Assessment of Dermal Exposure to Pesticide Residues During Re-entry

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ABSTRACT: Currently, the determination of health risks to pesticide applicators from dermal exposure to these chemicals is assessed using either a concentrate of the compound, or a relevant aqueous dilution. Neither of these conditions reflects a normal exposure of an individual when re-entering an area after pesticide application, i.e., contact with dried residue of the diluted product on foliage. Methodology has therefore been developed to determine a relevant estimate of this potential dermal re-entry exposure from pesticide residues. Potential delivery platforms have been characterized for the transfer of pesticide residue to skin. Spin coating has been used to deposit uniform pesticide layers on to each platform. Five pesticides have been chosen to encompass a wide range of physicochemical properties: atrazine, 2,4-dichlorophenoxyacetic acid (2,4-D), chlorpyrifos, monocrotophos and acetochlor. *In vitro* (Franz diffusion cell) experiments have been performed to monitor the transfer of these pesticides from the delivery platforms onto and through excised porcine skin. Parallel experiments were also conducted with aqueous pesticide dilutions for comparison, and a final *in vivo* measurement using ibuprofen (as a model compound) complemented the *in vitro* data. The results demonstrate that transfer of chemical residue onto and subsequently through the skin is dependent on the physical attributes of the residue formed. Thus, assessing dermal exposure to pesticides based on skin contact with either the chemical concentrate or a relevant aqueous dilution may incorrectly estimate the risk for re-entry scenarios.

KEYWORDS: Pesticides, skin permeation, dermal exposure, percutaneous absorption, risk assessment.

BRIEFS: Methodology is proposed to determine a relevant estimate of the potential dermal re-entry exposure from pesticide residues.

INTRODUCTION

The U.K. Pesticides Safety Directorate (PSD) has recently identified an issue concerning so-called dermal re-entry [1]: specifically, that individuals (e.g. workers/ramblers), when allowed to re-enter an area previously treated with a pesticide, are typically exposed to dried residues of the diluted product. However, because there is essentially no reliable way to estimate dermal absorption of pesticide from this dried and diluted residue, significant uncertainty exists in the resulting human health risk assessment. Pesticide absorption data across the skin are normally available for the concentrate (liquid, powder, granule) and for an aqueous dilution (1:10 to 1:1000 or more), and PSD currently uses the higher, measured value as a precautionary approach. This conservative strategy may, of course, result in refusal of applications for certain uses, and, in turn, this has led to the question as to whether an alternative methodology should be considered.

The principal objective of this project, therefore, was to develop and examine a new approach to determine potential dermal re-entry exposure to pesticide residues. The initial stage involved characterization of potential 'delivery platforms' which simulate surfaces from which pesticides may be transferred to human skin in a re-entry exposure. Both metallic and polymeric substrates were investigated, all of which were inert and impermeable to pesticide uptake. Spin coating was employed to deposit uniform layers of pesticides onto the platform materials. It is important to emphasise at the outset that, because plant surface properties vary considerably between different species, the selection of a reproducible/representative "model" is very challenging, and this is why an artificial substrate was targeted in this work.

Once a reliable spin coating technique had been established, the *in vitro* transfer of five representative pesticides from the delivery platforms into and through excised mammalian skin was examined. Presently, a notable source of fallibility in predicting dermal absorption arises because the majority of studies are performed on rats.[2,3] It has been reported that rodent skin often overestimates the

penetration rate in human skin of topically applied chemicals.[4,5] Thus, *in vitro* studies were performed using porcine skin, which is widely recognized as an excellent model for the human barrier.[6]

Experiments were conducted *in vitro* using Franz-type diffusion cells. Adhesive tape stripping allowed a disposition profile to be generated for each pesticide.[7] Complementary studies were conducted involving aqueous pesticide donor solutions for comparison. To assess the applicability of the method developed *in vitro*, the project was extended to include an *in vivo* study of the transfer of ibuprofen residue from a platform to the ventral forearm of a healthy human volunteer.

For the study, five pesticides were chosen which encompass a wide range of physicochemical properties: 2,4-dichlorophenoxyacetic acid (2,4-D), acetochlor, atrazine, chlorpyrifos and monocrotophos. Table 1 shows relevant properties of the five pesticides, along with ibuprofen.[8] The table also includes the predicted maximum flux (J_{max}) of each chemical, calculated from the octanol-water partition coefficient (P) and molecular weight (MW) with a commonly used algorithm:[9-11]

$$J_{max} = k_p^{corr} \cdot C_{sat}$$

where

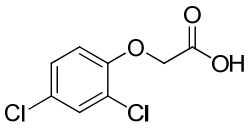
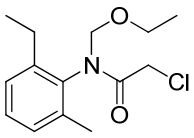
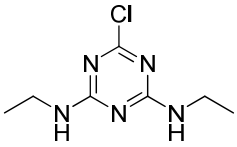
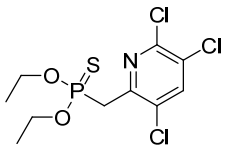
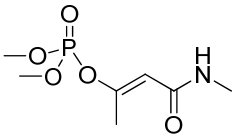
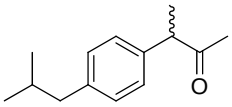
$$\log k_p = -2.7 + 0.71 \cdot \log P - 0.0061 \cdot MW$$

and

$$k_p^{corr} = k_p / \{1 + [(k_p \cdot \sqrt{MW})/2.6]\}$$

(units of the permeability coefficient (k_p) are cm/hr), and C_{sat} is the chemical's solubility in water. The model equation for predicting k_p assumes that the rate-limiting barrier to percutaneous absorption is skin's outermost layer, the lipophilic stratum corneum (SC).[9,10] For very hydrophobic compounds, however, the underlying, much more aqueous in nature, viable epidermis contributes significantly to the control of dermal penetration and the correction to k_p (k_p^{corr}) acknowledges this fact.[11]

Table 1: Structure and properties of the five representative pesticides, and ibuprofen.

Pesticide/Drug	Structure	MW	M.Pt. (°C)	log P	Water Solubility (mg mL^{-1})	Predicted J_{max} (µg cm^{-2} hr^{-1})	pK_a
2,4-D		221.1	140	2.81	0.680	5.72	2.73
Acetochlor		269.8	< 0	3.03	0.096	0.59	-
Atrazine		215.7	175	2.61	0.035	0.23	1.70
Chlorpyrifos		350.6	42	4.96	1.1 x 10^{-3}	0.04	-
Monocrotophos		223.2	55	-0.2	1000	62.5	-
Ibuprofen		206.3	75	3.97	0.021	1.09	4.91

EXPERIMENTAL METHODS

SPIN COATING: Spin coating of pesticide solutions was conducted on a variety of platform materials (12 mm diameter discs): glass cover slips (SPM Accessories, TAAB, Aldermaston, UK), acetate transparency film (Impega, Lyreco, Telford, UK), steel atomic force microscopy (AFM) discs (SPM specimen discs, TAAB, Aldermaston, UK) and a selection of polyethyleneterephthalate (PET) (Hostaphan, Mitsubishi, Frankfurt, Germany), which were uncoated, aluminized or siliconized. Pesticide solutions/suspensions (50 mg mL^{-1}) were prepared in acetonitrile and sonicated for 10 minutes to ensure homogeneity. Platforms were cleaned with ethanol prior to spin coating (KW-4A, Chemat Technology, Amersham, UK). The required aliquot of pesticide suspension was applied to the platform material and spun to generate a uniform coverage. The total material on each platform was determined by solvent extraction and subsequent high performance liquid chromatography (HPLC) quantification. Uniformity of the residue coverage was confirmed both by eye and by scanning electron microscopy (SEM). Residue-coated platforms that had visually inferior uniformity were discarded. Trials revealed that platforms could be reproducibly coated with loading levels consistently within ±15% of the target value. To produce a platform loading level of 1000 μg cm^{-2}, an aliquot of 24 μL of pesticide stock solution was pipetted onto the center of the disc, which was subjected to an initial spin speed of 650 - 700 rpm for 18 seconds, followed by 850 - 900 rpm for one minute. Platforms were also prepared with 100 μg cm^{-2} pesticide loadings. This was achieved by application of a diluted quantity of pesticide stock solution (5.3 μL of 50 mg mL^{-1}pesticide mixed with 4.7 μL acetonitrile). For this loading dose, a higher spin speed was required of 1500 rpm for 18 seconds, followed by 2350 rpm for one minute. After spin coating, platforms were left to air dry overnight before use. The pesticide loadings chosen were intended (i) to provide an essentially infinite ‘dose’ of chemical, and (ii) to mimic a ‘worst-case’ exposure scenario.

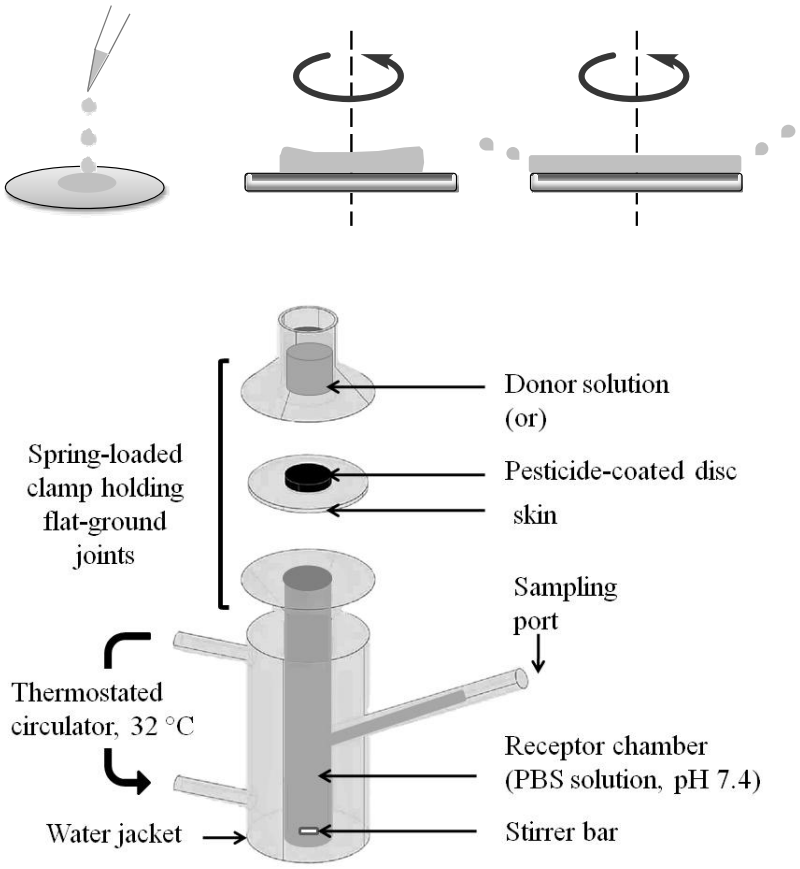


Figure 1: Top: Illustration of the principle of spin coating. Bottom: Franz diffusion cell set-up using either a platform or a donor solution to deliver pesticide.

IN VITRO EXPERIMENTS were performed using porcine abdominal skin, dermatomed (Zimmer, Warsaw, IN, USA) to 750 μm. Porcine skin was used because it is an excellent model for the human counterpart,[6] and (unlike human tissue) readily and economically available. The excised skin was carefully trimmed with scissors to remove hairs prior to cleansing with ultrapure water (NANOpure® Diamond™ Life Science Ultrapure Water System, Barnstead International, Dubuque, Iowa, USA). Experiments were performed in vertical Franz diffusion cells (Permegear, Hellertown, PA, USA) (Figure 1) which were magnetically-stirred and thermostated to 32°C; the receptor chamber was filled with phosphate-buffered saline solution (7.5 mL, pH 7.4). The tissue was clamped between the donor and receptor compartments exposing a diffusion area of 2 cm^2. The pesticide residue-coated platforms were applied against the skin once the cell temperature had fully equilibrated, and were weighted down

lightly with a small glass HPLC vial (approximate combined weight of vial and disc = 3.2 g). Alternatively, for the complementary set of experiments, a 1 mL aqueous pesticide donor solution (2 mg mL^{-1}) was applied to the skin *via* the donor chamber, which was covered with Parafilm®. Donor solutions were prepared by sonicating the pesticide with ultrapure water to produce a uniform suspension. This concentration was larger than the saturation concentration for all but monocrotophos.

At least six replicates were performed for each set of conditions; (three using skin taken from Pig A, three with skin from Pig B); a number previously demonstrated to provide an adequate statistical sample for data interpretation.[12] Transfer of the pesticide from the platform onto and (possibly) through the skin was permitted to proceed for 24 hours. A 750 µL sample of the receptor solution was withdrawn for analysis at 6 hours (replaced with fresh solution), and again at 24 hours. The rationale for the timings of the samples was as follows. Once any pesticide product residue has transferred to the skin, there will be the possibility of absorption until the contact site is cleaned. Even then, chemical which has diffused beyond the surface layers may be difficult or impossible to remove and uptake will continue over a much longer period of time than the original contact. For this reason, it was considered prudent to consider a 'worst-case' scenario and, for this reason, a 24-hour total exposure time was chosen. Sampling at 6 hours offered a small 'window' on the kinetics of penetration to be examined. This sample was filtered (Cronus syringe filter, Nylon 13 mm 0.45 µm, Labhut, Maisemore, Gloucestershire, UK) into HPLC vials for analysis. At 24 hours, the platform/donor solution was removed, the cell was dismantled, and the receptor solution was collected for analysis. The cumulative mass of chemical permeation is reported per exposed area, which was 1.1 cm^2 for platforms and 2 cm^2 for the aqueous solutions. Any visible material remaining on the skin was removed from the surface with a cotton bud. The SC was then removed by adhesive tape stripping.[13] The first tape was not included in the results and served to remove any excess residue remaining on the surface of the skin. Controls were performed on skin from each pig used for each experiment, by either the application of a chemical-free platform, or a chemical-free aqueous donor solution. Analysis was carried out using the same extraction conditions as for the chemical of investigation.

IN VIVO EXPERIMENT: Ibuprofen-coated platforms (steel AFM discs only) were fixed against the skin of a healthy female volunteer (aged 27 years, with no history of skin disease) using adhesive tape (Mefix, Mölnlycke Healthcare, Gothenburg, Sweden) for 24 hours, at which point they were removed, the area cleaned with a cotton bud, and the SC removed by adhesive tape stripping for analysis. The pressure with which the tapes were adhered to the skin matched that used in a previous investigation[13] and was such that complete removal of the SC could be achieved with no more than 20-25 strips. The study was approved by the Bath Research Ethics Committee and written informed consent was obtained from the participant.

TAPE STRIPPING: Squares of 2 x 2 cm adhesive tapes (transparent Scotch No.845 Book Tapes; 3M Media, Broken, Germany) were cut and pre-weighed on a 0.1 μg precision balance (SE2-F; Sartorius). In order to delimit a fixed area for tape stripping, a template was fabricated by self adhesion of two sheets of tape (transparent Scotch No.845 Book Tapes; 3M Media, Broken, Germany) with a 16 mm diameter aperture. For *in vitro* experiments, this was pinned onto the skin and, sequentially, tapes were adhered, pressed firmly onto the delimited area of skin, and then removed in a swift movement (for the *in vivo* experiment, the template was taped onto the skin directly (Mefix, Mölnlycke Healthcare, Gothenburg, Sweden)). After collecting 10 tapes for each sample, the tapes were again weighed, so that the amount of SC removed by each strip could be determined. As has been previously described,[13] given that the density of the SC (ρ) is known, and the stripped area of the skin (A) is well-defined, then knowing the weight of SC removed (m) on each strip allows the corresponding effective thickness of tissue (x) to be determined, i.e., $x = m/(\rho.A)$. It is then possible to display the uptake of chemical into the SC as a profile of concentration against distance, or depth (which is calculated from the cumulative mass of skin removed), into the barrier. The tapes were subsequently individually solvent-extracted (1 mL per tape) on an orbital shaker overnight. The solvent was chosen to ensure 100% recovery of the pesticide/drug with minimal interference of other skin/tape substances which may have interfered with

HPLC analysis. The extraction solution used for each pesticide is shown in Table S1 in the Supplementary Information. The extract was filtered (Cronus syringe filter, Nylon 4mm 0.45 μm, Labhut, Maisemore, Gloucestershire, UK) into HPLC vials for analysis.

HPLC ANALYSES were performed using a Jasco (Tokyo, Japan) system (comprised of a PU-2080 pump, UV-2075 UV detector, AS-2051 autosampler and column oven) controlled with Chromeleon software. Chromatographic separation was achieved using a HiQ Sil C18W column (Kya Tech, Tokyo, Japan) of dimensions 4.6 mm diameter by 250 mm long (300 mm for acetochlor) operated at 25°C and a flow rate of 1 mL min^{-1}. The 250 mm column was used in conjunction with a pre-column guard cartridge (HiChrom, Theale, Berkshire, UK). HPLC methods were developed and validated for pesticide detection and quantification; the details are shown in Table S1 in the Supplementary Information. The methods for ibuprofen and monocrotophos are based on published procedures.[13,14] For tape extractions, all pesticides were quantifiable to at least 0.05 μg mL^{-1}. This was also the case for most receptor solution analyses; however, for chlorpyrifos, receptor solution analysis was less sensitive and the limit of quantification was 0.75 μg mL^{-1}. For ibuprofen, the limit of quantification was 0.5 μg mL^{-1}, for both tape extractions and receptor solutions.

RESULTS AND DISCUSSION

Spin coating was successfully utilized to coat platforms with different loading levels of pesticide. Platforms could be consistently coated with the required loading level of pesticide to within at least ±15%, and usually within ±10%. A number of different platform materials were found to be suitable for this purpose. Visually, the most uniform films were produced on steel discs, acetate and uncoated PET. The PETs which were aluminized and or siliconized were found to produce less uniform films due to 'beading' on their surfaces. Satisfactory films were produced on glass (cover slips); however, their

fragile nature created difficulty in handling. Steel (AFM) discs were eventually chosen for the skin experiments owing to their ease of handling, regular shape and superior residue coverage.

When considering the results presented in this section, it is useful to acknowledge the differences in residue characteristics exhibited by the different chemicals. Atrazine and 2,4-D had formed dry residues by the end of the spin coating procedure. In contrast, acetochlor (a viscous liquid) and monocrotophos (a hygroscopic solid), never formed a solid state, instead remained as sticky residues. Chlorpyrifos and ibuprofen appeared to be 'wet' immediately after spin coating; however these substances eventually solidified over a number of hours. Thus different chemicals gave rise to residues with very different properties. For example, atrazine residues (Figure 2) had the consistency of a fine dry crystalline powder, in contrast to the 'glassier' solid ibuprofen residue.

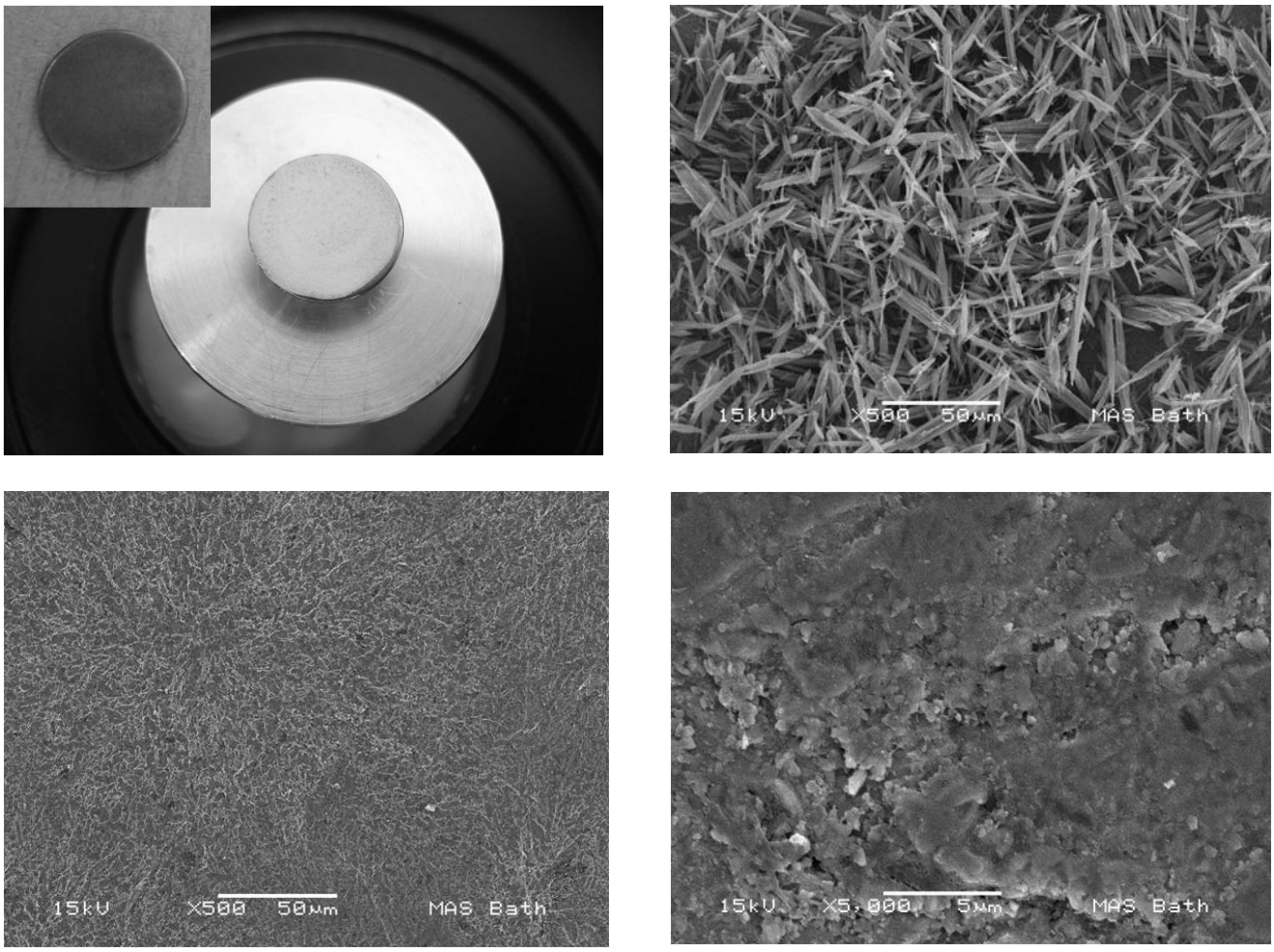


Figure 2: Top left panel: photograph of an atrazine-coated steel disc immediately after spin coating (inset, a blank disc). Remaining panels show SEM images of steel AFM discs coated with 1000 μg cm^{-2}

pesticide: Top right: atrazine (x 500 magnification); Bottom left: 2,4-D (x 500 magnification); Bottom right: chlorpyrifos (x 5000 magnification). SEM images were obtained using a JEOL 6480LV SEM (Japan) and analyzed using INCA software (Oxford Instruments, UK).

For all the experiments described, an equivalent set of control experiments were conducted using clean, chemical-free discs (or for the case of aqueous donor solution, a water donor solution). These control experiments were replicated in the same manner as for the pesticide-loaded case; receptor solution samples were withdrawn and adhesive tape stripping was performed. The tapes were then extracted in the chosen extraction solvent to ensure that no other compounds in the skin or tape could be responsible for any peak observed in HPLC spectra with a similar retention time to the analyte.

Chlorpyrifos was the only pesticide that was not detected in the receptor solution, neither from a platform nor from an aqueous donor solution (however, it should be noted that the limit of detection for this compound was 0.5 $\mu g\ mL^{-1}$, a factor of more than 10 higher than that for the other pesticides and only half of the solubility limit, so it would not be detectable as long as the receptor solution satisfies the requirements for sink conditions). This pesticide has very low solubility, and the maximum flux prediction using the Potts-Guy algorithm[10] multiplied by the solubility concentration was lower than for any other pesticide investigated (by nearly 10-fold). The pesticide was however detectable in each tape extract of the SC (Figure 3).

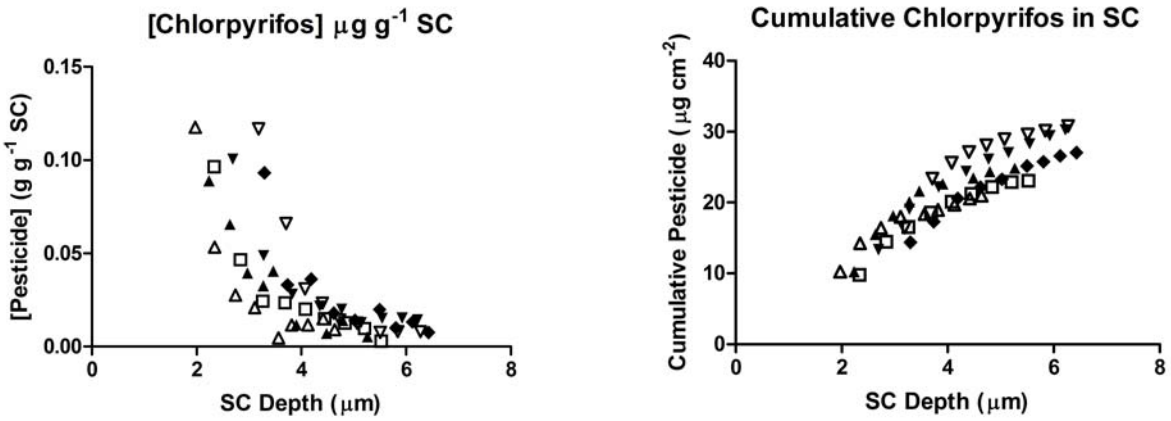


Figure 3: Chlorpyrifos concentrations present in the SC after 24 hours in two different subjects. Each symbol represents data from a different cell. Solid symbols indicate data from pig A, open symbols represent data from pig B.

The skin was also found to be relatively impermeable to atrazine. Receptor solution levels were below the limit of detection when atrazine was transferred from a platform; however, when applied as a solution, the receptor levels reached 4 μg cm^{-2} of application area during the 24 hours. Many studies in the literature are not easily cross-comparable owing to differences such as the use of solvents, penetration enhancers or skin from different species. However, a study by Moody on human skin reports very low permeation (0.9%) of atrazine from a saturated aqueous donor solution, which we calculate to be within approximately a factor of 5 of our experimental J_{max}.[15]

2,4-D was able to penetrate the skin of pig A but not pig B. There were no detectable levels of 2,4-D present in any of the latter receptor solutions after 24 hours, whereas for the former, the cumulative mass transfer was 2.15 μg cm^{-2} application area over 24 hours (a sample at 6 hours was below the limit of detection). These observations are in accordance with the findings of tape stripping analysis. When the data were separated by pig, it was found that across the skin of pig B, 2,4-D did not penetrate further than ~3 μm into the SC, whereas for pig A, there were detectable levels of 2,4-D in every tape throughout the SC. Experiments were also conducted at the 100 μg cm^{-2} loading level, and receptor solution concentrations were found to be even higher than for the 1000 μg cm^{-2} experiment. However,

these experiments were conducted on skin from a third pig, implying that permeability to 2,4-D is particularly dependent on the skin source used. A similar observation has been reported before: 2,4-D permeation was 2.5 times higher in a reconstructed skin equivalent compared to pig skin, yet for the three other compounds tested, there was no notable permeation difference between the two models.[16] It is possible that this observed variability in permeation is related to the low pK_a value for 2,4-D, a weak acid, which would suggest its nearly complete ionization at skin pH. Small variations in pH could therefore give rise to substantial differences in permeation.

When the platform was substituted by an aqueous donor solution of 2,4-D, a considerably larger amount of this chemical was able to permeate the skin into the receptor phase in 24 hours: 84 ± 38 μg cm^{-2}. Note that these experiments were conducted on pig B, for which no 2,4-D was found to penetrate the skin in the platform residue experiments described above.

Acetochlor is the only liquid among the five pesticides investigated. The quantity reaching the receptor solution after 24 hours was 59 ± 24 μg cm^{-2} when applied as a residue *via* a platform, and 44 ± 5 μg cm^{-2} when applied as an aqueous donor solution. These values are not statistically significantly different (t-test, 95% confidence interval). No significant difference in permeability was found between pigs A and B. Because this pesticide is a liquid at ambient temperature, it is not surprising that delivery *via* a platform resulted in a similar flux since the thermodynamic activity of the chemical in the two cases is about the same. The platform experiment was also performed with a pesticide loading of 100 μg cm^{-2}; in this case, the amount recovered in the receptor solution (after 24 hours) was 35 μg cm^{-2} which was somewhat lower than that observed at the 1000 μg cm^{-2} loading level. This is consistent with there having been a significant depletion of the applied chemical (approximately 15-20%).

Monocrotophos was purchased as a crystalline solid; however the residue generated by spin coating failed to solidify owing to high hygroscopicity. The penetration of this pesticide into the receptor solution over 24 hours was 61 ± 38 μg cm^{-2} for delivery *via* a platform, yet only 2.2 ± 1.5 μg cm^{-2} when applied as an aqueous donor solution. This is consistent with the nature of the residue, which was essentially a highly concentrated version of the aqueous donor solution. For monocrotophos, the

aqueous donor solution was a fraction of the concentration required for saturation, since this pesticide is extremely soluble in water. In contrast, the other chemicals investigated in this study have poor solubilities in water and were therefore saturated at the 1000 μg mL^{-1} aqueous donor solution loading level.

When applied as a solid residue, ibuprofen transport was extremely low. It had only reached detectable levels (mean of 1.65 μg cm^{-2}) in two of the six receptor solution samples by 24 hours. However, when ibuprofen was applied to the skin as an aqueous donor solution, a comparatively high level of penetration over 24 hours (118 ± 15 μg cm^{-2}) was recorded.

Experimental data recorded for the five pesticides and ibuprofen at 1000 μg cm^{-2} applied both as an aqueous donor solution and as a residue-coated platform are summarized in Figure 4, and receptor solution values at both 6 and 24 hours are tabulated in the Supplementary Information, S2. The left panel in Figure 4 shows the total quantity of substance in the receptor solution after 24 hours, and the right panel shows the total quantity of pesticide/drug present in the SC as determined by tape stripping at 24 hours (these values are tabulated in the Supplementary Information, S3).

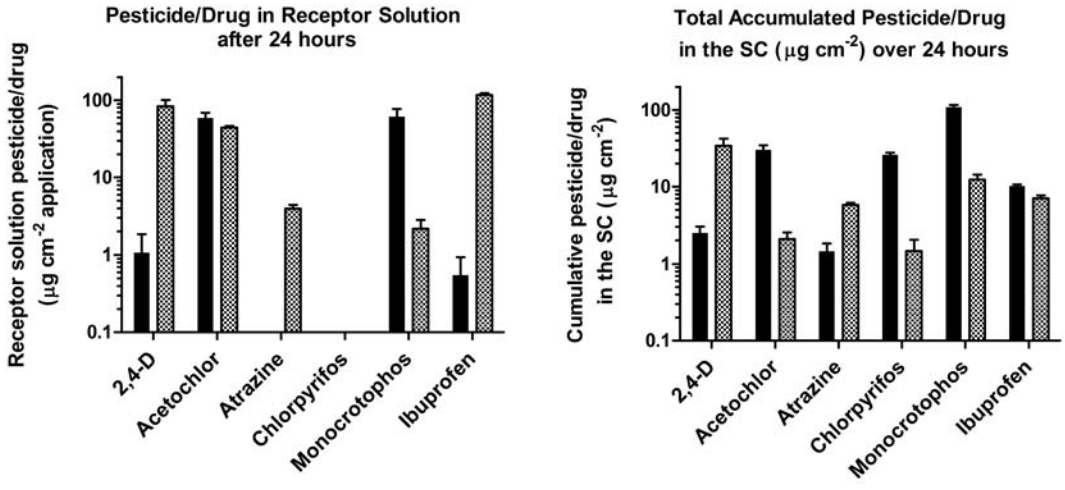


Figure 4: Average pesticide/drug levels present in the Franz cell receptor solutions and in the SC (from tape stripping) after 24 hours. Solid black bars represent residue application *via* a 'platform'; hashed bars indicate application of an aqueous donor solution (error bars depict +1 standard deviation).

As illustrated in Figure 4, there was a large disparity in skin permeation of chemicals between the residue-coated platform and the aqueous donor solution. It was found that substances which formed solid residues had very low permeation compared to their aqueous solutions. However, acetochlor (a liquid) and monocrotophos (highly hygroscopic) did not form solid residues, instead remaining 'liquidy' on the surface of the platform, and penetrated the skin significantly more readily than their aqueous donor solutions. Overall, the behaviour observed is quite consistent with the inverse correlation well-known in the skin penetration literature between melting point and transport (i.e., compounds with lower melting points permeate the skin more rapidly).

Table 2: Comparison of predicted and experimental maximal fluxes (J_{max}) following topical application of chemicals in aqueous solution.

Pesticide/Drug	Predicted J_{max} ($\mu g\ cm^{-2}\ hr^{-1}$)	Experimental J_{max}[a] ($\mu g\ cm^{-2}\ hr^{-1}$)	Ratio (Experiment/Prediction)
2,4-D	5.72	3.48	0.61
Acetochlor	0.59	1.85	3.14
Atrazine	0.23	0.16	0.70
Chlorpyrifos	0.04	0 (undetected)	0
Monocrotophos	62.5	0.09[b]	0.001
		45[c]	0.72
Ibuprofen	1.09	4.90	4.49

[a]Value determined from the cumulative amount of chemical penetrating 1 cm^2 of skin in 24 hours (but not including that recovered from the SC).
[b]From a solution at 2 mg mL^{-1}.
[c]Scaled using experimental solubility value.

Table 2 compares the originally predicted J_{max} values (given in Table 1) with those obtained experimentally from the aqueous donor solutions. The latter were approximated from the receptor

solution content (per cm^2) of the chemical after 24 hours of skin contact, and provide a value with which an estimate of exposure can be made for any skin contact area, and of any duration. This approach does not, however, take into account chemical which has entered, but not yet exited from the skin (as measured in the present study in the tape-strips) and might, therefore, underestimate potential risk in chronic exposure situations. Good correlation with the predicted J_{max} results is observed and, for the most part, the ratio of experimental to predicted fluxes falls within an order of magnitude of 1. It was not possible to confirm whether chlorpyrifos (the most lipophilic and least water-soluble chemical studied) was predicted to within a factor of 10, because the amount which had reached the receptor solution after 24 hours was below the limit of detection. Even had this molecule diffused at its predicted J_{max}, this would have resulted in only about 2 μg arriving in the receptor solution in 24 hours. Given that the volume of the receiver phase was 7.5 mL, the maximum concentration which the pesticide may have reached would have been less than 0.3 μg mL^{-1}, i.e., less than its limit of detection by the analytical method employed. The experimentally determined aqueous solubility of monocrotophos (1000 mg mL^{-1}) is 500-fold greater than that which was applied in the *in vitro* diffusion cell study (i.e., 2 mg mL^{-1}). Unlike all the other compounds considered, therefore, which were administered as aqueous suspensions, monocrotophos was delivered to the skin as a weak dilution compared to its solubility; that is, at much lower thermodynamic activity. The measured flux must therefore be multiplied by a factor of 500 (=1000/2) to be compared to J_{max} and this results in an acceptable agreement between the two values.

In addition to the results presented in Figure 4, the study was extended to incorporate an *in vivo* experiment involving ibuprofen residue-coated platforms. The drug-coated platform was applied to the ventral forearm of a healthy subject for the same 24 hour duration. During this period no washing, or other activity was undertaken which might have affected the results. At the end of the experiment, the SC was removed by adhesive tape stripping. The tapes were extracted as described previously for the *in vitro* study. Ibuprofen was found to be below the limit of detection within the SC, and subsequent extraction of the platform confirmed that no significant loss of drug from the platform had occurred.

This result is particularly illustrative, since ibuprofen is a widely used drug approved for topical administration: in an aqueous form it readily permeated the skin, but when the same dose was applied as a dry residue *via* a platform *in vitro*, the amount of ibuprofen detected in the receptor solution was considerably lower, by a factor of approximately 100.

It should be noted that, while the experiments presented in this study do not consider the situation where the exposed individual might be perspiring, or the surface on which the residue of the pesticide product remains is wet, or the humidity is high enough to cause a potential hydration layer between the skin and the pesticide residue, there is no doubt that coverage of the skin with the platform discs employed (as well as contact with the aqueous receptor phase below and the high humidity in donor chamber) ensure that the SC is fully hydrated. Further work might employ artificial sweat on the surface of the skin to confirm the relevance of this scenario. For example, fruit harvesting often takes place during early morning when plants are wet, which would increase dermal exposure.[17-19] An additional experimental refinement would include evaluation of smaller, 'finite' doses of chemical, to more closely approach likely, real-world exposures. Such a study would also allow a proper mass balance of the pesticide considered to be undertaken (impossible in the current investigation because of the very low percentage of the applied material which enters and penetrates the skin).

Taken together, these findings confirm and address the need for a more reliable method for assessing health risks associated with dermal re-entry exposure. Further, as commercially available pesticide formulations often contain many additives to improve function (such as surfactants) which would affect the properties of residues, and given that we have demonstrated that the physical properties of the residue formed are central to its ability to transfer from the delivery platform onto and through the skin, it follows that a more accurate assessment should involve coating platforms with the commercially available formulations.

Supporting Information: HPLC methods and *in vitro* skin permeation data. This material is available free of charge via the Internet at http://pubs.acs.org.

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SYNOPSIS TOC: