

ORIGINAL ARTICLE

Analysis of finite dose dermal absorption data: Implications for dermal exposure assessment

H. Frederick Frasch¹, G. Scott Dotson², Annette L. Bunge³, Chen-Peng Chen⁴, John W. Cherrie⁵, Gerald B. Kasting⁶, John C. Kissel⁷, Jennifer Sahmel⁸, Sean Semple⁹ and Simon Wilkinson¹⁰

A common dermal exposure assessment strategy estimates the systemic uptake of chemical in contact with skin using the fixed fractional absorption approach: the dermal absorbed dose is estimated as the product of exposure and the fraction of applied chemical that is absorbed, assumed constant for a given chemical. Despite the prominence of this approach there is little guidance regarding the evaluation of experiments from which fractional absorption data are measured. An analysis of these experiments is presented herein, and limitations to the fixed fractional absorption approach are discussed. The analysis provides a set of simple algebraic expressions that may be used in the evaluation of finite dose dermal absorption experiments, affording a more data-driven approach to dermal exposure assessment. Case studies are presented that demonstrate the application of these tools to the assessment of dermal absorption data.

Journal of Exposure Science and Environmental Epidemiology (2014) **24**, 65–73; doi:10.1038/jes.2013.23; published online 29 May 2013

Keywords: risk assessment; skin absorption; percent absorption; fractional absorption; dermal load; maximum flux; evaporation

INTRODUCTION

The potential for adverse systemic health effects resulting from dermal contact with a chemical is broadly recognized. In situations where dermal contact may contribute significantly to the total body burden, assessment of the systemic uptake of the chemical following skin contact allows for greater accuracy in the estimation of total absorbed dose, hence, a more comprehensive understanding of the risks of systemic toxicity. Therefore, it is incumbent upon the risk assessor to consider the dermal absorption potential of a chemical. This in turn requires a reasonable estimate of the dermal absorbed dose, that is, the amount of chemical that is systemically absorbed following contact with skin.

A common strategy¹ practiced in dermal exposure assessment estimates the systemic uptake of chemical by the dermal route using the fixed fractional absorption approach, in which the dermal-absorbed dose is related to some measure of exposure times the fraction of applied chemical that is absorbed. As an example, in the US EPA Standard Operating Procedures for Residential Pesticide Exposure Assessment,² the dermal absorbed dose is calculated as:

$$D = \frac{E \cdot AF}{BW} \quad (1)$$

where D is the absorbed dose (mg/kg/day), E is the exposure (mg/day), BW is body weight (kg), and AF is the fractional absorption factor. This factor—or its equivalent expressed as a percent absorption—is typically an empirical quantity that is assumed to be a fixed value specific to a given chemical regardless of exposure conditions. The fractional absorption factor

is commonly determined from finite dose *in vitro* or *in vivo* dermal absorption studies. In these experiments, at the end of a specified exposure duration, the dermal-absorbed dose is measured and the fractional absorption factor is calculated as the dermal-absorbed dose divided by the applied load.

The fractional absorption approach has been advocated by regulatory and advisory agencies in North America and Europe. In the United States, the EPA guidance for Superfund remediation³ as well as the Office of Pesticide Programs,^{2,4} adopt this approach. Additionally, the US Army,^{5,6} the Department of Homeland Security,⁷ and the National Institute for Occupational Safety and Health⁸ have incorporated fractional absorption in dermal risk assessment strategies. In Europe, the European Commission's technical guidance for risk assessment⁹ emphasizes the primacy of fractional absorption for dermal risk assessment. The EC guidance document on dermal absorption¹⁰ provides a process for setting dermal absorption percentages using default values or, preferably, values determined experimentally.

There are two main advantages of the fractional absorption approach to estimating dermal absorption. First, the finite dose experiment can represent a good model for realistic environmental and occupational exposure scenarios. In-use conditions, including the use of vehicle, expected dose loading, and exposure duration can be manipulated to mimic realistic exposures. The approach is also appealing in its simplicity. The fractional absorption factor is readily determined from dermal absorption experiments, and this factor is easily slotted into simple algebraic expressions such as Eq. (1) to derive the total dermal-absorbed dose from a given exposure.

¹Health Effects Laboratory, National Institute for Occupational Safety and Health, Morgantown, WV, USA; ²Education and Information Division, National Institute for Occupational Safety and Health, Cincinnati, OH, USA; ³Department of Chemical and Biological Engineering, Colorado School of Mines, Golden, CO, USA; ⁴Department of Occupational Safety and Health, College of Public Health, China Medical University, Taichung, Taiwan; ⁵Institute of Occupational Medicine, Edinburgh, UK; ⁶Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH, USA; ⁷Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA; ⁸ChemRisk, Boulder, CO, USA; ⁹Environmental and Occupational Medicine, Division of Applied Health Sciences, University of Aberdeen, Aberdeen, UK and ¹⁰Medical Toxicology Centre, Institute for Cellular Medicine, University of Newcastle, Newcastle Upon Tyne, UK. Correspondence: Dr. H. Frederick Frasch, Health Effects Laboratory, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA.

Tel.: +1 304 285 5755. Fax: +1 304 285 6041.

E-mail: HFrasch@cdc.gov

Received 22 October 2012; accepted 28 February 2013; published online 29 May 2013

However, this apparent simplicity is belied by complicating factors. It has been observed that an inverse relationship often exists between dermal loading and fractional absorption.^{11–13} As the loading increases, the fraction of chemical that is absorbed diminishes. Thus, the fractional absorption factor is not a constant for a given chemical, and it is typically highest for low dermal loads that are characteristic of environmental and occupational exposures. For dermal absorption testing of a quality sufficient for regulatory submission, a range of loads are often required to be investigated to span the range of expected exposures.¹⁴ However, peer review reports in the open literature typically cover a narrow range or just one load. Experiments at low loads present technical complications for the investigator.^{11,15} The uniform application of a thin homogeneous layer of the chemical of interest is challenging and the evaluation of absorbed dose under low loading may stress the limits of chemical detection (The term “low loads” is somewhat ambiguous, but its meaning will become clearer through the information presented in this manuscript.). The OECD Guideline for the Testing of Chemicals¹⁶ recommends a load of 1–5 mg/cm² for solids and up to 10 µl/cm² for liquids for applications that mimic human exposure, with considerations for using the appropriate vehicle, for example, neat, diluted, or formulated material containing the test substance. This recommendation does not account for differences in dermal absorption rates. These loads may realistically approximate the infinite dose regime for poorly absorbed materials, whereas those that are more readily absorbed may exhibit absorption profiles that are more characteristic of a finite dose application.

Other complicating factors are related to the disposition of the applied load. For example, a volatile chemical will both penetrate the skin and evaporate from the skin's surface. Evaporation will diminish the dermal absorbed amount, but it is not evident how to account in any quantitatively meaningful manner for the competing kinetic processes of absorption and evaporation. The exposure duration, or the time during which the skin remains in contact with the chemical, is clearly a factor in determining the absorbed amount and, hence, the fractional absorption factor. An 8-h exposure might be most relevant for occupational exposures, but low-level environmental exposures, for example, from indoor air contaminants, may persist indefinitely. For the experimental design and evaluation of finite dose dermal absorption data, it is therefore important to consider the relationship between the dermal absorption kinetics of a given compound and the exposure duration, in order to evaluate whether a calculated fractional absorption represents a steady state or if additional absorption would be expected from a longer exposure duration.

Despite the prominence of the fractional absorption approach in dermal exposure assessment, there is little guidance on the proper evaluation of dermal exposure and absorption data derived from experimental observations. The purpose of this paper is to present a systematic approach to the evaluation of finite dose *in vitro* dermal absorption experiments. Limitations of the fixed fractional absorption approach are presented and analyzed. The order of presentation is not necessarily a function of their importance; rather for a given set of experimental conditions, one or more may represent important considerations. We present a quantitative framework for the analysis of finite dose dermal absorption data that can aid in the evaluation of the potential of a chemical to be dermally absorbed and contribute to the systemic dose. The objective is to provide those tasked with evaluating dermal absorption data with a sensible, data-driven framework for dermal exposure assessment. Another goal is to lay the framework for researchers to use in the future when designing, conducting, and interpreting dermal absorption studies. More detailed theoretical analyses of finite dose absorption kinetics may be found elsewhere.^{17–20}

LIMITATIONS OF THE FIXED FRACTIONAL ABSORPTION APPROACH FOR ASSESSING SYSTEMIC TOXICITY FROM DERMAL EXPOSURE

Loading Affects Fraction Absorbed

Loading conditions can have enormous effects on fractional or percent absorption.¹³ Figure 1 presents a simplified case to elucidate the effect of loading on measured percent absorbed. This simple illustration demonstrates the crucial effect of loading on the measured result when absorption is reported solely as the fraction or percent of the applied amount. The example on the right is a case where load depletion is small relative to the applied load. This condition can readily be extrapolated to the case of an infinite load, whereby the percent absorbed must be practically zero. Thus, absorbed amount may range from zero to 100% for the same chemical, depending on the applied load. There are several reasons why 100% absorption may not be achieved in a practical setting. Chemical may bind to skin components or it may be removed from the skin by volatilization, debridement, sweating, or washing. This simple illustration, nevertheless, demonstrates that the use of a specified fixed cutoff value to evaluate the dermal absorption potential of a chemical seems unwarranted.

Given this, it is possible to draw any conclusions regarding the absorption potential of a chemical based on the knowledge of

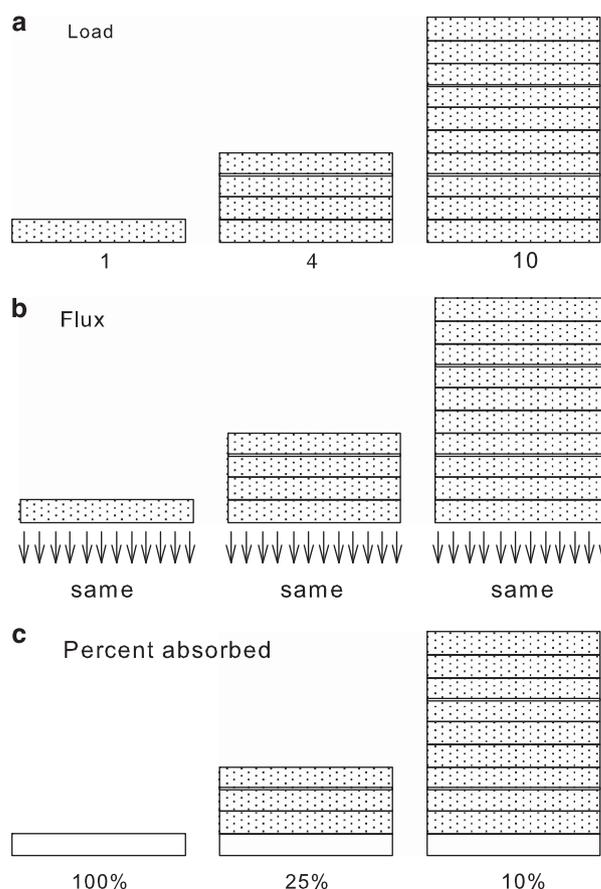


Figure 1. Illustration of how applied load affects percent that is absorbed. (a) Three examples of different loads (1, 4, and 10 units) of a given chemical on skin. (b) As long as absorption has not depleted the amount of chemical on the skin too much, flux of the chemical is the same under all conditions. (c) If the experimental duration is such that one load unit penetrates the skin into receptor fluid then on the left, 100% of the load is absorbed; in the middle, the value is 25%; and on the right it is 10%.

the fraction of applied finite dose that is absorbed? Kissel¹³ proposed a dimensionless ratio, N_{derm} , that may prove useful in the evaluation and interpretation of finite dose experiments. The dermal number quantifies the ratio of applied load and absorbable amount:

$$N_{\text{derm}} = \frac{\text{experimental load}}{\text{steady-state flux} \times \text{experimental duration}} \quad (2)$$

Experimental load is the mass of chemical applied per unit area of exposed skin, and steady-state flux (J_{ss}) is the steady-state absorption rate (mass/area/time) for the chemical at a specified dilution. J_{ss} can be experimentally determined using infinite doses of chemical at the dilution of interest as donor. In the absence of experimental knowledge of J_{ss} , it may be estimated by using any number of theoretical models that predict the steady-state permeability coefficient ($k_{\text{p,v}}$, length/time) of a compound from a given vehicle (typically, water), along with the knowledge of the concentration (C_{v} , mass/volume) of the compound in that vehicle (providing the compound or other components in the vehicle do not significantly alter the skin's barrier):

$$J_{\text{ss}} = k_{\text{p,v}} \cdot C_{\text{v}} \quad (3)$$

We note that this definition of N_{derm} (Eq. (2)) differs from that of Kissel¹³ in that Kissel uses maximum flux in place of steady-state flux. Maximum flux represents the steady-state flux from neat chemical or saturated solution, and its use is appropriate for such donors. Because in practice diluted solutions of chemical are often studied, N_{derm} as defined here is more generally applicable.

The distinction is made between *maximum* flux and *peak* flux. The latter is defined as the highest flux achieved from a given dose. Any *in vitro* diffusion experiment will exhibit a peak flux, the magnitude of which depends on donor concentration and loading. As loading increases, so too will peak flux until a value is reached, which corresponds to the highest flux obtainable from a given donor. If the donor is a neat chemical or a saturated solution, the peak flux will equal maximum flux (once again, provided the chemical or other components in the vehicle do not significantly alter the skin's barrier).

High values of N_{derm} signify an experiment that is flux limited; that is, one where there is ample load that is not significantly depleted through the time course of the experiment. Under this condition, % absorption varies inversely with load. In the simplified case described in Figure 1, a load of 10 units resulted in an absorption of 10% of the applied load. Consider a load of 100 units. Under the described conditions, only 1% of the applied load will be absorbed; for a load of 1000 units, 0.1% is absorbed; and so on. This inverse relationship between dermal load and relative dermal absorption has been noted in recent reviews of the literature,^{11–13} suggesting that a substantial proportion of finite dose experiments are performed in the high N_{derm} regime. Generally, high values of N_{derm} are not representative of the low loads typically encountered in occupational or environmental exposures and the reported % absorption under these conditions is not a useful indicator of the potential of a chemical to be dermally absorbed.

If the entire time course of dermal absorption is provided by the report, then important information can be obtained from experiments with high N_{derm} values. This regime allows the most reliable estimates of maximum flux and lag time. Because load depletion does not limit flux, it is likely that the experimental flux under high N_{derm} conditions approaches J_{max} of the chemical, if the chemical is applied neat or in a saturated solution. An exception to this rule may occur if a solid chemical is applied without a solvent, particularly, if it is coarsely divided or highly crystalline; in this case, absorption may become dissolution limited.

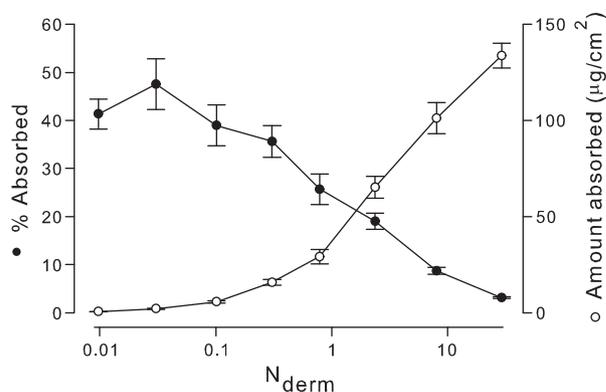


Figure 2. Percent absorption and total amount absorbed for the chemical vanillylnonanamide. Previously published *in vitro* human skin absorption data¹² has been recast as a function of the dimensionless dermal number N_{derm} , which quantifies the ratio of applied load and dermal absorption. Data demonstrate that % absorption diminishes with increasing load. Absorption does not include the skin depot amount.

A low value of N_{derm} signifies a delivery-limited state, where significant load depletion is expected to occur. This condition is a more proper application of the term “finite dose” and the measured peak flux under this condition will be less than the maximum steady-state flux possible for the specified donor, owing to depletion of the load.

Figure 2 displays experimental data that illustrate how N_{derm} affects both the percent of applied load and the total amount that are absorbed. Previously published¹⁷ *in vitro* split thickness human cadaver skin absorption data of the model compound vanillylnonanamide, a synthetic capsaicin, have been recast to display percent absorption and total absorbed amount as functions of the parameter N_{derm} . For an experimental duration of 72 h and maximum flux (measured) of $2 \mu\text{g}/\text{cm}^2/\text{h}$, the load equivalent to an N_{derm} of 1 was $144 \mu\text{g}/\text{cm}^2$. For small loads ($N_{\text{derm}} < 0.1$) of this particular compound, absorption plateaus at ~40–50% of the applied dose, whereas for larger loads ($N_{\text{derm}} > 10$), absorption is less than 10%. On the other hand, as dose is increased the total amount of the compound that is absorbed also increases, as expected. We emphasize that absorption here refers to the amount of chemical that has passed through the skin and into the receptor compartment, and does not include the amount deposited within the skin. This may contribute to the observation that absorption does not approach 100% at low loads. Another possibility is that the experimental duration was not long enough to observe 100% absorption.

It is difficult to assign a precise cutoff value of N_{derm} to distinguish between flux-limited and delivery-limited absorption. A complicating issue is the fact that the experimental duration (a variable in the denominator of Eq. (2)) may have been selected without consideration for the kinetics of the absorption process for the particular chemical investigated. For the data displayed in Figure 2, flux limitation does not appear to be reached at the maximum N_{derm} studied (~30). There exists a near linear relationship between dermal loading and total absorbed amount for $N_{\text{derm}} \sim 1$ and greater, but a plateau in total absorbed amount is not reached.

Case study 1 provides an example from the literature of a failure to consider the effect of loading on fractional absorption and demonstrates how a critical analysis, as advocated herein and by Kissel,¹³ may be applied in the evaluation of finite dose absorption data.

Case study 1: Pentabromodiphenyl ether

The European Union has published a risk assessment for pentabromodiphenyl ether.²¹ In that document, dermal exposure was evaluated using a fixed fractional absorption approach. In the worker exposure scenario, delivery of 0.1 mg/cm²/day to exposed skin was predicted using the Estimation and Assessment of Substance Exposure model. Of this amount, up to 60% was assumed to be absorbed based on experimental results obtained for PCBs in guinea pigs.²² The flux required to sustain the equivalent systemic dose can be calculated as:

$$\frac{0.1 \text{ mg}}{\text{cm}^2 \text{ day}} \times \frac{1000 \mu\text{g}}{\text{mg}} \times \frac{1 \text{ day}}{24 \text{ h}} \times 60 \% = 2.5 \mu\text{g}/\text{cm}^2/\text{h}$$

Roper et al.²³ conducted *in vitro* (human cadaver skin) absorption experiments using ¹⁴C-labeled tetrabromodiphenyl ether as a surrogate for pentabromodiphenyl ether. A purpose of the study was explicitly stated to be “to challenge the default value of 60%”. Observed uptake (skin plus receptor fluid) was 3.13 ± 1.15% (*n* = 10) and the absorbed dose (receptor fluid) was 1.94 ± 0.98% over a 24-h exposure. Because both fractions are much less than 60%, Roper et al. concluded that the ECB risk assessment was unduly conservative. However, in the Roper et al. experiments, the applied load was 10,000 μg/cm². The corresponding average systemic flux can be calculated as at least:

$$\frac{10,000 \mu\text{g}}{\text{cm}^2} \times \frac{1}{24 \text{ h}} \times 1.94 \% = 8.1 \mu\text{g}/\text{cm}^2/\text{h}$$

Therefore, the experimentally observed flux was actually over three times greater than that implicitly assumed in the “worst-case” ECB risk assessment. The comparatively low experimental absorption efficiency is simply an artifact of the 100 × greater surface load used experimentally than assumed in the risk assessment. If this measured flux is the maximum flux for this chemical, a reasonable assumption given that the authors applied neat compound and a steady-state appeared to have been reached within 4–6 h, then

$$N_{\text{derm}} = \frac{10,000 \mu\text{g}/\text{cm}^2}{8.1 \mu\text{g}/\text{cm}^2/\text{h} \times 24 \text{ h}} \approx 50,$$

and the experiment clearly lies within the flux-limited regime.

Additional experimental details, including the use of ethanol as the receptor fluid, might have been used to argue that the observed results were conservatively high, but Roper et al. did not do so. They simply noted that 1.94 and 3.13% were much less than 60%, revealing failure to consider the effect of loading on fractional absorption.

Evaporation or Sublimation of Volatile Compounds Affects Fraction Absorbed

Volatility of the test material is another variable that requires consideration. Fractional absorption of a volatile compound will depend not only on the experimental load, but also on the rate of evaporation compared with the rate of absorption. A highly volatile compound will evaporate from an unoccluded donor and will not be available for dermal absorption. Although small doses of a volatile compound may well represent a reasonable in-use exposure to splashes of chemical, and as such does yield useful data, this scenario may not provide much information regarding the permeability potential of the compound.

The N_{derm} parameter described above (Eq. (2)) does not take into consideration the volatility of the chemical. Therefore, in analogy with N_{derm} , we propose a dimensionless number that quantifies the balance between applied load and depletion through evaporation. The evaporation number is defined as:

$$N_{\text{evap}} = \frac{\text{Experimental load}}{\text{Steady-state evaporation flux} \times \text{exposure duration}} \quad (4)$$

Units for load, flux, and duration are the same as those for N_{derm} . Measured values of evaporation flux are unlikely to be available for most chemicals of interest. In these cases, approximations can be made using empirical or semi-theoretically based models. An example is the US EPA equation for estimating evaporation flux from the surface of chemical spills.²⁴ This

equation has also been applied to estimate evaporation from the donor compartment of diffusion cells^{25–27} and is presented here in the Appendix.

High values of N_{evap} signify the flux-limited condition in which there is no significant load depletion through evaporation. Low values of N_{evap} suggest a delivery-limited condition, whereby significant evaporative losses reduce the observed flux. In contrast with low values of N_{derm} , the percent of applied dose that is dermally absorbed may be quite low for low N_{evap} . Owing to substantial evaporation, less chemical is available for dermal absorption.

The dimensionless flux number (N_{flux}) should prove useful in the evaluation of dermal absorption studies using volatile chemicals. It quantifies the balance between evaporation and absorption:

$$N_{\text{flux}} = \frac{\text{Steady-state dermal flux}}{\text{Steady-state evaporation flux}} \quad (5)$$

Both dermal flux and evaporation flux should be evaluated under the same experimental donor conditions (e.g., neat or diluted). If both fluxes represent maximum fluxes, then N_{flux} is exactly equal to the inverse of the parameter χ described by Kasting and Miller.¹⁸ Low values of N_{flux} indicate a condition in which the measured dermal absorption will be diminished by evaporative losses. For $N_{\text{flux}} \ll 1$, the permeant will largely evaporate from the skin surface. Conversely, large values of N_{flux} are indicative of a compound that will primarily be absorbed. The time scale over which these competing processes occur depends on the membrane lag time, as demonstrated by Kasting and Miller.¹⁸ In brief, surface evaporation commences immediately following application of the load to the skin, whereas dermal flux requires some amount of time, related to the membrane lag time, to become established.

Another useful parameter can be used in the evaluation of finite dose absorption data from volatile compounds. One may estimate the time for evaporation of the applied dose to occur using the evaporation time, defined as:

$$t_{\text{evap}} = \frac{\text{Experimental load}}{\text{Steady-state evaporation flux}} \quad (6)$$

The comparison of t_{evap} with the exposure duration of the applied dose is key. If t_{evap} is much greater than the exposure duration, the experiment is flux limited. That is, there is insignificant load depletion due to evaporation. If t_{evap} is less than the exposure duration, this implies a delivery-limited condition, in which evaporation will limit the load available for dermal absorption, and experimental flux values will be reduced by evaporation.

Figure 3 displays model-based predictions of the % of applied dose that is absorbed and the total amount absorbed as functions of N_{derm} for a broad range of N_{flux} values. Calculations were made using the Finite Dose Skin Permeation Calculator,²⁸ which solves for the disposition of an applied surface load and is based on research undertaken by Kasting's group.^{17,18} These simulations are based on a hypothetical model compound for which the vapor pressure was arbitrarily varied to achieve the specified values of N_{flux} . Modeled maximum flux was 10.8 μg/cm²/h and lag time was 1.33 h. The vapor pressure required to achieve an N_{flux} of 1 was 0.48 Pa at 32 °C. Simulated experimental duration was 8 h.

For N_{derm} less than about 10, Figure 3 shows that percent absorbed increases with N_{flux} . Frasch showed²⁹ through theoretical analysis of Kasting's finite dose model that for low loads, the total absorbed fraction of applied dose at infinite time after exposure may be estimated as:

$$AF = \frac{f + 2N_{\text{flux}}}{2 + 2N_{\text{flux}}} \quad (7)$$

Here, f is the fractional thickness of the desquamating layer of the stratum corneum; a reasonable value for f is 0.1.¹⁸ Equation (7)

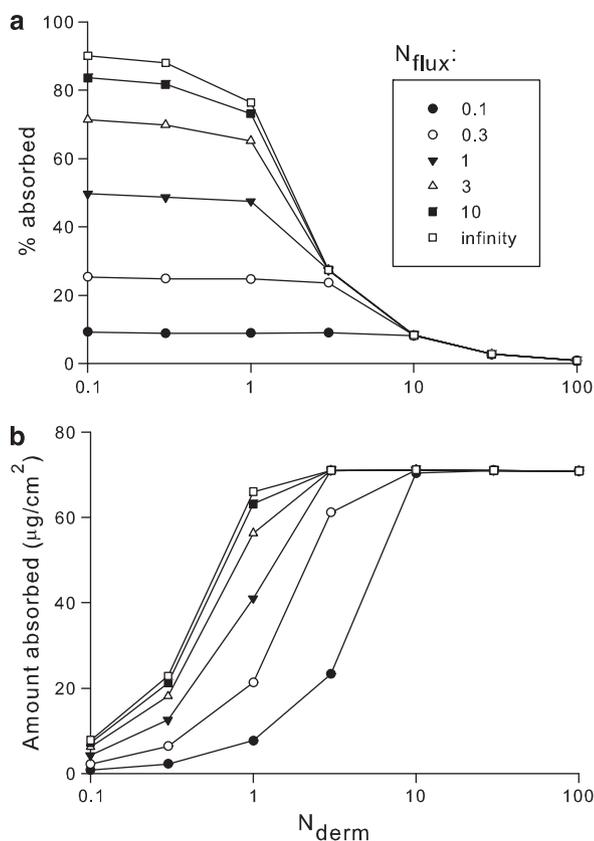


Figure 3. Model-based data showing how % absorption (a) and total absorbed amount (b) are modified by volatility of the compound for selected values of N_{derm} . The dimensionless flux number, N_{flux} , quantifies the balance between absorptive and evaporative fluxes. Dermal absorption depends both on volatility and load. Symbols represent calculated values; the lines are a guide to the eye.

may be used to estimate fractional absorption under low-load conditions.

Not only does % absorbed increase with N_{flux} , but so does the total amount that is absorbed (Figure 3b). For a non-volatile compound ($N_{\text{flux}} \rightarrow \infty$), flux limitation appears at $N_{\text{derm}} \sim 1$. Total absorbed amount approaches its maximum value for $N_{\text{derm}} \geq 1$, indicating that absorption is approaching the regime of infinite loading. Any additional loading beyond this point is excess; it does not contribute to absorption but does diminish the observed percent of load that is absorbed. For a high-volatility compound ($N_{\text{flux}} = 0.1$), this flux-limited regime is not revealed until $N_{\text{derm}} \sim 10$ (see Figure 3).

Although the data shown in Figure 3 are model based, the models have been validated with experimental data.^{17,26,30} For any given compound, *in vitro* absorption data may not be accurately predicted by this family of curves, but we would expect the trends to conform to these modeled data.

Case study 2 presents an application of concepts outlined here to the evaluation of dermal toxicity and absorption data from a volatile compound. This study demonstrates the importance of considering concurrent effects of both loading and evaporation in analyzing the presented data. As suggested by the data in Figure 3, it is important to consider both N_{derm} and N_{evap} for volatile compounds. As a general approach, it may be prudent to consider evaporation for cases where $N_{\text{flux}} \leq 10$; that is, where evaporation contributes 10% or more to applied load losses.

Case study 2: 1-Bromopropane (1-BP)

Elf Atochem undertook a dermal toxicity study, in which they applied 2000 mg/kg of 1-BP neat to shaved skin of rats and covered the application area with gauze for 24 h.³¹ After 2 weeks, there were no signs of dermal toxicity and the study concluded that the dermal LD₅₀ exceeds 2000 mg/kg. The American Conference of Governmental Industrial Hygienists cited this study in their conclusion that “there is no basis for a skin notation” for 1-BP.³² The maximum flux of 1-BP has been measured²⁷ at $\sim 1 \text{ mg}/\text{cm}^2/\text{h}$. Assuming a typical rat weight of 0.25 kg and a dermal application area of 20 cm² gives

$$N_{\text{derm}} = \frac{2000 \text{ mg}/\text{kg} \times 0.25 \text{ kg}/20 \text{ cm}^2}{1 \text{ mg}/\text{cm}^2/\text{h} \times 14 \text{ days} \times 24 \text{ h}/\text{day}} = 0.074$$

This low value indicates delivery limitation where significant load depletion is expected. Consideration of N_{derm} alone would indicate that a substantial fraction of the applied dose was absorbed by the rats. However, 1-BP is a highly volatile halogenated short-chain alkane with a reported vapor pressure (P_{vap}) of 111 mm Hg at 20 °C.³³ The evaporation flux of this chemical can be estimated using the equations in the Appendix. Because a typical skin surface temperature is 32 °C, Eq. (A2) is used to estimate P_{vap} at this temperature. A value of 32,130 J/mol for ΔH_{vap} of 1-BP has been reported³⁴ yielding an estimate of 163 mm Hg for P_{vap} at 32 °C. Using this in Eq. (A1) with a wind speed of 0.3 m/s yields:

$$J_{\text{evap}} \approx 600 \text{ mg}/\text{cm}^2/\text{h},$$

$$t_{\text{evap}} = \frac{2000 \text{ mg}/\text{kg} \times 0.25 \text{ kg}/20 \text{ cm}^2}{600 \text{ mg}/\text{cm}^2/\text{h} \times 60 \text{ min}/\text{h}} = 2.5 \text{ min}$$

Thus, the entire applied load is estimated to have evaporated within minutes after application. Also we have

$$N_{\text{evap}} = \frac{2000 \text{ mg}/\text{kg} \times 0.25 \text{ kg}/20 \text{ cm}^2}{600 \text{ mg}/\text{cm}^2/\text{h} \times 14 \text{ days} \times 24 \text{ h}/\text{day}} = 0.0001$$

This low value indicates a delivery-limited state with substantial load depletion from evaporation. The N_{flux} number differentiates which process, absorption or evaporation, dominates:

$$N_{\text{flux}} = \frac{1 \text{ mg}/\text{cm}^2/\text{h}}{600 \text{ mg}/\text{cm}^2/\text{h}} = 0.002$$

The rapid evaporation of applied 1-BP in the rat dermal toxicity study meant that very little of the applied dose was available to penetrate the skin. The reported low dermal toxicity of this compound could simply be a result of its rapid evaporation. An interpretation of the dermal LD₅₀ data based on an intrinsically low absorption rate and/or low systemic toxicity is not supported by this study.

This analysis is supported by recent experiments comparing the human epidermal absorption of 1-BP following finite dose and infinite dose exposures.²⁷ The authors report minimal (<0.2%) absorption of the applied unoccluded finite dose, despite a substantial maximum steady-state dermal flux of $\sim 1 \text{ mg}/\text{cm}^2/\text{h}$. Despite the considerable rate at which 1-BP penetrates skin, it pales in comparison with the rate at which it evaporates.

Experimental Duration Affects Measured % Absorbed

Another variable that needs to be considered in the evaluation of finite dose experiments is the experimental duration in relationship to the dermal absorption kinetics. The experimental duration is a term in the expressions for both N_{derm} (Eq. (2)) and N_{evap} (Eq. (4)), and therefore merits some discussion.

The experimental duration is generally established at the discretion of the investigator and it may be influenced by a number of considerations, including an attempt to mimic a defined occupational exposure duration, concern for tissue deterioration in an *in vitro* setting, and convenience. For the experimental duration to be a meaningful metric in the equations for N_{derm} and N_{evap} , its relationship to the kinetics of the absorption process is important. Consider a case where the finite dose is fully depleted, through dermal absorption, evaporation, or some combination. Any increase in experimental duration beyond this time will result in lower values of N_{derm} and N_{evap} , but

this decrease is misleading because the dynamic interplay of the diffusion/evaporation process has already abated. One means of evaluating this is to compare the experimental duration with the diffusion lag time (t_{lag} or τ) using the time number:

$$N_{time} = \frac{\text{Experimental duration}}{\text{Lag time for diffusion}} \quad (8)$$

The lag time is generally measured from an infinite dose *in vitro* permeation experiment as the time axis intercept of the asymptote of the absorption curve; typical values range from several minutes to several hours. Lag time measurements for the chemical of interest may be reported in the literature or estimated from the absorption profile, if it has been presented. However, their measurement may be problematic and there could be large variance in the reported quantity. Lag time estimates predicted from physico-chemical descriptors have had limited success.

If the lag time is not reported, an evaluation of whether steady-state absorption has been achieved is possible if the entire time course of dermal absorption is available. If a plateau in the absorption profile has not been reached by the end of the experimental duration, one may infer that additional absorption is to be expected over additional time.

Figure 4 presents model-based data (again, using the Finite Dose Skin Permeation Calculator) for a hypothetical non-volatile compound (vapor pressure = 0) that demonstrate the effect of experimental duration on absorbed amount as a function of N_{derm} . The same model parameters that were used to generate the data for Figure 3 were used here as well, except that experimental duration and applied dose were modified to achieve the specified values of N_{derm} and N_{time} . For small values of N_{derm} , 100% absorption is possible but very long experimental durations are required. According to the data in Figure 4, experimental durations in excess of about 10 lag times would be required to approach 100% absorption. Depending on the chemical of interest, this may range from hours to days, during which time surface removal of the chemical through sweating, washing, and debridement will reduce absorption. For experimental durations approximately equal to the lag time, one can expect no more than ~20% of the applied dose to be absorbed. For these short experimental durations, most of the applied dose resides on the skin surface and within the stratum corneum.

Note that for a given value of N_{derm} , the applied load increases with N_{time} for a given chemical, in accordance with Eq. (2). It is important to keep in mind the interplay among these different parameters when evaluating finite dose absorption data. Figure 4b shows the total mass absorbed for the hypothetical model chemical. This amount increases substantially with experimental duration. For all values of N_{time} , a plateau is reached at $N_{derm} \geq 1$, indicating flux limitation, but the height of the plateau increases for a given compound with N_{time} because both total load and also the time over which absorption is measured increase with this parameter.

Absorption Continues After Removal of Load

If a load is applied to the skin's surface and later removed, chemical will continue to penetrate the skin for some time afterward, even with 100% efficiency in skin residue removal. Some chemical remains in the skin after washing. This reservoir has a higher concentration near the surface, which drives transport through the skin. For volatile compounds, evaporation through the skin surface competes with this process so that post-exposure absorption diminishes with increasing volatility. For *in vitro* exposures designed to mimic a specific scenario, for example, 8 h followed by wash to mimic workplace exposures, it is therefore appropriate to follow permeation beyond the exposure duration to account for absorption from the skin reservoir. This phenomenon has been studied theoretically with some

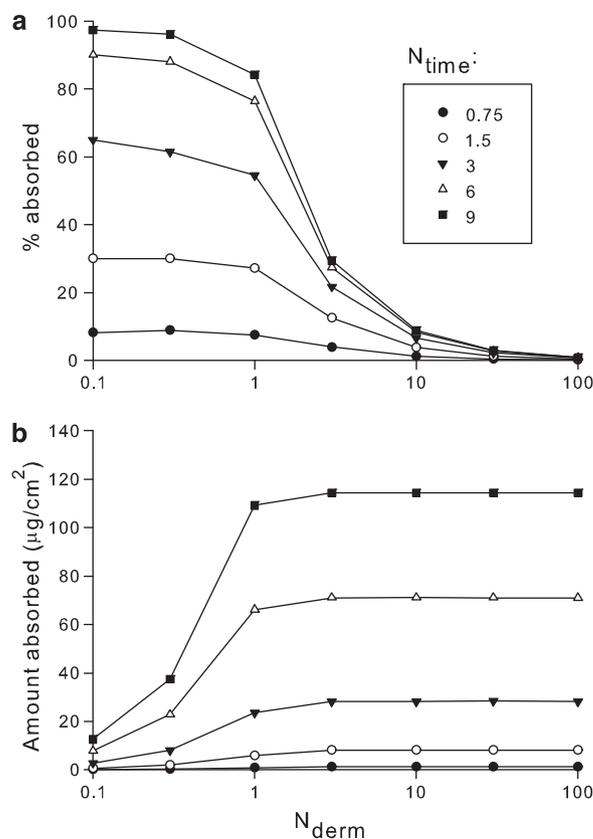


Figure 4. Model-based data showing how % absorption (a) and total absorbed amount (b) are modified by the experimental duration for selected values of N_{derm} . The dimensionless time number, N_{time} , is the ratio of experimental duration to the lag time for diffusion. For a given compound, increasing experimental duration increases the applied load amount that defines a specific N_{derm} , in accordance with Eq. (2). Symbols represent calculated values; the lines are a guide to the eye.

experimental validation.²⁰ The authors propose the following to estimate the total mass absorbed, per unit area of exposed skin, for a transient exposure to a non-volatile permeant.^{20,35}

$$m_{abs} = k_{p,v} \cdot C_v (t_{exp} + 2\tau) \quad (9)$$

with $k_{p,v}$ the permeability coefficient for the permeant in the given vehicle, C_v the concentration in that vehicle, and t_{exp} the exposure duration. Steady-state flux may be substituted for the product $k_{p,v} \cdot C_v$ (Eq. (3)). Equation (9) gives the total absorption that occurs through the duration of the exposure period, plus that which occurs after removal of the compound from the skin surface. The equation is valid if load depletion has not diminished absorption before removal of the compound. Volatility of the applied permeant will lead to evaporative losses through the skin surface upon removal of the compound, reducing the absorbed amount. For highly volatile compounds (say, $N_{evap} \leq 0.1$), the following may be used:²⁰

$$m_{abs} = k_{p,v} \cdot C_v \cdot t_{exp} \quad (10)$$

Equation (9) thus represents the maximum possible absorbed amount and can be used as a conservative estimate, if a reasonable estimate of τ is available.

Figure 5 displays the time course of absorption of the model compound, diethyl phthalate,²⁰ presented here to demonstrate that dermal absorption continues after removal of the load. Following a 40 min exposure (represented by the hashed box on the time axis) to dermatomed hairless guinea pig skin, the

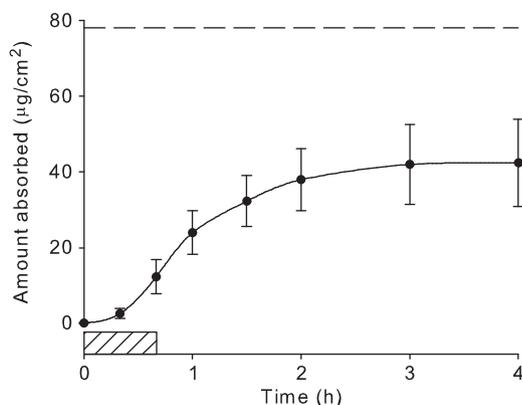


Figure 5. Time course of *in vitro* dermal absorption of diethyl phthalate. Following a 40-min exposure (hashed box on time axis), the chemical was removed and skin was thoroughly rinsed. Absorption continues well after the exposure duration. Mass accumulation at 4 h ($42 \mu\text{g}/\text{cm}^2$) was over three times more than that at 40 min ($13 \mu\text{g}/\text{cm}^2$). Dashed line at $78 \mu\text{g}/\text{cm}^2$ represents the total absorbed amount predicted by Eq. (9).

chemical was removed and the skin was thoroughly rinsed. Absorption continues for almost three more hours, and over three times more chemical was absorbed ($42 \mu\text{g}/\text{cm}^2$) than had been at the time the load was removed ($13 \mu\text{g}/\text{cm}^2$). The dashed line at $78 \mu\text{g}/\text{cm}^2$ represents the total mass absorbed predicted by Eq. (9) using independently measured k_p and τ for this non-volatile compound. Equation (9) overestimates the measured quantity by about a factor of 2 in this case. Binding of the chemical to skin components may explain the discrepancy, and Eq. (9) may thus represent a conservative estimate as there is some question as to whether this bound fraction is potentially bioavailable.

SUMMARY AND CONCLUSIONS

Finite dose skin permeation experiments serve to model “real world” exposures and thus provide important information that can be used in evaluating the risk associated with dermal exposures. However, care must be taken in analyzing and interpreting results from such experiments. This manuscript has described four limitations to the fixed fractional absorption paradigm. Our analyses of the first two pose strategies for evaluating whether loading effects or evaporation effects might be expected, and if so they alert to the possibility that the reported values of fractional absorption require consideration. The analyses outlined in the case studies are exemplary of strategies to approach the evaluation of finite dose absorption data. These studies suggest an informative strategy for the evaluation of the dermal absorption potential of a chemical based on careful assessment of the dermal number, flux number, evaporation number, time number, and auxiliary equations as presented here. We propose that an evaluation of finite dose dermal absorption data consider the following questions.

Is the Load or Range of Loads Investigated Appropriate?

The fraction or percent of applied dose that is absorbed from finite dose experiments depends on the amount of chemical that is initially loaded onto the skin surface. The dependence is so strong that it is conceivable that the absorbed amount can range from 0 to 100% for a given chemical. Experimental observations derived at large N_{derm} (Eq. (2)) may under represent the fractional absorption that would be expected at realistic exposures.

Thus, a single, fixed fractional absorption factor may be inadequate to indicate the true extent of systemic absorption.

Consideration of the dimensionless dermal number N_{derm} can guide the risk assessment of finite dose exposure data. Low values of N_{derm} are likely to be representative of exposures that can be expected in occupational and environmental settings. Therefore, absorption from experiments at low N_{derm} can provide important information regarding the risk for systemic uptake. High values of N_{derm} place the experiment in the flux-limited regime, where excess loading results in low percent absorption that is not representative of the absorption potential of the applied chemical. On the other hand, this flux limited, “infinite dose” regime enables the measurement of steady-state flux, permeability, and lag time and in our proposed strategy of evaluation its significance is not overlooked.

Does Evaporation Play a Significant Role?

For volatile chemicals, the complication of the competing interplay between absorption and evaporation must be considered. Evaporation will diminish the applied load such that less permeant is available for absorption. The dimensionless evaporation number N_{evap} (Eq. (4)) and related t_{evap} (Eq. (6)) may be used to evaluate whether load depletion through evaporation is significant. Additionally, the flux number N_{flux} (Eq. (5)) quantifies the balance between evaporation and absorption, and fractional or percent absorption may be estimated based on the knowledge of this parameter using Eq. (7). For highly volatile chemicals, the percent of applied dose that is absorbed may not achieve a significant level, even for small applied loads. Assessment of the risk of dermal exposure should then consider the types of exposures that are likely in the occupational or environmental setting. Exposures to unoccluded splashes may not lead to significant systemic uptake, but submersion or occluded exposures may contribute to a greater uptake, and thus are better characterized by considering the maximum flux obtainable for the given chemical.

Is the Experimental Duration Appropriate?

Dermal absorption from a given load of a permeant with a given volatility depends on the kinetics of the absorption and evaporation processes. Therefore, the exposure duration is an essential consideration in the evaluation of finite dose exposures. Exposure duration may be established at the discretion of the investigator or may be prescribed for regulatory submission but in either cases it is a significant variable in the measured absorption. The dimensionless time number N_{time} (Eq. (8)) can be used to help evaluate results from finite dose exposures in terms of evaluating the impact of experimental duration.

Has the Study Considered the Absorption that Occurs Following the Exposure time?

We have proposed algebraic expressions to estimate the total absorption of a non-volatile (Eq. (9)) or volatile (Eq. (10)) chemical for a given exposure duration or contact time, as long as depletion of load has not significantly limited absorption over the time course of exposure. These equations may be used in the evaluation of experiments in which the load is removed after a given time by washing. The investigator may continue to measure absorption following removal, or removal may represent the end point of the experiment. In the latter case, Eqs. (9) and (10) address the question of whether significant absorption may be expected after the exposure time. As demonstrated in Figure 5, post exposure absorption can be substantial.

For investigators undertaking finite dose experiments, the preceding analysis suggests strategies for the design of experiments that would enhance the utility of the reported results. The entire absorption profile should be reported, rather than simply any specified end point. The kinetics of the absorption process

provide important information, for example, whether a plateau in absorption has been reached or if additional absorption would be expected over additional time. It would also be beneficial if a range of loads were applied to span a broad range of N_{derm} . This provides additional information for evaluating the absorption potential of the chemical. Although there is an increasing emphasis on exposure periods and donor conditions that reflect anticipated occupational exposures,³⁶ the role of infinite dose exposure data remains important. These provide the most reliable measurements of lag time and steady-state flux, which not only can be used to predict absorption kinetics from arbitrary loads, but also provide means of evaluating and interpreting finite dose absorption data as outlined herein.

The analysis presented in this paper provides a strategy for the evaluation of dermal absorption data that acknowledges the important contribution of finite dose dermal absorption experiments, and addresses the limitations of a dermal risk assessment strategy that relies on the single, fixed fractional absorption paradigm. The application of this strategy should serve to better characterize the dermal absorption of industrial and environmental chemicals.

GLOSSARY

Absorption, the process of chemical transport from the outer surface of skin and into the receptor compartment in an *in vitro* experiment, or into the systemic circulation from an *in vivo* exposure; **Delivery limited**, a condition where dermal absorption is limited by the supply of chemical applied to the surface (dose). Peak flux will be diminished from its highest value attainable from a given donor. Compare with *flux limited*; **Dermal absorbed dose**, the total amount of chemical that is absorbed (for *in vitro* experiments, mass/area; in an *in vivo* setting, units are consistent with exposure units); **Donor**, the substance applied to the skin surface. It may consist of a solution or mixture containing the chemical of interest or the neat (undiluted) chemical; **Dose**, related to the amount of chemical applied to skin, dose is used more loosely than load, and may refer to either an amount (mass/area) of chemical or a specified volume of a concentration (mass/volume) of chemical; **Exposure**, the mass of chemical in contact with skin, typically normalized by time (mass/time) and possibly body mass (mass/mass/time) or exposed area (mass/area/time) of the organism; **Exposure duration**, the amount of time the chemical is in contact with the skin; **Experimental duration**, the amount of time that absorption is measured in an experiment. It may be longer than the exposure duration; **Finite dose**, a defined, limited dose; **Flux**, the rate of mass accumulation per area of exposed surface (mass/area/time); **Flux limited**, a condition where dermal absorption is limited only by the peak steady-state flux attainable from a given donor. Compare with *delivery limited*; **Fractional absorption**, the fraction of applied dose that is absorbed, calculated as the dermal absorbed dose divided by the applied load. It may equivalently be expressed as *percent absorption*; **Infinite dose**, an unlimited dose; in practice one where the applied dose is minimally depleted through absorption or evaporation; **Lag time**, a function of the finite time it takes for a chemical to permeate the skin, it is typically calculated as the time-axis intercept of the asymptote of the steady-state absorption curve (time); **Load**, amount of chemical that is in contact with the skin (mass/area); **Maximum flux**, the highest flux attainable from a given chemical, most reliably measured as the slope of the steady-state absorption curve using an infinite dose of neat chemical; **Peak flux**, the highest flux attainable from a given dose; **Steady-state flux**, the equilibrium flux that is achieved from an infinite dose; **Systemic uptake**, the quantity of chemical that enters the systemic circulation from a given skin exposure (units are consistent with exposure units); **Vehicle**, the solvent or agent mixed with the target chemical in contact with the skin surface

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DISCLAIMER

The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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APPENDIX

Estimation of Evaporation Flux

The US Environmental Protection Agency²⁴ suggests the use of the following equation (their Eq. (D-1)) to estimate evaporation from the surface of a pool of neat liquid, at or near the ambient temperature, for risk management of chemical spills. It may also be used to estimate maximum evaporation flux from the donor compartment of a diffusion cell,²⁵ or from the skin surface in an *in vivo* experiment:

$$J_{\text{evap}} = \frac{101,610 P_{\text{vap}} MW^{2/3} u^{0.78}}{T + 273} \quad (\text{A1})$$

where J_{evap} is evaporation flux ($\mu\text{g}/\text{cm}^2/\text{h}$), P_{vap} is the vapor pressure of the chemical at the ambient temperature (mmHg), MW is molecular weight, u is wind speed above the liquid surface (m/s), and T is liquid temperature ($^{\circ}\text{C}$). Typical values of u for indoor air range from 0.1 to 0.5 m/s.

Vapor pressure depends on temperature. If it is known at one temperature (T_1), it can be estimated at a different temperature (T_2) using a form of the Clausius–Clapeyron equation:³⁷

$$\ln\left(\frac{P_{\text{vap},2}}{P_{\text{vap},1}}\right) = \frac{\Delta H_{\text{vap}}}{R} \left(\frac{1}{T_1 + 273} - \frac{1}{T_2 + 273}\right) \quad (\text{A2})$$

where ΔH_{vap} is the molar enthalpy of vaporization (J/mol) and R is the gas constant (8.314 J/mol/K); temperatures T_1 and T_2 are in $^{\circ}\text{C}$. If no experimental data on vapor pressure are available, a calculated value may be used, for example, using Episuite.³⁸

In Eq. (A1), J_{evap} represents the maximum evaporation flux of the neat chemical. For diffusion cell experiments using diluted compound, when a chemical is present at a concentration (C_v) less than the saturation limit in the same vehicle (S_v), the evaporation flux will be less than the maximum flux by an amount that often is proportional to the saturation ratio (SR), defined as:

$$SR = \frac{C_v}{S_v} \quad (\text{A3})$$

and the evaporation flux under this subsaturated condition may be estimated:

$$J_{\text{evap,sub}} = J_{\text{evap}} \times SR \quad (\text{A4})$$

Equations (A3) and (A4) are appropriate for solutions in which thermodynamic activity is proportional to concentration. Although this approximation is often a good one, significant departures from this behavior are possible.³⁹