Reassessment of the experimental skin permeability coefficients of polycyclic aromatic hydrocarbons and organophosphorus pesticides

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Highlights

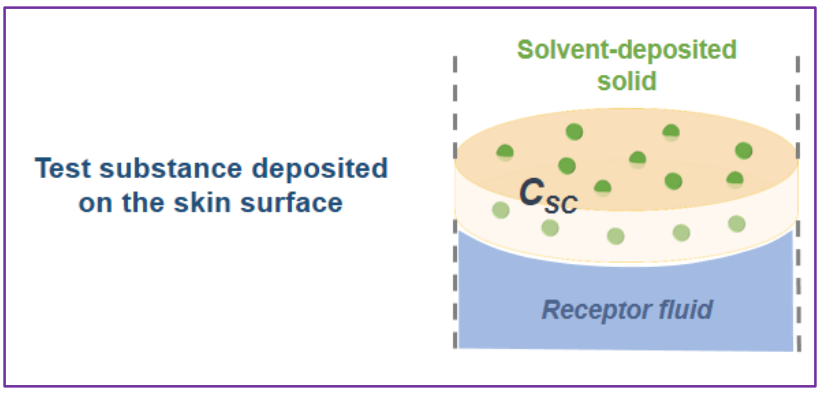
-The k_P values of PAHs and OPPs reported in some experimental studies result from assays with the solvent-deposited substance;

-A method for determination of k_Ps from these relevant type of skin permeation assays is not established;

-The (aqueous) k_Ps of solvent-deposited substances can be calculated using the maximum flux and water solubility;

-Reanalyzed fluxes and recalculated k_Ps of PAHs and OPPs are systematized for researchers and risk assessors;

-The k_P calculation method can be easily generalized to other vehicle-deposited toxicants and drugs.



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Abstract

Human exposure to polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides (OPPs) by dermal route is a continuing concern in environmental and occupational toxicology. Diverse authors have measured *in vitro* the absorption flux and permeability coefficient (k_P) of those compounds delivered on skin surface using volatile solvents. However, there isn't a harmonized method to obtain k_P when the test substance is deposited on the skin as a solid. Consequently, varied experimental k_Ps have been reported for PAHs and OPPs, most in clear disagreement with the values predicted by well-established mathematical models. In this work, we collected the permeation fluxes reported for these toxicants through human skin and calculated the (aqueous) k_Ps using a method based on the maximum flux and water solubility. The reanalyzed fluxes and recalculated k_Ps show improved consistency between the different experimental works and mathematical models. Notably, the recalculated k_P of benzo[a]pyrene, among others, was approximately 100 times higher than it had been previously considered. Suggestions are given to generalize the method in studies with other solvent-deposited toxicants and drugs.

Keywords

Skin permeability coefficients; Environmental toxicants; Chlorpyrifos; Skin permeation; Risk assessment.

1. Introduction

Dermal exposure to polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides (OPPs) is an important concern (Beitel et al., 2020; Fent et al., 2020; Peckham et al., 2017; Thredgold et al., 2019).

1.1. Skin *in vitro* permeation assays

Skin permeation of chemicals can be evaluated *in vitro* using diffusion cells with excised skin from human or animal sources (Franz, 1975). The test substance is applied to the surface of a skin sample separating the two compartments (donor and receptor) of the cell, and the substance or metabolites are monitored in the receptor fluid throughout the experiment (Figure 1). The absorption rate or flux (J, mg/cm^2/h) is calculated from the linear part of the absorption *versus* time curve (OECD, 2004). The permeability coefficient (k_P, cm/h) is a general representation of the rate at which a chemical penetrates the skin (OECD, 2019) and is used by regulatory agencies for dermal risk assessment (Mitragotri et al., 2011; Stepanov et al., 2020). This important skin absorption parameter is the infinite-dose steady-state flux (J_{ss}) divided by the difference in concentrations of donor and receptor compartment (ΔC). If sink conditions are maintained in the receptor fluid, ΔC is equal to the substance concentration in the vehicle present at the donor compartment, C_v (Hopf et al., 2020; Mitragotri et al., 2011):

$$J_{SS} = k_P \cdot \Delta C \cong k_P \cdot C_v \quad (1)$$

And k_P is then calculated as:

$$k_P = \frac{J_{SS}}{C_v} \quad (2)$$

Assuming the skin as a single pseudo-homogeneous membrane, k_P is defined as:

$$k_P = \frac{K_{SC} \cdot D}{h} \quad (3)$$

where K_{SC} is the stratum corneum/vehicle partition coefficient of the chemical, D is the effective diffusion coefficient and h the thickness of the diffusion barrier (Mitragotri et al., 2011; Zhang et al., 2009).

K_{SC} is the ratio of solute concentrations in the stratum corneum (C_{SC}) and in the vehicle (C_v). In the case a saturated solution of the test substance is maintained in the donor, $C_v=S_v$, and assuming the partition to the stratum corneum is fast, $C_{SC}=S_{SC}$:

$$K_{\mathrm{SC}} = \frac{C_{\mathrm{SC}}}{C_{\mathrm{v}}} = \frac{S_{\mathrm{SC}}}{S_{\mathrm{v}}} \tag{4}$$

where S_{SC} and S_v are the solubilities of the permeant in the stratum corneum and in the vehicle contacting the skin, respectively (Zhang et al., 2009).

A common approach in permeation experiments is to load the test substance via a (super)saturated solution or suspension in the donor compartment (Figure 1), which easily guarantees an infinite-dose of the permeant available for permeation through time ($J=J_{ss}$). Importantly, these conditions are also anticipated to saturate the stratum corneum and obtain the maximum flux of the permeant ($J=J_{max}$). For these saturating conditions, Equation 1 can be rewritten (Mitragotri et al., 2011; Zhang et al., 2009):

$$J_{\max} = k_{\mathrm{P}} \cdot S_{\mathrm{v}} = \frac{D}{h} \cdot S_{\mathrm{SC}} \tag{5}$$

J_{max} is independent of the vehicle considered (if not affecting skin properties) and a useful parameter for assessing the skin penetration potential of chemicals. D/h and S_{SC} are difficult to determine experimentally, but, alternatively, J_{max} of a chemical can be estimated knowing k_P and the solubility of the chemical in the same vehicle (Equation 5). Since k_P depends on the vehicle (see K_{SC} in Equation 3), it is imperative to be combined only with S_v values in the same vehicle, e.g. water (Hopf et al., 2020; Mitragotri et al., 2011).

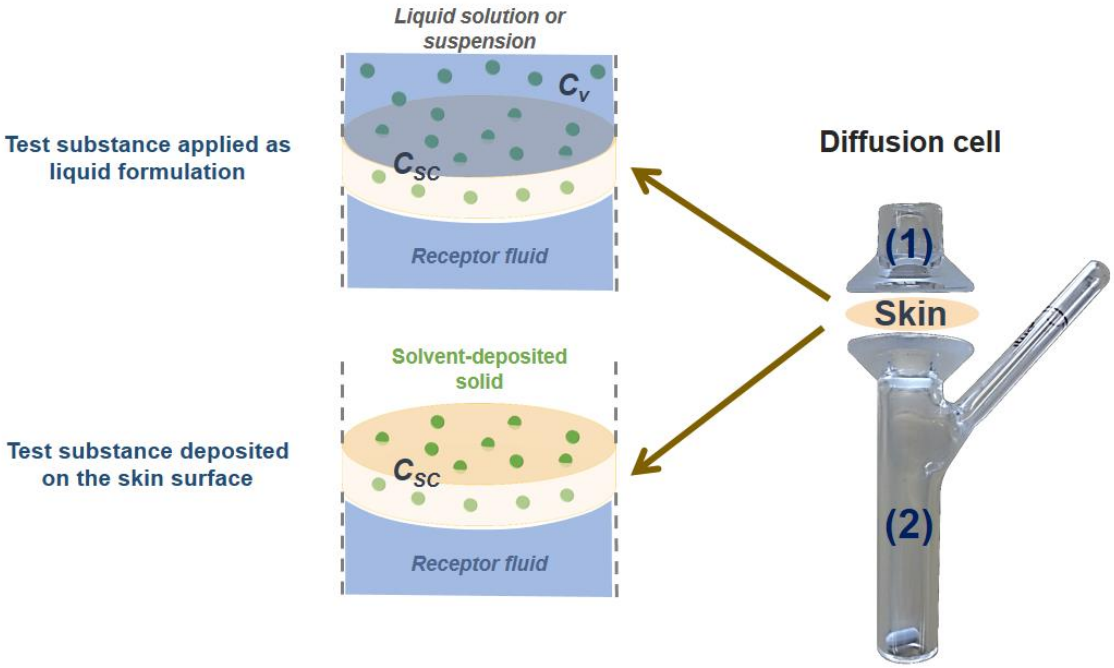

Figure 1. Illustration of alternative methods to apply the test substance on skin for permeation assays. The substance is loaded in the donor compartment (1) of the diffusion cell and permeates through the skin into the receptor compartment (2). The test substance can be presented as a liquid formulation (upper panel) or deposited on the skin surface as a solid using a volatile solvent that rapidly evaporates at the beginning of the assay (below panel). In the case a liquid formulation is loaded and maintained in the donor compartment during the assay, the concentration of the chemical actually available for skin permeation is C_v, *i.e.* the concentration of the test chemical dissolved in the liquid vehicle. C_{SC} is the concentration in the stratum corneum and represents the driving force for the permeation flux, independently of the method of application of the test substance.

However, there are different ways to apply the test substances on the skin for dermal absorption studies (Figure 1). Depending on the purpose of the study, the substance can be applied as solid/granule or liquid formulation in an aqueous or organic solution or suspension, the option that best represents the real exposure scenarios or the most suitable for obtaining the skin absorption parameters of the chemical (*e.g.*, the k_P). When the substance is insoluble in water, such as PAHs and many pesticides, the k_P values measured with aqueous vehicles are not recommended for risk assessment, but the alternative assay methodologies pose extra challenges for determination of the dermal absorption parameters (Hopf et al., 2020).

1.2. Human skin permeation data for PAHs and OPPs

Sartorelli et al. (1998) provided kinetic curves of permeation of several PAHs and OPPs through monkey skin and calculated the corresponding k_P values (Table I). The authors have also studied the phenoxy herbicides 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4-dichlorophenoxyacetic acid (2,4-D). In the permeation assays, all the compounds were applied on the skin in a small volume of acetone solutions (dermal loads from 6.9 to 626 nmol/cm^2). Since the donor compartment was left unoccluded, it can be expected that the vehicle solvent evaporated and the compounds became deposited onto skin. In a subsequent work, Sartorelli et al. (2001) used human skin to study the dermal absorption of benzo[a]pyrene (BaP) and other PAHs, but k_Ps were not reported in the article.

More recently, Hopf et al. (2018) presented the permeation kinetics and k_P values of major PAHs measured with human skin. In this study, the compounds were also applied under the form of an acetone mixture with PAH concentrations 5 mg/mL, and the reported k_P values are similar to those in Sartorelli et al. (1998) accepting a tolerance of one order of magnitude (Table I). However, the k_P values reported in both works seem very high for the polar compounds, such as omethoate, and very low for the more lipophilic compounds such as chlorpyrifos or BaP. Surprisingly, another work (Griffin et al., 2000) reported an even lower k_P for chlorpyrifos (log$K_{o/w}$ =4.96) (Table I), and this work serves as dataset supporting query results delivered by the recent skin permeation database HuskinDB (Stepanov et al., 2020).

Facing these apparent inconsistencies in the experimental data, we employed two mathematical models to estimate the k_Ps of the PAHs and OPPs studied. The empirical model of Potts and Guy (Potts and Guy, 1992) and the mechanistic Mitragotri model (Mitragotri, 2002) are relatively simple models that were shown to predict reasonably well the k_P of a great diversity of compounds (Mitragotri et al., 2011), except for highly hydrophilic log$K_{o/w}$ <-2 compounds (Lian et al., 2008). The values of log$K_{o/w}$ required to apply these models were obtained by the XLOGP3 method (Cheng et al., 2007) and

retrieved at the SwissADME web tool (http://www.swissadme.ch/). It can be observed in Table I and Figure 2 that the k_P values resulting from both mathematical models are substantially different from the values reported in the experimental works, namely for the more polar and apolar compounds. Also noticeable is the fact that the reported k_P values show an inverse correlation with the $\log K_{o/w}$ of the tested substances (Figure 2), and are in poor accordance with results from other experimental works (Peckham et al., 2017; Thredgold et al., 2019). For an additional example, the top k_P value obtained by Sartorelli et al. (1998) for dimethoate (Table I) contrasts with the results by Nielsen et al. (2004) which observed no permeation of this pesticide through human skin during 48-hour assays.

In the experiments by Sartorelli et al. (1998) and Hopf et al. (2018) the tested substances were loaded onto the skin as acetone solutions, and Griffin et al. (2000) used ethanol to deposit chlorpyrifos. Particulate or dissolved solids can be applied to the skin in acetone or other volatile solvent that rapidly evaporates and has no significant effects on skin permeability (Franz, 1975; Hopf et al., 2020). This procedure is regarded as the best one, mimicking the actual conditions of occupational and environmental exposure to some chemicals (Moore et al., 2014). However, following this experimental method there is no actual solution of the test substance present at the donor in the course of the permeation experiment (Figure 1), and the guidance documents do not state what should be the C_v or equivalent to consider in the determination of k_P (Equation 2). In consequence, various authors follow different criteria for a surrogate of C_v, such as using the applied dose per area or the initial concentration in the solvent (before evaporation). A further misunderstanding which gives rise to errors in the determination of k_P are the incoherent units of permeation flux, concentration and k_P. Kissel and Bunge (2003) have previously commented on the incorrect k_P values reported by Sartorelli et al. (1998) and the difficulties in calculating the k_P in experiments in which the test compound is deposited onto the skin instead of being delivered in solution.

Table I. Skin permeability coefficients (k_P) of polycyclic aromatic hydrocarbons, organophosphorus and phenoxy pesticides reported in different experimental works and predicted by mathematical models. The experimental data were measured at 32 or 37 ºC.

Compound	**Experimental k_P (cm/h)**		**Predicted k_P (cm/h)**	
	(Sartorelli et al., 1998)	**Other works**	**(Mitragotri, 2002)**	**(Potts and Guy, 1992)**
Naphthalene	5.12×10^{-3}	2.60×10^{-3} (a)	6.01×10^{-2}	6.57×10^{-2}
Anthracene	3.44×10^{-3}	3.46×10^{-4} (a)	1.36×10^{-1}	2.13×10^{-1}
Pyrene	1.69×10^{-3}	5.54×10^{-4} (a)	1.71×10^{-1}	3.07×10^{-1}
Chrysene	2.2×10^{-4}	2.92×10^{-4} (a)	4.73×10^{-1}	9.75×10^{-1}
Benzo[a]pyrene	N/A	1.94×10^{-4} (a)	3.99×10^{-1}	9.03×10^{-1}
Acenaphthene	6.33×10^{-3}	N/A	9.38×10^{-2}	1.26×10^{-1}
Fluorene	6.26×10^{-3}	N/A	1.12×10^{-1}	1.62×10^{-1}
Phenanthrene	1.96×10^{-3}	N/A	1.38×10^{-1}	2.17×10^{-1}
Benz[a]anthracene	1.5×10^{-4}	N/A	4.36×10^{-1}	8.98×10^{-1}
Omethoate	4.81×10^{-3}	N/A	1.62×10^{-5}	2.69×10^{-5}
Dimethoate	4.04×10^{-2}	N/A	1.40×10^{-4}	2.58×10^{-4}
Methamidophos	1.54×10^{-2}	N/A	6.13×10^{-5}	6.72×10^{-5}
Acephate	1.64×10^{-2}	N/A	2.40×10^{-5}	3.43×10^{-5}
Chlorpyrifos	1.28×10^{-3}	1.1×10^{-4} (b)	1.54×10^{-2}	4.36×10^{-2}
Fenitrothion	6.85×10^{-3}	N/A	3.51×10^{-3}	8.09×10^{-3}
2,4-D (2,4-Dichlorophenoxyacetic acid)	5.92×10^{-3}	N/A	4.29×10^{-3}	8.00×10^{-3}
MCPA (2-methyl-4-chlorophenoxyacetic acid)	9.60×10^{-3}	N/A	1.27×10^{-2}	2.19×10^{-2}
Dichlorvos	N/A	1.5×10^{-4} (c)	4.64×10^{-4}	8.39×10^{-4}

(a) k_P values reported by Hopf et al. (2018).

(b) k_P value reported by Griffin et al. (2000).

(c) k_P value reported by Thredgold et al. (2019).

N/A – Not Available.

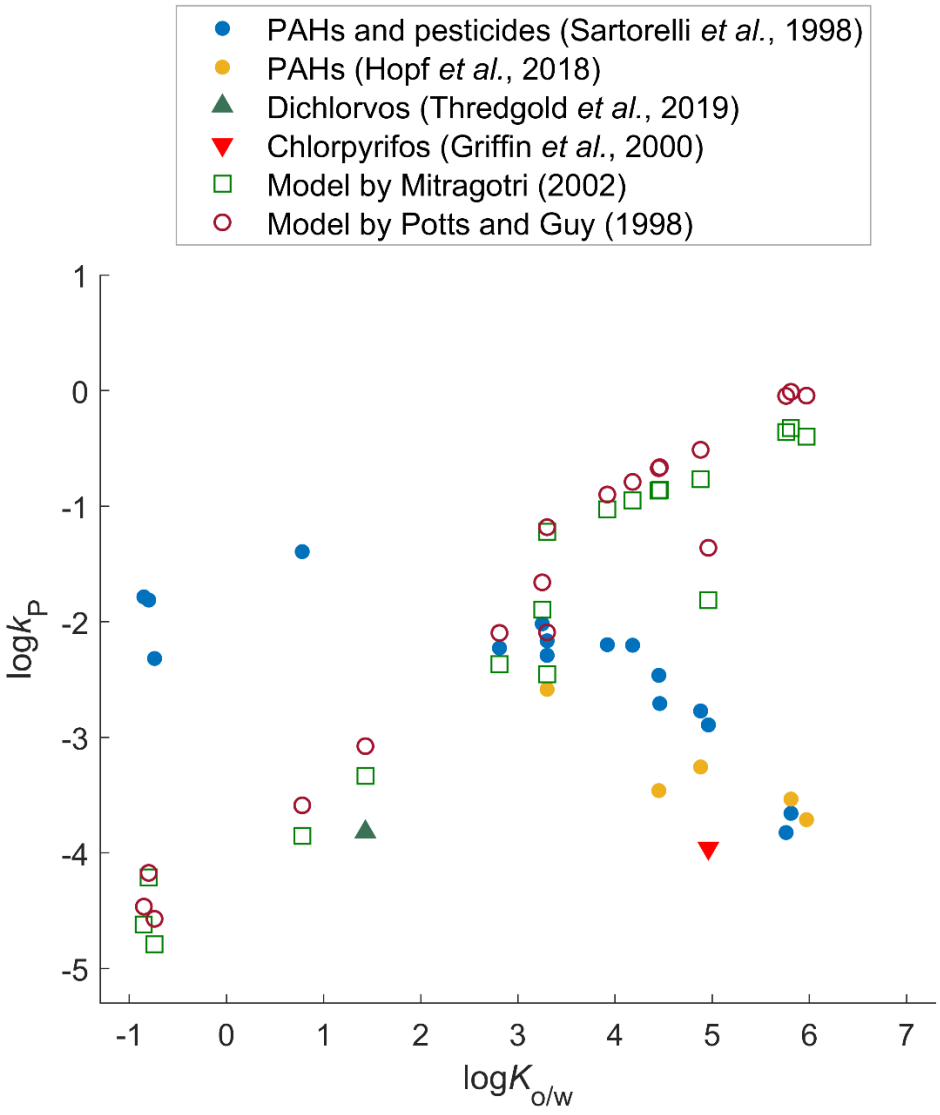

Figure 2. Representation of the logarithm of the skin permeability coefficients (k_P values in cm/h) from Table I, plotted against the logarithm of the octanol/water partition coefficient ($\log K_{o/w}$) of each substance.

1.3. Method harmonization

Guidelines are available from OECD for dermal absorption studies, namely the Guidance Document 28 for the conduct of skin absorption studies (OECD, 2004), the Test Guideline 428 for *in vitro* assays and, later, the Guidance Notes 156 document on dermal absorption that is currently under review (OECD, 2019). Meanwhile, varied authors have provided practical insights for revision of the skin absorption guidance documents and defended further harmonization to improve the quality of *in vitro* permeation studies (Hopf et al., 2020; Sullivan et al., 2017).

For the harmonization of the method of calculation of k_P from experiments where the test substance is deposited on the skin surface, instead of being presented in a liquid maintained in the donor compartment, we suggest the use of the maximum flux and the water solubility of the substance (Equation 5). This principle will be applied in the following section to recalculate the k_P values of PAHs and OPPs. A broader goal of this work is to reconcile available data on absorption fluxes and k_Ps for these important environmental and occupational toxicants.

2. Reevaluation of the fluxes and permeability coefficients of PAHs and OPPs

As presented in the previous section, published k_P values of PAHs and OPPs show diverse inconsistencies. However, the fluxes measured in Sartorelli et al. (1998), Hopf et al. (2018) and Griffin et al. (2000) might be useful to calculate valid k_P values.

2.1. Analysis of the fluxes

Dichlorvos is another important OPP, not studied in those 3 experimental works, but recently investigated by Thredgold et al. (2019) that reported a k_P of 1.5×10^{-4} cm/h at 37ºC. As illustrated in

Figure 2, this value is in good agreement with the model predictions considering dichlorvos MW 221 g/mol and log$K_{o/w}$ 1.4. Once again, the k_P values reported by Sartorelli et al. (1998) are surprisingly high for other OPPs with molecular size similar to dichlorvos, and even more polar ones, such as dimethoate and omethoate (log$K_{o/w}$ = 0.8 and -0.8, respectively). Nevertheless, the absorption fluxes reported for these OPPs were lower than the ones measured with dichlorvos (Thredgold et al., 2019), as predictable from the difference in their polarity. In reality, if we analyze the absorption profiles presented in the publications by Sartorelli et al. (1998) and Hopf et al. (2018), the expected correlation with the polarity of the diverse compounds tested can be perceived. Moreover, collecting the fluxes of PAHs from Sartorelli et al. (2001), which also applied the compounds in an acetone mixture, a good correlation with the data in Sartorelli et al. (1998) and with the compounds' polarity was found (Table II and Figure 3).

Analyzing in more detail the case of BaP, the flux reported by Hopf et al. (2018) is comparable to other similar experimental work which studied the permeation of acetone-deposited BaP through human skin in flow-through diffusion cells (Peckham et al., 2017), and in excellent agreement with the absorption profile established by Sartorelli et al. (2001) (Table II). In the case of chlorpyrifos, the flux of ethanol-deposited chlorpyrifos measured by (Griffin et al., 2000) is higher, possibly because ethanol is less volatile or alters skin permeability. Nevertheless, the flux of chlorpyrifos applied to human skin in another volatile solvent, isopropanol (Moore et al., 2014), is close to the one reported by Sartorelli et al. (1998), 3.1 and 18.89 ng/cm^2/h, respectively (Table II). To sum up, the comparison of fluxes from diverse studies indicates that, in spite of the k_P values calculated being incorrect, the absorption fluxes presented in Sartorelli et al. (1998), Hopf et al. (2018) and Griffin et al. (2000) are still meaningful.

On this basis, we decided to collect the fluxes from the several publications and proceed with the recalculation of the k_P values of the PAHs and OPPs (Table II). The recalculation depends on settling two additional points: only steady-state or maximum fluxes (J_{ss} or J_{max}) are valid to determine k_Ps, and a measure of C_v or S_v at the skin surface needs to be specified in Equation 2 or 5.

Table II. Recalculation of the skin permeability coefficients (k_P) of solvent-deposited polycyclic aromatic hydrocarbons, organophosphorus and phenoxy pesticides from the absorption fluxes measured in different experimental works (at 32ºC). The k_P values were obtained using the experimental fluxes and the water solubility of each substance. The values in parenthesis correspond to fluxes measured in experimental conditions that do not ensure an infinite dose or constant skin permeability throughout the permeation assay. The water solubilities were calculated by FILTER-IT program and retrieved at the SwissADME web tool (http://www.swissadme.ch/).

Compound	**Experimental Flux (ng/cm²/h)**		**Water solubility (ng/cm³)**	**Recalculated k_P (cm/h)**	
	(Sartorelli et al., 1998)	**Other works**		**(Sartorelli et al., 1998)**	**Other works**
Naphthalene	96.79	13.01 (a)	1.19×10^{4}	8.13×10^{-3}	1.09×10^{-3} (a)
Anthracene	6.87	1.73 (a)	3.20×10^{2}	2.15×10^{-2}	5.41×10^{-3} (a)
Pyrene	3.01	2.77 (a)	5.22×10^{1}	5.76×10^{-2}	5.31×10^{-2} (a)
Chrysene	0.35	1.46 (a)	8.46×10^{0}	4.10×10^{-2}	1.73×10^{-1} (a)
Benzo[a]pyrene	N/A	0.97 (a) 1.00 (b)	1.38×10^{0}	N/A	7.03×10^{-1} (a) 7.26×10^{-1} (b)
Acenaphthene	123.58	N/A	3.80×10^{3}	3.25×10^{-2}	N/A
Fluorene	(22.16)	N/A	1.10×10^{3}	(2.01×10^{-2})	N/A
Phenanthrene	(4.02)	N/A	3.20×10^{2}	(1.26×10^{-2})	N/A
Benz[a]anthracene	0.30	0.72 (b)	8.46×10^{0}	3.52×10^{-2}	8.51×10^{-2} (b)
Benzo[b]fluoranthene	N/A	0.42 (b)	1.38×10^{0}	N/A	3.03×10^{-1} (b)
Benzo[k]fluoranthene	N/A	0.10 (b)	1.38×10^{0}	N/A	7.05×10^{-2} (b)
Dibenzo[a,h]anthracene	N/A	0.12 (b)	2.23×10^{-1}	N/A	5.55×10^{-1} (b)
Omethoate	63.17	N/A	1.84×10^{7}	3.43×10^{-6}	N/A
Dimethoate	(616.86)	N/A	1.24×10^{7}	(4.97×10^{-5})	N/A
Methamidophos	(227.56)	N/A	1.03×10^{8}	(2.21×10^{-6})	N/A
Acephate	225.60	N/A	2.25×10^{7}	1.00×10^{-5}	N/A
Chlorpyrifos	18.89	(1718) (c) (3.1) (d)	4.84×10^{3}	3.90×10^{-3}	(3.55×10^{-1}) (c) (6.40×10^{-4}) (d)
Fenitrothion	(106.57)	N/A	1.16×10^{6}	(9.19×10^{-5})	N/A
2,4-D (2,4-Dichlorophenoxyacetic acid)	(711.59)	N/A	1.54×10^{5}	(4.62×10^{-3})	N/A
MCPA (2-methyl-4-chlorophenoxyacetic acid)	(1206.03)	N/A	2.38×10^{5}	(5.07×10^{-3})	N/A

(a) flux data from Hopf et al. (2018).

(b) flux data from Sartorelli et al. (2001).

(c) flux data from Griffin et al. (2000).

(d) flux data from Moore et al. (2014).

N/A – Not Available.

Regarding the fluxes, we analyzed the absorption fluxes presented in the original publications (Griffin et al., 2000; Hopf et al., 2018; Moore et al., 2014; Sartorelli et al., 2001, 1998) for the linearity of the temporal profiles and the relative dose that permeated in the duration of the assays. For all the compounds studied, it was possible to observe that the linearity of the absorption kinetics was maintained during several hours, enabling the determination of steady-state fluxes. However, the amount of chemical that permeated to the receptor until the end assays, in some cases, was a significant proportion of the dose initially delivered on the skin, risking the condition of an infinite-dose present during the assay. For example, in the tests of fluorene and acenaphthene by Sartorelli et al. (1998), more than 50% of the dose applied permeated the skin during the assay. In contrast, PAHs permeation in Hopf et al. (2018) experiments did not reach 1% of the initial dose, so the dose in the donor side can be regarded as practically constant throughout the assay (infinite-dose). The cases where more than 15% of the dose applied permeated during the assay were considered not to ensure infinite-dose condition. The fluxes measured in these assays are presented in Table II in parentheses.

Moreover, diverse results in this dataset of permeation fluxes indicate they are maximum absorption fluxes (J=J_{max}). For instance, compounds structurally different as pyrene and chlorpyrifos, but having similar $K_{o/w}$, showed similar fluxes in diverse works, despite differences in the doses applied (Hopf et al., 2018; Moore et al., 2014; Sartorelli et al., 1998). Also chrysene, benz[a]anthracene (BaA) and benzo[b]fluoranthene (Hopf et al., 2018; Sartorelli et al., 2001, 1998) – compounds with $logK_{o/w}$ approximately 5.8 – returned fluxes in a narrow range, 0.30 to 1.46 ng/cm^2/h (Table II and Figure 3). BaP ($logK_{o/w}$ 5.97) flux through human skin measured by Sartorelli et al. (2001) and Hopf et al. (2018) was practically the same (1.0 ng/cm^2/h), regardless of the doses being 100 times different. The doses of PAHs applied in Sartorelli et al. (2001) (0.35 to 2.62 nmol/cm^2) were also substantially lower than in Sartorelli et al. (1998), but it did not affect significantly the experimental permeation fluxes of the compounds with similar hydrophobicity, which suggests that they correspond to J_{max} values. Maximum

fluxes are steady-state fluxes observed for saturated solutions in the donor compartment or other condition that saturates the stratum corneum (Figure 1, C_{SC}=S_{SC}), providing the maximum driving force for the chemical's permeation through the skin. However, the permeant molecules need to cross different polarity regions in their path, including the polar heads groups and the tails of intercellular lipid, the matrix of corneocytes and the aqueous viable epidermis (Zhang et al., 2009). According to membrane permeation principles based on chemicals' partitioning between series of aqueous and lipid compartments:

$$\log J_{\max} = a + b \log K_{\mathrm{o/w}} + c \log(dK_{\mathrm{o/w}} + 1) \quad (6)$$

Zhang et al. (2009) noticed that logJ_{max} of a group of 10 phenols with similar molecular weight, as well as their S_{SC}, could be related with log$K_{o/w}$ by a bilinear function with maximum values for log$K_{o/w}$ between 2 and 3. As plotted in Figure 3, the fluxes of PAHs and OPPs from Table II show the same type of bilinear relationship, reinforcing that the experimental fluxes can be assumed as J_{max} values. A perfect correlation would not be expected in this plot as different size molecules are represented, but only one of the results for chlorpyrifos seems to clearly diverge from the general relationship. This flux corresponds to chlorpyrifos applied on skin in ethanol, and the receptor fluid contained 50% ethanol (Griffin et al., 2000), a condition that promotes the absorption of hydrophobic compounds and that could have exaggerated the permeation flux (the value is presented in Table II in parentheses). The other studies made with chlorpyrifos and with all the other compounds did not include ethanol in their assays.

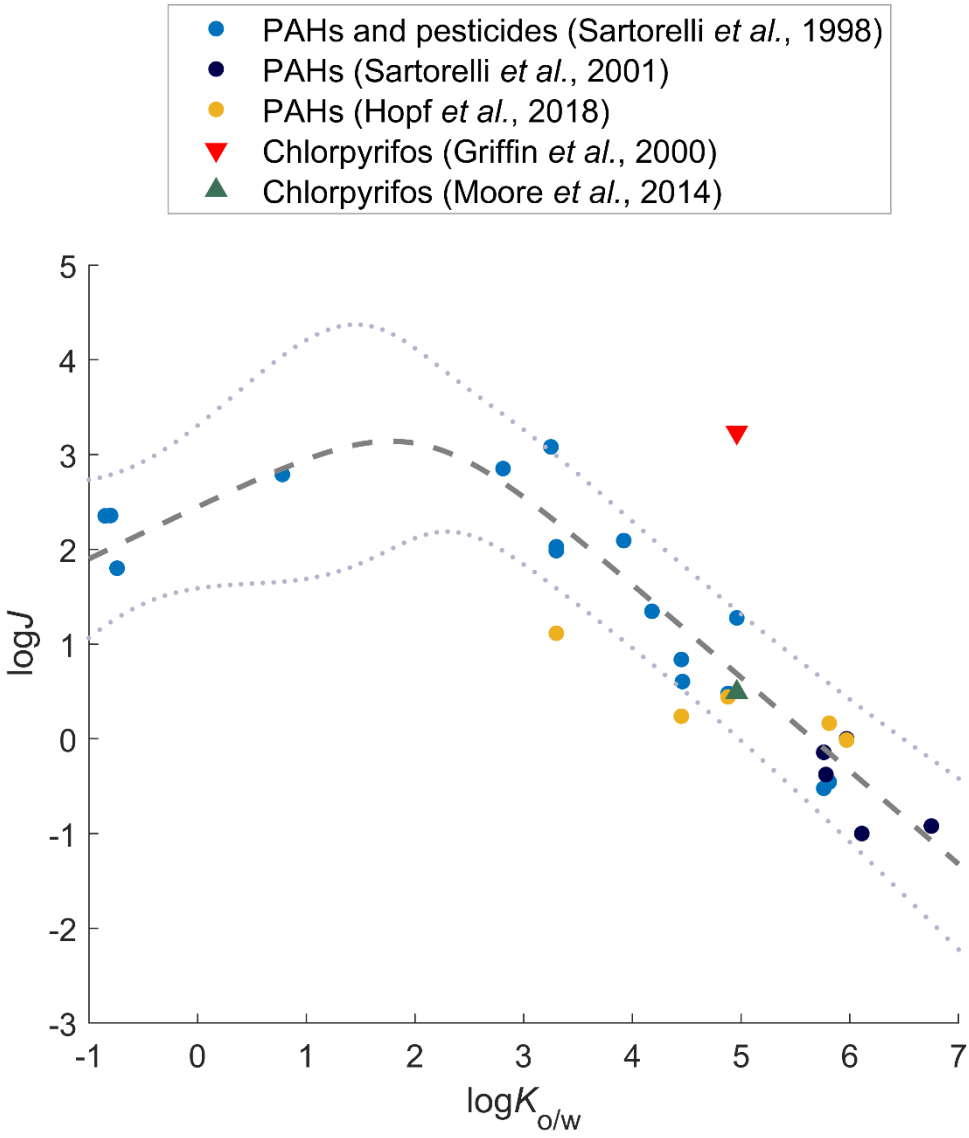

Figure 3. Representation of the logarithm of the fluxes (J, in ng·cm^{-2}·h^{-1}) in Table II plotted against the logarithm of the octanol/water partition coefficient (log$K_{o/w}$) of each substance. The dashed line is the best weighted regression fit of the experimental data to the bilinear Equation 6 (R^2=0.812), and the dotted lines limit the 95% confidence bands. The point for chlorpyrifos with log$J$ equal to 3.2 (red triangle) was at a distance larger than 1.5 standard deviations from the model, so it was excluded from the regression fit.

2.2. Recalculation of the permeability coefficients

The reanalyzed flux data in Table II can be used to recalculate the k_Ps of the PAHs and OPPs, if we specify the C_v to consider in Equation 2 or following Equation 5. The C_v or surrogate measure should be related to the thermodynamic activity of the chemical at the skin surface to inform on the driving force for permeation. Such measures, namely, the thermodynamic activity or concentration of the compound in the skin or stratum corneum (C_{SC}) are not easily available (Mitragotri et al., 2011). In alternative, if the fluxes were obtained in saturating conditions, *i.e.*, if they consist of J_{max}s of the chemicals, then k_P values can be calculated by a rearrangement of Equation 5 giving:

$$k_{\mathrm{P}} = \frac{J_{\mathrm{max}}}{S_{\mathrm{v}}} \tag{7}$$

As J_{max} is independent of the vehicle, the flux values can be used to calculate the (aqueous) k_Ps of the PAHs and OPPs considering their water solubilities (S_v). The water solubility of each substance was obtained by fragmental method and the k_P values calculated following Equation 7 are presented in Table II. The Filter-IT method was used to predict the water solubilities as it is based on 16 fragmental contributions, including the number of different halogen atoms (among other properties), in addition to the compound MW, so it can model the diversity of molecules under study (SILICOS-IT, 2014). It is clear that the recalculated k_Ps approximate to the values estimated by the mathematical models (Table I). Moreover, as evidenced in Figure 4.A, the k_Ps now recalculated follow the expected tendency of increasing value for more hydrophobic compounds (log$K_{o/w}$), in contrast to the less plausible variation observed in Figure 2. Also for the compounds which experimental fluxes are not guaranteed to be infinite-dose fluxes (e.g., fluorene), the recalculated k_Ps are more in accordance with the model estimates than the previously reported values, but the corresponding values were kept in parenthesis in Table II and omitted in Figure 4. For these compounds, additional experimental data is needed including for chlorpyrifos.

The consistency of the newly calculated k_Ps here presented is strengthened by the similar values obtained with data from different works, namely for pyrene or BaP, and including for different

compounds like chrysene and BaA with very close structural features, so it is expectable that they present similar permeability properties (Table II). For these cases, as well as for other PAHs – for which slightly different values (one order of magnitude range) are provided in Table II – it is not possible to endorse the more reliable absorption flux and k_P, as they seem equally valid.

Nevertheless, a significant difference can still be observed between the recalculated k_Ps and the model estimates (Figure 4.A and B). In spite of being derived in different ways, these two mathematical models offered the best predictions of k_Ps for 124 varied substances, and they share common features (Lian et al., 2008). Both models assume that the rate-limiting transport barrier of the skin is the stratum corneum lipid matrix, but additional pathways for transdermal permeation can differently affect the permeability of hydrophilic and lipophilic compounds (Lian et al., 2008; Mitragotri et al., 2011). Figure 4 (panel B) shows that the models up-estimate the k_Ps of most compounds in approximately 0.5 to 1.5 orders of magnitude, and the divergence seems independent of the hydrophobicity of the substance. Therefore, the possible additional contribution of other permeation pathways, such as transport through appendages (hair follicles and sweat ducts) can be discarded. Since the coefficients of hydrophilic compounds are equally distant (Figure 4.B) and the experimental fluxes were measured to diffusion cell receptors containing bovine serum albumin or PEG-20 oleyl ether, solubility limitations in the receptor fluid don´t seem to be a critical factor. The k_P calculation is however dependent on the water solubility considered for the tested substance (Equation 7). In this work, the water solubility of each substance was calculated using a recognized prediction model (Table II), but the solubility depends on the experimental conditions, such as temperature and pH, and the precision of prediction models is still challenging (Rognan, 2017). The experimental determination of the solubility in the assay conditions can be recommended for more accurate k_P calculation.

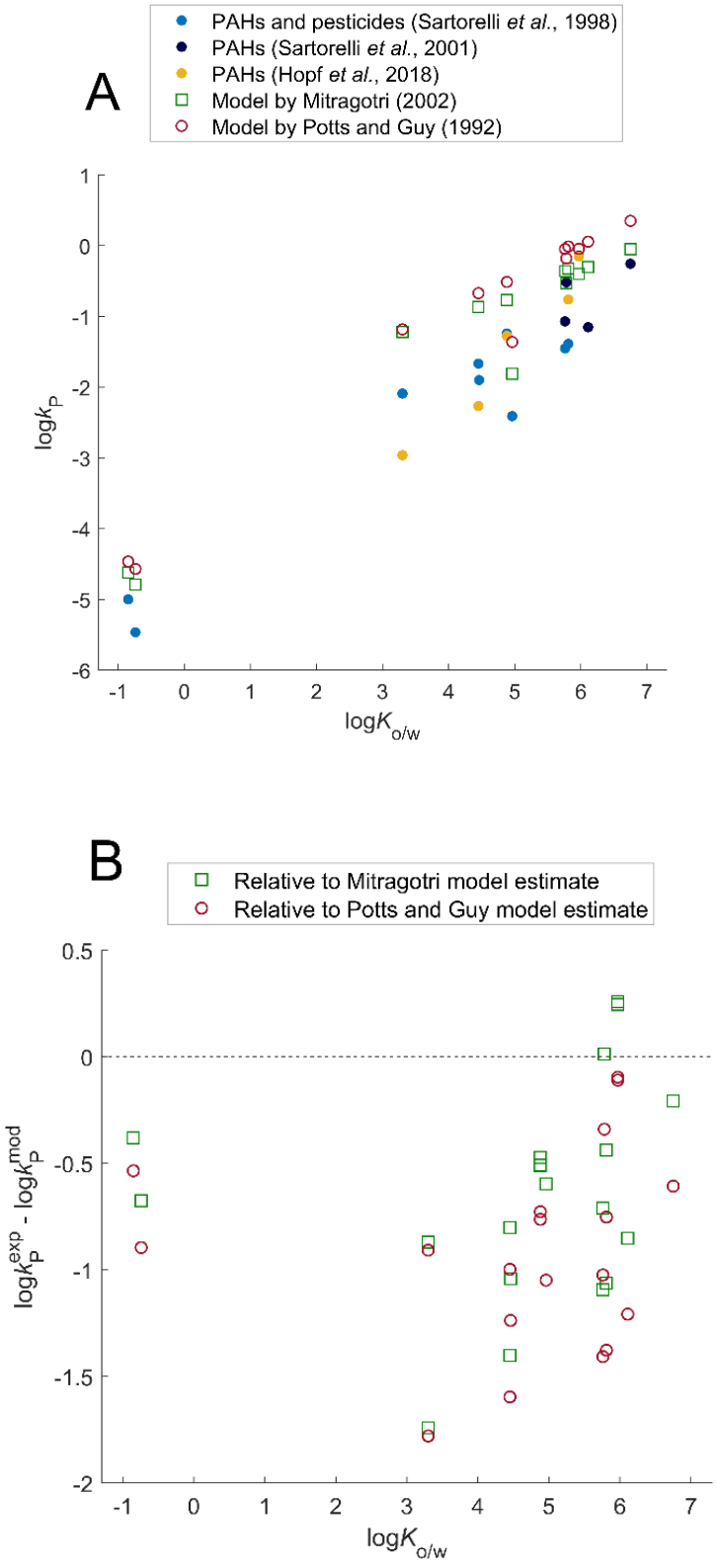

Figure 4. (A) Representation of the recalculated skin permeability coefficients (logk_P) from Table II (values in parenthesis excluded), plotted against the logarithm of the octanol/water partition coefficient (log$K_{o/w}$) of each substance. (B) Difference between the values calculated from experimental fluxes (k_P^{exp}) and the estimates (k_P^{mod}) by Mitragotri model and Potts and Guy model.

3. Conclusions

PAHs and OPPs are major environmental and occupational toxicants and human exposure to these chemicals, including by dermal route, receives critical attention. The skin absorption fluxes, namely J_{max}, and the (aqueous) k_P are the most useful permeability parameters for evaluating the risks of dermal absorption. Although k_P depends on the vehicle (for example commercial formulations of pesticides that contain ingredients altering the K_{SC}), it remains a key information for researchers and risk assessors to quantify how likely a chemical will penetrate the skin to enter systemic circulation.

Fluxes and k_Ps of PAHs and OPPs through excised human skin have been studied by various authors, but implausible k_P values are found in the bibliography (Griffin et al., 2000; Hopf et al., 2018; Sartorelli et al., 1998). This can be justified because the permeation assays were carried with solvent-deposited substances and there´s no established method to determine the k_P in these conditions. The deposition of particulate or dissolved solids on the skin for diffusion cell experiments is accepted to mimic specific conditions of toxic exposures, but determination of the k_P has been handled in different ways and given rise to ambiguous data. The need for stronger theoretical basis and harmonization in the determination of dermal absorption parameters was recently emphasized (Hopf et al., 2020).

In this work, it is proposed to calculate the (aqueous) k_P from the J_{max} measured in assays with solvent-deposited substance using the corresponding water solubility of the substance. In contrast to the reported k_P values, the flux data of PAHs and OPPs collected from diverse experimental studies show good coherence, even when comparing assays using monkey and human skin or the application of different doses of the test substance. Remarkably, the recalculated k_Ps are in greater accordance to estimates by mathematical models and to the general understanding of chemicals' skin permeability. For some compounds, such as phenanthrene, methamidophos, fenitrothion and chlorpyrifos, more studies are warranted albeit the recalculated k_Ps also appear more valid than those previously available.

The recalculated k_P values here provided can be useful for assessment of health risks from exposure to PAHs and OPPs. It should be noticed that the k_Ps of dangerous PAHs like BaP, BaA and benzofluoranthene(s) were recalculated in the order of 10^{-1} cm/h, much higher than previously indicated. These k_p values must be considered for an updated risk assessment mainly for exposures where these compounds are found at greater concentrations like on the firefighters' skin and personal protective equipment after a fire event (Stec et al., 2018).

The method of determination of (aqueous) k_Ps has the potential to be generalized for studies with other toxicants and drugs deposited on skin in solid form or via a volatile solvent. However, in the cases solid/granule forms are directly applied in the diffusion cell, it may be impossible to obtain a uniform coverage of the whole area of the skin and mass transfer limitations may additionally prevent from reaching the maximum permeation flux. For the best practices, when using a volatile solvent with fine particles or a dissolved solid that precipitates dispersed on the skin surface, it is recommended to assay different doses of the test substance to assure the J_{max} is reached. Determination of the water solubility of the chemical, in conditions as close as possible to the permeation measurements (e.g. temperature), is also important to achieve the more reliable k_P values.

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