MIXTURE EFFECTS ON THE DERMAL ABSORPTION OF BIOCIDES
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LIST OF ABBREVIATIONS

MWF	Metal-working fluids
MCF	Membrane-coated fibers
PDMS	Polydimethyl siloxane
PA	Polyacrylate
CW	Carbowax
PANI	Polyaniline
ANOVA	Analysis of variance
Log $K_{O/W}$	Log octanol/water partition coefficient
Log $K_{pdms/w}$	Log PDMS/water partition coefficient
MO	Mineral oil
PEG	Propylene glycol 200
SC	Stratum corneum
SLS	Sodium lauryl sulfate
Kp	Skin permeability
Log $K_{(Skin/Astrocut)}$	Log skin permeability in Astrocut formulation

ABSTRACT:

Workers in the metal-machining industry are frequently reported to develop occupational dermatitis amongst other adverse health effects following exposure to metal-working fluids. The *primary focus* of this research project was to quantify physicochemical interactions modulating dermal absorption of industrial irritants (e.g., biocides/preservatives) in metal working fluid (MWF) mixtures following dermal exposure. Our laboratory developed a novel membrane-coated fiber (MCF) technique that quantifies not only the partitioning behavior of these toxicologically important solutes but also other solvatochromatic descriptors (e.g., polarity, hydrogen bonding) that more accurately reflect the mechanistic interaction between the irritant, industrial formulation/mixtures, and skin. This approach allowed for quantification of mixture-membrane interactions that can be used as a predictive tool of dermal disposition of irritating biocides or preservatives relevant to worker exposure in the metal machining industry. Another major goal of the study was to evaluate the utility of the membrane-coated fibers (MCF) to capture simple mixture-induced effects and to correlate these changes to dermal permeability using a solvatochromatic approach to quantify physicochemical and biological interactions. Our major research findings can be summarized as follows:

(1) ***Calibrated a series of membrane coated fibers*** (MCFs) with a limited data set, and demonstrated solvent, surfactant, and MWF formulation effects and dilution effects on solute/biocide partitioning into these chemically diverse MCFs.

(2) The solvatochromatic or linear solvation energy relationship (LSER) approach ***adequately predicted the dermal permeability*** of several solutes and phenolic biocides in soluble oil and synthetic MWF. This research demonstrated that formulation-specific strength coefficients (*r p a b v*) predicted (R^2 = 0.75 to 0.83) changes in the dermal permeability of phenolic biocides when formulated with commercial metalworking fluid (MWF) formulations or 50% ethanol.

(3) MWF biocide permeability in skin can be significantly reduced as the MWF concentration increases. This can be of occupational concern as MWF dilutions in the work place can range from 1-20% with the ***more dilute formulation enhancing permeability*** of some classes of biocides. Permeation of chemicals was higher in generic synthetic MWF when compared to a soluble oil MWF. This suggests that a soluble oil MWF may be safer than a synthetic MWF in regards to dermal permeation of phenolic biocides/solutes to allow for an increased potential of systemic toxicity. Therefore, one may conclude that a synthetic type of formulation has more potential to cause contact dermatitis and possibly induce systemic toxicological effects.

(4) Demonstrated that large and hydrophobic biocides tend to be retained in the commercial MWFs while the more basic biocides tend to permeate through skin

(5) Demonstrated a ***strong correlation between changes in skin permeability and the changes in partitioning in an MCF-array*** for simple mixtures containing either ethanol or the surfactant, SLS. This MCF-array, which is a high-throughput and reproducible system may be applicable to predicting skin permeability of chemicals from industrial formulations such as metal-working fluids.

The data from this research can be used by regulators, manufacturers, and users of metal working fluids (MWF) and biocides to ***amend their formulations and recommend dilutions*** such that dermal absorption of biocides and other additives are least likely to occur and result in contact dermatitis and/or other systemic health effects.

SIGNIFICANT FINDINGS:

NIOSH estimates that more than 1.2 million workers/year are potentially exposed to metal working fluids (MWFs). The listed industries with the highest incidence rates of skin disorders all involved MWF exposure and MWF dermatitis in a variety of machine shops and ranged from 36-67% (NIOSH, 1998). The primary objective of this project **(RO1-OH-03669, 2005-2008)** was to identify significant physicochemical and chemico-biological interactions that influence the dermal disposition of various solutes including MWF biocides that have been identified as causing ***occupational irritant dermatitis*** amongst workers who are exposed to formulations containing these additives. Two membrane models were used to identify these interactions; namely, a membrane-coated fiber system (MCF) and a porcine skin in vitro system. The novelty of the membrane coated fiber (MCF) approach is that it can *link linear solvation energy relationship (LSER) and partitioning behavior* of polar and nonpolar solutes individually and as mixtures using specific chromatographic tools. This provided quantitative information that was amenable to extrapolation for ***risk assessment purposes***. The final component of this integrated physicochemical approach involved evaluation of chemical mixture effects on solute and biocide permeability in a calibrated *in vitro* skin model system.

These scientific findings are related to the following 3 Specific Aims of the Project:

Specific Aim 1: Identify and quantify solute partitioning behavior in MCF/Water systems and to determine molecular descriptors of 12 irritant biocides representing 5 major classes of biocides frequently used in the cutting fluid industry.

Specific Aims 2: Identify and quantify mixture interactions in the MCF/Water system that influence dermal disposition of industrial biocides.

Specific Aims 3. Identify chemical-biological interactions in a calibrated acute and chronically exposed biological membrane system; namely, porcine skin flow-through diffusion cells.

The most significant findings from this research are as follows:

- This research resulted in the first ever reporting of the calibration of chemically diverse membrane coated fibers (MCF) within a linear solvation energy relationship (LSER). These experiments were able to quantify partitioning behavior between a diverse series of solutes and metal-working fluid (MWF) biocides.
- The calibrated MCFs were able to determine the effect of (1) simple mixtures (e.g., ethanol or SLS), (2) various metal-working fluid (MWF) formulations, and (3) MWF dilution. Dilution appeared to more likely affect biocide partitioning into MCFs of soluble oil MWF than semisynthetic and synthetic MWFs.
- Solute and MWF biocide permeability in skin followed the trends observed in the MCFs. Analysis of the strength coefficients revealed that hydrophobicity and hydrogen bond donor interactions played important roles in explaining the reduced permeability of biocides in some MWFs compared to other MWF that were more hydrophilic. Ultimately, large and hydrophobic biocides will tend to be retained in the commercial MWFs while the more basic biocides tend to permeate through skin.
- We also demonstrated a strong correlation between (1) skin permeability and partitioning in the MCF-array of 3 fibers and (2) changes in skin permeability and the changes in partitioning in an MCF-array for simple mixtures containing either ethanol or the surfactant, SLS. Our research has therefore demonstrated that the MCF technique has the advantages of quickly making these assessments in the machine shop/workplace and

provided much needed estimates of how a chemical mixture or industrial formulation can significantly alter the dermal absorption of hazardous chemicals.

TRANSLATION OF FINDINGS:

Workers in the metal fabrication industry are more often exposed to metal working fluids (MWF) and its components such as biocides via the skin that can cause harm to the skin and/or the entire body if absorbed by the dermal route. Many of these workers are exposed to more than one chemical additive in any given MWF formulation, and there is little or no means of estimating what class of MWF formulations can result in increased or decreased absorption of biocides across skin. Our research describes a novel technique that models biocide absorption in skin on the basis of quantitative changes in physicochemical properties associated with the formulation interacting with a model membrane and then validated in skin *in vitro* to determine validity of these models in an occupational dermal exposure.

Under the auspices of this **grant (RO1-OH-03669)**, we demonstrated that the effects of different metal working fluid (MWF) formulations and concentrations can influence dermal permeability of phenolic biocides and other chemicals. Our research was also able to develop and eventually validate a novel membrane-coated fiber (MCF) array system that can predict the effect of solvents and surfactant mixtures on the dermal permeability of chemicals relevant to occupational health. In ***summary***, our novel MCF array is another approach to assessing dermal absorption and represents a ***paradigm shift*** within which toxicologist can begin to accurately model toxicant disposition in skin following dermal exposure to complex chemical mixtures in the work place. This physicochemical approach should ***advance the science of human health risk assessment*** that currently relies on incomplete data sets and limited quantitative and mechanistic models with which to accurately predict single chemical absorption, and needless to say chemical mixtures. Our approach allows **experimental** assessment of mixture interactions in a LSER framework that previously has only been used as a mathematical tool to predict single chemical absorption.

These *models will be applicable to actual workplace situations as* risk assessors, formulators, chemists, and regulators will be able to predict what MWF(s) would increase dermal uptake. For example, a 10% increase in relative dermal absorption may identify the biocide or additive as an occupational hazard in a new formulation that was of no concern in the original formulation. We further established that biocide permeability in skin can be significantly reduced as the MWF concentration increases. This can be of occupational concern as MWF dilutions in the work place can range from 1-20% with the more dilute formulation enhancing permeability of some classes of biocides. In summary, this research was the first to demonstrate that MWF phenolic biocides were more likely to penetrate the skin of workers exposed to synthetic MWFs than to soluble oil formulations. Furthermore, dilution with water, as often recommended, can actually increase biocide permeability and not the expected opposite effect. Regression models were developed to demonstrate these formulation effects and they were also validated with repeatable *in vitro* studies.

SCIENTIFIC REPORT:

BACKGROUND FOR THE PROJECT:

Current dermal risk assessments are plagued by the many conflicting experimental protocols and internal dose calculations that limit characterization of solute or biocide absorption in skin. Calculating solute absorption has been based on (1) percent dosed absorbed into the systemic circulation and/or (2) steady state flux which can be used to calculate solute permeability (McDougal, 2002). The latter is more useful primarily because *solute permeability is concentration independent*, whereas expressing penetration as a fraction of applied dose may cause large errors associated with variations in external dosing and exposure times. Permeability is therefore ***preferred for extrapolating across dose*** in dermal risk assessment and also better suited for assessing and ultimately extrapolating across formulation and mixture effects which is the purpose of this grant application. Assuming that solutes obey Fick's first law of diffusion as they diffuse across the human epidermal membrane, **skin permeability** can be defined by the equation,

Permeability (cm/hr), kp = J_{ss}/C_v **Equation 1**

where J_{ss} represents solute steady state flux and C_v the solute dosing concentration. Solute permeability is dependent on solute diffusivity, D, (cm^2/hr) in the membrane and its ability to ***partition*** from the dosing solution to the stratum corneum layer of skin. The latter is referred to as the stratum corneum-vehicle partition coefficient, K_{sv}, and is often correlated to octanol-water partition coefficients, Ko/w. Permeability can therefore be re-defined by equation 2, where l = membrane thickness.

$$Kp = \frac{D * K_{s/v}}{l} \qquad \textbf{Equation 2}$$

It is quite conceivable that in some chemical mixtures scenarios that not only will the physicochemical properties of the targeted solute be altered, but also the biological membrane. *Therefore solute diffusivity and/or partitioning behavior can be influenced by chemical mixtures.* These interactions have **not** been adequately addressed in many of the current mathematical models, and our research attempted to derive these parameters in a chemical mixture scenario; namely, metal-working fluid (MWF) formulations used in the metal machining industry. Workers are exposed to hundreds of these formulations and it would be more prudent to utilize robust and mechanistically-defined models to predict what additive or additives will influence these dermatopharmacokinetic parameters rather than performing extensive *in vivo* and *in vitro* toxicokinetic studies to estimate the dermal disposition of these industrial irritants.

Quantitative Structure Permeability Relationship (QSPR) Models.

QSPR models can be used to provide physicochemical understanding about the diffusion behavior of individual solutes. The main objective of our research was to further develop this approach using chromatographic and statistical methodologies to better characterize biocide-membrane interactions in skin following chemical mixture exposure. In simplistic terms, the aim here is to obtain and define physicochemical descriptors that would in different combinations model solvation in the various phases; that is, in a membrane system (MCF or skin) and in a chemical mixture solution.

QSPR models have been used to relate physicochemical parameters to dermal permeability, but they have limitations because of inadequate statistical fit and/or not being

directly applicable to chemical mixtures or formulations. Several of the earlier models demonstrated a linear and/or parabolic relationship between hyrophobicity and skin permeability, Kp (Scheuplein and Blank, 1971, Roberts *et al.*, 1977)). Because these models were derived from a homologous series of solutes or particular class of solutes, co-linearity between descriptors (e.g., MW and log Ko/w) can exist and disparate physicochemical characteristics are not represented in these models. Many of the more recent QSPR models have been based on permeability (Kp) data compiled by Flynn (1990) for 94 compounds from numerous sources and experimental protocols that can however be described as being more heterogeneous than other chemical clusters or series previously analyzed for QSPR. This data was utilized to generate the now widely cited Potts and Guy (1992) model (**Equation 3**) which however reported a poor fit ($r^2 = 0.67$), and there was no thorough statistical analyses of the variance.

Log* Kp = *0.71 log Ko/w – 0.0061MW – 6.3 ***Equation 3***

The US EPA has refined this model by excluding several experimental data points, and they have recommended that this refined model be utilized in predicting permeability (Kp) values. It should be recognized that it is based on small hydrocarbons and pharmaceutical drugs that *bear little resemblance to hazardous toxicants workers are exposed to in the chemical industry*. The more recent QSPR approaches now utilize such physicochemical descriptors as hydrophobicity (e.g., log Ko/w) as well as electronic properties (e.g., H-bonding), and steric properties (e.g., MW, MV) that are really *solvation energy descriptors*.

While **Equation 3** establishes a strong link between permeability (***Kp***) and molecular partitioning and distribution between an organic/lipid phase and an aqueous phase, the solute partitioning behavior can be better described at the molecular level in terms of the ***free energy of solute transport***. The latter is a function of solute size, polarity, and hydrogen bonding ability, and it is this ***solvatochromatic approach*** which has been used to determine a variety of physicochemical solute properties such as solubility (Kamlet *et al.*, 1986; Abraham and Le, 1999), partition coefficents (Kamlet *et al.*, 1988), and more recently bio-membrane permeability (Abraham *et al.*, 1994; 1995). In this approach, a small set of solvatochromatic descriptors were combined in a linear fashion to correlate solute properties which results in a ***linear solvation energy relationship (LSER)***. Any given LSER is basically a model of solute solvation in various solvent phases (solute-solvent interactions), and can by extension model solute-membrane interactions. It should be noted that the role of hydrogen bonding plays a significant role in the LSER, and has thus received the most attention in QSPR modeling. A more recent Potts and Guy (1995) model (**Equation 4**) clearly demonstrated its importance as reflected in its good fit ($r^2 = 0.94$) even in the absence of the hydrophobic descriptor, log Ko/w, but inclusion of a different steric descriptor, molecular volume (MV).

Log *Kp* = 0.0256 MV – 1.72 $\Sigma\alpha_2^H$ - 3.93 $\Sigma\beta_2^H$ - 4.85 **Equation 4**

The descriptors $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ reflect solute hydrogen donor capacity and solute hydrogen acceptor bond capacity, respectively, and they measure the exoergic effects. In essence, these two descriptors can measure the ability to donate or accept a proton in a solute-solvent hydrogen bond. This model also provides basic ***physicochemical insight*** into epidermal permeability as hydrogen bonding activity is inversely related to permeability, and where hydrogen bond donor activity ($\Sigma\alpha_2^H$) can be more important than hydrogen bond acceptor activity ($\Sigma\beta_2^H$).

This QSPR work was then followed-up by Abraham *et al.* (1999), and as can be gleaned from **Equation 5** below, this model was not only an excellent fit ($r^2 = 0.96$) for a more diverse set of the Flynn (1990) 53 solutes, but now includes other descriptors such as: molar fraction

(R_2), which can be obtained from refractive index of solutes that are liquid at 20°C; solute dipolarity/polarisability (π_2^H) which measures endoergic effects of solute-solvent dipole-dipole and dipole-induced dipole interactions; and McGowan characteristic molecular volume, V_x, which can be calculated from bond and atom contributions. When comparing earlier QSPR models with **Equation 4 and 5**, it is clear that molecular size and hydrogen bonding are correlated with hydrophobicity, log Ko/w.

$$\text{Log } Kp = 0.44R_2 - 0.49\,\pi_2^H - 1.48\,\Sigma\alpha_2^H - 3.44\,\Sigma\beta_2^H + 1.94\,Vx - 5.13 \qquad \textbf{Equation 5}$$

This equation can now be rewritten in general terms to relate a given solute transport property (SP) such as $K_{o/w}$ to a set of 5 independent descriptors as in **Equation 6** below. *This model was utilized in this research* project to assess *physicochemical processes*; namely, solute partitioning between a solution and an inert or biological membrane and solute partitioning between a solution and surfactant micelles present in a cutting fluid formulation.

$$\text{Log SP} = c + r \bullet R_2 + s \bullet \pi_2^H + a \bullet \Sigma\alpha_2^H + b \bullet \Sigma\beta_2^H + v \bullet V_x \qquad \textbf{Equation 6}$$

In an analysis of the several proposed QSPR models by Roberts *et al.* (1995), the above fundamental solvatochromatic approach (size+hydrogen bonding) was proven to provide better regression fits and better insights into the intermolecular forces driving solute permeability. These authors subsequently demonstrated that solvatochromatic parameters for individual H-bonding groups also gave better regressions for diffusivity than did number of H-bonding groups (Roberts *et al.*, 1996). However, it must be reiterated the addition of successive hydrogen bonding groups reduces the diffusivity by an order of magnitude per hydrogen bonding functional group.

The physicochemical nature of the stratum corneum (SC) and the effect of chemical additives on its inherent physicochemical properties or membrane environment (Pugh *et al.*, 1996) will obviously influence membrane diffusivity and thus permeability. The SC has been reported to be a predominantly H-bonding donor (α_2^H) rather than acceptor (β_2^H) with a α_2^H/β_2^H ratio of 0.6/0.4 (Pugh *et al.*, 1996), although this ratio has been debatable amongst various investigators. Intuitively, the presence of additives, diluents, and/or surfactants as proposed in this application can influence this ratio and thus enhance or retard biocide diffusion across a membrane. Impregnation of a PDMS membrane (an inert barrier) with octanol to mimic the hydrogen bonding environment of the SC demonstrated that this solvent and not toluene (which has no H-bonding properties) significantly reduced the flux of various acids and alcohols. (Du Plessis *et al.*, 2002). *This observation strongly suggests that hydrogen bonding interaction was responsible for altered permeability and is thus an integral and relevant solvatochromatic parameter in our research findings.*

It must be emphasized that the ***5 descriptors*** described thus far are a small set of independent variables. However when combined in a linear fashion to correlate with membrane partitioning or skin permeability, they allow for a quantitative and qualitative description of ***biocide-mixture interactions*** that we demonstrated in this research. The initial experiments utilized a selected training set of solutes with known inherent molecular descriptors used to calibrate the membrane-coated fiber (MCF)/water system. This MCF/water system was used to experimentally determine the **SP** parameter in **Equation 6; i.e., a value describing partitioning of the biocide between the MCF and water (also called $\text{LogK}_{\text{MCF/Water}}$ value).**

The ***system/strength coefficients*** in these equations represent the unique system coefficients for each system, and they can be obtained from multiple linear regression analysis. ***These coefficients refer to the interaction properties of the solute with the aqueous phase, and***

if there is more than one phase as is the case with skin, it encodes the difference in interaction properties of the two phases. These two phases can be octanol + water or can be MCF+water or skin+ dosing vehicle which are the primary systems that were evaluated in this research. This utility in mixtures and formulations research cannot be overemphasized. These important system or strength coefficients are thought to characterize the following physicochemical and biochemical properties of the system as described below:

a-strength coefficient: the phase hydrogen-bond acceptor strength,

b- strength coefficient: the phase hydrogen-bond donor strength;

s- strength coefficient: the phase or solution polarizability/dipolarity;

v- strength coefficient: the phase hydrophobicity.

r- strength coefficient: the tendency of the solvent phase to interact with π- and n-electron pairs;

The choice of membranes (MCFs) is critical, as the resulting systems should have system coefficients in **Equation 6** that are as diverse as possible in order to ***capture as many physicochemical attributes of skin***. Skin is a complex heterogeneous membrane whose physicochemical properties will NOT be reflected in a single MCF and by extension interactions between solute and membrane; thereby necessitating the use of multiple and physicochemically diverse MCFs. Finally, ***it is these system coefficients that will change in value by simply changing the "system"; i.e., by adding MWF additives*** such as formulation diluents, surfactants, or other performance enhancing components to the aqueous solution. It is these changes in strength/system coefficients that provide quantitative information on the magnitude of the interactions.

Metal Working Fluid (MWF) Mixture Effects

In recent years, straight or neat cutting oils have been replaced by two major types of cutting fluids; namely, soluble oils and synthetic fluids. These MWFs have also become more complex with the addition of various additives (***diluents, emulsifiers, anticorrosive agents, biocides,*** and ***lubricants***) to improve the performance of the MWFs in metal machining operations. At the same time however, these additives could have been selected based on their ability to modulate percutaneous absorption.

Diluents and Solvent Effects: Our previous research efforts were focused on the effect of surrogate soluble oil (mineral oil) and surrogate synthetic fluids (polyethylene glycol, PEG), and have determined that these two diluents can significantly influence additive effects on permeability and deposition of the biocide, triazine (Baynes et al., 2003). Until recently, the comparative effects of these diluents have not been reported in the literature, but we have been more aware of the effect of industrial solvents on the epidermal membrane barrier. Industrial organic solvents can cause ***delipidization*** of the stratum corneum (Abrams *et al.*, 1993), however, the influence of diluents or solvents on the intermolecular forces described by the LSER have not been assessed. Of further concern, workers routinely use solvents such as trichloroethylene (TCE) to remove the MWF from the fabricated metal as well as from their skin surface. Chronic exposure to these cleansing or related solvents significantly reduces the epidermal barrier as evidenced microscopically by extensive disorganization of intercellular lipid bilayers (Tsai et al., 2001). Interestingly, solvent extraction and reordering of lipids in the stratum corneum (SC) reduces the lipophilicity of the SC and appears more likely to enhance penetration of more hydrophilic molecules. Our laboratory has recently demonstrated enhanced permeability of (i) triazine following a 4-day pre-treatment with TCE (Baynes et al., 2004b) and

(ii) aromatic vs. aliphatic jet fuel components in porcine skin pretreated daily with jet fuel for 5 days (Muhammad et al., 2004). ***These kind of interactions will impact dermal permeability of biocides with varying LogKo/w values as we demonstrated in our research***. Mathematically, this enhanced permeability in compromised skin can be due to increased partitioning and/or diffusivity through the epidermis (Rosado et al., 2003).

Metal-working Fluid (MWF) Additives: Surfactants, alkanolamines, and sufated fatty acids are widely used in many industrial applications, and consequently serve as an additional mixture component that can alter biocide diffusion across skin (Baynes et al., 2003). There is significant evidence that alkanolamines and fatty acids that are frequently formulated with topical pharmaceuticals and cosmetics can enhance dermal disposition (Hadgraft and Wotton, 1985). Therefore our recent observations with MWFs were not surprising, and strongly supports more of a physicochemical interaction than chemical-biological effect.

Anionic surfactants such as sodium lauryl sulfate (SLS) and linear alkylbenzene sulfonate (LAS) are on the other hand more potent in the way they interact with the solute and membrane. They can cause swelling and disrupting of the stratum corneum and extension of the α-keratin structure resulting in spatial expansion and altered diffusion pathways for chemicals (Rhein *et al.*, 1986, Scheuplein and Ross, 1970). Surfactants can also enhance solute penetration by causing fluidization of intercellular lipids in the stratum corneum (Ribaud *et al.*, 1994). The dermal irritation, penetration, and enhancer effect of SLS on drug absorption has been well documented (Wilhelm *et al.*, 1991) and also previously demonstrated in our earlier mixture studies in skin (Baynes *et al.*, 1996, Qiao *et al.*, 1996) and more recently with our MWF mixtures (Baynes et al., 2002c). Researchers however often overlook the fact that ***surfactants can generate micelles that retain solutes on the skin surface and effectively modulate solute penetration into skin***. Several of our recent studies strongly suggest that surfactant concentrations above the critical micelle concentration (CMC) can influence solute absorption and that the octanol-water partition coefficient of the solute may be indicative of whether surfactants increase or decrease solute absorption. These observations were part of the stimulus for this research project that was focused on using a solvatochromatic approach (i.e., LSER) to understanding these interactions in MWFs.

Specific Aims

The following 3 Specific Aims of the Project were:

- **Specific Aim 1:** Identify and quantify solute partitioning behavior in MCF/Water systems and to determine molecular descriptors of 12 irritant biocides representing 5 major classes of biocides frequently used in the cutting fluid industry.
- **Specific Aims 2:** Identify and quantify mixture interactions in the MCF/Water system that influence dermal disposition of industrial biocides.
- **Specific Aims 3.** Identify chemical-biological interactions in a calibrated acute and chronically exposed biological membrane system; namely, porcine skin flow-through diffusion cells.

PROCEDURES & METHODOLOGY

Calibration of MCF/Water Systems (Specific Aims#1 & 2)

Chemicals. Acetone (GC grade), ethanol (200 proof) and sodium lauryl sulfate (99% in purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was prepared from a Picotech Water System (Research Triangle Park, NC, USA). A set of probe compounds having purity better than 98% were purchased from Sigma-Aldrich (**Table 1**).

Table 1. Molecular descriptors of training solutes and test* solutes from ADMA Boxes software (Pharma Algorithms, Toronto, Canada)

Peak#	Compounds	R	π	α	B	V
1	Toluene	0.60	0.52	0.00	0.14	0.857
2	Chlorobenzene	0.72	0.65	0.00	0.07	0.839
3	Ethylbenzene	0.61	0.51	0.00	0.15	0.998
4	p-Xylene	0.61	0.52	0.00	0.16	0.998
5	Bromobenzene	0.88	0.73	0.00	0.09	0.891
6	Propylbenzene	0.60	0.50	0.00	0.15	1.139
7	4-Chlorotoluene	0.71	0.67	0.00	0.07	0.980
8	Phenol*	0.81	0.89	0.60	0.3	0.775
9	Benzonitrile	0.74	1.11	0.00	0.33	0.871
10	4-Florophenol*	0.67	0.97	0.63	0.23	0.793
11	Benzyl alcohol	0.80	0.87	0.39	0.56	0.916
12	Iodobenzene	1.19	0.82	0.00	0.12	0.975
13	Pheny acetate	0.66	1.13	0.00	0.54	1.073
14	Acetophenone	0.82	1.01	0.00	0.48	1.014
15	*m*-Cresol	0.82	0.88	0.57	0.34	0.916
16	Nitrobenzene	0.87	1.11	0.00	0.28	0.891
17	Methyl benzoate	0.73	0.85	0.00	0.46	1.073
18	4-Chloroanisole	0.84	0.86	0.00	0.24	1.038
19	Phenylethyl alcohol	0.78	0.83	0.30	0.66	1.057
20	3-Methyl benzyl alcohol	0.82	0.90	0.33	0.59	1.057
21	4-Ethylphenol*	0.80	0.90	0.55	0.36	1.057
22	3,5-Dimethylphenol*	0.82	0.84	0.57	0.36	1.057
23	Ethylbenzoate	0.69	0.85	0.00	0.46	1.214
24	Methyl 2-methyl benzoate	0.77	0.87	0.00	0.43	1.214
25	Naphthalene	1.36	0.92	0.00	0.20	1.085
26	3-Chlorophenol*	0.91	1.06	0.69	0.15	0.897
27	4-Chloroaniline	1.06	1.13	0.30	0.31	0.939
28	4-Nitrotoluene	0.87	1.11	0.00	0.28	1.032
29	4-Chloroacetophenone	0.96	1.09	0.00	0.44	1.136
30	3-Bromophenol*	1.06	1.15	0.70	0.16	0.950
31	1-Methyl naphthalene	1.34	0.90	0.00	0.20	1.226
32	Biphenyl	1.36	0.99	0.00	0.22	1.324
33	4-*Tert*-amylphenol*1	0.79	0.80	0.50	0.44	1.479
34	*o*-Phenylphenol*	1.55	1.40	0.56	0.49	1.383

Individual stock solutions with a concentration of 10.0 mg/mL in acetone were prepared for each of the probe compounds. A standard mixture in acetone containing the probe compounds with a concentration of 100 μg/mL for each component was prepared from the individual stock solutions. A series of standard solutions in acetone were prepared from the standard mixture to be used as external calibration standards for GC/MS analysis. Some of the probe compounds are volatile and some of them are toxic. All of the solution preparation processes should be conducted in a fume hood with gloves and goggles.

Metalworking Fluid Formulations (Specific Aims# 2 & 3)

In order to demonstrate formulation effects in "real-world" industrial formulations, MCFs and porcine skin sections were exposed to industrial metal working fluid (MWF) formulations (Astrocut-C® from Monroe Fluid Technology, NY or Tapfree-2® from Winfield Brooks Company, Inc., MA), and 17% methanol. Astrocut is a commercial soluble oil MWF (pH 9.2) and Tapfree is a commercial synthetic MWF (pH = 8.0). Comparable generic soluble oil, semisynthetic, and synthetic MWFs were obtained as kind donation from Milacron Inc. These latter generic formulations were prepared at 3 concentration levels to represent the range of dilutions used in the metal-working industry. These formulations were also spiked with a test set of commercial phenolic biocides; namely, phenol, 4-florophenol, 4-ethylphenol, 3,5-dimethylphenol, 4-*tert*-amylphenol, *o*-phenylphenol, and 3-chlorophenol.

Membrane-Coated Fibers (MCFs). (Specific Aims# 1 & 2)

The following MCFs, 100-μm polydimethylsiloxane (PDMS), 85-μm polyacrylate (PA) and 50-μm carbowax/template resin (Wax) fiber assembles were purchased from Supelco (Bellfonte, PA, USA). A fourth MCF, PANI fiber, was developed as follows by laboratory and recently published (Yeatts et al., 2007):

A scanning electron microscope (Model JSM-6360LV, SEM, JEOL Ltd. Tokyo, Japan) was used to characterize the surface and thickness of the polyaniline (PANI) coating on the metal core fiber. Bare metal core SPME assemblies were custom ordered from a commercial source (Supelco, Bellefonte, PA). The composition of the metal fiber was proprietary. An EG&G Princeton Applied Research Potentiostat/Galvanostat Model (Princeton Applied Research, Oak Ridge, TN, USA) interfaced to a Dell Optiplex GX Pentium 4 computer (Dell corp., USA) by a GPIB-USB-HS interface (National Instruments, Austin, TX,USA) was used to electrochemically coat the metal fiber. The platinum wire counter electrode (CHI 115) and calomel reference electrode (CHI 150) were purchased from CH Instruments, Inc. (Austin, TX, USA). The SPME bare metal fiber assembly was used as the working electrode. Model 250 Research Electrochemistry software (M270) version 4.41 (Perkin Elmer Instruments and Princeton Applied Research, Oak Ridge, TN, USA) was used to control and acquire data from the potentiostat/galvanostat. Solid-phase microextraction (SPME) and analysis of the 37 compounds of interest was performed by an automated SPME system (Combi-PAL CTC analytics) coupled to a Gas chromatograph/quadrupole ion trap mass spectrometer (Varian 4000 GC/MS/MS, Varian Inc., Walnut Creek, CA, USA).

Polyaniline (PANI) SPME fiber preparation. Cyclic voltammetry (CV) was used to coat the metal fiber on the SPME assembly. A 0.1M solution of aniline in 1.0M sulfuric acid served as the electrolyte solution. The electrolyte solution was degassed by bubbling ultra-high purity nitrogen through the solution for about 3.5 minutes. The platinum wire and calomel electrodes served as the counter and reference electrodes respectively. The SPME bare metal fiber assembly

served as the working electrode. After completion of the CV experiments, the fiber was rinsed with DI water and preconditioned at 80°C for 30 minutes followed by 200°C for 1 hour in a GC injector port with Helium flowing at 1.0 ml/min. The CV program consisted of one initial scan from -0.2V to +1.1V followed by one set of 10 scans, four sets of 20 scans and one set of 10 scans from -0.2V to +0.8V with a 15 second equilibration time at -0.2V between each set of scans for a total of 101 scans. The scanning rate was 50 mV/sec. Figure 1 shows the SEM photograph of the polyaniline fiber.

Determination of the partition coefficients. The partition coefficients of the probe compounds were determined with three MCFs (100-μm PDMS, 85-μm PA and 50-μm Wax). The 100-μm PDMS fibers were conditioned at 250°C for 30 min and 85-μm PA fiber at 300°C for 2 h as recommended by the manufacture. The 50-μm Wax fibers were pre-conditioned at 220°C for 30 min. A Combi PAL automatic sampler (CTC Analytics, Switzerland) was used to perform the partitioning experiments. The detailed procedures for measuring partition coefficients using the MCF technique were described elsewhere (Xia et al., 2003). Here briefly, a glass vial containing 8.0 mL of the working solution was transferred into an incubator and shaken at 500 rpm for 5 min to equilibrate the sample temperature to 37°C. A preconditioned MCF was immersed into the working solution to start the absorption experiment under constant stirring at 400 rpm and 37°C. For a given period of time, the fiber was removed from the vial and transferred into the injector of a gas chromatograph (GC) for quantitative analysis (see below). The concentrations of the probe compounds in the aqueous working solution were optimized for quantitative analysis in the range of 0.01- 2 μg/mL. The partition coefficients of the probe compounds were also measured using the 3 MCFs (PDMS, PA and Wax) in aqueous solutions containing 0, 10, 25, 40 and 50% of ethanol or 0, 0.1, 0.5, 1, 2 and 5% of SLS.

Quantitative analysis. The quantitative analysis of the chemicals were performed with a Varian GC/MS 4000 equipped with ion trap mass selective detector. A Combi PAL automatic sampler was used for liquid injection, fiber absorption and headspace experiments. The injection port was maintained at 280°C for using PDMS and PA fibers, 250°C for Wax fibers and 270°C for PDMS/DVB fibers. These temperatures were selected for optimal thermal desorption and useful life of the fibers. The analytical conditions were improved to reduce analytical time and increase analytical sensitivity. Separation was performed on a 30 m x 0.25 mm (i.d.) x 0.25 μm (df) HP-5MS capillary column (Agilent, Palo Alto, CA, USA). The column oven was programmed as follows: the initial temperature was 40°C and held for 1 min, ramped at 20°C/min to 60°C and 3°C/min to 97°C, held at 97°C for 3.5 min, then ramped at 20°C/min to 200°C and 40°C/min to 250°C, and finally held at 250°C for 5 min. An electronic pressure control was used to maintain a carrier gas flow of 1.00 mL/min helium.

Flow-through diffusion cell experiments. (Specific Aims# 3)

The skin permeability of all compounds was measured by using a flow-through diffusion cell system (Bronaugh and Stewart, 1985). Porcine skin was obtained from the dorsal area of weanling female Yorkshire pigs. The skin was dermatomed to a thickness of 350 μm with a Padgett Dermatome (Kansas City, MO, USA). Each circular skin section was punched out and placed into a two-compartment Teflon flow-through diffusion cell. The skin membranes were perfused using Krebs-Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin (4.5%). The temperature of the perfusate and flow-through cells was maintained at 37°C using a Brinkman circulator (Westbury, NY, USA). The pH was maintained between 7.3 and 7.5. The

flow rate of the receptor solution was 4.0 mL/h. After careful release of any air-bubbles underneath the skin in the receptor compartments, blank samples were collected before dose. The chemical mixture in water, 1%SLS and 50% ethanol (500 μL) were dosed on the skin in the donor compartment, and sampled every 30 min throughout the 8 h diffusion experiments. The concentrations of different probe compounds in dose vehicles were in the range of 6 – 160 μg/mL in consideration of their solubility and analytical sensitivity known from pre-experimental measurements. The concentrations of the probe compounds in the collected samples were analyzed by a headspace/SPME and GC/MS method.

Calculations and Statistics (Specific Aims #1, 2, and 3)

Partition Coefficient (MCF/Mixture): The partition coefficient of a calibration compound in a given absorption system ($\log K_F$) was calculated from the equilibrium absorption amount ($n°$) by the definition of the partition coefficient (Xia et al., 2003):

$$K_F = \frac{C_{pe}}{C_{me}} = \frac{n^\circ V_m}{V_p(V_m C_0 - n^\circ)} \qquad \textbf{Equation 7}$$

where C_0 is the initial concentration of the compound in the working solution; V_m is the volume of the working solution; V_p is the volume of the MCF membrane (V_p=0.612 μL for 100-μm PDMS, 0.520 μL for 85-μm PA and 0.330 μL for 50-μm Wax); C_{pe} is the equilibrium concentration in the membrane ($C_{pe} = n°/V_p$) and C_{me} is the equilibrium concentration in the working solution ($C_{me} = C_0 - n°/V_m$).

Skn permeability (Kp): The permeability coefficient of a chemical through the skin membrane is calculated via the following equation;

$$Kp = \frac{J_{ss}}{AC_d}$$

where J_{ss} is the steady-state flux (μg/h), A is the dose area (0.64 cm^2) and C_d is the concentration of the chemical in the donor solution (μg/mL). The resulting unit for the permeability coefficient (*Kp*) is cm/h. The steady-state flux was the slope of the accumulation absorption amounts at different time-points (Addicks et al., 1987).

The compounds having a complete set of logKp and $\log K_F$ values were used for statistical analysis. The multiple linear regression analysis was performed by using SAS Analyst from SAS Institute Inc (Cary, NC, USA).

Changes in System Coefficients

A LSER equation matrix is generated from all of the probe and test compounds. In this study, we selected 32 probe compounds (Table 1) with a wide spectrum of molecular descriptor values:

$$LogKp^n = c + rR^n + p\pi^n + a\alpha^n + b\beta^n + vV^n \qquad (n=1, 2, 3, \ldots 32) \qquad \textbf{(Equation.8)}$$

where n is the number of probe compounds. The system coefficients of the absorption system [r p a b v] and the regression constant (c) can be obtained by multiple linear regression analysis of the LSER equation matrix (Eq.2).

When the vehicle or major components change in composition or proportion, the system coefficients will be altered. Therefore, the changes in the system coefficients were used to study the skin absorption from varying chemical mixtures. If the chemical mixture has a small change

in the vehicle, this change will be reflected in the system coefficients, i.e., a small change will be introduced into the system coefficients [$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v$]. The changes in the system coefficients can be obtained by subtraction of the system coefficients of the chemical mixture from those after the change:

$$[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v] = [r\ p\ a\ b\ v]_x - [r\ p\ a\ b\ v]_o = [r_x\text{-}r_o\ p_x\text{-}p_o\ a_x\text{-}a_o\ b_x\text{-}b_o\ v_x\text{-}v_o] \quad \textbf{(Equation 9)}$$

where $[r\ p\ a\ b\ v]_o$ are the system coefficients of the original chemical mixture; $[r\ p\ a\ b\ v]_x$ are the system coefficients after the change of a major component in the chemical mixture and [$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v$] are the changes of the system coefficients.

In our analyses thus far, we modified the skin and MCF systems by adding a solvent (50% ethanol), a surfactant, 1% sodium lauryl sulfate, or a commercial/generic MWF formulation. Thus far we have used regression analyses to (1) model skin permeability using partitioning coefficients in the MCF-array system and (2) model changes in system coefficient in skin with changes in system coefficients in the MCF-array system. The methods for the latter is described in more detail below:

Correlation of the Changes in System Coefficients

The MCF array approach for predicting skin permeability is based on the fact that both permeability coefficient ($\log K_p$) and MCF partition coefficients ($\log K_F$) are free energy related quantities, which are linearly additive from the contribution components (**Eq.1**). The changes in the system coefficients of the skin permeability for a given vehicle change in skin [$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]_{skin}$, will be reflected in alteration of system coefficients of multiple MCFs [$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]_{F1}$, [$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]_{F2}$, ... [$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]_{Fm}$, where m is the number of diverse MCFs)

It is hypothesized that a quantitative correlation exist between the changes in system coefficients of skin permeability and those of MCF partition coefficients under varying chemical mixtures (**Equation 4**)

$$\begin{pmatrix} \Delta r \\ \Delta p \\ \Delta a \\ \Delta b \\ \Delta v \end{pmatrix}_{SKIN} = a_0 + a_1 \begin{pmatrix} \Delta r \\ \Delta p \\ \Delta a \\ \Delta b \\ \Delta v \end{pmatrix}_{F1} + a_2 \begin{pmatrix} \Delta r \\ \Delta p \\ \Delta a \\ \Delta b \\ \Delta v \end{pmatrix}_{F2} + \bullet\bullet\bullet + a_m \begin{pmatrix} \Delta r \\ \Delta p \\ \Delta a \\ \Delta b \\ \Delta v \end{pmatrix}_{Fm} \qquad \textbf{(Eq.10)}$$

The molecular interaction properties of the two systems are described by a set of system coefficients [$r\ p\ a\ b\ v$]. In this paper we will use difference of system coefficients (DSC) technique in order to study the difference of system coefficients of skin. In this study three diverse fibers were polydimethylsilicone (PDMS), polyacrylate (PA) and carbowax (WAX). The DSC was calculated by subtracting the coefficients of each fiber absorbed in water from either 50% SLS or ethanol. We denote the DSC by [$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v$] for these three diverse fibers to access the DSC for skin. Each Δ value represents the difference of each system coefficient for three fibers when partitioned from water and when partitioned from either 50%ethanol-water or 1% SLS-water.

Multiple Regression Analysis

The multiple regression techniques give the coefficients a_1, a_2 and a_3 with intercept a_0. We use stepwise regression with all three independent variables. The independent variables are the array *[Δr Δp Δa Δb Δv]* for the difference of system coefficients (mixture – water) for WAX, PDMS and PA respectively. The dependent variable is *[Δr Δp Δa Δb Δv]* the difference of system coefficients (mixture – water) for skin permeability. We can express this in the form of equation (4) as

$$\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{SKIN} = a_0 + a_1\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{WAX} + a_2\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{PDMS} + a_3\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{PA} \qquad \textbf{Equation 11}$$

The above matrix equation can be written in form $y = a_0 + a_1x_1 + a_2x_2 + a_3x_3$. Where x_1, x_2 and x_3 are the difference of ***mixture*** coefficients of either a mixture of 50% ethanol or mixture of 1% SLS for WAX, PDMS and PA respectively. For simplicity in understanding we will interchangeably use the terms WAX, PDMS and PA for x_1, x_2 and x_3. First we performed the statistical analysis of the data when each fiber is absorbed in water and 1% SLS. We used two set of coefficients *[r p a b v]* values for 1%SLS and water mixtures. Stepwise regression is a technique for choosing the variables, i.e., terms, to include in a multiple regression model. For each term on y-axis, the plot describes the regression coefficients as a point with horizontal bars indicating confidence intervals. Blue points represents the terms that are in the model, while red points indicate terms that are not currently in the model. The R-square value is one minus the ratio of the error sum of squares to the total sum of squares. R-square indicates that the model accounts for a certain percentage of the variability in the observations and the adjusted R-square — the R-square statistic adjusted for the residual degrees of freedom and the RMSE denotes root mean squared error of the current model.

These analyses were performed with Matlab (The MathWorks, Inc. Novi, MI) by using the packages stepwise, regstat and polytool.

Standard errors were determined for all data sets. For analysis of flux, permeability, diffusivity,and absorption, multiple comparison tests were performed using ANOVA with significance level at 0.05. All analyses were carried out using SAS 8.01 for Windows software (SAS Institute Inc., Cary, NC). A least significant difference (LSD) procedure was used for multiple comparisons on all parameters assessed.

RESULTS & DISCUSSION

I. Development and Calibration of MCF system: (Specific Aims #1)

LSERs were developed for three commercial MCFs and one MCF prepared within our laboratory. The system coefficients along with the solute descriptor values (*E,S,A,B,V*) from **table 1** were used in the LSER equations (13) through (15) below to plot (**Figure 1)** the Log K values for all solutes. The correlation coefficients, R_2, ranged from 0.90 for the polyacrylate/water (pa/w) system to 0.99 for the polydimethylsiloxane/water (pdms/w) system.

$\text{Log } K_{pani/w} = 0.40 + 0.61E + 0.21S - 0.69A - 1.45B + 1.22V$ (Equation 12)
$R2 = 0.94$, $SE = 0.11$, $F = 84$, $n = 32$

$\text{Log } K_{pdms/w} = 0.59 + 0.68E - 1.38S - 2.27A - 3.51B + 3.26V$ (Equation 13)
$R2 = 0.99$, $SE = 0.11$, $F = 680$, $n = 31$

$\text{Log } K_{pa/w} = 0.30 + 1.35E - 0.49S - 0.15A - 2.98B + 2.18V$ (Equation 14)
$R2 = 0.90$, $SE = 0.26$, $F = 46$, $n = 31$

$\text{Log } K_{wax/w} = -0.51 + 1.34E - 0.27S + 0.44A - 3.56B + 3.14V$ (Equation 15)
$R2 = 0.96$, $SE = 0.18$, $F = 107$, $n = 31$

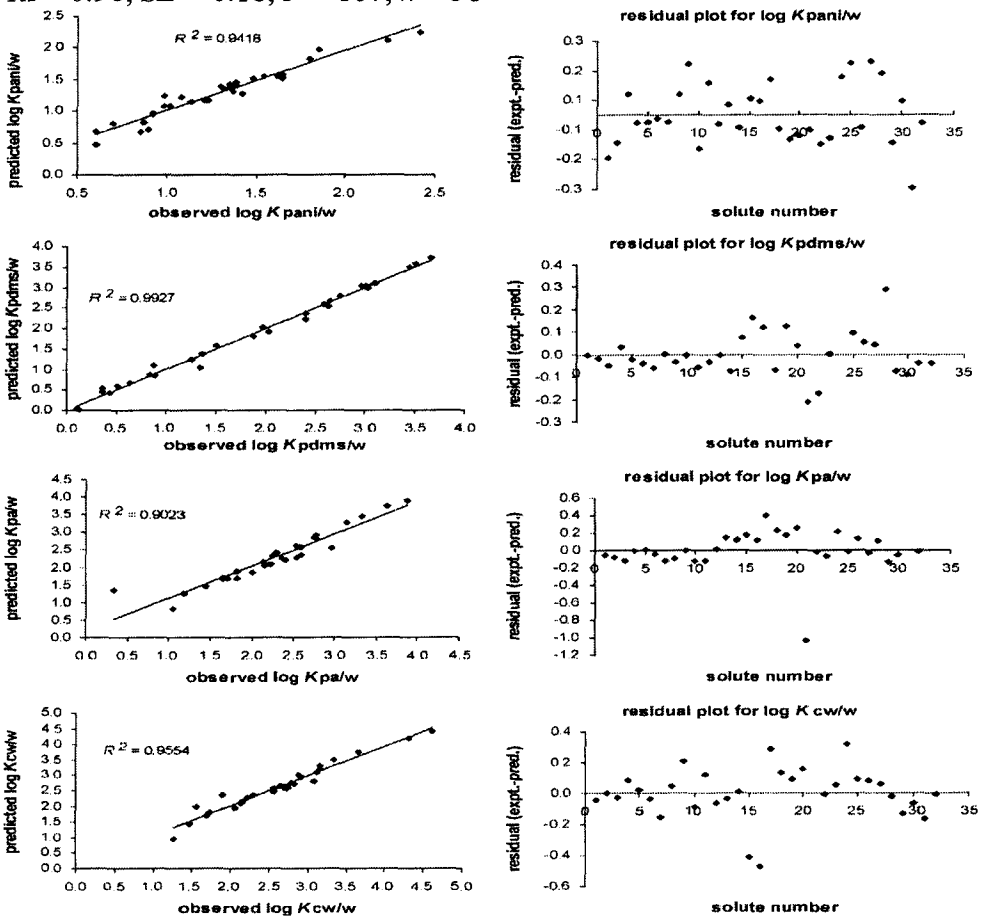

Figure 1. Predicted vs. observed partitioning coefficients for MCF/Water consisting of either PANI, PDMS, PA, or carbowax as well as respective residual plots.

Development and calibration of PANI MCF

The custom-made PANI-coated fiber was prepared with a commercially available proprietary inert metal alloy fiber. Coating this metal fiber with PANI saved time in not having to make the SPME assemblies. Furthermore, the PANI-coated bare metal SPME assemblies could be used with an automated SPME system. Previously, PANI has been electrochemically deposited on platinum, gold, and stainless steel wire and placed into homemade SPME devices that could only be used manually. To our knowledge, this is the first time that the PANI fiber was constructed with an inert metal alloy fiber from a commercial source that allows SPME with the PANI fiber to be automated. Unfortunately, the exact composition of the metal alloy was proprietary. After some trial and error with the number of scans, we chose to use 101 scans. The first scan was used as a preconditioning scan to make sure that the coating process was started correctly. This number of scans allowed us to prepare at least two to three fibers within a 6–8-h-period including solution preparation and electrochemical cell setup. The observed porous structure should theoretically increase the overall surface area of the PANI coating. The SEM photos obtained in our lab were similar in appearance to those published elsewhere.

Reproducibility of the PANI fiber. To show the reproducibility in the preparation of the PANI fiber, the coating thickness for five separate PANI fibers was measured by SEM. The average PANI coating thickness was 3±1_m (n = 5). Furthermore, the SPME experiments were repeated six times with six different PANI fibers all prepared under the same conditions. The average RSD for all 37 compounds was 9.4±4.3% (n = 37). Only four out of 37 of the compounds showed RSDs greater than 15% with the highest RSD being 23.2% for phenethyl alcohol. The average RSD for the five MWF biocides was 7.3±1.4% (n = 5). Therefore, the construction of the PANI fiber can be controlled to give acceptable reproducible data.

Sensitivity of the PANI fiber. SPME with the PANI fiber was used to determine partition coefficients of various probe compounds for LSERs and not for quantitative purposes, therefore, the detection limit for the PANI fiber was not experimentally determined. However, earlier tests (data not shown) performed with just the biocides alone in water showed that the PANI fiber was able to extract the biocides from plain water, under the same experimental conditions as in this report, at 1 ppm. The reason for the low sensitivity by the PANI fiber may be due to the smaller volume of the PANI-coating in comparison to other fibers. Furthermore, the detection limit of the PANI fiber might also be improved by adjusting such experimental parameters as the pH, adding salt or changing the extraction temperature. Since the initial goal of our research was to examine the extraction of the 32 probe compounds and biocides in plain water, those parameters such as pH, and salt content were not examined. All 37 compounds were extracted from plain water at 5 ppm by the PANI fiber.

Prediction of logK for five Metal Working Fluid biocides

The above LSER equations (12-15), derived from linear regression of the 32 probe compounds, was then used to predict partition coefficients of five biocides in PANI/W plus the two extremes, PDMS/W andCW/W, respectively. The biocides were not included in building the LSER model. **Figure 2** shows that the LSER equations predicted partitioning of the 5 biocides into PANI (R^2 = 0.80) and PDMS (R^2 = 0.89) well. The LSERs did not predict partitioning of the five biocides into the CW (R^2 = 0.15) very well. Even though the log K values for all the five biocides were not modeled very well by the LSERs derived for the CW/W system, a correlation (R^2) of 0.79 was obtained when the biocide 2-benzyl-4-chlorophenolwas left out. This confirms

that even though LSER analysis is a useful technique for shedding light upon the types of chemical interactions involved in bulk two phase partitioning processes, it does not always account for all the specific interactions that are present. The source of this lack of predictability deserves further study.

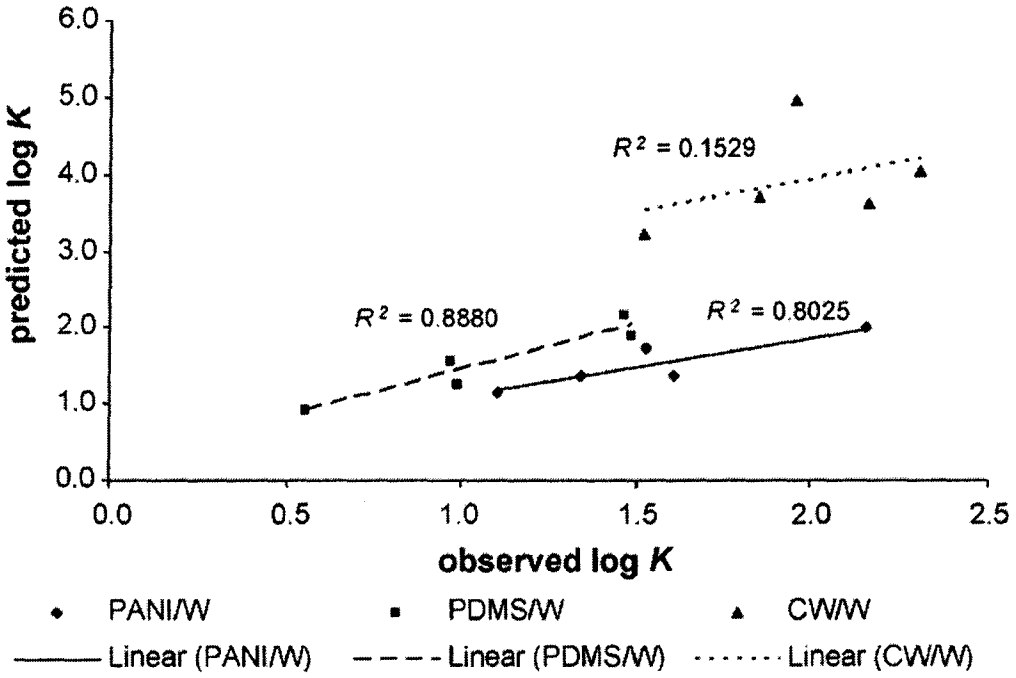

Figure 2. Plot of predicted vs. observed partitioning of 5 MWF biocides from an aqueous solution into PANI, PDMS, and CW fibers.

In conclusion, the LSER approach can be used for molecular interaction characterization of the several MCF fibers. In comparison to the three commercial fibers, PDMS, PA, and WAX, the LSER approach showed that the PANI fiber was the least hydrophobic yet the strongest hydrogen bond donor. This implies that the PANI fiber partitioning is higher for solutes which are strong hydrogen bond acceptors as compared to the other fibers all else being held constant. Furthermore, dipole-dipole and dipole-induced dipole interactions were strongest for the PANI fiber. The custom-made PANI fiber offers a unique selectivity for extracting a wide variety of compounds from an aqueous environment. This added diversity may help to improve our ability to understand and predict partitioning by incorporating the PANI fiber into our array of three fibers previously published.

II. Mixture and Metal working fluid (MWF) formulation effects on MCF partitioning (Specific Aims #2)

MCFs exposed to 50% ethanol resulted in a significant decrease in partitioning of solutes into each of the 3 MCFs which have chemically diverse membrane properties. These membranes were selected because they display absorption kinetics (Xia et al., 2004) and not adsorption properties (Vaes et al., 2000). **Figure 3** below depicts the solvent effects for the PA fiber alone as ethanol concentration increased from 0% to 50%. This was demonstrated for the most part in the other 2 fibers (data not presented). The predictive models for PA/50% Ethanol systems are:

$$\text{Log } K_{PA/50\%Ethanol} = -0.131 + 0.39R + 0.053\pi - 0.035\alpha - 2.025\beta + 1.29V \qquad \textbf{Equation 16}$$
$$(n = 32;\ R^2 = 0.97)$$

The greatest changes in system coefficients for all MCF membranes were for the "*b*" and "*v*" coefficients when ethanol was present. The addition of ethanol to water appears to change hydrogen bond acidity (Δb) and hydrophobicity (Δv) more so than changes in other system coefficients. These changes in system coefficients are consistent with the hypothesis that the presence of ethanol in water increases the hydrophobicity of the solution and consequently reduces the hydrophobicity difference (Δv) between the MCF and water. Previous MCF studies (Xia et al., 2007) with only the PDMS fiber demonstrated the importance of hydrogen bonding contribution and the hydrophobicity difference between the MCF and formulation as the ethanol concentration increased from 0% to 25% to 35% ethanol. Based on preliminary studies in our laboratory, one can assume that this concentration effect on specific molecular descriptors is comparable across all 3 MCFs. However, further work needs to be completed to confirm these specific interactions in MCF and skin. Having established these tentative relationships, our final objective was to demonstrate whether the ethanol induced changes in skin permeability can be predicted from a single or any combination of MCFs as was proposed in Specific Aims #3. However, the figure below is an example of how the MCFs are sensitive enough to assess changes in partitioning of suite of solutes including metal-working fluid biocides.

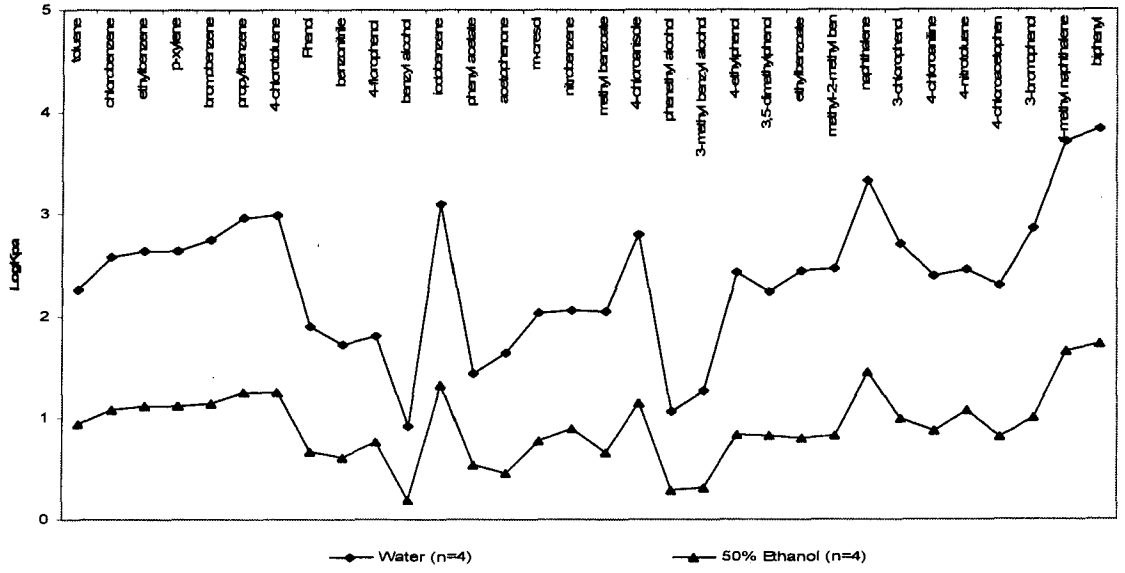

Figure 3. Distribution coefficients ($LogK_{PA}$) for solutes #1 to #32 (Table 1) measured with PA membrane coated fiber (MCF) in water or 50% ethanol solutions.

To date, our group has focused primarily on probing/screening for formulation effects on solute partitioning by exposing one of the MCFs, that is, PDMS, to three generic MWF formulations (soluble oil, semisynthetic, and synthetic MWF) and their corresponding surrogate mixtures (mineral oil or PEG-200) spiked with a 32-training solute set and phenolic biocides. The data summarized in **Figure 4** below demonstrates several interesting trends suggestive of formulation effects and the advantages of using a MCF-array as a screening system to predict biocide partitioning and permeability in skin. There was a general increase in solute partitioning into the PDMS fiber from the formulations as the Log Ko/w values of the solutes increased from 1.1 to 3.99. This infers that chemicals with relatively high lipophilicty could more readily be absorbed across the skin than the less lipophilic compounds. Solute partitioning into the PDMS fiber from generic soluble oil, as indicated by the regression ($R^2 = 0.77$) for the training set of solutes, generally increased as the concentration of MWF decreased (5.0% < 0.5% < 0.05%). It is possible that dilution of MWF formulation with water could potentially increase chemical absorption across the skin. These observations were less obvious with PEG-200 and mineral oil concentrations.

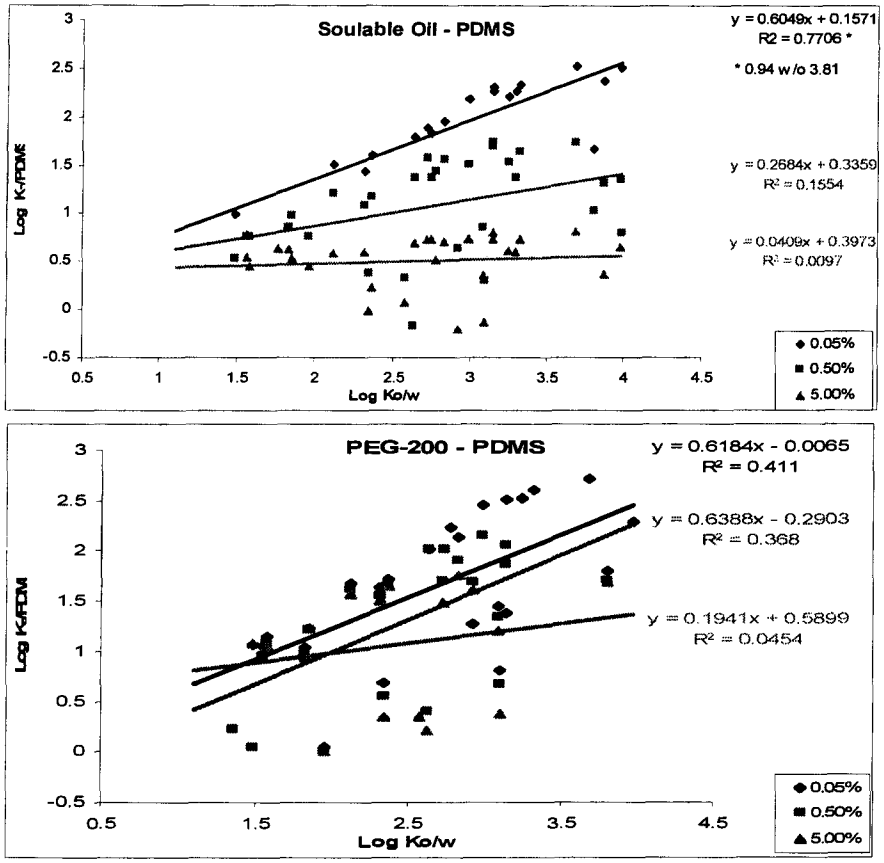

Figure 4. Partitioning behavior of training solutes and MWF biocides in a generic soluble oil formulation and synthetic formulation at 3 concentrations, 0.05, 0.5, and 5.0%.

The next phase of this research was to evaluate the partitioning of a phenolic biocide (4-tetriary amylphenol), in two generic formulations (a soluble oil or synthetic MWF) and 2 surrogate mixtures (mineral oil or PEG-200) at different biocide concentrations and three different MWF concentrations. **Figure 4 below** consists of 4 large graphs having the same scale on the Y-axis, to emphasize the partitioning differences between synthetic and soluble oils, while

the embedded graphs for the two synthetics have an expanded scale to show that the same trend appears in the generic synthetic MWF but no concentration effect in PEG-200. For this specific phenolic biocide, the greatest partitioning occurred in PEG-200 (log Ko/w = 1.81), followed by mineral oil, synthetic, and soluble oil (log Ko/w = 1.6). Note that these are preliminary data and semi-synthetic MWF was not analyzed at publication time.

Figure 4 below also depicts changes in AUC response from the fiber with respect to changes in biocide concentrations ranging from 0, 0.05, 0.10, 0.50, 1.00, and 5.00 ppm. These AUC values reflect GC-MSD responses following direct exposure of PDMS to the MWF followed by direct injection into the GC-MSD. Again, the same trend observed with the training set was observed with the biocide. In this application, biocide partitioning increased with decreasing MWF concentration across a biocide concentration range from 5.00 ppm to 0.05 ppm.

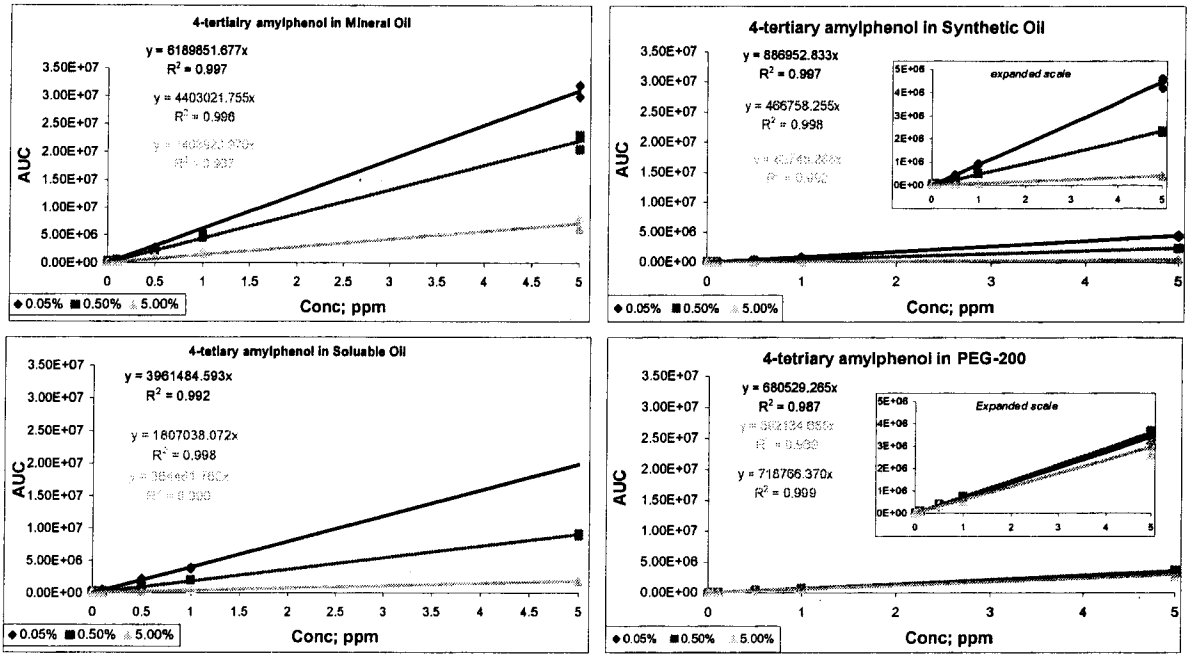

Figure 4. Total amount of 4-tertiary amylphenol in PDMS (AUC) following exposure to 5 different concentrations of amylphenol in generic MWF formulations or surrogate MWF mixtures.

In conclusion, these formulation studies with the MCF fiber clearly demonstrated the effects of different MWF formulations on solute/bioicde partitioning and by extension, solute/biocide permeability in skin. MWF concentrations can influence solute and biocide partitioning into MCFs such as the PDMS fiber. The long terms goal is to be able to determine if this screening process with the MCF reflects *in vitro* permeability in a viable skin model system used in our laboratory. While the focus of this research has thus far targeted phenolic biocides, another long-term goal is to be able to generate robust QSARs that can predict skin permeability of penolics and other biocide classes from a larger chemical space. These quantitative models attempt to capture permeability changes across soluble oil, semi-synthetic, and synthetic MWFs

III. Dermal permeability of solutes and MWF biocides and use of MCF-array to predict biocide permeability (Specific Aims #3).

Solute/Biocide permeability and developed LSER models:

The permeability (log Kp) for the diverse 25 solutes dosed in water or 50% ethanol ranged from -1.91 cm/hr for phenylethyl alcohol to -0.50 cm/hr for naphthalene in water and from -3.13 cm/hr for 3-bromophenol to -1.61 cm/hr for p-xylene in 50% ethanol. These data demonstrated that the presence of the ethanol in water significantly reduced the permeability of the 25 solutes by as much as 4.0 –fold for several solutes (e.g., naphthalene and biphenyl).

Almost similar formulation effects were observed in skin exposed to either 17% methanol, a soluble oil metal-working fluid (MWF) formulation (Astrocut), or a synthetic MWF formulation (Tapfree) (**Figure 5**).

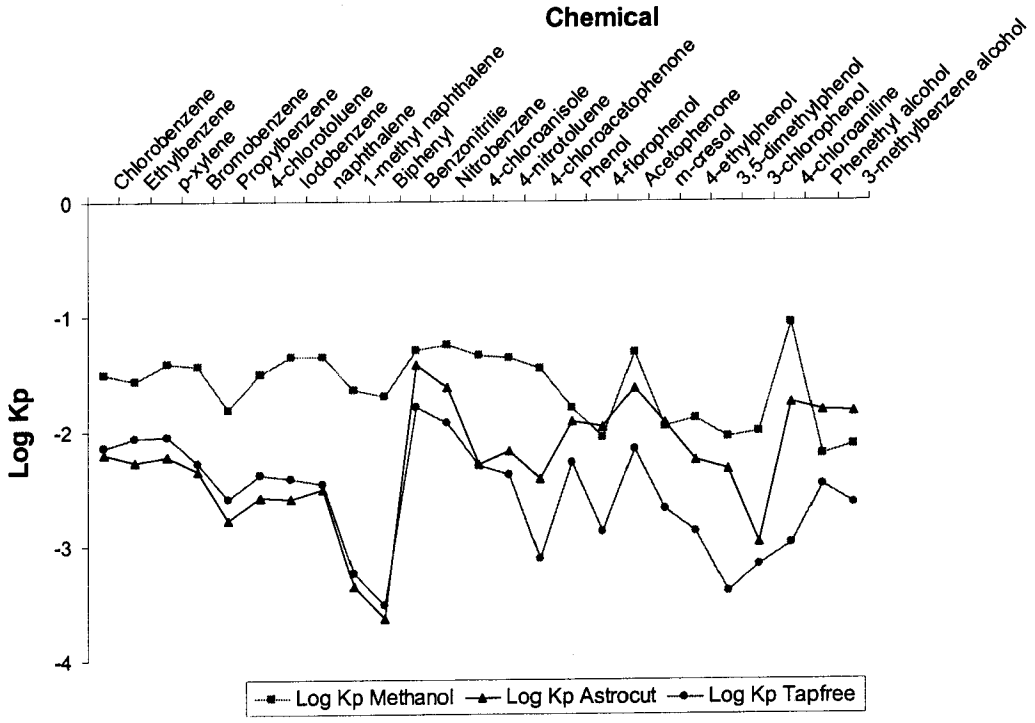

Figure 5. Porcine skin permeability (log Kp) (cm/hr) of 25 diverse solutes dosed in either methanol, Astrocut MWF formulation, or Tapfree MWF formulation topically applied to *in vitro* porcine skin sections.

When compared to an aqueous formulation such as 17% methanol, these commercial MWF formulations significantly reduced solute permeability. Furthermore, solute permeability was for the most part greater in the more aqueous MWF formulation (Tapfree) than in the soluble oil MWF formulation. These findings suggest that these specific formulations generally reduce solute permeation in skin; this runs contrary to our original hypothesis that formulation additives enhance solute permeation. Similar observations were reported in our laboratory and others for solute permeation in simpler formulations with defined solvent and surfactant

compositions. Data from these studies led to the hypothesis that lipophilic pesticides topically applied to the skin in formulations that enhance solubility in the formulation are least likely to partition from the formulation into the skin. This partitioning behavior was demonstrated with the dermal permeability of the biocide, triazine, in a chemically-defined soluble oil MWF or synthetic MWF. Compared to the phenolic biocides described in this report, triazine is more water soluble and therefore more permeable in a soluble oil formulation than an aqueous and less hydrophobic formulation. That is, because triazine is less soluble in soluble oil formulations, it will more likely partition from the soluble oil formulation into the skin membrane. In the present study, phenolic biocide permeability in the more hydrophobic formulation (Astrocut) is less than in the more aqueous formulation (Tapfree). It should be noted that Astrocut contains heavy naphthenic oil and Tapfree contains polyalkylene glycol and fatty acids. While the 50% ethanol formulation shows a similar inhibitory effect on skin permeability as the MWF formulations, the magnitude of the differences between the ethanol formulation and the MWF formulations underscores the complexity of the interaction within the MWF formulation and skin which is difficult to predict. By grouping the above data into training solute sets and test solute sets, regression analysis described below attempts to generate predictive models for each of the formulations.

The following predictive models were obtained for skin permeability of a subset of 20 training solutes in 50% ethanol, Astrocut, and Tapfree formulations:

Skin/Astrocut system

$$\text{Log } K_{(\text{Skin/Astrocut})} = 0.96 - 0.47\,R + 0.34\,\pi - 0.35\,\alpha + 1.95\,\beta - 3.54\,V \qquad \textbf{Equation. 17}$$

($R^2 = 0.94$; s = 0.15, n=20)

Skin/Tapfree system

$$\text{Log } K_{(\text{Skin/Tapfree})} = 1.27 - 0.19\,R - 0.67\,\pi - 1.5\,\alpha + 1.21\,\beta - 3.14\,V \qquad \textbf{Equation. 18}$$

($R^2 = 0.84$, s = 0.21, n=20)

Skin/50% Ethanol system

$$\text{Log } K_{(\text{Skin/50\% Ethanol})} = 0.04 + 0.02\,R - 0.53\,\pi - 1.20\,\alpha - 0.35\,\beta - 1.59\,V \qquad \textbf{Equation. 19}$$

($R^2 = 0.92$, s = 0.12, n=20).

The training set of 20 solutes have log octanol-water partition coefficients (log Ko/w) ranging from 1.05 to 3.98. The test set consisted only of the following phenols which are most likely formulated with commercial MWFs and therefore of occupational concern: phenol, 4-florophenol, 4-ethylphenol, and 3,5-dimethylphenol evaluated in all 3 formulations with 3-chlorophenol, 4-tert-amylphenol and *o*-phenylphenol also being evaluated in the two MWF formulations and 3-bromophenol evaluated in 50% ethanol formulation. The predictive model for the Astrocut formulation appears to be the most optimal ($R^2 = 0.83$) for predicting skin permeability of phenols compared to the other predictive models ($R^2 = 0.74 - 0.76$) for their respective formulations. These phenols have log Ko/w values ranging from 1.50 to 3.83 which is inclusive of log Ko/w range for the 20-solute training set. It is possible that inclusion of a diverse series of phenols in the training set would improve the predictive model and the robustness of the three formulation models could have been better evaluated with a larger and more diverse series of phenolic compounds. However, these findings are suggestive that a model specific for a

named formulation can be predictive of skin permeability of phenolic biocides used in MWF formulations that reflect dermal exposure to complex chemical formulations.

Development of MCF-array to predict skin permeability

The permeability coefficients of all 25 probe compounds in a dose vehicle containing 50% ethanol (log $K_{Skin/E50}$) were correlated with the partition coefficients of the compounds measured with three MCFs in 50% ethanol solutions (log $K_{PDMS/E50}$, log $K_{PA/E50}$, and log $K_{Wax/E50}$). A predictive model for the skin permeability of chemicals from 50% ethanol was established via multiple regression analysis of the matrix [log $K_{Skin/E50}$: log KPDMS/E50, log $K_{PA/E50}$, log $K_{Wax/E50}$];

$$\text{Log } K_{Skin/50\%Ethanol} = -1.18 + 0.36 \log K_{PDMS/50\%Ethanol} + 0.80 \log K_{PA/50\%Ethanol} - 1.32 \log K_{Wax/50\%Ethanol}$$

$(n = 25, R^2 = 0.91, F = 75, s = 0.133)$ **Equation 20.**

The absence of one or two of the MCF fibers in this array resulted in less than optimal fits with R^2 values ranging from 0.39 to 0.61 for correlations between skin permeability and partitioning into any single MCF

Figure 6 shows the experimental log k values versus predicted log k values with the three MCFs in 50% ethanol solutions. A linear correlation is observed with a R2 of 0.91.

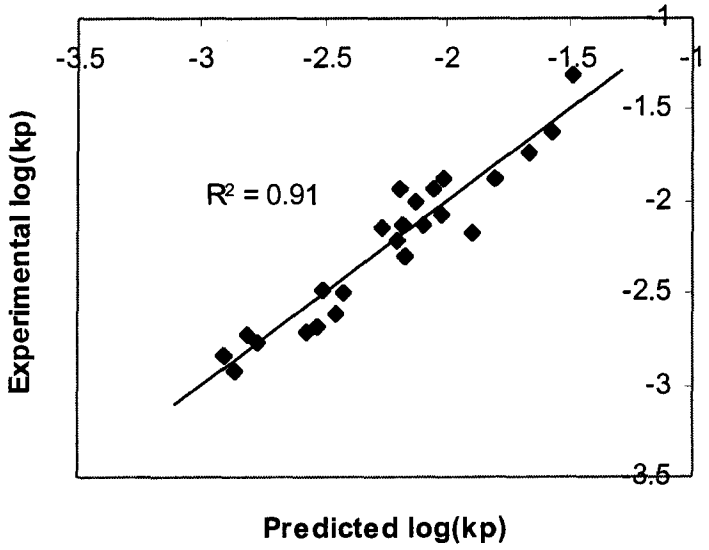

Figure 6. Correlation of experimental skin permeability (logKp) with predicted skin permeability (Kp) from the three MCF array in 50% ethanol.

Figure 7 below demonstrates that the greatest changes in system coefficients for both skin and all MCF membranes were for the "*b*" and "*v*" coefficients when ethanol was present. The addition of ethanol to water appears to change hydrogen bond acidity (Δb) and hydrophobicity (Δv) more so than changes in other system coefficients.

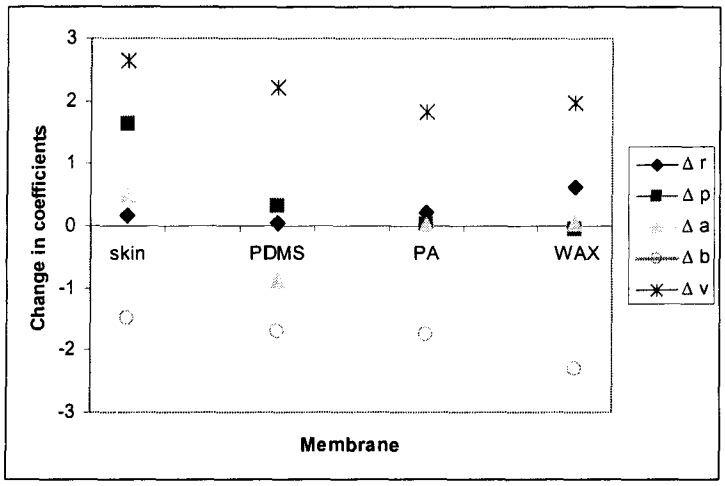

Figure 7. Changes in system coefficients (*r, p, a, b, v*) in Skin and PDMS, PA, and WAX fibers when comparing exposure to 50% ethanol and water

Regression analysis demonstrated that changes in dermal permeability in the presence of 50% ethanol or 1% SLS was strongly correlated to changes in partitioning in the MCF array of 3 fibers. R-square values ranged from 0.59 to 0.98 when changes in both systems were compared in either ethanol or SLS mixtures. The best correlations were observed when the full MCF array was included in the regression analysis.

The regression equation for ethanol mixtures is $y = 0.619 - 1.076x_1 + 0.288x_2 + 2.128x_3$ with x_1, x_2 and x_3 representing delta (Δ) values for WAX, PDMS, and PA fibers, respectively. The regression equation for the 1% SLS mixtures is $y = 0.548 - 0.931x_1 + 3.81x_2 - 2.66x_3$ with x_1, x_2 and x_3 representing delta (Δ) values for WAX, PDMS, and PA, respectively.

Figure 8 below demonstrates the strong relationship (R-squared = 0.984) between the 5 observed changes in system coefficients (*Δr Δp Δa Δb Δv*) and those predicted delta values from the polynomial model used in the previously described matrix .

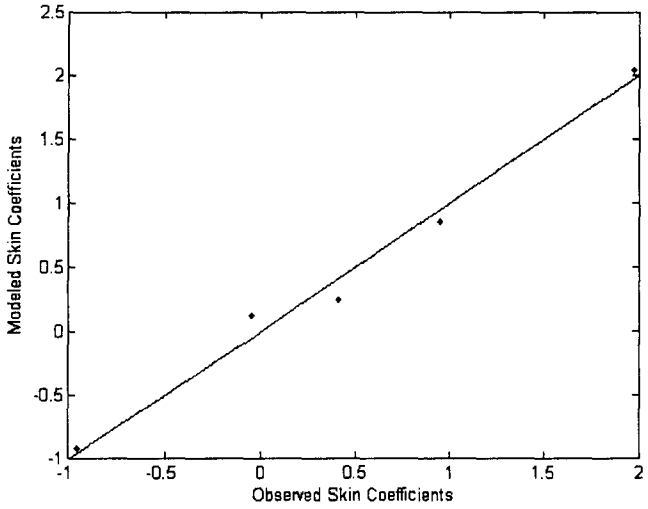

Figure 8. The predicted versus observed Δ system coefficients for skin for the full MCF array exposed to 1% SLS.

This study demonstrated that a MCF array of 3 physicochemically diverse fibers can accurately predict changes in dermal permeability. Our novel approach utilized several well accepted technologies (MCF) in analytical chemistry as well as physicochemical properties defined by differences in system coefficients or DSCs *[Δr Δp Δa Δb Δv]* in a LSER that captured these mixture interactions *independently* in both skin and the MCF array. We used the Abraham LSER model as it is generally accpeted as a represenation and robust QSAR approach. Multilinear regression was able to statistically analyze these matrices of DSCs to determine the significant contributions of individual MCF or combination of MCFs to accuately reflect mixture-induced permeability changes in the skin. This is the first study to link the quantitative changes in skin permeability with quntitative changes in solute partitioning into a MCF array on the basis of changes in a matrix of molecular descriptores. The use of five solvatochromatic parameters described in the LSER allowed for this linkage to be made between physicochemical interactions in skin and an inert membrane system. These data support the theory that PA, PDMS, and WAX possess functional groups that are collectively influenced in the same proportion to similar or related functional groups in skin when exposed to SLS or ethanol.

CONCLUSIONS

This body of research has resulted in 7 *peer-reviewed publications*, *1 book chapter,* and *6 abstracts* presented at toxicology and occupational health meetings. The data demonstrated formulation effects with simple mixtures (50% ethanol) as well as complex commercial metal-working fluid (MWF) formulations used in the metal machining industry. The effects of MWF formulations on the dermal permeability of phenolic biocides were characterized within a linear solvation energy relationship (LSER). MWF formulations consist of hundreds of performance additives which can potentially influence solute/biocide permeability via various mechanisms described above, therefore several if not all of these effects on skin permeability can be predicted within the context of a LSER model. This research was the first to demonstrate that the permeation of biocides and other aromatic chemicals is higher in synthetic metalworking fluids when compared to soluble oil, which indicates that a soluble oil type of MWF may be safer than a synthetic fluid with respect to dermal permeation of biocides. Therefore, there would be less potential to cause contact dermatitis. Furthermore, dilution with water, as often recommended, can actually increase biocide permeability and not the expected opposite effect. Regression models were developed to demonstrate these formulation effects and they were also validated with repeatable *in vitro* studies. The limitations of the proposed models include formulation specificity and biocide selection. This study also demonstrated the use of an LSER approach and membrane-coated fiber (MCF) array to predict permeability changes for solutes exposed to 50% ethanol solution. The model was optimal for MCF array and not for individual MCFs or pairs of MCFs for predicting changes in skin permeability when ethanol is added to an aqueous solution. Future work is focused on determining whether the MCF approach, which is a highly reproducible and high through-put system, can be predictable of skin permeability in more complex formulations such as MWF formulations. Future work will also be aimed at improving on our MCF calibration by using a more statistically robust training set of solutes from a more complete data set with complete molecular descriptors. The models developed from these assessments will be instructive to formulators and risk assessors concerned with limiting dermal absorption of related MWF chemicals when workers are continually exposed to these dermal irritants.

PUBLICATIONS GENERATED FROM THIS GRANT:

Peer-Reviewed Publications.

Xia, XR., Baynes, R. E., Monteiro-Riviere, NA., and Riviere, JE: [2007]. An experimentally based approach for predicting skin permeability of chemicals and drugs using membrane-coated fiber array. Toxicology and Applied Pharmacology. 221(3): 320-328. **(Specific Aim 1)**

Riviere, J.E., Baynes, R. E., and Xia, X.R: [2007]. Membrane-coated fiber array approach for predicting skin permeability of chemical mixtures from different vehicles. Toxicological Science. 99(1): 153-161. **(Specific Aim 2 & 3)**

Vijay, V., Yeatts, J., Riviere, JE., Baynes, RE: [2007]. Predicting dermal permeability of biocides in commercial cutting fluids using a LSER approach. Toxicology Letters 175: 134-143. **(Specific Aim 3)**

Yeatts, JL., Baynes, RE., Xia, XR., and Riviere, JE: [2008]. Application of linear solvation energy relationships to a custom-made ployaniline solid-phase microextraction fiber and three commercial fibers. Journal of Chromatography. *A*. 1188(2):108-117. **(Specific Aim 1)**

Baynes, R.E., Xia, XR., Imran, M., and Riviere, JE. [2008]. Quantification of chemical mixture interactions modulating dermal absorption using a multiple membrane fiber array. Chemical Research in Toxicology (21): 591-599. **(Specific Aim 3)**

Baynes, RE, Xia, XR., Vijay, V., and Riviere, J: [2008]. A solvatochromatic approach to quantifying formulation effects on dermal permeability. SAR QSAR Environmental Research, in press. **(Specific Aim 2 & 3)**

Vijay V, White EM, Kaminski MD, Riviere JE, and Baynes RE: [2008]. Dermal permeation of biocides and aromatic chemicals in three generic formulations of metal-working fluids. Journal of Toxicology and Environmental Health A, submitted. **(Specific Aim 3)**

Book Chapters

Baynes RE., and Vijay, V: [2008]. Dermal absorption and toxicity of metal-working fluids. In: *Toxicology of the Skin*: *Target Organ Series*, (ed. NA. Monteiro-Riviere), Informa Healthcare. in press.

Poster Presentations & Abstracts

Vijay, V., Baynes, RE. [2008]. Interpretation of molecular interactions influencing dermal permeation of biocides in commercial metal removal fluids using LSER approach. The Industrial Metalworking Environment: The 3rd Symposium on the Assessment and Control of Metal Removing Fluids. Dearborn, MI. October 5th – 8th.

Imran, M., Baynes, RE., Xia, X., and Riviere, JE. [2008]. Use of multi-fiber approach to quantify chemical mixture interactions modulating dermal absorption. *Toxicologist*. 102: 1556. Society of Toxicology 47th Annual Meeting, Seattle, WA, March 15th –19th.

Vijay, V., and Baynes, RE. [2008]. Solvatochromatic interactions influencing dermal permeation of biocides in commercial cutting fluids. *Toxicologist*. 102: 1557. Society of Toxicology 47th Annual Meeting, Seattle, WA, March 15th –19th.

Vijay, V., Barlow, B., Yeatts, J., and Baynes, RE. [2007]. Predicting cutaneous permeability of biocides using LSER approach. *Toxicologist*. 96: 2092 Society of Toxicology 46th Annual Meeting, Charlotte, NC, March 25th –29th.

Yeatts, JL., Baynes, RE., and Riviere, JE. [2007]. Custom made PANI fiber to assess dermal partitioning and absorption of biocides. *Toxicologist*. 96: 2099. Society of Toxicology 46th Annual Meeting, Charlotte, NC, March 25th –29th.

Xia, RR., Baynes, RE., Monteiro-Riviere, NA., and Riviere, JE. [2007]. Predicting skin permeability of molecules from chemical mixtures using an inert membrane-coated fiber array. *Toxicologist*. 96: 2100. Society of Toxicology 46th Annual Meeting, Charlotte, NC, March 25th –29th.