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Percutaneous Absorption of Chemical Mixtures

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I. INTRODUCTION

A primary route of occupational and environmental exposure to toxic chemicals is often through the skin. Although exposure to complex chemical mixtures is the norm, only mechanisms of absorption for single chemicals have been studied and most risk-assessment profiles are based on the behavior of single chemicals. Effects of co-administered chemicals on the rate and extent of absorption of a topically applied systemic toxicant may determine whether toxicity is ever realized.

The application of risk assessment to dermal absorption by U.S. regulatory agencies (Environmental Protection Agency, Occupational Safety and Health Administration, Agency for Toxic Substance and Disease Registry) is varied and highly dependent upon available data (1–3). A similar concern over lack of data exists for overall risk assessment of chemical mixtures (4–7). A congressional Commission of Risk Assessment and Risk Management (8) recommended moving beyond individual chemical assessments and focusing on the broader issues of toxicity of chemical mixtures. Current approaches are based on assigning toxicological equivalent units to similar chemical congeners (e.g., dioxins) or assessing toxicity after exposure to the complete mixture. It is recognized (4) that the dose–response curves of individual mixture components should be characterized, and then a "no-interaction" hypothesis for these components in a mixture tested. With complex mixtures of hundreds of components, these approaches become exceedingly complex. Finally, mixture component interactions that involve modulation of a known toxicant's absorption, and thus systemic bioavailability, have not been defined.

This problem 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) and not for their effects on absorption or toxicity of the potential toxicant.

The appreciation of the importance of chemical mixture interactions to effect chemical and drug disposition, pharmacokinetics, and activity has been well recognized for many years and is extensively reviewed elsewhere (4,5,9–13). Despite the widespread knowledge base of the importance of drug–drug interactions and the importance of chemical interactions in systemic pharmacology and toxicology, very little attention outside of the dermatological and transdermal formulation arenas have been paid to interactions that may occur after topical exposure to complex mixtures. The focus of this chapter is to overview the potential mechanisms operative in topical chemical mixtures as well as to illustrate these interactions with data from our laboratory.

II. RISK ASSESSMENT

Dermal risk assessment of individual chemicals is based on knowledge of the permeability characteristics of specific chemicals through skin, with extrapolations being made to potential absorption in humans (14). Numerous contributions in the present text discuss this field. A great deal of emphasis is appropriately placed on calculating potential exposure, with less attention focused on the actual permeability of the exposed compound through skin, which is required to estimate systemic exposure. Collection of this latter data is preferably done in a controlled and validated laboratory animal model, although one could argue that even quality data in a laboratory rodent might not be optimal for predicting human skin absorption due to well-known species differences. Unfortunately, very little human data exist to support these estimates and it is unethical to expose humans to hazardous materials to generate these parameters. When data are not present, extrapolations of potential absorption are made based on physical chemical parameters (e.g., molecular volume and water solubility) or surrogates such as partition coefficient (PC) (concentration ratio between vehicle and membrane) that correlates to permeability of individual chemicals primarily through in vitro skin models. A great deal of effort has been spent on developing these permeability estimates. However, it is evident from a close review of these approaches that the combination of dermal absorption and mixture guidelines has not yet occurred, despite broad acceptance that the skin is a primary route of exposure for many chemicals, and that most chemical exposure occurs in mixtures.

It is impossible to assess all potential combinations of chemicals in order to determine which have the greatest potential to modulate absorption of a known toxic entity topically exposed in a chemical mixture. The present state of knowledge in this area is particularly weak since the significance of specific interactions has not been quantified, let alone in many cases even identified. In many ways, this same concern continues to define the very nature of chemical mixture toxicology (5,9,10,12,13). 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 (15). 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 has to offer, one cannot define the links between absorption and the effects seen. Could the

observed toxicity be exerted because a specific toxicant was in the mixture, because two synergistic toxicants were absorbed, *or* was it exerted simply by the presence of a mixture component (e.g., alcohol, surfactant, and fatty acid) that enhanced the absorption of a normally minimally absorbed toxicant? In this latter scenario, if the enhancer were not present, absorption would have fallen below the toxicological threshold. We have demonstrated such an interaction with the putative toxins involved in the Gulf War Syndrome, where systemic pyridostigmine bromide or co-exposure to jet fuel was shown to greatly enhance the dermal absorption of topical permethrin (16,17). Would other pesticides be similarly affected? How does one take into account such critical interactions so that a proper risk assessment may be conducted?

One recently reported approach to address this problem assesses potential interactions in dermal absorption by fractionating the effects of a vehicle on drug penetration onto the two primary parameters describing permeation according to Fick's law: partitioning (PC) and diffusivity (D) [see below; permeability (K_p) = D × PC/membrane thickness] (18). 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.

A more inclusive approach to this problem is to define chemicals on the basis of how they would interact with other components of a mixture as well as 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? Unlike pharmaceutical formulation additives in a dermal medication, chemical components of a mixture are not classified by how they could modulate percutaneous absorption of simultaneously exposed topical chemicals. They are either 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 (cosmetic followed by topical insect repellent), *accidentally* because they were disposed of simultaneously as waste, or they are *coincidentally* associated as part of a complex occupational or environmental exposure.

III. 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 on the basis of specific mechanisms of action involved as well as 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 properties (e.g., solubility, volatility, and critical micelle concentration)
- Altered rates of surface evaporation
- Occlusive behavior
- Binding or interaction with adnexial structures or their products (e.g., hair, sweat, and 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 on 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, super-saturation, 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 (19–22). For some of these effects, chemicals act independent of one another. However, for many the presence of other component chemicals may modulate the effect seen.

Figure 1 illustrates the effects of the surfactant sodium lauryl sulfate (SLS) on the absorption of methyl and ethyl parathion in perfused porcine skin. Despite differences in the overall absorptive flux of both compounds administered in these aqueous vehicles, SLS decreased the absorption of both.

Chemical interactions may further be modulated by interaction with adnexial structures or their products such as hair, sebum, or sweat secretions. The result is

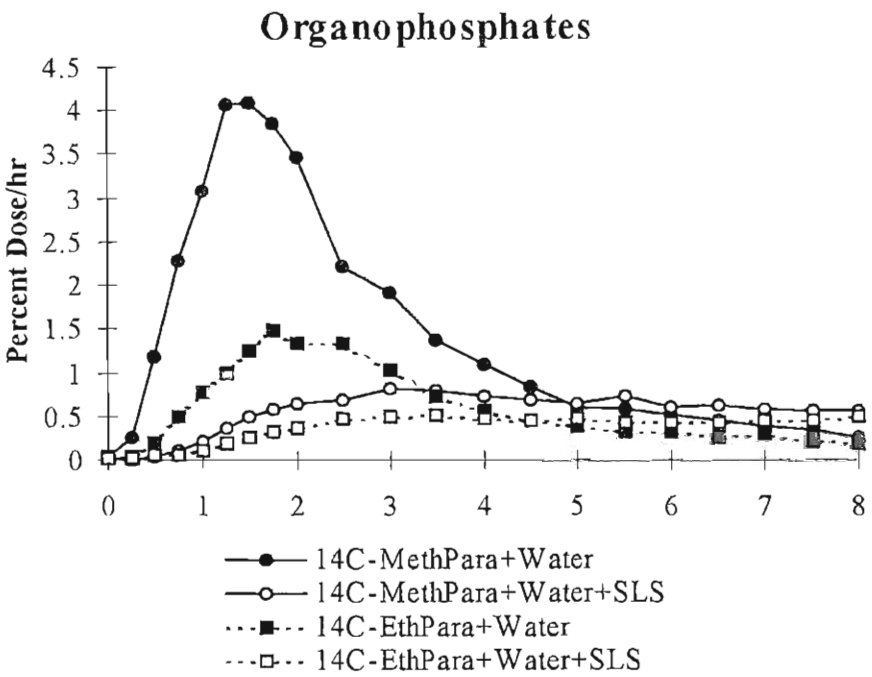

Figure 1 Effect of sodium lauryl sulfate (SLS) on mean absorption profiles of ethyl- and methyl-parathion in isolated perfused porcine skin.

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.

Second levels of potential interaction are 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 (22). 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 (23,24). Compounds may also bind to stratum corneum constituents forming a depot.

The PC between the drug in the surface dosing vehicle and stratum corneum lipids may be altered if chemical components of the mixture also partition and diffuse into the lipids and thus alter their composition. This provides a potential mechanism to assess the effects of a mixture interaction on subsequent absorption. Figure 2 illustrates the PC of pentachlorophenol (PCP) into porcine stratum corneum administered in a series of six mixtures (water, water + ethanol + methyl nicotinate, water + ethanol, water + SLS, ethanol + methyl nicotinate, ethanol). Figure 3 compares PCP absorption in perfused porcine skin dosed in these same mixtures against PC, illustrating that PC determined from the mixture of concern does correlate to absorption across viable skin.

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 (25,26). 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

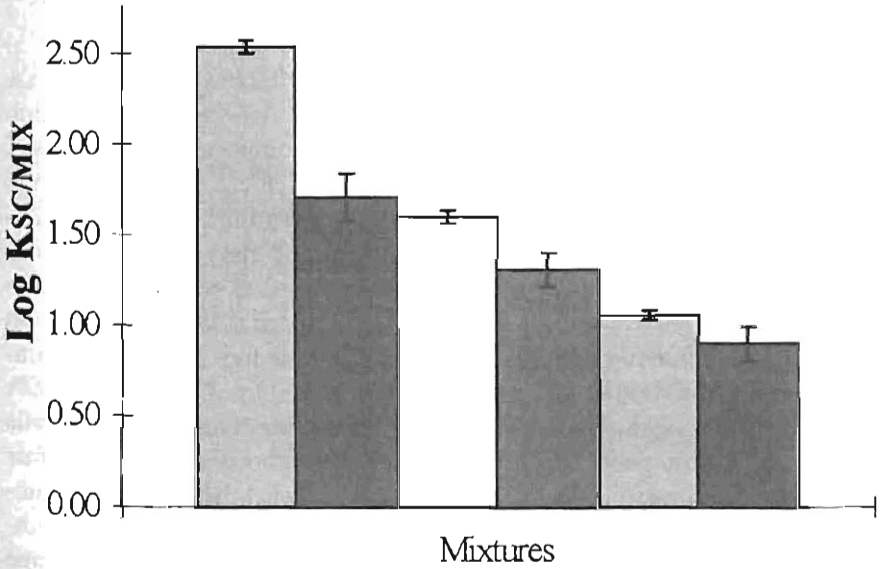

Figure 2 Isolated porcine stratum corneum/vehicle partiton coefficients (log $K_{SC/MIX}$) for pentachlorophenol (PCP) across six different chemical mixtures.

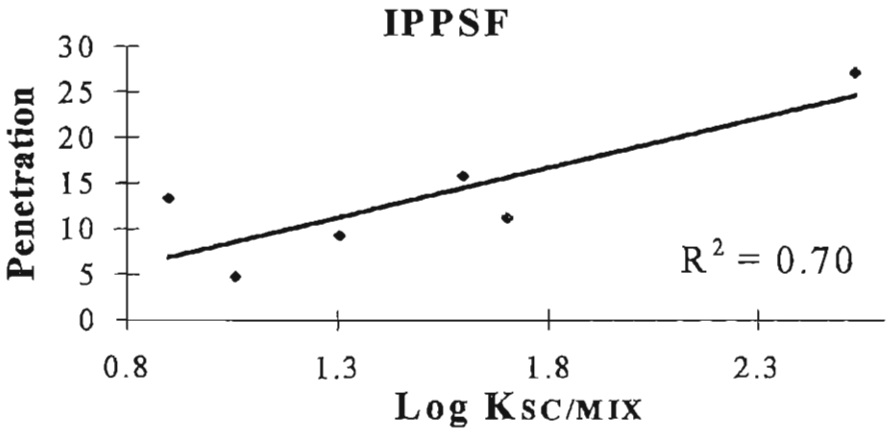

Figure 3 Correlation of pentachlorophenol (PCP) log $K_{SC/MIX}$ and absorption in isolated perfused procine skin.

(e.g., lipid synthesis enzymes) which would modify barrier function (27). A chemical could also induce keratinocytes to release cytokines or other inflammatory mediators (28–30), which could ultimately alter barrier function in the stratum corneum or vascular function in the dermis. Alternatively, cytokines may modulate biotransformation enzyme activities (31).

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 (32,33). In addition to modulating transdermal flux of chemical, such vascular modulation could also affect the depth and extent of toxicant penetration into underlying tissues.

IV. 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 (34) to be observed when the individual interactions are finally summed in the intact system, in our case in vivo skin. For example, assume that mixture component A decreases absorption of a chemical across skin due to increase binding to skin components. In contrast, mixture component B increases its absorption due to an enhancer effect on stratum corneum lipids. When the mixture components A and B are administered in combination, the transdermal flux of the chemical being studied may not differ from control. This is illustrated by the effect of two different jet-fuel performance additives metal deactivator additive (MDA) and butylated hydroxytoluene (BHT) on dermal absorption of naphthalene administered from the base fuel JP-8 not containing these additives or in combination (Fig. 4). In this case we hypothesize that MDA increases surface retention of naphthalene, thereby decreasing its absorption, while BHT functions more like a penetration enhancer. 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 (35,36).

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

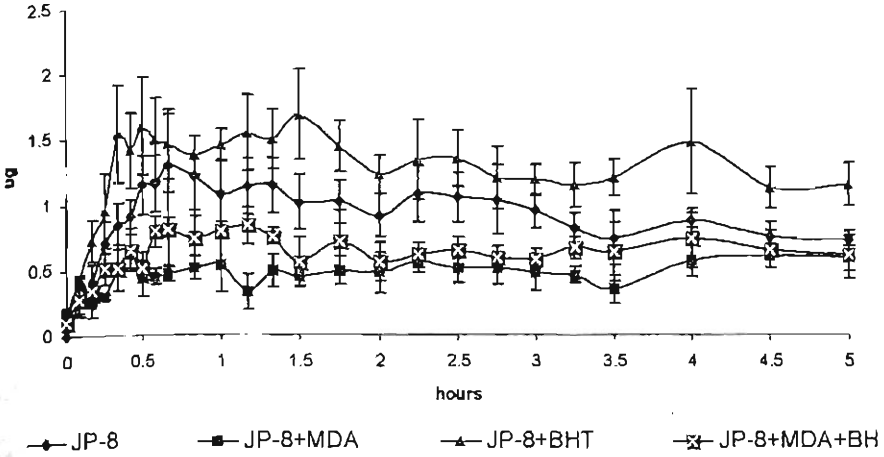

Figure 4 Effects of fuel performance additives butylated hydroxytoluene (BHT) and metal deactivator additive (MDA) on dermal absorption of naphthalene in isolated perfused porcine skin.

diffusion can be used to illustrate this. In the base situation (ø), compound flux would equal:

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

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:

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

One can appreciate how different factors that would interact with these altered parameters could drastically change dermal flux compared to the baseline scenario.

V. CONCLUSIONS

This brief overview of mixture absorption illustrates the complexity involved when trying to extrapolate single interactions seen with binary mixtures onto absorption from more complex mixtures. However, strategies aimed at quantitating potential interactions in the framework of mechanisms of absorption would seem to be the most promising approach to put order into this complex problem. The data that indicate that measured stratum corneum PC correlates to subsequent absorption through intact skin is encouraging as it provides an approach to experimentally assessing the effects of complex mixtures of K_p.

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