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An *in vitro* method to quantify dermal absorption of pesticide residues.

James F. Clarke¹, Sarah F. Cordery¹, Neil A. Morgan², Peter K. Knowles² and Richard H. Guy¹

¹University of Bath, Department of Pharmacy & Pharmacology, Bath, BA2 7AY, U.K.

²Syngenta, Jealott's Hill International Research Centre, Bracknell, RG42 6EY, U.K.

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ABSTRACT: All pesticides must go through a rigorous risk assessment process in order to show that they are safe for use. With respect to dermal risk assessment for re-entry workers, the absorption value applied to predict systemic dose from this external exposure is obtained by testing liquid forms of the pesticide *in vivo* and/or *in vitro*. However, in a real exposure scenario, the worker would be exposed to a dried residue, for which little or no absorption data are available. This study has developed a novel methodology for assessing the dermal absorption of pesticides from dried residues, and aims ultimately to use this methodology to obtain more realistic absorption values for the risk assessment.

A principal function of the skin is to act as a barrier, both to the loss of endogenous water and to absorption of exogenous compounds¹. The skin comprises two major components: the innermost dermis and the superficial epidermis. Barrier function resides in the outer layer of the epidermis, the stratum corneum (SC), the thickness of which is typically on the order of 20 µm.

When a crop is treated with pesticide, a residue is left behind on surfaces such as leaves. An individual who subsequently enters the area may then be exposed to these residues via contact with their skin; this is most common with 're-entry workers' who may enter the treated area after application of the pesticide formulation to carry out tasks such as crop inspection or manual harvest².

A risk assessment must be carried out for these re-entry workers and their Potential Dermal Exposure (PDE, µg/day) calculated²:

$$\text{PDE} = \text{DFR} \times \text{TC} \times \text{T}$$

where DFR is the Dislodgeable Foliar Residue (µg/cm²), the quantity of substance remaining on the surface of the leaf that can be dislodged and transferred to skin; TC is the Transfer Coefficient (cm²/h), which is specific to the particular re-entry task and refers only to the amount of contact between skin and the contaminated surface; T (h/day) is the Exposure Time, typically 2 hours for crop inspection and 8 hours for harvest.

Once the potential exposure has been calculated, the percentage of the applied 'dose' that becomes available systemically is estimated. *In vitro* skin absorption studies are carried out for most pesticides, determining the compound's uptake from a finite dose of both the concentrate

and from a relevant in-use spray dilution (described below). The higher of the calculated percentage absorption values (generally the most dilute solution) is then used to represent a worst-case scenario for the re-entry worker. To pass risk assessment, this value must be below the maximum acceptable value identified for the compound during toxicology testing³.

In a real exposure scenario, the re-entry worker would most likely come into contact with a dried residue rather than a liquid form of the product. Unfortunately, no acceptable methodology exists for the acquisition of absorption data from such residues. It is likely that the use of data from liquid applications represents an overestimate and that the dried residues left on plant surfaces, to which workers are exposed, would not be absorbed to the same extent. This may lead to the pesticide failing the risk assessment process meaning that safe and effective products may not be approved for use. Previous work⁴ has shown that pesticide absorption from a residue, when applied in the form of a coated disk pressed against the skin, was different to that of an aqueous solution. However, this occlusive and long-term exposure was not fully representative of a re-entry worker scenario, where only a brief contact between skin and foliage would occur. Furthermore, the 'doses' used (100-1000 µg/cm² of pesticide) were an order of magnitude higher than would occur in a re-entry exposure scenario and were delivered as neat active ingredients either in solution or as a suspension (as opposed to a commercially relevant formulation)

The aim of this study, therefore, is to develop a robust methodology for assessing the dermal absorption of pesti-

cides from dried foliar residues that addresses these limitations and is more relevant to the re-entry scenario. The ultimate aim is to use this approach to obtain more realistic absorption values for risk assessment. It is important that this method is as close to a real exposure scenario as possible and is easily reproducible.

A standard *in vitro* protocol was used to measure pesticide dermal absorption^{5,6}. Experiments (n = 4-5) were performed in static Franz diffusion cells (PermeGear, Hellertown, USA), with dermatomed porcine skin (diffusion area = 2 cm²) maintained at 32°C. The receptor chamber contained 7.4 ml of a 6% (w/v) solution of polyoxyethylene glycol (10) oleyl ether (Sigma, UK) in phosphate-buffered saline at pH 7.4. The pesticide Trinexapac-ethyl (TXP, Syngenta plc, Jealott's Hill, U.K.) was applied to the skin as an emulsifiable concentrate (10% w/w) diluted 100-fold in water, or as a dried residue (see below).

In the case of the liquid formulation, 20 µl (1 µg/µl) were applied directly and evenly to the skin surface. For the residue, 40 µl of the diluted concentrate were first applied to a 12 mm diameter steel disc (SPM specimen discs, TAAB Laboratories Equipment Ltd., Aldermaston, U.K.) and allowed to dry for 24 hours to a dried residue. The disc was then attached to a weighted vial (~10 g) that was rotated on the skin surface. The procedure involved three complete rotations in both the clockwise and anticlockwise directions, followed by moving the disc laterally in a "+" configuration (see Supporting Information 1). After application, non-transferred residue remaining on the disk was extracted and quantified to confirm the amount actually transferred to skin, specifically 21.7 ± 3.3 µg, (mean ± S.D.; i.e., ~54% of that applied to the disk) with the aim being to match the 20 µg application from the liquid. Post-application of the formulations, the receptor solution was sampled at 2, 4, 6 and 8 hours. The skin surface was washed at 8 hours (to represent a typical working day) with 100 µl of a mild (0.1% w/v) soap solution and dried with two cotton buds. An additional receptor solution sample was taken at 24 hours, after which the stratum corneum was sequentially removed by adhesive tape stripping⁷. The first two tape-strips were not discarded and the chemical thereon was quantified; however, the quantities found were not included in the total absorption calculations as this material is generally not assumed to be bioavailable³.

The skin uptake and absorption of TXP (Table 1; Figure 1) was determined following HPLC analysis (see Supporting Information 2) of the receptor solution samples, the SC tape-strips, the washing solution, viable tissue and cotton buds. The pesticide was efficiently extracted from the SC using 60:40, acetonitrile: water.

Total TXP absorption (i.e., quantity of pesticide in tape strips 3-15 + skin extraction + quantity permeated to receptor) was significantly lower for the residue than for the liquid; permeation of pesticide into the receptor solution continued after skin was washed. It is noteworthy that, while significantly more TXP permeated into the receptor phase

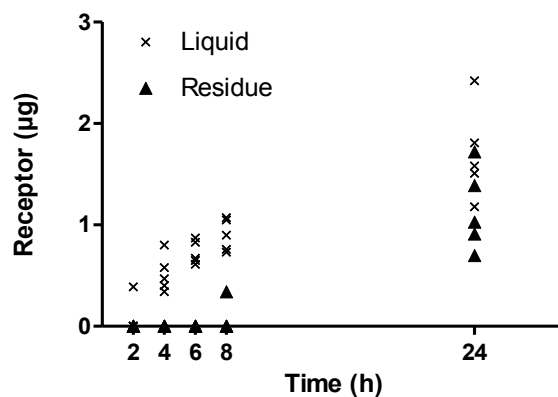
in 8 hours following liquid application, there was no significant difference after 24 hours between the liquid and dried residue exposures.

Table 1: Skin uptake of TXP (mean±SD); surface cleaned at 8 hr.

	Liquid (µg)	Residue (µg)	p-value
Receptor 2 hr	0.08±0.17	0.00±0.00	0.35
Receptor 4 hr	0.52±0.18	0.00±0.00	< 0.01
Receptor 6 hr	0.72±0.12	0.00±0.00	< 0.01
Receptor 8 hr	0.90±0.16	0.07±0.15	< 0.01
Receptor 24 hr	1.70±0.46	1.15±0.40	0.08
Tapes 1 & 2	0.86±0.45	0.56±0.16	0.19
Tapes 3-15	0.33±0.11	0.23±0.12	0.29
Surface wash	11.0±1.78	12.2±2.67	0.42
Skin	1.08±0.66	0.30±0.10	0.03
Total absorbed	3.11±0.86	1.68±0.56	0.015
% 'dose' absorbed	15.54±4.36	7.63±1.65*	< 0.01

* Expressed as a percentage of the estimated 'dose' applied for each replicate.

Figure 1: Permeation of TXP into receptor (µg) as a function of time. Skin surface cleaned at 8 hr.



It was unclear why pesticide absorption from the residue began slowly but then appeared to 'catch up' with that from the liquid between 8 and 24 hours. To investigate the possibility that the washing procedure somehow aided pesticide absorption from the residue, further experiments were conducted with the wash procedure carried out at 24 hours (i.e., at the termination of the entire experiment) instead of at 8 hours⁸.

Table 2 compares the results from the liquid and residue applications for the 24 hour surface wash. At 4 and 6 hours, as before, more pesticide had penetrated to the receptor phase from the liquid application. Notably, at 8 and 24 hours, there was no significant difference between the amounts of TXP that had reached the receptor from the

liquid and residue applications. At the 8 hour time point, the protocol in this experiment is identical to that described above and all of the 8 hour receptor solution quantities, from the two experiments, were therefore analysed together. The amount that had penetrated the skin from the liquid was found to be significantly higher ($p < 0.001$).

For the residue, total absorption was significantly higher when washing was performed at 24 hours instead of 8 hours. Therefore, there is no evidence of a possible “wash-in” effect. Intriguingly, at 24 hours, there is no significant difference between total absorption from the liquid and that from the residue. This may be due to (some) TXP residue dissolving into skin surface moisture (TXP is relatively water-soluble, 10 mg/ml). Alternatively, when applied as a solution, evaporation may transform the vehicle into a residue. As a result, as time progresses, the uptake/penetration of TXP from the dilute formulation and the dried residue are similar.

Table 2: Skin uptake of TXP (mean±SD); surface cleaned at 24 hr.

	Liquid (µg)	Residue (µg)	p-value
Receptor 2 hr	0.12±0.17	0.00±0.00	0.20
Receptor 4 hr	0.55±0.19	0.00±0.00	< 0.01
Receptor 6 hr	0.80±0.30	0.06±0.11	< 0.01
Receptor 8 hr	0.91±0.33	0.51±0.17	0.06
Receptor 24 hr	2.21±0.61	1.95±0.46	0.51
Tapes 1 & 2	0.68±0.16	0.76±0.19	0.54
Tapes 3-15	0.36±0.10	0.36±0.10	0.74
Surface wash	7.22±2.14	8.62±1.19	0.28
Skin	0.89±0.12	0.77±0.18	0.24
Total absorbed	3.45±0.57	3.10±0.70	0.41
% ‘dose’ absorbed	17.27±2.87	15.05±2.24*	0.22

* Expressed as a percentage of the estimated ‘dose’ applied for each replicate.

In summary, a novel, *in vitro* method has been developed with which to measure dermal exposure from dried pesticide residues under relevant ‘in-use’ conditions. Further work is required to fully validate the approach for a range of typical pesticide formulations and for a range of ‘actives’ of diverse physicochemical properties. It should also be emphasized that, for regulatory purposes, mass balance would be an essential requirement; for example, full washings of the diffusion cell would be required. In this regard, it is noted that the EFSA ‘guidance on dermal absorption’³ recommends, when low chemical recovery is observed, that uptake data should be normalised and ‘expressed as a percentage of the total amount recovered’ for each replicate. When this procedure is carried out with the data in

tables 1 and 2, and the normalised % absorption values statistically analysed, the results point to exactly the same conclusions as described above. Additionally, the impact of environmental moisture and concomitant skin hydration upon the degree of exposure would need to be assessed.

AUTHOR INFORMATION

Corresponding author

James F. Clarke – j.f.clarke@bath.ac.uk

Author contributions

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ABBREVIATIONS

SC, stratum corneum; DFR, Dislodgeable Foliar Residue; TC, Transfer Coefficient; T, Exposure Time; TXP, Trinexapac-ethyl; HPLC, high-performance liquid chromatography; EFSA, European Food Safety Authority.

Supporting Information

1. HPLC method for TXP analysis. 2. Video demonstrating method used to transfer pesticide to skin. This material is available free of charge via the internet at <http://pubs.acs.org>.

REFERENCES

- (1) WHO. (2006) Dermal Absorption, In *Environ. Health Criter.* World Health Organisation, Geneva.
- (2) EFSA. (2011) Scientific Opinion on the Science behind the Revision of the Guidance Document on Dermal Absorption. *EFSA J.* 9.
- (3) EFSA. (2012) Guidance on Dermal Absorption. *EFSA J.* 10.
- (4) Belsey, N. A., Cordery, S. F., Bunge, A. L., and Guy, R. H. (2011) Assessment of dermal exposure to pesticide residues during re-entry. *Environ. Sci. Technol.* 45, 4609-4615.
- (5) OECD. (2004) Guidance Document For the Conduct of Skin Absorption Studies. *OECD Series on Testing and Assessment, No 28*, 1-31.
- (6) OECD. (2004) *Test No. 428: Skin Absorption: In Vitro Method.* OECD Publishing.
- (7) Herkenne, C., Naik, A., Kalia, Y. N., Hadgraft, J., and Guy, R. H. (2007) Dermatopharmacokinetic prediction of topical drug bioavailability in vivo. *J. Invest. Dermatol.* 127, 887-894.
- (8) Moody, R. P., and Maibach, H. I. (2006) Skin decontamination: Importance of the wash-in effect. *Food Chem. Toxicol.* 44, 1783-1788.

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