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The mismeasure of dermal absorption

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The results of dermal absorption experiments are routinely and often exclusively reported in terms of fractional absorption. However, fractional absorption is not generally independent of skin loading conditions. As a consequence, experimental outcomes are commonly misinterpreted. This can lead in turn to poor estimation of exposures under field conditions and inadequate threat assessment. To aid interpretation of dermal absorption-related phenomena, a dimensionless group representing the ratio of mass delivery to plausible absorptive flux under experimental or environmental conditions is proposed. High values of the dimensionless dermal number (N_{DERM}) connote surplus supply (i.e., flux-limited) conditions. Under such conditions, fractional absorption will generally depend on load and should not be assumed transferable to other conditions. At low values of N_{DERM}, dermal absorption will be delivery-limited. Under those conditions, high fractional absorption is feasible barring maldistribution or depletion due to volatilization, washing, mechanical abrasion or other means. Similar logic also applies to skin sampling and dermal toxicity testing. Skin surface sampling at low N_{DERM} is unlikely to provide an appropriate measure of potential dermal dose due to depletion, whereas dermal toxicity testing at high N_{DERM} is unlikely to show dose dependence due to saturation. *Journal of Exposure Science and Environmental Epidemiology* (2011) **21,** 302–309; doi:10.1038/jes.2010.22; published online 28 April 2010

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Introduction

Dermal absorption is a less obvious route of chemical exposure than either ingestion or inhalation. As a result, methods for assessment of dermal exposure developed later than, and were influenced by, conventional approaches for assessment of those routes. However, exposure to skin differs in important ways from exposure by ingestion or inhalation. As a consequence, descriptive approaches that are satisfactory for ingestion or inhalation assessment many not be similarly useful when applied to dermal absorption. In particular, description of dermal absorption in terms of fixed fractional availability is problematic. For purposes of illustrating relevant issues and informing analysis to avoid pitfalls, application of a dimensionless group representing the ratio of mass delivery to potential absorptive flux is proposed here.

Background

Experimental investigations of the dermal absorption of environmental contaminants, when conducted in human skin, often yield fractional efficiencies that are less than 10% of the applied dose. In contrast, experimental oral absorption efficiencies of the same compounds can approach 100%. As a consequence, the significance of dermal exposure is frequently discounted. Consider the following quotation from the Third National Report on Human Exposures to Environmental Chemicals (CDC, 2005):

> "Chlorpyrifos is not well absorbed through the skin but is rapidly absorbed once ingested."

This judgment is offered despite the fact that dermal absorption of chlorpyrifos (CPS) is a well-known occupational hazard meriting "skin" notation in the NIOSH Pocket Guide to Chemical Hazards (NIOSH, 2005). The basis for the statement in question above can be found in the much cited experiments of Nolan et al. (1984). Those investigators delivered CPS to human volunteers in both oral and dermal dosing experiments and collected urine samples that were analyzed for the primary metabolite of CPS, 3,5,6-trichloro-2-pyridinol (TCP). Nolan et al. estimated that a mean of 70% of the orally administered dose was excreted (molar equivalents as TCP) whereas less than 1% of the dermal dose was collected in urine. Taken at face value, these results appear supportive of the conclusion cited above. However, it is useful to examine Nolan et al.'s results in more detail. In the oral experiments, a dose of 0.5 mg/kg was administered. Participants were adult males with average body weight of approximately 80 kg. The effective surface area of the small intestine of an adult, considering microstructure, is estimated to be on the order of 300 m^2 (Vander et al., 1985). The average surface loading can therefore be estimated as:

$$\frac{0.5\ \text{mg/kg} \times 80\ \text{kg}}{300 \times 10^4\ \text{cm}^2} = 13\ \text{ng/cm}^2 \qquad (1)$$

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Assuming a residence time in the small intestine of 4 h (ICRP, 1979), a time-averaged flux into the gastrointestinal wall can be estimated as:

$$\frac{70\% \times 13\ \text{ng/cm}^2}{4\ \text{h}} = 2.3\ \text{ng/cm}^2\text{h} \qquad (2)$$

In Nolan et al.'s dermal experiments, a higher dose of 5 mg/kg was used (for 5 of 6 subjects) and this amount was spread over only 100 cm^2 of forearm skin. Loading on skin in the dermal experiments can be approximated as:

$$\frac{5.0\ \text{mg/kg} \times 80\ \text{kg}}{100\ \text{cm}^2} = \frac{400\ \text{mg}}{100\ \text{cm}^2} = 4000\ \mu\text{g/cm}^2 \qquad (3)$$

Participants were instructed not to wash for at least 12 h and reported washing at 12–20 h. Given that forearms were not guarded, that the vehicle chosen (dipropyleneglycol monomethylether) would have evaporated relatively slowly (and hence been subject to loss by wipe-off), and that the efficacy of the eventual washing is unknown, the effective duration of these experiments is not well defined. Assuming a 16-h duration, the average flux in the 5-mg/kg dermal dosing experiments can be estimated as:

$$\frac{1\% \times 4000\ \mu\text{g/cm}^2}{16\ \text{h}} = 2.5\ \mu\text{g/cm}^2\text{h} \qquad (4)$$

Comparing Eq. 4 result to that of Eq. 2, the observed average flux into the skin in Nolan et al.'s experiments was roughly three orders of magnitude larger than the observed average flux through the intestinal wall. Even if it is assumed that absorption of residue left on the skin continued after the first washing, no credible exposure period would lead to a ratio less than two orders of magnitude. Hence an assumption that the skin presents a rigorous barrier to absorption of relatively low molecular weight, moderately lipophilic, semi-volatile compounds such as CPS is not supported by Nolan et al.'s results.

It is useful to note that the gastrointestinal tract has evolved to absorb nutrients. The dimensions of the small intestine provide adequate retention time and relatively large surface area for transport. These conditions have not arisen without cause. Increasing transport surface area to increase absorption efficiency confers an evolutionary advantage, hence the convoluted structures of intestines and lungs. By contrast, conduct of dermal absorption experiments at high surface loads is a good strategy for minimizing apparent uptake efficiency. By limiting the skin area exposed and applying a higher dermal than oral dose, Nolan et al. effectively predetermined that the results would give an appearance of relatively low dermal availability on a fraction absorbed basis.

Note also that in this case the ramifications of incomplete excretion are potentially much more important with respect to the dermal results than the oral results. CPS is a lipophilic compound that, in the absence of metabolism, would likely be stored in human fat for long periods. Mass accounting for the oral dose was roughly 70%. Whatever the disposition of the other 30%, the estimated intestinal flux would not change dramatically if it were assumed absorbed. Mass accounting for the dermal dose, conversely, was limited to the roughly 1% recovered in urine as no attempt was made to recover unabsorbed residue from the skin. Sequestration of a small portion of the unaccounted for CPS in blood or tissue in that case could lead to a several fold increase in the estimated average flux across the skin. This would shift the ratio of average dermal flux to average intestinal flux still further upward.

The consequences of dependence on fractional absorption rather than flux to evaluate the significance of dermal exposure are illustrated in calculations in a recent review of the toxicology of CPS presented by Eaton et al. (2008). After discussing dietary exposures and reviewing literature reporting measured surface loads of CPS in residences and day care facilities, Eaton et al. present the following example calculation:

> "For example, if one assumed that 'surface loading' occurred directly to a child's skin at a rate of 10 ng/cm^2, and 100 cm^2 of skin were in contact at that rate, and further assumed that 5% of the exposed dose was absorbed over the course of a day, the daily dose for a 20-kg child would be 0.0025 μg/kg-day."

They further assert (emphasis added):

> "These represent *conservative* assumptions that are likely to *substantially overestimate* dermal exposure to a child, but are useful in assessing the contribution of dermal exposure to aggregate exposure to children, and suggest that dermal exposure is unlikely to contribute significantly to urinary (TCP) values, relative to other sources."

In mathematical terms, Eaton et al.'s argument is as follows:

$$\frac{5\% \times 100\ \text{cm}^2 \times 10\ \text{ng/cm}^2}{20\ \text{kg} \times 1\ \text{day}} = 0.0025\ \mu\text{g/kg day} \qquad (5)$$

Eaton et al. presumably consider this computation conservative as 5% is larger than Nolan et al.'s average estimate of 1% absorption and because they specify a potential dose by setting the skin load equal to a value taken from the upper range of loads on inanimate residential surfaces typically reported in the literature. Neither component of the dose estimate is conservative, but the assumption regarding dermal absorption efficiency is of primary interest here. As this is a daily estimate, average flux through the skin can be computed as:

$$\frac{5\% \times 10\ \text{ng/cm}^2}{24\ \text{h}} \approx 20\ \text{pg/cm}^2\text{h} \qquad (6)$$

This result can be compared to that shown in Eq. 4 above. Eaton et al. have declared conservative an estimated flux that is roughly 100,000 times smaller than that actually observed in the human *in vivo* experiments of Nolan et al. (1984). In this case the projected rate of delivery of CPS to the skin is so low relative to the demonstrated ability of human skin to absorb it that the only estimate that could confidently be considered conservative would be 100% absorption.

Mathematical Argument

Reliance on fixed fractional absorption continues despite the fact that the logical basis for predicting higher absorption efficiency at lower surface loading is straightforward. Dermal absorption is best conceptualized as gradient-driven mass transfer through a membrane. The driving force for absorption is the gradient in thermodynamic activity across the membrane (skin). If a pure compound is applied to the skin in amounts exceeding the rapid sorption capacity of the outermost cells and sebum, its thermodynamic activity in the external layer will be independent of the mass of the chemical applied. (Mass loads on surfaces are often inappropriately referred to as concentrations. In science concentration is defined as amount per amount [(mass or volume)/(mass or volume)] and is widely used as a surrogate for thermodynamic activity. Mass loading [mass/area] is not concentration and is not an appropriate surrogate for thermodynamic activity.) Hence, as long as coverage is complete, initial flux into the skin should also be independent of the surface load. This condition is illustrated in Figure 1 and written as follows:

$$J_{\text{thin}} = J_{\text{thick}} \tag{7}$$

where J is flux (mass area^{-1} time^{-1}). It is also true per the problem statement that:

$$\text{SL}_{\text{thin}} < \text{SL}_{\text{thick}} \tag{8}$$

where SL is the surface load (mass area^{-1}). It follows directly that:

$$\left(\frac{J}{\text{SL}}\right)_{\text{thin}} > \left(\frac{J}{\text{SL}}\right)_{\text{thick}} \tag{9}$$

As the ratio of flux to surface load (time^{-1}) represents fractional uptake per unit time, it is clear that, for a given experimental duration insufficient to produce significant depletion of the external source or saturation of the skin, fractional uptake would be expected to be higher from the thinner surface load.

Supporting Empirical Evidence

Increasing fractional absorption with decreasing loading is, in fact, routinely observed in the literature in data sets describing absorption experiments conducted at different loads for the same compounds by the same investigators. Thongsinthusak et al. (1999b) and Zendzian (2000) reviewed rat data submitted to regulatory agencies for pesticide registration purposes and noted such an effect. More recently, Buist et al. (2009) reviewed the broader literature and found substantial evidence for increasing fractional absorption with decreasing load for a wide range of organic compounds.

In limited cases, linear inverse proportionality has been observed. Hughes et al. (2001) applied decabromodiphenyl oxide (DBDPO) to mouse skin *in vitro* at 9, 45 and 90 μg/cm^2 and observed roughly the same average flux (receptor plus depot after solvent cleaning) at all three loads. Comparing results at the upper and lower limits of the range, one-tenth the loading produced a little more than ten times the fractional absorption. Meuling et al. (2005) applied CPS to the arms of human volunteers at loads of 54 and 161 μg/cm^2. Uptake, on the basis of TCP collected in urine, was estimated as 3.5 times greater in the lower load experiment (i.e., at one-third the load). More often an inverse loading effect is observed but strict proportionality is not. At least one simple explanation exists for this finding. Among the greatest difficulties encountered by dermal experimentalists is inability to achieve uniform distribution of target agent on skin whether *in vivo* or *in vitro*. This is illustrated in Figure 2. If mass is not uniformly distributed, effective interfacial area can be less than assumed in the experimental design leading to reduction in the apparent average flux.

If compounds are not applied neat or in rapidly dispersed volatile solvents, chemical mass load is directly related to vehicle mass load and driving force will depend on concentration in the external phase. With allowance for those differences, the logic behind Eqs. 7–9 still applies. As would be expected, investigations of dermal absorption from contaminated soil, for instance, do show decreasing fractional absorption with increasing (supra-monolayer) soil load

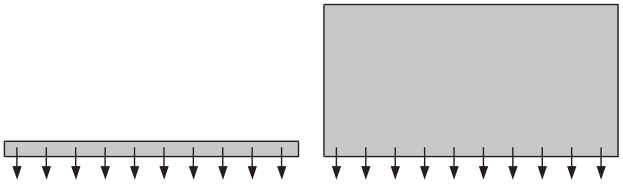

Figure 1. A schematic representation of flux from thin versus thick uniform surface loads. Short-term (pre-depletion) relative efficiency of absorption would be expected to be inversely proportional to loading.

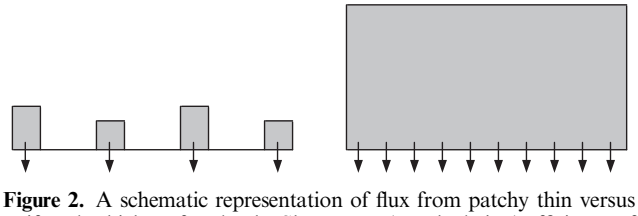

Figure 2. A schematic representation of flux from patchy thin versus uniformly thick surface loads. Short-term (pre-depletion) efficiency of absorption would be expected to be greater in the thin case, but not in a manner linearly and inversely proportional to average loading.

at constant soil concentration (Duff and Kissel, 1996; Spalt et al., 2009).

Further evidence for increased fractional uptake at lower surface loads can be found in experiments intended to gauge recoveries from or transfer to skin rather than absorption. Fenske and Lu (1994) put known amounts of CPS on a test tube, which was then gripped by a volunteer as it was twisted. The resulting average loads on skin were estimated as approximately 0.02 to 10 $\mu g/cm^2$. Volunteers' hands were rinsed in 10% isopropanol/water within 1 min or at about 1 h and recovery was reported. Generally lower recoveries were observed at lower initial loadings and with longer delay until washing. The results were interpreted as evidence of relatively rapid binding/absorption of the missing CPS mass. Fenske et al. (1998) reported incomplete recovery of captan using similar methods. Campbell et al. (2000) applied four pesticides (glyphosate, alachlor, trifluralin and methyl parathion) to porcine skin patches and attempted recovery at 90 min by wiping with gauze pads impregnated with one of four solvents. Pesticide loads were 0.5, 2 and 8 $\mu g/cm^2$. Recoveries from the lowest load were significantly lower than recoveries from the highest load in 14 of 16 comparisons.

Results from some of the absorption and transfer/recovery experiments discussed above are presented in Figure 3. The x-axis scale of Figure 3 is deliberately broad. Actual residential exposures occur at the far left side of the x-axis. Deliberate experimentation typically occurs at higher loads and sometimes at much higher loads. (Some experiments reported by Buist et al. (2009) fall well off the graph to the right.) Increasing efficiency of absorption and/or reduced efficiency of recovery is generally observed at lower loadings in the selected studies.

Still more evidence of higher efficiency of uptake at lower surface loads can be found in comparison of absorption efficiencies required to explain observed biomonitoring in exposure studies that permit mass balance to be attempted. Geer et al. (2004) investigated five CPS handler exposure studies submitted to USEPA in which both dosimetry and biomonitoring were conducted. They found that assumption of 3% dermal availability for CPS (an ostensibly conservative estimate on the basis of the Nolan et al. (1984) experiments) led to under-prediction of urinary TCP excretion. As average CPS loads on handlers' skin would generally be expected to be much less than the 4000 $\mu g/cm^2$ employed by Nolan et al., higher fractional efficiency is very plausible. However, even though Geer et al. explicitly concluded that fractional absorption in excess of the EPA default of 3% was required to explain observed excretion of TCP in the cases they studied, they were subsequently criticized by Mage (2006):

> "…the expectation from Nolan's data is that 1.3% would be absorbed into the skin. Consequently, the authors' usage of 3% for a DAF (dermal absorption fraction) is very likely to lead to a gross over-prediction of the amount entering the body."

As failure to consider the effect of loading on dermal absorption can lead to misinterpretation of experimental results or field observations, it is reasonable to seek a strategy to identify conditions under which loading dependence might be expected.

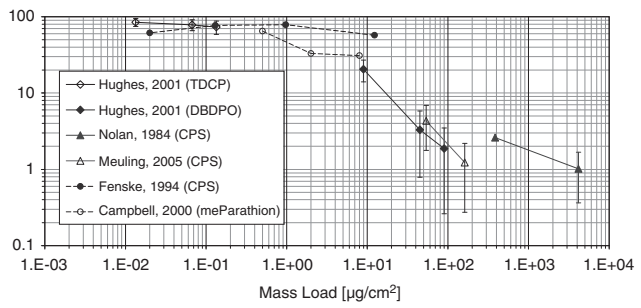

Figure 3. Fraction absorbed or unrecovered versus initial mass load in selected studies (see text). The lines connect data points from the same study. Solid lines denote absorption experiments. Dashed lines denote transfer/recovery experiments.

Methods

Dimensionless parameter groups are used in many branches of science to elucidate relative magnitudes of competing phenomena. In this case it is reasonable to characterize the relative balance between supply and demand (i.e., absorptive flux) at the skin surface by calculating a dimensionless ratio or dermal number. In the context of designed experiments, this number can be written:

$$N_{\mathrm{DERM,exp}} = \frac{\text{experimental load (mass/area)}}{\text{maximum flux (mass/area} \times \text{time)} \times \text{duration (time)}} \tag{10}$$

In symbolic notation and using commonly encountered units, Eq. 10 can be rewritten as:

$$N_{\mathrm{DERM,exp}} = \frac{\mathrm{SL}\ (\mu g/cm^2)}{J_{\max}\ (\mu g/cm^2\ h) \times \mathrm{ED}\ (h)} \tag{11}$$

where SL is surface load, $J_{\max}$ is maximum plausible flux (on the basis of prior experimental results or theoretical considerations) and ED is exposure duration. High values of the experimental dermal number, $N_{\mathrm{DERM,exp}}$, connote surplus load (i.e., flux-limited) conditions. Under those conditions absorbed dose cannot be assumed to be proportional to applied dose and hence observed fraction absorbed cannot be assumed to be transferable to other loading conditions. Low values of $N_{\mathrm{DERM.exp}}$ connote supply-limited conditions. Under those conditions efficient absorption is plausible.

Similarly, for scenarios that occur outside of the laboratory or controlled environment and that are not designed, an environmental dermal number can be defined as:

$$N_{\mathrm{DERM,env}} = \frac{\left(\frac{\text{delivery rate (mass/time)}}{\text{exposed skin area (area)}}\right)}{\text{maximum flux (mass/area}\times\text{time)}} \quad (12)$$

In one common conceptualization, delivery to the skin is estimated as the product of a transfer coefficient and a dislodgeable residue. Eq. 12 then becomes:

$$N_{\mathrm{DERM,env}} = \frac{\mathrm{TC}\,(\mathrm{cm^2/h}) \times \mathrm{DR}\,(\mu\mathrm{g/cm^2})/\mathrm{SA}\,(\mathrm{cm^2})}{J_{\max}\,(\mu\mathrm{g/cm^2h})} \quad (13)$$

where TC is the transfer coefficient, DR is dislodgeable residue and SA is area of skin exposed. Interpretation of $N_{\mathrm{DERM,env}}$ with respect to absorption efficiency is the same as for $N_{\mathrm{DERM,exp}}$. A high value of $N_{\mathrm{DERM,env}}$ indicates a system that is flux-limited, which might show misleadingly low fractional availability. A low value of $N_{\mathrm{DERM,env}}$ indicates a system that is delivery-limited (i.e., delivery is slow relative to absorption). In that case residence time at the surface should be short and skin sampling would be expected to be of little value in estimating potential dose. Conversely, a high value of $N_{\mathrm{DERM,env}}$ indicates surplus delivery, in which case skin sampling might be useful for purposes of defining potential dose.

Results

Relationships embodied in Eqs. 10–13 are readily applied to the dermal exposure scenarios discussed above as well as additional cases. Meuling et al. (2005) found the same flux at loadings of 54 and 161 μg/cm^2. At the lower loading, N_{DERM} can be calculated (using the flux apparently observed in Nolan et al.'s experiments) as:

$$N_{\mathrm{DERM,exp}} = \frac{54\ \mu\mathrm{g\,cm^2}}{2\ \mu\mathrm{g/cm^2h}\times 4\ \mathrm{h}} \approx 7 \quad (14)$$

This result suggests the system was flux-limited at even the lower experimental load. In the absence of depletion or maldistribution, similar fluxes at the lower and higher loads, and therefore inversely proportional fractional absorptions, would be expected. Similarly, the DBPDO experiments of Hughes et al. (2001) discussed earlier have experimental dermal numbers of roughly 5–50.

Calculation of N_{DERM} in the case of the recovery experiments of Fenske and Lu (1994) is problematic due to difficulty of assigning an appropriate value of $J_{\max}$ given a protocol in which some transfer may have been by mechanical embedding rather than passive chemical transport. However in the experiments of Campbell et al. (2000), the target compounds were distributed on porcine skin samples in a manner similar to that used in traditional absorption experiments (i.e., deposition from volatile solvent). Estimating a plausible flux for methyl parathion of 0.4 μg/cm^{2}h on the basis of porcine skin results reported for the structurally similar ethyl parathion (Chang et al., 1994) gives the following result:

$$N_{\mathrm{DERM,exp}} = \frac{0.5\ \mu\mathrm{g/cm^2}}{0.4\ \mu\mathrm{g/cm^2h}\times 1.5\ \mathrm{h}} \approx 0.8 \quad (15)$$

A value less than one indicates delivery-limited conditions at the lowest load tested. Therefore the low wipe recovery reported (c. 25% at 90 min) is consistent with expectation. At 8 μg/cm^2, N_{DERM} would be 16 times greater (i.e., >10) suggesting those experiments were conducted in the flux-limited regime. Higher efficiency of wipe recovery at that load is again consistent with expectation.

Dermal experimentation is not limited to characterization of permeation or transfer/recovery. The arguments presented here apply as well to dermal toxicity studies. If experiments intended to define a dermal No Observed Effect Level (NOEL) are conducted under flux-limited conditions, results may be misleading. For example, a dermal toxicity study submitted to EPA in support of registration of amitraz (ORNL, 2004) used doses of 8, 16 and 24 mg/kg distributed on 80 cm^2 of skin on adult male subjects (mean mass 77 kg). The resulting average surface loads were approximately 8,000, 15,000 and 23,000 μg/cm^2. Results from a rat study submitted to the California Department of Pesticide Registration (Thongsinthusak et al., 1999a, b) permit the estimation of an average flux of nearly 6 μg/cm^{2}h at the highest load tested. This value should be conservative for humans due to the generally greater permeability of rat skin. An experimental dermal number can therefore be calculated at the lowest dose as:

$$N_{\mathrm{DERM,exp}} = \frac{8000\ \mu\mathrm{g/cm^2}}{<6\ \mu\mathrm{g/cm^2h}\times 6\ \mathrm{h}} > 200 \quad (16)$$

By contrast, Cole et al. (2005) applied doses of 50, 100, 125 and 150 mg/kg of CPS to 4-cm^2 areas on the backs of mice. Using results reported by Shah et al. (1981) to estimate CPS flux through mouse skin, the experimental dermal number at the highest dose in the Cole et al. experiments is approximately:

$$N_{\mathrm{DERM,exp}} = \frac{(150\ \mathrm{mg/kg}\times 0.02\ \mathrm{kg}\times 1000\ \mu\mathrm{g/mg})/4\ \mathrm{cm^2}}{\approx 20\ \mu\mathrm{g/cm^2h}\times 24\ \mathrm{h}} \approx 1.6 \quad (17)$$

This suggests that the highest dose is on the boundary of supply-limited and flux-limited conditions. Interestingly, Cole et al. found a clear dose response (for brain acetylcholinesterase inhibition, see Figure 4a in Cole et al.) up to the 125 mg/kg dose, but little or no difference between the 125 and 150 mg/kg doses.

Estimates of experimental N_{DERM} for multiple trials in the cases discussed above are presented in Table 1. For the Hughes et al. (2001) experiments, values of $J_{\max}$ were estimated from observed results of those same experiments.

Given the high overall absorption efficiencies seen for tris-(1,3-dichloro-2-propyl)phosphate (TDCP), observed average flux probably underestimates J_{max}. The values of N_{DERM} shown for those experiments should therefore be overestimates.

The conditions reflected in Eqs. 14-17 were imposed deliberately. The dimensionless dermal number can also be applied to interpretation of results observed under uncontrolled conditions. Morgan et al. (2005) have described environmental measurements of CPS and TCP obtained in EPA's CTEPP NC study. In that study, hard surface wipes produced median CPS loads on the order of $10^{-5}\,\mu g/cm^2$. For the CPS case, a TC of roughly $5000\,cm^2/h$ gives reasonable correspondence between predicted aggregate exposure and observed urinary excretion of TCP (unpublished results, author's laboratory). Assuming that contact can occur over any part of a child's body and using the CPS flux estimate corresponding to traditional interpretation of the Nolan et al. experiments, the corresponding environmental dermal number would be:

$$N_{DERM,env} = \frac{(5000\ cm^2/h \times 10^{-5}\ \mu g/cm^2)/8,000\ cm^2}{2\ \mu g/cm^2 h} \approx 3 \cdot 10^{-6} \quad (18)$$

This very low dermal number implies that, under conditions indicated by the CTEPP sampling, uptake would be delivery-limited (i.e., absorption efficiency could be very high) and that therefore skin sampling would provide a very poor estimate of potential dermal dose.

Discussion

In all cases shown in Table 1, J_{max} was estimated on the basis of a reported empirical result. For some compounds, it may not be possible to find useful empirical data. Alternatively, maximum steady-state flux can be roughly estimated as the product of aqueous solubility and an appropriate permeability coefficient (from water). The latter quantity can in turn be estimated for human skin using one of several available regressions on molecular weight and the octanol–water partition coefficient. This approach has been described in a recent NIOSH (2009) publication. The resulting estimate of J_{max} has large uncertainty, but can nevertheless be useful for calculating N_{DERM} if the result is interpreted accordingly.

The values of N_{DERM} derived above show that both flux- and delivery-limited conditions are encountered in dermal experimentation, sampling and exposure assessment. Consideration of this fact is necessary if observations are to be interpreted appropriately. The review of Buist et al. (2009) provides ample evidence that fractional dermal absorption does increase with decreasing loading for many compounds. Failure to observe a loading effect in some experiments was attributed to depletion due to volatilization or alteration of the barrier function of the skin by the agent being tested. These are certainly plausible explanations that may hold in some cases. However, Buist et al. failed to note the obvious possibility that in some experiments no loading effect was seen simply because there was no difference in actual loading as opposed to nominal loading across trials (i.e., maldistribution resulted in very incomplete coverage at the lower loadings attempted). Failure to initially distribute the test chemical uniformly could also explain a less than proportional effect even when a loading effect is observed. Meuling et al. (2005), who found inverse linear proportionality as noted above, used a glass microscope slide, rather than the more common pipette tip, to spread loads on their volunteers' arms.

The review of Buist et al. (2009) also shows a shortage of experiments at low loads relevant to common exposure conditions and of particular interest here given prediction of increasing absorption efficiency at low loads. Only a handful of absorption experiments have been conducted at loads under $200\,ng/cm^2$. These include the *in vitro* mouse TDCP experiments of Hughes et al. (2001), the *in vitro* human DEET experiments of Santhanam et al. (2005) and some early dioxin/dibenzofuran experiments using rats *in vivo* (Brewster et al., 1989; Banks and Birnbaum, 1991). Santhanam et al. (2005) found relatively low fractional absorption at low loads, a result that is attributable to the relatively high vapor pressure of DEET and the long duration of the experiments. By contrast, relatively high fractional absorptions were reported for TDCP and dioxins/dibenzofurans in low-load trials despite aggressive post-exposure washing using solvents that either likely would or clearly did strip off the stratum corneum. In addition, in the dioxin experiments material in the solvent-washed application site epidermis/dermis was excluded from the definition of absorption. Hence both sets of experiments likely produced underestimates of rodent skin penetration. Experiments that show high absorption efficiency inevitably entail depletion of the external phase. As a consequence, late-stage flux may be very slow and material may accumulate in the stratum corneum. Assessment of low-level exposure generally occurs in the context of chronic exposures not well replicated by batch experiments. Replenishment of the external phase by subsequent exposure could drive material left in the stratum corneum into lower skin layers and ultimately internal circulation. Therefore the appropriateness of exclusion of skin depots is doubtful. Additional experimentation at low levels and under repeated exposure conditions is needed.

Miller and Kasting (2010) investigated dermal absorption of parathion in human skin *in vitro*. Experiments were run for 96 h. Under occluded conditions, fractional absorption (receptor plus depot after washing) exceeded 70% even at the highest load tested ($117\,\mu g/cm^2$). These experiments illustrate the fact that, in the absence of volatilization, wash-off or other loss, relatively low-molecular-weight, moderately lipophilic compounds will continue to be absorbed as long as

Table 1. Estimated values of experimental N_{DERM} for selected studies.

Reference	Compound	Species	*Vivo/vitro*	Dose (mg/kg)	BW kg	SA (cm^2)	Load ($\mu g/cm^2$)	Duration (h)	Estimated J_{max} ($\mu g/cm^2 h$)	N_{DERM}	% Absorbed
Absorption experiments											
Nolan et al. (1984)	CPS	Human	*Vivo*	0.5	80	100	400	16	2	13	2.6
				5	80	100	4000	16	2	125	1.0
Meuling et al. (2005)	CPS	Human	*Vivo*			100	54	4	2	7	4.3
						100	161	4	2	20	1.2
Hughes et al. (2001)	DBDPO	Mouse	*Vitro*			0.64	9	24	0.08	5	20.5
						0.64	45	24	0.08	23	3.3
						0.64	90	24	0.08	47	1.9
Hughes et al. (2001)	TDCP	Mouse	*Vitro*			0.64	0.013	24	>0.004	<0.1	85
						0.64	0.067	24	>0.004	<0.7	78
						0.64	0.134	24	>0.004	<1.4	73
Recovery experiments											
Campbell et al. (2000)	Methyl parathion	Pig	*Vitro*			25	0.5	1.5	0.4	0.8	65[a]
						25	2	1.5	0.4	3.3	33[a]
						25	8	1.5	0.4	13.3	31[a]
Dermal toxicity experiments											
ORNL (2004)	Amitraz	Human	*Vivo*	8	77	80	7700	6	<6	>214	
				16	77	80	15,400	6	<6	>428	
				24	77	80	23,100	6	<6	>642	
Cole et al. (2005)	CPS	Mouse	*Vivo*	50	0.02	4	250	24	20	0.5	
				100	0.02	4	500	24	20	1.0	
				125	0.02	4	625	24	20	1.3	
				150	0.02	4	750	24	20	1.6	

Abbreviations: BW, body weight; CPS, chlorpyrifos; DBDPO, decabromodiphenyl oxide; SA, surface area; TDCP, tris-(1,3-dichloro-2-propyl)phosphate.
[a]Mean fraction not recovered by wiping.

a positive thermodynamic gradient is maintained at the surface of the skin. Observed fractional absorption is clearly dependent upon experimental conditions. The commonly encountered assumption that fractional absorption can be definitively capped at values much less than 100% through limited experiments conducted under unrepresentative conditions is not well founded. Movement away from default reporting of fractional absorption in favor of observed flux has the potential to greatly improve general understanding of dermal absorption phenomena.

Multiple misinterpretations of dermal absorption phenomena are evident in examples presented here. Mage's (2006) insistence that Geer et al. (2004) ignore the evidence they assembled using a mass balance approach in favor of deference to the unfounded assumption that the high-load experiments of Nolan et al. (1984) in five individuals delimited for all time the potential dermal availability of CPS is of interest primarily as an example of the consequences of unfamiliarity with the logic of Eqs. 7–9.

By contrast, there is opportunity for more serious error if the loading effect is ignored in the design and interpretation of dermal toxicity studies. If, in an oral dosing study, tubes were inserted into the subjects' mouths and threaded all the way to their colons, shunting the target chemical past the small intestine, the results would be viewed as illegitimate. Nevertheless dermal dosing studies conducted at high loads on a very small fraction of the total skin surface area, which similarly involve artificial suppression of dermal availability, are apparently commonly viewed as acceptable. The amitraz study discussed above (ORNL, 2004) was approved by toxicologists employed by both an EPA contractor and EPA before running afoul of EPA's Human Studies Review Board (HSRB, 2006). At $N_{DERM} > 200$, the amitraz experiments were flux-limited, hence the higher nominal doses did not represent higher absorbed doses. The potential for generation of misleading results is obvious. If the area of the dosing site is so small that no effect is seen even if it is saturated, the apparent toxicity of the compound in question can be artificially reduced (as reflected in increasing dermal NOEL) by simply loading more chemical onto the target area. The results of dermal toxicity studies conducted at $N_{DERM} > 1$ are therefore of dubious value.

Shortcomings are also evident in Eaton et al.'s (2008) assessment of dermal CPS exposure to children. Those authors arbitrarily assumed that children could only be dermally exposed through approximately 25% of the surface area of their hands (approximately 1% of their body surface area) and that children are passive receptors. They then coupled their non-conservative estimate of potential dose with a likely underestimate of fractional absorption justified by uncritical reading of Nolan et al. (1984). This confluence of poor assumptions can be found in many prior dermal exposure assessments. In the case of CPS, the practice of multiplying an assumed 3% dermal availability by a surface

load obtained from skin wipes can be reasonably expected to lead to compounded non-conservatism (i.e., multiplication of underestimates of both potential dose and fraction absorbed) in many circumstances. Note however, that the issues raised here are not compound-specific. CPS is featured prominently in this work simply because it is, relatively speaking, a very well studied compound and numerical examples involving CPS are easily produced.

In summary, the prediction that absorption efficiency should, for many compounds of interest, increase with decreasing mass load stems from logical application of fundamental principles of physics, chemistry and mathematics. This prediction is further supported by observations from evolutionary biology, and by results of absorption experiments, transfer and recovery experiments, and human exposure studies that encompass both dosimetry and biomonitoring (and hence permit mass balance to be attempted). Therefore, the assumption that fractional dermal uptake is independent of mass loading on skin is not well founded. Application of a dimensionless dermal number, N_{DERM}, representing the ratio of mass delivery to skin to potential absorptive flux has been proposed and shown here as an aid to design of experimental procedures and interpretation of observed outcomes.

Conflict of interest

The author declares no conflict of interest.

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