Characterization of Dermal Absorption Following Decontamination via Washing

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This is to certify that I have examined this copy of a master's thesis by

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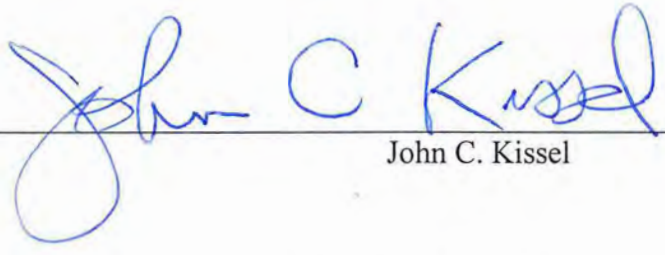

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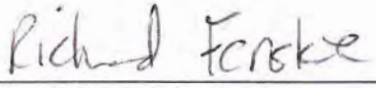

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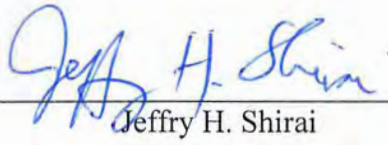

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Chapter 1. The Effect of Washing on Dermal Absorption in the Published Literature

1.A. Introduction and Background

Contact with contaminants such as pesticides in the environment can have various and potentially severe impacts on human health. Therefore, it is important to understand and minimize the entry of such chemicals into the body. Exposure can occur via the inhalation, ingestion and dermal absorption routes. Of these, dermal absorption is the least understood. One aspect of dermal absorption that is particularly uncertain is the effect of washing.

In both personal and occupational hygiene, washing is the primary method recommended for dealing with dermal exposure to hazardous chemicals. The Worker Protection Standard (CFR 2008a, 2008b) states that employers of pesticide handlers must provide "decontamination supplies" in the form of water, soap and towels sufficient for washing. These are the only required decontamination supplies under this standard. Therefore, it is implicit in the regulations that washing with soap and water is an effective way to remove dermal contamination. Despite its regular usage in personal and industrial hygiene and its widely assumed effectiveness as a decontaminating agent for toxic substances, at present there is no widely accepted and well-founded theory that explains the removal of chemicals from the skin via washing. This review aims to compile relevant information from published studies and elucidate any patterns in the effects of washing on dermal absorption. A better understanding of the current available data is a necessary step toward developing a mathematical model that incorporates this information with general chemical principles into a theoretical framework. Implementation of both a review of the published literature and development of a mathematical model will aid in increasing understanding of dermal exposure in general and effective washing practices in particular.

1.A.1. Skin Physiology

A basic understanding of the physical structure of the skin is necessary to appreciate the effects of washing. Full thickness human skin consists of several layers: the outermost layer, the epidermis, followed by the dermis and subcutaneous fat layers (Figure 1). The topmost layer of the epidermis is the stratum corneum (SC). It is made up of proteinaceous dead skin

cells called corneocytes surrounded by intercellular lipids and is thought to be the primary barrier to the penetration of most compounds into the skin (Maibach and Feldman 1974, EPA 1992, Lademann 2010). The epidermis below the stratum corneum is called the viable epidermis. Sweat glands and hair follicles pass through the epidermis and terminate in the dermis layer of the skin, which contains the vasculature that perfuses the skin and leads to the systemic distribution of contaminants. Sweat glands are separate structures from hair follicles that extend from the skin surface to approximately the dermis-adipose tissue boundary, but are not shown in Figure 1 for simplicity. The lipid concentration decreases and the water concentration increases with increasing skin depth (EPA 1992).

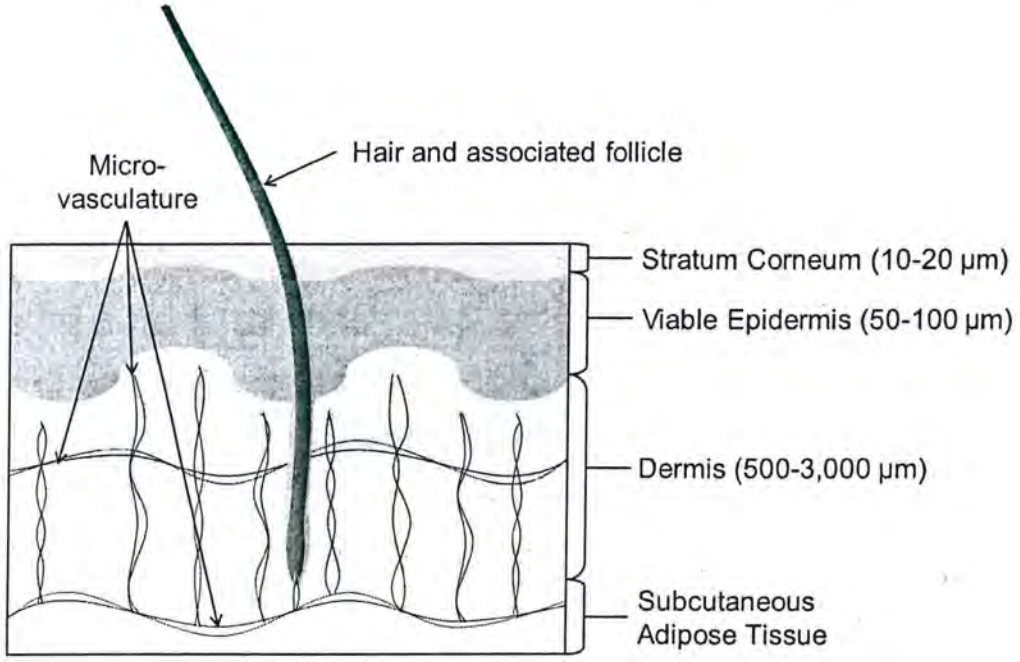

Figure 1. Skin Anatomy

In humans, full thickness skin averages about 1 to 2 mm thick, with a range between 0.5 mm to 6 mm. Thicker skin is found on the back and callused areas such as the sole of the foot (Woodburne 1969). The depth of the stratum corneum is estimated to be between 10 and 20 µm thick in areas commonly used for dermal absorption studies, such as the torso, arms and legs (Scheuplein and Blank 1971). The viable epidermis is between 50 and 100 µm thick, followed by the dermis at 500 to 3,000 µm (EPA 1992). The number of hair follicles also varies across anatomical sites. Human skin samples used for dermal absorption studies usually have fine, vellus hair follicles of the kind typically found on the body outside of the

scalp and pubic areas. These vellus follicles have an average total depth of 646 ± 140 μm (Vogt et al. 2007). Terminal hair follicles, such as those found on the scalp, are much deeper at 3864 ± 605 μm thick. The bulk of the micro-vasculature is located in the dermis, with the top of the capillaries reaching about 200 μm from the surface of the skin (EPA 1992).

1.A.2. Effect of Physicochemical Properties on Movement of Compounds in Skin

Dermal absorption of chemical compounds is related to the interaction between physicochemical properties of the contaminant and the physiology of the skin, among other factors. It should be noted that in this review, absorption and penetration are used interchangeably to describe movement into and through the skin. This differs from the vocabulary defined in some of the literature. Therefore, when these terms are utilized here, further information will be provided to help the reader determine if the compound is potentially recoverable or may not reach the bloodstream.

Chemicals with small molecular volumes penetrate through the skin at a higher rate (ECETOC 1993). Molecular volume can be approximated by the compound's molecular weight, although halogenated compounds tend to have a smaller volume and higher rates of penetration compared to a non-halogenated organic compound of similar weight (EPA 2004). The molecular volume influences penetration into the skin because smaller compounds are more easily able to diffuse into the skin membrane and move through the small lipid-filled spaces between the corneocytes. EPA (1992) notes that compounds with a molecular weight above 400 tend to be less able to penetrate into the skin due to their size.

Diffusion through the skin can also be influenced by the interaction of the compound with the skin membrane. Compounds can move through the stratum corneum by either the intercellular or transcellular route. Highly lipophilic compounds move preferentially through the lipid-rich intercellular spaces, while hydrophilic compounds usually penetrate more slowly via the partially hydrated corneocytes in the transcellular pathway (EPA 1992). The primary barrier to dermal absorption for hydrophilic compounds is the stratum corneum. However, lipophilic compounds easily penetrate the lipid-rich layer. For these compounds, the watery viable skin layers (e.g., epidermis and dermis) are the primary barrier to penetration (EPA 1992). Therefore, compounds that are fairly soluble in both water and the

lipid-rich stratum corneum are best able to penetrate through all layers of the skin and become systemically absorbed (EPA 1992, ECETOC 1993).

In a simplified understanding, the movement of a compound through the skin is thought to be driven by the gradient of the contaminant in the membrane. When the skin is exposed to a compound, some amount is assumed to instantly saturate a thin portion of the external stratum corneum (Kasting & Miller 2006). The absorbed compound begins to diffuse through the stratum corneum and toward the viable epidermis. During the beginning of this diffusion phase, the absorption into the stratum corneum is at its most rapid (Cleek & Bunge 1993). As diffusion continues, the gradient of the compound in the skin builds, eventually resulting in permeation of the compound into the more water-rich layers of the skin. When the compound first reaches the bloodstream, the flux (mass/[area·time]) into the systemic compartment is slow. However, if enough compound is on the surface of the stratum corneum to replace that which has penetrated deeper into the skin, the flux out of the stratum corneum will increase to eventually match the flux in (Cleek & Bunge 1993). This steady state flux through the skin will be maintained until the compound on the surface of the skin runs out or is removed. At that time, the driving force maintaining the gradient is no longer present and the flux through the skin will decrease. When the unbound compound on the skin surface has been fully depleted, some amount of contaminant remains in the skin. This reservoir can continue to move through the skin and into the bloodstream at slower rates (Cleek & Bunge 1993, Roberts et al. 2004). Figure 2 illustrates the profile typically observed for situations in which a finite dose of compounds without extreme lipophilicity or hydrophilicity is applied to the skin surface.

For most dermal absorption studies, flux (mass/[area·time]) or penetration (mass/time) are plotted on the y-axis, while time is plotted on the x-axis. Graphs of penetration or flux exhibit similar curve shapes but different magnitudes. Both flux and penetration are rates at which mass of a compound is transmitted through the skin. However, flux is normalized over the exposed surface area and therefore more helpful when attempting to compare between different protocols.

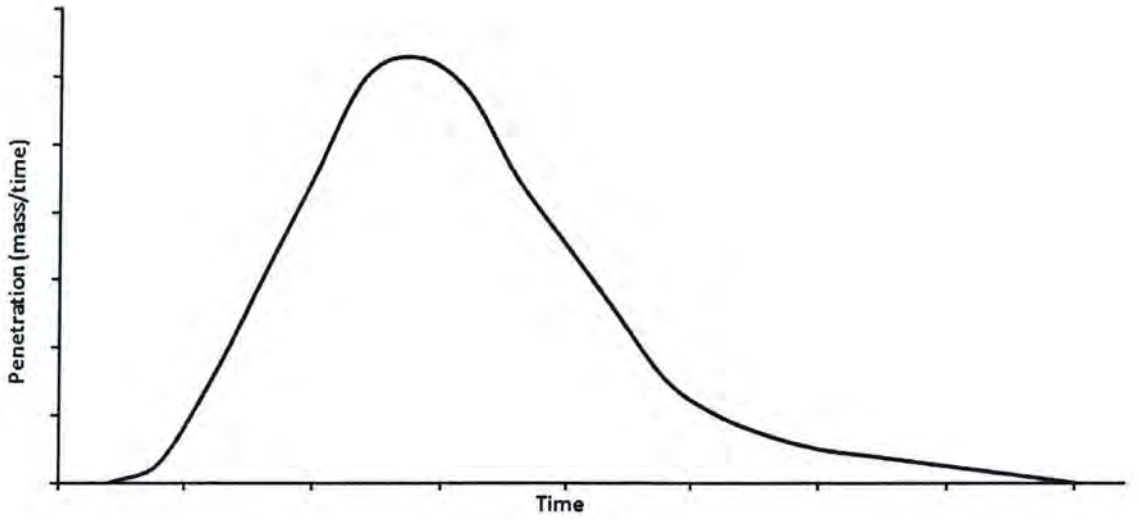

Figure 2. Expected Penetration with Finite Dose. Adapted from experimental data reported by Riviere et al. (2010) for penetration of pentachlorophenol applied in water.

In Figure 2, the penetration is increasing until a peak is achieved, at which point the rate of mass through the skin begins to decline. This decline is due to the depletion of the compound on the skin surface, either through loss to the environment or penetration into the skin. This example assumes the skin was not washed. As the chemical remaining in the skin becomes depleted, the driving force behind chemical penetration through the skin lessens and the rate of decline in penetration slows. This eventually leads to the characteristic "tail" of the curve as time progresses.

Figure 2 shows an example in which the reservoir on the skin surface is depleted prior to reaching the maximum flux or penetration. This type of curve is more likely to occur for low loadings of compounds that are fairly lipophilic and hydrophilic and can move more rapidly through the skin. For compounds that have a lower maximum penetration or flux through the skin, the penetration profile for á typical experimental trial is illustrated in Figure 3.

If the penetration in this example was monitored for a longer period of time, the reservoir of compound on the skin would slowly be depleted and a decline in penetration would occur similar to that observed in Figure 2.

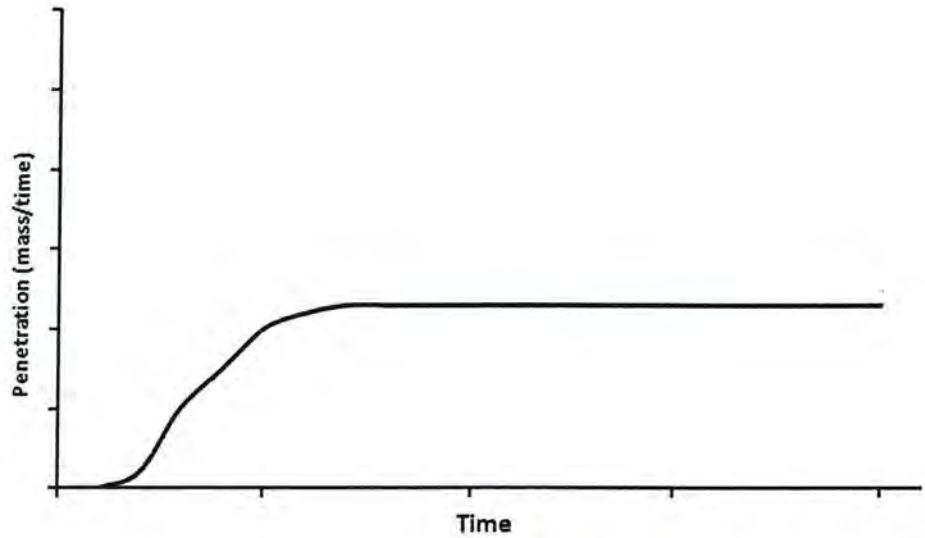

Figure 3. Expected Penetration Profile for Compound Reaching its Maximum Penetration. Adapted from experimental data reported by Riviere et al. (2010) for penetration of parathion in water and sodium lauryl sulfate.

The area under the penetration curve represents the total mass absorbed through the skin, while the area under a flux curve normalizes this mass by exposed area. However, not all studies plot a rate of mass through the skin on the y-axis. Some plot only the mass or concentration recorded at various times. In these cases, the area under the curve does not approximate the total mass absorbed unless receptor fluid was collected at a constant flow rate.

Compound remaining in the top of the stratum corneum may be slowly lost as the top layers of dead skin cells are naturally shed and sebum is produced and excreted. This is especially true of highly lipophilic compounds to which the viable epidermis is the primary barrier to absorption or highly hydrophilic compounds that move slowly through the lipid-rich stratum corneum (EPA 2004). As discussed above, some extremely lipophilic compounds can easily penetrate the stratum corneum but do not partition easily into the more aqueous viable epidermis. In these cases, higher concentration of compound must form at the stratum corneum-epidermis barrier in order to drive compound through the viable skin and into the bloodstream. This can result in the build-up of compound in the top-most layer, with little compound entering the systemic compartment after depletion or removal of the external reservoir and driving force. Similarly, highly hydrophilic compounds only partition slowly into and through the stratum corneum, but are quickly transmitted through the viable

epidermis and into the bloodstream. These types of compounds can take longer to penetrate the stratum corneum than the two to three weeks required to completely shed this layer (EPA 1992). The penetration curve for these types of compounds might be expected to be much smaller in peak magnitude and spread out over a longer period of time.

1.A.3. Potential Impacts of Washing

In most cases of skin contamination, exposed persons wash their skin at some time after the exposure in an attempt to remove the contaminant. Even in cases where exposure occurs without the knowledge of the person, typical personal hygiene practices result in the skin being washed within 24 hours. Therefore, washing can frequently occur prior to complete absorption of the applied dose into the skin. For this reason, it is important to understand the possible effects of washing on dermal absorption.

Washing is expected to remove most if not all of the external reservoir of unbound chemical. Some amount of the unbound reservoir may be left behind by washing with water or dilute soap and water solutions if the compound is particularly lipophilic or applied in a greasy vehicle. In these cases, the wash solution may not be able to solubilize enough of the compound to completely remove it, although washing with an excess of solution would likely dislodge a large portion of the dose. More uncertain is the question of what happens to the compound that has entered into the stratum corneum and the deeper skin layers prior to washing. Some evidence suggests that washing could potentially remove some compound absorbed into the top portion of the stratum corneum by exfoliation of the contaminated corneocytes (Reifenrath et al. 1984). By removing the external reservoir, washing can also remove the driving force for the diffusion gradient through the skin. This could potentially slow the penetration of compound remaining in the skin.

On the other hand, washing has also been hypothesized to reduce the ability of the skin to act as a barrier. This change in the skin's barrier is not fully understood and could be due to hydration via the washing solution or changes to the stratum corneum caused by the rubbing of the skin during washing.

As discussed previously, the stratum corneum layer has the highest lipid and lowest water content of the skin layers, containing about 15% water at its external surface and between 40

and 70% at the junction with the viable epidermis (Warner et al. 1988, Caspers et al. 2000). The stratum corneum can absorb up to 70% of its normal water content via binding to polar groups found in the extracellular lipids and intercellular kertin, with the greatest increases in water content observed in the external, less hydrated portion (Jacobi 1958, Kasting & Barai 2003). Aqueous washing solutions are capable of hydrating the skin. This hydration could have one of two potential effects. First, increased hydration of the skin has been shown to increase its permeability to a variety of compound types (Roberts & Walker 1993). This change has been attributed to the plasticizing effect of hydration on the stratum corneum. On the other hand, aqueous wash solutions increase the water content of the lipophilic stratum corneum, which in turn makes the usually highly lipophilic layer a less favorable place for storage of hydrophobic contaminants while simultaneously allowing hydrophilic compounds to move through more quickly. The ability of washing to increase the moisture content of the skin is minimal in most cases due to the short duration of typical washing. Soaking or extensive washing potentially would be more likely to reduce the skin's barrier function via hydration of the skin.

Some studies have suggested that use of soap in the wash solution could result in irritation of the skin. Wilhelm et al. (1991) applied the surfactant sodium lauryl sulfate (SLS) to skin for 24 hours and observed irritation as a result. Subsequent application of four drugs to the skin irritated by SLS resulted in an increase in penetration relative to skin that had not been exposed to the surfactant. Because washing usually takes place following exposure to a contaminant and is usually of a limited duration, soap in washing solutions would not be expected to cause the sort of irritation observed by Wilhelm et al. (1991) after long-term exposure to SLS. Riviere et al. (2010) applied compounds in either water or surfactant solution (linear alkylbenzene sulfonate [LAS] or SLS) to viable skin *in vitro.* Greater penetration was observed for compound applied in water compared to 11 out of the 12 trials in which compound was applied in one of the surfactant solutions. This is consistent with the hypothesis that soap increases the affinity of lipophilic compounds for the application solution, which in turn reduces partitioning to the lipid-rich skin. This hypothesis is further supported by the fact that the largest reduction in penetration was observed for the most lipophilic compound. Soap may have caused some damage to the skin (for example, by removing lipids from the stratum corneum) despite the reduction in penetration observed in

most trials. However, the damage was not extensive enough to increase the penetration for any compound. Therefore, washing with soap for a limited duration could potentially cause skin damage but would not be expected to decrease the barrier provided by the stratum corneum except when washing for an excessive duration.

The mechanical action of washing could also have an impact on the barrier function of skin. Coarse materials such as harsh sponges, cloths or gritty exfoliants could mechanically remove corneocytes or cause abrasions through the stratum corneum during washing. If compound remained on the skin following this abrasion, it could enter the skin through tears in the stratum corneum or more quickly penetrate through areas in which the thickness of the layer had been reduced. Lademann et al. (2010) also suggest the massaging action of rubbing the skin during washing could potentially move the hair in such a way as to allow deeper access into follicles for foreign particles. This could provide a pathway for compounds past the stratum corneum and into the viable epidermis and dermis, where the hair follicles terminate. This effect would be more pronounced in skin with high concentrations of hair follicles. However, EPA (1992) suggests that for most human skin, the low number of hair follicles would make this pathway of absorption negligible.

The removal of compound and the impacts of the washing solution and method on the skin could have two different types of effects on dermal absorption. Washing exposed skin could increase or decrease the total mass of contaminant absorbed compared to not washing or washing at a later time. This effect would be observed over a duration that was long enough to account for the final fate of most of the applied compound. In the short term, decontamination could increase or decrease the flux of compound through the skin compared to not washing. When washing to remove chemical contamination on the skin, the desired outcome is a decrease in both temporary penetration and long-term overall percutaneous absorption. This decrease could result from the removal of an unbound reservoir of compound on the skin surface or some amount of compound in the top layers of the stratum corneum that would otherwise continue to penetrate into the skin.

However, some have suggested that unintentional side-effects of washing as outlined above could actually enhance percutaneous absorption via the "wash-in" effect (Moody & Maibach

2006, Klingner 2007). Even though washing can remove unbound compound on the skin surface, this "wash-in" hypothesis suggests that it also can "mobilize" compound remaining in the skin into the blood supply. This hypothesis is in particular championed by Moody & Maibach. They state in their 2006 review, "The potential importance of the [wash-in] effect cannot be overly stressed." Figure 4 provides an illustration of a hypothetical short-term increase in flux post-washing.

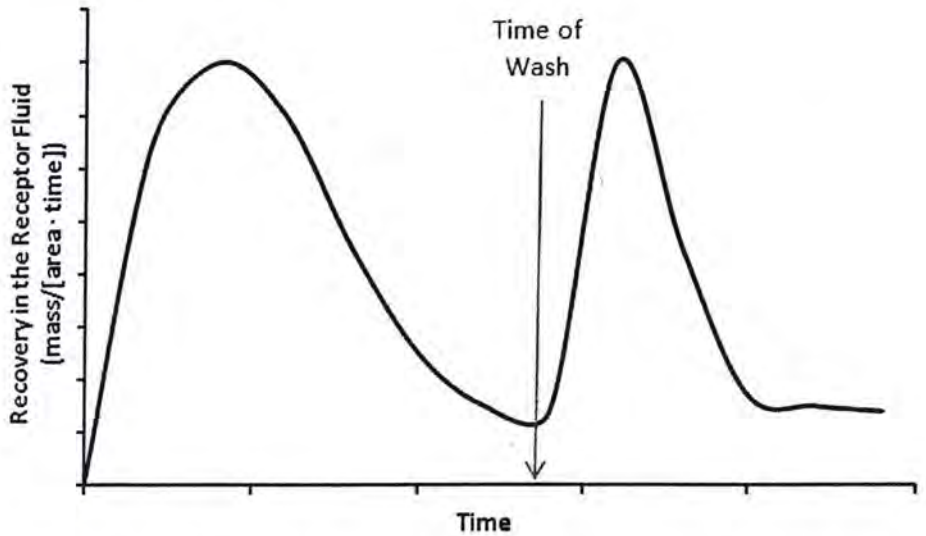

Figure 4. Hypothetical Post-Washing Spike in Flux. Adapted from experimental data reported by Moody & Nadeau (1997) for 2,4-D amine applied to guinea pig skin.

In this illustration of the "wash-in" effect, the time of wash precedes an increase in penetration or flux into the receptor fluid. This post-washing peak in penetration could be smaller or larger than the maximum penetration observed prior to washing. In this example, the washing produced an increase in flux that was about equal to the flux of the contaminant through the skin prior to decontamination. However, proper interpretation of this post-washing peak requires an in-depth knowledge of the relevant experimental conditions for the trial in which it was observed.

It is important to note that many of the studies used to support the "wash-in" hypothesis attempt to wash the exposed skin after the normal flux through the skin has peaked and is beginning to decline. This is so any post-washing peak is easily discernable from the normal penetration profile and can be evaluated separately. One shortcoming of this technique is that even when researchers wait for many hours prior to washing, the timing of the wash may

still overlap with the peak normal flux through the skin for compounds that penetrate at a slower rate (e.g., large, very lipophilic or hydrophilic compounds). Without a prior knowledge of the normal penetration curve and an unwashed control with which to compare the washed sample, it is difficult to say with certainty that any peak in penetration following washing is due solely to the effects of washing alone.

The short- and long-term impacts of washing could be positively correlated if, for example, the increase in post-wash flux is so large in size or duration that it leads to an increased overall absorption compared to an unwashed control. However, an increase in short-term flux does not always result in an increase in the total mass absorbed. These are two separate effects. An increase in flux post-washing may be followed by a reduction in flux for the rest of the trial or a shorter duration of excretion if washing performs its desired function and removes contaminant from the system, as illustrated in Figure 5.

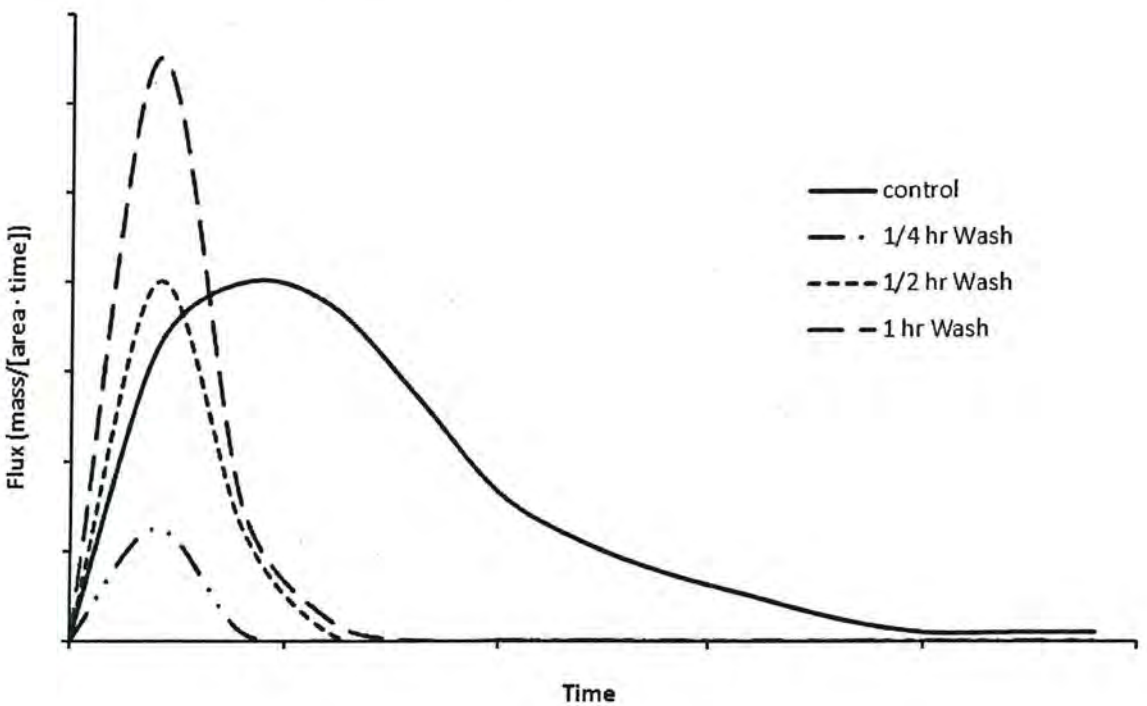

Figure 5. Possible Influence of Washing Time on Flux and Area Under the Curve. Adapted from experimental data reported by Loke et al. (1999) for diethyl malonate applied to skin and then washed with 2% sodium dodecyl sulphate solution.

In this example, the area under the curve is the total mass that penetrates through the skin over the duration of the experiment. Therefore, less compound was dermally absorbed in the

samples washed at 15 or 30 minutes or 1 hour compared to the unwashed control, despite the fact that washing increased the short-term flux in the sample washed at 1 hour.

1.B. Methodology of Published Dermal Absorption Studies

In order to better understand these potential effects of washing on dermal absorption, a review of the published literature is warranted. Trials have been conducted both *in vivo* in living subjects and *in vitro* on excised skin that has been placed on an experimental apparatus designed to replicate *in vivo* conditions. *In vivo* and *in vitro* trials both have positive features and limitations that influence their ability to provide data about the effects of washing on dermal absorption. In general, *in vivo* human work is preferred, but is limited by ethical and/or practical considerations. Therefore, both *in vivo* and *in vitro* trials are included in this review.

It should be noted that due to the limited duration of experimental protocols, there is some uncertainty about the fate of compound recovered in the skin upon termination of each trial. It is generally accepted that the mass of compound recovered in the receptor fluid *in vitro* and in the excreta and non-exposed tissues *in vivo* should be considered systemically absorbed and potentially capable of causing health impacts. Guidance documents also suggest that compound recovered in the viable epidermis and lower skin layers of the exposed site should also be considered to have been irreversibly absorbed (OECD 2004). Often, the duration of dermal absorption experiments is not long enough to observe the migration of all applied compound out of the dosed skin. Additionally, *in vitro* artifacts such as reduced solubility in the receptor fluid and damaged micro-circulation can also result in higher levels of compound retained in the viable skin layers than in *in vivo* trials. The normal shedding of layers in the stratum corneum is also difficult to replicate *in vitro* due to limitations on the duration of trials and the loss of viability in the excised skin (Yourick et al. 2004). Some guidance documents suggest that including compound in the SC *in vitro* as part of the systemically absorbed dose may overestimate the amounts that would be eventually absorbed *in vivo* (Diembeck et al. 1999, SCCP 2006). Others suggest the compound recovered in the SC has the potential to be absorbed further into the skin (ECETOC 1993). Some studies attempt to determine the amount of compound in the stratum corneum using a method called tape stripping, which consists of repeated application and removal of plastic tape to the skin

surface. The adhesive on the tape removes one or several layers of corneocytes, depending on the hydration of the skin. In this review, compound recovered in the stratum corneum, when reported separately from the amount recovered in the viable skin layers, is not considered absorbed, while compound in the viable epidermis and dermis is considered unrecoverable.

Many published dermal absorption studies have incorporated washing into their experimental protocol. Sometimes decontamination of the skin is an afterthought that occurs at the termination of the trial in an attempt to determine the amount of free compound remaining on the surface of the skin. Of greater interest to this review are the studies designed to investigate the effect of washing on dermal absorption. However, there is no standardization in experimental protocol for these types of trials. Therefore, this review attempts to classify trials in the relevant studies and characterize how differences in protocol could impact their results. Overall trends in results will also be highlighted and areas for further study discussed.

Studies that investigated dermal absorption of compounds and washed the exposed skin were reviewed with an emphasis on the literature published between 1990 and 2011. Sixty-six studies found to include washing were reviewed. Washing was defined as decontamination utilizing some sort of fluid or solution as at least one component of the protocol. Dry wiping only was not considered washing for the purposes of this review. Partial information from each study's experimental protocol and results were summarized. Key points included:

Background

- Purpose of study

Methods

- *in vivo* or *in vitro*
- Chemical(s) of interest
- Skin type used (species, anatomical location)
- Skin preparation (hair removal)
- Skin damage (tape strip, disease)
- Area of skin exposed

Compound application (loading amount, total mass applied)
Vehicle (volume, duration of contact with skin)
Timing (of washing, end of trial)
Washing solution, method
Sample size

For *in vitro* trials only:
Skin thickness
Skin handling (length of storage, storage method)
Diffusion cell apparatus (flow rate, temperature, receptor fluid collection frequency)

Results

Statistics (measure of central tendency, variance)
Recovery in washing fluid (mass or % dose)
Recovery in skin (intact or in each layer/tape strip) (mass or % dose)
Recovery in *in vitro* receptor fluid/ *in vivo* excretion (mass or % dose)
Mass balance (mass or % dose)
Change in concentration or flux over time

Each study was classified based on the information recoverable from that particular combination of experimental designs, including whether the study could provide information regarding the effect of washing on the short- and/or long-term movement of compounds through skin. As discussed previously, there is currently minimal standardization across dermal absorption studies, especially in regards to the timing and method of the washing step. Therefore, this review attempts to classify the various protocols and determine the information recoverable from a wide variety of experiments described in the published literature.

Twenty-five studies provide information about the effect of washing on total mass absorbed and/or change in flux. Studies that cannot provide information on either of the effects of interest are summarized in minimal form in Appendix A.

It should be noted that in several of these studies, the description of methods was limited and instead the reader was informed that the experimental design was "identical" to prior experiments. This claim may not always be valid, especially in studies utilizing or combining several past protocols where changes to the procedures might have been made and not explained fully. Therefore, although the best effort was made to carefully outline the methods used, there is the potential that the descriptions contain unknown inaccuracies that could impact the results.

1.B.1. Impact of Washing on Both Total Absorption and Flux

Pelletier et al. (1989)

This *in vivo* study characterized the dermal and oral absorption, distribution and elimination of 2,4-dichlorophenoxy acetic acid dimethylamine salt (2,4-D amine). Pelletier et al. applied radiolabeled 2,4-D amine in an aqueous solution containing dimethylamine and dispersing and wetting agents to rats. Hair was removed from skin via clippers and a depilatory cream. Sixty µl of solution was applied to a 9.36-cm^2 area for a dose of 10 mg 2,4-D amine per kg body weight. Rats weighed between 240 and 260 g. An average loading of 267 µg/cm^2 was calculated using the above information provided by the study authors. The exposed area was covered by a non-occlusive "pack". Animals were washed by rubbing twice with a gauze sponge soaked in 1 mL of 0.25% liquid Ivory soap solution and then wiping once with 1 mL of water, with a dry sponge wipe following each wet step. All samples were washed a total of 3 ml of wash solution, or 0.3 ml of solution per cm^2 of exposed skin. Washing was conducted at 30 minutes, 1, 2, 4 or 6 hours. Four animals were washed at each time point, after which the animals were immediately sacrificed. Additional animals were washed at 7 hours only and then four were sacrificed at subsequent times of 8, 10, 13, 16, 24, 48 or 72 hours. For trials that continued past the time of washing, protective guards were reapplied to the area following washing. Urine and feces were collected every 24 hours for the duration of each trial and analyzed. Upon sacrifice, the blood was removed and analyzed, as was the carcass and skin from the application site. Recoveries in the whole skin and wash solution were presented as mean and standard error of the mean (SEM) percent applied dose. Mass balance was presented as mean cumulative percent dose and recoveries in urine, blood, liver,

gastro-intestinal tract, kidneys and muscle were plotted as graphs of percent applied dose recovered at each termination time.

No statistical testing was conducted for this study. Therefore all comparisons are only nominally different. Earlier washing was reported to remove a larger fraction of chemical than later washing. An average of 71.5% was removed from samples washed within 6 hours while an average of 62.8% was removed for samples washed at 7 hours post-exposure. The percent of the dose found in the skin was about the same (an average of 12%) for the animals sacrificed immediately post-washing at 6 hours or before. Washing at 7 hours resulted in a peak skin recovery of 16.6% of the dose observed at 8 hours which declined the longer the animals were followed. Mass balance was between 79.3% and 91.2% for all dermal absorption trials. The percent dose recovered in the blood was highest in the trials terminated at 30 minutes (about 1%), plateaued to a fairly similar percent recovery for trials terminated between 2 and 8 hours (about 0.1%) and then declined for trials terminated after 10 hours to less than 0.01% at 72 hours.

Ritter et al. (1990)

This *in vivo* study utilized different methods of preparing application sites in rats as well as characterized the effects of different cleansing agents on dermal absorption of 2,4-dichlorophenoxy acetic acid dimethylamine salt (2,4-D amine). Part one of the study examined dermal absorption following various hair-removal techniques. These samples were washed at 7 hours with an 8-hour termination or at 7 and 23 hours with a 24-hour termination. This protocol did not provide any useful information for this review.

In part 2, Ritter et al. applied 2,500 µg of radiolabeled 2,4-D amine in 40 µl of unspecified vehicle to 9.4 cm^2 of mid-dorsal rat skin (from which hair had been removed by clipping and shaving with an electric razor) for a loading of 266 $\mu g/cm^2$. A non-occlusive "pack" was applied over dosed skin to prevent rats from ingesting any chemical. The exposed area was either washed at 7 hours with termination at 8 or 24 hours, washed at 7 and 23 hours with termination at 24 hours or washed at 23 hours and ended at 24 hours. Animals were washed by rubbing twice with gauze soaked in a washing solution. Solutions were either 0.25% Ivory soap in water, water, 70% isopropyl alcohol in water or Rad-Con® foaming hand

cleaner, a product designed to "lift" radioactive contaminants off skin prior to wiping or rinsing. Advertisements for the Rad-Con® product state it contains no skin irritants or abrasives, but the MSDS for the compound recommends avoiding skin contact and states the product contains propane and butane in addition to paraffin oil with a neutral pH of 7 (Nuclear Associates 2002, MXR 2010). Skin was wiped with dry gauze between each washing step. Following the wiping series, the area was wiped a final time with water-soaked gauze. A fifth group was washed by wiping three times with gauze soaked in acetone and wiping with a dry wipe only after the third acetone wipe. All samples were washed a total of 3 ml of wash solution, or 0.3 ml of solution per cm^2 of exposed skin. Each condition had four replicates. Recovery in the wash solutions and the 21.8-cm^2 area surrounding and including the dosed area of skin was presented as bar graphs of the percent applied dose. Recovery in blood at time of termination and cumulative urine were presented as mean and SEM of percent applied dose. Mass balance was not presented.

Delaying washing from 7 to 23 hours reduced the percent of the total applied dose of 2,4-D recovered by all wash solutions from an average of 60% for the 7-hour wash to 45% for 23 hours. This difference was significant ($p<0.05$) for all washing solutions except acetone. Cleaning at 7 hours also reduced the percent dose recovered in the skin at 24 hours to approximately 14% compared to 19% for decontaminating at 23 hours and ending at 24 hours. However, this reduction was significant ($p<0.05$) only for the isopropanol and acetone wash solutions. Washing the same animals at both 7 and 23 hours resulted in about 10% of the applied dose being recovered in the skin at 24 hours. Blood levels were not significantly different one hour after cleaning between the 7 or 23 hour cleaning times ($p>0.05$) and ranged between 0.12 and 0.28% of the applied dose. Rats cleaned at 7 hours and sacrificed at 24 hours had significantly ($p<0.05$) lower blood levels (0.018 to 0.030% of the applied dose) than either those washed at 7 hours and sacrificed at 8 or washed at 23 and sacrificed at 24 hours. Cleaning at 7 and 23 hours and sacrificing at 24 hours gave higher blood levels (0.033 to 0.080% of the applied dose) than cleaning at 7 and sacrificing at 24 hours, although the difference was not significant for the soap and water wash solution. In urine, cleaning at 23 hours only and sacrificing at 24 hours resulted in the highest percent dose excreted in urine (between 16% and 26%) for samples washed with all solutions and ended at 24 hours, although the difference was only significant ($p<0.05$) for isopropanol. There was no

significant difference ($p<0.05$) in urinary recovery of 2,4-D amine for samples washed as 7 hours (12.4% to 16.4%) or 7 and 23 hours (15.4% to 19.3%) and terminated at 24 hours, regardless of solution, although washing twice resulted in nominally higher values.

Loke et al. (1999)

This *in vitro* study tested the hydration effects of washing on the skin's ability to act as a barrier to the nerve agent simulant diethyl malonate. Loke et al. applied 4 µl of 99.9% pure diethyl malonate (specific gravity of 1.04 g/ml) via microsyringe to 0.8 cm^2 of human cadaver thigh skin for a loading of 5,200 µg/cm^2. Skin was dermatomed to 600 µm thick and stored at -30 °C until used within 1 month of harvest. The skin was placed on a diffusion cell apparatus with a constant flow rate of 8 ml/h that maintained a skin temperature of 32 °C. Chemical was applied and skin was washed at 15, 30 or 60 minutes post-exposure without removing the skin from the cell. Skin was washed for 1 minute with a constant flow of 2% (w/v) sodium dodecyl sulphate (SDS), 2% (w/v) Hyamine, 2% (w/v) RM21 (NATO standard cleaning solution), deionized water, 0.9% (w/v) saline or 9% (w/v) saline solution for a total of 0.5 L of washing solution. This resulted in the application of a total of 625 ml of wash solution per cm^2 of exposed skin. Controls were not washed. The receptor fluid was collected for 24 hours in 2-hour increments. Sample size was three for each delay time/washing solution combination but not specified for controls. Information about penetration rates into the receptor fluid were graphed or presented as a mean and SEM "enhancement". "Enhancement" was the increase in penetration in µg/hr between washed samples and the average of all of the controls at two hours. Decontamination efficiency, defined as the amount of compound that penetrated through the control skin minus the amount that penetrated washed skin divided by the amount that penetrated the control, was presented as a bar graph. The mass balance and the amount of chemical recovered in the skin were not presented. The stratum corneum was not analyzed separately via tape stripping.

Authors noted that 45 to 50% of the dose evaporated. They also noted that decontamination efficiency was better if the skin was washed at an earlier time. Authors state no significant differences ($p>0.05$) were observed in decontamination efficacy between washing solutions at each time point. No additional statistical testing was reported. Therefore, differences

discussed below may not be statistically significant. Washing at 30 and 60 minutes resulted in an increased penetration rate for the first two hours compared to the control for all washing solutions. The mean penetration enhancement at 2 hours observed for samples washed at 1 hour differed between wash solutions, although all demonstrated an increase over the average penetration rate of 99 μg/h observed in unwashed controls. The increases in mean penetration enhancement ranged from 141 μg/h for SDS to 23 μg/h for the 9% saline solution wash. The maximum penetration rate as estimated from a graph was 24 μg/h for samples washed with SDS at 15 minutes, 148 μg/h for samples washed at 30 minutes, 284 μg/h for samples washed at 1 hour and 154 μg/h for the relevant control samples. The maximum penetration was observed at 2 hours for the washed samples and at 4 hours for the controls. The maximum penetration for controls as estimated from the graph did not exactly match the penetration for the controls which was used to calculate the penetration enhancement. This is likely due to authors averaging all of the control samples for each wash solution to calculate the value of 99 μg/h while the mean value of 154 μg/h was obtained only from the controls for the samples washed with SDS.

Monteiro-Riviere et al. (2001)

This study compared the efficacy and impact on dermal absorption of several decontamination methods for phenol. The first half of the study used *in vivo* methods to determine the effect of washing on phenol's physical damage to the skin. The decontamination methods that were most effective *in vivo* at preventing damage were tested *in vitro* against unwashed control samples. A freshly-harvested isolated perfused porcine skin flap system of unspecified thickness was placed on a flow-through diffusion cell apparatus that collected receptor fluid at 30 minute intervals for 8 hours, with additional samples collected at 5, 15 and 105 minutes. Receptor fluid flow rate and skin temperature were not provided. Skin viability was tested by sampling for glucose metabolism for one hour prior to exposure and during the trials. An area of 2.8 cm^2 of skin was exposed to 400 μl of 89% aqueous phenol on an occluded cotton pad for one minute resulting in an unknown loading since not all of the compound in the pad reached the skin. Skin was removed from the diffusion cell for chemical application and subsequent immediate washing in decontaminated samples. Skin was washed by alternating rinsing with water at 1 L/min (360

ml/[min·cm^2]) for 1 minute and a treatment wash (water, polyethylene glycol (PEG) 400 or isopropanol (IPA)) at 100 ml/min (36 ml/[min·cm^2]) for one minute for a total of 15 min, beginning and ending with a higher speed water wash. This washing protocol resulted in a total of 3,107 ml of wash solution being applied per cm^2 of exposed area. Sample size was four per condition. Compound recovered in the receptor fluid was plotted as concentration in μg/ml over time. The mean and standard error of the mean (SEM) were also provided for the area under the curve. No other results were displayed graphically.

The peak concentration observed in the receptor fluid was nominally higher for the control unwashed sample (19.9 μg/ml) than the samples washed with water only (15.4 μg/ml), IPA and water (12.8 μg/ml) and PEG 400 and water (10.2 μg/ml). The samples washed with PEG 400 and water peaked at the first sampled time point (23 minutes) while samples washed with IPA and the controls peaked at 45 minutes. The area under the curve (μg·min/ml) was significantly ($p<0.05$) larger for the control than any of the washed samples.

Nielsen (2010)

Nielsen's *in vitro* study examined the effect of washing on dermal absorption for compounds with a wide range of molecular weight (122 to 330 g/mole) and water solubility (0.15 to 22 g/L). Radiolabeled benzoic acid, glyphosate, caffeine and malathion were applied via an unspecified method in 106 μl aqueous solution with 0.9% sodium chloride and 2% ethanol to 2.12 cm^2 of human breast or abdominal skin for a loading of 100 μg/cm^2 for malathion and 200 μg/cm^2 for the other compounds. Skin was stored at -20 °C for up to 12 months prior to use and then subcutaneous fat was removed, resulting in a skin thickness of between 600 and 1000 μm. Skin was kept at 32 °C on a diffusion chamber without continuous flow for 18 hours prior to the start of each trial. Skin was not removed from the apparatus for washing or when collecting each 0.2 ml sample of receptor fluid (accomplished at unspecified intervals through a port). However, cells were covered with parafilm. Twelve to thirteen samples were washed at six hours via wiping with cotton swabs soaked in pH neutral hand soap and then rinsing twice with isotonic water. At 48 hours, all previously washed samples as well as 14 unwashed controls were wiped 4 times with cotton swabs soaked in 50% acetonitrile in water and then twice with dry swabs. Data were reported as mean and SEM for maximal

flux, lag time and for both percent of the applied dose and mass recovered in the wash solutions, skin and receptor fluid.

Overall, mass balance was good, with an average between 90.2% and 102.9% for washed samples and 79.8% and 95.9% for unwashed samples. The maximum fluxes of the contaminants through skin were approximately equal for washed and unwashed samples treated with glyphosate (0.0155 $\mu g/cm^2$-hr) and malathion (0.9 $\mu g/cm^2$-hr) and slightly greater ($p<0.05$) in the unwashed samples for the caffeine (1.8 vs 1.0 $\mu g/cm^2$-hr) and benzoic acid (20.4 vs 15.8 $\mu g/cm^2$-hr). The timing of the observed maximal flux was not clearly provided, but appears to occur prior to 12 hours post-exposure for all glyphosate samples, prior to 8 hours for all benzoic acid samples, prior to 10 hours for all malathion samples, prior to 8 hours for washed caffeine and prior to 24 hours for unwashed caffeine samples. Total mass that was recovered in the skin at the termination of the trial was greater ($p<0.05$) in unwashed samples compared to washed samples for glyphosate (2.3 vs 0.6 μg), caffeine (19.4 vs 2.3 μg), benzoic acid (5.1 vs 3.5 μg) and malathion (23.4 vs 12.7 μg). A similar pattern of greater absorption ($p<0.05$) in unwashed samples was observed for the total mass recovered in the receptor fluid over the course of the study for glyphosate (1.3 vs 0.4 μg), caffeine (101.8 vs 34.1 μg), benzoic acid (380.6 vs 327.2 μg) and malathion (43.9 vs 38.8 μg). This effect was most pronounced for the more hydrophilic compounds. The amount recovered by washing was greater for samples washed at 6 and 48 hours than for samples decontaminated only at 48 hours.

1.B.2. Impact of Washing on Total Absorption

Maibach & Feldman (1974)

Maibach & Feldman (1974) looked at many aspects of dermal absorption of radiolabeled pesticides *in vivo* by applying compounds via pipette to 4 to 6 male human subjects per trial and collecting urine for 5 days. Four $\mu g/cm^2$of azodrin, ethion, lindane or baygon, 4 or 40 $\mu g/cm^2$ of 2,4-D and 4, 40 or 400 $\mu g/cm^2$ of malathion or parathion were applied in the hydrophilic vehicle acetone to forearm skin and washed for two minutes with soap and hot water. Four $\mu g/cm^2$ of parathion in acetone was also applied to the forehead or palm of volunteers and washed with soap and water. Four $\mu g/cm^2$ of malathion or parathion were

applied in acetone to forearm skin but washed with an "excess" of rubbing alcohol at 15 minutes, 1 or 4 hours. Four $\mu g/cm^2$ of malathion, parathion or baygon were also applied in acetone to the forearm, forehead or palm and then washed via full-body showering at 4 hours post-exposure. Finally, 4 $\mu g/cm^2$ of parathion or baygon were applied in the lipophilic vehicle xylene to forearm skin and washed with soap and water at 15 minutes, 4 or 24 hours. Samples without a specified wash time were washed at between 2 and 8 of the following times: 1, 5, 15 or 30 minutes or 1, 2, 4, 8 or 24 hours. Area of skin exposed and volume of vehicle or wash solution were not provided. Recoveries were presented as percent absorbed, which was the percent of the original dose recovered in urine from dermal exposure divided by percent in urine from exposure via injection.

Authors did not present information on statistical analyses between any of the analyzed samples. Therefore, the following discussion refers only to nominal differences. In general, for all chemicals, loading amounts and application locations, the percent penetration increased with longer time between application of the chemical and soap and water washing. Some chemicals did not show an increase at every time point, but all chemicals washed with soap and water showed greater penetration at the final observed time point versus the earliest. As an example of a compound with relatively low overall percent penetration, 4 $\mu g/cm^2$ 2,4-D resulted in a penetration of 0.5% following washing at 1 minute and a penetration of 5.8% following washing at 24 hours. On the other hand, 4 $\mu g/cm^2$ of parathion applied to forehead skin resulted in the highest overall percent penetration. In subjects washed at 1 minute, 8.4% of the original dose penetrated the skin while 36.3% of the dose penetrated in subjects washed at 24 hours. The condition with the greatest variability was the application and washing of 4 $\mu g/cm^2$ parathion on the palm. In this case, the percent penetration doubled from about 6% to about 14% between 5 and 15 minutes wait time to washing. The penetration then decreased at each subsequent time point (30 min, 1, 2 and 4 hours) to about 8% at 4 hours before increasing again to about 12% at 24 hours. Attempting to remove malathion and parathion with rubbing alcohol had a different pattern, where washing at 15 minutes resulted in greater absorption than washing at 1 hour, while washing at 4 hours led to the greatest percent penetration.

Whole body showering at 4 hours post-exposure was reported to be slightly more effective at removing malathion and baygon than soap and water washing, reducing percent penetration from 12.1% to 8.8% for malathion and from 15.5% to 9.9% for baygon. In contrast, showering was slightly less effective at removing parathion from arm, forehead and palm skin than soap and water washing, with an increase of percent penetration from 8% to 16.5% for compound applied on the arm.

Applying parathion and baygon in a xylene vehicle resulted in increased penetration compared to applying parathion in acetone for subjects washed at 15 minutes or 4 or 24 hours and baygon applied in acetone vehicle when washed at 15 minutes. Baygon applied in xylene and washed at 4 or 24 hours showed a decrease in percent penetration than when applied in acetone. Baygon applied in xylene resulted in an increase in penetration with longer time to washing while parathion applied in xylene showed a spike in penetration at 4 hours of 31% from about 20% at 15 minutes, followed by a decrease back to 20% at 24 hours.

Nitsche et al. (1984)

This *in vitro* study characterized how lindane emulsion partitions into skin layers in intact and damaged skin under various washing conditions. Radiolabeled lindane was applied via pipette followed by 90 seconds of rubbing in 5 µl of commercial Jacutin emulsion to 2 cm^2 of whole human mammary skin with 1 to 2 mm of subcutaneous fat still attached. The lindane was applied as 0.3% of the solution (7.5 $\mu g/cm^2$) or 1% of the solution (25 $\mu g/cm^2$). Information on the source, storage and handling of the skin was not provided. Some samples had the stratum corneum removed via tape stripping prior to application of the lindane. Skin was mounted on static glass cells and maintained at a temperature of 32 °C. Receptor fluid was not sampled over time. Sample size was not provided.

Skin was removed from the glass cells during the washing procedures. Washing with "hard soap solution" or water was conducted via rubbing with soaked cotton swabs for 10 seconds. Samples washed with soap solution were then rubbed with cotton swabs soaked in water to remove soap residue. Wash solution volume was not provided. Trials were ended at 3 and 10 hours with no wash. Trials were also washed with soap and water at 3 hours and ended at

10 hours or washed with either soap solution or plain water at 10 hours and ended at 24 hours. At the end of each trial, the skin was removed from the glass cells, rubbed with dry cotton and sectioned into 40-μm layers to determine the distribution of the lindane. These data were presented as the concentration (μg/mL tissue) in each layer of skin (SC, epidermis, upper and lower dermis and subcutaneous fat). Recovery in the receptor fluid, wash solution and total mass recovered were not published. For this review, concentration data were converted to total mass recovered in each layer by approximating the volume of skin exposed for each layer and multiplying that value by the concentration. This approximation was less accurate for subcutaneous fat because the reported thickness ranged from 1 to 2 mm. An average thickness of 1.5 mm was used in calculations. The percent of the original applied dose recovered in each layer was also calculated by dividing the total mass recovered in each layer by the total mass applied.

Authors did not present information on statistical analyses between any of the analyzed samples. Therefore, the following discussion refers only to nominal differences. At 10 hours, larger amounts of lindane were found in the lower skin layers (the epidermis, dermis and fat layers) of the intact samples washed at 3 hours (total of 0.196 μg) than in the intact samples that were not washed (total of 0.043 μg). However, washing at 3 hours did reduce the mass of chemical in the stratum corneum at 10 hours when compared to not washing (1.03 μg for washed skin vs 2.98 μg for unwashed). For samples washed at 10 hours and analyzed at 24 hours, washing with soap and water reduced the mass of lindane recovered in the skin compared to washing with just water (1.5 μg in the SC and 0.4 μg in the viable skin layers when washed with water vs 0.7 μg in the SC and 0.3 μg in the viable skin layers when washed with soap and water).

When simulating damaged skin via removal of the stratum corneum prior to lindane exposure, washing at 3 hours reduced the mass of lindane recovered in the skin at 10 hours (0.7 μg) compared to not washing (1.4 μg). This washing resulted in a reduction in mass of lindane in the epidermis and dermis compared to no wash, while the mass of lindane recovered in the subcutaneous fat for both the wash and no wash scenarios were similar.

Wester et al. (1990b)

This study investigated the dermal absorption of dichlorodiphenyltrichloroethane (DDT) and benzo[a]pyrene (b[a]p) into and through skin from soil and acetone. In the *in vivo* part of the study, compounds were applied to the abdomens of rhesus monkeys which were then washed at 24 hours. Urine was collected for 7 days total and only the cumulative recovery in the urine at the end of the 7 days was reported. This provided no information about excretion of the compounds over time.

Wester et al. also utilized an *in vitro* study design. Radiolabeled DDT (104 mCi/mmol) and b[a]p (60 mCi/mmol) was applied to 500-μm thick intact human cadaver skin. Skin was placed on flow-through diffusion cells with a flow rate of 3 mL/hr and human plasma at 37 °C as a receptor fluid. Skin was stored at 4 °C and used within 5 days. For both DDT and b[a]p, 0.04 g of soil were applied to 1 cm^2 of skin and spread with a glass rod for a loading of 0.4 $\mu g/cm^2$. Compounds were also applied via pipette in 5 μl acetone solution at concentration of 0.023 μCi/μl to 1 cm^2 of skin. This resulted in a loading of 0.49 $\mu g/cm^2$ for b[a]p and 0.40 $\mu g/cm^2$ for DDT. Washing was conducted at the time of termination (25 minutes or 24 hours) using 1 mL Ivory liquid soap followed by washing twice with 1 mL of distilled water. This washing protocol resulted in a total of three ml of wash solution being applied per cm^2 of exposed area. Compound was applied while skin was on the diffusion cells but washing occured after removal from the apparatus. Sample size was 6 per condition for the 24-hour wash and 3 per condition for the 25-minute wash. Recoveries were presented as the mean and standard deviation (SD) of the percent of the original applied dose for the skin, wash solution and receptor fluid. Recovery in the receptor fluid was presented as total cumulative only and recovery in skin was not analyzed separately by layers.

Authors did not present the results of statistical analyses between the different washing times for any of the analyzed samples. Therefore, the following discussion refers only to nominal differences. *In vitro* trials showed that less of either compound could penetrate into skin and more could be removed via washing for both times when compounds were applied in soil versus acetone. Very little compound penetrated through the skin into the receptor fluid under any of the experimental conditions (between 0.01% and 0.09% for the 24-hour trials

while none was detected for the 25-minute trials). When applied in acetone, 24% of b[a]p was recovered in the skin washed at 24 hours compared to 5% from skin washed at 25 minutes. Additionally, washing recovery for b[a]p applied in acetone was higher in samples washed at 25 minutes (92%) than for samples washed at 24 hours (53%). Similar patterns were observed when both compounds were applied in soil. For DDT applied in acetone, washing earlier resulted in a slight decrease in percent recovery in the skin (17% at 25 minutes vs 18% at 24 hours) while also resulting in a slight decrease in washing recovery (62% at 25 minutes compared to 64% at 24 hours). Mass balance was between 77% and 115% for all samples.

Koizumi (1991)

This *in vivo* study characterized the dermal absorption, including the effects of washing, of hexachlorobenzene (HCB) in rats as an approximation for human workers. Koizumi (1991) applied radiolabeled HCB to 4 cm^2 of the shaved back skin of rats via a glass gas-tight syringe and allowed it to evaporate under a heat lamp. The dosed area was then covered by a Teflon film and protective jacket. The amount of chemical and volume of the tetrachloroethylene vehicle were dependent on the weight of the rat, which was reported as approximately 230 g (SD of 6). An average of 620 $\mu g/cm^2$ was applied to unwashed rats and an average of 660 $\mu g/cm^2$ was applied to animals that were washed. Compound was applied in about 100 µl of solution. Washing at 6 hours consisted of wiping 20 times with a cotton swab soaked in an unspecified amount of 0.5% (w:w) soap and water solution and then applying a new Teflon film and jacket. All rats were washed at the termination of the trial with tetrachloroethylene soaked cotton swabs. Urine and feces were collected until rats were sacrificed at 6, 24 and 72 hours for unwashed rats or 72 hours only for washed rats. The distribution of HCB in the carcass was determined. Sample size was three rats per set of conditions. Results were presented as mean and SD µg equivalents or mean percent of the applied dose recovered in the wash, urine, feces, carcass, skin, cage wash and mass balance. The recovery from the skin was split into recovery from a 16-cm^2 area around the 4-cm^2 exposed area and recovery from the remaining unexposed whole skin. Wash recovery included recovery from the covering Teflon film and jacket. Cumulative excretion at 6, 24, 48 and 72 hours was also graphed for both washed and unwashed animals.

The 6-hour washing of the skin and protective coverings recovered about 34% of the applied dose. At 72 hours, animals that were washed had significantly less ($p<0.05$) HCB recovered from a 16-cm^2 area surrounding the dosed skin (1% vs 5%) and less considered absorbed (5% vs 10%) than control animals that were not washed. Compound recovered in the carcass and excreta minus compound in the dosed area was considered absorbed. Authors state the decreases in recovery for these components in washed animals were only significant ($p<0.05$) for compound recovered in the feces and carcass, while the observed decreases in recovery were not significant between mass recovered in the liver or urine. Mass balance calculations by the authors demonstrated between 92 and 97 total percent recovery of the applied dose of HCB. Washed animals excreted a nominally smaller cumulative amount at 6, 24, 48 and 72 hrs than unwashed animals. The blood concentration at 72 hours was also nominally lower in washed versus non-washed animals.

Weber et al. (1992)

This *in vitro* study characterized the effectiveness and impact on dermal absorption of several decontamination methods for removing 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) from skin. Weber et al. state they applied 2.5 µg of radiolabeled TCDD in 40 µl acetone by spreading the vehicle with a flattened glass rod for 30 seconds on 3.8 cm^2 of human cadaver skin, for a loading of 0.65 µg/cm^2. Skin was dermatomed to around 1 mm thick and stored at -20 °C for an unspecified amount of time. The stratum corneum was removed from half of the skin samples via tape stripping 15 to 30 times prior to chemical application. Authors note that the removal of the stratum corneum was to simulate damage to the skin. After application, skin was placed on gauze soaked in sterile saline and incubated at 32 °C for 100 minutes. At that time, three samples of both intact and damaged skin were removed from the gauze and washed by either rubbing six times with four dry cotton balls, rubbing with dry cotton followed by soaking with 0.2 ml of mineral oil and more dry rubbing, rubbing with dry cotton followed by a mineral oil soak and then rubbing with cotton balls soaked in acetone or rubbing with cotton balls soaked in water and rubbed in soap. Skin samples were then returned to the gauze and incubated for another 200 minutes. A control was left in incubation for 300 minutes total and then rubbed with dry cotton. Skin was then frozen and sectioned and the percentage of the total applied dose in each skin layer determined. Sample

size was three for each experimental condition. The percent of the applied dose recovered in each skin layer as well as the mass of TCDD per exposed area recovered in all layers to 800 μm were presented as means and standard errors. Mass balance was not presented, nor was the amount or percent compound recovered in the wash or gauze.

In intact skin, rubbing with dry cotton resulted in a similar total percent dose ($p>0.05$) recovered in the skin compared to the control. In damaged skin, rubbing with dry cotton actually caused a significant ($p<0.05$) increase in percent dose recovered in the epidermis. However, for intact skin, all three wet decontamination methods, whether they used oil, acetone or water, resulted in a decrease in percent dose recovered in the stratum corneum (16.6 - 29.2%), epidermis (1.08 - 2.17%) and upper dermis (0.51 - 0.91%) compared to the stratum corneum (43.7%), epidermis (4.06%) and upper dermis (2.14%) in the intact control samples. This difference was significant ($p<0.05$) for all samples except recovery in the epidermis for samples washed with soap and water. A similar significant ($p<0.05$) reduction was observed in the epidermis (6.33 - 7.49%) and upper dermis (1.12 - 1.36%) for the damaged samples washed with the wet decontamination methods compared to the damaged controls (37.5% for epidermis and 4.19% for upper dermis). No significant change was observed in the lower dermis between any decontamination method and the control for either intact (0.23 - 0.51%) or damaged skin (0.17 - 0.73%).

Wester et al. (1992b)

This study characterized dermal absorption of isofenphos in human volunteers when washed at different times. In the first two *in vivo* portions of this study, radiolabeled isofenphos was applied to the forearms of human volunteers. In part 1, subjects had 158.3 μg of isofenphos in 60 μl of acetone applied to 12 cm^2 of skin for a loading of 13.2 $\mu g/cm^2$. Four of these subjects were washed at 24 hours and four were washed at 72 hours. The washing procedure consisted of first using a 50% (v:v) liquid Ivory soap and water solution followed by plain water, a repeat of the soap solution and two more rinses with plain water. The same washing protocol was used for each volunteer, regardless of exposure duration. Volume of washing solution and duration of washing were not provided. Urine was collected for 7 days and each 24-hour sample analyzed. Skin was tape stripped 10 times at the end of the trial for subjects

washed at 24 hours only. Results were presented as mean and SD percent of the applied dose for overall urinary, wash and stratum corneum recovery. A bar graph also showed urinary excretion per hour for each 24-hour sample collected. Percent dose absorbed was also calculated for part 1 by intravenously (IV) dosing rhesus monkeys with isofenphos and dividing urinary ^{14}C excreted after topical application by urinary excretion following the IV dose.

In part 2, four subjects had 16.6 µg of isofenphos in 5 µl of acetone applied to six 1-cm^2 areas on their forearms with a loading of 16.6 µg/cm^2 per area. Each participant then had an exposed area washed at each of the following times: 0, 60, 120, 240, 480 and 1440 minutes. Areas were washed first with 50% (v:v) liquid Ivory soap and water solution and then twice with plain water. The experimental design of part 2 did not allow for absorption or mass balance data to be collected. Washing recovery only was presented as the mean and SD percent of the applied dose. Skin was not tape stripped.

Part 3 was an *in vitro* cadaver skin trial that washed and terminated the trial at 24 hours and reported overall recovery in the receptor fluid, intact skin and wash solution. This part was not further summarized.

Authors did not present the results of any statistical analyses. Therefore, the following discussion refers only to nominal differences.

In part 1, the percent dose excreted showed a general declining pattern following a peak in excretion of about 0.71% per hour in the first 24 hours for subjects washed at 24 hours. The peak excreted percent dose per hour was similar in magnitude to the 24-hour wash peak that was observed between 24 and 48 hours for subjects washed at 72 hours, with declining percent dose excretion on the following days. The urinary excretion (1.8%) and the adjusted percent absorbed (3.6%) was similar for both washing times. A very small amount (0.003% dose) was recovered via tape stripping 10 times at 7 days following the 24-hour wash. Washing recovery was greater when subjects were washed at 24 hours (0.75%) than at 72 hours (0.17%).

In part 2, washing at earlier times resulted in a greater washing efficacy than washing at later times, with 61.4% recovered at 0 hours, 54.1% at 1 hour, 30.3% at 8 hours and 0.53% at 24 hours.

Hewitt et al. (1995)

This *in vitro* study tested the effectiveness of different decontamination methods for the industrial carcinogens 4,4'-methylenebis[2-chloroaniline] (MbOCA) and 4,4'-methylene-dianiline (MDA). Hewitt et al. (1995) applied 5 µl of radiolabeled compound in ethanol with a microsyringe to 0.32 cm^2 of fresh, full thickness human breast skin with "subcutaneous tissue" removed or to fresh, clipped rat skin of unspecified thickness. Skin was placed on flow-through diffusion cells with a constant flow rate of 1.5 ml/h, maintained at 32 °C and allowed to equilibrate for 30 minutes prior to the start of each trial. Three ml of receptor fluid was collected every 2 hours for the 72-hour duration of the trial. In part 1 of the study, some cells were occluded with a teflon cap and compound was applied at loadings of between 9.7 and 17.7 µg/cm^2 for MbOCA and between 17.6 and 21.6 µg/cm^2 for MDA. All cells were washed at only 72 hours by rubbing the skin for 10 seconds twice with swabs soaked in an unspecified amount of ethanol, water or 1 or 10% v:v water/soap solution. Sample size was different for each condition, but ranged between 3 and 10. Washing recovery was presented as mean and SD percent of applied dose. Part 2 of the study used only unoccluded cells with the same range of loadings for MbOCA and between 17.4 and 25.3 µg/cm^2 for MDA. These samples were washed at either 3 or 30 minutes or 1, 2, 3, 4 or 6 hours with ethanol-soaked swabs 3 times for 10 seconds. Sample size was three for all washed samples except for MDA applied to rat skin which had a sample size of two. Controls had between 19 and 27 replicates for each condition. Percent of the applied dose recovered in the receptor fluid over the entire 72-hour duration and in the whole skin at termination of the trial was presented as means and SD for each set of experimental parameters. No washing recovery was presented in part 2 and recovery in the receptor fluid over time was not presented for either part of the study.

In part 1 for human skin, authors did not conduct relevant statistical analyses and therefore, the following discussion refers only to nominal differences. All four wash solutions were

able to recover similar percents of the total applied dose at 72 hours for unoccluded samples dosed with either MbOCA (33 to 43%) or MDA (44 to 47%). Similarly, no difference in the total percent recovery in the wash was observed between any of the same four solutions used on the occluded samples for MbOCA (25 to 30%) or MDA (21 to 31%). For rat skin, 10% soap solution and ethanol were significantly ($p<0.05$) more effective at removing either compound in the wash at 72 hours than washing with water only. In part 2, washing at 3 or 30 minutes resulted in a decrease in percent of the applied dose recovered in the receptor fluid or skin compared to the unwashed controls. This decrease was significant ($p<0.05$) for all skin types and compounds except for MbOCA applied to rat skin. Washing at later time points (1, 2, 3, 4 or 6 hours) resulted in a general decrease in compound recovered in the skin or receptor fluid compared to the control, but this decrease was not significant for any sample except MDA applied to human skin and washed at 4 hours ($p<0.05$).

Nielsen et al. (2004)

This study attempted to determine the percutaneous absorption of 5 different pesticides with a wide range of physicochemical properties by applying compounds to 2.12 cm^2 of human skin on static *in vitro* diffusion cells. The vehicle was 600 µl of aqueous solution containing 0.9% NaCl and 2% ethanol. Compounds were applied in 0.2 mM concentrations for prochloraz, methiocarb, paclobutrazol, pirimicarb and 0.1 mM dimethoate, which resulted in loadings of 21.3, 12.7, 16.6, 13.5 and 64.9 µg/cm^2, respectively. Skin was healthy human breast skin of 600 to 900 µm thick obtained from living donors before freezing at -20 °C for up to 12 months. The diffusion cell apparatus was maintained at 32 °C. Skin was placed atop the apparatus and allowed to equilibrate with the receptor fluid for 18 hours prior to the start of each trial. During the trial, cells were occluded with parafilm. At 6 or 24 hours, the remaining dosing solution was removed and the skin washed via an unspecified solution and method. One milliliter of receptor fluid was sampled and replaced with fresh fluid without removing the skin at "appropriate" times for 48 hours prior to termination, likely at 6, 12, 24, 30 and 48 hours post-exposure. Each condition had 7 to 9 replicates. Total penetration into the receptor fluid and wash recoveries at 6 or 24 hours and at the trial's end were presented as mean and SD in mass and percent of the applied dose. Tape stripping and analysis of the skin as a whole or by layers was not conducted. Authors attempted to estimate the amount of

compound in the skin by subtracting the receptor fluid and wash recoveries from the total applied dose.

Percent recovery of the total applied dose in the receptor fluid was nominally reduced in samples washed at 6 hours compared to those washed at 24 for methiocarb (7.1% vs 8.7%), paclobutrazol (4.3% vs 6.9%) and pirimicarb (5.3% vs 13%). Percent recovery was nominally higher in the receptor fluid at 48 hours for samples washed earlier for prochloraz (3.8% vs 2.9%) and negligible for dimethoate (0% vs 0%). Wash recovery was higher at the first wash time of 6 hours (between 34.8% and 102.6%) than at the later wash time of 24 hours (between 4.6% and 97.3%) for all compounds. The difference in the wash recovery was significant ($p<0.01$) only for methiocarb and prochloraz. The maximum flux was on the order of 0.09 $\mu g/cm^2h$ for the four compounds that showed dermal penetration. Washing earlier nominally decreased flux for methiocarb and pirimicarb and increased it very slightly for prochloraz.

Zhai et al. (2007)

This *in vitro* study tested the effects of different decontamination solutions on dermal absorption of formaldehyde through human skin. Zhai et al. applied of 0.25 μg of radiolabeled formaldehyde in 10 μl aqueous solution via HPLC syringe to 3 cm^2 of 500 μm thick human cadaver skin for a loading of 0.083 $\mu g/cm^2$. Skin was stored at 4 °C for up to 5 days. Skin was placed onto static glass diffusion cells at an unspecified temperature and exposed for either 1, 3 or 30 minutes and then washed three times with 4 mL of either tap water, isotonic (0.9%) saline or hypertonic (1.8%) saline solution. This washing protocol resulted in a total of 12 ml of wash solution being applied, or 4 ml per cm^2 of exposed area. Washing method and sample size were not specified. Radioactivity was measured in the receptor fluid only following completion of the protocol. Skin was then tape stripped twice to "remove residual chemical" and this tape was subsequently analyzed separately from the remaining skin. Washing and tape stripping occurred while the skin was still on the diffusion cell. Recoveries in the wash solution, tape strips, receptor fluid and skin were presented as mean and SD of the total mass recovered in μg as well as the percent of the original applied dose.

Authors did not conduct statistical analyses between time points for recovery in any of the analyzed samples. Therefore, the following discussion refers only to nominal differences. For all washing solutions, less mass was recovered in the wash and more mass was recovered in the skin and tape strips as the exposure time increased. Washing at 1 minute could recover between 83% and 98% of the applied dose compared to between 27% and 42% of the dose at 30 minutes. About 1% of the applied dose remained in the skin washed at 1 minute versus between 11% and 14% remaining following washing at 30 minutes. Minimal amounts (less than 1.5% of applied dose) were recovered in the receptor fluid for all trials. When analyzed separately from the rest of the skin, the stratum corneum removed by tape stripping showed the same pattern of retaining more chemical at later washing times, with about 0.5% of the applied dose in the stratum corneum after washing at 1 minute compared to between 6% and 8% of the dose at 30 minutes. Mass balance decreased with increasing times to washing due to the volatility of the compound, with between 48% and 64% of the applied dose recoverable at 30 minutes.

Zhai et al. (2008)

This *in vitro* study tested the effects of washing human skin with different decontamination solutions to remove a model herbicide. Zhai et al. (2008) applied 10 µl of radiolabeled gylphosate in a 1:1 aqueous solution to 3 cm^2 of 500-µm thick human cadaver skin via a high performance liquid chromatography (HPLC) syringe. Authors state this resulted in an application of 375 µg to the dosed area, which is a loading of 125 $\mu g/cm^2$.

It should be noted that the molecular weight (MW) for glyphosate given by the authors (405.2) is not the actual MW for pure glyphosate (169.1). An internet search revealed that glyphosate-sesquisodium has a MW of 405.2. No such compound or any other glyphosate formulations with a MW of 405.2 were sold radiolabeled by Sigma-Aldrich or other suppliers. Based on this information, it is likely that authors utilized an incorrect MW in their original calculations. Therefore, loading amounts and the mass recovered were recalculated using the pure MW of 169.1 which resulted in an estimated loading amount of 53.17 $\mu g/cm^2$. Percent recoveries should remain unchanged.

Skin was stored at 4 °C for up to 5 days. Skin was placed onto static glass diffusion cells at an unspecified temperature and exposed for either 1, 3 or 30 minutes prior to being washed three times with 4 mL of either tap water, isotonic (0.9%) saline or hypertonic (1.8%) saline solution. Wash solution was placed on skin, rubbed with a teflon policeman scrubber for 15 seconds and removed via pipette. This washing protocol resulted in a total of 12 ml of wash solution being applied, or 4 ml per cm^2 of exposed area. Sample size was not specified. Radioactivity was measured in the receptor fluid only following completion of the protocol. Skin was then tape stripped twice to "remove residual chemical" and this tape was subsequently analyzed separately from the remaining skin. Washing and tape stripping occurred while the skin was still on the diffusion cell. Recoveries in the wash solution, tape strips, receptor fluid and skin were presented as mean and SD of the total mass recovered in µg as well as the percent of the original applied dose.

Authors did not conduct statistical analyses between time points for recovery in any of the analyzed samples. Therefore, the following discussion refers only to nominal differences. For both saline solutions, less mass was recovered in the wash and more mass was recovered in the skin and receptor fluid as the exposure time increased. Washing at 1 minute with saline solutions could recover approximately 100% of the applied dose compared to between 80% and 85% of the dose at 30 minutes. Between 0.7% and 2.2% of the applied dose remained in the skin washed at 1 minute versus between 5.1% and 9.8% remaining following washing at 30 minutes. A similar pattern was observed in the receptor fluid, with between 0% to 0.1% recovered in the fluid at 1 minute and between 2.3% and 5.4% recovered at 30 minutes. For skin washed with either saline solution at 3 minutes, the wash, skin and receptor fluid recoveries fell between the respective values observed following washing at 1 and 30 minutes. For tap water, this pattern was observed only when comparing washing at 1 minute to washing at 30 minutes, as the washing recovery dropped from 94% to 85% of applied dose, the amount in the skin increased from 2.2% to 7.5% and the amount in the receptor fluid increased from 0% to 2.3%. However, washing at 3 minutes recovered a higher percent of the total applied dose in the wash (95%) and less in the skin (0.7%) than washing at 1 minute. A small increase was observed in the receptor fluid at 3 minutes (0.1%). When analyzed separately from the rest of the skin, the stratum corneum removed by tape stripping retained more chemical following washing at 30 minutes than washing at

earlier times. The mass of gylphosate recovered by tape stripping was less than that retained in the skin under all conditions. In general, mass balance decreased slightly with increasing times to washing but all conditions had a greater than 94.8% total recovery.

1.B.3. Impact of Washing on Flux

Moody & Ritter (1992)

This study characterized dermal absorption of the herbicide fenoxaprop-ethyl (FPE) while validating *in vitro* against *in vivo* methods. Moody and Ritter utilized radiolabeled FPE in both their *in vivo* and *in vitro* experiments. In the *in vivo* portion of the study, FPE was applied to rats, washed at 24 hours and urine was collected for 10 days. This experimental design was not helpful in this review and therefore not summarized further here.

For the *in vitro* part of this study, authors state that at least part of the experimental design was taken from Moody & Martineau (1990) in lieu of providing a detailed description. Therefore, the explanation of the protocol provided here is based on the assumption that the experimental methods were the same unless otherwise specified. In the *in vitro* part of this study, radiolabeled FPE in 10 µl acetone was applied to 0.2 cm^2 of freshly excised (n = 20) or frozen (n = 19) 600- to 900-µm thick intact skin from the same rats used previously in the *in vivo* trials for a loading of 1.4 $\mu g/cm^2$. Acetone was volatized from the skin via cool air from a fan for five minutes. Frozen skin was stored in liquid nitrogen for 7 or 25 days. Skin was placed on diffusion cells maintained at 37 °C with a constant flow rate of 40 µl/min. Ringer's receptor fluid was collected for a total of 48 hours in 2-hour increments. Skin was washed at 24 hours by rinsing at 5 ml/minute with 5 ml of 1:1 (v:v) Radiac soap and water solution followed by 5 ml distilled water. This washing protocol resulted in a total of 50 ml of wash solution being applied per cm^2 of exposed area at an adjusted flow rate of 25 $ml/(min \cdot cm^2)$. Sample size was four per rat. Data were presented separately for each rat skin donor. Results were presented as the percent of applied dose recovered in the wash, skin, air traps (volatilization) and receptor fluid (skin permeation). Percent of the applied dose recovered in the receptor fluid was also graphed for the fresh and frozen (25 days) skin of one rat over time.

Percent total recoveries averaged 95% for fresh skin and 81% for frozen. Similar percent of the applied dose was recovered for fresh and frozen skin in the wash solution (30% for fresh and 23% for frozen), skin (17% for fresh and 15% for frozen) and receptor fluid (27% for fresh and 25% for frozen).

Percent dose recovered for fresh skin peaked at about 10.5 hours with a maximum flux of 0.016 $\mu g/cm^2/hr$. A nominally similar maximum flux (0.02 $\mu g/cm^2/hr$) was observed for the frozen skin at 12 hours post-exposure. An increase in percent dose recovered post-washing was visually estimated from the graphs for one cell in fresh skin and two cells for frozen skin. The change was much more pronounced and prolonged for the frozen skin, but was still fairly minor compared to the variable percent recovery observed over time in all the cells.

Moody & Nadeau (1993)

This study utilized animal models for the lipophilic compound n,n-diethyl-m-toluamide (DEET) and compared *in vivo* and *in vitro* dermal absorption of the chemical. DEET was applied to rats and hairless guinea pigs *in vivo*, washed at 24 hours and urine and feces were collected for 14 days. This experimental design was not helpful in this review and therefore not summarized further here.

For the *in vitro* part of this study, authors state that at least part of the experimental design was taken from Moody & Martineau (1990) and Moody & Ritter (1992) in lieu of providing a detailed description. Therefore, the explanation of the protocol provided here is based on the assumption that the experimental methods were the same unless otherwise specified. In the *in vitro* part of this study, radiolabeled DEET was applied in 10 µl of acetone to 0.2 cm^2 of mouse, rat, guinea pig, pig or human abdomen skin or to testskin, a cultured human skin product, for loadings of 33.3, 38.7, 12.5, 19.4, 44.7 and 27.9 $\mu g/cm^2$, respectively. Rat and guinea pig skin were excised from an unexposed area on the same animals used in the *in vivo* part of this study following completion of those experiments. Mouse skin was full thickness (500 µm) and testskin was cultured to 300 µm thick. All other skin samples were dermatomed to 500 µm. Testskin was used within 24 hours of arrival from the supplier, pig and human skin were used within 1 hour of excision and the rat, guinea pig and mouse skin

were used immediately following excision to preserve viability. This skin was placed on diffusion cells maintained at 37 °C with a flow rate of 40 µl/minute. Ringer's receptor fluid was collected in 2-hour increments. Air was also passed over the apparatus at a constant rate (12 ml/min). Skin was washed at 24 hours by rinsing at 5 ml/minute with 5 ml of 1:1 (v:v) Radiac soap and water solution followed by 5 ml distilled water. This washing protocol resulted in a total of 50 ml of wash solution being applied per cm^2 of exposed area at an adjusted flow rate of 25 ml/(min·cm^2). Receptor fluid was collected for a total of 48 hours. Sample size was four for each condition. Results were presented as the percent of applied dose recovered in the wash, skin, air traps (volatilization) and receptor fluid (skin permeation). Percent of the applied dose recovered in the receptor fluid was also graphed over time. Data were graphed separately for each diffusion cell.

Total percent recoveries of between 70.0 and 92.7% were obtained in the *in vitro* trials. The different skin types had a range of recoveries in the skin (0.4% human, 0.7% testskin, 2.8% mouse, 13.7% pig, 27.6% guinea pig and 43.5% rat) and receptor fluid (10.9% guinea pig, 13.1% testskin, 15.3% pig, 21.4% rat, 27.7% human and 36.2% mouse).

The penetration at various times was visually estimated from the graphs. Graphs showed an initial peak in penetration following exposure which returned to background levels by about 20 hours for mouse skin, 14 hours for human skin and 10 hours for testskin. Pig, rat and guinea pig skin also had a pronounced initial peak which declined to slightly above background just prior to washing at 24 hours. The magnitude of these initial pre-washing peaks was extremely variable between both skin types and diffusion cells using the same skin. Rat skin was the only type for which reported pre-washing peak flux was similar between all four diffusion cells (3.5%/hr or 1.4 µg/[cm^2·hr]). Other skin types were much more variable, with the largest inter-cell variability observed for mouse (between 1 and 13%/hr [0.34 to 4.3 µg/(cm^2·hr)]), human (between 3.5 and 6.5%/hr [1.6 to 2.9 µg/(cm^2·hr)]) and testskin (between0.5 and 8%/hr [0.1 to 2.2 µg/(cm^2·hr)]).

Following the 24-hour wash, no change in the recovery of compound was observed for mouse or human skin. In rats, a small second peak (<0.5%/hr or <0.19 µg/[cm^2·hr]) was noted for all cells. In guinea pigs, a small second peak (<0.5%/hr or <0.06 µg/[cm^2·hr]) was

seen in only two cells. For pigs, a small second peak appears immediately before the soap and water wash at 22 hours (<0.25%/hr or <0.05 μg/[cm^2·hr]) followed by a very minor third peak (<0.15%/hr or <0.03 μg/[cm^2·hr]). This was observed in all four cells. For testskin, the relative size of the second peak is the largest noted (approximately 1.5%/hr or 0.4 μg/[cm^2·hr]) but only observed in one cell out of four. Recovery in all skin types was nearly undetectable at 48 hours.

Moody & Nadeau (1994)

This study compared *in vivo* and *in vitro* methods and their ability to determine the dermal absorption of diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) as a model for occupational exposure. Diazinon was applied to rats and hairless guinea pigs *in vivo*, washed at 24 hours and urine and feces were collected for 14 days. This experimental design was not helpful in this review and therefore not summarized further here.

For the *in vitro* part of this study, authors state the experimental design was almost identical to Moody & Nadeau (1993) in lieu of providing a detailed description. Therefore, the explanation of the protocol provided here is based on the assumption that the experimental methods were the same unless otherwise specified. Radiolabeled diazinon was applied in 10 μl of acetone to 0.2 cm^2 of rat, guinea pig, pig or human abdomen skin or testskin, a cultured human skin product, for loadings of 16.7, 12.4, 9.5, 10.9 and 9.5 μg/cm^2, respectively. Rat and guinea pig skin were excised from an unexposed area on the same animals used in the *in vivo* part of this study following completion of those experiments. Testskin was cultured to 300 μm thick. All other skin samples were dermatomed to 500 μm. Testskin was used within 24 hours of arrival from the supplier, pig and human skin were used within 1 hour of excision and the rat and guinea pig skin were used immediately following excision to preserve viability. This skin was placed on diffusion cells maintained at 37 °C with a flow rate of 40 μl/minute. Ringer's receptor fluid was collected in 2-hour increments. Air was also passed over the apparatus at a constant rate (12 ml/min). Skin was washed at 24 hours by rinsing at 5 ml/minute with 5 ml of 1:1 (v:v) Radiac soap and water solution followed by 5 ml distilled water. This washing protocol resulted in a total of 50 ml of wash solution

being applied per cm^2 of exposed area at an adjusted flow rate of 25 ml/(min·cm^2). Receptor fluid was collected for a total of 48 hours. Sample size was four for each condition. Results were presented as the percent of applied dose recovered in the wash, skin, air traps (volitilzation) and receptor fluid (skin permeation). Percent of the applied dose recovered in the receptor fluid was also graphed over time for only human skin and testskin. Data were graphed separately for each diffusion cell.

Total percent recoveries of between 87.6 and 94.1% were obtained in the *in vitro* trials. The different skin types had a range of recoveries in the skin (9.2% pig, 13.6% human, 24.2% guinea pig, 27% testskin and 33.5% rat) and receptor fluid (5.8% pig, 6% human, 8.3% guinea pig, 9% testskin and 13.6% rat).

The penetration at various times was visually estimated from the graphs. Graphs showed an initial peak in penetration following exposure of 0.2%/hr (0.02 μg/[cm^2·hr]) for human skin and 0.56%/hr (0.05 μg/[cm^2·hr]) for testskin. By 24 hours, this peak had fallen to about half of its maximal value for human skin and about a third of the value for testskin. A post-wash peak that was approximately equivalent to the pre-wash peak was observed for both skin types. This post-wash peak had higher variability in percent recovery between diffusion cells than the initial peak, with penetration values of between 0.35 and 0.85%/hr (0.034 to 0.08 μg/[cm^2·hr]) for testskin and between 0.12 and 0.25%/hr (0.01 to 0.03 μg/[cm^2·hr]) for human skin. None of the cells for either skin type had returned to background levels at the end of 48 hours. Authors state they observed this post-washing peak in all skin types, including those which were not plotted for this paper, but especially human and testskin.

Moody et al. (1994a)

This study determined the ability of an *in vitro* method to predict *in vivo* dermal absorption of 2,4-dichlorophenoxyacetic acid (2,4-D). 2,4-D was applied *in vivo* to rats and hairless guinea pigs, washed at 24 hours and urine and feces were collected for 14 days. This experimental design was not helpful in this review and therefore not summarized further here.

For the *in vitro* part of this study, authors state that many of their methods are "identical" to Moody & Nadeau (1993) in lieu of providing a detailed description. Therefore, the

explanation of the protocol provided here is based on the assumption that the experimental methods were the same unless otherwise specified. These trials used ADIA or Moody diffusion cells with either Hanks' or Ringer's receptor fluid. Radiolabeled DDT was applied in 10 µl of acetone to 0.2 cm^2 of rat, guinea pig, pig or human skin or testskin, a cultured human skin product, for loadings of 6.9, 7.5, 7.5, 7.7 and 8.4 $\mu g/cm^2$, respectively. Rat and guinea pig skin were excised from an unexposed area on the same animals used in the *in vivo* part of this study following completion of those experiments. Testskin was cultured to 300 µm thick. All other skin samples were dermatomed to 500 µm. Testskin was used within 24 hours of arrival from the supplier. Human and pig skin were used within 1 hour of excision and the rat and guinea pig skin were used immediately following excision to preserve viability. Skin was placed on diffusion cells maintained at 37 °C with a flow rate of 40 µl/minute. Receptor fluid was collected in 2-hour increments. Ringer's receptor fluid was used with the pig skin samples while all other cells contained Hanks' receptor fluid in order to better maintain tissue viability. Air was also passed over the apparatus at a constant rate (12 ml/min). Skin was washed at 24 hours by rinsing at 5 ml/minute with 5 ml of 1:1 (v:v) Radiac soap and water solution followed by 5 ml distilled water. This washing protocol resulted in a total of 50 ml of wash solution being applied per cm^2 of exposed area at an adjusted flow rate of 25 $ml/(min \cdot cm^2)$. Receptor fluid was collected for a total of 48 hours. Sample size was four for each condition. Results were presented as the percent of applied dose recovered in the wash, skin, air traps (volitilzation) and receptor fluid (skin permeation). Percent applied dose recovered in the receptor fluid for human and rat skin was also graphed over time. Data were graphed separately for each diffusion cell.

Fairly high total percent recoveries of between 91.5 and 97.5% were obtained *in vitro*. The different skin types had a range of recoveries in the skin (7.4% pig, 12% testskin, 12.2% guinea pig, 17.9% human and 28.2% rat) and receptor fluid (1.2% human, 1.4% guinea pig, 6.1% pig, 11.1% rat and 35.4% testskin).

Graphs were only provided for rat and human skin and showed an initial peak in penetration following exposure which returned to slightly above background by 24 hours. The penetration at various times was visually estimated from the graphs. The pre-wash peak in human skin was about 0.15%/hr (0.01 $\mu g/[cm^2 \cdot hr]$). Three of the cells with rat skin had pre-

wash peak recoveries of about 0.38%/hr (0.03 μg/[cm^2·hr]) while the fourth cell had a peak recovery of 1%/hr (0.07 μg/[cm^2·hr]). The post wash peak (between 0.75%/hr and 1.38%/hr or 0.05 to 0.096 μg/[cm^2·hr]) was higher than the first peak for all but one rat cell while the post wash peak in human skin was very minor (<0.05%/hr or <0.004 μg/[cm^2·hr]). Authors state they observed the post-wash peak in all skin types but especially rat. Following the post-wash peak, the human skin cells returned to background levels by 48 hours while three of the rat skin cells maintained an elevated percent recovery of about 0.25%/hr (0.02 μg/[cm^2·hr]) for the remainder of the trial. The fourth rat cell dropped back to almost background levels by 48 hours.

Moody et al. (1994b)

This study determined the validity of using two different *in vitro* flow-through diffusion cells to approximate *in vivo* dermal absorption of 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT). DDT was applied to rats and hairless guinea pigs *in vivo*, washed at 24 hours and urine and feces were collected for 14 days. This experimental design was not helpful in this review and therefore not summarized further here.

For the *in vitro* part of this study, authors state that many of their methods are "identical" to prior studies (Moody & Martineau 1990, Collier & Bronaugh 1991, Moody & Nadeau 1993) in lieu of providing a detailed description. Therefore, the explanation of the protocol provided here is based on the assumption that the experimental methods were the same unless otherwise specified. Two diffusion cell apparatuses were used. One was the ADIA or Moody diffusion cell described in Moody & Nadeau (1993). In this setup, radiolabeled DDT was applied in 10 μl of acetone to 0.2 cm^2 of pig skin or testskin, a cultured human skin product, for loadings of 23.2 and 16.2 μg/cm^2, respectively. Testskin was cultured to 300 μm thick. Pig skin samples were dermatomed to 500 μm. Testskin was used within 24 hours of arrival from the supplier while pig skin was used within 1 hour of excision. This skin was placed on diffusion cells maintained at 37 °C with a flow rate of 2.5 ml/hr (similar to the 40 μl/minute used in the ADIA cells in prior Moody et al. experiments). Ringer's receptor fluid was collected in 2-hour increments. Air was also passed over the apparatus at a constant rate (12 ml/min). Skin was washed at 24 hours by rinsing at 5 ml/minute with 5 ml of 1:1 (v:v)

Radiac soap and water solution followed by 5 ml distilled water. This washing protocol resulted in a total of 50 ml of wash solution being applied per cm^2 of exposed area at an adjusted flow rate of 25 ml/(min·cm^2). Receptor fluid was collected for a total of 48 hours.

Alternatively, skin was placed on a Bronaugh *in vitro* diffusion cell. In this setup, the same volume of radiolabeled DDT was applied to 0.64 cm^2 of rat, guinea pig, pig, human skin and testskin for loadings of 23, 27.1, 19.9, 26.3 and 22.3 μg/cm^2, respectively. Pig and testskin thickness and preparation were the same as described above for the Moody cells. Rat and guinea pig skin were excised from an unexposed area on the same animals used in the *in vivo* part of this study following completion of those experiments. All other skin samples were dermatomed to 500 μm. Human skin was used within one hour of excision and the rat and guinea pig skin were used immediately following excision to preserve viability. Skin was then placed on diffusion cells maintained at 32 °C with a flow rate of 1.5 ml/hr. Hanks' receptor fluid was collected in 2-hour increments. Information about air flow was not provided and volatilization was not measured. Skin was washed using the same solutions and cotton tipped swabs. This washing protocol resulted in a total of 15.6 ml of wash solution being applied per cm^2 of exposed area. Further information regarding the washing method was not discernable from this or cited studies. Receptor fluid was collected for a total of 48 hours.

Sample size was four for each condition. Results were presented as the percent of applied dose recovered in the wash, skin, air traps (volitilzation for Moody/ADIA apparatus only) and receptor fluid (skin permeation). Percent of the applied dose recovered in the receptor fluid for human and rat skin on the Bronaugh diffusion cells was also graphed over time. Data were graphed separately for each diffusion cell.

No statistical analysis was presented for this study. Total percent recoveries of between 75.3 and 100.8% were obtained in the *in vitro* trials. For the Bronaugh cells, the different skin types had a range of recoveries in the skin (13.2% pig, 21.7% testskin, 27.1% human, 33.6% rat and 39% guinea pig) and receptor fluid (0.3% testskin, 0.4% rat, 0.5% human, 1.1% pig and 2.6% guinea pig). For the Moody cells, the recoveries were nominally higher in the skin

(17.4% pig and 27.7% testskin) and nominally lower in the receptor fluid (0.2% testskin and 0.2% pig) than the same skin type on the Bronaugh cell.

Graphs were only provided for rat and human skin on Bronaugh cells with no further information provided in the text regarding the flux over time for the other trials. The flux at various times was visually estimated from the graphs. Neither of the presented graphs had a pronounced initial peak in penetration following exposure. Instead both skin types rapidly reached a plateau of about 0.005%/hr (about 0.001 $\mu g/[cm^2 \cdot hr]$ for both rat and human skin) for the first 24 hours. A post wash peak was observed for both skin types. The peak percent recovery was between 0.05 and 0.075%/hr (0.01 to 0.02 $\mu g/[cm^2 \cdot hr]$) for rat skin and between 0.02 and 0.03%/hr (0.005 to 0.009 $\mu g/[cm^2 \cdot hr]$) for human skin. Following the post-wash peak, two of the human skin cells maintained an elevated percent recovery of between about 0.018%/hr and 0.028%/hr (0.005 to 0.007 $\mu g/[cm^2 \cdot hr]$) for the remainder of the trial. The rest of the cells in both skin types dropped back to the original plateau levels of around 0.005%/hr (0.001 $\mu g/[cm^2 \cdot hr]$) for the rest of the 48 hours.

Moody et al. (1995a)

This study compared the dermal absorption between *in vivo* and *in vitro* studies for n,n-diethyl-m-toluamide (DEET) in three different commercial formulas. DEET was applied to rats *in vivo*, washed at 24 hours and urine and feces were collected for 7 days. This experimental design was not helpful in this review and therefore not summarized further here.

For the *in vitro* part of this study, authors stated that many of their methods are "identical" to several previous studies in lieu of providing a detailed description. Therefore, the explanation of the protocol provided here is based on the assumption that the experimental methods were the same unless otherwise specified. These trials used Bronaugh diffusion cells with Hanks' receptor fluid. Radiolabeled compound was applied in 50 µl of commercial formula to 0.64 cm^2 of rat, guinea pig or human abdomen skin. Commercial formulas originally contained 14% DEET in OFF!, 24% DEET in DEEP WOODS or 95% DEET in Muskol. Additional radiolabeled compound was applied to these over-the-counter concentrations. This resulted in loadings of 14.5, 26.2 and 95.5 $\mu g/cm^2$ on rat skin, 12.5,

22.1 and 84.9 μg/cm^2 on guinea pig skin and 16.7, 25.3 and 97.3 μg/cm^2 on human skin for OFF!, DEEP WOODS and Muskol formulations, respectively. Rat skin was excised from an unexposed area on the same animals used in the *in vivo* part of this study following completion of those experiments. All skin samples were dermatomed to 300 μm. Human skin was used within two hours of excision and the rat and guinea pig skin were used immediately following excision to preserve viability. Skin was placed on diffusion cells maintained at 32 °C and a flow rate of 1.5 ml/hr. Receptor fluid was collected in 2-hour increments. Information regarding air flow was not provided. Skin was washed at 24 hours using cotton tipped swabs and 5 ml of 1:1 (v:v) Radiac soap and water solution followed by 5 ml distilled water. This washing protocol resulted in a total of 15.6 ml of wash solution being applied per cm^2 of exposed area. Further information regarding the washing method was not discernable from this or cited studies. Receptor fluid was collected for a total of 48 hours. Sample size was four for each condition. Results were presented as the percent of applied dose recovered in the wash, skin and receptor fluid (skin permeation). Mean mass per exposed area recovered in the receptor fluid for all skin types was also graphed over time.

High total percent recoveries of between 93.4 and 104% were obtained in the *in vitro* trials. The different skin types had a range of recoveries in the skin (between 13.8 and 40.5% for human, 3.4 and 8.2% for guinea pig and 12.8 and 21.6% for rat skin) and receptor fluid (between 2.8 and 11.7% for human, 10.5 and 42.9% for guinea pig and 28.2 and 32.6% for rat skin).

The flux at various times was visually estimated from the graphs. In the rat skin cells, a small pre-washing peak or plateau was observed prior to a pronounced post-washing peak for all dosing formulations. In the 95% DEET test, the permeation remains elevated at 48 hours to about one third of the peak value. The maximum flux estimated for any formulation applied to rat skin was about 1.6 mg/(cm^2·hr). Guinea pig skin appears to plateau at about one third of observed peak levels prior to washing. Peak recovery in the receptor fluid was observed post-washing at 26 hours, after which permeation drops to less than half of the pre-wash recovery levels. The maximum flux estimated for any formulation applied to guinea pig skin was about 0.6 mg/(cm^2·hr). Human tests show a small first peak followed by a

large post-washing peak and elevated levels post-wash. The maximum flux estimated for any formulation applied to human skin was about 0.3 mg/(cm^2·hr).

Moody et al. (1995b)

This study compared the dermal absorption between *in vivo* and *in vitro* studies for benzo[a]pyrene (b[a]p). B[a]p was applied to rats and hairless guinea pigs *in vivo*, washed at 24 hours and urine and feces were collected for 14 days. This experimental design was not helpful in this review and therefore not summarized further here.

For the *in vitro* part of this study, authors state that many of their methods are "identical" to several previous studies in lieu of providing a detailed description. Therefore, the explanation of the protocol provided here is based on the assumption that the experimental methods were the same unless otherwise specified. These trials used Bronaugh diffusion cells with Hanks' receptor fluid. Radiolabeled b[a]p was applied in 10 µl of acetone to 0.64 cm^2 of rat, guinea pig, 32-year-old human abdominal skin, 50-year-old human abdominal skin or testskin, a cultured human skin product, for loadings of 11, 13.3, 9.8, 8.4 and 9.1 µg/cm^2, respectively. Rat and guinea pig skin were excised from an unexposed area on the same animals used in the *in vivo* part of this study following completion of those experiments Testskin was cultured to 300 µm thick. All other skin samples were dermatomed to 500 µm. Testskin was used within 24 hours of arrival from the supplier. Human skin was used within 1 hour of excision and the rat and guinea pig skin were used immediately following excision to preserve viability. Skin was placed on diffusion cells maintained at 32 °C with a flow rate of 1.5 ml/hr. Receptor fluid was collected in 2-hour increments. Information regarding air flow was not provided. Skin was washed at 24 hours using cotton tipped swabs and 5 ml of 1:1 (v:v) Radiac soap and water solution followed by 5 ml distilled water. This washing protocol resulted in a total of 15.6 ml of wash solution being applied per cm^2 of exposed area. Further information regarding the washing method was not discernable from this or cited studies. Receptor fluid was collected for a total of 48 hours. Sample size was four for each condition. Results were presented as the percent of applied dose recovered in the wash, skin and receptor fluid (skin permeation). Percent applied dose recovered in the receptor

fluid for all skin types was also graphed over time. Data were graphed separately for each diffusion cell.

High total percent recoveries of between 97.4 and 102.1% were obtained in the *in vitro* trials. The different skin types had a range of recoveries in the skin (20.1% 32-year-old human, 23.1% guinea pig, 34.1% testskin, 42.5% 50-year-old human and 44% rat) and receptor fluid (0.2% testskin, 1.5% 50-year-old human, 3.3% 32-year-old human, 27.5% guinea pig and 51.1% rat).

The penetration at various times was visually estimated from the graphs. Graphs showed a large initial peak in penetration (around 3.5%/hr or 0.39 $\mu g/[cm^2 \cdot hr]$) followed by a smaller post-washing peak (around 1.8%/hr or 0.2 $\mu g/[cm^2 \cdot hr]$) for rat skin. Cells had returned to less than 0.5%/hr (0.06 $\mu g/[cm^2 \cdot hr]$) recovery by 48 hours. In guinea pig skin, a slow increasing percent recovery to about 0.75%/hr (0.1 $\mu g/[cm^2 \cdot hr]$) prior to washing was observed along with a post wash peak of about 3%/hr (0.4 $\mu g/[cm^2 \cdot hr]$) which declined to less than 0.25%/hr (0.03 $\mu g/[cm^2 \cdot hr]$) by 48 hours. Thirty-two-year-old human skin also had a slowly increasing percent recovery to <0.1%/hr (<0.01 $\mu g/[cm^2 \cdot hr]$) prior to washing and a post-wash peak of about 0.13%/hr (0.013 $\mu g/[cm^2 \cdot hr]$) for two cells and 0.26%/hr (0.025 $\mu g/[cm^2 \cdot hr]$) for the other two. Percent recoveries for all four cells remained slightly elevated at 48 hours, with values of about 0.1%/hr (0.01 $\mu g/[cm^2 \cdot hr]$). Fifty-year-old human skin showed extremely variable percent recovery over time, partially due to the very low penetration of b[a]p into the skin (<0.13%/hr or 0.01 $\mu g/[cm^2 \cdot hr]$ at the highest peak). Overall, it showed a general pattern of increasing over time for all cells. Testskin also had recoveries that were very low but more uniform between cells, with a small initial peak of about 0.0038%/hr (0.0003 $\mu g/[cm^2 \cdot hr]$) which then remained at a plateau of between 0.0013 and 0.0038%/hr (0.0001 to 0.0003 $\mu g/[cm^2 \cdot hr]$) until 24 hours. A post-wash peak of about 0.008%/hr (0.0007 $\mu g/[cm^2 \cdot hr]$) was observed in all cells. None of the cells returned to background levels post-wash, with some percent recoveries continuing to increase up to 48 hours.

Moody & Nadeau (1997)

This study compared the dermal absorption between *in vivo* and *in vitro* trials for 2,4-dichlorophenoxacetic acid dimethyl amine (2,4-D amine) in two commercial formulas. 2,4-D amine was applied to rats *in vivo*, washed at 24 hours and urine and feces collected for 7 days. This experimental design was not helpful in this review and therefore not summarized further here.

For the *in vitro* part of the study, authors stated that many of their methods are "identical" to several previous studies in lieu of providing a detailed description. Therefore, the explanation of the protocol provided here is based on the assumption that the experimental methods were the same unless otherwise specified. These trials used Bronaugh diffusion cells with Hanks' receptor fluid. Radiolabeled 2,4-D amine was applied in 50 µl of the commercial formulas Clean Crop (CC) or Wilbur-Ellis (WE) to 0.64 cm^2 of rat, guinea pig or human abdomen skin. This resulted in loadings of 760 and 771 µg/cm^2 on rat skin, 760 and 760 µg/cm^2 on guinea pig skin and 760 and 756 µg/cm^2 on human skin for CC and WE formulations, respectively. Rat skin was excised from an unexposed area on the same animals used in the *in vivo* part of this study following completion of those experiments. All skin samples were dermatomed to 300 µm. Human skin was used within two hours of excision and the rat and guinea pig skin were used immediately following excision to preserve viability. Skin was placed on diffusion cells maintained at 32 °C with a flow rate of 1.5 ml/hr. Receptor fluid was collected in 2-hour increments. Information regarding air flow was not provided. Skin was washed at 24 hours using cotton tipped swabs and 5 ml of 1:1 (v:v) Radiac soap and water solution followed by 5 ml distilled water. This washing protocol resulted in a total of 15.6 ml of wash solution being applied per cm^2 of exposed area. Further information regarding the washing method was not discernable from this or cited studies. Receptor fluid was collected for a total of 48 hours. Sample size was four for each condition. Results were presented as the percent of applied dose recovered in the wash, skin and receptor fluid (skin permeation). Mean mass per exposed area recovered in the receptor fluid for all skin types was also graphed over time.

High total percent recoveries of between 94.6 and 100.3% were obtained in the *in vitro* trials. The different skin types had a range of recoveries in the skin (between 14.8 and 17% for human, 10.1 and 13.6% for guinea pig and 19.9 and 21.8% for rat skin) and receptor fluid (between 10.3 and 10.9% for human, 5.9 and 7.5% for guinea pig and 5.8 and 8.3% for rat skin).

As visually estimated from the graphs, there appears to be a small pre-washing peak in the rat skin cells that is about one sixth of the average maximal post-washing peak. Both dosing formulations resulted in a pronounced post-washing peak for rat skin of about 6 $\mu g/(cm^2 \cdot hr)$ for the WE formulation and 15 $\mu g/(cm^2 \cdot hr)$ for the CC formulation. The permeation returns to nearly background levels almost immediately following the post-wash peak. Guinea pig skin demonstrated a pre-wash peak for both application formulations that was similar in size to the post-washing peak with elevated levels recovered in the receptor fluid until 48 hours. Peak recovery in the receptor fluid was about 3.5 $\mu g/(cm^2 \cdot hr)$ for the WE formulation and 4.5 $\mu g/(cm^2 \cdot hr)$ for the CC formulation. Human tests show a large pre-washing peak of about 7.5 $\mu g/(cm^2 \cdot hr)$ for the WE formulation and 9 $\mu g/(cm^2 \cdot hr)$ for the CC formulation followed by a smaller second, post-washing peak (about 5 $\mu g/(cm^2 \cdot hr)$ for both vehicles) and elevated levels post-wash falling to about 1 $\mu g/(cm^2 \cdot hr)$ at 48 hours.

Yourick et al. (2004)

This study characterized the dermal absorption and metabolism of the cosmetic ingredients, dihydroyacetone (DHA) and 1,4,5,8-tetraaminoanthraquinone (also known as disperse blue 1 or DB1) and the industrial chemical, 7-(2H-naphtho[1,2-d]triazol-2-yl)-3-phenylcoumarin (7NTPC) using both the standard 24-hour and extended *in vitro* trials. Trials for DHA and 7NTPC were washed and ended at 24 hours only and therefore are not summarized further here.

For DB1, a mixture of non-radiolabeled and radiolabeled compound was applied in either 9.6 µl of ethanol or 5 mg hair dye formulation to 0.64 cm^2 of either fuzzy rat or human abdominal skin such that each vehicle contained 0.52% DB1. This percentage was assumed to be 0.52% of the total volume for ethanol and 0.52% of the total weight for the hair dye formulation based on how authors presented the application amounts of the vehicles. Using

these assumptions, an approximate loading of 122 μg/cm^2 was calculated for skin samples dosed with ethanol while a loading of 41μg/cm^2 was estimated for samples dosed with the hair dye. Rat skin was excised, clipped to remove hair, dermatomed to between 250 and 350 μm thick and then placed on the flow-through diffusion cell apparatus with a flow rate of 1.5 mL/hr. Human skin was dermatomed to between 200 and 280 μm thick immediately following surgical removal and then placed on the same apparatus. Receptor fluid was maintained at 32 °C and collected in 6-hour increments except for the first two 3-hour increments. Skin was washed at 30 minutes and the trial ended at 24 hours for DB1 applied in hair dye. The washing time for DB1 applied in ethanol was not stated clearly by the authors and may have been at 30 minutes or 24 hours. Trials with DB1 applied in ethanol ended at 24 hours. In the extended trial, rat skin was washed at 24 hours and the trial ended at 72 hours, but the vehicle and loading are uncertain. To wash, 0.1 ml of 10% v:v liquid detergent solution was applied and rubbed with cotton swabs. This step was repeated 3 times. Then skin was rinsed twice with 0.2 mL of distilled water. This washing protocol resulted in a total of 1.1 ml of wash solution being applied per cm^2 of exposed area. Skin was tape stripped at the end of each trial to determine the compound present in the stratum corneum. Sample size was unclear but potentially between 2 and 20 per condition. Results were presented as graphs of percent dose recovered in the receptor fluid over time as well as mean and standard error (SE) of the percent applied dose at the termination of the trial in the receptor fluid, skin and wash for the 24 hour trials.

At 24 hours, a significantly larger fraction of applied compound ($p<0.05$) was recovered in the combined skin and receptor fluid when applied in ethanol (9.5% total for rats and 11.3% for humans) than in hair dye (approximately 3% total for either skin). The majority of this was recovered in the skin, with less than 0.7% recovered in the receptor fluid for any skin/vehicle combination. The extended trials showed a maximum penetration (about 0.015%/hr) occurred in the first 3-hour collection time. Penetration declined from there until about 24 hours. From 24 to 72 hours, approximately 0.02 percent was recovered in each 6-hour increment for a penetration of 0.003%/hr. Flux cannot be calculated for this example due to the lack of clear loading information.

Hughes & Edwards (2010)

This *in vitro* study characterized the dermal absorption of the insecticides bifenthrin, deltamethrin and permethrin in rat and human skin. The majority of the trials in this study were washed and ended at 24 hours. These do not provide relevant information for this review and are not summarized further here. One hundred nmol of radiolabeled compound was applied to 0.32 cm^2 intact skin in 15 µl acetone, which resulted in a loading of 132.2 $\mu g/cm^2$ for bifenthrin, 157.9 $\mu g/cm^2$ for deltamethrin and 122.3 $\mu g/cm^2$ for permethrin. Rat skin was clipped to remove hair 24 hours prior to excision. It was dermatomed to 350 µm thick and used immediately. Human cadaver skin was an average of 360 µm thick and stored at -70 °C for an unspecified length of time prior to use. Skin was placed on a flow-through diffusion cell and allowed to equilibrate for 30 minutes prior to each trial. Each cell had a receptor fluid flow rate of 1.5 ml/hr and was maintained at 35 °C. Receptor fluid increments were collected at approximately 4-hour intervals over the 48-hour course of the trial. At 24 hours, samples were washed 6 times in the intact diffusion cell by applying and removing each 0.5 ml aliquot of 1:1 soap and water solution 20 times. This resulted in a total of 9.4 ml of wash solution being applied per cm^2 of exposed area. The sample size was 8 for rat and 3 for human skin trials. Recoveries were presented as a graph of cumulative percent dose in the receptor fluid over time as well as mean cumulative percent recovered at 24 and 48 hours. Recoveries in the skin and wash for these trials were not reported, nor was the mass balance.

For human skin, 0.6% of bifenthrin, 0.5% of deltamethrin and 1% of permethrin was recovered in the first 24 hours pre-wash while an additional 0.4% of bifenthrin, 0.5% of deltamethrin and 0.4% of permethrin was recovered in the second 24 hours. A similar recovery of compound in the pre- and post-wash receptor fluid was observed in rat skin for bifenthrin (1%), deltamethrin (2%) and permethrin (3%). The graphs show a very small increase, as estimated visually, in the slope following washing for all trials except permethrin on human skin. This trend is more obvious in rat versus human skin and is not quantified by data in the paper.

Selected experimental parameters from the relevant trials within these reviewed studies are summarized for clarity in tables 1, 2, 3 and 4.

Table 1a. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Both Total Mass Absorbed and Flux.

Citation	Chemical Tested	Skin Type	Skin Thickness (μm)	Skin Storage		Diffusion Cell Apparatus					
				Store Temp (°C)	Duration (days)	Receptor Fluid	Set-up Temp (°C)	Static / Flow-through	Cell Vol (ml)	Flow Rate (ml/hr)	Skin removed for Wash?
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed* and *Flux*											
Loke et al. (1999)	Diethyl malonate	Human	600	-30	<31	Saline	32	Flow	0.32	8	No
Monteiro-Riviere et al. (2001)	Phenol	Swine	NR	n/a	Fresh[1]	NR	NR	Flow	NR	NR	Yes
Nielsen (2010)	Multiple[2]	Human	600-1000	-20	<365	Saline & BSA	32	Static	10	Stir + Sample	No

Table 1b. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Both Total Mass Absorbed and Flux.

Citation	Chemical Tested	Skin Type	Load of Chemical					Time on Cell Pre-Trial (min)	Time to End (min)	Time to Wash (min)	Washing Solution	Mechanism of Washing
			Loading (μg/cm^2)	Skin Area (cm^2)	Vehicle	Vehicle Vol (μl)	Vehicle Loading (μl/cm^2)					
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed* and *Flux*												
Loke et al. (1999)	Diethyl malonate	Human	5250‡	0.8	None	4	5‡	5	1440 1440	n/a 15,30,60	None (Control) 2% SDS	None Rinse 1 min w 0.5L
Monteiro-Riviere et al. (2001)	Phenol	Swine	16814‡[3]	2.8	Water	4001	143‡[3]	1	480 480	n/a 0	None Water, Water or PEG 400 or IPA	None Rinse[4]
Nielsen (2010)	Multiple[2]	Human	100-200	2.12	Water w NaCl and Ethanol	106	50‡	1080	2880 2880	n/a 360	None Hand soap, Water	None Wipe, Rinse 2x

Table 1c. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Both Total Mass Absorbed and Flux

Citation	Chemical Tested	Skin Type	Length of Washing (min)	Vol of Wash Sol'n / area (ml/cm^2)	Flow Rate of Sol'n / area ($ml/min \cdot cm^2$)	Washing Solution (repeated)	Mechanism of Washing (repeated)
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed* and *Flux*							
Loke et al. (1999)	Diethyl malonate	Human	n/a 1	n/a 625	n/a 625	None (Control) 2% SDS	None Rinse 1 min w 0.5L
Monteiro-Riviere et al. (2001)	Phenol	Swine	n/a 15	n/a 3,110	n/a Alternate 36 and 360	None Water, Water or PEG 400 or IPA	None Rinse[4]
Nielsen (2010)	Multiple[2]	Human	n/a NR	n/a NR	n/a NR	None Hand soap, Water	None Wipe, Rinse 2x

BSA = bovine serum albumin; IPA = isopropanol; n/a = not applicable; NR = not reported by authors; PEG = polyethylene glycol; SDS = sodium dodecyl sulphate; ‡ Calculated from data provided in the relevant study; [1] Authors stated skin was excised immediately prior to use; [2] Multiple = Glyphosate, Caffeine, Benzoic Acid, Malathion; [3] Compound was applied via gauze soaked in the application solution, therefore this is a maximal loading and vehicle amount. The majority of the compound likely stayed in the gauze.; [4] Rinse = rinsed for 15 minutes alternating every 1 minute between water at 1 L/min and alternate solution at 100 ml/min

Table 2a. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Total Mass Absorbed

Citation	Chemical Tested	Skin Type	Skin Thickness (μm)	Skin Storage		Diffusion Cell Apparatus					
				Store Temp (°C)	Duration (days)	Receptor Fluid	Set-up Temp (°C)	Static / Flow-through	Cell Vol (ml)	Flow Rate (ml/hr)	Skin removed for Wash?
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed*											
Nitsche et al. (1984)	Lindane	Human (intact or damaged)	>2mm[1]	NR	NR	Saline	32	Static	NR	NR	Yes
Wester et al. (1990b)	DDT, B[a]p	Human	500	4	<5	Human Plasma	37	Flow	3	3	Yes[2]
Weber et al. (1992)	TCDD	Human (intact or damaged)	About 1000	-20	NR	Saline Soaked Gauze	32	n/a	n/a	n/a	Yes
Hewitt et al. (1995)	MbOCA, MDA	Human, Rat	Full[3]	n/a	Fresh[4]	Hanks	32	Flow	0.13	1.5	No
Nielsen et al. (2004)	Multiple[5]	Human	600-900	-20	<365	Saline & BSA	32	Static	10	Stir + Sample	No
Zhai et al. (2007)	Formalde-hyde	Human	500	4	<5	Saline	NR	Static	6	NR	No
Zhai et al. (2008)	Glyphosate	Human	500	4	<5	Saline	NR	Static	6	NR	No

Table 2b. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Total Mass Absorbed

Citation	Chemical Tested	Skin Type	Load of Chemical					Time on Cell Pre-Trial (min)	Time to End (min)	Time to Wash (min)	Washing Solution	Mechanism of Washing
			Loading ($\mu g/cm^2$)	Skin Area (cm^2)	Vehicle	Vehicle Vol (µl)	Vehicle Loading ($\mu l/cm^2$)					
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed*												
Nitsche et al. (1984)	Lindane	Human (intact or damaged)	7.5‡	2	Jacutin ® emulsion	5	3‡	NR	600 600	n/a 180	None Soap and Water	Rub[6] Wash[6]
Wester et al. (1990b)	DDT, B[a]p	Human	0.4, 0.49‡	1	Acetone	5	5‡	NR	1440 25	1440 25	Ivory Liquid Soap 1x, Water 2x	Wash
Weber et al. (1992)	TCDD	Human (intact or damaged)	0.65	3.8	Acetone	40	11‡	NR	300	300 100 100 100 100	None (Control) None None Acetone Water and Soap	Wipe (control)[7] Wipe[7] Wipe, oil, wipe[7] Wipe, oil, wipe[7] Wipe, wipe[7]
Hewitt et al. (1995)	MbOCA, MDA	Human, Rat	9.7 – 21.6	0.32	Ethanol	5	16‡	30	4320	3 - 360 4320	Ethanol Ethanol (Control)	Rub w/ swabs for 10 sec 3x except control rub 2x
Nielsen et al. (2004)	Multiple[5]	Human	12.7 - 64.9‡	2.12	Water w NaCl and Ethanol	600	283‡	1080	2880	360, 1440	NR	"Washed"
Zhai et al. (2007)	Formalde-hyde	Human	0.083‡	3	Water	10	3‡	NR	1,3,30	1,3,30	Water 0.9% Saline 1.8% Saline	"Rinse"
Zhai et al. (2008)	Glyphosate	Human	53.17‡	3	Water	10	3‡	NR	1,3,30	1,3,30	Water 0.9% Saline 1.8% Saline	Rub w/ teflon scrubber, Pipette off 3x

Table 2c. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Total Mass Absorbed

Citation	Chemical Tested	Skin Type	Length of Washing (min)	Vol of Wash Sol'n / area (ml/cm^2)	Flow Rate of Sol'n / area ($ml/min \cdot cm^2$)	Washing Solution (repeated)	Mechanism of Washing (repeated)
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed*							
Nitsche et al. (1984)	Lindane	Human (intact or damaged)	n/a NR	n/a NR	n/a n/a[8]	None Soap and Water	Rub[6] Wash[6]
Wester et al. (1990b)	DDT, B[a]p	Human	NR	3	NR	Ivory Liquid Soap 1x, Water 2x	Wash
Weber et al. (1992)	TCDD	Human (intact or damaged)	n/a n/a 10 NR NR	n/a n/a 0.05 NR NR	n/a n/a n/a[8] n/a[8] n/a[8]	None (Control) None None Acetone Water and Soap	Wipe (control)[7] Wipe[7] Wipe, oil, wipe[7] Wipe, oil, wipe[7] Wipe, wipe[7]
Hewitt et al. (1995)	MbOCA, MDA	Human, Rat	$^{10}/_{60}$	NR	n/a[8]	Ethanol Ethanol (Control)	Rub w/ swabs for 10 sec 3x except control rub 2x
Nielsen et al. (2004)	Multiple[5]	Human	NR	NR	NR	NR	"Washed"
Zhai et al. (2007)	Formalde-hyde	Human	NR	4	NR	Water 0.9% Saline 1.8% Saline	"Rinse"
Zhai et al. (2008)	Glyphosate	Human	0.75	4	n/a[9]	Water 0.9% Saline 1.8% Saline	Rub w/ teflon scrubber, Pipette off 3x

b[a]p = benzo[a]pyrene; BSA = bovine serum albumin; DDT = dichlorodiphenyltrichloroethane; MbOCA = 4,4'-methylenebis[2-chloroaniline]; MDA = 4,4'-methylene-dianiline; n/a = not applicable; NR = not reported by authors; TCDD = 2,3,7,8-tetrachloro-dibenzo-p-dioxin; ‡ Calculated from data provided in the relevant study; [1] Authors stated they used full thickness skin with 1 to 2 mm of subcutaneous fat attached; [2] Removed at termination of trial; [3] Authors stated they used full thickness skin; [4] Authors stated skin was excised immediately prior to use; [5] Multiple = Prochloraz, Methiocarb, Paclobutrazol, Pirimicarb, Dimethoate; [6] None = Rubbed with dry cotton at termination of trial, Wash = Rubbed 10 sec w/ solution on cotton. For samples washed with soap and water, additional rubbing with water on cotton occurred after the initial rubbing with soap solution; [7] All samples were first wiped 3x with each of 4 dry cotton balls. The samples marked oil had gauze soaked in 200 ul of mineral oil placed on them for 10 minutes. The samples marked wipe a second time were wiped again 3x with each of 4 cotton balls that were either dry or soaked in the indicated wash solution (and then rubbed in soap, in the case of the water and soap wash solution); [8] Washing solution was applied with soaked gauze; [9] Washing solution was applied and/or removed via pipette

Table 3a. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Flux

Citation	Chemical Tested	Skin Type	Skin Thickness (µm)	Skin Storage		Diffusion Cell Apparatus					
				Store Temp (°C)	Duration (days)	Receptor Fluid	Set-up Temp (°C)	Static / Flow-through	Cell Vol (ml)	Flow Rate (ml/hr)	Skin removed for Wash?
Potentially Provides Information for the Evaluation of the Effect of Washing on *Flux*											
Moody & Ritter (1992)	Fenoxaprop-ethyl	Rat	600-900	n/a -196‡	Fresh[1] 7-25	Ringer	37	Flow	0.09	2.4	No
Moody & Nadeau (1993)	DEET	Mouse Rat Guinea Pig Pig Human Testskin	500 500 500 500 500 300	NR	Fresh[1] Fresh[1] <0.04 <0.04 <0.04 >1	Ringer	37	Flow	0.09	2.4	No
Moody et al. (1994a)	2,4-D	Rat Human	500 500	NR	Fresh[1] <0.04	Hanks & BSA	37	Flow	0.09	2.4	No
Moody et al. (1994b)	DDT	Rat Human	500 500	NR	Fresh[1] <0.04	Hanks & BSA	37	Flow	0.4	1.5	No
Moody & Nadeau (1994)	Diazinon	Human Testskin	500 300	NR	<0.04 >1	Ringer	37	Flow	0.09	2.4	No
Moody et al. (1995a)	DEET	Rat Guinea Pig Human	300	NR	Fresh[1] Fresh[1] <0.08	Hanks & BSA	32	Flow	0.4	1.5	No
Moody et al. (1995b)	B[a]p	Rat Guinea Pig Human 32 yrs Human 50 yrs Testskin	500 500 500 500 300	NR	Fresh[1] Fresh[1] <0.04 <0.04 >1	Hanks & BSA	32	Flow	0.4	1.5	No
Moody & Nadeau (1997)	2,4-D Amine	Rat Guinea Pig Human	300	NR	Fresh[1] Fresh[1] <0.08	Hanks & BSA	32	Flow	0.4	1.5	No
Yourick et al. (2004)	Disperse blue 1	Fuzzy Rat	250-300	n/a	Fresh[1]	HHBSS & BSA	32	Flow	0.4	1.5	No
Hughes & Edwards (2010)	Multiple[2]	Rat Human	350 360	n/a -20	Fresh[1] NR	Hanks & BSA	35	Flow	0.4	1.5	No

Table 3b. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Flux

Citation	Chemical Tested	Skin Type	Load of Chemical					Time on Cell Pre-Trial (min)	Time to End (min)	Time to Wash (min)	Washing Solution	Mechanism of Washing
			Loading ($\mu g/cm^2$)	Skin Exposed (cm^2)	Vehicle	Vehicle Vol (μl)	Vehicle Loading ($\mu l/cm^2$)					
Potentially Provides Information for the Evaluation of the Effect of Washing on *Flux*												
Moody & Ritter (1992)	Fenoxa-propethyl	Rat	1.4	0.2	Acetone	10	50‡	NR	2880	1440	Soap and Water; Water[3]	Rinse[4]
Moody & Nadeau (1993)	DEET	Mouse Rat Guinea Pig Pig Human Testskin	33.3 38.7 12.5 19.4 44.7 27.9	0.2	Acetone	10	50‡	NR	2880	1440	Soap and Water; Water[3]	Rinse[4]
Moody et al. (1994a)	2,4-D	Rat Human	6.9 7.7	0.2	Acetone	10	50‡	NR	2880	1440	Soap and Water; Water[3]	Rinse[4]
Moody et al. (1994b)	DDT	Rat Human	23 26.3	0.64	Acetone	10	16‡	NR	2880	1440	Soap and Water; Water[3]	Rub[4]
Moody & Nadeau (1994)	Diazinon	Human Testskin	10.9 9.5	0.2	Acetone	10	50‡	NR	2880	1440	Soap and Water; Water[3]	Rinse[4]
Moody et al. (1995a)	DEET	Rat Guinea Pig Human	High[5]	0.64	Off,DW, MK	50	78‡	NR	2880	1440	Soap and Water; Water[3]	Rub[4]
Moody et al. (1995b)	B[a]p	Rat Guinea Pig Human 32 yrs Human 50 yrs Testskin	11 13.3 9.8 8.4 9.1	0.64	Acetone	NR	16‡	NR	2880	1440	Soap and Water; Water[3]	Rub[4]
Moody & Nadeau (1997)	2,4-D Amine	Rat Guinea Pig Human	760-771 760 756-760	0.64	CC, WE	50	78‡	NR	2880	1440	Soap and Water; Water[3]	Rub[4]
Yourick et al. (2004)	Disperse blue 1	Fuzzy Rat	40.6‡[6]	0.64	Hair Dye[6]	5 mg	n/a	NR	4320	1440	Soap and Water; Water[7]	Rub, Rinse[7]
Hughes & Edwards (2010)	Multiple[2]	Human, Rat	122 - 158‡	0.32	Acetone	15	47‡	30	2880	1440	Soap and Water	Via Pipette[8]

Table 3c. Summary of Selected Protocol for *In Vitro* Studies that Provide Information on the Effect of Washing on Flux

Citation	Chemical Tested	Skin Type	Length of Washing (min)	Vol of Wash Sol'n / area (ml/cm^2)	Flow Rate of Sol'n / area ($ml/min \cdot cm^2$)	Washing Solution (repeated)	Mechanism of Washing (repeated)
Potentially Provides Information for the Evaluation of the Effect of Washing on *Flux*							
Moody & Ritter (1992)	Fenoxa-propethyl	Rat	2	50	25	Soap and Water; Water[3]	Rinse[4]
Moody & Nadeau (1993)	DEET	Mouse Rat Guinea Pig Pig Human Testskin	2	50	25	Soap and Water; Water[3]	Rinse[4]
Moody et al. (1994a)	2,4-D	Rat Human	2	50	25	Soap and Water; Water[3]	Rinse[4]
Moody et al. (1994b)	DDT	Rat Human	NR	15.6	n/a[9]	Soap and Water; Water[3]	Rub[4]
Moody & Nadeau (1994)	Diazinon	Human Testskin	2	50	25	Soap and Water; Water[3]	Rinse[4]
Moody et al. (1995a)	DEET	Rat Guinea Pig Human	NR	15.6	n/a[9]	Soap and Water; Water[3]	Rub[4]
Moody et al. (1995b)	B[a]p	Rat Guinea Pig Human 32 yrs Human 50 yrs Testskin	NR	15.6	n/a[9]	Soap and Water; Water[3]	Rub[4]
Moody & Nadeau (1997)	2,4-D Amine	Rat Guinea Pig Human	NR	15.6	n/a[9]	Soap and Water; Water[3]	Rub[4]
Yourick et al. (2004)	Disperse blue 1	Fuzzy Rat	NR	1.1	n/a[10]	Soap and Water; Water[7]	Rub, Rinse[7]
Hughes & Edwards (2010)	Multiple[2]	Human, Rat	NR	9.4	n/a[10]	Soap and Water	Via Pipette[8]

2,4-D = 2,4-dichlorophenoxacetic acid; 2,4-D amine = 2,4-dichlorophenoxacetic acid dimethyl amine; b[a]p = benzo[a]pyrene; BSA = bovine serum albumin; CC = Clean Crop formulation; DDT = dichlorodiphenyltrichloroethane; DEET = n,n-diethyl-m-toluamide; DW = Deep Wood formulation; MK = Muskol formulation; n/a = not applicable; NR = not reported by authors; WE = WilburEllis formulation; ‡ Calculated from data provided in the relevant study; [1] Authors stated skin was excised immediately prior to use; [2] Multiple = Bifenthrin, Deltamethrin, Permethrin; [3] 5 mL 1:1 v:v Radiac soap and water solution, 5 mL distilled water; [4] Rub = Rubbed with cotton tipped swabs; Rinse = Rinsed w/ syringe infusion pump at 5 mL/min; [5] For rat skin, loading of 1.5-9.6 mg/cm^2, 1.3-8.5 mg/cm^2 for guinea pig skin and 1.7-9.7 mg/cm^2 for human skin; [6] Information uncertain based on vague explanation of methods; [7] Pipetted on 0.1 ml 1:1 liquid detergent and water solution and rubbed with cotton swab 3x, rinse with DI water 2x; [8] Skin was washed 6 times by applying and removing each 0.5 mL 20 times via pipette; [9] Washing solution was applied with soaked gauze; [10] Washing solution was applied and/or removed via pipette

Table 4. Summary of Selected Protocol for *In Vivo* Studies.

Citation	Chemical Tested	Skin Type	Protection Dosed Area	Load of Chemical					Time to End (min)	Time to Wash (min)	Length of Washing (min)	Vol of Wash Sol'n / area (ml/ cm^2)	Flow Rate of Sol'n / area (ml/ min· cm^2)	Washing Solution	Mechanism of Washing
				Loading (µg/cm^2)	Skin Exposed (cm^2)	Vehicle	Vehicle Vol (µl)	Vehicle Loading (µl/cm^2)							
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed* and *Flux*															
Pelletier et al. (1989)	2,4-D amine	Rat	unocclusive "pack"	267‡	9.4	Water	60	6	30-360 480-4320	30-360 420	NR	0.32	n/a[1]	Liquid Ivory soap and water	sol'n+dry wipe x2, water wipe x1
Ritter et al. (1990)	2,4-D amine	Rat	unocclusive "pack"	266	9.4	Water	60	6	1440	420, 1380, 420+1380	NR	0.32	n/a[1]	Ivory soap, isopropyl, Rad-con sol'n or water Acetone x3	sol'n+dry wipe x2, water wipe x1 Rinse, dry wipe
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed*															
Maibach & Feldman (1974)	Azodrin	Human	NR	4	NR	Acetone	NR	NR	7200	15-1440	2	NR	NR	Soap and water	2 min wash
	Ethion	Human	NR	4	NR	Acetone	NR	NR	7200	15-1440	2	NR	NR	Soap and water	2 min wash
	Malathion	Human	NR	4-400 4 4	NR	Acetone Acetone Acetone	NR	NR	7200	1-1440 15-240 240	2 NR NR	NR	NR	Soap and water Rubbing Alcohol Water	2 min wash Excess Full Body Shower
	Parathion	Human	NR	4-400 4 4 4	NR	Acetone Xylene Acetone Acetone	NR	NR	7200	1-1440 15-1440 240 15-240	2 2 NR 2	NR	NR	Soap and water Soap and water Water Rubbing Alcohol	2 min wash 2 min wash Full Body Shower Excess
	Lindane	Human	NR	4	NR	Acetone	NR	NR	7200	5-1440	2	NR	NR	Soap and water	2 min wash
	Baygon	Human	NR	4	NR	Acetone Xylene Acetone	NR	NR	7200	1-1440 15-1440 240	2 2 NR	NR	NR	Soap and water Soap and water Water	2 min wash 2 min wash Full Body Shower
	2,4-D	Human	NR	4-40	NR	Acetone	NR	NR	7200	1-1440	2	NR	NR	Soap and water	2 min wash
Koizumi (1991)	HCB	Rat	occlusive teflon film + "jacket"	619‡ 660‡	4	Tetra-chloro-ethylene	approx 100	25	4320	n/a 360	NR	NR	n/a[1]	None Soap and Water	None Wipe 20x w/ soaked cotton
Wester et al. (1992b)	Isofen-phos	Human	None	13.2	12	Acetone	60	5	10080 10080	1440 4320	NR	NR	NR	Liquid Ivory soap, H20, soap, H20, H20	Wash

‡ Calculated from data provided in the relevant study; 2,4-D = 2,4-dichlorophenoxacetic acid; HCB = hexachlorobenzene; n/a = not applicable; NR = not reported by authors; [1] washing solution was applied with soaked gauze

1.C. Literature Classification

Following the review of the literature, applicable portions of studies were classified based on when the samples were washed, when the trials were ended, the relationship in time between washing and termination of the trials and whether the concentration in the receptor fluid or an equivalent measure such as urinary excretion of the chemical of interest was measured over time. These classifications are summarized in Table 5.

Table 5. Classification of Reviewed Literature.

Type #	Time to wash	Time to End	Trial extends past washing time	Receptor fluid surveyed over time	Potential Information Provided	
					Impact on total mass absorbed	Impact on flux over time
1A	Trials washed at various times	Trials ended at the same time	Yes	Yes	+	+
1B			Yes	No	+	-
1C/D			No	Yes/No	-[a]	-[a]
2A	Trials washed at the same time	Trials conducted for various times	Yes	Yes	-	+
2B			Yes	No	-	≈[b]
3A	Trials washed at various times	Trials conducted for various times	Yes	Yes	≈[c]	+
3B			Yes	No	≈[d]	≈[d]
3C			No	Yes	≈[e]	-
3D			No	No	≈[e]	-
4A	Trials washed at the same time	Trials ended at the same time	Yes	Yes	-	+
4B			Yes	No	-	-
4C			No	Yes	-	-
4D			No	No	-	-

+/- classification types that can/cannot provide this information; [a] classification types with one wash time and one control sample that was not washed; [b] classification type that can provide information via comparison between trials of total absorption at end; [c] classification type can contain a subset of trials that fall into categories 1A or 2A; [d] classification type that provides little information in its entirety but can contain a subset of trials that fall into categories 1B or 2B; [e] classification type that can only provide information if compound recovered in the skin post-washing is considered absorbed.

Figure 6 provides a visual representation of the relationship between the time of washing and termination of the trial for the study types that can provide relevant information to this review. Types 1C, 1D, 4C and 4D are not included in this figure because they cannot

provide any relevant information. In both cases there is only one washed trial that is ended immediately following washing.

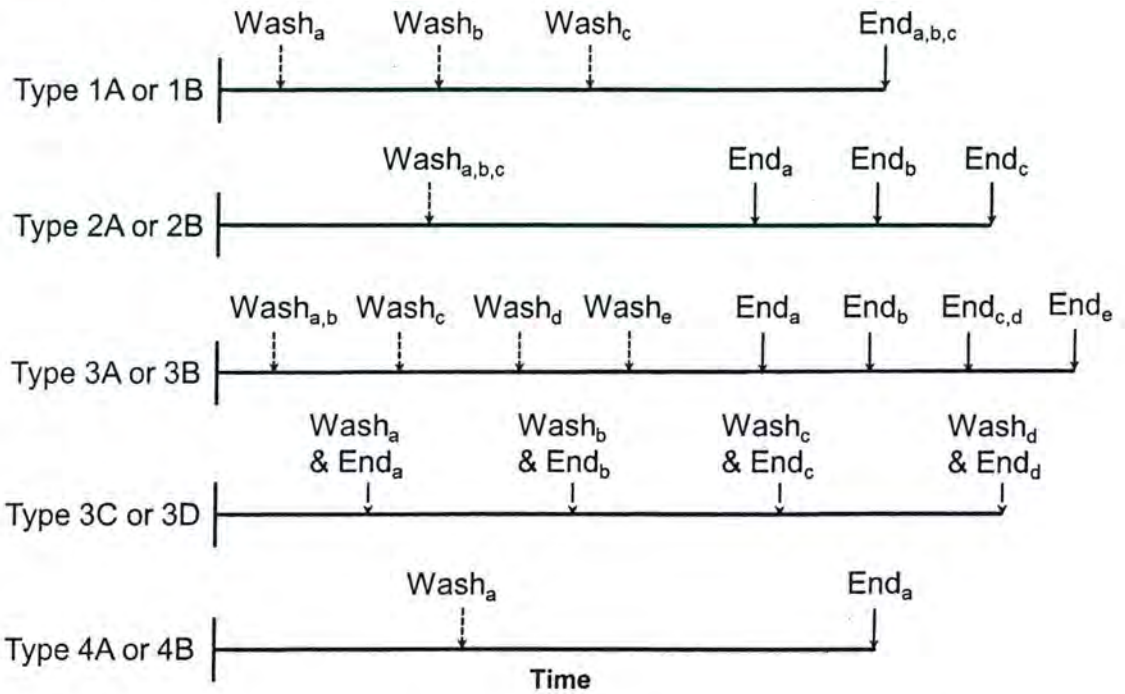

Figure 6. Relationship between Time of Washing and Termination for Relevant Classification Types. Subscripts denote groups of samples.

The parameters used to classify the relevant literature were chosen because they divided trials into groups with differing abilities to detect the short- and long-term effects of washing on dermal absorption. For clarity, several terms will be used consistently in this discussion. Each published article will be referred to as a "study". Within each study, multiple combinations of experimental methods may exist. Each set of conditions that uses the same methods is referred to as a "trial". Multiple trials may be compared to each other within a "classification type" to obtain information about washing. Classification types may also consist of only one trial. Each trial is made up of several "runs" with identical experimental conditions. Runs are usually combined to provide data for each trial, although occasionally results for individual runs are published separately.

The clearest data characterizing the effect of washing on total mass absorbed over time were obtained from classification types that compared a series of trials varying in exposure

duration. Exposure duration was defined as the time between the application of the contaminant and washing. However, this comparison is meaningful only if all the trials were ended at the same time at some point after washing (types 1A and 1B). Otherwise, differences in total absorption of compound could be due to one sample having more overall time for the chemical to penetrate the skin barrier, which in general leads to a greater systemic dose for compounds that can penetrate the viable epidermis (ECETOC 1993).

Classification types that compare between trials that wash at various times and end immediately following washing (types 3C and 3D) can estimate the effect of washing on overall absorption if the total amount of compound recoverable in the skin and receptor fluid at the termination of each trial is provided. The amount remaining in the skin following washing provides an estimate for the total amount of compound that remains to be absorbed systemically. When added to the total amount penetrated into the receptor fluid prior to washing, the resulting value is an estimate of the total mass that would have been absorbed if the trial had been allowed to continue indefinitely following decontamination. This is based on the assumption that all compound in the skin post-washing (or a similar percentage between compared trials) will eventually move into systemic circulation, given enough time. This is a reasonable assumption for non-volatile compounds. Frequently, groups of trials classified as a type 3C or 3D only report washing recovery data. However, the comparison between these values is not always an accurate indicator of the total mass of contaminant that has moved into and through the skin. For low washing recoveries, compound not in the wash could have been absorbed into the skin or systemic circulation or it could have been lost to the environment due to volatilization or mechanical transfer.

Sampling the receptor fluid for the duration of the trials allows researchers to determine the amount of chemical moving through a defined area of skin per unit of time, or flux. Usually this is accomplished *in vitro* by continuously pumping receptor fluid through a diffusion cell and collecting a pre-defined quantity of the perfusate over subsequent time increments. These aliquots of receptor fluid can be collected over any chosen time period, but most trials collect them for about two hours per sample, depending on the timing of the washing step and the flow rate of the receptor fluid. In *in vivo* trials, urine, feces or blood can be collected to determine flux. The most common choice is urine due to the fact that its collection is

relatively non-invasive. The one drawback is that it usually has to be collected in longer time increments, due to the batched nature of voiding. Often it is collected in 6-, 12- or 24-hour time increments. Classification types containing trials that collect receptor fluid prior to and following washing (types 1A, 2A, 3A and 4A) can provide information regarding the effect of washing on flux, with *in vitro* trials generally producing clearer data than *in vivo*, in which compound is influenced by other metabolism, storage or excretion mechanisms in the body prior to collection in larger batches. Type 1A is the preferable design to examine the effect of washing on flux because it has the potential to compare the change in flux in a washed trial against the flux in an unwashed control trial. In classification types 2A, 3A and 4A, the interpreted effects of washing on flux are a best guess based on comparing results to an expected unwashed flux profile. It is impossible to know the true effect of washing on flux with certainty unless a comparison can be made to a similar experimental trial that was not washed.

Type 2B trials can approximate the effect of washing on flux. In this classification type, all samples are washed at the same time post-exposure. A series of subgroups are then ended at increasing times post-washing. If the amount of compound recovered in the receptor fluid at each end time is determined and these end times are frequent and close enough to the time of washing, the total receptor fluid recoveries can be plotted over time as an estimate of the changing cumulative absorption through the skin. A change in flux would be visible as a change in slope between values. This method is not as desirable as classification types 1A, 2A, 3A or 4A because it does not determine the pre-washing flux of compound through the skin and the different data points are obtained using a number of animals or diffusion cells, which incorporates additional variability.

In their entirety, classification types 3A and 3B contain a variety of trials that may or may not be able to be compared to each other to provide clear information regarding the effects of washing. Type 3A can be helpful if it contains a subset of trials that fall into the type 1A category, which could provide information regarding the change in total mass absorption following washing. Similarly, type 3B can contain subsets of trials that conform to the type 1B or 2B characteristics. In these cases, the overall classification type may be noted in the

summaries but the collections of trials that are capable of providing useful information are classified as the type relevant to that subgroup.

Trials classified as types 1C, 1D, 4B, 4C and 4D are not capable of providing data regarding the short- or long-term effects of washing on dermal absorption. For types 1C, 1D, 4C and 4D, this is because the trials are ended immediately following washing with no additional trials to provide context to the recoveries in the various compartments. However, studies that are not helpful in this review can still potentially provide information regarding the washing recovery and distribution of the compound immediately following washing, which can be helpful in validating dermal absorption models.

The classifications of all 83 groups of trials across the 66 reviewed studies are presented in Table 6.

Table 6. Classifications of Reviewed Trial Groupings by Type

Type #	# Relevant Trial Groups	# Unhelpful Trial Groups
1A	6	4
1B	5	1
1C	0	0
1D	0	1
2A	0	1
2B	1	1
3A	0	0
3B	0	3
3C	0	0
3D	4	1
4A	10	10
4B	0	11
4C	0	3
4D	0	21
Total	26	57

It should be noted that while a study may contain trials that are classified as having the potential to provide insight into the effects of washing on dermal absorption, they do not always do so. Problems with other aspects of experimental design or reporting of results can make an otherwise helpful study design unable to provide information needed for this review.

A total of 83 groups of trials were classified out of the 66 reviewed studies. This is because a single reviewed study may have contained several independent experimental protocols or a protocol that was divided into helpful and extraneous trials. Data obtained from 26 trials groups in 25 studies that provided information about the effect of washing on total mass absorbed or change in flux are outlined below.

1.D. Effect of Washing on Total Mass Absorbed and Flux

1.D.1. The Effects of Washing on Total Mass Absorbed

Fifteen studies provided information regarding the change in total mass absorbed due to washing. Only the relevant trials from each study that provided information about the effect of washing on total mass absorbed are presented in Table 7.

The total mass absorbed in trials washed at an earlier time was compared to trials washed at a later time or an unwashed control and the assessment recorded in the column titled "Impact of Washing on Total Mass Absorbed." The notation "Down" indicates that the total mass absorbed decreased significantly ($p<0.05$) for all the trials washed at an earlier time, while "Up" indicates an increase and "Minimal" denotes no significant change in total mass absorbed between the samples regardless of washing time. Results are displayed separately for each skin type within a study. Differences in chemical of interest, washing time and solution between trials also resulted in varied outcomes. If this variation in results between groups of trials was noted for a study, the number of results in each category was considered. If the majority of trial groups demonstrated a significant ($p<0.05$) decrease in total mass absorbed following washing at an earlier time, the study was deemed to have resulted in general decrease, notated as "Generally Down". Similarly, "Generally Up" refers to a study in which the majority of trial groups demonstrated a significant increase in total mass absorbed for samples washed at an earlier time, while "Generally Minimal" indicates a study that observed no significant difference between washed and unwashed samples in most trials.

Table 7. Effects of Washing on Total Mass Absorbed.

Paper	in vivo/ in vitro	Skin Used	Type	Impact of Washing on Total Mass Absorbed
Koizumi (1991)	in vivo	Rat	1A	**Generally Down**
Wester et al. (1992b)	in vivo	Human	1A	Nominally Minimal[a]
Loke et al. (1999)[b]	in vitro	Human	1A	Nominally Down[a]
Monteiro-Riviere et al. (2001)[b]	in vitro	Swine	1A	**Down**[a, c]
Nielsen et al. (2004)[b]	in vitro	Human	1A	Nominally Down[a]
Nielsen (2010)[b]	in vitro	Human	1A	**Down**
Maibach & Feldman (1974)	in vivo	Human	1B	Nominally Down[a]
Nitsche et al. (1984)	in vitro	Human (intact) Human (no SC)	1B	Nominally Up[d] Nominally Down[d]
Ritter et al. (1990)[b]	in vivo	Rat	1B[e]	**Generally Minimal**
Weber et al. (1992)	in vitro	Human (intact, no SC)	1B	**Down**[d]
Hewitt et al. (1995)	in vitro	Human, Rat	1B	**Generally Down**[f]
Pelletier et al. (1989)[b]	in vivo	Rat	3D	Nominally Down[g]
Wester et al. (1990b)	in vitro	Human	3D	Nominally Down[g]
Zhai et al. (2007)	in vitro	Human	3D	Nominally Down[g]
Zhai et al. (2008)	in vitro	Human	3D	Nominally Down[g]

[a] estimated from receptor fluid only; [b] studies also provide information on flux through skin as presented in Table 8; [c] assumes constant receptor fluid volume and flow rate; [d] estimated from recovery of compound in skin only due to lack of receptor fluid in design or analysis; [e] classification was a unique type 1B in that one group of animals was washed twice prior to termination; [f] comparison between various wash times and control shows a stronger association between decreased absorption and earlier washing than comparison between earlier and later times to wash, although a general decrease is apparent for all; [g] absorption estimated via recovery of compound from skin and receptor fluid at termination immediately following washing.

However, not all studies conducted or reported statistical testing between the trials of interest. In these studies, different values may have been observed between trials washed at an earlier time compared to those washed later or not at all. However, it is impossible to say for certain without statistical testing that these differences were not the result of random chance. Therefore, any observed trends in the data for these studies are prefaced with the qualifier "Nominally" which indicates that any perceived decrease or increase in total mass absorbed due to washing may or may not have been statistically significant.

Of the 15 relevant studies noted in the table above, only six conducted and presented the results of statistical testing between the trials of interest. Five of those six studies observed a significant ($p<0.05$) decrease in total mass absorbed between samples washed at an earlier time and those not washed or washed at a later time.

Down

The overall trend from three of the studies was a significant decrease in the total mass absorbed in all samples that were washed earlier compared to an unwashed control or sample washed at a later time. Two of these studies contained groups of trials classified as type 1A and one contained trials classified as type 1B.

> *Weber et al. (1992)* washed with oil, acetone or water and observed a significant decrease in percent dose of TCDD recovered in skin recovery compared to the unwashed control. This pattern was apparent for the intact skin regardless of whether the stratum corneum was considered absorbed as well as for damaged skin. Dry wiping only was not considered "washing" for the purposes of this review and the samples decontaminated in this manner were not included in the summary of washing effects.
>
> *Monteiro-Riviere et al. (2001)* provided data as concentration over time, but did not supply the flow rate of the receptor fluid. The effect of washing on total mass of phenol absorbed could only be calculated from the provided data if the receptor fluid flow rate and volume were constant. Assuming this fairly conventional protocol was the case, washing with all three solutions would result in a significant decrease in total mass absorbed over the control.
>
> *Nielsen (2010)* showed a significant decrease in total mass absorbed for any washed sample when compared to unwashed trials. This was true for all of the chosen compounds (malathion, benzoic acid, glyphosate and caffeine), which spanned the spectrum of physicochemical properties.

All of these studies support the hypothesis that washing decreases the total mass absorbed compared to not washing.

Generally Down

Two additional studies showed a significant decrease in the total mass absorbed when samples were washed earlier in the majority of relevant trials. One of these studies contained trials in a classification type 1A while one contained a group of trials classified as type 1B.

Koizumi (1991) demonstrated that washing of the skin at six hours resulted in reduced recovery of hexachlorobenzene in the dosed skin, excreta and carcass as well as lower blood concentration values compared to the unwashed controls at the termination of the trials. This decrease was significant for the recoveries in the exposed skin area, feces, non-dosed skin, subcutaneous tissue and remaining carcass. It was not significant for recoveries from the liver and urine, although a nominal decrease in both was observed.

Hewitt et al. (1995) observed that washing at 30 minutes or sooner resulted in a significant decrease in percent recovery of 4,4'-methylenebis[2-chloroaniline] (MbOCA) or 4,4'-methylene-dianiline (MDA) in the skin for 6 out of 8 trials and in the receptor fluid for 8 out of 8 trials. Washing between 1 and 6 hours resulted in a significant decrease in the receptor fluid for 1 out of 18 trials and in the skin for 0 out of 8 trials. All washed trials except two that produced values that were not significantly different from the 72 hour controls demonstrated a nominal decrease in total mass recovered in the skin and receptor fluid of washed samples. Therefore, this trial is considered generally down due to the significant decrease in total mass absorbed observed when trials were washed at an earlier time compared to the controls. The designation would change to generally minimal if the trials washed at the later times between 1 and 6 hours were considered. However, these trials washed at a later time were not included in order to highlight the ability of washing to significantly reduce the total mass absorbed for samples washed quickly. The cut-off time at which washing is less effective at decreasing the total mass absorbed is likely related to the loading of compound on the skin surface and its physicochemical properties. A nominal reduction in dermal absorption with earlier washing was also observed between most any two trials washed at different times that utilized the same

skin type and compound, although this trend was not as prominent as the comparison to the unwashed controls.

Therefore, although there was some variability in the data from these groups of trials, the majority support the hypothesis that washing, especially washing quickly, decreases the total mass absorbed compared to not washing.

Generally Minimal

Of the one remaining study that conducted statistical testing, no significant difference in total mass absorbed was observed between samples washed at different times. The trials in this study were compared as a classification type 1B.

> *Ritter et al. (1990)* showed that washing with a variety of solutions at 7 hours significantly reduced the recovery of 2,4-D amine at 24 hours in the skin for 1 out of 5 trials and in urine for 1 out of 5 trials compared to washing at 23 hours only. A nominal decrease in total mass absorbed was observed in all non-significant trials. Therefore, washing earlier nominally reduced the total amount of 2,4-D absorbed and available for absorption for most of the trials.

While this study did not show a significant decrease in total absorption for most samples washed at an earlier time, it still observed either a nominal or significant increase in all trials. The lack of significance could have been due to the length of the exposure time prior to washing the earlier trials (7 hours).

Nominally Down

Out of the nine studies that did not conduct relevant statistical testing, 7 observed a nominal decrease in total mass absorbed for most or all of the washed trials when compared to a trial washed at a later time or an unwashed control. Two of these studies contained trials in a classification type 1A, one in a classification type 1B and four in a classification type 3D.

> *Maibach & Feldman (1974)* showed that under most observed conditions for a variety of compounds (azodrin, ethion, malathion, parathion, lindane, baygon and 2,4-D), washing at an earlier time resulted in a nominally smaller percent penetration. All

except 1 of the 18 trials showed a nominal decrease in percent penetration between the earliest washing time and the 24-hour wash for the control. Malathion washed with rubbing alcohol had higher excretion at the earliest washing time compared to the latest washing time. Three of the soap and water wash trials had the nominally highest percent recovery observed prior to the 24-hour control, while three other trials, one of which was applied in xylene and one washed with rubbing alcohol, had the nominally lowest percent recovery occur later than the first washing time. Overall, 56 out of 63 trials showed a nominal decrease when compared to the subjects washed at the latest time.

Pelletier et al. (1989) conducted trials that were simultaneously washed and ended between 30 minutes and 6 hours post-exposure. The trends in the mass recovered in the exposed skin at different washing times differed from the trends observed in the total mass recovered in the receptor fluid. However, these trials followed the general pattern that washing at the earliest time removed nominally more 2,4-D amine and nominally reduced the total mass recovered in the skin and systemic absorption compared to washing at the last time (6 hours). Therefore, in this group of trials, the nominal decrease of absorbed compound observed at earlier washing times is apparent, although it may not be statistically significant.

Wester et al. (1990b) observed the same pattern that washing earlier nominally reduced the total mass recovered in the intact skin for both DDT and b[a]p compared to washing at a later time. This would also result in a nominal reduction of total mass of compound remaining in the system for further penetration. Slightly more compound was recovered in the receptor fluid at 24 hours than at 25 minutes, which would be expected due to the longer duration of the 24-hour trial and should not be considered to be due to washing since washing occurred immediately prior to termination of the trials.

Loke et al. (1999) did not report total mass absorbed. However, utilizing area under the curve (an approximation of the total mass of the compound that penetrated through the skin over the 24-hour exposure period) showed that washing at earlier

times resulted in a nominal decrease in total absorption of diethyl malonate for all samples washed with sodium dodecyl sulphate (SDS). Curves for the other washing solutions were not provided, but Loke et al. stated similar but less pronounced patterns were observed for all solutions. It follows from that statement that other wash solutions would likely have similar but less pronounced results on total mass absorbed.

Nielsen et al. (2004) showed that washing at a later time nominally decreased washing recovery and nominally increased total penetration into the receptor fluid for all compounds (methiocarb, paclobutrazol, pirimicarb and dimethoate) except the most lipophilic (prochloraz, log K_{ow} 4.4). Authors also estimated recovery in the skin by subtracting recovery in the wash and receptor fluid from the total applied amount. If the estimated skin recovery is added to the receptor fluid recovery, total penetration in samples washed at 6 hours nominally decreases for all compounds including prochloraz compared to samples washed at the later time. However, this may not be an accurate estimate due to the potential loss of compound via an uncharacterized pathway such as irreversible binding to tools or matrices used to apply compound or wash the skin. Volatilization would be expected to be minimal since the cells were occluded.

Zhai et al. (2007) observed that washing at an earlier time nominally removed more formaldehyde and nominally reduced the total mass recovered in the viable skin and stratum corneum for all wash solutions and times. This would result in a nominal reduction in the total mass of compound remaining in the system for further penetration.

Zhai et al. (2008) observed a nominal decrease in absorption of glyphosate into the skin and receptor fluid for all wash times and solutions compared to later washing. This would result in a nominal reduction in the total mass of compound remaining in the system for further penetration.

Without statistical testing, it is not possible to make clear statements about the effects of washing on total mass absorbed. However, in some of these cases, the nominal differences

could have potentially been significant, which would have supported the overall observed pattern that washing earlier results in a decrease in total mass absorbed compared to a sample washed at a later time or one not washed at all.

Nominally Minimal

One study containing trials in a classification type 1A did not conduct significance testing but obtained the same mean for two trials washed at different times.

> *Wester et al. (1992b)* did not show decontamination to be either advantageous or damaging since washing earlier did not nominally change the total mass of isophenfos absorbed. Washing earlier did nominally increase the amount recovered by the wash and resulted in an earlier approximate peak recovery in urine. However, both raw and adjusted percent recoveries in urine were similar for both wash times.

Without statistical testing it is difficult to speculate on the effects of washing in this case, although decontamination appears to have made very little impact on the total mass absorbed.

Nominally Variable

The final study that did not conduct statistical testing noted a nominal increase in total mass absorbed in trials using intact skin. This study consisted of trials in a type 1B configuration.

> *Nitsche et al. (1984)* washed both intact and "damaged" skin at 3 hours and compared samples against an unwashed control with a similar skin condition at 10 hours. In the trials using damaged skin, washing resulted in a nominal decrease in total mass of lindane absorbed into the skin compared to not washing. In the intact samples, washing resulted in nominally higher concentrations of lindane in deeper layers of the skin but a nominal reduction in recovery from the stratum corneum compared to the control. If the compound in the stratum corneum was not considered absorbed, data from these trials indicate that washing resulted in a nominal increase of total mass absorbed into the skin.

As mentioned above, this study did not conduct statistical testing and therefore little can be said with certainty about the results. The nominal results obtained did not appear to conform

to the established pattern for intact, healthy skin while the expected nominal decrease was observed for skin when the stratum corneum was removed prior to exposure.

Conclusions Regarding Total Mass Absorbed

After reviewing the data from the 15 studies that provided information regarding the effect of washing on total mass absorbed, the results from a majority of the trials that conducted statistical testing (5 out of 6) illustrated a significant decrease in total mass passing into and through the skin for most or all washed trials when compared to unwashed controls or trials washed at a later time. This significant decrease in total mass absorbed due to washing is more prominent for samples decontaminated at an earlier time than in cases when washing was delayed. Additionally, 7 out of 9 studies that did not present results from any potential statistical testing observed a nominal decrease in most or all of the trials washed at an earlier time compared to trials washed later or not at all. This provides strong evidence that washing does reduce the total mass absorbed through the skin, especially when washed soon after the initial exposure, while even later washing may still result in a nominal decrease. No significant increase in total mass absorbed was observed in any washed trial. This supports the statement that, despite its potential limitations as outlined above, washing can have a beneficial effect by reducing the total systemically absorbed dose.

Further discussion regarding the differences in protocols between these studies and their potential effects on the data presented are outlined in section 1.E titled "Additional Considerations Regarding Study Protocol".

1.D.2. The Effects of Washing on Flux

Fifteen studies were found that could provide information regarding the change in flux over time due to washing. Five of these also supplied information about the effect of washing on total mass absorbed, as outlined above. Only the results from relevant groups of trials from each study are presented in Table 8.

Table 8. Effects of Washing on Flux.

Paper	in vivo/ in vitro	Skin Used	Type	Impact of Washing on Overall Peak Flux	Impact of Washing on Post-Wash Flux Only
Loke et al. (1999)[a]	in vitro	Human	1A	Nominally Up	n/a[b]
Monteiro-Riviere et al. (2001)[a]	in vitro	Swine	1A	Nominally Down[c]	n/a[b]
Nielsen (2010)[a]	in vitro	Human	1A	**Generally Down**	Nominally Minimal
Ritter et al. (1990)[a]	in vivo	Rat	1B[d]	n/a	**Generally Up**
Pelletier et al. (1989)[a]	in vivo	Rat	3B[e]	n/a	Nominally Down[f]
Moody & Ritter (1992)	in vitro	Rat (Fresh, Frozen)	4A	n/a	Nominally Up[f]
Moody & Nadeau (1993)	in vitro	Mouse, Human	4A	n/a	Nominally Minimal[f]
		Rat, Pig, Guinea pig, Testskin		n/a	Nominally Up[f]
Moody et al. (1994a)	in vitro	Rat, Human	4A	n/a	Nominally Up[f]
Moody et al. (1994b)	in vitro	Rat, Human	4A	n/a	Nominally Up[f]
Moody & Nadeau (1994)	in vitro	Human, Testskin	4A	n/a	Nominally Up[f]
Moody et al (1995a)	in vitro	Rat, Guinea Pig, Human	4A	n/a	Nominally Up[f]
Moody et al (1995b)	in vitro	Rat, Guinea Pig, Testskin, Human (32 yr old)	4A	n/a	Nominally Up[f]
		Human (50 yr old)		n/a	Nominally Minimal[f]
Moody & Nadeau (1997)	in vitro	Rat, Guinea Pig, Human	4A	n/a	Nominally Up[f]
Yourick et al. (2004)	in vitro	Fuzzy Rat	4A	n/a	Nominally Minimal[f]
Hughes & Edwards (2010)	in vitro	Rat, Human	4A	n/a	Nominally Up[f]

[a] studies also provided information regarding the effect of washing on total mass absorbed through the skin; [b] pre- and post-wash peaks were simultaneous because samples were washed immediately following exposure; [c] total mass estimation based on assumptions that receptor fluid volume and flow rate were constant; [d] classification was a unique type 1B in that one group of animals was washed twice prior to termination; [e] comprised of a type 3D group of trials that provided information about receptor fluid concentration pre-wash and type 2B group of trials that provided the same information post-washing; [f] change estimated by comparison to predicted ideal flux through the skin following exposure without washing.

As discussed in the previous section, results are displayed separately for each skin type within the relevant part of each study, although differences in chemical of interest, washing time and washing solution between trial also resulted in varied results. For clarity, short-term changes in flux due to washing are divided into two different categories. Protocols that are able to differentiate between the increase in penetration that normally occurs following exposure and the potential change in flux observed post-washing can provide data on that post-washing peak in flux separately. Usually, these are trials washed several hours after the initial exposure to allow the applied dose to penetrate into the receptor fluid and start to decline prior to initiating decontamination. The change or estimated change in flux occurring post-washing is presented in Table 8's column titled "Impact of Washing on Post-Wash Flux Only." Notations for the results of each group of trial are similar to those previously

described, with "Down" indicating a significant ($p<0.05$) decrease in maximal flux compared to control, "Up" indicating an significant increase and "Minimal" denoting no significant difference between trials. "Generally" can signify variation in results between groups of trials within a study or skin type. However, some studies provided penetration profiles for individual diffusion cells only within each trial. Lacking the raw data, it is not possible to average the trends from these cells. Therefore, "Generally" can also refer to variation within a trial in the results from each run.

It should be noted that 11 out of the 13 reviewed studies that provided information about post-washing flux through the skin did not have an unwashed control sample. Therefore, the effects of washing on flux in those studies are a best guess based on comparing results to an expected unwashed flux profile. As mentioned previously, this can be especially problematic for compounds that move through the skin and into the receptor fluid at a slower rate. It is possible that, for such a compound washed at a later time, the flux through the skin observed post-washing corresponded with the increase in flux as part of their expected, unwashed penetration profile. In such a case, it would be inaccurate to attribute all of a post-washing peak to the influence of washing on the movement of compound through the skin. The variable nature of the raw unaveraged data presented in the graphs adds additional difficulties when attempting to distinguish change in flux or compare results against an expected flux profile for unwashed controls. This further highlights the fact that it is impossible to know the true effect of washing on flux without comparing it to a similar experimental trial that was not washed. Therefore, all results from studies without a control have a "Nominally" qualifier. Only two of the relevant reviewed studies estimated the change in post-washing flux by comparison against an unwashed sample (Ritter et al. 1990, Nielsen 2010). However, only one of those studies conducted or reported statistical testing between the trials of interest. Therefore, any observed trends in the data for these studies is also prefaced with the qualifier "Nominally" which, as discussed previously, indicates that any perceived decrease or increase in total mass absorbed due to washing may or may not have been statistically significant.

The second type of change in flux is the overall peak flux observed over the duration of the entire trial, both pre- and post-washing. In order to determine the effect of washing on the

maximal flux for each trial, it must be compared to an unwashed control. This information is presented in Table 8's column titled "Impact of Washing on Overall Peak Flux." A group of trials classified as 1A that included an unwashed sample is the only study design that would be able to provide information on both categories of flux.

Results for the overall peak flux are designated in the table in a manner similar to that for the two preceding discussions. "Down" indicates a significant decrease in flux following washing as compared to expected or observed results from an unwashed control, while "Up" indicates a significant increase in flux was observed following washing compared to an unwashed control, "Minimal" denotes no significant difference from the expected or observed control outcome and the "Generally" modifier indicates varying results between the trials. Again, very few (1 out of 3) studies that can provide this information conducted any statistical testing. Therefore, the remaining two were given the "Nominally" qualifier to indicate that any perceived increase or decrease in overall peak flux due to washing may or may not be statistically significant.

Uninformative Types of Studies

As noted in the discussion of classification types, type 1A experimental designs were able to provide information on both flux and total mass absorbed. However, flux through the skin is difficult to quantify in *in vivo* trials that use collection of urine or feces to determine percutaneous absorption. Urine and feces are usually collected and averaged over longer intervals, due to the batched nature of voiding. Additionally, prior to recovery in the urine or feces, the fate of the compound in the body is influenced by metabolism, storage or excretion mechanisms. Moody and Ritter (1992) note that estimating dermal flux via percent dose excreted in urine may not be accurate due to body storage of compounds which could slow the appearance of the compound in the urine. Therefore, it is not appropriate to extrapolate these *in vivo* excretion results to determine the effect of washing on flux. Koizumi (1991) and Wester et al. (1992b) were *in vivo* trials and unable to provide clear information about flux despite being a type 1A. Additionally, groups of *in vivo* trials classified as type 2A, 3A or 4A were not summarized in this portion of the review as they were unable to provide relevant information about flux.

Nielsen et al. (2004) used an *in vitro* type 1A experimental design that was unable to provide information regarding the impact of washing on maximal flux due to the lack of unwashed control samples. Additionally, data from Nielsen et al.'s (2004) trials could not provide information regarding the post-washing change in flux for several reasons. Receptor fluid was collected over longer time periods (every six hours), compounds were observed to have a longer lag time so that only three samples demonstrated percutaneous penetration prior to washing and washing appeared to occur during what would have been the normal initial peak in flux following exposure. Data were also provided only via a cumulative penetration curve which made visual estimation difficult. Therefore, this study was deemed to provide no helpful information regarding washing's effect on flux through the skin.

Overall Peak Flux: Results

Three studies containing type 1A groups of trials can provide information regarding the effect of washing on the overall peak flux. One observed a significant decrease in maximal flux for most samples.

> *Nielsen (2010)* observed a significant decrease increase in maximal flux as calculated by the study authors for half of the washed samples when compared to unwashed trials. The timing of the maximal flux was not clearly provided and it could have occurred prior to or following washing. However, it appears to have occurred at similar times for both washed and unwashed samples. The maximal flux was significantly reduced ($p<0.05$) in washed samples compared to controls for caffeine and benzoic acid, while it was about equivalent between washed and unwashed samples exposed to malathion or glyphosate. A very small nominal decrease was noted for glyphosate while a similarly tiny nominal increase was noted for malathion.

Those studies that did not conduct statistical testing noted a nominal decrease in all washed samples compared to unwashed controls in one and a nominal trend towards increasing maximum flux over time for the other.

> *Loke et al. (1999)* observed a nominal increase in the peak penetration of diethyl malonate for samples washed at one hour compared to the control. The peak flux for

the samples washed at 30 minutes was nominally equivalent to the peak for the control, while washing at 15 minutes nominally decreased the peak compared to all other samples. The peak flux observed at all three wash times was also prior to the peak flux for the control. While only one trial out of three in this study demonstrated a nominal increase in maximal flux over the control, Loke et al. (1999) was classified as illustrating an overall pattern of "Nominally Increasing". This was the result of the expected continuance of the trend that washing later resulted in increasing maximum flux.

Monteiro-Riviere et al. (2001) noted a nominal decrease in estimated maximal peak flux of phenol for washed samples compared to controls for all decontamination solutions. The peak flux was estimated from chemical concentration recovered in the receptor fluid. Therefore, estimation of the effect of washing on flux assumes that the flow rate and volume was constant for the receptor fluid.

Conclusions Regarding Overall Peak Flux

It is difficult to make generalizations about the effect of washing on overall peak flux due to the small number of relevant studies and the lack of statistically significant results. Nominal effects seemed to be linked to time of washing, with earlier washing decreasing maximal flux and later washing potentially increasing it. Further study is needed before any conclusions on this topic can be made.

Information about post-washing flux is also not available from either Loke et al. (1999) or Monteiro-Riviere et al. (2001) because samples were washed during the collection of the first sample of receptor fluid. Therefore, the pre- and post-wash peaks were simultaneous.

Post-Wash Peak Flux: Generally Up

Of the 13 studies that can provide information on the post-wash peak in flux, one observed a significant increase in flux following decontamination. This study contained trials classified as type 1B.

Ritter et al. (1990) was a unique protocol that washed one set of samples twice with either water, soap and water, Rad-Con® cleanser, isopropanol or acetone. Data indicated that washing at 7 and 23 hours and sacrificing at 24 hours resulted in higher blood levels of 2,4-D amine than washing at 7 hours and sacrificing at 24 hours for all wash solutions, although the difference was not significant ($p>0.05$) for the soap and water wash, which is the most commonly used decontamination solution when exposed persons attempt to clean their skin. Two of the wash solutions that produced a significant ($p<0.05$) increase in blood levels (isopropanol and acetone) are not representative of normal washing procedure and could have caused skin damage due to their ability to remove skin lipids and compromise the stratum corneum layer. This suggests that washing at 23 hours increased the flux of compound possibly remaining in the skin following the first wash into the blood compared to not washing.

This study supports the hypothesis that washing can result in a secondary post-washing increase in flux through the skin, although the multiple washes may have influenced the results.

Post-Wash Peak Flux: Nominally Up

Nine additional studies containing classification type 4A trials estimated a nominal increase in flux post-washing in at least one run for most or all of the trials. All peaks discussed below are visually estimated and lack any control for comparison.

Moody & Ritter (1992) showed a potential peak in percutaneous penetration of fenoxapropethyl at 10 hours and what could possibly be interpreted as a very small second peak at about 26 to 28 hours for one run out of four using fresh rat skin and two runs out of four using frozen rat skin. The post-washing changes are difficult to distinguish from high levels of variation in the flux over time.

Moody & Nadeau (1993) noted a small but distinct nominal increase in flux of DEET post-washing for all replicates utilizing rat skin. A nominal post-washing peak in flux was also observed for two out of four guinea pig cells, one out of four pig cells and one out of four testskin cells. The nominal increase was barely distinguishable in pig

skin due to its small magnitude. No change in flux post-washing was estimated in any runs using mouse or human skin. A nominal increase in flux was observed post-washing in at least one run for 4 out of 6 skin types.

Moody et al. (1994a) noted a nominal increase in flux of 2,4-D post-washing starting at about 24 hours in all skin types but with the most pronounced nominal increase in flux for rat skin. Graphs demonstrating this change were only provided for human and rat skin. The secondary peak shown for rat skin was nominally larger than the original peak. In the graph of percent recovery over time for human skin, a very small potential post washing peak is observed for three of the four cells. The fourth cell could have had a small nominal increase in flux post-washing, but it is visually indistinguishable from background variability.

Moody et al. (1994b) observed a relatively strong nominal post-wash increase in flux of DDT into the receptor fluid starting at about 24 hours in human and rat skin on the Bronaugh cells, with no pre-washing peak noted for either of the presented skin types. No information was presented in the text or data on the post-wash flux for any of the other skin or cell types.

Moody & Nadeau (1994) stated that they observed a nominal increase in flux of diazinon into the receptor fluid post-washing starting at about 24 hours in all skin types. However, graphs are only provided to illustrate this change in human and testskin. The secondary peaks shown for human and testskin samples are fairly pronounced and are nominally equivalent in size to the original peaks.

Moody et al. (1995a) also noted a nominal increase in flux of DEET into the receptor fluid post-washing at about 26 hours for all three skin types regardless of application vehicle. The average estimated value of the post-washing peak observed in the receptor fluid was potentially twice as large as any pre-washing peak for all conditions.

Moody et al. (1995b) observed a pronounced nominal increase in flux of b[a]p into the receptor fluid post-washing at about 24 hours for all rat and guinea pig cells. A

moderate nominal increase was also observed at the same time for all testskin cells but only in two out of four cells for 32-year old human skin. No peak in percent recovery was estimated post-washing for any runs with 50-year old human skin. A nominal increase in flux was observed post-washing in at least two runs for 4 out of 5 skin types.

Moody & Nadeau (1997) observed a nominal increase in flux of 2,4-D amine into the receptor fluid post-washing at about 26 hours for all three skin types regardless of application vehicle. The average value of the post-washing peak was nominally larger than the pre-washing peak for both formulations on rat skin and for the Clean Crop formulation on guinea pig skin, while the post-washing peak was nominally smaller than the pre-washing peak for the remaining conditions.

Hughes & Edwards (2010) demonstrated a nominal increase in flux post washing for the samples that washed at 24 hours and ended at 48 hours. This increase in slope post-wash was visually estimated from the graphs of cumulative recovery over time and was more obvious for rat than human skin. Rat skin showed a nominal increase in flux for all three compounds of interest, while a very minor increase in post-wash flux was observed in only two compounds (bifenthrin and deltamethrin) applied to human skin while no change was estimated for permethrin.

These studies demonstrated a nominal small secondary post-washing increase in flux in at least some of their relevant trials.

Post-Wash Peak Flux: Nominally Minimal

In contrast to the majority of the reviewed studies, two studies that conducted no statistical testing observed nominally minimal changes in flux. One of these studies contained trials in a type 4A classification, while the other was classified as containing type 1A trials. All peaks discussed below are visually estimated and lack any control for comparison.

Yourick et al. (2004) applied Disperse Blue 1 (DB1) to samples ended at 24 hours as well as samples washed at 24 hours and ended at 27 hours. The trials ended at 24 hours are not helpful in this review because the time of washing is unclear. In the

extended 72-hour trial, a peak and subsequent decline in receptor fluid recovery of DB1 was visually estimated as occurring prior to washing. Following the wash, the decline in flux appeared to level out and recovery and flux potentially remained constant for the rest of the trial. However, it is not apparent whether this nominal change in flux is due to washing or the natural leveling out usually observed as the last of the compound penetrates the skin in unwashed dermal absorption studies, especially since the percent of overall applied dose recovered was already so low (less than 0.05%). Therefore, any potential nominal changes in flux post-washing are difficult to ascertain from the provided information but are likely to be minimal.

Nielsen (2010) did not observe any nominal increase in post-washing flux, as estimated from plots of cumulative mass collected over time. In one of the four chemicals (caffeine), the flux through the skin nominally declined following washing compared to the unwashed trial. The other three compounds (benzoic acid, malathion and glyphosate) observed nominally similar fluxes following the time of washing in both the washed and unwashed trials. Authors noted that the two-hour intervals between sampling may have missed a peak of shorter duration, but many other studies noted a post-wash increase in flux using the same collection timing.

In these studies, the overall post-washing effect on dermal flux appears to be nominally minimal.

Post-Wash Peak Flux: Nominally Down

Finally, one study with a group of trials classified as type 3B estimated a nominal decrease in flux post-wash compared to the expected flux in an ideal unwashed sample. Again, all peaks discussed below are visually estimated and lack any control for comparison.

Pelletier et al. (1989) washed and ended some trials between 30 minutes and 6 hours and washed additional trials at 7 hours but terminated them between 8 and 72 hours. There was no visually estimated peak in blood concentration of 2,4-D amine at 8 hours (1 hour after washing at 7 hours). At that time, blood concentration was about similar to that found in animals immediately post-washing at 2, 4 or 6 hours.

Following that, blood concentration nominally declined. The lack of a visually estimated peak in blood concentration following washing suggests that a nominally minimal change in flux through the skin occurred immediately following washing. The nominal decrease observed a short while later could have been part of the normal decline observed following depletion or removal of the external reservoir of compound.

Interpretation of the percent dose recovered in the blood is difficult. The percent dose recovery in the blood can be generally correlated to the concentration in blood since the rats all weighed about the same and therefore contained similar volumes of blood (UCSD IACUC 2004). However, these data were obtained by washing and then killing rats at various time points to obtain their blood, not by taking blood samples from a group of rats over time, during which they were washed. This, combined with a lack of control, make the data in this study difficult to interpret.

Conclusions Regarding Post-Washing Peak Flux

After reviewing the data from the 13 studies that provided information regarding the post-washing peak flux through the skin, only one was able to provide statistically significant results. That study did note a significant increase in post-washing flux, but the design was such that additional information regarding the flux profile as not obtained while multiple washes also could have contributed to the observed effect. In studies without any statistical testing, the overwhelming trend illustrated a nominal increase in flux following washing when hypothetically compared to an expected unwashed flux profile. This trend was slightly less obvious but still apparent if only trials using human skin were considered. It should also be noted that out of the nine studies that contained at least some trials in which a nominal post-washing peak in flux was observed, 8 were from the laboratory run by Moody and coworkers. Therefore, this body of evidence is not as robust as if the effect had been observed by 10 different research groups. Support for the post-washing peak in flux and therefore Moody & Maibach's (2006) "wash-in" hypothesis stems primarily from these type 4A studies without statistical testing or controls.

It should also be noted that there are additional issues with the primary studies cited in support of the "wash-in" hypothesis. Several of the studies in the Moody et al. series presented graphs of flux over time for only a selection of the skin types (Moody et al. 1994a, b, Moody & Nadeau 1994). Two of these studies chose to present the trials that demonstrated the most pronounced post-washing increase in flux (Moody et al. 1994a, Moody & Nadeau 1994) while one neglected to state in the text whether a post-washing increase was observed in the skin samples that were not plotted (Moody et al. 1994b). Therefore, some number of the 11 trials these studies chose not to plot might have shown a peak in flux following washing in only some or none of the diffusion cells. Some of the studies in the Moody et al. series also have results that are not consistent with the laws of physics that govern diffusion of compound through the skin membrane. For example, in Moody & Nadeau (1997), the flux through human skin peaks quickly at a value 10 times less than the expected maximal flux before quickly declining. A second peak is observed post-washing that was smaller than the first, and the majority of compound recovered in the wash. This is problematic because the washing recovery showed that a large reservoir of compound remained on the skin surface until the time of wash. This should have resulted in an increase in flux until the maximal flux was reached. The reservoir would have then maintained that maximal flux until it was depleted. The fact that this was not observed suggests that there was a potential problem with the experiment. The formation of an air bubble between the skin and receptor fluid that could have been dislodged during mechanical washing could be one explanation. Other changes in protocol over time as discussed below may also have increased the likelihood of observing a post-washing peak in flux regardless of whether or not it would be present *in vivo*. The fact that the "wash-in" hypothesis is based primarily on these problematic data weakens it. While it may still occur *in vivo*, further study is needed to characterize the frequency of its occurrence as well as the magnitude of the change in flux.

Further discussion regarding the differences in protocols between these studies and their potential effects on the data presented are outlined below.

1.E. Additional Considerations Regarding Study Protocol

In addition to the timing of washing and termination of trials and the monitoring of receptor fluid, there are other noteworthy experimental design characteristics that can have an impact

on the results of dermal absorption washing studies. Because there is little standardization of protocol across dermal absorption studies, these differences in protocol can make comparisons between washing studies tricky. Some of these discussions apply only to the *in vitro* protocols, but most are relevant to the *in vivo* trials as well.

1.E.1. Skin Type

In most cases, dermal absorption studies are done in order to characterize potential exposure via skin contamination to workers and people throughout their daily lives. However, not all studies use human volunteers or skin due to the ethical issues of *in vivo* work and the practicality of obtaining viable human skin *in vitro*. Much work has been done to identify alternate species with similar skin properties. However, most animal models tend to have differences in skin morphology, including the thickness of skin and number of hair follicles that can influence dermal absorption. Animal skin has been shown to overestimate human skin exposure, although pig and monkey skin is a closer match than other species (EPA 1992, OECD 2004, SCCP 2006). Therefore, if the use of human materials is feasible, it is preferable to reduce any potential confounding. Cultured skin products have not been shown to adequately mimic the barrier function found in full *in vivo* skin and therefore should be used with caution (SCCP 2006).

The majority of the relevant reviewed studies used human skin in at least some of their trials. Ten studies used only human and nine used human along with a variety of animal species. Only six studies used solely rat (Pelletier et al. 1989, Ritter et al. 1990, Koizumi 1991, Moody & Ritter 1992, Yourick et al. 2004) or pig skin (Monterio-Riviere et al. 2001). Other skin types included guinea pig, mouse and testskin, a cultured skin product made from human foreskin. Of the nine studies that used a mix of animal and human skin, three showed a weaker or minimal post-wash peak in flux through the human skin compared to strong post-washing peaks in at least some of the other skin types (Moody & Nadeau 1993, Moody et al. 1994a, 1995b). Total absorption was also different between human and animal skin. In seven of the nine mixed studies, rat skin yielded higher total absorption than human skin while pig skin under-predicted human skin total absorption in all relevant trials. Guinea pig and testskin showed greater total absorption than human skin in only about half of the trials. This highlights the uncertainty inherent in using non-human skin and suggests that the

studies utilizing only rat skin above could be over-predicting total absorption and exaggerating post-wash change in flux when compared to human skin.

Dermal absorption can also vary by body region. In humans, the limbs, torso, palm of the hand and ball of the foot are better able to prevent movement of compound through the skin than other sites. For example, a three to four-fold increase in penetration of compound above forearm skin was observed in sites on the scalp and face, a seven-fold increase for the underarms and up to a 20-fold increase for scrotal skin (Maibach & Feldman 1974, Bronaugh & Collier 1991). All summarized studies that specified the body region from which skin was obtained or exposed used skin from the limbs or torso. While possible, it is unlikely that one of the studies which did not provide this information used skin from a more permeable site. Not only are the differences in dermal absorption between skin sites widely known, the most easily accessible skin is also from the less permeable sites such as the torso and limbs. Therefore, if a study had used facial, axial, or genital skin, it is likely researchers would have noted their use of this non-standard location. For these reasons, in this review it is assumed that the variability in permeability between body regions would have contributed minimally to the differences in dermal absorption between studies.

1.E.2. Skin viability and barrier function

Skin storage

Ideally, *in vitro* skin should retain its *in vivo* ability to act as a barrier to the dermal penetration of contaminants. The barrier function of skin can be compromised by harsh storage methods. Some studies have shown that skin can be frozen for over a year at -20 °C with no increase in permeability (Diembeck et al. 1999, OECD 2004), while another study showed a reduced barrier function in some samples frozen between 2 and 12 months (Bronaugh et al. 1986). This uncertainty means that, when frozen skin is used, it should be checked to ensure it has similar permeability compared to freshly excised skin. Skin should never be frozen at extremely low temperatures (-80 °C) and dermatoming should occur prior to freezing to avoid damaging the skin barrier (EPA 1992, OECD 2004). Viability can also be influenced by the method of skin storage prior to the start of the trial, with frozen skin

quickly becoming non-viable. Refrigerating skin at 4 °C has also been shown to maintain viability for up to 8 days (Wester et al. 1998).

Some studies froze skin for anywhere between 7 days and up to 12 months (Moody & Ritter 1992, Loke et al. 1999, Nielsen et al. 2004, Nielsen 2010). Weber et al. (1992) and Hughes & Edwards (2010) also froze human skin samples for unspecified amounts of time. Others refrigerated the skin for up to 5 days (Wester et al. 1990b, Zhai et al. 2007, Zhai et al. 2008) in an attempt to maintain viability. However, the remaining majority of the studies attempted to use skin excised as quickly as possible. In one study, rat skin that had been frozen at very low temperatures in liquid nitrogen for 7 or 25 days showed a more pronounced post-washing increase in flux than freshly excised skin (Moody & Ritter 1992). Freezing combined with the longer duration of the study (48 hours) could have damaged the skin barrier to a greater extent than was observed in fresh skin, although the total amount penetrated was similar between the two trials.

It should be noted that living skin is capable of metabolizing significant portions of certain compounds. This metabolism can influence the penetrant's physical properties (lipophilicity, molecular volume) which in turn can change how quickly the metabolite moves through the skin (EPA 1992). Therefore, living skin should be used for dermal absorption studies in which the compound of interest is significantly altered by skin metabolism.

Skin has been estimated to have enzyme activity between 2 and 80% of that of the liver for polycyclic aromatic hydrocarbons such as benzo[a]pyrene (b[a]p), although some studies have shown actual metabolism of b[a]p in the skin is minor (EPA 1992). Extensive hydrolysis of diethyl malonate in the skin has also been documented (EPA 1992). Wester et al. (1990b) and Moody et al. (1995b) utilize b[a]p as a test compound, although both avoid freezing the skin in an attempt to maintain skin viability. Loke et al. (1999) froze skin samples for up to one month prior to applying diethyl malonate. If metabolism was a significant occurrence in viable skin, Loke et al. (1999) might have obtained results that were not reflective of the dermal absorption expected to occur *in vivo*.

Skin preparation method

Harsh hair removal protocols could abrade or damage the skin. Rat skin is often treated prior to the start of the protocol to remove hair (Pelletier et al. 1989, Ritter et al. 1990, Koizumi 1991, Hughes & Edwards 2010). Ritter et al. (1990) treated *in vivo* rat skin with a variety of preparation methods including shaving, clipping, depilatory application and washing 24 hours prior to the dermal absorption trial. No difference was observed in the dermal absorption between rats treated with the various hair removal methods. This supports ECETOC's (1993) and OECD's (2004) assertion that as long as these treatments are separated in time by at least 16 hours from the actual trial of interest, they should have little effect *in vivo*. Of the relevant studies, Koizumi (1991) and Yourick et al. (2004) clipped the skin to remove hair immediately prior to the start of the trials. In the Yourick et al. protocol, skin was clipped immediately after euthanizing the animals. This could have slightly increased the dermal penetration, but less so than the use of other potential hair removal methods that wetted or abraded the skin or exposed it to harsh hair removal chemicals. For *in vitro* samples, any additional manipulation of the skin increases the risk of manual damage to the skin barrier and therefore, hair removal should be attempted *in vivo* prior to skin excision if possible.

Trial duration

Existing *in vitro* trials published in the literature can last anywhere from one minute to seven days. The ideal trial lasts long enough to observe most of the excretion of the compound but not so long that the skin begins to lose its viability and barrier function and is no longer reflective of healthy *in vivo* skin. Additionally, if the goal is to separately identify the initial penetration peak usually observed immediately post-exposure from any peak in flux caused by washing, the trial must be long enough to allow the first peak in percutaneous absorption to fall prior to decontamination. Currently, there is no consensus regarding the accepted time at which skin is considered compromised. Some researchers attempt to determine skin viability by testing for glucose metabolism throughout the trial (Monteiro-Riviere et al. 2001). Others test to ensure the barrier function is still intact by exposing the skin to H_30 (Hughes & Edwards 2010) or measuring capacitance (Nielsen et al. 2004, Nielsen 2010).

Any trial that continues past the point at which skin loses viability or barrier function risks obtaining questionable results that potentially over-predict the penetration or flux of the compound into the skin. Guidance from cosmetics and chemical regulatory agencies (Diembeck et al. 1999, OECD 2004, SCCP 2006) suggest that point occurs sometime after 24 hours, depending on other factors influencing skin viability and barrier function such as storage method or receptor fluid composition.

Of the relevant *in vitro* studies reviewed here, 11 lasted for 48 hours. Of those 11, Nielsen et al. (2004) and Nielsen (2010) had an additional 18-hour period prior to the start of each trial where the skin was allowed to come to equilibrium with the receptor fluid. Hewitt et al. (1995) and Yourick et al. (2004) had even longer durations with each trial terminated at 72 hours. All but one of these studies containing longer duration trials were able to provide information regarding the effect of washing on flux. Loke et al. (1999) and Monteiro-Riviere et al. (2001) were the only trials with sub-48-hour trial lengths that were able to illustrate the effect of washing on flux (24 and 9 hours, respectively). This raises some questions about the post-washing peak observed in trials that washed at 24 hours and followed to a later point. If the skin's barrier function was already becoming compromised at 24 hours, washing may have been able to drive compound through the skin that would have been removed or retained in the stratum corneum layer in healthy *in vivo* skin. This could have resulted in an exaggerated post-washing peak compared to what would have been observed *in vivo* for these studies of longer duration. The *in vitro* studies that provided information regarding washing's effect on total absorption mostly avoided this problem, with only 3 (Hewitt et al. 1995, Nielsen et al. 2004, Nielsen 2010) of the 10 studies containing trials lasting longer than 24 hours.

Washing protocol

As discussed in the introduction to this review, washing can influence the barrier properties of skin. This is especially true in *in vitro* trials where the skin is already over-hydrated or delicate. Harsh mechanical washing processes such as rubbing with dry materials or high-powered rinsing could potentially contribute to skin damage. For example, Weber et al. (1992) washed with both wet and dry cotton balls in their decontamination processes and the

mechanical action of the dry cotton ball actually forced compound deeper into the skin in skin that was already damaged by removal of the stratum corneum. This was not observed in samples that were rubbed with cotton soaked in any of the different types of wash solutions. This negative effect of dry wiping was not observed in intact skin. This supports the idea that skin that was already damaged in some way could be more sensitive to harsh washing methods.

The majority of the reviewed trials washed by rubbing the skin with cotton or some other absorbent material soaked in the washing solution. The solution on the swab would be expected to reduce the friction associated with dry wiping. Pelletier et al. (1989) and Ritter et al. (1990) rubbed with both wet and dry cotton, but damage to the skin would be reduced since the studies were *in vivo*. Some studies rinsed with the washing solution instead of wiping or rubbing the skin (Loke et al. 1999, Monteiro-Riviere et al. 2001, Hughes and Edwards 2010). It is difficult to say whether rinsing could cause more or less damage than wiping. Of the rinsing methods, Monteiro-Riviere et al. (2001) washed at rates of 0.1 to 1 liters per minute for 15 minutes and therefore had the greatest risk of damage to the skin due to the high flow rates and long duration of washing. However, their chosen washing method may have less impact on the results for this study due to the fact that fresh, viable skin was washed after only one hour of perfusion on the diffusion cell. On the other hand, the Moody et al. series of studies which washed at 24 hours used two washing methods. The earlier trials rinsed samples while later trials were rubbed with cotton swabs. The authors state they believed the rubbing protocol potentially disrupted the barrier function of the skin more so than the rinsing procedure (Moody et al. 1994b). This could partially explain why later studies utilizing the mechanical washing procedure showed stronger post-washing increases in flux (Moody et al. 1994b, 1995a, b, Moody & Nadeau 1997). It is difficult to cite this change in washing procedure as the sole reason for the stronger pattern observed in later studies as other components of the trial protocols, including receptor fluid and skin thickness, were changed as well.

Guidance documents suggest replicating the hand soap and water washing solutions used in the field (OECD 2004). However, in the reviewed trials, a variety of washing solutions were used. Twenty-one studies used some sort of surfactant or soap and water solution in at least

some trials. In addition, six studies used plain water, five used a solvent or alcohol solution and three trials used saline solution in some or all of their trials. Weber et al. 1992 used a unique wiping procedure incorporating mineral oil for two trials. Almost all the trials used some sort of aqueous solution for washing, with a few exceptions that used pure ethanol, rubbing alcohol or a combination of oil, wiping and acetone (Maibach & Feldman 1974, Weber et al. 1992, Hewitt et al. 1995). The majority of the soap solutions used hand, liquid or "Ivory" soap, which would be expected to be the type of soap most people use to decontaminate their skin. These should have resulted in minimal injury to the skin, as discussed previously. Studies that used unusual soaps not designed for human skin potentially could cause some irritation, but that would be unlikely due to the short duration of washing and the fact that most trials rinsed samples with water following a soap and water wash. Additionally, washing with solvent, saline or alcohol solutions also may decontaminate or damage skin to a greater or lesser degree than what would be expected under normal washing conditions.

1.E.3. Skin thickness

It is also important that skin is of the correct thickness. Dermatoming skin too thin can result in a greater chance the barrier function will be compromised. OECD (2004) states this can occur in skin around 200 μm thick. Recently, there has also been some concern that the transport of compound through the skin via hair follicles has been underestimated (Lademann et al. 2008). Dermatoming skin could open up the follicles to create a passageway through the skin especially if the skin is also subjected to additional damage, such as harsh mechanical washing (Lademann et al. 2010). Skin samples used for dermal absorption studies usually have vellus hair follicles that terminate in the mid-dermis (EPA 1992, Vogt et al. 2007). Therefore, thinly dermatomed skin, especially skin dermatomed to remove the dermis, should attempt to use mild washing procedures to avoid opening up the potentially compromised follicles. OECD (2004) states that allowing skin to hydrate on an *in vitro* apparatus prior to the start of a trial can cause the skin to swell, thus closing off any open hair follicles. No studies are cited in support of this claim. Diembeck et al. (1999) and OECD (2004) also advise against using skin thicker than about 1000 μm while EPA (1992) suggests removing most of the dermis, especially for lipophilic compounds. *In vitro* preparations of

skin destroy the micro-circulation that perfuses normal *in vivo* skin with blood to solubilize and transport absorbed compound (SCCP 2006). Therefore, using skin samples that are too thick could potentially result in a reduction in the rate or mass of percutaneous penetration into the receptor fluid (EPA 1992).

Skin thickness in the relevant *in vitro* trials reviewed here ranged from between 200 μm to over 2 mm. Studies that used thinner skin included the most recent studies in the Moody et al. series (Moody et al. 1995a, Moody & Nadeau 1997) (300 μm thick for all species), Yourick et al. (2004) (200 - 280 μm for human samples) and Hughes & Edwards (2010) (350 - 360 μm). The thinner skin used in these protocols could be more susceptible to damage and result in greater percutaneous absorption. The rest of the Moody et al. series used 500 μm thick skin except for Moody & Ritter (1992) which used skin between 600 and 900 μm thick. The differences in skin thickness between these protocols could also contribute to the fact that a stronger pattern of post-wash increase in flux was observed in the later studies than in the earlier ones. The thickest skin samples were used by Nitsche et al. (1984) (full thickness with 1 to 2 mm of subcutaneous fat still attached) and Weber et al. (1992) (about 1000 μm). This could have resulted in a decreased driving force for compound through the skin for these trials that used thicker skin, although the effect might be minimal for Weber et al. (1992) as they were on the upper edge of the recommended thickness cited above. This reduced driving force could potentially have a greater impact for Nitsche et al.'s (1984) results since recovery in the receptor fluid was not reported for trials in that study and only recovery in the viable skin layers was used to estimate total penetration. The reduced driving force could potentially have caused more compound to stay in the upper skin layers, specifically the stratum corneum, than would have been the case if the trial had used thinner skin samples. This could have led to the under-estimation of total percutaneous absorption in the unwashed trial which had nominally more compound in the upper layer than the washed trial.

1.E.4. *In vitro* Diffusion Apparatus

Removal of Skin

When conducting an *in vitro* study, removing the skin from the diffusion cell during a trial can impact the movement of applied compound into the receptor fluid and is inadvisable (Diembeck et al. 1999). If an external surface reservoir is present, it may be mechanically transferred to the diffusion cell or the side of the skin directly contacting the receptor fluid. This could allow more compound to enter the receptor fluid than would otherwise penetrate through the skin alone. Excessive handling of the skin may also damage the skin or result in the transfer of compound to other implements and reduce the total amount of compound recovered overall for the trial.

Of the 20 relevant *in vitro* studies reviewed here, 4 removed the skin from the apparatus in order to wash it. This would have little impact on the results of Wester et al. (1990b) since the skin was washed at the termination of the trial and Weber et al. (1992) since the skin was resting on gauze instead of placed in a diffusion cell. However, it could have had a larger influence on the results of the two other studies. Monteiro-Riviere et al. (2001) removed skin from the diffusion cell where it had been equilibrating prior to the start of the trial to apply compounds and then immediately wash for 15 minutes. Therefore, data are not available regarding the flux through the skin during the application and 15-minute wash times because samples were removed from the diffusion cell during those tasks. This could have resulted in the loss of some compound that might have penetrated through the skin before its return to the diffusion cell, especially since the washing method was rather vigorous and long in duration. Additionally, the samples that produced their peak receptor fluid recoveries immediately after replacing the skin on the diffusion cell potentially could be observing the peak flux late if it occurred during washing or the observed peak could have been elevated due to accidental mechanical transfer of the compound into the receptor fluid. Nitsche et al. (1992) also removed the skin from the diffusion cell for washing, but did so near the midpoint of the trials (3 hours for the 10-hour trial and 10 hours for the 24-hour trial). The extra handling of the skin could have caused some damage due to its more hydrated state following perfusion. This potentially could have resulted in increased movement of compound into the lower levels of the washed sample compared to the unwashed sample. As

discussed above, this could have resulted in an overestimation of absorption in the washed trial since skin recovery in the viable layers was used as an approximation of total percutaneous absorption.

Composition of Receptor Fluid

One of the limitations of *in vitro* trials is that perfusing dermatomed skin with receptor fluid does not always accurately replicate the same chemical sink provided by perfusion of live *in vivo* skin with blood (OECD 2004). Therefore, care must be taken in choosing a receptor fluid that mimics the ability of blood to solubilize compounds. According to various guidance documents (EPA 1992, Diembeck et al. 1999, OECD 2004, SCCP 2006), saline solution can be used if the compound of interest is not very lipophilic and if skin viability is not necessary. Otherwise, a receptor fluid such as a growth medium that provides nourishment for viable skin preparations is required. Components such as bovine serum albumin or ethanol can be added to help solubilize lipophilic compounds of interest. If a receptor fluid additive such as ethanol is chosen that could negatively affect the barrier function of the skin, the potential risks and benefits should be weighed and alternative options considered (SCCP 2006). Receptor fluids can have varying abilities to solubilize compounds with different octonal/water partition coefficients as well as varying capabilities of maintaining skin viability and barrier function.

The reviewed *in vitro* studies used a variety of the above receptor fluid combinations. For example, Moody et al.'s series of studies (Moody & Ritter 1992, Moody et al. 1994a, b, 1995a, b, Moody & Nadeau 1993, 1994, 1997) used Ringer's saline solution with glucose and antibiotics in the early studies. Later studies switched to Hanks' HEPES buffered growth medium with BSA, $NaHCO_3$ and antibiotics. The Hanks' medium was better able to maintain skin viability and also contained BSA to increase the solubility of lipophilic compounds in the receptor fluid. This could partially explain the smaller peaks in penetration in the earlier trials that did not have a compound like BSA in the receptor fluid but applied the lipophilic compounds fenoxaprop-ethyl (log K_{ow} of 4.58) or diazinon (log K_{ow} of 3.81). Outside of the Moody et al. series, six other *in vitro* trials used saline solution without any additive to help solubilize lipophilic compounds (Nitsche et al. 1984, Weber et

al. 1992, Hewitt et al. 1995, Loke et al. 1999, Zhai et al. 2007, 2008). Therefore, the receptor fluid used in these studies was not representative of the *in vivo* condition. The lack of solubility for lipophilic compounds would be expected to have a larger impact on dermal absorption for the trials that used compounds such as MbOCA (log K_{ow} of 3.91), lindane (log K_{ow} of 3.72) and TCDD (log K_{ow} of 6.8) and less effect on more hydrophilic compounds such as glyphosate (log K_{ow} of -3.4) and formaldehyde (log K_{ow} of 0.35). This is especially important to note for the studies that estimated total percutaneous absorption via skin or receptor fluid recovery only (Nitsche et al. 1984, Weber et al. 1992, Loke et al. 1999). The saline solution used as the receptor fluid in these trials may have resulted in retention of more compound in the skin than would otherwise be expected and could have slowed the flux from the topmost layers into the lower layers as the concentration gradient declined. This could have in turn resulted in an underestimation of dermal penetration in these studies. Monteiro-Riviere et al. (2001) did not indicate the receptor fluid used, but since the compound of interest was phenol (log K_{ow} of 1.46), saline solution would likely be sufficiently able to solubilize the compound. The lack of consistency in choice of receptor fluid makes comparing the effects of washing on various *in vitro* studies more difficult, especially when combined with the variety of other potential differences in protocol.

Flow Rate of the Receptor Fluid

In addition to receptor fluid content, flow-through diffusion cells usually utilize a flow rate that is designed to move saturated receptor fluid away from the dermal surface of the skin (OECD 2004). This allows for a concentration gradient to form across the skin as compound moves into the receptor fluid and provides a driving force for percutaneous uptake into the fluid similar to that seen *in vivo* into the blood (EPA 1992). OECD (2004) suggests a flow rate that results in at least 3 changes of the receptor fluid per hour for diffusion cells with a volume of 3 ml and 5 to 10 changes an hour for cells that contain 0.15 to 0.3 ml of fluid, although they state that a flow rate that results in 1 full change per hour can be acceptable in certain cases. Static cells attempt to create a similar gradient by utilizing larger volumes between 2 and 20 ml and incorporating a stirring mechanism to fully mix the fluid in the chamber (OECD 2004, SCCP 2006). Therefore, when designing a study, the volume and

flow of receptor fluid to which the skin is exposed over the duration of the trials should be large enough to maintain a sink.

Of the reviewed studies, most were flow through designs that adhered to OECD's (2004) suggestions for flow rates. Monteiro-Riviere et al. (2001) did not provide the necessary information to determine if the flow-through diffusion apparatus maintained a proper sink. Zhai et al. (2007, 2008) used a static diffusion cell design which did not sample receptor fluid until the end of each trial. However, the short duration of these trials and minimal penetration into the skin may have minimized the need for a greater sink. Nielsen (et al. 2004, 2010) also used a static diffusion cell but noted that they stirred the receptor fluid in each cell as well as sampled and replaced it at various intervals using a port, which helped to maintain a sink for compound. The static design could have been of greater concern for Nitsche et al. (1984). They do not mention stirring the receptor fluid or sampling and replacing it even in the longer 10- or 24-hour trials. This could have reduced the ability of the fluid to act as a sink for lindane (log K_{ow} 3.7) and could have resulted in less compound penetrating to and through the deeper skin layers. Finally, Weber et al. (1992) did not have receptor fluid at all and instead placed skin samples on saline-soaked gauze. Therefore, it was not possible to determine if any compound passed completely through the skin and the lack of a moving liquid also means a smaller sink may have been present into which the compound could move. Both Nitsche et al. (1984) and Weber et al. (1992) recovered compound in the lowest layers of the skin sample, and therefore were of long enough duration for compound to penetrate through the skin sample and potentially into the receptor fluid.

Temperature

Guidance documents suggest conducting all *in vitro* experiments at normal skin temperature (Diembeck et al. 1999, SCCP 2006). *In vivo* healthy skin is maintained at a temperature of approximately 32 to 34°C (OECD 2004). *In vitro* protocols that elevate the skin temperature may also elevate the dermal absorption of the compound of interest (Chang & Riviere 1991). Unless this is a desired effect to mimic dermal absorption in strenuous or hot work environments, skin temperature should be maintained as close to the normal range as

possible. Additionally, *in vitro* set-ups should be designed to heat the receptor fluid in such a manner as to achieve a similar skin-surface temperature to help maintain skin viability and to attempt to accurately replicate any volatilization that may occur *in vivo*.

The majority of the relevant reviewed studies maintained skin within the normal *in vivo* temperature range. Hughes & Edwards (2010) kept skin slightly warmer at 35 °C while temperature of the *in vitro* apparatus was not provided by three of the studies (Monteiro-Riviere et al. 2001, Zhai et al. 2007, 2008). Of note, increased absorption of compound through the skin may have occurred in the six studies that maintained the *in vitro* skin at the internal body temperature of 37 °C (Wester et al. 1990b, Moody & Ritter 1992, Moody & Nadeau 1993, 1994, Moody et al. 1994a, b). These samples appear to have been kept in an oven or incubator. However, Wester et al. (1990b) may have only used receptor fluid heated to 37 °C, which, when run through an otherwise room temperature setup, could produce a skin surface temperature of 32 °C (Bhatt et al. 2008).

1.E.5. Air flow

To accurately reproduce the normal circumstances surrounding dermal exposure, air should be allowed to move across the skin in both *in vivo* and *in vitro* trials unless attempting to replicate occlusive conditions. This is especially important since occlusion can result in excessive hydration and reduced loss to volatilization, which results in increased dermal penetration (EPA 1992, OECD 2004). If the compound is volatile, the air should also be collected in order to calculate the mass balance or the total percent of the applied dose recovered at the end of a trial. This mass balance is an important check to ensure a trial was well designed and the penetration of the compound was accurately determined. The mass of compound lost due to evaporation off the skin surface at experimental temperatures can be a large percentage of the applied compound for volatile chemicals (EPA 1992). Therefore, it is important to quantify this loss along with the amount recovered in the wash, skin and receptor fluid.

In the reviewed studies, the formaldehyde used by Zhai et al. (2007) is quite volatile but loss to the air was not quantified. Following the completion of the trials, Zhai et al. (2007) attempted to determine the volatilization from plastic disks over time to adjust for their low

percent recoveries at later times. The earlier studies in the Moody et al. series also used a device to maintain air flow and quantify volatilization (Moody & Ritter 1992, Moody & Nadeau 1993, 1994, Moody et al. 1994a, b). They recovered non-trivial percentages for DEET and diazinon in the air traps. Some studies also prevented air from flowing over the skin surface by capping the diffusion cell apparatus (Nielsen et al. 2004, Nielsen 2010). Koizumi (1991) used a protective Teflon film and covering *in vivo* to prevent grooming of the exposed site by the animals. This covering was most likely occlusive and impeded air flow. The resultant skin hydration caused by the lack of exposure to moving air would have artificially increased dermal penetration of the compound compared to a non-occlusive situation.

1.E.6. Compound Application

Vehicle Volume and Type

The amount and type of vehicle chosen can influence the dermal penetration of the compound of interest. OESC (2004) and SCCP (2006) guidance documents suggest applying compound in a vehicle loading less than 10 $\mu l/cm^2$ to avoid the vehicle pooling on or running off of the skin, resulting in an over-estimation of applied dose. Volatile solvents or small amounts of water can also quickly evaporate, leaving behind pure compound and minimizing hydration of the stratum corneum. This is more representative of exposures from physical contact with contaminated items. On the other hand, the use of an excess of water or other non-volatile compound such as commercial formulations or lotions as a vehicle could change skin properties. Utilizing an *in vitro* diffusion cell that maintains occluded conditions and especially that holds fluid in the donor chamber against the stratum corneum will hydrate and change the barrier function of the skin, potentially increasing dermal absorption (EPA 1992, ECETOC 1993). Therefore, this choice of vehicle is more reflective of absorption of compounds from bathing or swimming. Some thicker lotion-like commercial formulas can also occlude the skin. When attempting to determine the effects of washing on dermal absorption, protocols that utilized vehicles that could damage or hydrate the skin could be more difficult to interpret. This is especially true if the effects of washing are due to temporary hydration or damage to the skin, conditions that would be exaggerated for skin exposed to these vehicles.

Of the reviewed studies, 18 applied compound in volatile solvents (5 to 50 μl/cm^2) or small amounts of water (3 to 6 μl/cm^2). Nielsen (2010) applied compound in 50 μl of water per cm^2 of exposed skin and some was still remaining on the skin at the 6-hour wash. Nielsen et al. (2004) applied compound in 283 μl of water per cm^2 of exposed skin. This excess of water remained in contact with the skin until it was removed at 6 or 24 hours for the washing step. Therefore, in these studies, the skin had hydrating aqueous solution in contact with some portion of the stratum corneum for a non-trivial amount of time. This could have resulted in the over-hydration of the skin and a greater potential for damage via the mechanical aspects of washing. Monteiro-Riviere et al. (2001) used 400 μl of 89% aqueous phenol to dose the skin, but applied the solution via soaked gauze. Therefore, it is unlikely any liquid remained on the skin after the gauze was removed at one minute and any effect of the vehicle volume would have been minimal. Four additional studies applied compound in 3 to 78 μl or 8 mg of commercial formulation per cm^2 of exposed skin (Nitsche et al. 1984, Moody et al. 1995a, Moody & Nadeau 1997, Yourick et al. 2004). The potential effects of using these formulations as vehicles is uncertain, as the thicker formulas could have partially occluded the skin while other ingredients interacted with the skin or compound of interest to slow or increase its absorption.

Protection of the Dosed Area

In *in vivo* trials, the exposed area should be protected prior to washing so experimental animals will not groom themselves and ingest compound and so that human subjects will not accidentally rub off part of the dose onto clothing or other objects in their environment. Most experiments using animal subjects apply a guard that protects the dosed area without actually touching the exposed skin (Pelletier et al. 1989 Ritter et al. 1990, Koizumi 1991). This is more difficult for human subjects and can influence experimental results. For example, in the relevant portion of Wester et al. (1992b), isofenphos was applied to subjects, allowed to dry and then subjects permitted to go about their normal activities without any device protecting the skin from rubbing against clothing or other items. This could have resulted in some portion of the applied dose being removed or spread over a larger surface area via mechanical action, especially if the contact occurred while an unbound surface reservoir of the compound was still present. This could explain the fact that there was very

little difference between the total percent absorption in the two washing groups – most of the superficial dose could have been lost prior to even the 24-hour wash. In this case, the compound could have been removed at similar times between the two groups, resulting in shorter, similar contact times instead of the expected windows of exposure (24 versus 72 hours). This is supported by the very low total percent recoveries in the cumulative urine, wash and (when applicable) tape strip samples for the 24- and 72-hour wash groups (2.55% and 1.97%, respectively) compared to data from a washing efficacy portion of the study, where recoveries of 61.4% to 30.3% were observed in the wash alone for subjects who were washed at 0 to 8 hours in the laboratory without allowing the dosed skin to contact any clothing. A similar issue could have occurred in Maibach & Feldman's (1974) trials.

Table 9 and Table 10 provide an overview of the methods used in the reviewed studies that could potentially influence the experimental results obtained and therefore deserve further consideration.

In these tables, "X" denotes a study in which all relevant trials used protocols that potentially could have influenced the results obtained for dermal absorption following washing. If only some of the trials in the study used methods discussed above as potentially of concern, an "S" is used. Finally, a "?" denotes trials that did not provide information relating to that particular component of their methods. The aspects of the experimental protocol highlighted in these tables reflect those discussed above and are not meant to address all potential issues that could arise from the choice of various experimental designs.

More potential areas of concern are found in *in vitro* protocols than *in vivo* experimental designs. Additionally, many of these problems are exacerbated by a trial of longer duration. Therefore, protocol design problems have a greater chance of impacting the post-washing peak flux through the skin, since that particular effect of washing is best observed in an *in vitro* trial that extends beyond the time at which the normal penetration peak has passed prior to washing.

Table 9. Overview of Additional Considerations for *In Vitro* Studies

Citation	Skin Type	Skin Thickness	Skin Prep.	Skin Storage	Diffusion Set up				Air Flow/ Occlusion	Commercial Vehicle	Vehicle Loading	Duration of Trial	Wash Sol'n Volume	Solvent Wash Sol'n
					Receptor Fluid Type	Temp	Receptor Fluid Flow	Skin removed for Wash						
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed* and Flux														
Loke et al. (1999)				X	X								X	
Monteiro-Riviere et al. (2001)	X	?			?	?	?	X					X	S
Nielsen (2010)				X					X		X	X	?	
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed*														
Nitsche et al. (1984)		X		?	X		X	X		X			?	
Wester et al. (1990b)						X								
Weber et al. (1992)		X		X	X		X							S
Hewitt et al. (1995)	S	X			X							X	?	X
Nielsen et al. (2004)				X					X		X	X	?	?
Zhai et al. (2007)					X	?	X							
Zhai et al. (2008)					X	?	X							
Potentially Provides Information for the Evaluation of the Effect of Washing on *Flux*														
Moody & Ritter (1992)	S			S	X	X						X		
Moody & Nadeau (1993)	S				X	X						X		
Moody et al. (1994a)	S					X						X		
Moody et al. (1994b)	S					X						X		
Moody & Nadeau (1994)	S				X	X						X		
Moody et al. (1995a)	S	X								X		X		
Moody et al. (1995b)	S											X		
Moody & Nadeau (1997)	S	X								X		X		
Yourick et al. (2004)	X	X	X							X		X		
Hughes & Edwards (2010)	S	X		S		X						X		

Table 10. Overview of Additional Considerations for *In Vivo* Studies

Citation	Skin Type	Skin Prep.	Protection of Dosed Area	Air Flow/ Occlusion	Vehicle	Vehicle Loading	Wash Sol'n Volume	Solvent Wash Sol'n
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed* and *Flux*								
Pelletier et al. (1989)	X							
Ritter et al. (1990)	X							S
Potentially Provides Information for the Evaluation of the Effect of Washing on *Total Mass Absorbed*								
Maibach & Feldman (1974)			?				?	S
Koizumi (1991)	X	X	?	X			?	
Wester et al. (1992 b)			X				?	

1.F. Impacts of Findings and Conclusions

All relevant reviewed studies had differences in protocol that potentially could have influenced their results. Despite these differences, a few overall patterns emerged from their data. The current published literature suggests that washing skin exposed to contaminants will result in a significant decrease in total mass of compound absorbed into the body. This trend is particularly strong when exposed skin is washed quickly. Delaying the wash may still nominally decrease the total mass absorbed, and definitely does not increase the systemic dose over the control. However, the most pronounced decrease is observed for samples washed earlier. Washing's ability to decrease the total mass absorbed should assist in reducing the risk of chronic health effects to exposed persons who wash their skin. The published literature also suggests that washing contaminated skin could potentially temporarily increasing flux through the skin post-washing. While the data that support the temporary spike in flux following decontamination are weak, there is the potential that it could exist under some circumstances. Even if washing does increase flux temporarily, the health consequences of this effect are questionable, especially for compounds for which low-level, chronic exposures are of primary concern. The impact of the temporarily increased blood levels is likely to be of greatest concern for very acutely toxic compounds like nerve agents for which peak concentrations have been correlated with negative health outcomes. However, immediate washing with soap and water may be preferable over delaying decontamination even for nerve agents. Hassen et al. (2006) demonstrated that washing with a 1% soap and water or 0.5% bleach and water solution following a 2-minute exposure to the nerve agents Soman, VX and VR in guinea pigs (*in vivo*) was more protective than not washing. Therefore, when specialized cleaning agents are not available, water washing would be expected to be able to mitigate the effects of the nerve agents.

The observed differences in protocol and somewhat conflicting data highlight the importance of quantifying exactly how washing influences the movement of chemicals through living skin in order to accurately assess occupational health risks and advise workers. Further research is needed in this area. A standardized protocol for use in all washing studies would assist in comparisons between studies. Future studies investigating the effects of washing would provide the most helpful information if type 1A designs were used. For type 1A *in*

vivo trials, subjects (preferably human) would ideally be washed at multiple times and the recoveries reported in the washing solution, urine (and feces if applicable) excretion as well as any other potential loss of the compound to attempt a mass balance. *In vitro* trials would also ideally attempt to determine the normal flux through the skin for a compound if possible using the above guidelines to preserve barrier function and wash the samples immediately following or during the decline of the normal flux curve. This would help determine if the post-washing peaks observed in the reviewed studies were artifacts of reduced skin barrier function.

Because so few *in vivo* human studies were found in the published literature, an *in vivo* human type 1A study was designed and implemented as part of this thesis work to help fill that gap. Experimental methods are detailed in Appendix B, pending results.

Chapter 2. Application of Dermal Absorption Modeling to *in vivo* Human Experimental Results

2.A. Introduction and Background

Important patterns regarding the effects of washing on dermal absorption through skin can be inferred from data found in the published literature. Just as vital are the mathematical models that are used to explain the causes behind the observed trends. These models, once developed and evaluated against existing data, can then be applied to predict exposures via the dermal route for which no empirical evidence exist.

The movement of chemicals through the skin is modeled as diffusion into and through a membrane. The differential equations required for this classical approach were originally solved into analytical solutions by Crank in his text *The Mathematics of Diffusion*, first published in 1950. These solutions have been interpreted and applied to dermal absorption problems by many subsequent researchers. One family of applied equations as written by Herkenne et al. (2007) and Bunge (personal communication, 2011) can be used to model a simple example case of dermal absorption. Herkenne et al.'s (2007) equations provide predictions which can be used to estimate the concentration profile in the stratum corneum at a particular point in time while Bunge's (personal communication, 2011) equations can be used to predict the mass of the compound that has penetrated into and out of the stratum corneum over time. Bunge's model provides two different equations for mass out of the skin layer – one for a situation in which an excess of compound is on the skin surface and one that models the penetration of compound out of the skin once that external reservoir of chemical has been removed.

As discussed in Chapter 1, skin is a series of several layers: the stratum corneum, viable epidermis and dermis. However, as implemented in this section, Herkenne et al. and Bunge's equations assume that the stratum corneum is the only barrier to diffusion. The amount of compound that passes into and out of this layer is thought to represent the mass in and out of the entire exposed skin area. This assumption is most applicable to compounds that are of low or middling lipophilicity and can easily pass through the viable skin compared to the stratum corneum. This model also does not take into account hydration of the skin due to application vehicles or washing solutions. Therefore, the model should only be employed for

cases in which compounds are applied in volatile vehicles or extremely small amounts of water that evaporate before hydration can occur. Washing should also be of shorter duration to limit skin hydration if modeling extends past that point. Finally, loading of a compound on the skin surface is not a term incorporated into this particular basic model. Instead, the mass term in both Herkenne et al.'s and Bunge's equations assume a constant external driving force provided by an excess of compound on the external boundary of the skin. This is most applicable to cases in which exposure duration is short, the compound is less volatile and loading of compound is high.

The predictions of these equations are compared to the results from three experimental studies (Selim et al. 1995, 1999, Meuling et al. 2005), and the differences between predicted and observed data are discussed. The models are then used to predict the results expected from the *in vivo* human DEET exposure study completed by this laboratory and described in Appendix B. To summarize, either 500 or 3,000 μg of DEET in 400 μl of ethanol were applied to 100 cm^2 of forearm skin. Skin was washed after either 10 or 40 minutes by either deionized (DI) water, 5% (v:v) soap and DI water solution or 10% (v:v) ethanol and DI water solution. Skin was wetted with 6 ml of one of the aforementioned solution, rubbed for 10 seconds with a dry cotton ball and rinsed with 25 ml of DI water for the water or soap solution or 25 ml of ethanol solution for subjects washed with the same. Urine was collected for 5 days or 120 hours post-exposure (24 hours pre-exposure background sample plus 96 post-exposure).

2.B. Methods

Both Herkenne et al. and Bunge rely on several basic relationships between the physicochemical properties of compounds and their movement through the skin membrane in their equations. For instance, the diffusivity (D_{sc}; $\mu m^2/hr$) of a compound through the stratum corneum (Eq. 1) is estimated here by the permeability of said compound from the application vehicle into the stratum corneum ($P_{sc/w}$; cm/hr), the depth of the stratum corneum (L; μm) and the stratum corneum-vehicle partition coefficient ($K_{sc/w}$; unitless). In examples for which a compound is applied in a volatile vehicle that quickly leaves pure compound remaining on the skin, these permeability and partition coefficients assume water is the vehicle. Both the permeability and partition coefficients are functions of the

physicochemical properties of the compound and the characteristics of the skin membrane. The permeability coefficient (Eq. 2) is calculated here from the octanol/water partition coefficient (K_{ow}; unitless) and the molecular weight (MW; g/mol) via the modified Potts-Guy equation (Potts & Guy 1992). Therefore, the permeability coefficient is related to a compound's lipophilicity and molecular weight, which is a surrogate for molecular volume (EPA 2004). The relationship between these physicochemical properties and the modified Potts-Guy permeability coefficient has been characterized in the form of a regression on the K_{ow} and molecular weight of permeability coefficients determined using experimental results for 90 compounds. The Potts-Guy equation is most defensively applied to compounds that have physicochemical properties within the "effective predictive domain", a range of values that is similar to those found in the original dataset. Even for compounds within that domain, there is a large amount of uncertainty incorporated into the Potts-Guy relationship. The stratum corneum-water partition coefficient (Eq. 3) is calculated from the octanol-water partition coefficient only.

$$D_{sc} = \frac{P_{sc/w} * CF * L}{K_{sc/w}} \quad (1)$$

$$\log P_{sc/w} = -2.8 + 0.66 * \log K_{ow} - 0.0056 * MW \quad \text{[cm/hr]} \quad (2)$$

$$\log K_{sc/w} = 0.74 * \log K_{ow} \quad (3)$$

A conversion factor (CF) of 10^3 μm/cm is utilized to obtain the diffusivity in the desired units ($\mu m^2/hr$).

2.B.1. Stratum Corneum Concentration Profiles

Herkenne et al. utilized simplified equations that considered the composition of the stratum corneum to be a homogenous membrane. These equations provided the stratum corneum concentration (C_x; $\mu g/cm^3$) at various normalized depths (x/L) in the stratum corneum at a chosen time t (hours), where x is the number of specified slices at which a concentration is calculated (Eq. 4).

$$C_x = K_{sc/v} C_v \left\{ \left(1 - \frac{x}{L}\right) - \frac{2}{\pi} \sum_{m=1}^{\infty} \frac{1}{m} \sin\left(\frac{m\pi x}{L}\right) \exp\left(\frac{-D_{sc} m^2 \pi^2 t}{L^2}\right) \right\} \quad (4)$$

As discussed above for this analysis, compounds are assumed to be applied in volatile vehicles to avoid hydration effects on the stratum corneum. Herkenne et al.'s equations convert the concentration in the vehicle to the stratum corneum concentration using the stratum corneum-vehicle partition coefficient. In the case of pure compound on the skin, the stratum corneum concentration is saturated. This condition is best modeled by defining the concentration of compound in the vehicle as the saturation concentration of the compound in water and $K_{sc/v}$ as the stratum corneum-water partition coefficient.

The area under the curve (AUC; $\mu g/cm^3$) for the chemical concentration profile in the stratum corneum is estimated by equation 5. This AUC is the average concentration of the compound in the stratum corneum at time t.

$$AUC = K_{sc/v} C_v \left\{ \frac{1}{2} - \frac{4}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(- \frac{(2n+1)^2 \pi^2 D_{sc} t}{L^2} \right) \right\} \qquad (5)$$

The total mass in the stratum corneum (M_{sc}; μg) at time t can also be calculated for the area under the curve if the exposed area (A_{ex}; cm^2) is known (Eq. 6).

$$M_{sc} = AUC \cdot L \cdot CF \cdot A_{ex} \qquad (6)$$

Here a conversion factor required of 10^{-3} cm/μm is required, given concentration in the units outlined above. Herkenne et al.'s equations only provide information on the mass and profile of the contaminant in the stratum corneum at one particular point in time. Other equations are required to determine the cumulative mass that has penetrated into and out of the layer over time.

2.B.2. Cumulative Penetration of Mass Over Time

Bunge's equations provide such a solution by determining the total mass into and out of the stratum corneum over time. This approach utilizes non-dimensional time and mass in order to produce a general form of the model for various situations and compounds. Non-dimensional time (τ; unitless) is calculated in equation 7 by dividing each time point by the characteristic time of diffusion (t_{Dc}; hours), which is a function of the depth of the stratum corneum (L; μm) and the diffusivity (D_{sc}; $\mu m^2/hr$) of that particular compound (Eq. 8).

$$\tau = \frac{t}{t_{Dc}} \qquad (7)$$

$$t_{Dc} = \frac{L^2}{D_{sc}} \qquad (8)$$

Non-dimensional mass into the stratum corneum ($\tilde{M}_{in}$; unitless) over non-dimensional time (τ) can be calculated for situations in which a constant concentration of compound is maintained on the skin surface by a reservoir of chemical (Eq. 9). Therefore, this equation only applies prior to the non-dimensional time of washing (τ_c).

$$\text{when } \tau \le \tau_c,\ \tilde{M}_{in} = \tau + \frac{1}{3} - 2\sum_{m=1}^{\infty} \frac{\exp\left(-m^2\pi^2\tau\right)}{m^2\pi^2} \qquad (9)$$

In this analysis, it is assumed that no mass enters the stratum corneum post-washing. It also assumes that no mass that has been absorbed into the skin is lost to the wash solution or external environment during or after the wash. However, Bunge does provide equations to calculate the movement of compound out of the stratum corneum and into the systemic circulation, again assuming no resistance from the viable epidermis. Equation 10 assumes a constant external driving force and therefore was only utilized prior to washing. On the other hand, equation 11 assumes no external driving force and therefore was used to determine the absorption of the compound remaining in the skin post-washing.

$$\text{when } \tau \le \tau_c,\ \tilde{M}_{pre-out} = \tau - \frac{1}{6} - 2\sum_{m=1}^{\infty} \frac{(-1)^m \exp\left(-m^2\pi^2\tau\right)}{m^2\pi^2} \qquad (10)$$

$$\text{when } \tau > \tau_c,\ \tilde{M}_{post-out} = \tau_c + \frac{1}{3} - 2\sum_{m=1}^{\infty} \frac{(-1)^m \exp\left(-m^2\pi^2\tau_c\right)}{m^2\pi^2} \qquad (11)$$

$$-4\sum_{n=0}^{\infty} \frac{(-1)^n \left(1 - \exp\left[-\lambda_n^2(\tau - \tau_c)\right]\right)}{\lambda_n} \left\{ \sum_{m=1}^{\infty} \frac{\exp\left(-m^2\pi^2\tau_c\right)}{m^2\pi^2 - \lambda_n^2} \right\}$$

$$-2\sum_{n=0}^{\infty} \frac{(-1)^n \exp\left[-\lambda_n^2(\tau - \tau_c)\right]}{\lambda_n^3}$$

where

$$\lambda_n = \frac{(2n+1)\pi}{2} \qquad (12)$$

The resultant non-dimensional cumulative mass ($\tilde{M}_x$; unitless) can be converted to the appropriate units (M_x; μg) for a relevant example using the stratum corneum concentration at equilibrium with pure compound ($K_{sc/v}C_v$; μg/cm^3) and the volume of the exposed area (Eq. 13).

$$M_x = \tilde{M}_x \cdot K_{sc/v}C_v \cdot A_{ex} \cdot L \cdot CF \qquad (13)$$

This equation can be used to convert non-dimensional mass into units for either mass moving into or out of the membrane (where subscript "x" is "in" OR "pre-out" OR "post-out"). A conversion factor of 10^{-3} cm/μm is also required in this case to produce the appropriate units.

Prior to the time of wash, subtracting $M_{pre\text{-}out}$ from M_{in} was equivalent to the mass in the stratum corneum (M_{sc}) as calculated from Herkenne et al.'s equation for the AUC (Eq. 6).

2.B.3. Comparison of the Model to Published Studies

The above equations were entered into MATLAB® version R2009b by MathWorks. Detailed code is provided in Appendix C. Physicochemical properties and experimental conditions from three studies were entered into the model for three successive runs. All three studies were *in vivo* human designs that collected excreta to determine total absorption of the compound of interest through the skin. Meuling et al. (2005) applied either 5,390 or 16,150 μg of chlorpyrifos in 500 μl of ethanol to 100 cm^2 of forearm skin. Skin was washed after 4 hours by wiping 10 times with cotton wool soaked in water. Urine was collected for 5 days or 120 hours total post-exposure. Recoveries in the urine and wash solution were determined.

Selim et al. (1999) applied 2983 μg of piperonyl butoxide in 100 μl of isopropyl alcohol to 24 cm^2 of forearm skin. Skin was washed after 8 hours by wiping with isopropyl alcohol-soaked cotton swabs and likely rinsing with isopropyl alcohol (washing protocol not explicitly stated but a rinsing step is assumed based on context clues and references to previous studies). Urine and feces were collected for just over 5 days or 128 hours post-

exposure. Recoveries in the wash solution, stratum corneum, dosing materials and excreta were determined, as well as the total percent of the applied dose recovered.

Selim et al. (1995) applied 15,000 μg (15 μl) of pure DEET or 12,000 μg of DEET in 100 μl of ethanol to 24 cm^2 of forearm skin. Skin was washed after eight hours by wiping with isopropyl alcohol-soaked cotton swabs and rinsing with isopropyl alcohol. Urine and feces were collected for just over 5 days or 128 hours post-exposure. Recoveries in the wash solution, stratum corneum, dosing materials and excreta were determined, as well as the total percent of the applied dose recovered. The physicochemical properties of these three compounds are presented in Table 11.

Table 11. Physicochemical Properties of Selected Compounds

Compound	CAS No.	$logK_{ow}$ (--)	MW (g/mol)	C_w ($\mu g/cm^3$)
chlorpyrifos	2921-88-2	4.94	350.6	0.925
piperonyl butoxide	51-03-6	4.75	338.4	14.3
DEET	134-62-3	2.18	191.3	1911

Model predictions were plotted and compared to the experimental data presented in the studies. Plots of the concentration profile and cumulative mass in and out of the stratum corneum were also generated to predict the results expected for the *in vivo* human study completed as part of this work.

2.C. Results

2.C.1. Chlorpyrifos

Meuling et al. (2005) exposed human volunteers to chlorpyrifos for four hours prior to decontamination. For those experimental conditions, Bunge's equations predicted that 96 μg of chlorpyrifos would be present in the stratum corneum at the time of washing but none would have yet penetrated into the systemic circulation. Over the remaining 116 hours, the model predicts only 65 μg would pass through the skin and into the blood. This would leave

31 μg in the skin at 120 hours. Figure 7 shows the predicted change in mass into and out of the skin over the duration of the chosen experimental study.

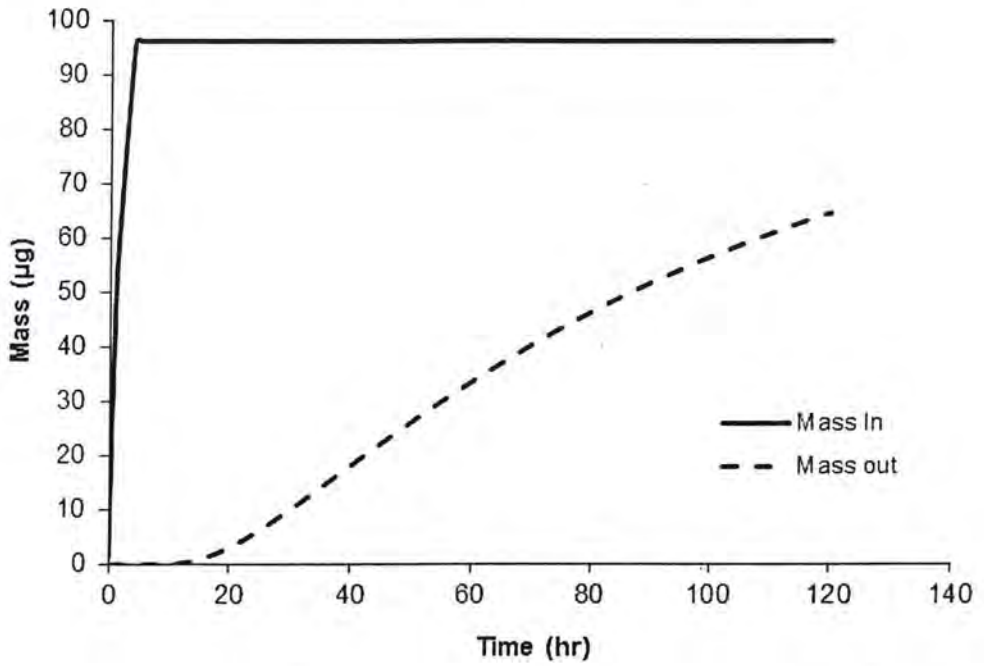

Figure 7. Cumulative Mass of Chlorpyrifos Into and Out of the Stratum Corneum Following Washing at 4 Hours. Estimated using Meuling et al. (2005) experimental conditions.

The cumulative mass into the stratum corneum layer increases rapidly up until the time of washing. At that point, no further mass enters the skin, resulting in a slope of zero for the remainder of the observation period. Compound begins to pass out of the stratum corneum membrane at about seven hours and slowly increases in penetration rate. By the end of the observation period, the rate at which the mass was moving out of the skin was beginning to slow despite the prediction that about one-third of the total mass was still remaining in the skin.

Using Meuling et al.'s (2005) experimental conditions, the results of Herkenne et al.'s equations predict that a steady state gradient had not yet been established at the time of washing. This is demonstrated by the concentration profile in the stratum corneum (Figure 8).

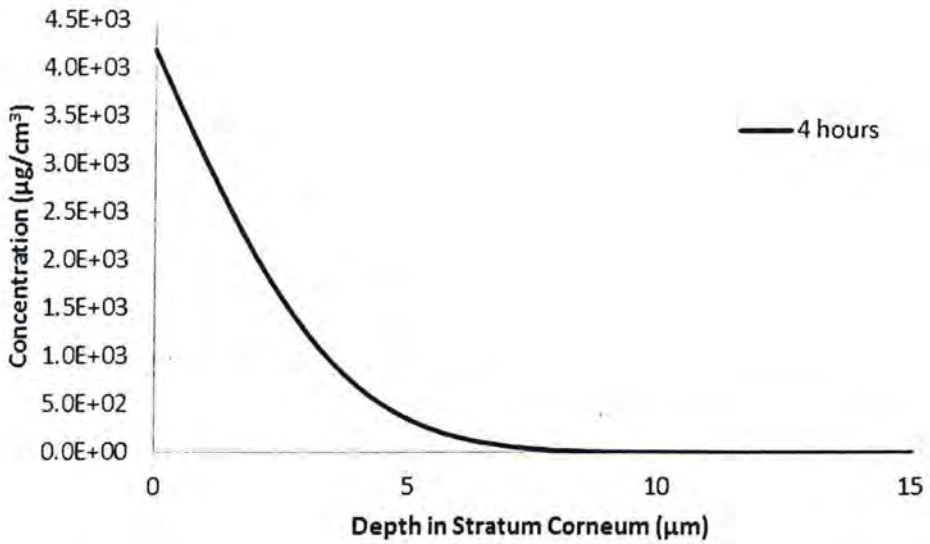

Figure 8. Concentration Profile of Chlorpyrifos in the Stratum Corneum at 4 hours. Estimated using Meuling et al. (2005) experimental conditions.

The concentration profile in the top layer of skin is predicted to reach nearly 0 µg/cm^3 at a depth of about 14 µm. Experimentally, 233 µg (4.3% of the applied dose) was recovered in the urine when 5,390 µg were applied and 204 µg (1.3% of the applied dose) was recovered in the excreta when 16,150 µg were applied. Similar values were recovered for both the high and low loadings, suggesting both were above the amount necessary to maintain a reservoir on the skin surface for the entire four hours prior to washing. Overall, the experimental data suffered from a low total percent recovery (46% for the low and 66% for the high loading), although the mass remaining in the skin and body cannot be determined due to the study's in vivo design. Further, the authors made no attempt to characterize the loss to volatilization. Experimental results are compared to the model output in Table 12.

Table 12. Comparison of Selected Predicted and Observed Chlorpyrifos Results from Meuling et al. (2005)

	Low Dose Results		High Dose Results	
	Predicted	Observed	Predicted	Observed
Original Applied Dose (µg)	5,390		16,150	
Total % Recovery	n/a	46%	n/a	66%
M_{in} (µg)	96.4	n/a	96.4	n/a
Wash Recovery (µg)	5,294	2,260	16,054	10,500
M_{out} (µg) @ End (120 hours)	64.7	233	64.7	204

The expected washing recovery as estimated by the model can be calculated by subtracting M_{in} (96 μg) from the original applied dose. Use of the modeled Potts-Guy permeability coefficient leads to underprediction the mass of chlorpyrifos that could penetrate completely through the skin and be available for excretion (65 μg via the model vs approximately 220 μg via experimental data). The model also overpredicts the mass that could be recovered in the wash solution.

2.C.2. Piperonyl butoxide

Selim et al. (1999) exposed human volunteers to piperonyl butoxide for eight hours prior to decontamination. For those experimental conditions and modified Potts-Guy permeability coefficient, Bunge's equations predicted that 403 μg of piperonyl butoxide would be present in the stratum corneum at the time of washing but only a very small amount (0.2 μg) would have penetrated into the systemic circulation. Over the remaining 120 hours, the model predicts 312 μg would pass through the skin and into the blood. This would leave 91 μg in the skin at 128 hours. Figure 9 is the predicted change in mass into and out of the skin over the duration of the chosen experimental study.

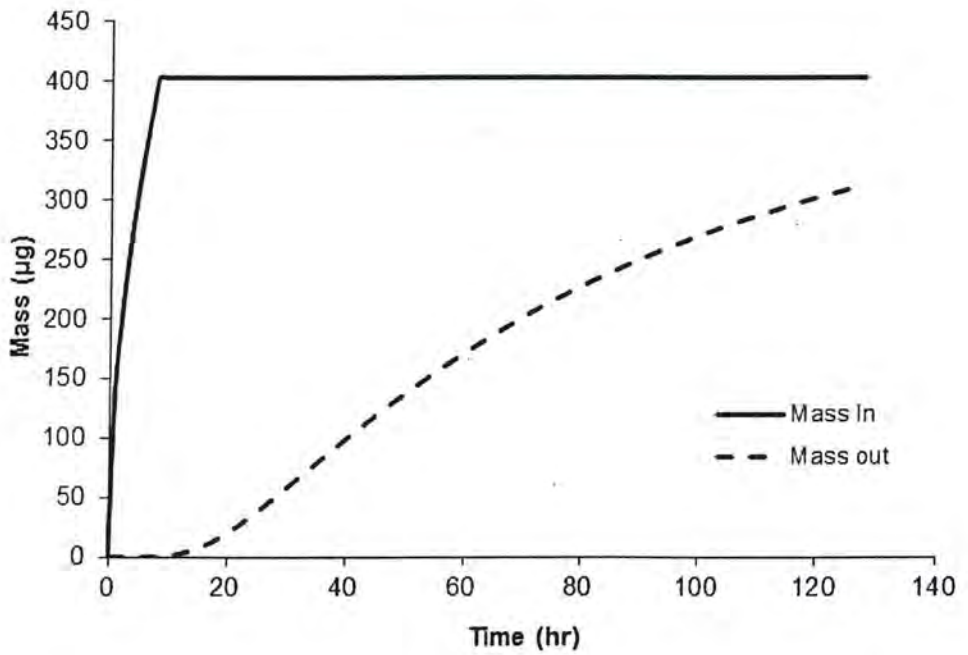

Figure 9. Cumulative Mass of Piperonyl Butoxide into and out of the Stratum Corneum Following Washing at 8 hours. Estimated using Selim et al. (1999) experimental conditions.

The cumulative mass of piperonyl butoxide into the stratum corneum layer was predicted to increase rapidly immediately following the initiation of exposure. Washing is at eight hours, by which time the rate of mass in is beginning to decrease slightly. As described above, no further mass enters the skin post-washing. The piperonyl butoxide begins to pass out of the stratum corneum membrane at about six hours and slowly increases in penetration rate. By the end of the observation period, the rate at which the mass was moving out of the skin was beginning to slow despite about a quarter of the total mass predicted to be still remaining in the skin.

When applied to Selim et al.'s (1999) experimental conditions, Herkenne et al.'s equations predict that a steady state gradient would not have been established at the time of washing. However, the curve for piperonyl butoxide at eight hours was closer to reaching steady state than the chlorpyrifos profile at four hours. The predicted concentration profile in the stratum corneum is provided in Figure 10.

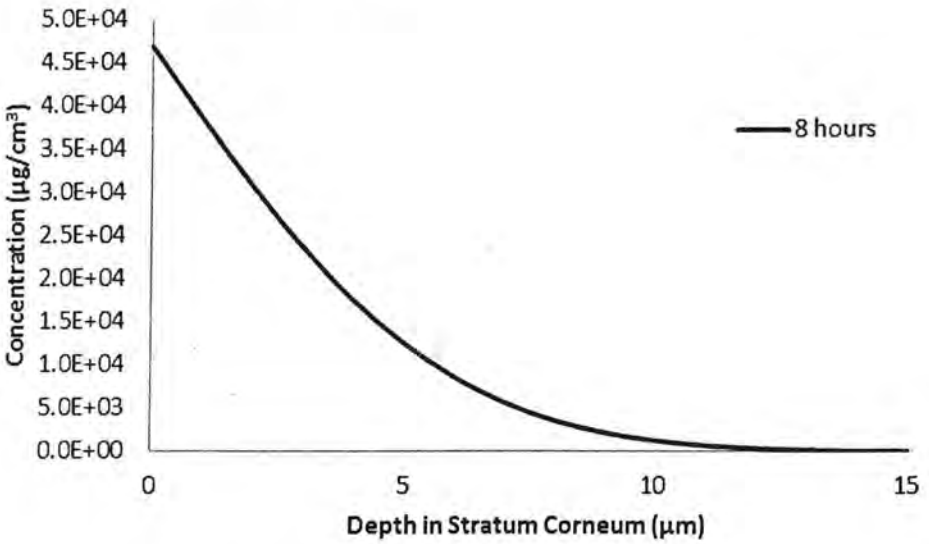

Figure 10. Concentration Profile of Piperonyl Butoxide in the Stratum Corneum at 8 hours. Estimated using Selim et al. (1999) experimental conditions.

The profile in the top layer of skin reaches a concentration of about 69 $\mu g/cm^3$ at a depth of 14 µm. Therefore, some non-significant amount of compound could have penetrated through and out of the skin. However, Bunge's equations predicted that piperonyl butoxide began exiting the skin only two hours prior to washing, which could explain why negligible

amounts of compound were predicted to penetrate out of the stratum corneum prior to washing.

Experimentally, 66.8 μg (2.2% of the applied dose) was recovered in the urine and feces when 2,982.6 μg were applied. An excellent total percent recovery of 100.9% was obtained. This suggests that no compound was retained in the skin and body of the volunteers at the conclusion of the study. The high percent of the applied dose recovered in the washing supports the model's conclusion that the experimental loading was above the amount necessary to maintain a reservoir on the skin surface for the entire eight hours prior to washing. Experimental results are compared to the model output in Table 13.

Table 13. Comparison of Selected Predicted and Observed Piperonyl Butoxide Results from Selim et al. (1999)

	Predicted Results	Observed Results
Original Applied Dose (μg)	2,983	
Total % Recovery	n/a	100.9%
M_{in} (μg)	402.8	n/a
Wash Recovery (μg)	2,580	2,845
M_{out} (μg) @ End (120 hours)	312.2	66.8

The expected washing recovery as estimated by the model was calculated as explained above for the chlorpyrifos example. The model overpredicts the mass that could penetrate completely through the skin and be available for excretion (403 μg via the model vs approximately 67 μg via experimental data). It underpredicts the mass of piperonyl butoxide that could be recovered in the wash solution.

2.C.3. DEET (Experimental)

Selim et al. (1995) applied 10,997 μg pure DEET or 8,602 μg DEET in ethanol to human volunteers for 8 hours. These dose amounts are adjusted by subtracting compound placed on the skin surface but then removed on application equipment or protective coverings. Under these conditions, Bunge's equations predicted that at the time of washing 1,402 μg of DEET would be present in the stratum corneum and 890 μg would have penetrated into the systemic circulation, for a total of 2,292 μg of DEET moving into the membrane. Over the remainder of the trial, the model predicts all compound in the skin at the time of washing would pass

into the blood, leaving no compound in the skin at 128 hours. Figure 11 shows the predicted change in mass into and out of the skin over the duration of the chosen experimental study.

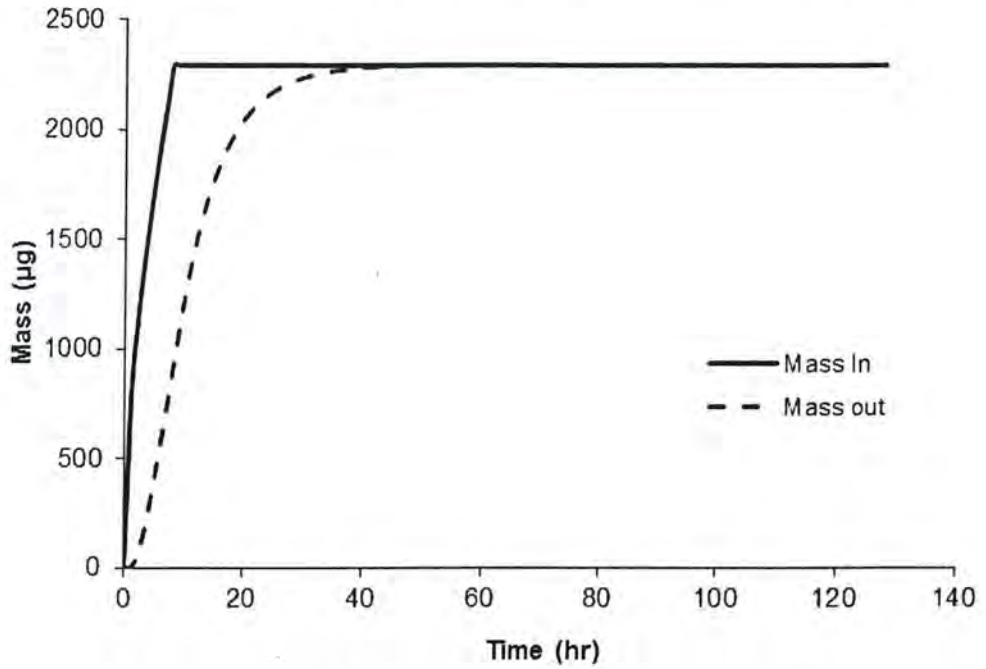

Figure 11. Cumulative Mass of DEET into and out of the Stratum Corneum Following Washing at 8 hours. Estimated using Selim et al. (1995) experimental conditions.

The cumulative mass of DEET into the stratum corneum layer was estimated to increase far more rapidly following the initiation of exposure than either of the prior compounds. Washing is at eight hours, by which time the rate of mass in is beginning to decrease very slightly. Assuming complete removal of the surface reservoir, no further mass enters the skin post-washing. Compound begins to pass out of the stratum corneum membrane in less than one hour post-exposure and rapidly increases in penetration rate. By 77 hours, all 2,292 µg remaining in the skin during the wash was predicted to have been systemically absorbed.

Using Selim et al.'s (1995) experimental conditions, the results of Herkenne et al.'s equations predict that a steady state gradient was established at the time of washing. The concentration profile in the stratum corneum is provided in Figure 12.

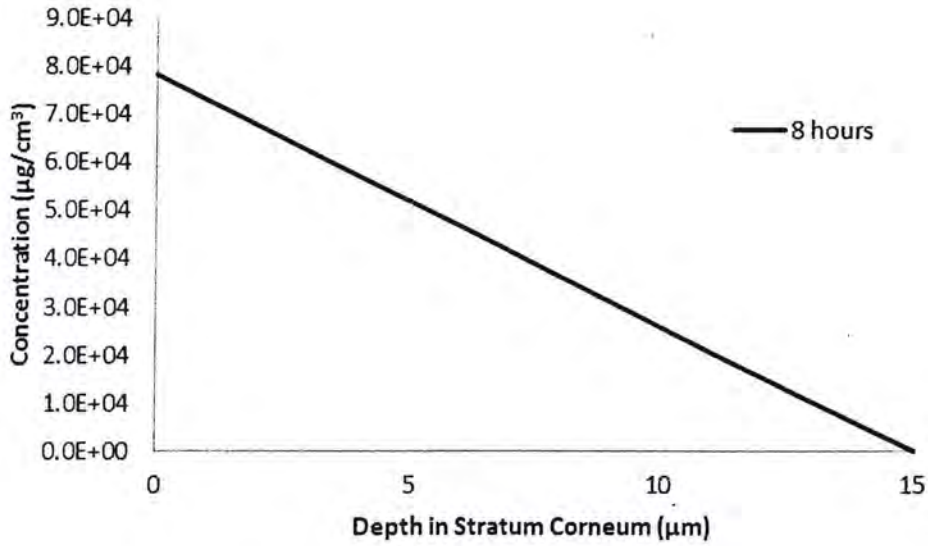

Figure 12. Concentration Profile of DEET in the Stratum Corneum at 8 hours. Estimated using Selim et al. (1995) experimental conditions.

The profile in the top layer of skin reaches a concentration of about 5,138 µg/cm^3 at a depth of 14 µm. This is consistent with the Bunge equations' prediction that a large amount of DEET would penetrate out of the stratum corneum prior to washing.

Experimentally, 1,008 µg (11.7% of the applied dose) was recovered in the urine and feces when 8,602 µg were applied in ethanol and 845 µg (7.7% of the applied dose) was recovered in the excreta when 10,997 µg were applied. Both dose amounts were above the amount modeled as necessary to maintain a reservoir on the skin surface for the entire eight hours prior to washing. The total percent recoveries were fairly high (88.7% for DEET applied in ethanol and 94.3% for pure DEET). If the models' predictions are valid and little to no compound remained in the skin after about half-way through the trial, the loss of compound could be mostly due to DEET's volatility rather than to retention of compound in the skin and body. Experimental results are compared to the model output in Table 14.

Table 14. Comparison of Selected Predicted and Observed DEET Results from Selim et al. (1995)

	In Ethanol Results		Pure Compound Results	
	Predicted	Observed	Predicted	Observed
Original Applied Dose (μg)	8,602		10,997	
Total % Recovery	n/a	88.7%	n/a	94.3%
M_{in} (μg)	2,292	n/a	2,292	n/a
Wash Recovery (μg)	6,310	6,233	8,705	9,291
M_{out} (μg) @ End (120 hours)	2,292	1,008	2,292	845

The model's expected washing recovery was again calculated by subtracting the mass in from the total dose applied. The model overpredicts the mass that could penetrate completely through the skin and be available for excretion (2,292 μg via the model vs either 1,008 μg or 845 μg via experimental data). It underpredicts the mass of DEET that could be recovered in the wash solution for DEET applied as pure compound, but only slightly overpredicts washing recovery for DEET applied in ethanol.

2.C.4. DEET (Predicted)

As discussed in Appendix B, an *in vivo* human study conducted by this laboratory applied either 3,000 μg or 500 μg of DEET in ethanol solution to human volunteers for either 10 or 40 minutes. These dose amounts are not adjusted for any compound that may have been removed by application equipment or volatilization. Bunge's equations predicted that for the subjects washed at 10 minutes, 1,328 μg of compound would be present in the stratum corneum and none would have penetrated into the systemic circulation at the time of decontamination. Over the remainder of the trial, the model predicts all compound in the skin at the time of washing would pass into the blood, leaving no compound in the skin at 120 hours. These predictions do not take loading amounts into account, and therefore are only valid for the high-loading scenarios. Figure 13 shows the change in mass into and out of the skin over the duration of the study for the subjects washed at 10 minutes.

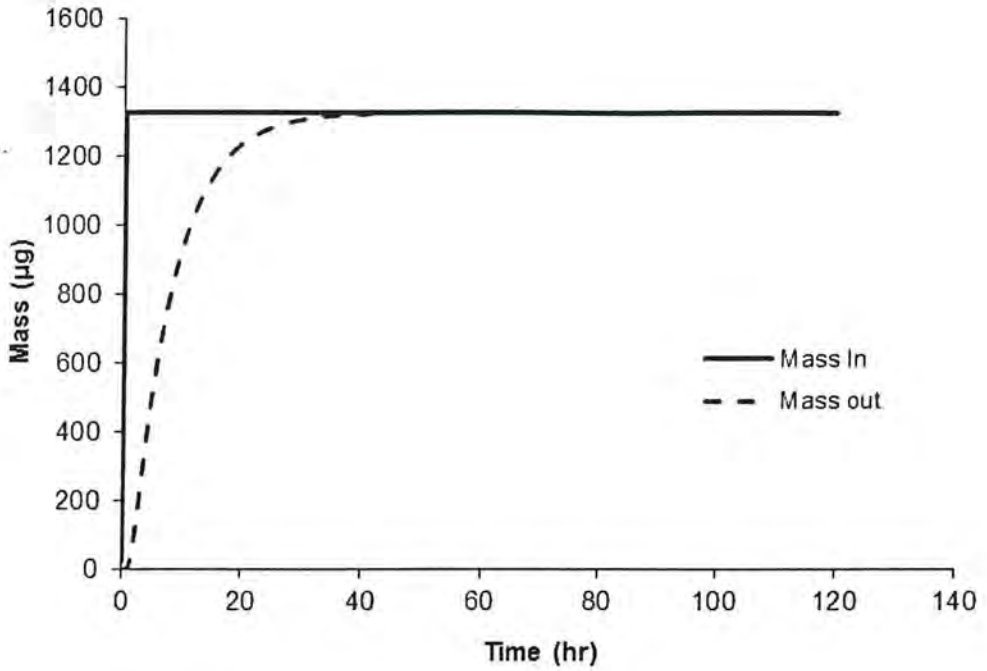

Figure 13. Cumulative Mass of DEET into and out of the Stratum Corneum Following Washing at 10 Minutes. Estimated using experimental conditions from an *in vitro* exposure study.

DEET is able to quickly penetrate into the stratum corneum, resulting in a non-trivial mass in the membrane at the time of washing, despite the short exposure duration. DEET begins to pass out of the stratum corneum membrane between 10 and 30 minutes post-exposure and rapidly increases in penetration rate. By 70 hours, all compound in the skin at the time of washing was predicted to have been systemically absorbed.

As expected, Bunge's equations predicted that more mass would move into the stratum corneum for subjects washed at 40 minutes. Following this longer exposure duration, 2,655 µg of compound would be present in the stratum corneum at the time of washing and only 0.7 µg would have penetrated into the systemic circulation. Over the remainder of the trial, the model predicts all compound in the skin at the time of washing would pass into the blood, leaving no compound in the skin at 120 hours. Figure 14 shows the change in mass into and out of the skin over the duration of the study for the subjects washed at 40 minutes.

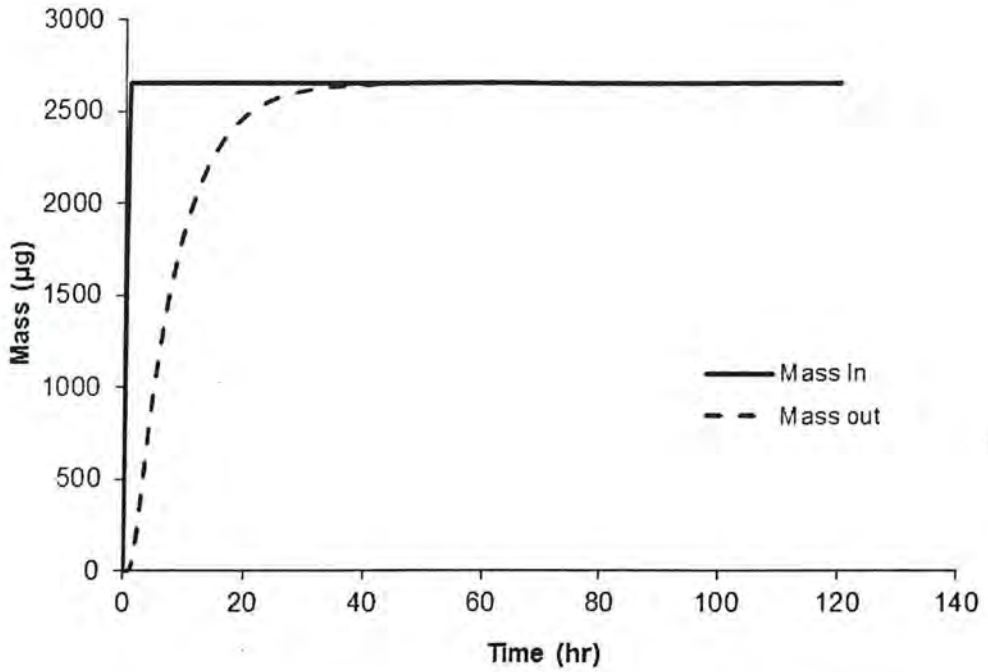

Figure 14. Cumulative Mass of DEET into and out of the Stratum Corneum Following Washing at 40 Minutes. Estimated using experimental conditions from an *in vitro* exposure study.

The plot of cumulative mass in and out of the skin is very similar in shape between subjects exposed to DEET and washed at either 10 or 40 minutes. Again, a non-trivial amount of DEET penetrates into the membrane by the time of washing and compound begins to pass out of the stratum corneum membrane between 10 and 30 minutes post-exposure. All compound in the skin at the time of washing was predicted to be systemically absorbed at 74 hours.

Both the shorter and longer exposure times resulted in the penetration of less than the applied dose for the high-loading scenario. However, the low-loading scenario resulted in a dose of 500 µg which was less than the amount expected to penetrate the skin at either wash time. Using Bunge's equations, the entire dose of 500 µg was modeled to have been absorbed into the stratum corneum within 85 seconds. Similar to the high-load cases, DEET began to penetrate out of the skin within 30 minutes post-exposure and no compound remained in the skin by 61 hours. Figure 15 shows the predicted change in mass into and out of the skin over the duration of the study when the 500 µg dose was depleted at 85 seconds.

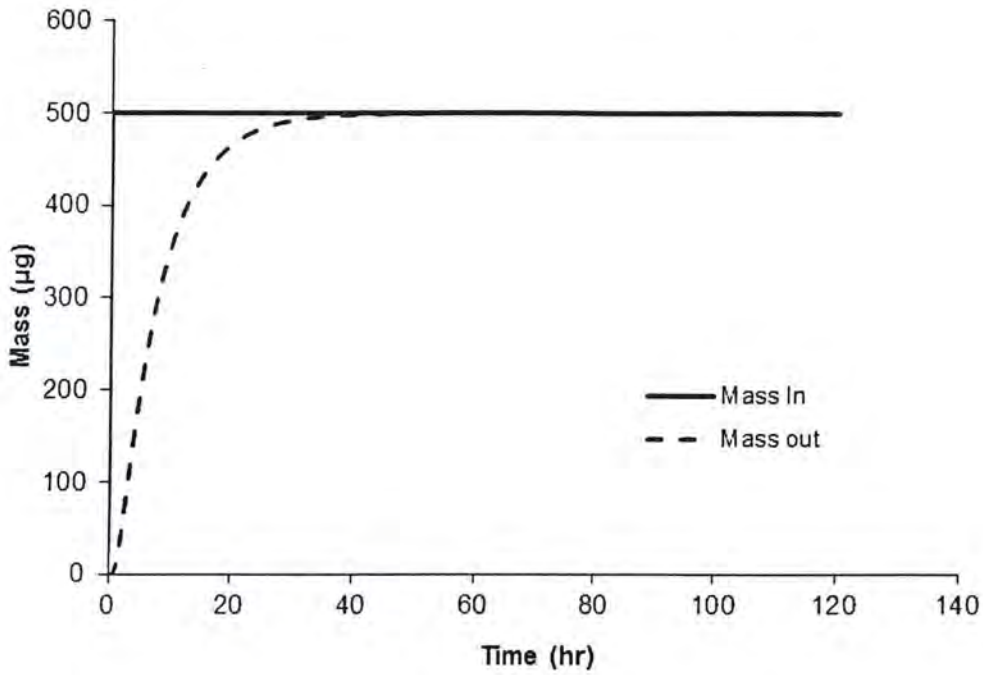

Figure 15. Cumulative Mass of DEET into and out of the Stratum Corneum Following Depletion of the Dose at 85 Seconds. Estimated using experimental conditions from an *in vitro* exposure study.

Because equations to calculate the concentration profile of DEET in the stratum corneum required only the physicochemical properties of the compound, depth of the membrane and time, the profiles for all four DEET scenarios can be plotted together in Figure 16. The fact that Selim et al. (1995) exposed a smaller surface area is not relevant for Herkenne et al.'s equations. The 8-hour exposure used by Selim et al. (1995) would be predicted to result in a steady state gradient by the time of washing barring any losses to volatilization. However, steady state was not reached for any subjects in the study conducted by this laboratory.

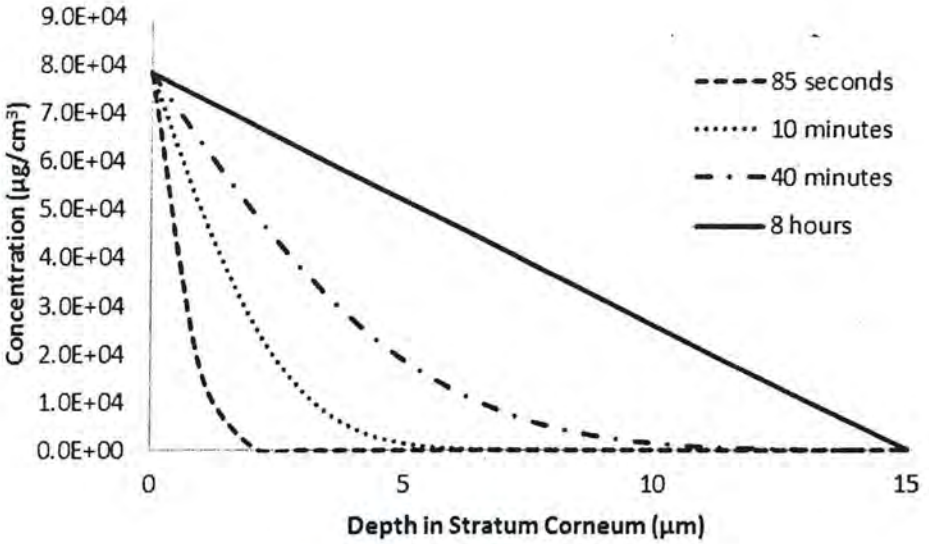

Figure 16. Concentration Profile of DEET in the Stratum Corneum at Various Exposure Times. Estimated using Selim et al. (1995) experimental conditions along with experimental conditions from an *in vitro* exposure study.

For the low loadings that resulted in the 500 µg dose that was predicted to have been absorbed into the skin in 85 seconds, the model estimates that the DEET would not have penetrated past 5 µm into the membrane at the time of depletion. Similarly, at 10 minutes, compound had only penetrated to a depth of 12 µm. At 40 minutes, the profile in the top layer of skin reaches a concentration of about 63 µg/cm^3 at a depth of 14 µm, which was much less than the value of 5,138 µg/cm^3 for steady state at the same depth. The low or non-existing concentrations in the deeper stratum corneum are consistent with the Bunge equations' prediction that little to no compound would penetrate out of the stratum corneum prior to washing for any of these wash times.

Selected model output for the three previously unpublished conditions is summarized in Table 15.

Table 15. Selected Predicted DEET Results for the Previously Unpublished Trials

	10 Minute Wash		40 Minute Wash	
	Predicted Results		Predicted Results	
Original Applied Dose (μg)	3,000	500	3,000	500
Total % Recovery	n/a	n/a	n/a	n/a
M_{in} (μg)	1,328	500	2,655	500
Wash Recovery (μg)	1,672	0	345	0
M_{out} (μg) @ End (120 hours)	1,328	500	2,655	500

The model's expected washing recovery was again calculated by subtracting the mass in from the total dose applied. Washing recovery for the low-load scenarios is expected to be negligible unless the washing solution recovers some amount of compound already in the stratum corneum.

2.D. Discussion

The equations from Herkenne et al. and Bunge contained several simplifications and approximations. Therefore, it is not surprising that there were discrepancies between the modeled results and the observed data. For the Meuling et al. (2005) study, the model underpredicted the amount of chlorpyrifos that penetrated through and out of the skin compared to the experimental data. This is likely due to the log K_{ow} of the compound of interest, chlorpyrifos (4.9). It is on the high end of the effective predictive domain for the Potts-Guy $P_{sc/w}$ (EPA 2004). There is a high degree of uncertainty in applying the Potts-Guy $P_{sc/w}$ to individual compounds and that could have resulted in the underestimation of flux observed in this case. However, the prediction is still within a factor of 3 of the experimental result, which is not unexpected considering that the 95% confidence intervals about the Potts-Guy permeability coefficient are much broader. If the ethanol vehicle increased the permeation of the compound into the skin, that too might have contributed to the underprediction by the model. For recoveries in the wash solutions, the differences between the experimental and modeled recoveries are unlikely to be fully explained by the model's prediction that some amount of compound remained in the skin following the completion of the 120-hour experimental trials.

For the Selim et al. (1999) analysis, the model overpredicted the mass of piperonyl butoxide that would penetrate out of the skin. The prediction was within a factor of 5 of the

experimental result. Again, a large portion of this overprediction should be attributed to the uncertainty inherent in the Potts-Guy permeability coefficient used in the model. It could also partially be due to the fact that the harsher washing method employed by the study authors actually was able to recover some non-trivial amount of the roughly 400 μg predicted to be present in the stratum corneum at the time of washing. If decontaminating with isopropyl alcohol recovered 245 μg from the exposed stratum corneum (or 61% of the total dose in the top layer of the skin at 8 hours), approximately 158 μg would be left in the membrane. If the skin still retained the predicted 91 μg at the termination of the trial, 67 μg would have moved into the systemic compartment. However, the nearly 100% recovery of the applied compound experimentally suggests that very little was retained in the skin or body of the volunteers despite the model's prediction that a quarter of the applied dose would be retained in the stratum corneum at 128 hours. If this was the case, 336 μg of the 403 μg predicted to be in the stratum corneum at the time of wash could have been removed while only 67 μg were left to eventually move out into the bloodstream. If 336 μg were in fact removed via decontamination, washing would be required to penetrate up to 1/3 of the total depth of the stratum corneum. Alternatively, if 245 μg were removed via washing, the washing would be estimated to penetrate up to 1/5 of the total depth, assuming complete stripping of DEET from the washed parts of the stratum corneum. The depth to which washing could recover compound was estimated by integrating equation 4 multiple times between a depth of 0 and L in intervals of 0.1 μm. This resulted in a curve showing cumulative mass in the skin as a function of skin depth.

Similarly for the Selim et al. (1995) review, the model overpredicted the amount of DEET that would penetrate through and move out of the skin. These predictions are within a factor of 2 to 3 of the experimental results, which is well within the precision of the Potts-Guy permeability coefficient. Some portion of this discrepancy is likely due to the uncertainty inherent in the permeability coefficient prediction. The model's overprediction might also be partially explained by the use of the harsh washing solution that may have recovered some amount of previously absorbed compound. If the discrepancy between the predicted and experimental results was assigned completely to the harsh washing solution, the washing would be required to remove all or most of the DEET in the stratum corneum for both pure compound and compound applied in ethanol. While washing with isopropyl alcohol is an

unusually harsh method that could potentially strip DEET from the stratum corneum, it is unlikely that it could recover such a significant portion of compound that had already penetrated into the top skin layer. Therefore, the majority of this overprediction is likely due to the chosen modified Potts-Guy permeability coefficient estimation. Some amount of compound could also have been lost experimentally to the environment, although the large percent recovery of the original applied dose suggests any experimental losses were minimal. Overall, the mass predicted by Bunge's equations as penetrated out of the stratum corneum at the time of washing is very similar to the amount recovered experimentally in the excreta.

The two loading levels used by Selim et al. (1995) produced slightly different recoveries in the excreta, despite both loadings likely being above the level assumed necessary to provide a constant external reservoir for the duration of the exposure. This could be due to the difference in application vehicle, as ethanol may have increased the penetration of the DEET into the skin. The slight overprediction of the wash recovery for the DEET applied in ethanol is could also be partially due to the fact that the model does not take volatilization into account. Up to 11% of the dose in that case was not recovered and could have been lost to the external environment prior to washing.

The comparison between Selim et al.'s (1995) DEET experimental and corresponding modeling results can be applied to the modeling results for the unpublished *in vivo* DEET study conducted by this laboratory. Some amount of volatilization and loss to the application equipment would be expected to occur, which would reduce the total applied dose to something less than 3,000 or 500 µg. Volatilization would be expected to have less impact on the lower dose and 10-minute washing times because the external reservoir of DEET on the skin surface would be maintained for a shorter duration. If ethanol did indeed function as a penetration enhancer, the rate into the skin might be slightly higher than expected by the modeling results. Finally, the washing solutions utilized in this study would be expected to be much milder than the isopropyl alcohol utilized by Selim et al. (1995, 1999), but potentially could recover some amount of compound that had already penetrated into the skin. Less overall mass would expect to be recoverable from the skin for the low-loading trials, although the percent removal from the skin might be higher, depending on how far into the skin the compound had penetrated.

Herkenne et al. and Bunge's equations produce a simplified but helpful model of dermal absorption. Although they are limited by uncertainty inherent in current methods of permeability coefficient estimation, they can still provide important insights into a large number of exposure scenarios. Using these equations also highlights the potential dramatic effects of washing on recovery of compound from the skin. A model that incorporated this removal while also characterizing any potential hydration effects could provide an even more useful prediction of a very common real-world situation.

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Appendix A. Summaries of Articles Unable to Provide Data on the Effects of Washing

Of the 66 studies reviewed, 41 were unable to illustrate further the effects of washing on the dermal absorption of contaminants. For some articles, this was the result of an experimental protocol that washed at only one time immediately prior to the termination of the trials, thus allowing no time to observe the impacts of washing. Other articles had experimental designs that were so complex that interpretation of the data was no longer possible. Finally, other studies lacked basic information necessary to interpret their results for our purposes. While some information can be obtained from these studies regarding dermal absorption or washing efficacy, they were not suitable for our review.

Wester et al. (1977)

Wester et al. (1977) applied radiolabeled hydrocortisone *in vivo* to rhesus monkeys and measured ^{14}C in their urine to characterize how repeated applications affect dermal absorption. Monkeys either had 13.3 $\mu g/cm^2$ of chemical applied 1 or 3 times (at 0, 5.5 and 12 hours post-initial exposure) or 40 $\mu g/cm^2$ applied 1 time to their forearms. Monkeys were washed with soap and water once at 24 hours or, for some of the animals exposed multiple times, immediately prior to the 5.5- and 12-hour doses in addition to the 24-hour wash. Urine was collected for 5 days in 24-hour increments. The data showed that single or multiple doses of 13.3 $\mu g/cm^2$ resulted in less total absorption compared to a single dose of 40 $\mu g/cm^2$. Washing the skin between each of the multiple lower doses resulted in a statistically significant increase in penetration through the skin compared to not washing the skin between the multiple doses.

This was a type 1A study but was not utilized in the more extensive washing review because the multiple doses and washing times complicated the interpretation of the data. In the samples that were washed multiple times just prior to the application of the next dose, the observed effect could be caused by the subsequent dose penetrating the skin more rapidly. This could have been due to the reduced barrier provided by the temporarily hydrated skin. It also could have been due to any previously applied compound being driven into the skin.

The long timeframe between application and washing also means washing would be less effective in general.

Lange (1981)

In this study, lindane was applied in a commercial formula to the entire body of human subjects, both with and without skin damage from scabies. Subjects were exposed and washed with either water or soap and water three times. The sample size was two for most conditions.

This was a type 1A study, but due to the small sample size, multiple wash times and reporting of results in terms of serum concentration of lindane only, often averaged across different washing types, results were uninterpretable in the context of the more extensive washing review.

Wester et al. (1983)

This study evaluated the dermal absorption, systemic elimination, and dermal wash efficiency for polychlorinated biphenyls (PCBs). Radiolabeled 42% and 54% PCB were applied *in vivo* to both rhesus monkeys and guinea pigs in 1:1 v:v benzene: hexane vehicle. Animals were washed with either soap and water or water followed by acetone immediately following exposure or at 24 hours. Trials washed immediately were also terminated following washing and only washing recovery reported. This was a type 4D trial and not helpful in this review. Excretion was measured for animals washed at 24 hours for 15, 16 or 28 days. Washing recovery was presented for guinea pigs. Recovery in the first 5 24-hour urine collections and then in the subsequent 5- or 10-day collection periods were reported for both animals. This was a type 4A trial, but was not helpful to the more extensive washing review because the long collection periods for urine would mask any potential short-term changes in flux immediately following washing, as well as the fact that compound excretion *in vivo* is not always reflective of the flux through the skin due to metabolism and storage in the body.

Reifenrath et al. (1984)

This *in vitro* study assessed the various parameters of a shower decontamination prototype in removing chemical warfare agents. Pure and thickened (with acrylate polymer) soman and the less toxic potential surrogate, diethyl malonate were applied to pig skin. At 15 minutes, samples were washed by either a 2-second shower with distilled water followed by scrubbing with a 1% aqueous surfactant solution on a cotton ball and then rinsed with the same 1% aqueous surfactant solution or just the scrubbing and rinsing with surfactant solution. Recoveries in the showering step, rinsing step, skin and receptor fluid were reported at the termination of the trials at 15 minutes. This study was type 4D, and does not provide any information about the effect of washing on the absorption of compounds through the skin.

Bucks et al. (1985)

Bucks et al. (1985) applied radiolabeled malathion to guinea pigs to examine how the combination of repeated application and washing influenced *in vivo* dermal absorption. In part 1, animals were exposed, half were washed and then all were followed to a later endpoint. Urine was collected to estimate the amount absorbed into the skin, with no significant difference observed in percent recovery between washed and unwashed animals. This was a type 1A design but was so lacking in information describing its methods that it is not usable. In part 2, malathion was applied every 24 hours for 15 days, with only some doses radiolabeled, and urine collected for 21 days. Half of these animals were washed 1 hour prior to each application of compound. Higher percent recoveries of radiolabeled doses were observed in the urine of washed animals.

This is a type 1A study as well since urine was collected over time and all trials were ended at 21 days. However, repeated washing and exposure of the same site caused visible irritation to the skin. This and the timing of the washing step 1 hour prior to the next dose make this a difficult study to interpret. Therefore, it is not included in the more extensive washing review.

Wester et al. (1987)

This study compared methods of determining binding and absorption of contaminants in water to skin. Radiolabeled p-nitroaniline, benzene and 54% polychlorinated biphenyls (PCBs) were applied in 1.5 mL of water to 1.5 mg powdered human stratum corneum in part 1, 5.7 cm^2 of intact human skin using an *in vitro* protocol with continuously flowing water reservoir in part 2, and *in vivo* to 5.7 cm^2 of skin on the abdomen of rhesus monkeys after which urine was collected for 5 days in part 3. All samples were washed at 30 minutes with plain water except part 1, which was not relevant. Part 2 reported recoveries in the receptor fluid, skin and wash at the termination of the trial as percent of applied dose. Part 3 reported only the amount excreted. Part 2 was a type 4D and part 3 was a type 4B. None of the trials in this study provided any information about the effect of washing on the absorption of compounds through the skin.

Bucks et al. (1989)

Radiolabeled alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide) was applied to 5.7 cm^2 of human skin in either 1:20, 1:40 or 1:80 v:v dilutions with water to determine how dermal absorption was impacted by concentration of the compound in the vehicle. Either 23, 11.8 or 5.98 mg of alachlor per ml of water was applied in 0.5 and 1 ml of solution. Receptor fluid was collected continuously until 8 hours post-exposure when the skin was washed three times with distilled water and the trial discontinued. It is unclear whether the skin was also washed at 30 minutes and 4 hours post exposure for the 0.5 ml applications and at 1 and 4 hours for the 1-ml applications. The authors state those were additional "exposure times". Only results for the eight-hour exposures were reported. Alachlor was also mixed with powdered callus to determine binding to the stratum corneum and the ability to recover the compound with water or soap and water. The percent applied dose recovered in the receptor fluid per hour is graphed for eight hours. Additionally, the percent dose applied and the mass recovered in the receptor fluid, wash solutions and skin were reported as mean and standard deviation.

This study type is potentially a type 4C if the data reflects only washing and termination at 8 hours. If each sample was washed multiple times, it would be a type 4A. However, the

methods are unclear and therefore this study is not helpful in the more extensive washing review.

Wester et al. (1990a)

This study evaluated the dermal absorption and distribution of polychlorinated biphenyls (PCBs). In part 1, Aroclor 1242 in mineral oil was soaked *in vitro* with powdered human stratum corneum for 15 minutes before adding 1 ml of either water, soap and water, ethanol or mineral oil. The mixture was then allowed to sit for an additional 0, 1, 10, 60, 240 or 480 minutes before it was centrifuged. In part 2, Aroclor 1242 was applied in mineral oil or tricholorbenzene *in vivo* to the abdominal skin of rhesus monkeys. Monkeys had multiple 1-cm^2 areas demarcated on their abdomens which were dosed and then washed after 15, 25, 75, 195, 375 or 1455 minutes with either 20% (v/v) soap and water, trichlorobenzene, mineral oil or ethanol. Washing was conducted by rubbing five times with a soaked cotton-tip swab. Washing earlier removed a larger percentage of the applied dose. Washing recovery was the only parameter reported for parts 1 and 2. These trials were type 4D, which were not able to provide any information about the effect of washing on the absorption of compounds through the skin.

In part 3, Aroclor 1242 or 1254 was applied in mineral oil or trichlorobenzene to rhesus monkeys. Skin was washed at 24 hours and urine and feces collected for 30 days. Urine and feces recovery was reported in 10-day blocks. This was a type 4A study, but was not helpful to this review because of the long collection periods for urine which would mask any potential short-term changes in flux immediately following washing, as well as the fact that compound excretion *in vivo* is not always reflective of the flux through the skin due to metabolism and storage in the body.

Wester et al. (1991)

This *in vivo* study characterized the dermal binding and absorption of radiolabeled glyphosate. In part 1 of the study, glyphosate was applied in the diluted commercial formulation Roundup to a 1-cm^2 area of abdominal skin on 4 rhesus monkeys. Each monkey had multiple 1-cm^2 areas demarcated on their abdomen. Each 1-cm^2 area was

decontaminated at either 0, 30, 180, 360 or 1440 minutes by rubbing one time with a cotton-tipped swab soaked in either a 50% (v:v) Ivory soap and water solution or water followed by two water-only rinses. Only washing recovery was reported. In general, decontamination at an earlier time resulted in a larger percentage of the dose recovered in the washing solution. This was a type 4D study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

In part 2 of the study, glyphosate in various dilutions of Roundup was applied to monkeys and washed at 12 hours. Urine and feces were collected for 7 days. Recovery in the urine and feces was reported for each 24-hour collection time. This was a type 4A trial. It was not helpful due to the long collection periods for urine which would mask any potential short-term changes in flux immediately following washing, as well as the fact that compound excretion *in vivo* is not always reflective of the flux through the skin due to metabolism and storage in the body.

Meuling et al. (1991)

This *in vivo* study assessed the health risks of dermal and oral exposure to the pesticide propoxur. Propoxur was applied to the forearm of human volunteers in either 50% (v:v) water and methanol or in a dilution of the commercial formulation, Unden, in water. Six volunteers had their forearms occluded by aluminum foil. After 6 hours, volunteers were washed with cotton soaked in the 50% (v:v) water and methanol solution before being rubbed with dry cotton. Urine was collected for 72 hours. Only recovery in the urine was reported. This was a type 4B study, and was not able to provide any information about the effect of washing on the absorption of compounds through the skin.

Brouwer et al. (1992)

This *in vivo* study was designed to facilitate the long-term estimates of pesticide exposure in bulb workers. Mancozeb was applied in the commercial formulation TRIDEX to the hands of 5 human volunteers for an estimated loading of 9.7 $\mu g/cm^2$. The surface area of the exposed skin was not provided by the authors. After 15 minutes of exposure, both hands were washed twice for 30 seconds with 500 mL of EDTA solution in a polyethylene bag.

Only washing recovery was reported. Approximately 81% of the applied compound was recovered in the wash. This was a type 4D study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

Wester et al. (1992a)

This *in vivo* study evaluated the percutaneous absorption of alachlor when diluted and the ability of water or soap and water to remove the compound over time. In part 1 of the study, alachlor was applied in diluted commercial Lasso formulation to 1 cm^2 of abdominal skin in 4 rhesus monkeys for a loading of 23 $\mu g/cm^2$. Each monkey had multiple 1-cm^2 application sites representing a variety of conditions. Only washing recovery could be determined. Application sites were washed three times at 0, 60, 180, 360 or 1440 minutes with a Q-tip soaked in either 50% Ivory soap and water (v:v) solution or water only. Overall, there was a decrease in percent of the applied dose removed with increase in time to wash for both water and soap and water washing. This was a type 4D study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

In part 2 of the study, alachlor in various dilutions of Lasso was applied to monkeys and washed at 24 hours. Urine and feces were collected for 7 days. Recovery in the wash, urine and feces was reported for each 24-hour collection time. This was a type 4A trial. It was not helpful due to the long collection periods for urine which would mask any potential short-term changes in flux immediately following washing, as well as the fact that compound excretion *in vivo* is not always reflective of the flux through the skin due to metabolism and storage in the body.

Wester & Maibach (1994)

This study quantified dermal absorption of contaminants via bathing or swimming in contaminanted waters. Cadmium 109 as a chloride salt was applied *in vitro* to human skin. In part 1, all trials were washed and terminated at 16 hours. This was a type 4D trial, and not helpful in this review. In part 2, exposed skin was washed at 30 minutes and followed for 48 hours. However, only recovery in the skin and receptor fluid was reported for the total duration of the study. Washing at 30 minutes left a reservoir in the skin that continued to

diffuse to the receptor fluid. This was a type 4B study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

Fenske & Lu (1994)

The objective of this *in vivo* study was to develop standard handwashing procedures. Chlorphyrifos in Dursban 4E was applied to test tubes and allowed to dry. Human subjects then gripped the tube to transfer the pesticide to their hand. Exposed skin was washed either immediately following exposure or 60 minutes later with either 10% isopropanol in water or ethanol. Only washing recovery was reported. Washing at later times resulted in decreased or similar washing recovery to immediate washing. This was a type 4D study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

Wester et al. (1994)

Wester et al. (1994) conducted an *in vivo* study with human volunteers to determine dermal absorption of pyrethrin and piperonyl butoxide (PBO), common treatments for head lice. Commercial formulations containing radiolabeled 0.3% pyrethrin or 3% PBO were applied to forearm skin. The sample size was six volunteers per condition. Four hundred and eighty seven μg of pyrethrin was applied in 200 μl of formulation to 88 cm^2 of skin for a loading of 5.53 $\mu g/cm^2$. Alternatively, 680 μg of PBO was applied to 9 cm^2 skin in 20 μl of vehicle for a loading of 75.8 $\mu g/cm^2$. All samples were washed with soap and water solution at 30 minutes and urine collected for 7 days in 24-hour increments except for the day of exposure during which it was collected in 4-hour intervals for the first 12 hours and then once in a 12-hour interval for the remaining time. The percent dose excreted in the urine was adjusted by dividing it by the excretion following a parenteral injection of the compound into rhesus monkeys.

Urinary excretion for each time period was reported as a bar graph. In general, the largest percent dose was excreted on day 1 for pyrethrin and day 2 for PBO. These relationships between values are estimated from the graph. More quantitative values are provided and difficult to estimate from the graph due to the authors' choice of y-axis scale and the small

percent recoveries. Authors seemed to forget the first few samples were collected over shorter time periods when comparing them in the discussion. Overall, 1.9 percent of the adjusted applied dose was recovered in urine for pyrethrin and 2.1 percent for PBO.

This was a study type 4A, but can provide little information regarding change in flux due to washing because of the fact that urine was collected in longer time periods. No increase in flux was observed for pyrethrin in that the estimated percent applied dose recovered per hour peaked and was similar between the 4- to 8-hour and 8- to 12-hour post-exposure samples. This indicated no immediate observable change in flux due to washing at 8 hours. A change might have been obscured due to the time it took for the compound to work its way out of the body. The estimated percent applied dose recovered per hour for PBO was similar for the 4- to 8-hour and 8- to 12-hour samples and then decreased slightly for the 12- to 24-hour sample before increasing to the peak percent dose recovered per hour during the 24- to 48-hour sample. This potentially could be due to an increased mobilization of compound via washing that took a while to show up in the urine.

Franz et al. (1996)

Franz et al. (1996) applied 10 ul/cm^2 of lindane in 1% lindane commercial lotion formulation and 5% permethrin commercial cream formulation to 0.8 cm^2 of human thigh skin *in vitro* and guinea pig skin *in vitro* and *in vivo*. *In vivo* trials were not washed. For the *in vitro* trials, receptor fluid was removed and replaced several times over the course of the 48-hour trial. Skin was washed two times with 0.5 ml of isopropanol for two minutes at the termination of the trial. Recoveries were reported in µg from the receptor fluid, dermis, epidermis and wash solution.

This study was a type 4C and therefore not helpful in the more extensive washing review. Washing efficacy and the distribution of compounds in the *in vitro* system at the time of washing could be determined, but was not helpful because there were no earlier or later washing times with which to compare the recoveries.

Geno et al. (1996)

This *in vivo* study was designed to develop a handwipe sampling procedure to measure dermal contact to pesticides. Chlorpyrifos and pyrethrin were applied to aluminum foil and allowed to dry. Human subjects pressed their hand against this foil and were decontaminated within 30 seconds by wiping with a propanol soaked sponge. Only wipe recoveries were reported. This was a type 4D study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

Hood et al. (1996)

This study was designed to determine the percutaneous absorption of the potentially carcinogenic compound musk xylol in order to characterize human exposure under normal use. Musk xylol was applied in either an oil and water mixture or methanol to human or hairless guinea pig skin *in vitro*. Most trials were washed and terminated at 24 hours, but one group was continued for an additional 7 days. Percent recovery in the wash, skin, receptor fluid and mass balance were reported. A graph of percent dose absorbed over time for the 7 days following a 24-hr wash was presented. That graph had only 1 peak in penetration at 24 hours as measured by 24 hour receptor fluid fraction collection. This was a type 3A trial overall, 4C for the guinea pig skin trials, 4D for the trials using human skin that were terminated at 24 hours and 4A for the human skin trials followed for 7 days. The type 4A study, the only type in this trial that could potentially provide useful data, was not helpful due to the long collection time for receptor fluid fractions which would mask any potential short-term changes in flux immediately following washing.

Baynes et al. (1997)

Baynes et al. (1997) attempted to characterize the dermal absorption of several compounds applied simultaneously that could potentially have resulted in Gulf War Syndrome. Permethrin was applied with DEET in either acetone, dimethyl sulfoxide (DMSO) or ethanol vehicles to rat, mouse or pig skin *in vitro*. Radiolabeled carbaryl was also applied alone or with DEET in either acetone or DMSO to *in vitro* pig skin. Receptor fluid was collected over time until the trials ended at 8 hours for rat and pig skin and 24 hours for mouse skin. At the

end of the trials, skin was washed with soapy solution and tape stripped 6 times. The percent of the applied dose recovered in the receptor fluid (absorption), skin, stratum corneum (tape stripping) and wash (skin surface) were reported for the various carbaryl trials only.

This study was a type 4C and therefore not helpful in the more extensive washing review. Washing efficacy and the distribution of compounds in the *in vitro* system at the time of washing could be determined for the carbaryl trials, but was not helpful because there were no earlier or later washing times with which to compare the recoveries.

Fenske et al. (1998)

This *in vivo* study was designed to determine the removal efficiency of pesticide via handwashing to assist in occupational exposure estimates. Captan was applied to test tubes and allowed to dry. Human subjects washed their hands and then gripped the tube to transfer captan to their skin. Exposed hands were then washed at 0 or 60 minutes. Only washing recovery was reported. Washing at earlier times improved washing recovery. This was a type 4D study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

Selim et al. (1999)

Selim et al. (1999) attempted to characterize the dermal absorption and excretion of piperonyl butoxide (PBO) *in vivo* using human volunteers. PBO was placed on 24 cm^2 of forearm skin in 100 µl of isopropyl alcohol for a loading of about 124 $\mu g/cm^2$ on 4 subjects or commercial formulation for a loading of approximately 157 $\mu g/cm^2$ on 4 additional subjects. All subjects were washed at eight hours with isopropyl alcohol soaked cotton swabs. Urine and feces were collected at 4, 8, 12 and every 12 hours after until 128 hours after the application of PBO. Blood was also collected throughout the exposure period from both the treated and untreated arm. One third of the exposed area was tape stripped 16 times at either 1, 23 and 45 hours post-washing. The percent of the applied dose recovered in urine and feces was reported for each averaged collection period, along with plasma concentrations of PBO. Wash recovery, tape stripping and other external recoveries were also reported.

Plasma concentrations were mostly below the limit of quantification (30 dpm) with all values above the limit occurring prior to washing at 8 hours. Concentration of PBO in the plasma of the exposed arm appeared to increase until about 6 hours for PBO applied in isopropyl alcohol and then decreased fairly steadily. For PBO applied in commercial formulation, concentration in the exposed arm peaked at about 2 hours post-exposure, declined until 8 hours, had another, smaller peak at 10 hours (2 hours post-wash) and then continued to decline for the duration of the experiment. The percent of the applied dose recovered in urine peaked in the 8 to 12 hour collection period for both formulations and no secondary peak was observed.

This study was a type 4A, which can provide information about the impact of washing on flux through the skin. Because it was an *in vivo* study, urine was collected over larger time periods. This could potentially obscure any changes in flux following washing, making urine values less instructive. Blood plasma concentration values are potentially interesting but problematic in that they are all (except the peak value in each trial) below the limit of quantification. In general, the peak value observed in the plasma of the exposed arm was observed prior to washing. In general, a very small percent of the applied dose was absorbed (between 94 and 98 percent was recovered in the wash), also making trends difficult to see. The fact that the study washed with isopropyl alcohol also does not make it a good approximation of what happens when skin is washed and potentially hydrated by soap and water.

Wester et al. (1999)

This *in vivo* study was designed to compare the effectiveness of washing with hydrophilic solutions such as soap and water or just water versus lipophilic wash solutions such as corn oil or polyglycols for the lipophilic contaminant methylene bisphenyl isocyanate (MDI). MDI was applied to rhesus monkeys as a pure compound and then washed at either 5, 60, 240 or 480 minutes post-exposure with one of the washing solutions. Only washing and tape stripping recovery was reported. This study concludes that quicker washing results in more effective recovery in the wash solutions. It also concludes that washing with water-based solutions is not as effective as lipophilic solutions for lipophilic compounds. At 4 and 8

hours, polypropylene, a "polyglycol-based cleanser" abbreviated PG-C and corn oil were significantly ($p<0.05$) better decontaminants than water or soap and water. A similar pattern was observed in the tape strips, with polypropylene, PG-C and corn oil leaving less MDI behind in the skin than water or soap and water. A full mass balance is not possible as the monkeys were not sacrificed. This study cautions that harsh organic solvents may cause problems of their own by damaging skin or causing toxicity. This was a type 4D study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

Brouwer et al. (2000)

This study was a review that included some original *in vivo* human data. Overall, its objective was to review existing hand wash and skin wipe studies to identify issues that impede comparison of results. Nine different compounds were applied to human skin in water for a variety of loadings. All subjects were washed at 30 minutes. Washing efficacy was the only data reported. Therefore, this is a type 4D study. It provides no helpful data for the more extensive washing review because all samples were washed at the same time and receptor fluid was not collected.

Campbell et al. (2000)

This *in vitro* pig study was designed to compare the ability of four solvents to remove pesticides via wiping and to provide information to interpret skin wipe sample results collected in the field. Glyphosate, alachlor, methyl parathion and trifluralin were applied in commercial formulations to 25 cm^2 of pig skin for three different loading amounts. Skin was placed on top of moistened gauze between two pieces of plexiglass with 28 openings in the top piece to create a template. Samples were exposed for 90 minutes and then wiped 15 times with 0.5 mL of 1-propanol, PEG (polyethylene glycol), 10% soap and water or D-TAM® (a cleansing product containing propylene glycol and surfactants) on soaked cotton gauze and then blotted with dry gauze. Some samples were treated with 0.5 mL of the wash solution 30 minutes prior to exposure to the pesticide. Only washing recovery was reported. Wiping recovered a significantly ($p<0.05$) larger percent of applied dose for the higher loading amounts in 13 out of 16 trial groups. The remaining three did not so a significant

difference between wiping recoveries from the different loading amounts. This was a type 4D study and therefore cannot provide any information about the effect of washing on the absorption of compounds through the skin.

Nielsen and Nielsen (2000)

This study applied 0.2 mM methiocarb, 0.2 or 0.1 mM paclobutrazol, and 0.2 mM pirimicarb to 2.04 cm^2 of human skin on static *in vitro* diffusion cells in 600 µl of unspecified vehicle. The cell was occluded with parafilm and then, in some samples, the remaining dosing solution was removed at 4.5 or 24 hours and then skin then washed with phosphate buffer 3 times. Receptor fluid was sampled and replaced with fresh fluid at unspecified times for 72 hours prior to termination. Results were graphed as concentration in µmol per liter recovered in sampled receptor fluid over time. Estimating from the graphs, it appears receptor fluid was only collected at most 2 times per 24 hour period, and therefore, detailed information regarding flux is not available. Washing at earlier time periods resulted in a reduction in compound absorbed and estimated flux through the skin compared to the control samples.

This was a study type 1A but was not helpful because it does not provide receptor fluid recovery data in a clear manner (line graphs only) and provides no information about the amount recovered in the skin or wash solutions. Additionally, in samples that were not washed or prior to washing in decontaminated samples, compounds were presented in an excess of some type of vehicle. Authors note that at 48 hours, unwashed samples still exposed to the donor fluid were at "equilibrium" with the receptor cell. Comparing cells that were exposed to a potential hydrating vehicle for different amounts of time is less helpful in understanding the hydrating effects of washing.

Baynes et al. (2002)

This study attempted to identify possible exposures that could have resulted in Gulf War syndrome. These exposures included various combinations of permethrin and DEET for insect control, pyridostigmine bromide (PB) as a prophylactic anti-nerve agent and nerve agents. Baynes et al. (2002) exposed porcine skin flaps *in vitro* with PB and the nerve agent stimulant diisopropyl fluorophosphates (DFP) in the receptor fluid for some samples.

Radiolabeled permethrin was applied to the skin in a variety of vehicles comprised of a combination of ethanol, water and various concentrations of DEET. Perfusate was collected over time and at 480 minutes all samples were washed with soapy solution, tape stripped and the experiment ended. Amounts recovered in the wash solution, tape strips, dosed skin, and receptor fluid were reported.

This study was a type 4C. It was not helpful in the more extensive washing review because all samples were washed and ended at the same time (480 minutes) and because of the variety of compounds applied to the skin and in the receptor fluid. A penetration curve over time was provided but could not give any information about the washing-in hypothesis because receptor fluid was not collected past the point of decontamination.

Cnubben et al. (2002)

This study applied ortho-phenylphenol (OPP) *in vivo* and *in vitro* to humans and rats. All samples were rubbed with cotton at 4 hours to remove remaining unbound dose and followed for 48 hours. Receptor fluid or urine was collected over time.

This is a type 4A study, but not relevant because no true washing with any sort of solution occurred.

Riviere et al. (2002)

This study examined how simultaneous exposure to a variety of compounds (sulfur mustard, JP-8 jet fuel and DEET) may have influenced dermal absorption of permethrin, a potential cause of Gulf War Syndrome. Radiolabeled permethrin was applied to 5 cm^2 of isolated perfused porcine skin flaps (IPPSF) in 100 µl solution for a loading of 40 $\mu g/cm^2$. Sample size was four replicates for each condition. Pyridostigmine bromide and/or diisopropyl fluorophosphate (DFP) was added to the receptor fluid in some samples to simulate systemic exposure to nerve gas or prophylactic anti-nerve gas treatment. Some samples were also occluded with fabric. Receptor fluid was collected over time until termination at 8 hours, when the samples were washed with ivory soap solution on cotton gauze and tape tripped 12 times. The percent of the applied dose recovered was reported for the receptor fluid

(absorption), wash solution (surface residue), stratum corneum (tape strips) and skin (penetration minus absorption). Flux (percent dose per minute recovered in the receptor fluid) was also graphed.

This study was a type 4C and therefore not helpful in the more extensive washing review. Washing efficacy and the distribution of compounds in the *in vitro* system at the time of washing could be determined, but was not helpful because there were no earlier or later washing times with which to compare the recoveries.

Marquart et al. (2002)

This *in vivo* human study evaluated the efficacy of washing with water and soap to remove pesticides from the skin of crop handlers. In parts 1 and 2, workers were exposed to propoxur/baygon, chlorothalonil and mancozeb via contact with treated plants. The exact loading was estimated. Subjects were asked to wash their hands with soap and water at their break in part 1 and after 2 hours in part 2. In part 3, subjects were exposed to propoxur/baygon and mancozeb in the laboratory in water for 30 minutes, following which they washed with soap and water. The study only reported washing recoveries. This is a type 4D study for all three parts and therefore not helpful in the more extensive washing review.

Curwin et al. (2003)

This *in vivo* human study was designed to measure the amount of acephate 75 SP on the hands of harvesters and to determine the effectiveness of washing in reducing compound. Workers were exposed to acephate occupationally while harvesting tobacco for 4 hours, after which one hand was wiped with isopropanol-soaked gauze. Following this wipe, workers were asked to wash their hands normally with soap and water. After the completion of this washing, the hand that was not originally wiped was wiped in the same manner as the first with isopropanol-soaked gauze. Exposure and washing was repeated four times per worker. This was a type 4D study in that all workers washed at about the same time (4 hours) and excretion was not measured. The study was not included in the more extensive washing

review for this reason. Additionally, the study could not determine actual loading of the skin, so the effect of washing on absorption could not be quantified.

Zendzian (2003)

Zendzian (2003) was a summary of *in vivo* rat studies submitted to the US EPA that examined the fate of chemicals remaining in the skin post-washing and characterized their potential contribution to dermal toxicity. In general, studies had several groups of rats that were washed and terminated simultaneously, usually within the first few hours of exposure. Several other groups of rats were all washed at a later time (10 or 24 hours typically) and subsections of that group terminated at subsequent times over the course of the next few days. Each group reported the average percent dose and nM/cm^2 of compound recovery in the wash, in the carcass, blood and tissues (systemic) and in the urine and feces (excreted).

Overall, this was a type 3B study. The author notes that compound continues to be absorbed from washed skin for almost all chemicals. This study was not helpful in the more extensive washing review because no groups of rats were terminated at the same time as any other group within a trial, making comparisons across groups impossible. Additionally, even though the recovery of compound in the systemic compartment increases in many cases between samples washed at a particular time when one is terminated at a later time, there are no data on the systemic absorption of the chemicals prior to washing, and so a peak post-washing could be the only peak observed for that chemical.

van de Sandt et al. (2004)

This study was a collaboration between multiple laboratories in Europe to compare various established *in vitro* dermal absorption protocols. Benzoic acid, caffeine and testosterone were applied to human skin (except for in one location which used rat skin) at a concentration of 4 mg/ml in a 1:1 ethanol/water solution for a loading of 100 ug/cm^2. Skin was exposed for 24 hours during which time receptor fluid was collected. Upon completion of the exposure period, the skin was washed and the amount of compound recovered in the skin and receptor fluid determined as well as the maximal absorption rate.

This is a type 4C study, therefore not helpful in our review. Washing efficacy cannot be determined, nor can the distribution of the compound in various skin layers.

Kraeling et al. (2004)

This study investigated the absorption of diethanolamine (DEA) through skin when applied in shampoo, hair dye and lotion. DEA was applied to human skin *in vitro* in a variety of vehicles and loading amounts. Most conditions were only replicated in one trial and receptor fluid was not collected, for a type 4B or 4D trial. However, 1 $\mu g/cm^2$ of DEA was applied in the lotion E vehicle and washed at 24 hours before being followed to either 24, 48 or 72 hours, for a type 2B study. These data were not able to provide an estimate for flux over time because the trials were terminated 24 hours apart, and any temporary increase in flux would be obscured by the longer collection time. Overall, this study was a type 3B.

Curwin et al. (2005)

This *in vivo* human study investigated the effectiveness of washing to reduce green tobacco sickness among harvesters who were dermally exposed to nicotine via picking tobacco. Harvesters were exposed to nicotine occupationally for 4 hours, after which one hand was wiped with isopropanol-soaked gauze. Following this wipe, workers were asked to wash their hands normally with soap and water. After the completion of this washing, the hand that was not originally wiped was wiped in the same manner as the first with isopropanol-soaked gauze. Exposure and washing was repeated four times per worker. This was a type 4D study in that all workers washed at about the same time (4 hours) and excretion was not measured. The study was not included in the more extensive washing review for this reason. Additionally, the study could not determine the actual loading of the skin, so the effect of washing on absorption could not be quantified.

Tomalik-Scharte et al. (2005)

This study applied permethrin *in vivo* to subjects to determine the dermal absorption of the compound during treatment for lice and scabies. In part 1, 215 mg of permethrin was applied to the just-washed scalp of 6 healthy men in 50 ml of ethanol. The exposed surface area was

not provided. The hair was rinsed with water 45 minutes later and urine collected in 8-hour increments for the first 24 hours and then at subsequent, non-continuous 8-hour collection periods prior to 168 hours. In part 2, 3 grams of permethrin were applied to the showered and dried body minus genitals and head of 6 healthy men in 60 grams of commercial formulation cream. Exposed skin was washed 12 hours later with shower gel and water. Urine was collected in 6-hour increments for the first 24 hours and again at subsequent, non-continuous 8-hour collection periods prior to 168 hours. Part 3 was identical to part 2, except 3 of the subjects were women and all of the subjects had mild scabies. Urinary excretion was graphed for each collection period as nanomoles of permethrin metabolite recovered per hour. For whole body exposure to subjects with healthy skin, the peak excretion rate occurred on average between 18 and 24 hours post exposure and no minor peaks were observed.

This is a study type 4A, but is not very helpful for the more extensive washing review in that subjects were washed just prior to application of permethrin and the subjects exposed on their scalps were occluded via a shower cap. Because it was an *in vivo* study, urine was collected over larger time periods. This could potentially obscure any short-term change in flux following washing, making urine values less instructive.

Hanssen et al. (2006)

This study was designed to determine the effectiveness of new decontamination product, RSDL, in skin decontamination of nerve agents compared to current cleaning methods. Soman, VX or VR nerve agents were applied to about 10 cm^2 of *in vivo* guinea pig skin. Skin was washed after 2 minutes and animals followed for 24 hours. One group remained unwashed as a control. Data were presented as a protective ratio (PR). PRs were calculated for each agent by dividing the LD50 for the decontamination method by the LD50 for the control. Washing with any decontamination solution including soap and water improved survival in guinea pigs. It is impossible to know if the improvement was due to a reduced peak in blood concentration or a reduction in total mass absorbed, and therefore this study cannot provide information about the mechanisms of washing. However, it does support the idea that washing is beneficial, even for nerve agents. This was a type 1B study although it

did not provide results in a way that was consistent with the other studies and therefore it was not included in the more extensive washing review.

Traynor et al. (2007)

Traynor et al. (2007) examined the dermal absorption of butoxyethanol and ethoxyethanol in various concentrations in a water vehicle. Compounds were applied *in vitro* to 0.64 cm^2 of human or rat skin as an "infinite" dose in 200 $\mu l/cm^2$ of solution or a "finite" dose in 20 $\mu l/cm^2$ of solution. Human skin was decontaminated at 4 hours by swabbing with 6 tissue swabs that were alternately dry or soaked in a 3% detergent water solution and then continued until 20 hours. Rat skin was washed and terminated at 20 hours. Receptor fluid was collected over time.

The human skin portion of this study was a type 4A while the rat skin portion was a 4C. The human portion of this study could have been helpful in our review but data was presented as cumulative absorption (mg/cm^2) over time on line graphs from which data regarding flux over time was impossible to estimate. The maximum absorption rate was provided but not the time at which it occurred.

Domesle et al. (2010)

This study applied chlorpyrifos and pentachlorophenol *in vitro* to human cadaver skin. Trials were washed and terminated at 90 minutes. This type 4D study provided no helpful information for the more extensive washing review.

Riviere et al. (2010)

Riviere et al. (2010) applied pentachlorophenol (PCP), 4-nitrophenol (PNP), parathion, fenthion, simazine, and propazine to isolated perfused porcine skin flaps (IPPSF) and a non-biological membrane coated fiber (MCF) system. Chemicals were applied in water, water plus 10% sodium lauryl sulfate (SLS), or water plus 10% linear alkylbenzene sulfonate (LAS). For the trials conducted using IPPSF, researchers applied 100 μl of solution to a 5-cm^2 area of pig skin, for a loading of 10 $\mu g/cm^2$ for all compounds. Skin was then washed at

8 hours by rubbing with cotton swabs soaked in 1% Ivory liquid soap solution and the trial immediately terminated.

All compounds penetrated into and through the skin in greater amounts when they were applied with SLS than with LAS solutions. Riviere et al. attributed this to the smaller micelle size found in the SLS mixture. All compounds penetrated into and through skin faster and in greater amounts when applied in plain water than when applied in either SLS or LAS, except for simazine. Simazine has a higher peak flux in water than either surfactant, but it had greater total absorption into and through the skin in SLS than in water. Absorption to the MCF showed the same general pattern of increased absorption from water than surfactant.

This study is a type 4C trial, and cannot provide any information regarding the effect of washing on dermal absorption. However, it still provides interesting data in regards to the effects of surfactants on the skin. Compounds applied in surfactant solutions had less penetration through skin than compounds applied in water for every case except simazine. This could be due to the low water solubility of the chosen compounds. Without surfactants, they would be attracted to the lipid rich stratum corneum. However, in the presence of surfactants, they remained solubilized in solution which resulted in less partitioning to the skin. This is contrary to the increased penetration that would be expected if surfactants caused a breakdown of the skin barrier. When the MCF system was soaked in the compound-containing solutions, the amount that partitioned to the fibers showed a similar pattern to the absorption observed in the pig skin system. This supports the idea that the variable penetration observed between the surfactant and water solutions was due to chemical properties instead of biological effects such as damage or a reduction in viability of the skin.

Lademann et al. (2010)

This *in vivo* study compared the effectiveness of mechanical washing versus passive uptake of contaminants into absorbent materials. Octylmethoxycinnamate (OMC) was applied in waterproof sunscreen formulation to 20 cm^2 of inner forearm skin for a loading of 60 $\mu g/cm^2$. Trials were ended at 10 minutes following either no decontamination or decontamination via pressing absorbent material 1 or 2 into the skin for 1 minute or by rinsing with running water for 30 seconds. Following decontamination, each area was tape stripped 10 times and each

strip was analyzed for corneocytes and OMC recovery to determine the profile of the compound in the stratum corneum. Profiles in the stratum corneum showed that washing with water resulted in deeper penetration but reduced total mass present in the sampled layers compared to control. Decontamination by the two absorbent materials resulted in stratum corneum profiles that were reduced in magnitude and depth of penetration compared to both water wash and control samples.

This is a type 1D study, where both the unwashed control and washed samples were ended at 10 minutes, which was the same time as the washing. This design provides no experimental data about the effect of washing on flux or total absorption into the receptor fluid, because the trial ended at washing and no plasma or urine was collected. However, this study is still interesting because washing with water showed as an immediate result a stratum corneum profile with some compound penetrating to the bottom of the sampled layers, which is much deeper than the control sample. If the trial was allowed to continue, this could result in an earlier peak of the compound in the systemic circulation from water wash samples compared to control. Additionally, if the remaining compound in the stratum corneum was absorbed instead of lost by shedding contaminated layers, washing with water would still result in an overall reduction of mass of contaminant available for absorption. Decontamination with the absorbent materials reduces the depth and mass of compound available for absorption, which would likely lead to a decrease in total mass absorbed and flux into the skin compared to control.

Reifenrath et al. (2011)

This study applied permethrin and piperonyl butoxide *in vivo* to rats and *in vitro* to rat and human skin. Most samples were washed and ended at 24 hours. However, some *in vivo* rat studies were extended an additional 4 days following the 24 hour wash. The recovery of the compound from receptor fluid or urine over time was not reported except on a poorly labeled graph.

Overall, this study was a type 2A study since all samples were washed at 24 hours. However, the samples washed and ended at 24 hours were a type 4D and provided no relevant information. The animals washed at 24 hours and followed for a total of 5 days

were part of a type 4A trial, but, as discussed previously, this information was not helpful due to the *in vivo* nature of the data that obscured any changes in flux.

Appendix B. Methodology for an *in vivo* Human Study Investigating the Effects of Washing on Dermal Absorption

Introduction

There is a dearth of human *in vivo* dermal absorption studies in the published literature that can address the effects of washing on the movement of compounds through the skin. Therefore, this study was performed to help fill this void. DEET (N,N-Diethyl-meta-toluamide) was chosen as the compound of interest due to its well-characterized, low toxicity which makes it feasible for use in human studies, its ability to function as a surrogate for other pesticides of interest, the fact that it has a moderate log K_{ow} of 2.18 and because biomonitoring of DEET and its metabolites can be conducted by previously published methods in urine, which is an easily sampled specimen.

Chemicals

DEET (CAS No. [134-62-3], MW = 191.3) at 98% purity was purchased from Ultra Scientific (North Kingstown, RI). Ethanol (94-96% purity) was purchased at EMD Chemicals (Gibbstown, NJ). Moisturizer- and antibiotic-free SoftSoap® brand hand soap was purchased from a retail outlet. Stock solutions of DEET in ethanol were prepared at target concentrations of 1.25 or 7.5 μg/μl.

Subjects

The study was designed and implemented under the oversight of the University of Washington's Human Subjects Division's Institutional Review Board (IRB) for the protection of human participants in research studies. Persons were excluded from participation if they were pregnant, nursing, prisoners, younger than 18 or older than 70, or if they had active skin disease or any visible cuts, abrasions, rashes or tattoos on their inner forearm. Eighteen subjects (nine male and nine female) between the ages of 19 and 47 years old were recruited and enrolled in the study. Pregnancy tests were administered to all female subjects immediately prior to each DEET exposure to ensure results were negative. Subjects were asked to avoid any products containing DEET or close contact with anyone using

DEET for the week prior to and the week following their exposure. Additionally, volunteers were asked to abstain from using lotion on their hands and arms the day of their exposure.

In vivo *Dermal Absorption Study*

A 50-cm^2 area (5 cm by 10 cm) was marked with an eyeliner pencil on the interior aspect of both forearms. One hundred µl of ethanol solution containing DEET was applied via pipette and spread over the demarcated area with a glass rod after which the ethanol was allowed to rapidly volatilize. This was repeated a second time, for a total application volume of 200 µl of dosing solution per exposed forearm. The entire application protocol was completed on the first arm before starting on the second arm. This resulted in the application of either 500 µg or 3,000 µg of DEET total to each subject, at a loading of either 5 µg/cm^2 or 30 µg/cm^2. Subjects were exposed for 10 or 40 minutes. During that time, subjects were watched by researchers to ensure the exposed skin did not contact anything to prevent loss of DEET via transfer. For the entire experimental procedure, subjects remained seated immediately adjacent to a laboratory fume hood, which maintained air flow across the exposed skin and prevented inhalation of any volatilized compound. Air flow was confirmed with an electronic smoke generator.

At the completion of the exposure time, the dosed area was wetted with 6 ml of either a 5% (v:v) SoftSoap® brand hand soap and deionized (DI) water solution, 10% (v:v) ethanol and DI water solution or DI water only. The area was then rubbed for 5 seconds with a dry cotton ball and rinsed with 25 ml of DI water for subjects wetted with either soap solution or DI water or rinsed with 25 ml ethanol solution for subjects wetted with the ethanol solution. The washing protocol was completed on the first arm before starting on the second arm. The order in which the forearms were exposed was randomized and washing was conducted in the same order as the preceding exposure.

Wetting and rinsing solutions were applied via a 30 ml Becton-Dickinson™ (Franklin Lakes, NJ) plastic blunt-tipped syringe to control the direction and force of the application and minimize splashing. The syringe never contacted the subjects' skin. Subjects rested their forearms above a stainless steel baking pan during the decontamination protocol to collect the wash solutions for analysis. Following the washing protocol, subjects' forearms were

blotted with a paper towel. Subjects were then asked to refrain from wetting or washing their forearms for 12 hours from the start of the exposure.

Each subject completed four different combinations of the two loadings and wait times, as outlined in Figure 17. Each subject was washed with only one of the washing solutions for all four trials that they completed. The order in which the loading and wait time combinations (5,10; 5,40; 30,10; 30,40) were completed was determined randomly at the time of scheduling. Trials completed by the same subject were spaced such that there was at least one week in between the end of their previous trial and the start of their next trial.

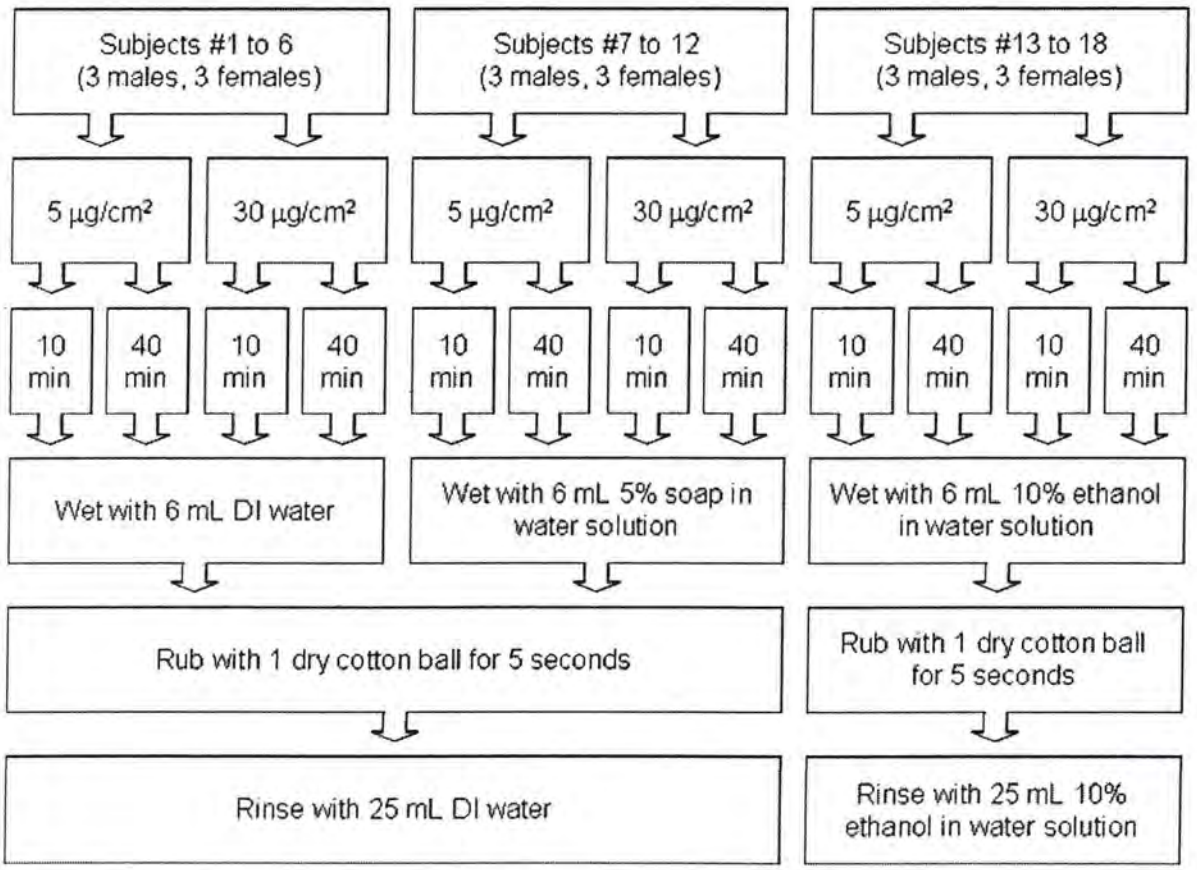

Figure 17. Flowchart of Study Procedures. Each subject completed four trials. The second row is the loading at which DEET was applied to the exposed area; the third row is the time between application of DEET and washing; the fourth row is the wetting solution and the sixth row is the rinsing solution.

This study was a split plot design with the time to wash and loading of DEET as the split variables. Each of the 18 volunteers completed 4 trials that differed for these variables, for a total of 72 trials. This will allow pair-wise comparisons and minimize discernable effect size

between loading and delay-dependent outcomes. Relative effect size required to determine differences among the washing solutions will be slightly higher.

Sample Collection

Subjects collected urine for 5 days: a 24-hour background sample for the day prior to each exposure, two 6-hour samples followed by a 12-hour collection on the day of exposure, and 24-hour samples for the remaining 3 days. Collected urine was stored in a cool, dark place (e.g., a home or work freezer) and transported in coolers with ice packs prior to placement in the laboratory freezers.

Sample Analysis

Wetting and rinse solutions were pooled for each trial. Aliquots of this solution along with the cotton balls used to rub the skin during washing and the ethanol rinse of the glass spreading rod were kept in a -20 °C freezer prior to extraction and analysis via a high-performance liquid chromatography (HPLC) technique. Extraction efficacy of DEET from cotton balls was established prior to HPLC analysis. External recovery of DEET during the application process and the amount recoverable in the wash following the relevant exposure times are the target outcomes. Aliquots of each urine sample were also frozen at -20 °C prior to analysis for DEET and its metabolites. Total and short-term mass of DEET excretion are the target outcomes. Samples were transferred to the University of Cincinnati (wash solution, spreading rod rinse, cotton ball) and Emory University (urine) for chemical analysis.

Appendix C. MATLAB® Code

The code provided in this appendix contains physiochemical properties and experimental conditions modeled on the study by Selim et al. (1999). Other studies were evaluated by using the same code but substituting different values for time to end, time to wash, molecular weight, the log octanol-water partition coefficient, solubility in water and the surface area exposed.

```
clear; clc; close all;
t=0:1:128;      %Time Vector
tcl=8;          %Wash Time
L=15;           %Overall Skin thickness (um)
x=0:1:L;        %Stratum Cornium Depth
MW=338.4;       %Molecular Weight (g/mol)
logKow=4.75;    %Octanol Water Partitiion Coefficient
Cwsat=14.3;     %Solubility in water (ug/cm^3)
Aex=24;         %Surface Area Exposed (cm^2)
n = 100;        %Number summation iterations

%Modified Potts-Guy permeability coefficient (cm-hr)
Pscw=10^(-2.80+0.66*logKow-0.0056*MW);

%Stratum corneum water partition coefficient)
Kscw=10^(0.74*logKow);

%Stratum corneum concentration in equilibrium with pure compond or
%saturated solution (ug/cm^3)
KcvCv=Cwsat*Kscw;

%Diffusivity
Dsc=10^4*Pscw*L/Kscw;

%Characteristic Time of Diffusion (non-dimensionalizing)
tDc=L^2/Dsc;

%Time Vector (non-dimensionalized)
tau = t./tDc;

%Time of Washing (non-dimensionalized)
tau_c=tcl/tDc;

%Mass (non-dimensionalized)
ndmass=KcvCv*Aex*L*10^-4;



%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Compute SC Concentration over depth and time (Cx)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Initialize Cx output
Cx=zeros(length(x),length(t));
for X=1:length(x);    %Compute Concentration for each slice
```

```
    trunc_sum=0;
    for N=1:n;       %Compute the truncation of the inifinite sum
      trunc_sum=trunc_sum+(1/N)*sin(N*pi*x(X)/L).*exp(-Dsc*N^2*pi^2*t/L^2);
    end

    Cx(X,:) = KcvCv*((1-x(X)/L)-(2/pi)*trunc_sum);

 end

 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
 %Compute the Area Under Portion of the Curve
 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
 x1=0;
 x2=0:.1:L;
 AUC_partial=zeros(length(x2),length(t));
 for(x2_idx=1:length(x2))

    for T=1:length(t)
      trunc_sum_a=0;
      for N=1:n;       %Compute the truncation of the inifinite sum
        trunc_sum_a=trunc_sum_a+(1/N)*(-L/(N*pi))*cos(N*pi*x1/L).*exp(-Dsc*N^2*pi^2*t(T)/L^2);
      end
      X1=x1-x1^2/(2*L)-(2/pi)*trunc_sum_a;

      trunc_sum_b=0;
      for N=1:n;       %Compute the truncation of the inifinite sum
        trunc_sum_b=trunc_sum_b+(1/N)*(-L/(N*pi))*cos(N*pi*x2(x2_idx)/L).*exp(-
Dsc*N^2*pi^2*t(T)/L^2);
      end
      X2=x2(x2_idx)-x2(x2_idx)^2/(2*L)-(2/pi)*trunc_sum_b;
      AUC_partial(x2_idx,T)=KcvCv*(X2-X1)/L;
   end
end
%Columns = Time values
%Rows = Cumulative Mass in SC at given depth (x2)
mass_in_sc_partial=AUC_partial*Aex*L*10^-4;


%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Compute Total Mass Stratum Corneum at each time  (mass_in_sc)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Initialize AUC Output
AUC=zeros(1,length(t));
for T=1:length(t)

  trunc_sum2=0;
  for N=0:n-1;       %Compute the truncation of the inifinite sum
    trunc_sum2=trunc_sum2+((1/(2*N+1)^2)*(exp(-1*(2*N+1)^2*pi^2*Dsc*t(T)/L^2)));
  end
  AUC(1,T) = KcvCv*((1/2)-(4/pi^2)*trunc_sum2);
end
mass_in_sc=AUC'*Aex*L*10^-4;
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Non Dimensional Mass In - Prewash (tau<=tau_c)  (MV1_pre)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
for T=1:length(t)
   trunc_sum3=0;
   for N=1:n;        %Compute the truncation of the inifinite sum
      trunc_sum3=trunc_sum3+exp(-N^2*pi^2*tau(T))/(N^2*pi^2);
   end
   MV1_pre(T)=ndmass*(tau(T)+1/3-2*trunc_sum3);
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Non Dimensional Mass Out - Prewash (tau<=tau_c)  (M1R_pre)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
for T=1:length(t)
   trunc_sum4=0;
   for M=1:n;        %Compute the truncation of the inifinite sum
      trunc_sum4=trunc_sum4+((-1)^M*exp(-M^2*pi^2*tau(T)))/(M^2*pi^2);
   end
   M1R_pre(T)=ndmass*(tau(T)-(1/6)-2*trunc_sum4);
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Non Dimensional Mass Out - Postwash (tau>tau_c) (M1R_post)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
for T=1:length(t)
  trunc_sum5=0;
  trunc_sum6=0;
  trunc_sum6a=0;
  trunc_sum7=0;
%   for(M=1:n)
%    lambda_m=((2*M+1)*pi/2);
%    trunc_sum6a=trunc_sum6a+exp(-M^2*pi^2*tau_c)/(M^2*pi^2-lambda_m^2);
%   end

  for N=0:n-1;%Compute the truncation of the inifinite sums
     M=N+1;
     lambda_n=((2*N+1)*pi/2);


     %First Term
     trunc_sum5=trunc_sum5+ (-1)^M*exp(-M^2*pi^2*tau_c)/(M^2*pi^2);

     %Second Term
    trunc_sum6a=0;
     for Ma=1:n
        trunc_sum6a=trunc_sum6a+exp(-Ma^2*pi^2*tau_c)/(Ma^2*pi^2-lambda_n^2);
     end
    trunc_sum6=trunc_sum6+(((-1)^N*(1-exp(-lambda_n^2*(tau(T)-tau_c))))./lambda_n)*trunc_sum6a;
     %trunc_sum6=trunc_sum6+(((-1)^N*(1-exp(-lambda_n^2*(tau(T)-tau_c))))./lambda_n);
```

```
    %Third Term
    trunc_sum7=trunc_sum7+(-1)^N*exp(-lambda_n^2*(tau(T)-tau_c))/lambda_n^3;

  end
  M1R_post(T)=ndmass*(tau_c+1/3-2*trunc_sum5-4*trunc_sum6-2*trunc_sum7);
  %M1R_post(T)=ndmass*(tau_c+1/3-2*trunc_sum5-4*trunc_sum6*trunc_sum6a-2*trunc_sum7);

end
```