Percutaneous absorption of thirty-eight organic solvents in vitro using pig skin

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Abstract

Percutaneous absorption is highly variable between chemicals but also within chemicals depending on exposure conditions and experimental set up. We tested a larger number of organic solvents with the same experimental set up, using skin from new-born piglets and static diffusion cells. Thirty-six common organic solvents were studied neat (and 31 of them also in water dilution): acetone, acetonitrile, n-butanol, 2-butanone, 2-butoxyethanol, 1-butoxy-2-propanol, n-butyl acetate, butyl acrylate, cyclohexane, cyclohexanone, 1,2-dichloroethane, dichloromethane, ethanol, 2-ethoxyethanol, ethyl acetate, ethyl acrylate, ethylbenzene, furfuryl alcohol, n-hexane, 2-hexanone, 2-isopropoxyethanol, methanol, 1-methoxy-2-propanol, methyl acrylate, 3-methyl-1-butanol, methyl tertiary butyl ether, 4-metyl-2-pentanol, methyl methacrylate, 2-propanol, 2-propen-1-ol, 2-propanyl alcohol, 1-propoxy-2-propanol, styrene, trichloromethane, toluene and m-xylene. In addition, a mixture of 2-methylbutyl acetate and n-pentyl acetate was tested. For most of the solvents, little or no percutaneous absorption data have been published. Lag times, steady-state fluxes and apparent permeability coefficients were obtained from the time courses of solvent appearance in the receptor medium, as measured by gas chromatography. The use of the same methodology and kind of skin resulted in small variability within experiments, underlining the need for consistent methodology for useful results for developing predictive models. Furthermore, a comparison of the neat and diluted data shows that water dilution affects all these variables and that the direction and magnitude of the effects vary between chemicals. This comparison strongly supports that prediction of percutaneous absorption of neat and water diluted chemicals requires different models.

Introduction

Understanding percutaneous absorption of organic solvents is important in many areas, such as prediction of the time-to-effect of topically administered medications, the retention and behaviours of cosmetics on the skin, or risk assessment of unintended exposures. The demand for percutaneous absorption data for risk assessment purposes has increased with the
introduction of the REACH legislation in the European Union, as manufacturers and producers of chemicals are required to evaluate the safe use of their products considering all relevant exposure routes, including dermal exposure.

For risk assessment of occupational exposures, solvents are of special importance due to extensive occupational use. Both inhalation and dermal exposure may contribute to systemic toxicity, meanwhile, successful reduction of ambient air levels increases the relative importance of dermal exposure. In the regulation of occupational exposure, the so-called “skin notation” is commonly used (in conjunction with occupational exposure limits, OELs) to identify chemicals that are easily taken up through the skin, and/or may cause or contribute to systemic toxicity. However, the criteria for skin notations, and how they are applied in practice, differ substantially between standard-setters [1–3]).

Among the difficulties to assign skin notation is the lack of relevant and reliable absorption data. A review of available studies for 108 different chemicals showed a huge variation in dermal permeability between as well as within chemicals [4]. This highlights the need for evaluating appropriateness of dermal absorption data. The kind of data most relevant for skin notations and other risk assessments of human skin exposure, i.e. human or in vivo dermal exposure toxicity, are rare due to ethical, practical and economical restrictions. Therefore, route-to-route extrapolation will be required for most chemicals. The same restrictions apply also to human and in vivo toxicokinetic data, underscoring the need for in vitro systems to evaluate percutaneous absorption and, in a longer perspective, to develop or improve computational predictive models.

The static diffusion cell, or Franz cell [5], is a commonly used tool to measure percutaneous absorption in vitro. The most common physical quantities used to describe the absorption process of a specific chemical can be derived from Fick’s laws of diffusion. Given a homogenous medium exposed to a chemical Fick’s first law can be used to calculate the steady-state flux \( J_{SS} \) and the permeability coefficient \( K_p \). These two physical quantities may then be used to develop predictive models, such as quantitative structure–activity relationship (QSAR) or quantitative structure-property relationship (QSPR) models [6,7]. Such models for percutaneous absorption have been the scope of a multitude of publications, e.g. [8–14], as well as evaluations of their performance [11, 15–18]. These models are mainly based on linear methods, which may not be sufficient for modelling the dynamics of percutaneous absorption. There are also few examples of different machine learning techniques [18–21], which show some promise. Deep learning methods in particular might be useful in future analysis of skin permeation data. Deep learning has been applied in a few cases of pharmaceutical and toxicological research [22] and performed well in the 2014 Tox21 challenge on prediction of nuclear receptor signaling and stress pathway assays [23].

A challenge in the development of linear as well as nonlinear approaches to predictive modelling of skin permeation is the limited availability of experimental data. Thus, the skin permeation models cited above are based on data for roughly 10 to 250 compounds whereas the Tox21 challenge participants had access to standardised data for 12 000 compounds [23]. Whichever predictive skin permeation model is chosen, the accuracy will depend on the input data and, as shown by e.g. Johanson and Rauma [4], these data are highly variable. For example, the donor species, location on the body and skin preparation all have a major impact on the results. Additionally, the experimental protocol, experimental and analytical equipment will influence study results [24,25]. For instance, experimental permeation data from diffusion cells using synthetic membranes, reducing the intra- and interspecies variability, may still comprise both inter- and intra-laboratory variability [26,27]. Comparisons of the static diffusion cells with flow-through diffusion cells, however, have shown that these two in vitro systems yield similar results [28–30].

Competing interests: MR is currently affiliated with Systecon AB, Stockholm, Sweden and MF is currently affiliated with Pharmteheus AB, Uppsala, Sweden. The authors confirm that these companies were not involved in the research in any manner. All work performed towards this paper was performed by the authors while employed at the Institute of Environmental Medicine, Karolinska Institutet.
The purpose of the present study was to evaluate the dermal penetration potential of a number of chemicals relevant for the occupational setting, namely organic solvents. Permeation studies of 38 common organic solvents were performed with skin from new-born piglets using the same experimental set up with static diffusion cells. Because the properties of neat chemicals and water dilutions may differ significantly, both these solutions were tested. Estimates of the lag time $t_{lag}$, the steady-state flux $J_{SS}$ and the apparent permeability coefficient $K_p$ are presented.

Materials and methods

2.1 Chemicals

Acetone (CAS 67-64-1, purity $\geq 99.5\%$), acetonitrile (75-05-8, $\geq 99.8\%$), n-butanol (71-36-3, 99.5%), trichloromethane (67-66-3, $\geq 99.0\%$), dichloromethane (75-09-2, $\geq 99.5\%$), ethyl acetate (141-78-6, $\geq 99.5\%$), 3-methyl-1-butanol (123-51-3, $\geq 99.0\%$), 2-butanone (78-93-3, $\geq 99.5\%$) and m-xylene (108-38-3, $\geq 99.0\%$) were obtained from Merck (Darmstadt, Germany).

2-Butoxyethanol (CAS 111-76-2, purity 99%), 2-ethoxyethanol (110-80-5, 98%) and toluene (108-88-3, $\geq 99.5\%$) were obtained from Kebo Lab (Stockholm, Sweden).

n-Butyl acetate (CAS 123-86-4, purity $\geq 99.0\%$), 1-butoxy-2-propanol (5131-66-8, $\geq 99.0\%$), butyl acrylate (141-32-2, $\geq 99.0\%$), cyclohexane (110-82-7, 99.9%), cyclohexanone (108-94-1, 99%), Furfuryl alcohol (98-00-0, 99%), 2-hexanone (591-78-6, 98%), 2-isopropanoxethanol (109-59-1, 99%), methanol (67-56-1, 99%), 1-methoxy-2-propanol (107-98-2, 98%), methyl acrylate (96-33-3, 99.0%), methyl tertiary butyl ether (1634-04-4, 99.8%), 4-methyl-2-pentanol (108-11-2, 98%), 2-propanol (67-63-0, $\geq 99.5\%$) and 2-propoxyethanol (2807-30-9, $\geq 99.0\%$) were obtained from Sigma Aldrich (Steinheim, Germany). A commercially available mixture of n-pentyl acetate (628-63-7) and 2-methylbutyl acetate (624-41-9) was obtained from Sigma Aldrich (Steinheim, Germany) in a 65%/35% mixture having 99% purity, proportions were 60% and 40% according to our analysis by gas chromatography.

Ethanol (CAS 64-17-5, purity 99%) was obtained from Kemetyl (Stockholm, Sweden).

2-Propen-1-ol (CAS 107-18-6, purity $\geq 99.5\%$), 1,2-dichloroethane (107-06-2, $\geq 99.5\%$), ethyl acrylate (140-88-5, $\geq 99.0\%$), ethylbenzene (100-41-4, $\geq 98.0\%$), n-hexane (110-54-3, $\geq 98.0\%$), methyl methacrylate (80-62-6, $\geq 99.0\%$), 2-propoxyethanol (2807-30-9, $\geq 99.0\%$) and styrene (100-42-5, $\geq 99.0\%$) were obtained from Fluka (Buchs, Switzerland).

For the dilution studies, the three solvents with limited miscibility with water, m-xylene, cyclohexane and ethylbenzene, were diluted in degassed phosphate buffered saline (1000–3, Sigma–Aldrich, Steinheim, Germany) containing 6% PEG-20 oleyl ether (P5641, Sigma–Aldrich, Steinheim, Germany). Remaining chemicals were diluted in laboratory grade deionized water. All reported percentages are calculated by volume.

2.2 Skin

Piglets (Duroc) that had died of natural causes (at birth or first week of life) were obtained from local commercial breeders. As this source of skin is categorised as slaughter waste, it is exempt from the Swedish Board of Agriculture’s requirements on ethical vetting of research involving animals.

Pig skin has been shown to be similar to human skin with respect to stratum corneum and epidermal thickness as well as permeability [4,25,31,32]. Skin specifically from new-born pigs that died of natural causes has also been shown to be a suitable replacement for human skin by Cilurzo et al. [33], as their experimentally derived in vitro fluxes for seven benzoxazinones were within a factor of 2 from previously published data on human epidermis.
For early experiments we employed dermatomed skin (n = 10, labelled in Table 1), and then proceeded to use full-thickness skin. In both cases, skin pieces measuring approximately 8 x 5 cm$^2$ were collected from the back and flank of the piglet. Each skin piece was stretched around the edges of a soft polyethylene plate (21 x 3 x 0.7 cm$^3$) and fastened to the sides of the plate with a staple gun. Another plate, wrapped in polyethylene film, served as a lid and was placed on top of the skin and firmly and evenly fixed to the bottom plate by bolts and nuts. The mounted skin pieces were stored at -20°C. In those cases skin was dermatomed, it was taken out after 12 h and the frozen piece dermatomed (Model C, Padgett Instruments, Inc., Kansas City, MO). Dermatomed skin pieces were wrapped in aluminium foil and polyethylene film, and then stored at -20°C until later use.

Twenty four hours before study the skin pieces were thawed for 15 minutes in room temperature. Thereafter the thickness was measured by using a micrometer (293-661-10, Mitutoyo) and integrity was checked by an ohm meter (Fluke 111, Fluke Corporation, Everett, WA, USA). Pieces with a resistance below 50 kΩ were discarded. This cut-off was validated against in house measurements of intact and damaged skin pieces. The skin pieces were stored overnight in saline at +8°C prior to permeation measurements.

2.3 Franz cell studies

Six jacketed static Franz cells (orifice diameter 9 mm, corresponding to a skin exposure area of 0.64 cm$^2$, receptor volume 5.0–5.4 mL, model number 4G-01-00-090-05, Permegear, Bethlehem, PA, USA) were mounted in a magnetic stirrer (HP 6 Variomag, H+P Labortechnik, Munich, Germany) and kept at 32°C [34] by means of circulating water from a thermostatted water bath (21 AT, Heto, Allrød, Denmark).

Degassed phosphate buffered saline (1000–3, Sigma–Aldrich, Steinheim, Germany) containing 6% PEG-20 oleyl ether (P5641, Sigma-Aldrich, Steinheim, Germany), according to OECD guidelines [34], was used as receptor fluid. The receptor compartment was kept well stirred using Teflon coated magnets. Skin pieces were mounted onto the Franz cells one hour before start of exposure.

At start of experiment, the donor compartment was filled with excess test chemical (approximately 1 ml, neat or diluted in water) and capped with a glass stopper. Experiments ran for 4 to 9 hours.

Aliquots of receptor fluid (50 μl) were sampled at predefined times (every 10 min first hour, every 20 min second hour, then every 30 min) using a gas-tight syringe (004250, SGE, Victoria, Australia). Samples were directly transferred to head-space glass vials, which were immediately capped and stored at +8°C for later analysis (within two days) by head-space gas chromatography.

2.4 Gas chromatographic analyses

The analyses were performed with a 6890+ GC (Hewlett Packard, Palo Alto, CA, USA), an 8700 GC (Perkin Elmer, Waltham, MA, USA) or a Clarus 500 (Perkin Elmer, Waltham, MA, USA) gas chromatograph equipped with a 10-m or 25-m Poraplot Q column and flame-ionization detector.

All studied chemicals were readily detected in the gas chromatographic analyses of receptor medium with limits of detection ranging from 0.1 μg/ml to 9.5 μg/ml (median 0.4 μg/ml) depending on chemical. To allow for quantitative analyses, standard curves were established for each test chemical. At least one concentration in each of the standard curves was well above the highest concentration achieved in the receptor fluid during the experiments. All
Table 1. Permeability data from static diffusion cell experiments using pig skin.

| Compound | CAS     | # C | MW (g mol\(^{-1}\)) | Log P  | \(c_{donor} (%)\) | Skin | N  | \(t_{lag}\) (min) | \(J_{lag}\) (g cm\(^{-2}\) h\(^{-1}\)) | \(J_{ss}\) (g cm\(^{-2}\) h\(^{-1}\)) | \(K_p\) (cm h\(^{-1}\)) | \(CV_Kp\) (%) |
|----------|---------|-----|---------------------|--------|-------------------|------|----|-----------------|----------------|-----------------|----------------|-------------|
| **Alcohols** |         |     |                     |        |                  |      |    |                 |                 |                 |                 |             |
| Methanol | 67-56-1 | 1   | 32.0               | -0.63  | 100.0             | Full | 6  | 40.1            | 6.5             | 7.55E-04       | 1.27E-02       | 9.35E-04     | 18           |
| Ethanol  | 64-17-5 | 2   | 46.1               | -0.14  | 100.0             | Split| 6  | 22.4            | 2.5             | 5.71E-03       | 1.59E-03       | 7.23E-03     | 68           |
| 2-Propanol | 107-18-6 | 3   | 58.1               | 0.21   | 100.0             | Full | 6  | 31.8            | 4.9             | 2.25E-03       | 1.08E-03       | 2.65E-03     | 108          |
| n-Butanol | 71-36-3 | 4   | 74.1               | 0.84   | 100.0             | Split| 6  | 32.5            | 4.7             | 2.11E-04       | 3.23E-03       | 2.60E-04     | 37           |
| 3-Methyl-1-butanol | 123-51-3 | 5   | 88.1               | 1.26   | 100.0             | Full | 6  | 68.8            | 8.5             | 4.43E-04       | 1.59E-04       | 5.46E-03     | 88           |
| 2-Propanol | 98-00-0 | 6   | 102.2              | 1.68   | 100.0             | Full | 6  | 40.9            | 5.3             | 1.30E-04       | 2.89E-05       | 1.61E-04     | 54           |
| **Chlorinated** |         |     |                     |        |                  |      |    |                 |                 |                 |                 |             |
| Dichloromethane | 75-09-2 | 1   | 84.9               | 1.34   | 100.0             | Full | 6  | 28.7            | 5.5             | 2.57E-04       | 4.66E-04       | 3.16E-04     | 43           |
| Trichloromethane | 119-4-6 | 2   | 119.4              | 1.52   | 100.0             | Full | 6  | 38.7            | 5.9             | 7.67E-03       | 7.66E-03       | 5.18E-03     | 44           |
| 1,2-Dichloroethane | 107-06-2 | 3   | 99.0               | 1.83   | 100.0             | Full | 6  | 30.7            | 1.8             | 3.80E-04       | 1.34E-04       | 9.66E-03     | 52           |
| **Aromatic** |         |     |                     |        |                  |      |    |                 |                 |                 |                 |             |
| Toluene  | 108-88-3 | 7   | 92.1               | 2.54   | 100.0             | Split| 6  | 27.4            | 1.8             | 3.80E-04       | 2.78E-05       | 4.36E-04     | 32           |
| Styrene  | 100-42-5 | 8   | 104.2              | 2.89   | 100.0             | Full | 6  | 90.3            | 14.6            | 7.11E-05       | 1.33E-05       | 7.86E-05     | 17           |
| Ethylbenzene | 100-41-4 | 9   | 106.2              | 3.03   | 100.0             | Full | 6  | 148.5           | 8.2             | 1.24E-04       | 1.39E-05       | 1.43E-04     | 24           |
| m-Xylene | 108-38-3 | 10  | 106.2              | 3.09   | 100.0             | Split| 6  | 69.0            | 1.8             | 6.27E-05       | 4.90E-06       | 7.29E-05     | 56           |
| **Esters** |         |     |                     |        |                  |      |    |                 |                 |                 |                 |             |
| Methyl acrylate | 96-33-3 | 11  | 86.1               | 0.73   | 100.0             | Full | 6  | 11.9            | 2.3             | 1.01E-03       | 4.49E-05       | 1.06E-03     | 47           |
| Ethyl acrylate | 141-78-6 | 12  | 88.1               | 0.86   | 100.0             | Full | 5  | 22.6            | 4.8             | 2.70E-03       | 6.48E-04       | 3.02E-03     | 54           |
| Ethyl acrylate | 140-88-5 | 13  | 100.1              | 1.22   | 100.0             | Full | 6  | 26.6            | 3.2             | 9.58E-04       | 2.35E-04       | 1.04E-03     | 60           |
| Methyl methacrylate | 80-62-6 | 14  | 100.1              | 1.28   | 100.0             | Full | 6  | 20.7            | 3.8             | 8.00E-04       | 8.90E-05       | 8.52E-04     | 47           |
| n-Butyl acrylate | 116-2-1 | 15  | 112.2              | 1.85   | 100.0             | Split| 6  | 24.3            | 4.1             | 4.75E-04       | 4.49E-05       | 5.39E-04     | 51           |
| Butyl acrylate | 128-2-2 | 16  | 128.2              | 2.20   | 100.0             | Full | 6  | 56.6            | 5.0             | 1.78E-04       | 1.57E-05       | 1.98E-04     | 74           |

(Continued)
standard curves were linear, showing that the maximum solubility of the receptor fluid was not exceeded for any of the tested chemicals.

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| Compound | CAS # | MW (g mol⁻¹) | Log P | cdonor (%) | Skin | N | tlag (min) | SElag (min) | Jsa (g cm⁻² h⁻¹) | SEJsa (g cm⁻³ h⁻¹) | Kp (cm h⁻¹) | SEkp (cm h⁻¹) | CV Kp (%) |
|----------|------|--------------|-------|------------|------|---|-----------|------------|----------------|----------------|-------------|-------------|---------|
| 2-Methyl butyl acetate* | 624-41-9 | 130.2 | 2.26 | 40.0 | Full | 6 | 59.0 | 3.9 | 2.23E-04 | 3.61E-05 | 6.37E-04 | 1.03E-04 | 40 |
| n-Pentyl acetate* | 628-63-7 | 130.2 | 2.34 | 60.0 | Full | 6 | 54.7 | 5.4 | 6.26E-04 | 8.21E-05 | 1.19E-03 | 1.56E-04 | 32 |

Glycol ethers

| Compound | CAS # | MW (g mol⁻¹) | Log P | cdonor (%) | Skin | N | tlag (min) | SElag (min) | Jsa (g cm⁻² h⁻¹) | SEJsa (g cm⁻³ h⁻¹) | Kp (cm h⁻¹) | SEkp (cm h⁻¹) | CV Kp (%) |
|----------|------|--------------|-------|------------|------|---|-----------|------------|----------------|----------------|-------------|-------------|---------|
| 2-Ethoxyethanol | 4 | 90.1 | -0.42 | 100.0 | Full | 6 | 58.5 | 2.5 | 9.19E-04 | 1.74E-04 | 9.88E-04 | 1.87E-04 | 46 |
| 1-Methoxy-2-propanol | 107-98-2 | 118.2 | 0.08 | 100.0 | Full | 6 | 60.7 | 5.9 | 12.24E-04 | 2.57E-04 | 1.96E-03 | 3.36E-04 | 42 |

Ketones

| Compound | CAS # | MW (g mol⁻¹) | Log P | cdonor (%) | Skin | N | tlag (min) | SElag (min) | Jsa (g cm⁻² h⁻¹) | SEJsa (g cm⁻³ h⁻¹) | Kp (cm h⁻¹) | SEkp (cm h⁻¹) | CV Kp (%) |
|----------|------|--------------|-------|------------|------|---|-----------|------------|----------------|----------------|-------------|-------------|---------|
| Acetone | 67-64-1 | 58.1 | -0.24 | 100.0 | Full | 6 | 13.1 | 3.2 | 9.86E-04 | 1.74E-04 | 9.96E-04 | 1.86E-04 | 56 |
| 2-Butanone | 78-93-3 | 72.1 | 0.26 | 100.0 | Full | 6 | 37.9 | 7.5 | 1.62E-03 | 3.34E-04 | 8.93E-04 | 1.67E-04 | 57 |
| Cyclohexanone | 108-94-1 | 98.1 | 1.13 | 100.0 | Full | 6 | 15.4 | 3.2 | 9.21E-04 | 1.82E-04 | 8.42E-04 | 1.62E-04 | 57 |

Miscellaneous

| Compound | CAS # | MW (g mol⁻¹) | Log P | cdonor (%) | Skin | N | tlag (min) | SElag (min) | Jsa (g cm⁻² h⁻¹) | SEJsa (g cm⁻³ h⁻¹) | Kp (cm h⁻¹) | SEkp (cm h⁻¹) | CV Kp (%) |
|----------|------|--------------|-------|------------|------|---|-----------|------------|----------------|----------------|-------------|-------------|---------|
| Acetonitrile | 75-05-8 | 41.1 | -0.15 | 100.0 | Full | 6 | 17.9 | 2.6 | 5.85E-04 | 1.68E-04 | 7.45E-04 | 1.23E-04 | 70 |
| Methyl tertiary butyl ether | 1634-04-4 | 88.2 | 1.34 | 100.0 | Full | 6 | 35.5 | 3.2 | 8.47E-04 | 2.05E-04 | 1.08E-02 | 2.61E-03 | 59 |
| Cyclohexane | 110-82-7 | 84.2 | 3.18 | 100.0 | Full | 6 | 65.4 | 14.7 | 6.90E-05 | 1.29E-05 | 9.19E-05 | 1.67E-05 | 52 |
| n-Hexane | 110-54-3 | 86.2 | 3.29 | 100.0 | Full | 6 | 63.1 | 5.0 | 9.15E-04 | 2.10E-04 | 1.41E-03 | 3.23E-04 | 56 |

CAS, Chemical Abstracts Service Registry Number; # C, number of carbon atoms; CV, Coefficient of Variation (same for \( J_{sa} \) and \( K_p \)); MW, molecular weight; \( c_{donor} \), volume concentration of chemical in donor compartment; N, number of experiments; \( t_{lag} \), lag time; \( J_{sa} \), flux at steady-state; \( K_p \), permeability coefficient; Log P, logarithm of the octanol:water partition coefficient (estimated using US EPA[73]); SE, standard error of the mean. Split, split-thickness skin; Full, full-thickness skin.

*2-Methylbutyl acetate (40%) and n-pentyl acetate (60%) were tested as a mixture.
2.5 Calculations
The steady-state flux, \( J_{SS} \) (g/cm\(^2\)/h), was calculated from the exposed area of the skin (i.e. 0.64 cm\(^2\)) and the slope of the steady-state region of the amount in the receptor medium versus time curve. From the steady-state flux the apparent permeability coefficient \( K_p \) was obtained as:

\[
K_p = \frac{J_{SS}}{c_{donor}}
\]

where \( c_{donor} \) is the concentration (g/cm\(^3\)) of the chemical in the donor compartment exposing the skin. The lag time, \( t_{lag} \) (min), is defined as the time-point where the extrapolated steady-state region intersects with the x-axis.

Data management and calculations were performed in Microsoft Excel (2010). To avoid data points from the pre-steady state part of the curve, data points with sampling times below the estimated value of \( t_{lag} \) were excluded. Furthermore, for some chemical the time-concentration curve appeared to flatten out and these "post-steady state" values were also omitted.

Correlations between experimentally derived permeability measures and physicochemical characteristics were investigated using regression analyses in the software R (version 3.3.2). As pentyl acetate and 2-methylbutyl acetate were tested as a mixture, they were not included in these analyses.

Results and discussion
The experimentally derived mean values of \( t_{lag} \), \( J_{SS} \), and \( K_p \) for each chemical and concentration are presented in Table 1. As expected, the tested solvents showed skin permeabilities (\( K_p \)) ranging from “moderate” (10\(^{-4}\) cm/h) to “very high” (10\(^{-2}\) cm/h) according to previously proposed classification schemes [35,36].

Comparable percutaneous absorption data using infinite dose of, or occluded exposure to, neat chemical have been published previously for 20 of the 38 chemicals investigated herein. One aim of in vitro permeation experiments is to predict percutaneous permeation for humans. Human in vivo data in the neat was available for nine substances [37–49]. We also wished to compare our data to other studies using similar experimental conditions, i.e. in vitro using human or animal skin [50–72]. The ratio between \( K_p \) values of the present study and \( K_p \) values from previous studies are plotted against log octanol:water partition coefficient (log P) in Fig 1. Values of log P were estimated using the EPI Suite software [73].

For nine of the 13 available human in vivo studies the \( K_p \) values were similar to those obtained in the present study (Fig 1, circles). Dutkiewicz and Tyras [37–39] reported very high and seemingly unrealistic fluxes and \( K_p \) values for toluene, styrene and ethyl (note 1 in Fig 1). As discussed above for ethylbenzene, the calculations were based on amount left on the skin after exposure. This method may easily overestimate the absorption. Mraz and colleagues [46] estimated the percutaneous absorption of cyclohexanone by quantification of its metabolite 1,2-cyclohexanediol in urine collected up to 72 h after exposure (note 2). The remaining human in vivo studies, covering six chemicals, are in reasonable agreement (within one order of magnitude) with our present study.

Estimates of \( K_p \) from previous in vitro studies on neat chemicals are available for 18 chemicals and are in most cases (37 out of 47 comparisons covering 15 chemicals) within one order of magnitude of the \( K_p \) for neat chemicals derived in the present study (Fig 1). These studies cover skin from several species (human, hairless rat/rat, guinea pig, pig/minipig), varying thickness and both static and flow-through diffusion cells. There are no discernible trends concerning species and agreement with our data. Although rat skin is expected to yield higher
permeability coefficients than human skin or pig skin, this seems not to be the case in most studies. The deviating results for ethanol and methanol (note 3) may be due to evaporation at the sampling stage [55] or during the exposures as Pendlington and colleagues [62] only recovered 40% of applied ethanol, although care was taken to avoid evaporation. The largest deviations were seen for the more hydrophobic chemicals (note 4; ethylbenzene, n-hexane, toluene). For n-hexane the ratios reached as high as 1400 [54] and 15000 [52]. The latter study employed physiological saline as receptor medium, which would reduce diffusion as compare
to our receptor solution, however, it is unclear whether these factors would reduce diffusion this much.

The experimental variability is comparably low across our performed experiments. For 71 cases listed in Table 1, the coefficient of variation ranged from 11% to 120%. This maximal factor of two is substantially slower than the many orders of magnitude seen in between studies for many substances [4]. Furthermore, the coefficient of variation was in most cases below 50% (49 out of 71) and only above 75% in 7 cases. The higher coefficients of variation were primarily found among alcohols (n = 6). Although we cannot offer any explanation for this pattern, we note that the cases cover both water dilutions and neat solvents as well as both full-and split-thickness skin.

In Fig 2, two substances attract attention due to long lag times, namely cyclohexane (165.5 min in neat and 205.1 min in 0.1% water dilution) and ethylbenzene (148.5 min, only tested neat). For cyclohexane experiments ran for 540 min (9h) and for ethylbenzene 360 min (6h). The long lag times decreases the confidence in the calculations of $J_{SS}$ and $K_p$ as steady state may not have been reached, in particular for ethyl benzene. For cyclohexane we have not found any other dermal permeation data. We found three studies presenting $J_{SS}$ values for neat ethylbenzene. Dutkiewicz and Tyras [37] performed 10–15 min occluded exposures of volunteers to ethylbenzene in vivo and obtained a $J_{SS}$ of 2.8 $\times$ 10^{-2} g/cm^2/h, i.e. 200 times higher than our value. In their study, the absorbed amount was defined as the difference between applied amount and amount remaining on the skin after exposure. This approach assumes that all chemical absorbed into skin is systemically absorbed, even though some may have evaporated after the occluded exposure ended. Tsuruta [52] used rat skin in vitro and physiological saline as the receptor medium. The resulting $J_{SS}$ was 6.3 $\times$ 10^{-6} g/cm^2/h, i.e. 20 times lower than our value. As ethylbenzene is hydrophobic, the use of physiological saline as receptor medium may have led to an underestimation of $J_{SS}$. Furthermore, the experiments ran for 3–6 hours and the $t_{lag}$ was 2 hours, hence as in our experiments, steady state may not have been reached. The third and most recent study with ethylbenzene is that of Susten et al. [74] who exposed hairless mice in vivo to ethylbenzene. We consider this study to be the most reliable of the three, and it reported a $J_{SS}$ of 2.2 $\times$ 10^{-3} g/cm^2/h, which is six-fold lower than our value.

Previous research indicates that molecular size is related to $t_{lag}$, for instance Nielsen et al. [16] found a general trend of increasing $t_{lag}$ with increased molecular weight in permeation experiments with 9 chemicals in aqueous solutions (MW 122 to 376.7 g/mol. Such a trend is obvious for the esters, where the lag time clearly increases with increasing MW (neat: intercept = -54.1, slope = 0.79, $r^2 = 0.71$ p = 0.04; dilute: intercept = -32.9, slope = 0.56, r = 0.94, p = 0.001). However, for the overall material there was no strong correlation between molecular weight (neat: intercept = 14.5, slope = 0.29, $r^2 = 0.04$ p = 0.2; dilute: intercept = 37.2, slope = 0.1, $r^2 = 0.005$, p = 0.7). For number of carbons the correlations were statistically significant, but relatively small (neat: intercept = 3.4, slope = 7.9, $r^2 = 0.22$ p = 0.004; dilute: intercept = 16.5, slope = 6.8, $r^2 = 0.14$, p = 0.04). Thus, although some of the variability in lag time can be attributed to molecular size, other factors, among them polarity and dilution, seem to have a significant influence. For chemicals where both neat and diluted solutions were tested (n = 31) the neat solutions had shorter time lags than the corresponding diluted mixtures in two thirds of the cases. Methanol stands out as the only alcohol with a clearly longer time lag for the neat chemical (Table 1). Although we assume stratum corneum to constitute the main barrier, we cannot exclude that the difference in skin thickness between experiments for neat and diluted methanol played a role.

The average steady-state fluxes ($J_{SS}$) for each chemical and concentration are presented in Table 1. For neat substances, decreased flux with increasing number of carbon atoms is evident for most solvents (Fig 3). This relationship was statistically significant for both neat and diluted
substances (neat: intercept = -2.0, slope = -0.25, $r^2 = 0.63$, $p < 0.0001$; dilute: intercept = -2.8; slope = -0.19; $r^2 = 0.35$ $p = 0.0004$; log $J_{SS}$). Diluted mixtures have in general a lower flux than neat chemicals, often only one tenth of the flux for the neat compound. As reported previously [49,69,75], 2-butoxyethanol is a striking exception with an opposite effect of dilution (Table 1). Bunge et al. identified that $J_{SS}$ of 2-butoxyethanol was proportional to thermodynamic activity up to a concentration of 80% (by weight), at higher concentrations the decreased flux is likely due to dehydration of the skin [71].

Of the neat chemicals, methanol had the highest apparent permeability coefficient ($K_p$) of $1.3 \times 10^{-2}$ cm/h, while m-xylene had the lowest of $7.3 \times 10^{-5}$ cm/h (Table 1). The $K_p$ values presented here are considered “apparent” as they are calculated using nominal concentrations

![Fig 3. Steady state flux (mg cm$^{-2}$ h$^{-1}$) plotted in log scale over number of carbon atoms in the molecule.](https://doi.org/10.1371/journal.pone.0205458.g003)

![Fig 4. Ratio of apparent permeability coefficient (dilute / neat, log scale) plotted over the log octanol: Water partition coefficient (log P, estimated using US EPA[73]).](https://doi.org/10.1371/journal.pone.0205458.g004)
and not thermodynamic activity. Because concentration and not activity was used in the
denominator, we expect these apparent $K_p$ values to be higher for diluted than for neat solvent,
which also was the case for all but methanol (Table 1, Fig 4). However, as seen in Fig 4, the log-
arithm of the $K_p$ ratio (dilute/neat) seems to increase with an increasing log P up to a log P of
about 1.5. In particular for the amphiphilic solvents in our selection, the water dilution vs neat
discrepancies in $K_p$ may be partly explained by the substance altering the skin barrier function.
This has been proposed for 2-buthoxyethanol by Bunge and colleagues [71] as well as for
2-hydroxypropyl acrylate by Frasch and colleagues [76]. For improved understanding of how
water affects the $K_p$, experimental data are needed on thermodynamic activity vs concentration
for the test substances.

The discrepancies in $K_p$ between neat and diluted solvent also hold implications for predic-
tive models. The present study allows comparison between predictions and experimental data
for both neat and water dilution for 31 substances, studied using the same experimental set up.
In Fig 5 we plot the EPISuite (US EPA [73]; similar to Potts and Guy[9]) predictions for the
chemicals tested in neat and in water dilution over the experimentally derived values from the
present study, log scale on both axes. The fit is poor (on a linear scale) for both neat (inter-
cept = 0.02, slope = -1.9, $r^2 = 0.018$, $p = 0.4$) and water diluted solvents (intercept = 0.009,
slope = -0.08, $r^2 = 0.009$, $p = 0.6$). Furthermore, the correlation has a negative slope, i.e. the
lower the predicted $K_p$, the higher the experimental $K_p$ (see also S1 Fig). A previous study com-
pared experimental permeation data for eleven substances in neat with outcomes for three dif-
ferent predictive models, finding that the model outcomes correlated well with each other but
less so with experimental data [17]. Fig 5, and the differences in regression lines, furthermore
illustrates that prediction of percutaneous absorption of neat and water diluted chemical
requires markedly different models.

Conclusions
We have measured lag times, steady-state fluxes, and apparent permeability coefficients for 38
organic solvents. For a number of them, this study contributes with the first, or one of few,
experimental data sets on skin permeability. The present study adds to the body of evidence
showing that $K_p$ is concentration dependent, and that the influence of water dilution varies sig-
ificantly between chemicals. Hence, dilution is a factor that needs to be considered in risk
assessment of dermal exposures as well as incorporated in QSAR and other modelling efforts.

The variability between experiments was minimized as we used the same kind of skin and
methodology, with minor variations, throughout. This is an important aspect, as the perme-
ability for the same chemical has been found to vary by up to six orders of magnitude between
different studies [4]. In the present study the coefficient of variation for our experiments ran-
ged from 11% to 120% with a median of 45%. Only seven experiments yielded a coefficient of
variation above 75%. Hence, overall, our experiments display low variability compared to that
seen between studies [4]. The lower variability underlines the importance of a standardized
and consistent methodology to achieve useful results e.g. for QSAR analyses. This aspect is
probably far more important than the choice of human over pig skin. These two species are
rather similar regarding stratum corneum and epidermal thickness as well as permeability
[4,31].

Furthermore, human skin is in itself a source of inter- and intra-individual variability [25,
77]. Human skin may be difficult to obtain and, when obtained, it may be not be possible to
control how it was sampled, from whom (donor age) and from where (body site). This is even
more problematic if a large number of chemicals are to be covered. In conclusion, although
human skin may seem preferable because of the intended application domain (i.e.
Percutaneous absorption and subsequent health risks to humans), well performed in vitro experiments with pig skin appear to be a better alternative.

Supporting information
S1 Fig. Predicted (EPISuite, US EPA [73]) versus experimental (present study) permeability coefficients (Kp, cm/h) for neat (n = 36) and water diluted organic solvents (n = 31). Note the log scale on both axes, S1 Fig shows the data on linear scale.

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References
1. Nielsen J.B., Grandjean P. Criteria for Skin Notation in Different Countries. Am J Ind Med. 2004; 45:275–280. https://doi.org/10.1002/ajim.10340 PMID: 14991854
2. Sartorelli P., Ahlers H.W., Alanko K., Chen-Peng C., Cherrie J.W., Drexler H., et al. How to Improve Skin Notations. Position paper from a workshop. Regul Toxicol Pharmacol. 2007; 49:301–307. https://doi.org/10.1016/j.yrtph.2007.08.006 PMID: 17919793
3. Sartorelli P., Ahlers H.W., Cherrie J.W., Kezic S., Johanson G., Filon F.L., et al. The 2008 ICOH Workshop on Skin Notation. Med Lav. 2010; 101:3–8. PMID: 20415043
4. Johanson G., Rauma M. Basis for Skin Notation. Part 1. Dermal Penetration Data for Substances on the Swedish OEL List. Arbete och Hälsa 42; 2008. ISBN 978-91-85971-02-2.
5. Franz T.J. Percutaneous Absorption on the Relevance of in Vitro Data. J Invest Dermatol. 1975; 64:190–195. PMID: 123263
6. Puzyzn T., Leszczynski J., Cronin M.T.D. Recent Advances in QSAR Studies: Methods and Applications. Springer, Dordrecht, New York; 2010.
7. Tsakovska I., Pajeva I., Al Sarif M., Alov P., Fioravanzo E., Kovarich S., et al. Quantitative Structure-Skin Permeability Relationships. Toxicology 2017; 387:27–42. https://doi.org/10.1016/j.tox.2017.06.008 PMID: 28645577
8. Fiserova-Bergerova V., Pierce J.T., Droz P.O. Dermal absorption potential of industrial chemicals: criteria for skin notation. Am J Ind Med. 1990; 17:617–635. PMID: 2337085
9. Potts R.O., Guy R.H. Predicting Skin Permeability. Pharm Res. 1992; 9:663–669. PMID: 1608900
10. Cleek R., Bunge A. A new method for estimating dermal absorption from chemical exposure. 1. General approach. Pharm Res. 1993; 10:497–506. PMID: 8489361
11. Wilschut A., ten Berge W., Robinson P.J., McKone T.E. Estimating skin permeation. The validation of five mathematical skin permeation models. Chemosphere. 1995; 30:1275–96. PMID: 7749723
12. Sartorelli P., Aprea C., Cenni A., Novelli M.T., Orsi D., Palmi S., Matteucci G. Prediction of percutaneous absorption from physicochemical data: a model based on data of in vitro experiments. Ann Occup Hyg. 1998; 42:267–76. PMID: 9713250
13. Patel H., ten Berge W., Cronin M.T.D. Quantitative structure–activity relationships (QSARs) for the prediction of skin permeation of exogenous chemicals. Chemosphere. 2002; 48:603–613. PMID: 12143935
14. ten Berge W. A simple dermal absorption model: Derivation and application. Chemosphere. 2009; 75:1440–1445. https://doi.org/10.1016/j.chemosphere.2009.02.043 PMID: 19304310
15. Lian G., Chen L., Han L. An evaluation of mathematical models for predicting skin permeability. J Pharm Sci. 2008; 97:584–598. https://doi.org/10.1002/jps.21074 PMID: 17722002
16. Nielsen J.B., Ahm Sørensen J., Nielsen F. The Usual Suspects- Influence of Physicochemical Properties on Lag Time, Skin Deposition, and Percutaneous Penetration of Nine Model Compounds. J Toxicol Environ Health Part A. 2009; 72:315–323. https://doi.org/10.1080/15287390802529872 PMID: 19184747
17. Korinth G., Schaller K.H., Bader M., Bartsch R., Göen T., Rossbach B., et al. Comparison of Experimentally Determined and Mathematically Predicted Percutaneous Penetration Rates of Chemicals. Arch Toxicol. 2012; 86:423–30. https://doi.org/10.1007/s00204-011-0777-z PMID: 22076108
18. Brown M. B., Lau C., Lim S. T., Sun Y., Davey N., Moss G. P., et al. An evaluation of the potential of linear and nonlinear skin permeation models for the prediction of experimentally measured percutaneous drug absorption. J Pharm Pharmacol. 2012; 64:566–577. https://doi.org/10.1111/j.2042-7158.2011.01436.x PMID: 22420662
19. Neely B.J., Madihally S.V., Robinson R.L. Jr, Gasem K.A.M. Nonlinear quantitative structure-property relationship modeling of skin permeation coefficient. J Pharm Sci. 2009; 98:4069–4084. https://doi.org/10.1002/jps.21678 PMID: 19189399
20. Değim T., Hadgraft J., İlbasmış S., Özkan Y. Prediction of Skin Penetration Using Artificial Neural Network (ANN) Modeling. J Pharm Sci. 2003; 92: 656–664. https://doi.org/10.1002/jps.10312 PMID: 12587127
21. Baba H., Takahara J., Mamitsuka H. In Silico Predictions of Human Skin Permeability using Nonlinear Quantitative Structure-Property Relationship Models. Pharm Res. 2015; 32:2360–2371. https://doi.org/10.1007/s11095-015-1629-y PMID: 25616540
22. Ekins S. The Next Era: Deep Learning in Pharmaceutical Research. S Pharm Res. 2016; 33:2594–2603
23. Mayr A., Klambauer G., Unterthiner T., Hochreiter S. DeepTox: toxicity prediction using deep learning. Front Environ Sci. 2016; 3:80.
24. Godin B., Touitou E. Transdermal Skin Delivery: Predictions for Humans from in Vivo, ex Vivo and Animal Models. Adv Drug Deliv Rev. 2007; 59:1152–1161. https://doi.org/10.1016/j.addr.2006.10.004 PMID: 17889400
25. Barbero A.M., Frasch H.F. Pig and Guinea Pig Skin as Surrogates for Human in Vitro Penetration Studies: A Quantitative Review. Toxicol in Vitro. 2009; 23:1–13. https://doi.org/10.1016/j.tiv.2008.10.008 PMID: 19013230
26. Hauck W.W., Shah V.P., Shaw S.W., Ueda C.T. Reliability and Reproducibility of Vertical Diffusion Cells for Determining Release Rates from Semisolid Dosage Forms. Pharm Res. 2007; 24:2018–2024 https://doi.org/10.1007/s11095-007-9329-x PMID: 17530388
27. Ng S.F., Rouse J.J., Sanders on F.D., Meidan V., Eccleston G.M. Validation of a Static Franz Diffusion Cell System for in Vitro Permeation Studies. AAPS PharmSciTech. 2010; 11:1432–1441. https://doi.org/10.1208/s12249-010-9522-9 PMID: 20842539
28. Bronaugh R.L., Stewart R.F. Methods for in Vitro Percutaneous Absorption Studies IV: The Flow-Through Diffusion Cell. J Pharm Sci. 1985; 74:64–67. PMID: 3981421
29. Clohesy H.M., Scott R.C., Heylings J.R. Skin Absorption—Flow-Through or Static Diffusion Cells. Toxicol in Vitro. 1994; 8:827–830. PMID: 20693022
30. Guth K., Schäfer-Korting M., Fabian E., Haltner-Ukomadu E., van Ravenzwaay B., Landsiedel R. Flow-Through versus Static Design for Dermal Absorption Experiments in Vitro. Toxicol. Lett. 2013; 221:S187.
31. Schmook F.P., Meingassner J.G., Billich A. Comparison of Human Skin or Epidermis Models with Human and Animal Skin in in-Vitro Percutaneous Absorption. Int J Pharm. 2001; 215:51–56 PMID: 11250091
32. Huong S.P., Bun H., Fourneron J.D., Reynier J.P., Andrieu V. Use of Various Models for in Vitro Percutaneous Absorption Studies of Ultraviolet Filters. Skin Res Technol. 2009; 15:253–261. PMID: 19630207
33. Cilurzo F., Minghetti P., Sinico C. Newborn Pig Skin as Model Membrane in In Vitro Drug Permeation Studies: A Technical Note. AAPS PharmSciTech. 2007; 8:E1–E4.
34. OECD. Test No. 428: Skin Absorption: In Vitro Method, OECD Guidelines for the Testing of Chemicals, Section 4, No. 428, OECD Publishing, Paris; 2004. http://dx.doi.org/10.1787/9789264071087-en.
35. Marzulli F.N., Callahan J.F., Brown D.W. Chemical structure and skin penetrating capacity of a short series of organic phosphates and phosphoric acid. J Invest Dermatol. 1965; 44:339–344.
36. Barber E.D., Hill T., Schum D.B. The percutaneous absorption of hydroquinone (hq) through rat and human skin in vitro. Toxicol. Lett. 1995; 80:167–172. PMID: 7482585
37. Dutkiewicz B., Tyras H. A Study of the Skin Absorption of Ethylbenzene in Man. Brit J Ind Med. 1967; 24:330–332.
38. Dutkiewicz B., Tytras H. The Quantitative Estimation of Toluene Skin Absorption in Man. Int Arch Gewerbepathol und Gewerbephyg. 1968; 24:253–257.
39. Dutkiewicz B., Tyras H. Skin Absorption of Toluene, Styrene, and Xylene by Man. Br J Ind Med. 1968; 25:243. PMID: 5663430
40. Engstrom K., Husman K., Riihimaki V. Percutaneous Absorption of m-Xylene in Man. Int Arch Occup Environ Health. 1977; 39:181–189. PMID: 924688
41. DiVincenzo G.D., Hamilton M.L., Kaplan C.J., Krasavage W.J., O'Donoghue J.L. Studies on the Respiratory Uptake and Excretion and the Skin Absorption of Methyl n-Butyl Ketone in Humans and Dogs. Toxicol Appl Pharmacol 1978; 44:593–604. PMID: 567391
42. Riihimaki V. Percutaneous Absorption of m-Xylene from a Mixture of m-Xylene and Isobutyl Alcohol in Man. Scand J Work Environ Health. 1979; 5:143–150. PMID: 472685
43. Dutkiewicz B., Konczalik J., Karwacki W. Skin Absorption and Per Os Administration of Methanol in Men. Int Arch Occup Environ Health. 1980; 47:81–88. PMID: 7429648
44. Berode M., Droz P.O., Guillemin M. Human Exposure to Styrene. VI. Percutaneous Absorption in Human Volunteers. Int Arch Occup Environ Health. 1985; 55:331–336. PMID: 400805
45. Johanson G., Boman A., Dysenius B. Percutaneous Absorption of 2-Butoxyethanol in Man. Scand J Work Environ Health. 1988; 12:499–503.
46. Mraz J., Galova E., Nohova H., Vítková D., 1994. Uptake, Metabolism and Elimination of Cyclohexane in Humans. Int Arch Occup Environ Health. 1994; 66:203–208. PMID: 7814101
47. Batterman S.A., Franzblau A., 1997. Time-Resolved Cutaneous Absorption and Permeation Rates of Methanol in Human Volunteers. Int Arch Occup Environ Health. 1997; 70(5):341–351 PMID: 9352338
48. Kezic S., Mahieu K., Monster A.C., de Wolff F.A. Dermal Absorption of Vaporous and Liquid 2-Methoxyethanol and 2-Ethoxyethanol in Volunteers. Occup Environ Med. 1997; 54:38–43. PMID: 9072032
49. Jakasa I., Mohammadi N., Kruse J., Kezic S. Percutaneous Absorption of Neat and Aqueous Solutions of 2-Butoxyethanol in Volunteers. Int Arch Occup Environ Health. 2004; 77:79–84. https://doi.org/10.1007/s00420-003-0456-3 PMID: 12915943
50. Scheuplein R.J., Blank I.H. Mechanism of Percutaneous Absorption. IV. Penetration of Nonelectrolytes (Alcohols) from Aqueous Solutions and from Pure Liquids. J Invest Dermatol. 1973; 60:286–296. PMID: 4758734
51. Tsuruta H. Percutaneous Absorption of Organic Solvents 2) A Method For Measuring The Penetration Rate Of Chlorinated Solvents Through Excised Rat Skin. Ind Health. 1977; 15:131–139.
52. Tsuruta H. Percutaneous Absorption of Organic Solvents III. On the Penetration Rates of Hydrophobic Solvents through the Excised Rat Skin. Ind Health. 1982; 20:335–345. PMID: 7153067
53. Dugard P.H., Walker M., Mawdsley S.J., Scott R.C. Absorption of Some Glycol Ethers through Human Skin in Vitro. Environ Health Perspect. 1984; 57:193–197. https://doi.org/10.1289/ehp.8457193 PMID: 6499804
54. Loden M. The In Vitro Permeability of Human Skin to Benzene, Ethylene Glycol, Formaldehyde, and n-Hexane. Acta Pharmacol Toxicol. (Copenh) 1986; 58:382–389.
55. Gummer C.L., Maibach H.I. The Penetration of [14C]Ethanol and [14C]Methanol through Excised Guinea-Pig Skin in Vitro. Food Chem Toxicol. 1986; 24:305–309. PMID: 3732976
56. Bartnik F.G., Reddy A.K., Klecak G., Zimmerman V., Hostynek J.J., Kunstler K. Percutaneous Absorption, Metabolism, and Hemolytic Activity of n-Butoxyethanol. Fundam. Appl. Toxicol. 1987; 8:59–70. PMID: 3556823
57. Barber E.D., Teetsel N.M., Kolberg K.F., Guest D. A Comparative Study of the Rates of In Vitro Percutaneous Absorption of Eight Chemicals Using Rat and Human Skin in Vitro. Fundam Appl Toxicol. 1992; 19:493–497. PMID: 1426706
58. Jacobs R.R., Phanprasit W. An In Vitro Comparison of the Permeation of Chemicals in Vapor and Liquid Phases Through Pig Skin. Am Ind Hyg Assoc J. 1993; 54:569–575. https://doi.org/10.1080/15298669319355071 PMID: 8237790
59. Ursin C., Hansen C.M., Van Dyk J.W., Jensen P.O., Christensen I.J., Ebbehoej J. Permeability of Commercial Solvents through Living Human Skin. Am Ind Hyg Assoc J. 1995; 56:651–660 https://doi.org/10.1080/15428199510166048 PMID: 7618604
60. Larese Filon F., Fiorito A., Adami G., Barbieri P., Coceani N., Bussani R., et al. Skin Absorption in Vitro of Glycol Ethers. Int Arch Occup Environ Health. 1999; 72:480–484. PMID: 10541914
61. Lockley D.J., Howes D., Williams F.M. Percutaneous Penetration and Metabolism of 2-Butoxyethanol. Arch Toxicol. 2004; 78:617–628. https://doi.org/10.1007/s00204-004-0581-0 PMID: 15455191
62. Betts C.J., Dearman R.J., Heylings J.R., Kimber I., Basketter D.A. Skin Sensitization Potency of Methyl Methacrylate in the Local Lymph Node Assay: Comparisons with Guinea-Pig Data and Human
Experience. Contact Dermatitis. 2006; 55:140–147. https://doi.org/10.1111/j.1600-0536.2006.00898.x PMID: 16918612

67. Frasch H.F., Barbero A.M., Alachkar H., McDougall J.N. Skin Penetration and Lag Times of Neat and Aqueous Diethyl Phthalate, 1,2-Dichloroethane and Naphthalene. Cutan Ocul Toxicol. 2007; 26:147–160. https://doi.org/10.1080/15569520701212274 PMID: 17612981

68. Korinth G., Göen T., Schaller K.H., Drexler H. Discrepancies Between Different Rat Models for the Assessment of Percutaneous Penetration of Hazardous Substances. Arch Toxicol. 2007; 81:833–840. https://doi.org/10.1007/s00204-007-0221-6 PMID: 17576541

69. Traynor M.J., Wilkinson S.C., Williams F.M. The Influence of Water Mixtures on the Dermal Absorption of Glycol Ethers. Toxicol. Appl. Pharmacol. 2007; 218:128–134. https://doi.org/10.1016/j.taap.2006.09.019 PMID: 17173944

70. Fasano W.J., McDougal J.N. In Vitro Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration: Summary and Evaluation of USEPA’s Mandated Testing. Regul Toxicol Pharmacol. 2008; 51:181–194. https://doi.org/10.1016/j.yrtph.2008.04.005 PMID: 18501488

71. Bunge A.L., Persichetti J.M., Payan J.P. Explaining Skin Permeation of 2-Butoxyethanol from Neat and Aqueous Solutions. Int J Pharm. 2012; 435:50–62. https://doi.org/10.1016/j.ijpharm.2012.01.058 PMID: 22330932

72. Dennerlein K., Schneider D., Göen T., Schaller K.H., Drexler H., Korinth G. Studies on Percutaneous Penetration of Chemicals—Impact of Storage Conditions for Excised Human Skin. Toxicol in Vitro. 2013; 27(2):708–13. https://doi.org/10.1016/j.tiv.2012.11.016 PMID: 23219852

73. US EPA. Estimation Programs Interface Suite for Microsoft Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA, 2012.

74. Susten A.S., Niemeier R.W., Simon S.D. In Vivo Percutaneous Absorption Studies of Volatile Organic Solvents in Hairless Mice II. Toluene, Ethylbenzene and Aniline. J Appl Toxicol. 1990; 10: 217–225. PMID: 2380484

75. Johansson G., Fernström P. Influence of Water on the Percutaneous Absorption of 2-Butoxyethanol in Guinea Pigs. Scand J Work Environ Health. 1988; 14:95–100.

76. Frasch H.F., Barbero A.M., Dotson G.S., Bunge A.L. Dermal Permeation of 2-Hydroxypropyl Acrylate, a Model Water-Miscible Compound: Effects of Concentration, Thermodynamic Activity and Skin Hydration. Int J Pharm. 2014; 460:240–247. https://doi.org/10.1016/j.ijpharm.2013.11.007 PMID: 24239832

77. Meidan V.M., Roper C.S., 2008. Inter- and Intra-Individual Variability in Human Skin Barrier Function: a Large Scale Retrospective Study. Toxicol in Vitro. 2008; 22:1062–1069. https://doi.org/10.1016/j.tiv.2008.01.009 PMID: 18321675