expression in skin after single and repeated irritation by sodium lauryl sulphate. *Exp. Dermatol*. 16:1032–1040.

Gregoire, S., Ribaud, C., Benech, F., Meunier, J. R., Garrigues-Mazert, A., and Guy, R. H. 2009. Prediction of chemical absorption into and through the skin from cosmetic and dermatological formulations. *Br. J. Dermatol*. 160:80–91.

Hargreaves, A. E. 2003. *Chemical formulation: An overview of surfactant-based preparations used in everday life*. London: Royal Society of Chemistry.

Idson, B. 1983. Vehicle effects in percutaneous absorption. *Drug Metab. Rev*. 14:207–222.

Khan, A. Y., Talegaonkar, S., Iqbal, Z., Ahmed, F. J., and Khar, R. K. 2006. Multiple emulsions: An overview. *Curr. Drug Delivery* 3:429–443.

Lavoue, J., Begin, D., and Geerin, M. 2003. Technical, occupational health and environmental aspects of metal degreasing with aqueous cleaners. *Ann. Occup. Hyg*. 47:441–459.

Monteiro-Riviere, N. A., Bowman, K. F., Scheidt, V. J., and Riviere, J. E. 1987. The isolated perfused porcine skin flap (IPPSF): II. Ultrastructural and histological characterization of epidermal viability. *In Vitro Toxicol*. 1:241–252.

Qiao, G. L., Brooks, J. D., Baynes, R. E., Monteiro-Riviere, N. A., Williams, P. L., and Riviere, J. E. 1996. The use of mechanistically defined chemical mixtures (MDCM) to assess component effects on the percutaneous absorption and cutaneous disposition of topically-exposed chemicals. I. Studies with parathion mixtures in isolated perfused porcine skin. *Toxicol. Appl. Pharmacol*. 141:473–486.

Riviere, J. E., and Brooks, J. D. 2005. Predicting skin permeability from complex chemical mixtures. *Toxicol. Appl. Pharmacol*. 208:99–110.

Riviere, J. E., and Brooks, J. D. 2007. Prediction of dermal absorption from complex chemical mixtures: Incorporation of vehicle effects and interactions into a QSPR framework. SAR and QSAR. *Environ. Res*. 18:31–44.

Riviere, J. E., and Monteiro-Riviere, N. A. 1991. The isolated perfused porcine skin flap as an *in vitro* model for percutaneous absorption and cutaneous toxicology. *Crit. Rev. Toxicol*. 21:329–344.

Riviere, J. E., Baynes, R. E., and Xia, X. R. 2007. Membrane coated fiber (MCF) array approach for predicting skin permeability of chemical mixtures from different vehicles. *Toxicol. Sci*. 99:153–161.

Riviere, J. E., Bowman, K. F., Monteiro-Riviere, N. A., Dix, L. P., and Carver, M. P. 1986. The isolated perfused porcine skin flap (IPPSF). I. A novel *in vitro* model for percutaneous absorption and cutaneous

toxicology studies. *Fundam. Appl. Toxicol.* 7:444–453.

Riviere, J. E., Monteiro-Riviere, N. A., and Baynes, R. E. 2002. Gulf War Illness-related exposure factors influencing topical absorption of ^{14}C-permethrin. *Toxicol. Lett.* 135:61–71.

Rosado, C., Cross, S. E., Pugh, W. J., Roberts, M. S., and Hadgraft, J. 2003. Effect of vehicle pretreatment on the flux, retention, and diffusion of topically applied penetrants in vitro. *Pharm. Res.* 20:1502–1507.

Sloan, K. B., Koch, S. A. M., Siver, K. G., and Flowers, F. P. 1986. Use of solubility parameters of drug and vehicle to predict flux through skin. *J. Invest. Dermatol.* 87: 244–252.

Somasundaran, P., Chakraborty, S., Qiang, Q., Deo, P., Wang, J., and Zhang, R. 2004. Surfactants, polymers and their nanoparticles for personal care applications. *J. Cosmet. Sci.* 55(suppl.):S1–S17.

U.S. Environmental Protection Agency. 2004. *Risk assessment guidance for Superfund*, vol. I, *Human health evaluation manual* (*Part E, Supplemental guidance for dermal risk assessment*), pp. 3-1–3-10. EPA/540/R/99/005, July. http://www.epa.gov/oswer/riskassessment/ragse/index.htm

Wester, R. C., Melendres, J., Sedik, L., Maibach, H. I., and Riviere, J. E. 1998. Percutaneous absorption of salicylic acid, theophylline, 2,4-dimethylamine, diethly hexylphthalic acid and *p*-aminobenzoic acid in the isolated perfused porcine skin flap compared to man. *Toxicol. Appl. Pharmacol.* 151:159–165.

Wilhelm, K. P., Surber, C., and Maibach, H. I. 1991. Effect of sodium lauryl sulfate-induced skin irritation on in vivo percutaneous penetration of four drugs. *J. Invest. Dermatol.* 97:927–932.

Xia, X. R., Baynes, R. E., Monteiro-Riviere, N. A., Leidy, R. B., Shea, D., and Riviere, J. E. 2003. A novel in vitro technique for studying percutaneous permeation with a membrane coated fiber and gas chromatography/mass spectrometry. I. Performance of the technique and determination of the permeation rates and partition coefficients of chemical mixtures. *Pharm Res.* 20:275–282.

Xia, X. R., Baynes, R. E., Monteiro-Riviere, N. A., and Riviere, J. E. 2004. A compartment model for the membrane-coated fiber (MCF) technique used for determining the absorption parameters of chemicals into lipophilic membranes. *Pharm. Res.* 21:1345–1352.

Yeatts, J. L., Baynes, R. E., Xia, X. R., and Riviere, J. E. 2008. Application of linear solvation energy relationships to a custom made polyaniline solid-phase microextraction fiber and three commercial fibers. *J. Chromatogr. A* 1188:108–117.