14
Dermal Chemical Mixtures

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14.1
Introduction

Drug and chemical dermal absorption typically involves experiments conducted using single chemicals making the mechanisms of absorption of individual chemicals extensively studied. Similarly, most risk assessment profiles and mathematical models are based on the behavior of single chemicals. A primary route of occupational and environmental exposure to toxic chemicals is through the skin; however, such exposures are often to complex chemical mixtures. In fact, the effects of coadministered chemicals on the rate and extent of absorption of a topically applied systemic toxicant may determine whether toxicity is ever realized. This dichotomy between the availability of data and the absorption models based on single chemicals with field exposure scenarios dominated by complex mixtures deserves further attention.

It is axiomatic that for a topically applied chemical to exert systemic toxicity, absorption across the dermal barrier is required. For a topically applied compound to be absorbed into the skin, it must first pass through the stratum corneum, continue through the epidermal layers and penetrate the dermis where absorption into the dermal microcirculation becomes the portal for systemic exposure. For a chemical with direct toxicity to the skin, systemic absorption is not required as the target cells could be any of those comprising the epidermis or dermis.

The application of risk assessment to dermal absorption by US regulatory agencies (EPA, OSHA, and ATSDR) is varied and highly dependent upon available data [25, 26, 44]. A similar concern over the lack of data exists for overall risk assessment of chemical mixtures [16, 23, 24, 45]. A Congressional Commission on Risk Assessment and Risk Management [20] recommended going beyond individual chemical assessments and to focus on the broader issue of toxicity of chemical mixtures. With complex mixtures of hundreds of components, current approaches border on the impossible. Importantly, interactions involving the modulation of dermal absorption, and thus systemic bioavailability, have not been addressed.

It is impossible to assess all potential combinations of chemicals to determine which has the greatest potential to modulate absorption of a known toxic entity

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topically exposed to a chemical mixture. The present state of knowledge in this area is particularly weak since the significance of specific interactions has not been quantitated, let alone in many cases even identified. In many ways, this same concern continues to define the very nature of chemical mixture toxicology. The appreciation of the importance of chemical mixture interactions to effect chemical and drug disposition, biotransformation, pharmacokinetics, and activity has been well recognized for many years and is extensively reviewed elsewhere [15, 16, 28–30, 45]. A large body of literature exists in the field of drug–drug interactions and a body of work is developing in the area of food additive interactions on drug absorption. Despite this widespread knowledge base of the importance of drug–drug interactions and formulation effects in pharmaceutical science, the potential role of chemical interactions in systemic toxicology has not garnered significant attention. This chapter provides an overview of the potential mechanisms operative in topical chemical mixtures and presents some of our laboratory's efforts to quantitate these effects.

In cases where the potential toxicity of a specific mixture is of concern (e.g., at a specific toxic waste site), the complete mixture is often tested [35]. However, how does one quantitate the absorption of a mixture consisting of 50 chemicals? How are markers selected? How are these data expressed? Unfortunately, even after a complete toxicological profile of a specific mixture (e.g., "standard" mixture of 50 environmentally relevant compounds, surrogate jet fuels, etc.) is defined using all the techniques modern toxicology and toxicogenomics have to offer, one cannot define the links between the absorption and the effects seen. Was the observed toxicity exerted because a specific toxicant was in the mixture or because two synergistic toxicants were absorbed *or* was it exerted simply by the presence of a mixture component (e.g., alcohol, surfactant, fatty acid) that enhanced the absorption of a normally minimally absorbed toxicant? We have demonstrated [9, 52] such an interaction with the putative toxins involved in the Gulf War Syndrome [1] where systemic pyridostigmine bromide or coexposure to jet fuel was shown to greatly enhance the dermal absorption of topical permethrin. Would other pesticides have this effect? How does one take into account such critical interactions so that a proper risk assessment may be conducted? An inclusive approach to this problem is to define chemicals on the basis of how they would interact both with other components of a mixture and with the barrier components of the skin. What are the physical–chemical properties that would significantly modify absorption and potentiate systemic exposure to a toxicant? What are the properties of molecules susceptible to such modulation?

One recently reported approach to address this problem assesses potential interactions in dermal absorption by fractionating the effects of a vehicle on drug penetration into two primary parameters describing permeation according to Fick's law: partitioning (PC) and diffusivity (D) (see below; permeability (K_p) = D·PC/membrane thickness) [56]. Although this study only reported on four compounds, one (diazepam) was not predictable using this approach as its physiochemical properties were already optimal for absorption, and only absorption enhancers were investigated. This study illustrates the difficulty of

making broad generalizations across compounds solely on physical–chemical properties.

The problem of dermal mixture absorption is conceptually similar to that of dermatological formulations in the pharmaceutical arena. The primary difference is that most pharmaceutical formulation components are added for a specific purpose relative to the delivery, stability, or activity of the active ingredient. In the environmental and occupational scenarios, additives are a function on either their natural occurrence or presence in a mixture for a purpose related to uses of that mixture (e.g., a fuel performance additive, stability), and not for their effects on absorption or toxicity of the potential toxicant. Unlike pharmaceutical formulation additives in a dermal medication, chemical components of a mixture are not classified on how they could modulate percutaneous absorption of simultaneously exposed topical chemicals. They are present *functionally* for specific purposes (e.g., performance additives, lubricants, and modulators of some biological activity), *sequentially* because they were applied to the skin independently at different times for unrelated purposes (cosmetics followed by topical insect repellent, sunscreens), *accidentally* because they were simultaneously disposed of as waste, or *coincidentally* associated as part of a complex occupational or environmental exposure.

14.2 Mechanisms of Interactions

Chemical interactions that may modulate dermal absorption can be conveniently classified according to physical location where an interaction may occur. The advantage of this approach is that potential interactions may be defined both on the basis of specific mechanisms of action involved and by the biological complexity of the experimental model required to detect it.

Surface of Skin:

Chemical–chemical (binding, ion-pair formation, etc.)

Altered physical–chemical and solvatochromatic properties (e.g., solubility, volatility, critical micelle concentration (CMC))

Altered rates of surface evaporation

Occlusive behavior

Binding or interaction with adnexial structures or their products (e.g., hair, sweat, sebum).

Stratum Corneum:

Altered permeability through lipid pathway (e.g., enhancer)

Altered partitioning into stratum corneum

Extraction of intercellular lipids

Epidermis:

Altered biotransformation

Induction of and/or modulation of inflammatory mediators

Dermis:

Altered vascular function (direct or secondary to mediator release)

The first and most widely studied area of chemical–chemical interactions is the surface of the skin. The types of phenomena that could occur are governed by the laws of solution chemistry and include factors such as altered solubility, precipitation, supersaturation, solvation, or volatility, as well as physical–chemical effects such as altered surface tension from the presence of surfactants, changed solution viscosity, and micelle formation [4, 32, 40, 61]. For some of these so-called solvatochromatic effects, chemicals act independent of one another. However, for many the presence of other component chemicals may modulate the effect seen.

Chemical interactions may further be modulated by interaction with adnexial structures or their products such as hair, sebum, or sweat secretions. The result is that when a marker chemical is dosed on the skin as a component of a chemical mixture, the amount freely available for subsequent absorption may be significantly affected. The primary driving force for chemical absorption in skin is passive diffusion that requires a concentration gradient of thermodynamically active (free) chemical.

A second level of potential interaction is of those involving the marker and/or component chemicals with the constituents of the stratum corneum. These include the classic enhancers such as oleic acid, Azone®, or ethanol, widely reviewed elsewhere [61]. These chemicals alter a compound's permeability within the intercellular lipids of the stratum corneum. Organic vehicles persisting on the surface of the skin may extract stratum corneum lipids that would alter permeability to the marker chemical [38, 48]. Compounds may also bind to stratum corneum constituents forming a depot.

Another level of interaction would be with the viable epidermis. The most obvious point of potential interaction would be with a compound that undergoes biotransformation [18, 43]. A penetrating chemical and mixture component could interact in a number of ways, including competitive or noncompetitive inhibition for occupancy at the enzyme's active site, or induction or inhibition of drug-metabolizing enzymes. Other structural and functional enzymes could also be affected (e.g., lipid synthesis enzymes) that would modify barrier function [22]. A chemical could also induce keratinocytes to release cytokines or other inflammatory mediators [3, 33, 37] that could ultimately alter barrier function in the stratum corneum or vascular function in the dermis. Alternatively, cytokines may modulate biotransformation enzyme activities [39].

The last level of potential interaction is in the dermis where a component chemical may directly or indirectly (e.g., via cytokine release in the epidermis) modulate vascular uptake of the penetrated toxicant [49, 62]. In addition to modulating

transdermal flux of chemicals, such vascular modulation could also affect the depth and extent of toxicant penetration into underlying tissues.

The optimal experimental approach to define such interactions is to conduct studies *in vivo* since all potential mechanisms of interactions are present. However, this approach is cost-prohibitive and is not amenable to determination of specific permeability parameters that would elucidate basic principles that could then be used to predict interactions in the future. Although the biologically intact skin seen in the *in vivo* setting might seem to be ideal, in reality it is difficult to dissect out interactions that occur in different phases of absorption. This is due to the confounding influence of multiple biological and surface chemical factors, as well as systemic effects, being present. This scenario is further aggravated by the high level of interindividual variability typical of *in vivo* studies that mask the detection of important interactions that might be exerted in other mixtures of slightly different composition. Most importantly, acute studies or those using extremely toxic chemicals could never be conducted in humans for ethical reasons or even in intact animals due to humane considerations, making an alternative approach such as outlined here a necessity.

14.3
Mixture Interactions in Skin

Our research program has focused on the effects of chemical mixture components on dermal absorption of select "marker" chemicals in the mixture [8, 47, 53, 54]. As varied as these potential interactions may be, experimentally isolating them is difficult due to confounding effects from simultaneously occurring multiple interactions (e.g., enhanced partitioning coupled with decreased diffusivity, solubility, or vascular uptake). To dissect out these processes, our laboratory's approach has been to use different model systems with increasing levels of biological complexity.

Silicone Membrane Flow-Through Diffusion Cells (SMFT): Sensitive to solvatochromatic processes
Porcine Skin Flow-Through Diffusion Cells (PSFT): Also sensitive to changes in lipid permeability
Isolated Perfused Porcine Skin Flaps (IPPSF): Also sensitive to irritation and vascular events

This hierarchy of experimental models allows interactions to be independently studied using efficient and humane *in vitro* model systems. These systems, as well as the basic principles of percutaneous absorption of single chemicals, have been extensively described in the literature [17, 50].

In order to investigate the nature of mixture interactions on chemical absorption, a series of studies were conducted on 12 diverse chemicals representing three chemical classes [58, 59]:

Substituted Phenols: nonylphenol, pentachlorophenol (PCP), phenol, *p*-nitrophenol (PNP)

Organophosphate Pesticides: chlorpyrifos, ethylparathion, fenthion, methyl parathion
Triazines: atrazine, propazine, simazine, and triazine

These compounds have molecular weights ranging from 94 to 350 and log octanol–water partition coefficients (log $K_{o/w}$) ranging from −1 to 5. Compounds were studied using a complete factorial experimental design. There were three vehicles and three binary-vehicle combinations: water, ethanol, propylene glycol (PG), water/ethanol, water/PG, and ethanol/PG. For each vehicle combination, a control (no additive) or one or both of two mixture components, the surfactant sodium lauryl sulfate (SLS) or vasodilator methyl nicotinate (MNA), were added. This resulted in 24 mixture combinations per chemical, each conducted in two flow-through diffusion cell systems: silastic membrane (SMFT) or porcine skin (PSFT). Porcine stratum corneum partition coefficient (log $K_{SC/MIX}$) across all vehicles was determined. Parameters determined in the diffusion cell studies included permeability and diffusivity. A restricted number of specific vehicles and compound combinations were then selected for study in the IPPSF. Data were initially analyzed using compass plots to determine mixture interactions. Compass plots have been useful to visually examine data to probe complex mixture interactions [6, 19, 41, 47]. Analysis of means (ANOM) is used to visualize statistical significance of effects seen [19].

The underlying hypothesis in this study was that permeability parameters are a function of log $K_{o/w}$ and molecular weight, which may be affected when a compound is administered in a chemical mixture. This forms the basis of recent EPA dermal absorption guidelines based on the work of Potts and Guy [46]. Comparisons where this relationship held confirm that diffusion processes remain rate limiting. However, when such relations break down, a significant interaction may be present that is not diffusion limited, and thus not directly related to log $K_{o/w}$. This would have obvious impact on risk assessment procedures as it would affect the mathematical form of any quantitative structure permeability relationship (QSPR) equation linking log $K_{o/w}$ to dermal absorption. These occurred when extrapolating across solvent systems (e.g., ethanol/water), when surfactant SLS was added to different systems, and occasionally when individual compounds deviated from their predicted fluxes.

14.3.1
Compound Susceptibility to Solvent Interactions

A consistent finding across all mixtures was that as expected, some compounds behave differently in specific solvent systems. This interaction tended to be consistent across SMFT and PSFT systems, a finding supporting our hypothesis that the interaction is solvatochromatic and does not involve interaction with other constituents of the stratum corneum or epidermis. Figure 14.1 is a series of compass plots illustrating permeability in PSFT of all 12 study compounds in 12 propylene glycol mixtures. Compounds are arranged clockwise around the plot in order of descending

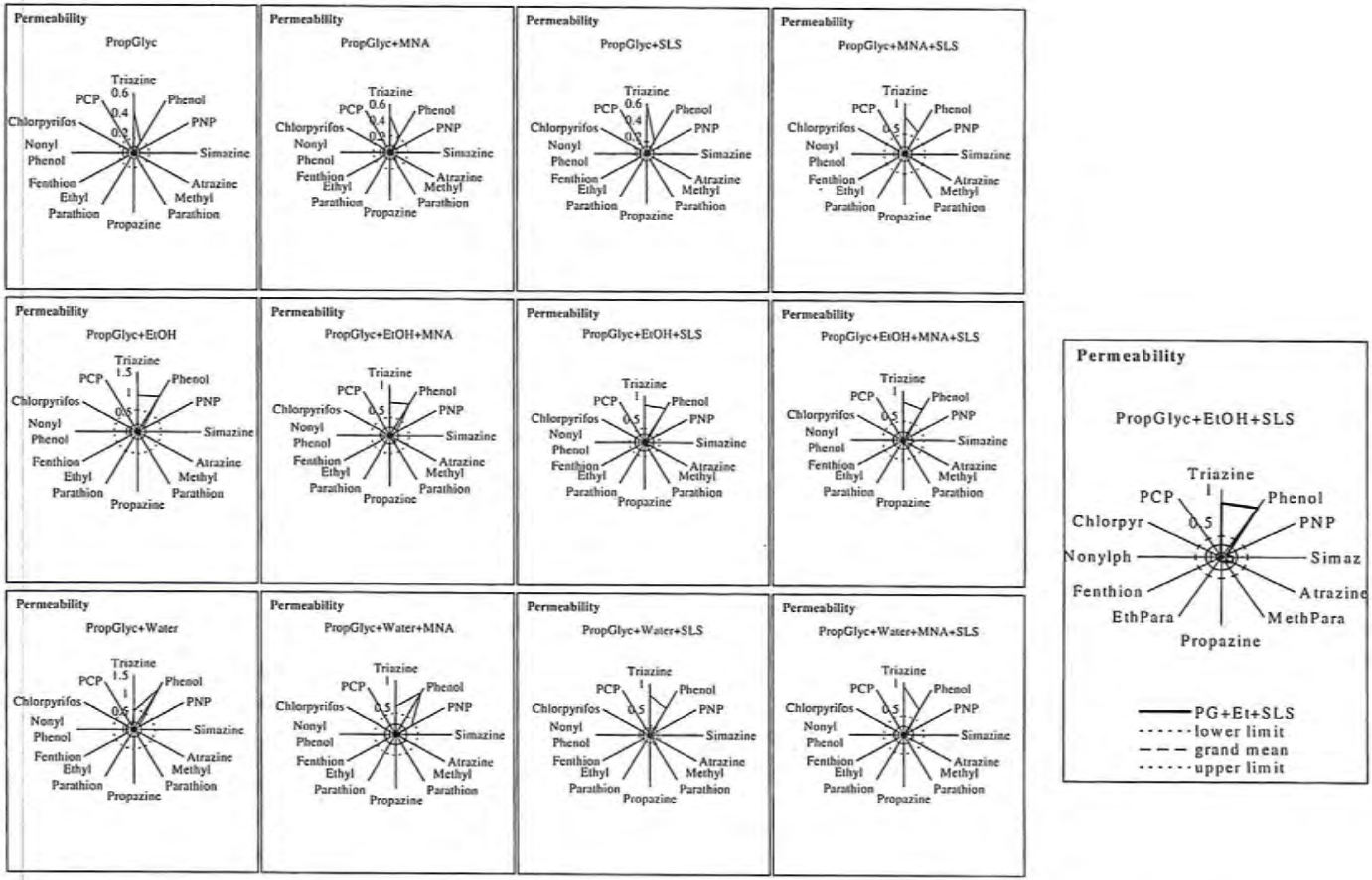


Figure 14.1 Compass plots for penetrants in propylene glycol mixtures in PSFT. The interactions noted in this plot at left for triazine and phenol were positive and outside of the upper confidence interval for significant interactions ($p < 0.05$). One cell is expanded above to illustrate upper and lower bounds.

log $K_{o/w}$ with the lowest (triazine, −0.9) at 12 o'clock and the highest (PCP, 5.12) at 11 o'clock. Permeability from flow-through diffusion cells are plotted on the radii that is further demarcated by mean, upper, and lower ANOM confidence intervals. These plots illustrate that in this system, compounds behave similarly except for triazine and phenol, two compounds with the lowest $K_{o/w}$. Exposure to these in PG would not be predicted from a log $K_{o/w}$ relationship. Ethanol modulates these fluxes, especially for phenol. Compass plots across SMFTs (data not shown) and PSFTs were comparable. Absorption in PG was low compared to the other solvents studied (except for low log $K_{o/w}$ compounds), and mixture interactions detected were opposite of what was seen in other systems (e.g., water/ethanol). These complex interactions make simple extrapolations difficult.

14.3.2 Mixture Interactions Across Model Systems

An analysis of compass plots detected a series of statistically significant ($p < 0.05$) interactions in some solvent systems that did not clearly extrapolate across model systems and demonstrated individual compound specificity. Those identified are illustrated in Figure 14.2 that depicts absorption flux (% dose/h) versus time profiles for four compounds in two chemical classes (PNP, PCP, atrazine, and propazine) exposed in water or water + SLS, studied in SMFT or PSFT models as well as IPPSFs. It must be noted that the shape of the absorption profiles across the three model systems is always different due to differences in model system structure. Flux through the SMFT is an order of magnitude greater than that of the PSFT making the time of peak flux to occur earlier in this system. It is the *correlation* of permeability between these systems, and not the absolute magnitude, that is important for assessing mixture effects on absorption. The delayed absorption seen in the PSFT compared to the SMFT reflects the greater lag time, and hence reduced diffusivity, for transport across porcine skin. Similarly, the shorter lag time generally seen in IPPSFs reflects the shorter diffusional distance seen when a model is perfused by dermal capillaries. A characteristic of these models, compound flux in the PSFT exceeds those in IPPSF that possesses a more complex membrane.

The first comparison is a relatively consistent SLS effect across compounds and models. The next comparison involved the substituted phenols PCP and PNP in the same solvent systems. In the SMFT, PCP flux was greater than PNP as predicted from its greater log $K_{o/w}$ of 5.1 versus 1.9. However, the fluxes were of a similar order of magnitude in the PSFT experiments, which carried forward into the IPPSF studies where the major difference was a slightly earlier peaking of PCP. These compounds have significantly different molecular properties (solubility, H-bonding indices) that could explain these differences both on the basis of stratum corneum penetration and subsequent dermal disposition. log $K_{o/w}$ alone, used in risk assessment models, would not predict this IPPSF response. Again, in all systems, SLS reduced flux compared to controls. This suggests that the SLS surfactant effect is detectable in the simplest model system; however, the actual shape of the transdermal flux profile is not directly predictable from the simpler systems. In

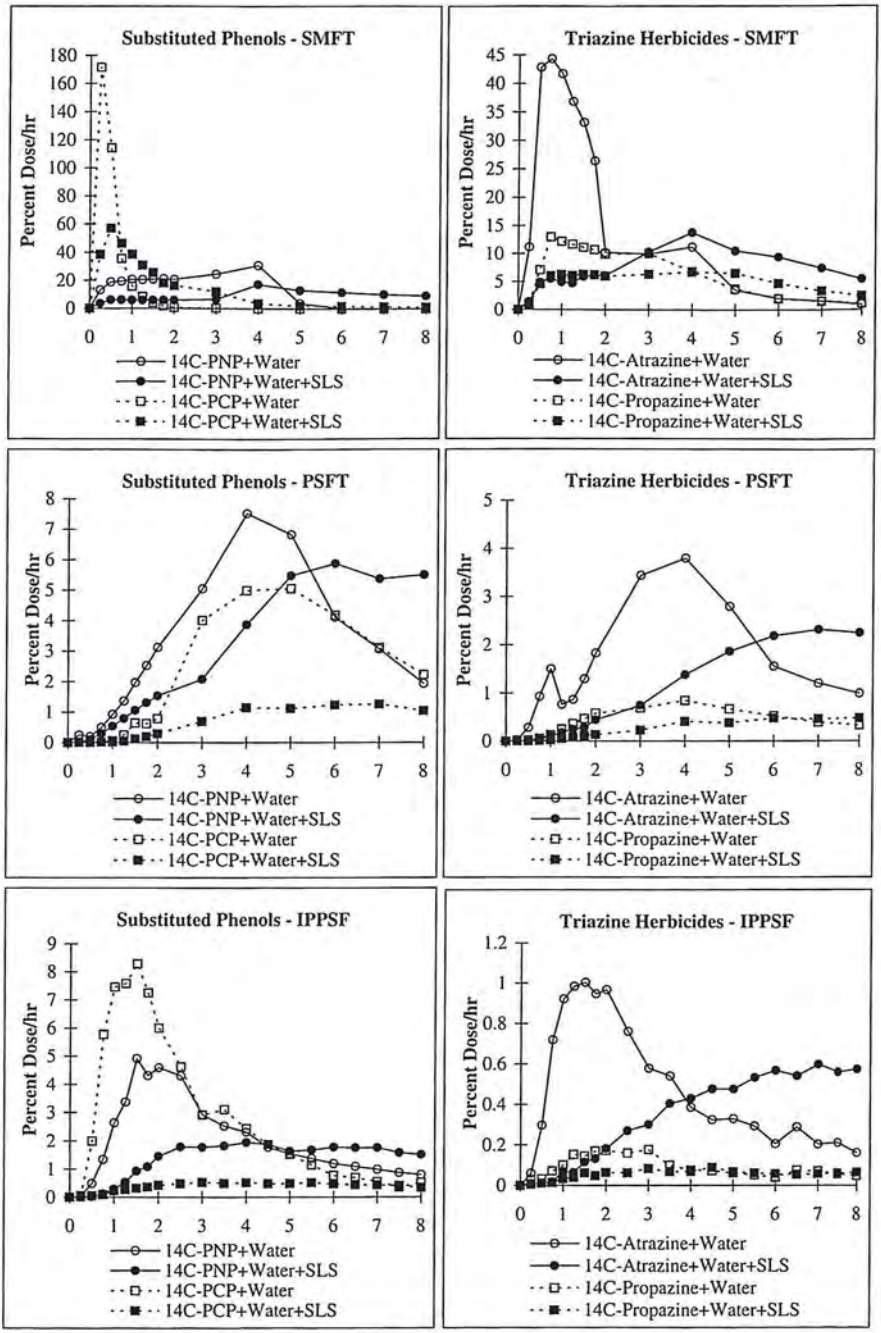


Figure 14.2 Effects of sodium lauryl sulfate on dermal flux profiles of four model penetrants (PNP, PCP, atrazine, and propazine) in SMFT, PSFT, and IPPSF model systems. Open symbols are control and filled symbols are SLS treatments.

fact, the broadening of the SLS absorption profiles may reflect modified dermal deposition within the stratum corneum that would be consistent with previously reported enhancing effects of SLS. We would expect the shape of the absorption profile to be a function of the kinetic model parameters describing these flux profiles. Second, it suggests that the SLS effect dominates over any log $K_{o/w}$ QSPR relationship for permeability of a single-compound absorption. This key finding provided the insight into developing the modified linear free energy relationship (LFER) approaches discussed below.

The mechanism most likely responsible for the flux reduction from SLS at this concentration (10%) under these aqueous solvent conditions may be via reducing the CMC in solution [7], an effect that reduces availability of compound for absorption independent of its initial permeability. We have shown that mixture interactions modulate CMC, a phenomenon detectable across all model systems. These SLS effects are fully documented in Refs [58, 59]. This effect would be present across all model systems. Once the compound is absorbed into the stratum corneum, SLS could affect its structure and further modulate absorption reflected in the altered shape profiles. It should be noted that in acetone or DMSO vehicles, parathion flux had been shown to increase with SLS [47], reflecting a differential response of this mixture additive depending upon the solvent system in which it is exposed. This dependency of mixture additive effects on the dosing vehicle was a major finding of this work.

Effects of these mixture interactions on IPPSF kinetic parameters are listed in Table 14.1 as they illustrate the most interesting mixture-specific data. SLS in water results in a decreased absorption rate (smaller *b*, *d*) compared to water alone. Ethanol in water reduces "*b*" for all compounds. Ethanol significantly reduces "*d*" except for propazine and simazine. These independent changes in rate parameters are consistent with different shape profiles seen under different mixture conditions.

14.3.3
Can Partition Coefficient Predict Mixture Behavior?

If log *K* is a primary determinant of diffusion, our hypothesis was that disposition from a mixture could be predicted from stratum corneum/mixture partition coefficient ($K_{SC/MIX}$). Validation of this relationship would offer strong support for using LFER equations to predict mixture absorption. This was addressed by

Table 14.1 Effects of SLS or ethanol on *b*/*d* mean IPPSF kinetic parameters in water.

	Atrazine	Chlorpyrifos	Ethyl-parathion	Methyl-parathion	PCP	PNP	Propazine	Simazine
Water	0.45/0.68	0.13/0.29	0.46/0.64	0.57/0.74	0.65/0.77	0.47/0.58	0.39/0.62	0.36/0.69
Water/SLS	0.06/0.11	0.02/0.10	0.11/0.26	0.18/0.31	0.17/0.33	0.16/0.22	0.18/0.38	0.14/0.46
Water/EtOH	0.23/0.46	0.09/0.22	0.15/0.46	0.28/0.65	0.26/0.34	0.36/0.50	0.14/0.72	0.28/1.28

comparing log $K_{SC/MIX}$ to permeability across SMFT and PSFT models, as well as to total IPPSF penetration (absorption + skin deposition). Figure 14.3 is a histogram of these parameters across all systems using PCP as a model penetrant to illustrate this effect. Chemical mixtures are ordered by decreasing log $K_{SC/MIX}$ (top panel). Permeability in SMFT and PSFT models, as well as IPPSF absorption, generally follows the order of descending log $K_{SC/MIX}$ with a few clear exceptions. SMFT permeability mirrored log $K_{SC/MIX}$ ($R^2 = 0.83$). A unique mixture was ethanol/water/MNA in PSFTs where permeability was less than predicted from log $K_{SC/MIX}$, an effect that carried into the IPPSF suggesting a potential interaction with epidermal cells or dermal components; the only consistent factors different between isolated stratum corneum and SMFT compared to PSFT and IPPSFs. This interaction was also seen with other compounds. For PCP, stratum corneum partitioning appears to be the dominant factor. These findings support the hypothesis that a mixture component effect (e.g., SLS) in a specific solvent system will reduce permeability across penetrants (independent of the compound-specific QSPR relation to log $K_{o/w}$) and can be estimated by partition coefficients in simpler system.

14.3.4 Solvent–Water Interactions

A significant interaction detected was the different effects seen for absorption across mixtures consisting of water, water/ethanol, and ethanol. Although log $K_{SC/MIX}$ correlated highly with log $K_{o/w}$ in water (system in which most dermal absorption QSPR analyzes are defined), there was no clear correlation between log $K_{o/w}$ across these other relatively simple solvents. This mechanism was explored in more detail using log $K_{SC/MIX}$ [58, 59]. Figure 14.4 depicts both log $K_{SC/MIX}$ for all 12 compounds ordered by log $K_{o/w}$ and the regression analyses for log $K_{SC/MIX}$ versus log $K_{o/w}$ in the three separate solvent mixtures. As expected, there is a reasonable correlation between these parameters in water. However, the correlation significantly weakened when water/ethanol and ethanol system were analyzed. Viewed from another perspective, rank order of log $K_{SC/MIX}$ was expected to be water > water/ethanol > ethanol, which held for compounds with log $K_{o/w}$ at the extremes. However for compounds with *mid-range* PCs (e.g., atrazine–ethylparathion), this clear-cut order was lost, suggesting molecular properties not predicted by log $K_{o/w}$ may modulate these relationships. Other researchers [56] showed that diazepam with mid-range log $K_{o/w}$ did not respond to chemical enhancement. It is evident that a mixture containing ethanol has a narrower range of altered log $K_{SC/MIX}$ than does a pure water system, potentially reflecting stronger molecular interactions seen with this solvent. Significantly, a 50/50 mixture could not be interpolated from data in pure solvents. Finally, one must mention that molecular weight was also an important covariate in these analyses; however, they did not add a significant ability to discriminate these interactions or predict absorption over that which partition coefficient already provided.

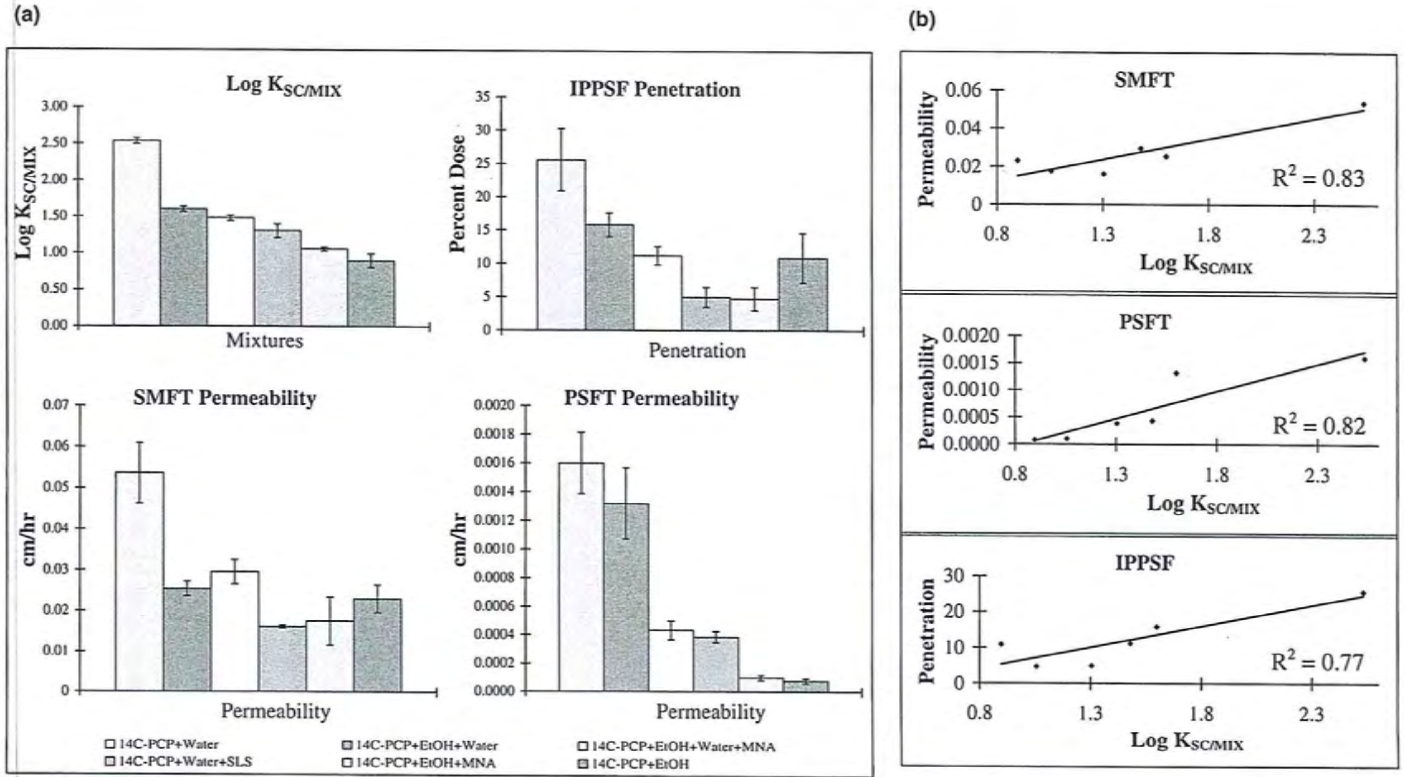


Figure 14.3 Comparative PCP partitioning (log $K_{SC/MIX}$) and permeability from six mixtures across all three model systems, SMFT, PSFT, and IPPSF (a) and correlation between PCP permeability and penetration from the six mixtures and log $K_{SC/MIX}$ (b).

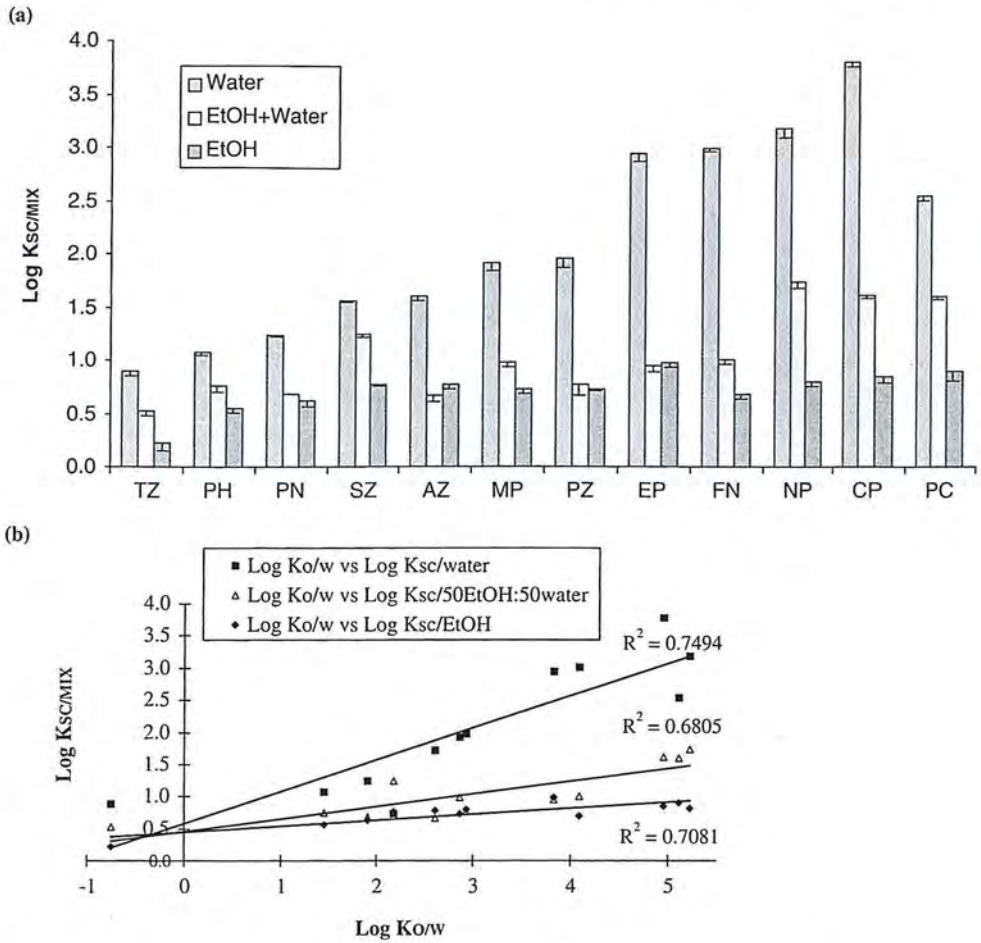


Figure 14.4 log $K_{SC/MIX}$ for 12 pesticides in water, 50% ethanol/water, and ethanol mixtures (a) and the correlation between these log $K_{SC/MIX}$ experimental values and literature values for log $K_{o/w}$ (b).

14.3.5
Modified QSPR Equations that Predict Chemical Absorption from a Mixture

The above analysis suggested that mixture interactions (e.g., vehicles, surfactants) modify the normal correlation seen between a parameter such as log $K_{o/w}$ and absorption. Such correlations are often embedded in the QSPR based on linear free energy relationship. For individual chemicals, there is often a strong correlation with parameters such as log $K_{o/w}$ and molecular weight [46] or with multiple molecular descriptors that allow a wider range of chemicals to be accounted for [2]. As can be seen from the above data, the addition of SLS or ethanol modified the correlation between absorption and log $K_{SC/MIX}$ or log $K_{o/w}$, and did not destroy this relationship. Such "mixture effects" were often constant across all model systems studied as was

demonstrated with the compass plot analyses. Finally, the fact that mixture effects seen in the IPPSF somewhat reflected changes in log $K_{SC/MIX}$ suggested that an LFER approach incorporating both molecular descriptors of the penetrants of interest (classical single chemical approach) and mixture effects described by physical descriptors of the mixture might hold promise.

We elected to use Abraham's LFER model as our base equation since it is representative of the dermal QSPR approaches presently available [27]. Preliminary analyses applying 16 different LFER equations reviewed by Geinoz *et al.* [27] to the entire PSFT (288 treatment combinations) and IPPSF data set (32 treatment combinations) demonstrated a superior fit of our data set to the Abraham equation compared to most other models reviewed. It must be stressed that the purpose of this research was neither to identify the *optimal* LFER for predicting dermal permeation nor to validate that this model is predictive of dermal absorption. Rather, we selected this model since it best described the data generated in this specific research and is widely accepted by the scientific community.

$$\log k_p = c + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + s\pi_2^H + rR_2 + vV_x,$$

where k_p is the permeability constant for the PSFT experiments, $\Sigma\alpha_2^H$ is the hydrogen-bond donor acidity, $\Sigma\beta_2^H$ is the hydrogen-bond acceptor basicity, π_2^H is the dipolarity/polarizability, R_2 represents the excess molar refractivity, and V_x is the McGowan volume. Molecular descriptor values for all these parameters were calculated for the 12 penetrants studied with ABSOLV® Solute Property Prediction Software (Sirius Analytical Instruments, Ltd, East Sussex, UK). The parameters a, b, s, r, and v are *strength coefficients* coupling the molecular descriptors to skin permeability in the specific experimental system (e.g., PSFT or IPPSF).

To incorporate mixture effects, another term called the mixture factor (MF) is added, yielding

$$\log k_p = c + mMF + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + s\pi_2^H + rR_2 + vV_x.$$

This concept allows to define an LFER equation across data collected from different mixtures. Hostynek and Magee [31] had used indicator variables embedded in LFER equations to allow analysis across exposures consisting of different vehicles or occlusive conditions. Unlike our approach, these indicator variables did not contain any information concerning the vehicles, but were a statistical regression tool to allow the base LFER model to be applied to penetrants dosed under different experimental conditions.

Figure 14.5 depicts the predicted versus observed permeability constants (log K_p) for all 288 treatment combinations studied without taking into account the specific mixtures these chemicals were dosed. The residuals of this model showed no further correlation with penetrant properties. However, when vehicle/mixture component properties were analyzed, trends in residuals became evident. An excellent single parameter explaining some variability of this residual pattern (R^2 of 0.44) was log(1/Henry Constant) (1/HC). Figure 14.6 depicts the modified LFER model including MF = log(1/HC).

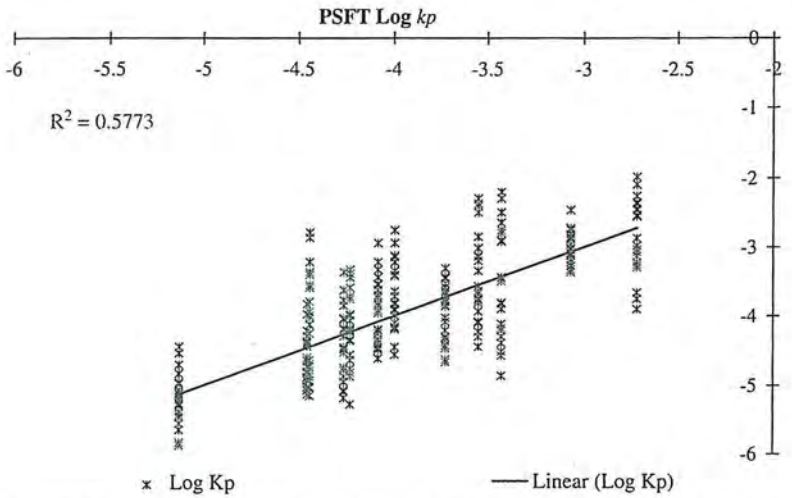


Figure 14.5 Observed versus predicted for pesticide permeability (log K_p) for 12 pesticides from various chemical mixtures. Data obtained from PSFT experiments and regression analysis performed without inclusion of a mixture factor.

The improvement on the prediction of PSFT permeability across all treatment is clearly evident. It must be stressed that an MF related to 1/HC is not the final form of this analysis but was the first property suggestive that this approach might work. Other physical parameters of the mixture similar to the molecular descriptors of the penetrant were also correlated. A similar approach was used for the smaller data sets

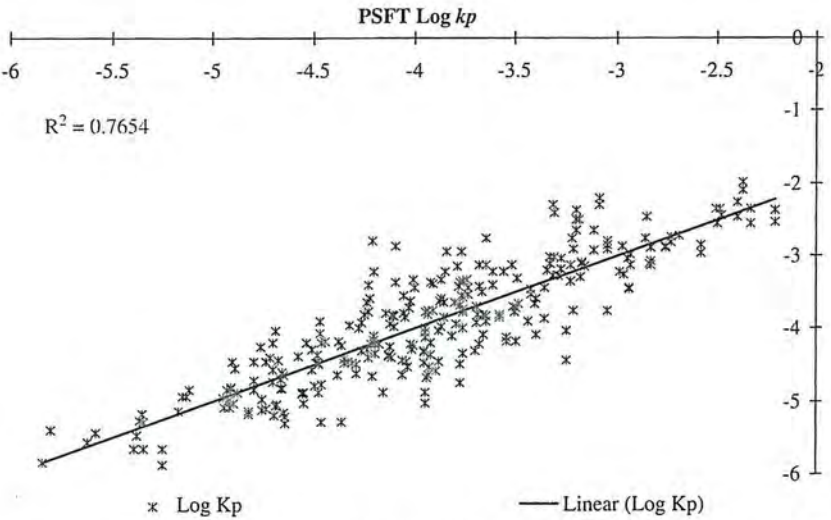
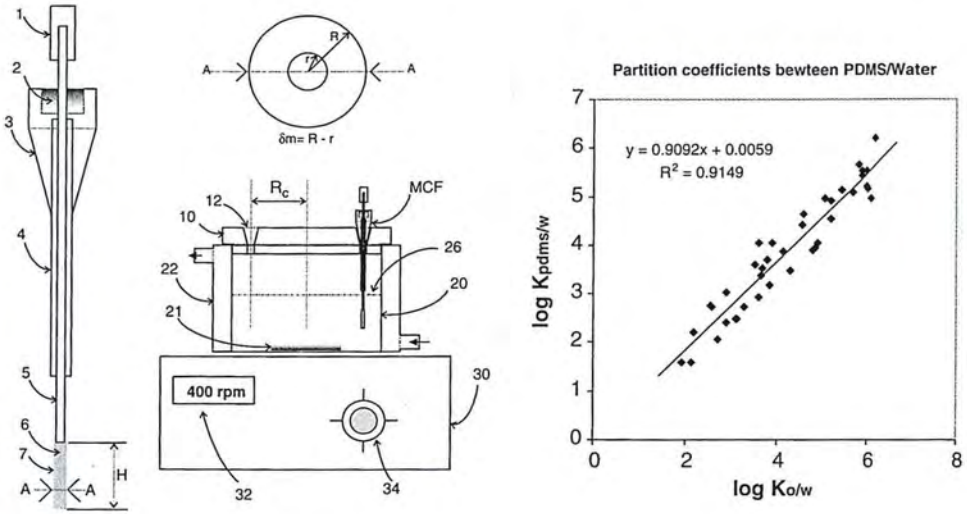


Figure 14.6 Observed versus predicted for pesticide permeability (log K_p) for 12 pesticides from various chemical mixtures. Data obtained from PSFT experiments and regression analysis performed with inclusion of a mixture factor.

available in the IPPSF mixture exposures, and inclusion of a mixture factor improved predictability. The final form of such a modified mixture LFER must await further research. This includes defining which physical–chemical property of the mixture best describes the mixture factor. However, the finding that an MF can be used to extrapolate chemical dermal absorption data across different vehicles and mixture combinations is significant that we believe would have an impact on the occupational risk assessment process. It would allow single-chemical data collected in multiple research environments, and resulting single-chemical LFER determined in different studies, to be modified on the basis of mixture component or vehicle properties.

14.3.6
Novel MCF Approach to Calibrate Dermal Absorption of Mixtures

The membrane-coated fiber (MCF) has been used as a solid-phase microextraction technique for chemical analysis of drug and environment samples [60, 64]. In brief, the MCF (Figure 14.7a) is immersed in the sample solution containing the analyte of interest, and after a given permeation time to allow solute equilibrium between the solution and the MCF, the MCF is injected directly into an LC or GC injector for dissolution or desorption and MS analysis to determine phase distribution of



(a) (b)

Figure 14.7 (a) MCF fiber system (left side) and experimental apparatus (right side) and (b) correlation of log $K_{PDMS/w}$ versus log $K_{o/w}$. (1) Holding tip, (2): sealing septum, (3) needle base, (4) piercing needle, (5) fiber attachment tubing, (6) inert fiber, (7) PDMS membrane, (10) needle holding cap, (12) MCF positioning holes, (20) solution container, (21) magnetic stirring bar, (22) water jacket, (26) donor solution, (30) magnetic stirrer, (32) tachometer, (34) stirring speed control. *r*: radius of the inert fiber; *R*: radius of the membrane, δ_m: thickness of the membrane, and R_c: hole radius on the cap.

analyte between the solution and the MCF membrane. The membrane coatings of commercially available MCF assemblies can range from 7 to 100 μm thick coated onto a fused silica or metal alloy fiber. There are at least seven commercially available coatings, of which only three MCFs display absorption characteristics (polydimethylsiloxane, PDMS; polyacrylate, PA; and carbowax, WAX) and the others display mixed absorption and adsorption characteristics (e.g., carboxen, divinylbenzene). PDMS, PA, and WAX have been demonstrated to display an equilibrium process based on Fick's first law of diffusion. Our laboratory was the first to capitalize on the unique physicochemical characteristics of these various three MCF membranes to develop mathematical models [65, 66] that predict solute partitioning and initial solute permeation rates following MCF exposure to a diverse series of 39 solutes (Figure 14.7b).

These MCFs were able to characterize partitioning behavior of the predominant aromatic components of jet fuels into human stratum corneum [13]. The purpose of these experiments was to demonstrate the reproducibility of the MCFs to predict octanol–water coefficients and by extension its versatility to assess chemical partitioning into skin from chemical mixtures. The latter is worth noting in that traditional approaches of assessing octanol–water partition coefficients are limited to single chemicals and not chemical mixtures. Solute partitioning into bilayers (e.g., skin, MCFs) differs from that into bulk isotrophic liquid hydrocarbons such as octanol [34, 36]. Furthermore, the octanol–water partition coefficient has sometimes been characterized as a poor descriptor of solute partitioning into the stratum corneum [2, 36] as the latter consists of a heterogenous matrix of diverse lipids and proteins that cannot be simply replaced with octanol. Thus, the use of multiple fibers (MCF array) with diverse physicochemical properties can theoretically better characterize solute partitioning into skin than octanol. The jet fuel data generated from our partitioning studies demonstrated that the more hydrophobic the jet fuel component is, the more likely it is able to partition into the MCFs. However, this partitioning ability was reduced in the presence of a solvent that in reality mimics partitioning behavior of these components from a jet fuel mixture into skin. The two MCFs, PDMS and PA, optimally predicted jet fuel component partitioning into skin ($R^2 = 0.86$–0.93) and demonstrated that the presence of a solvent in the mixture decreased component partitioning by a factor greater than 2. This finding is consistent with previous partitioning studies involving the use of stratum corneum and chemical mixtures [8, 58, 59] and can be explained by the fact that the presence of some solvents in some mixtures can increase solute solubility in the chemical mixture thereby reducing the solute thermodynamic activity in the membrane [21, 57].

Our experiments also utilized the 3-MCF array approach to predict skin permeability with three MCFs and was based on a multiple linear regression analysis of the permeability ($\log K_p$) data sets generated in our lab with porcine skin and the three log K_{MCF} data sets [67]. The mathematical model describing this relationship is

$$\log K_p = -2.34 - 0.124 \log K_{PDMS} + 1.91 \log K_{PA} - 1.17 \log K_{WAX}$$
$$(n = 25, R^2 = 0.93).$$

The 1-MCF or any combination of 2-MCF arrays performed poorly in predicting skin permeability. While the 3-MCF array approach resulted in an excellent correlation, the chemical space was limited to 25 solutes used in the calibration of the three MCFs and these solutes were limited to $K_{o/w}$ values ranging from 2 to 6. Future research efforts should be focused on exploring the utility of these regression models using the extremes of the physicochemical properties of chemicals, that is, chemicals outside of the defined chemical space and of specific occupational and environmental concern.

With this in mind, it was hypothesized that MCF array exposure to chemical mixtures containing either a solvent or a surfactant should result in changes in solute absorption into the MCF that are correlated with changes in skin permeability. When the MCF array was exposed to chemical mixtures of either 50% ethanol or 1% SLS (Figure 14.8), there was a decrease in absorption into all three fibers that is consistent with observations from previous dermal permeability studies [8, 53, 55]. Data from the Riviere *et al.* study [55] were used to develop a predictive model for the skin permeability of chemicals from 50% ethanol (E50) established by using multiple regression analysis of the matrix [log $k_{Skin/E50}$: log $K_{PDMS/E50}$, log $K_{PA/E50}$, log $K_{WAX/E50}$];

$$\log k_{Skin/E50} = -1.18 + 0.36 \log K_{PDMS/E50} + 0.80 \log K_{PA/E50} - 1.32 \log K_{WAX/E50}$$

$$n = 25, R^2 = 0.91, s = 0.133, F = 75.$$

This analysis demonstrated the feasibility of the MCF array to predict solute permeability in skin in the presence of 50% ethanol. The next step was to ascertain whether the chemical-induced mixture interactions in the MCF array are predictive of similar solvatochromatic interactions in skin. This first required correlating the log $K_{MCF/mixture}$ or the log $K_{Skin/mixture}$ experimental values with the five solvatochromatic

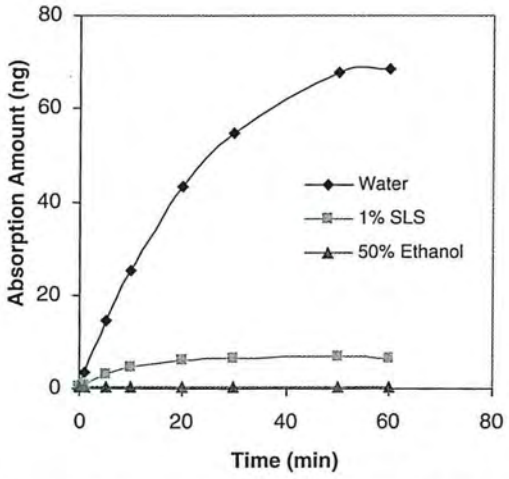


Figure 14.8 Partitioning of biphenyl into WAX fiber in water, 1% SLS and 50% ethanol solutions.

descriptors already described in this chapter (E, S, A, B, and V) to generate corresponding system or strength coefficients for skin and for each MCF exposed to water and either of the solvent or surfactant mixtures. The solvatochromatic relationships for each of the MCF fibers can be described by the following equations:

$$\log K_{\text{MCF/mixture}} = c + a\Sigma\alpha_2^{\text{H}} + b\Sigma\beta_2^{\text{H}} + s\pi_2^{\text{H}} + rR_2 + V_x$$

$$\log K_{\text{MCF/water}} = c + a\Sigma\alpha_2^{\text{H}} + b\Sigma\beta_2^{\text{H}} + s\pi_2^{\text{H}} + rR_2 + vV_x$$

and the solvatochromatic relationship for the skin can be described by the following equations:

$$\log K_{\text{Skin/mixture}} = c + a\Sigma\alpha_2^{\text{H}} + b\Sigma\beta_2^{\text{H}} + s\pi_2^{\text{H}} + rR_2 + vV_x$$

$$\log K_{\text{Skin/water}} = c + a\Sigma\alpha_2^{\text{H}} + b\Sigma\beta_2^{\text{H}} + s\pi_2^{\text{H}} + rR_2 + vV_x$$

The difference in system coefficients (Δ = mixture – water) for corresponding molecular descriptors following skin or MCF exposure to either 1% SLS or 50% ethanol can be calculated by utilizing the same data set as described above [55, 67, 68].

$$[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v] = [rpabv]_x - [rpabv]_o = [r_x - r_o\ p_x - p_o\ a_x - a_o\ b_x - b_o\ v_x - v_o],$$

where $[rpabv]_o$ are the system coefficients of the water mixture in either skin or MCF; $[rpabv]_x$ 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.

This generated a matrix of changes in system coefficients ($[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]$ for both the 3-MCF array and the skin permeability and these delta changes were correlated within a multilinear regression analysis framework as shown below [14].

$$\begin{pmatrix} \Delta r \\ \Delta p \\ \Delta a \\ \Delta b \\ \Delta v \end{pmatrix}_{\text{Skin}} = a_0 + a_1 \begin{pmatrix} \Delta r \\ \Delta p \\ \Delta a \\ \Delta b \\ \Delta v \end{pmatrix}_{\text{WAX}} + a_2 \begin{pmatrix} \Delta r \\ \Delta p \\ \Delta a \\ \Delta b \\ \Delta v \end{pmatrix}_{\text{PDMS}} + a_3 \begin{pmatrix} \Delta r \\ \Delta p \\ \Delta a \\ \Delta b \\ \Delta v \end{pmatrix}_{\text{PA}}$$

The WAX, PDMS, and PA in the above matrix refer to the carbowax, polydimethylsilaxane, and polyacrylate fibers, respectively, which make up the 3-MCF array system.

Multiple linear regression analysis relating the 3-MCF array to skin permeability for mixtures containing 1% SLS resulted in the following regression equation:

$$y = 0.548 - 0.931x_1 + 3.81x_2 - 2.66x_3$$

with x_1, x_2, and x_3 representing delta (Δ) values for WAX, PDMS, and PA, respectively. An R^2 of 0.9445 demonstrated a strong correlation between changes

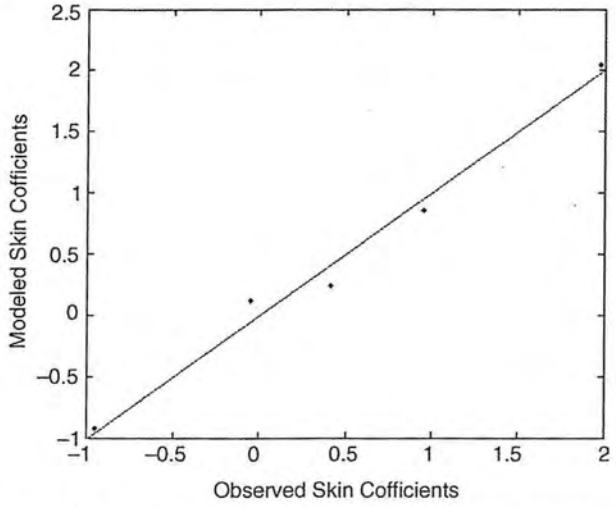


Figure 14.9 The predicted versus observed Δ system coefficients for skin for the full 3-MCF array exposed to 1% SLS.

in system coefficients across skin and the 3-MCF array. Very weak correlations were observed when Δ values from a single or pairs of MCFs were correlated with Δ values from skin permeability. Note that the above model accounts for over 94% of the variability in observations. Figure 14.9 demonstrates the strong relationship (R^2 0.984) between the five observed changes in system coefficients ($\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v$) and those predicted Δ values from the polynomial model used in the previously described matrix.

The MCF array therefore has the unique potential as a tool to not only simulate dermal permeability of individual chemicals but also assess how chemical mixtures can modulate solute permeability in skin. The above experiments demonstrated that the chemical mixture interactions can be quantified within a solvatochromatic framework. The solvatochromatic approach compares the physicochemical interactions between a solute and a diffusion barrier (e.g., skin or MCF) for one chemical mixture scenario with another mixture scenario. The presence of chemical mixtures can result in significant changes in physicochemical properties as described above for solvents and surfactants and where the MCF array can simulate these physicochemical interactions. For some occupational and environmental exposures, these changes may be significant as described above but there is the very likelihood that some exposure scenarios may result in little or no change in physicochemical properties. The MCF array may not be sensitive to such small or subtle changes and will require further calibration for these exposure scenarios and exposure scenarios involving higher levels of mixture interactions such as more complex solvent + surfactant mixtures. These interactions will also need to be assessed across different classes of solvents and surfactants to be of practical use.

14.4
Potential Impact of Multiple Interactions

The complexity occurs when one considers that the above interactions are all independent making the observed effect *in vivo* a vectorial sum of all interactions. This allows the so-called "emergent properties" of complex systems [5] to become evident when the individual interactions are finally combined in the intact system, in our case *in vivo* skin. For example, assume that mixture component A decreases the absorption of a chemical across skin due to increased binding to skin components. In contrast, mixture component B increases its absorption due to an enhancer effect on stratum corneum lipids. When A and B are administered together, the transdermal flux of the chemical under study may not differ from control. This is illustrated by the effect of two different jet fuel performance additives, *N,N*-disalyclidene-1,2-propanediamine (MDA) and butylated hydroxytoluene (BHT), on dermal absorption of naphthalene administered from the base fuel JP-8 not containing these additives, or in combination as often is the case with JP-8(100) jet fuel (Figure 14.10). BHT functions as an antioxidant additive that prevents formation of soluble gums and insoluble particulate deposits in jet fuel systems produced by oxidation, and MDA functions as a metal deactivator additive that suppresses the catalytic effect that some metals in fuels induce on the surfaces of fuel systems and tanks. In this case, we hypothesize that MDA increases surface retention of naphthalene thereby decreasing

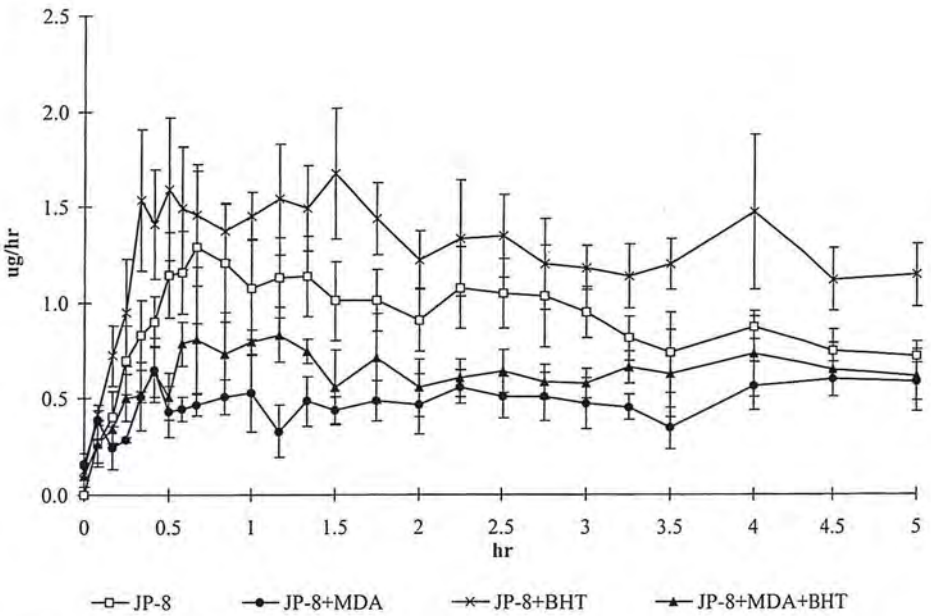


Figure 14.10 Dermal absorption of naphthalene through perfused porcine skin from JP-8 jet fuel administered with additives BHT and MDA alone and in combination.

its absorption, while BHT functions more like a penetration enhancer within skin [41]. When both are present, flux returns to base levels. We have previously seen similar effects with other combinations of additives on absorption of jet fuel hydrocarbons [6, 51].

It may be a mistake to assume that these opposite effects simply cancel one another out and that the flux of chemical is now equivalent to it being applied alone. The mechanisms behind the similarity in fluxes are different. Fick's first law of diffusion can be used to illustrate this. In the base situation (∅), compound flux would

$$\text{Flux}_{\varnothing} = (K_p)(\Delta C),$$

where K_p is the permeability coefficient and ΔC is the concentration gradient driving the absorption process. We will consider ΔC the effective dermal dose since increasing concentration on the surface of skin effectively increases ΔC. In the presence of additives, we had two scenarios where additive A decreased absorption by retaining chemical on the surface, effectively reducing ΔC:

$$\downarrow \text{Flux}_A = (K_p)\,(\downarrow \Delta C)$$

and scenario B where flux increased due to an increased K_p:

$$\uparrow \text{Flux}_B = (\uparrow K_p)\,(\Delta C).$$

When both A and B are present, the flux is now back to base levels, but is governed by a fundamentally different set of diffusion parameters:

$$\text{Flux}_{A+B} \cong \text{Flux}_{\varnothing} = (\downarrow K_p)(\downarrow \Delta C).$$

One can appreciate how different factors that would interact with these altered parameters could change dermal disposition patterns within skin compared to the baseline scenario.

In occupational exposure to metal working fluids (MWFs), workers are often exposed to complex chemical mixtures of performance additives such as biocides (e.g., triazine), surfactants (e.g., linear alkylbenzene sulfonate, LAS), anticorrosive agents (triethanolamine, TEA), and lubricants (e.g., sulfated ricinoleic acid, SRA) that can result in the previously described interactions. The dermal absorption of commonly used MWF biocide, triazine, is limited to 2.41–3.89% dose in PSFT and 12.61–18.63% dose in SMFT [10]. In a synthetic MWF formulation neither LAS nor SRA had an individual effect on triazine absorption, but TEA alone, SRA + TEA, and the complete surrogate MWF formulation significantly increased triazine permeability (Figure 14.11a). This trend was also demonstrated in a soluble oil-based MWF formulation where the physicochemical interactions are expected to be significantly different from those in a synthetic MWF. It should, however, be noted that triazine deposition in stratum corneum after an 8 h exposure was significantly reduced by the presence of additives in soluble oil-based MWF, but the trend was reversed in synthetic MWF. The presence of MWF additives can also significantly reduce SRA permeability in both types of MWFs (Figure 14.11b) with LAS having one level of the

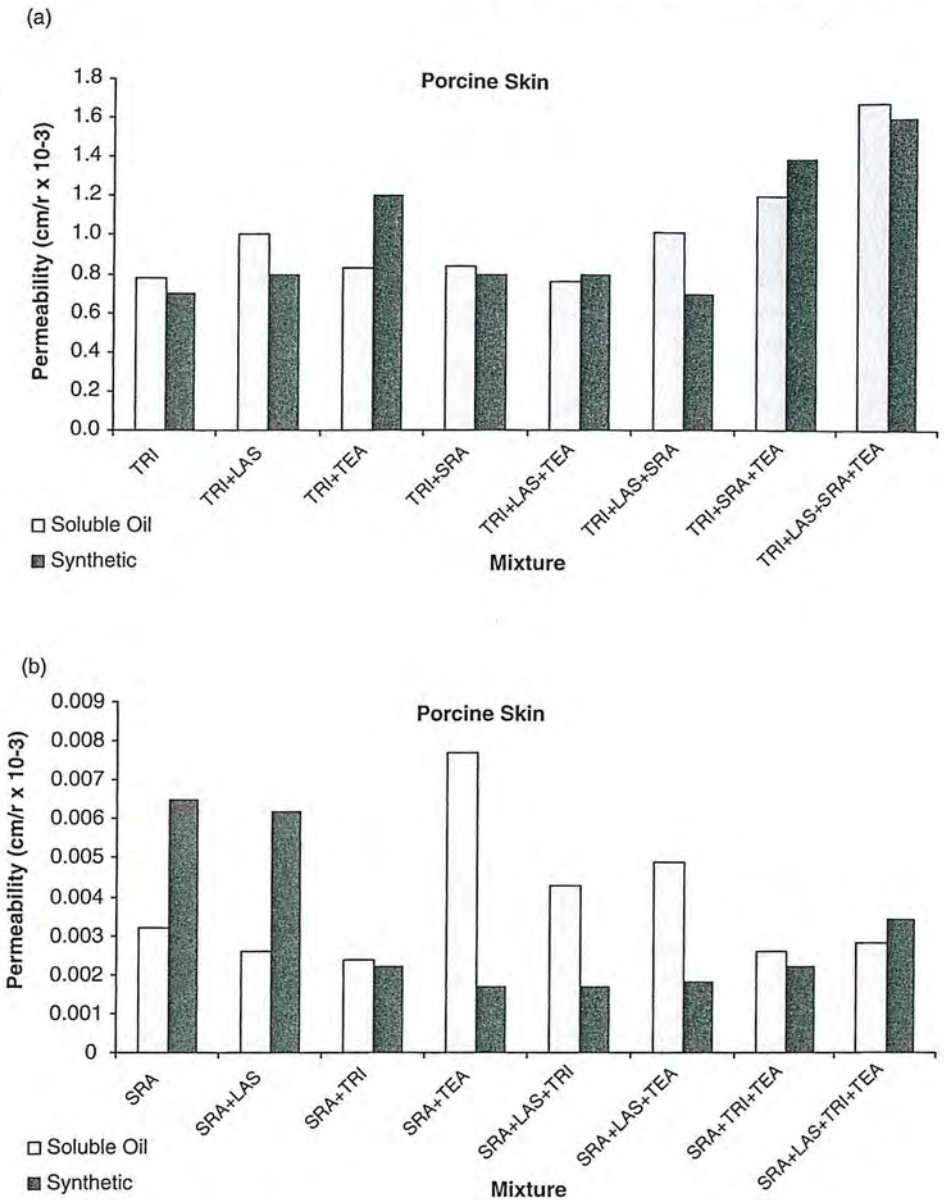


Figure 14.11 Permeability of (a) triazine and (b) sulfated ricinoleic acid in PSFT system in mixtures containing linear alkylbenzene sulfonate, triethanolamine, and/or SRA in either soluble oil mixtures (gray histograms) or synthetic fluid mixtures (black histograms).

inhibitory effect while other additives and combination of additives providing a more significant second-level inhibitory effect [11]. These inhibitory effects were apparently more evident in synthetic MWF (3.8-fold) than in soluble oil-based MWF (1.2-fold), and this was consistent with the less preferential partitioning of SRA into the stratum corneum from the synthetic MWF than from a soluble oil-based MWF. Not surprisingly, TEA by itself significantly increased SRA permeability in PSFT exposed to soluble oil-based formulations.

Our laboratory for the most part was able to reproduce these trends in a synthetic membrane system such as the SMFT where permeability for either triazine or SRA was approximately 6–20-fold greater than in the PSFT. Although these performance additives can potentially modulate the chemical–biological interactions in skin during solute permeability, these PSFT versus SMFT comparisons demonstrated that chemical–chemical interactions may play a more significant role in predicting mixture interactions that modulate dermal absorption. The dermal enhancer effects of alkanolamines such as TEA and fatty acids such as SRA are well documented in the literature [63] as it applies to transdermal delivery of cosmetics and pharmaceutics; however, these series of studies were the first to demonstrate how chemical interactions during an occupational scenario such as an exposure to MWF can influence the eventual delivery of potential toxicants into the systemic circulation. Data from our physicochemical evaluation of these soluble oil-based and synthetic MWF support this hypothesis as we have seen mixture-induced changes in viscosity, stratum corneum/mixture partition coefficients, changes in critical micelle concentration in the presence of SRA, and changes in additive solubility [7, 10, 11].

The above scenario becomes a bit more complicated when the worker's skin is repeatedly exposed to chemical mixtures that actually modify the skin biology. This is especially true for repeated exposures to industrial solvents that are known to either remove intercellular lipids or alter their structure and orientation in the epidermis [38]. *In vivo* pretreatment of porcine skin by the industrial solvent cleanser, TCE, for 4 days almost doubled triazine permeability and was more important in synthetic MWF than soluble oil-based MWF [12]. Simultaneous exposure to TCE did not, however, influence dermal permeability of triazine. Conversely, both classes of MWF caused a 4–10-fold increase in TCE absorption, which is significantly less than the effect the MWF additives had on triazine absorption. The differences here could be attributed to the relative polar nature of the MWF compared to a penetrant that is polar such as triazine and a penetrant that is nonpolar such as TCE. In similar simulations in our laboratory, we demonstrated that repeated occupational exposure to jet fuels can significantly increase dermal absorption of aromatic and aliphatic hydrocarbon components in jet fuel mixtures [42]. A twofold increase in absorption was observed after a 1-day pre-exposure and a fourfold increase was observed after a 4-day pre-exposure. These *in vitro* simulations highlight the fact that the skin biology is altered after repeated occupational exposure and this in addition to the complex chemical–chemical interactions previously described should be considered in any dermal absorption assessment for workers in these scenarios.

14.5 Summary

This chapter has reviewed the potential effects that topical exposure to a chemical mixture might produce compared to exposure to a single neat chemical. In fact, most single-chemical exposures are not to neat compound but rather to a chemical in a defined vehicle such as water, ethanol, or acetone in many toxicological studies. It is clear that there are significant vehicle effects that may overshadow differences between absorption of chemicals with different properties. Such vehicle effects have been previously noted. However, what is just as significant is the phenomenon that chemical interactions may also be vehicle specific depending upon the physiochemical properties of both the penetrating chemical and the vehicle/mixture in which it is dosed. The ability to dramatically improve the prediction of permeability for 12 diverse chemicals across multiple mixtures when the physical–chemical properties of the solvent/mixture are also taken into account presents an approach that may have future utility once fully validated. The MCF array approach provides a novel experimental tool to quantify these mixture interactions using solvatochromatic parameters. Even in situations where chemical permeability is similar across mixtures, it is possible that opposing mixture interactions are at work that would alter the toxicological interpretation of this permeability compared to control values where no mixture additives are present.

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