Regulatory Toxicology and Pharmacology 106 (2019) 55–67

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Assessing in vitro dermal absorption of dry residues of agrochemical sprays using human skin within OECD TG 428

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ARTICLE INFO

Keywords:
Dermal absorption
In vitro
Human skin
Plant protection products
Agrochemicals
Pesticides
Re-entry worker risk assessment
Dislodgeable foliar residues
DFR
Dermal exposure

ABSTRACT

We describe a novel experimental method that mimics exposure to dried agrochemical residues on contact surfaces during re-entry into crops. It includes the creation of dry dislodgeable residues and subsequent transfer to human skin for *in vitro* measurement of dermal absorption within a standard Organisation for Economic Co-operation and Development test guideline (OECD TG) 428 study. A pre-determined volume of spray containing ^{14}C-labelled active substance is transferred onto a polytetrafluorethylene-coated septum and air-dried. The septum is then gently placed onto the pre-wetted skin mounted in a flow-through Franz diffusion chamber. The septum is gently rotated thrice to transfer the dose. Preliminary tests determined transfer efficiency to ensure the appropriate test concentration on the skin. Then, a standard dermal absorption study is performed according to OECD TG 428. Results from 10 compounds indicate that the methodology can be robustly incorporated into a standard TG study. These data show that the dermal absorption from a dry dislodgeable residue is lower than that from the equivalent dose of the aqueous spray, regardless of formulation type or active substance. Studies following the scenario described above can be a suitable tool to better estimate dermal absorption from dry residues in re-entry worker and resident exposure assessment for agrochemicals.

1. Introduction

Today, dermal absorption of chemicals is predominantly estimated with *in vitro* dermal penetration studies using human skin, i.e., according to OECD TG 428. For agrochemicals, the active substance is tested within a representative formulation and data of hundreds of such studies have been published (Aggarwal et al., 2014; Aggarwal et al., 2015; EFSA, 2011; EFSA, 2017a). The operator ("user") of agrochemicals can be exposed to a concentrated product (e.g., during mixing and loading activities) or its spray dilution (e.g., while spraying in a field), which is reflected in the standard dermal absorption testing regime. However, exposure can also occur during re-entry scenarios when the agrochemical will be present as a dried spray residue on contact surfaces, which is not addressed in the available testing procedures.

In the current study, a novel methodological adaptation was developed to create ^{14}C-labelled dry dislodgeable residues and their subsequent transfer onto human skin samples *in vitro*. Thereafter, dermal absorption can be assessed with the standard OECD TG 428 (2004) methodology. The dermal absorption from dry dislodgeable residues of 10 example compounds were compared with the already available dermal absorption values from an equivalent skin dose applying spray and a concentrate for the selected compounds.

1.1. Dermal exposure scenario for agricultural workers

Within the context for European plant protection products, Regulation (EC) No. 1107/2009 (European Commission, 2009) defines five distinct exposure groups: consumers, operators (users), bystanders, residents and workers. Dermal absorption drives the exposure of the latter four. Operators can be dermally exposed to the concentrated

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https://doi.org/10.1016/j.yrtph.2019.04.016
Received 21 December 2018; Received in revised form 19 April 2019; Accepted 21 April 2019
Available online 25 April 2019
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product, for example during mixing and loading, or to the spray dilution during application in the field. Exposure patterns have notably changed: with today's modern application equipment and operator training, exposure in general has been reduced, and direct contact with the spray dilution during spraying plays a smaller role than before. These traditional risk assessment scenarios have been refined for many years by investigating the dermal absorption of the concentrate and the spray dilution.

The exposure scenario for re-entry workers is noticeably different: workers could -in the context of the regulation- enter areas of treated crops once the spray application has dried. Good agricultural practice (GAP or label document) dictates that the worker should not re-enter the crop until the spray is completely dry. Thus, the deposits to which they are exposed are likely to be significantly different to the physical form tested in the dermal absorption study (*i.e.* a liquid); the time point of worker entry can, mainly depending on the activities necessary within the respective crops, be between the day after spraying and months later (at harvest).

Exposure for workers, according to the European Food Safety Authority (EFSA) guidance on non-dietary exposure assessment (2014), primarily depends on dermal contact with dried residues on the treated crops.

While bystanders or residents near to sprayed areas can be accidently exposed *via* drift caused by the prevailing wind, they can also be exposed to residues when entering the treated field, or unintentionally contaminated surfaces such as private lawns.

1.2. Dermal absorption for agricultural worker risk assessment

Most of the regulatory human risk assessment models for agrochemicals require dermal absorption input values in percentage (of the applied dose from the study). Typically, dermal absorption measured from liquid spray dilutions is being used for estimating potential exposure for re-entry workers, as generically per cent dermal absorption from spray dilution is higher than that from concentrate. Hence, there is an obvious contradiction of study conduct and the use of the dermal absorption estimate in the realistic re-entry worker exposure scenario, i.e. the worker who is primarily exposed to dry residues on the treated surface.

There have been several arguments made regarding the applicability of dermal absorption data for the spray (or concentrate) to the re-entry worker exposure assessment. On one hand, it seems logical that when the spray dries, the evaporation of water and other diluents removes a vehicle that could potentially mediate penetration through the skin. If this is the key factor, then the dermal absorption for the concentrated product would appear to be more appropriate. On the other hand, it has often been observed that lowering the mass per unit area of an active substance on the skin will increase the proportion i.e. percent absorbed (Aggarwal et al., 2014, 2015). As the skin loading from exposure to dry residues is low compared to that resulting from exposure to the concentrate, this might suggest that the value for the spray dilution could be appropriate (obviously in absence of data from a dried spray). In fact, prior to the data reported in this paper, there were very few published data specifically relating to dermal absorption of dried spray residues, particularly for commercially formulated pesticides. Exploratory work by Belsey et al. (2011) indicated that absorption of pesticide technical material and its aqueous dilutions is different to that of residues formed on treated surfaces and that the physical attributes of the residue may be a determining factor.

Therefore, there is a need for increased realism and a requirement for a robust methodology to measure dermal absorption from dried residues, as also evidenced by other recently published work (Clarke et al., 2015, 2018). We describe an applicable and apply-able method, and directly compare dermal absorption of concentrates and sprays *vs* respective dry residues from commercially available pesticide formulations. Dermal absorption was assessed *in vitro* according to the internationally accepted OECD TG 428 in human skin. The only parameters that were changed and systematically investigated are the preparation and transfer of dried residues of the sprays onto the skin instead of a liquid matrix. This method supplement is proposed to be adopted as a refinement to the currently practiced methodology for pesticidal products for the generation of more realistic dermal absorption values for the risk assessments for re-entry workers, bystanders and residents from dried residues.

2. Materials and methods

From the existing database (Aggarwal et al., 2015), ten compounds were selected where the concentrate and at least one spray dilution had already been tested.

In brief, ^{14}C-labelled active substance contained within a spray dilution of the pesticide formulation was applied onto a polytetrafluorethylene-coated (PTFE) septum and air-dried. The septum was then applied onto the skin surface in the Franz diffusion cell; gently rotated to transfer the residues. To investigate differences in absorption from spray dilution *vs* dried residue, the amount (i.e. μg/cm^2) of the dried residue on the skin selected in this study corresponded to the amount μg a.i./cm^2 of the spray dilution tested previously. The methodological steps amended to the standard procedure of dermal absorption testing through human skin *in vitro* are illustrated in Fig. 1. After dose application, the dermal absorption study was performed according to the OECD TG 428 (OECD, 2004) and key elements of the study conduct were described in Sullivan et al. (2017).

2.1. Formulations and chemicals

An overview of the tested compounds, agrochemicals, their respective formulations, used concentrations and their physico-chemical properties are presented in Table 1. Blank formulations for preparation of the spray dilutions and corresponding dry residues were provided by Dow AgroSciences, USA and ADAMA, Israel. All other chemicals used were obtained from commercial suppliers. The chemicals and products were selected to cover different classes of plant protection products (i.e. fungi-, insecti- and herbicides) and different formulation types (i.e. EC, EW, SC, WG, SL and SE).

2.2. Radiolabels

Radiolabelled (^{14}C) Myclobutanil, Fenbuconazole, Metazachlor, Triclopyr 2-BEE and Propyzamide were obtained from Dow AgroSciences, USA; Ethofumesate, Tebuconazole, Folpet and Propiconazole from Izotop, Hungary; and Acetamiprid from Moravek Biochemicals, Inc, USA. The radiochemical purity of all radiolabelled compounds was > 95% as verified shortly before study start by using radio-High Performance Liquid Chromatography (HPLC).

2.3. Transfer device

A polytetrafluorethylene (PTFE) septum (Supelco® 8 mm Ø, double-faced PTFE coated silicone, Sigma Aldrich, product no. 27096-U) was used as a transfer device. To optimize handling, a bended disposable plastic rod (coffee stirrer) was glued opposite to the 'transfer' side with cyanoacrylate glue. To ensure adequate fixing, the respective side was pre-treated with a primer and left overnight. After fixing, devices were checked for adequate adherence of the septum to the plastic rod and the 'transfer side' was carefully cleaned with ethanol.

The use of double-sided PTFE septa was based on pre-tests with a set of different transfer septa, i.e. single-faced PTFE, stainless steel and titanium, which did not show the intended transfer efficiency (details of the pre-test are given in the Results section).

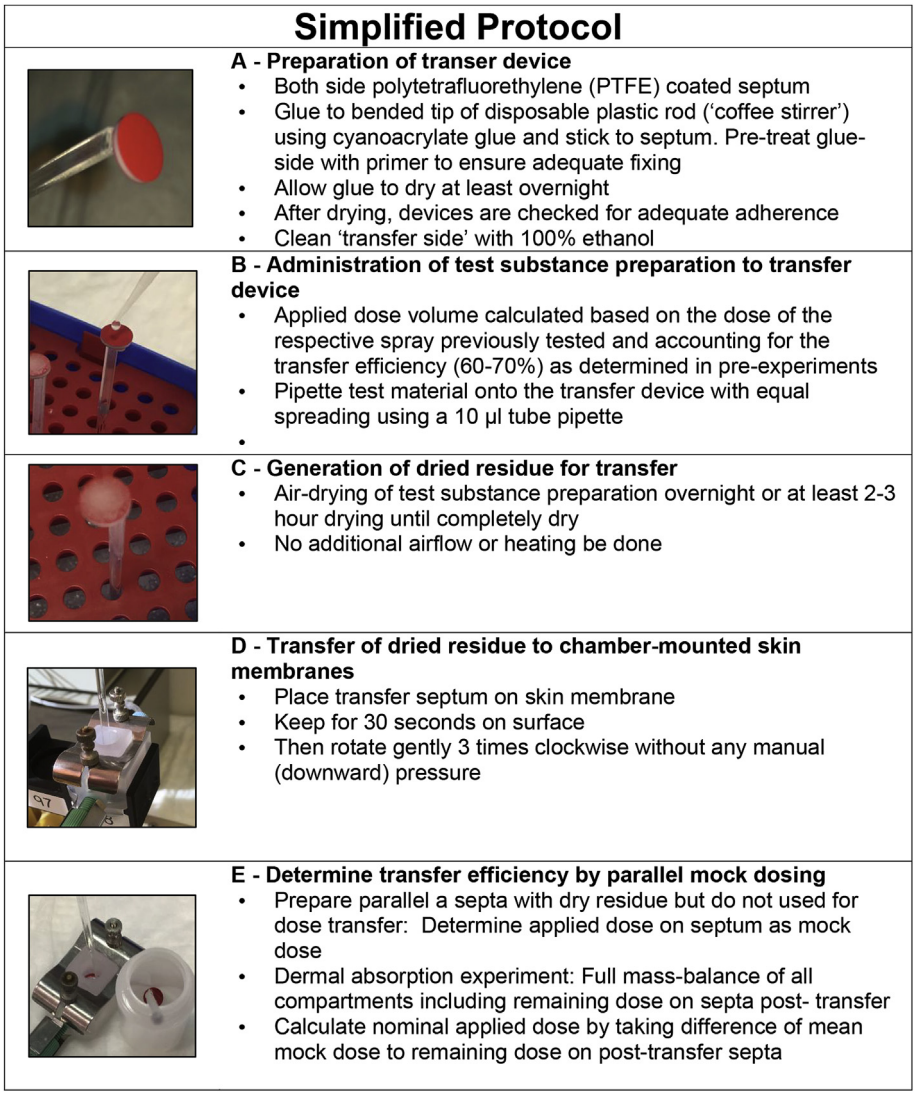

Fig. 1. Methodological steps to create and transfer dry residue of the spray dilution within the protocol of dermal absorption testing *in vitro* according to OECD TG 428.

2.4. Dose preparation

Radiolabelled test substances were used to prepare the respective spray dilutions. According to the intended concentration and specific activity ^{14}C-isotope was added to the diluted blank formulations. As an example, the dose formulation was prepared by serial dilution. First, a 30-times diluted blank formulation was prepared by mixing 0.5 mL semi-blank formulation with 14.5 mL demineralised water. Then 1 mL of the 30-times diluted blank formulation was mixed with 9 mL demineralised water for a 300-times diluted blank formulation.

The required amount of [^{14}C]-radiolabelled test item in organic solvent (*e.g.* acetonitrile) was transferred to a brown glass vial. The solvent was evaporated under nitrogen gas until near dryness. Then 80 µL of the 30-times diluted semi-blank formulation was added and stirred overnight. On the next day, 720 µL demineralised water was added to the dilution. After measuring the radioactive concentration, 300-times diluted semi-blank formulation was added to reach the target concentration.

Where needed, an ultra-sonication was used to obtain homogeneity of the formulation. Alternatively, part of the water volume in the dilution was replaced with methanol which was then evaporated upon air-drying to obtain the dry residue (compare discussion of the results for Metazachlor).

The concentration and homogeneity of the test preparations was checked in terms of radioactivity content by taking random aliquots in triplicate prior to application to the skin (10 µL/cm^2) or transferring a defined volume to the PTFE septum. For homogeneity, a coefficient of variation (i.e. ratio of standard deviation to mean) lower than 10% was considered sufficient. Preliminary tests showed that the transfer efficiency for transfer of the dried residues to the skin surface was *ca.* 60–70%. Therefore, the volume applied to the PTFE septum was appropriately increased to achieve the same applied concentration as was used for the spray dilution test.

[However, authors recommend to perform a transfer efficiency experiment for each formulation and adjust the transfer volume accordingly.]

The dried residue dose in this experiment was set to match with dose of the spray diluted tested earlier to compare the spray dilution to dried residue dermal absorption.

2.5. Preparation of dried residue

The septum with the glued-on holder was placed with the transfer side up in a test tube rack (see Fig. 1B). The required volume of the prepared dilution was applied to the septum by a disposable tip pipette. The pipette tip was used to gently distribute the volume over the whole

Table 1
Overview of the agrochemical test compounds and their respective formulations used in the study.

Test compound	Concentration of the test compound in the formulation	Formulation type	Water solubility[a] (g/L)	Log P[a]	Molecular weight[a] (g/mol)
Myclobutanil	45 g/L	Emulsion, oil in water (EW)	0.124 at pH 3–5; 0.132 at pH 7; 0.115 at pH 9–11 (all at 20 °C)	2.56	288.77
Ethofumesate	200 g/L	Suspo-emulsion (SE)	0.039 (at 20 °C)	2.7 (at pH 6.4, 20 °C); 2.7 (at pH 6.4, 25 °C)	286.34
Acetamiprid	200 g/L	Soluble concentrate (SL)	3.48 at pH 5; 2.95 at pH 7; 3.96 at pH 9 (all at 25 °C)	0.80 (at 25 °C)	222.68
Tebuconazole	250 g/L	Water dispersible granulate (WG)	0.036 at pH 7.2 (at 20 °C)	3.7 (at pH 7, 20 °C)	307.82
Fenbuconazole	25 g/L	Emulsion, oil in water (EW)	0.0026 at pH 4; 0.0025 at pH 7; 0.0022 at pH 10	3.79 (at pH 4); 3.79 (at pH 7); 3.76 (at pH 10)	336.82
Folpet	450 g/L	Suspension concentrate (SC)	0.0008 (at 25 °C)	3.017 (at 20 °C)	296.55
Metazachlor	500 g/L	Suspension concentrate (SC)	0.45 (at 20 °C); 0.63 (at 25 °C)	2.5 (at pH 7, 22 °C)	277.75
Triclopyr BEE	125 g/L	Emulsifiable concentrate (EC)	0.007 at pH 5, 7 and 9 (all at 20 °C)	4.75 (at pH 5); 4.62 (at pH 7); 4.31 (at pH 9)	356.7
Propyzamide	400 g/L	Suspension concentrate (SC)	0.0091 at pH 4; 0.0090 at pH 7; 0.0085 pH 9.6	3.0	256.13
Propiconazole	90 g/L	Emulsifiable concentrate (EC)	0.150 at pH 5.2 (at 20 °C)	3.72 (at pH 6.6, 25 °C)	342.22

[a] All information was taken from publications from EFSA or the European Commission, Health & Consumer Protection Directorate-General. Directorate E.

septum surface of the transfer side. The septum was left at room temperature until dry.

2.6. Supply and preparation of human split-thickness skin membranes

Human skin was derived from abdomen and/or breast from female donors (age 30–63) upon informed consent directly after reconstructive surgery from the local hospital. It was transported on ice to the lab as soon as possible after the procedure but within 24 h. Subcutaneous fat was removed, the skin surface carefully cleaned and then stored at a temperature below −18 °C but no more than a year.

The day before skin exposure to the test preparations, the skin was removed from storage and left to thaw at room temperature for *ca.* 1 h. It was cut to a thickness of *ca* 0.2–0.4 mm using a dermatome (25 mm, Nouvag GmbH, Germany). The skin preparations were then sized and cut to fit the diffusion cells. The thickness of each skin was measured using a digimatic micrometer (No. 293-521-30, MDC-25M, Mitutoyo Corporation, Japan).

2.7. Flow through diffusion cells and skin integrity test

Split-thickness skin membranes were placed in 9 mm flow-through (FT) automated diffusion cells (PermeGear Inc., Riegelsville, PA, USA) and allowed to hydrate prior assessment of skin membrane integrity. The skin surface temperature was 32 ± 1 °C, at ambient humidity (determined in an empty cell containing a non-treated skin membrane in each experimental run). Receptor fluid during the integrity test consisted of saline (0.9% sodium chloride (w/v) containing 0.01% sodium azide (w/v)). After hydration, the integrity of human skin membranes was evaluated by measuring the permeability coefficient (Kp) for tritiated water. A volume of 200 µL of saline containing tritiated water was applied to the skin in the donor compartment and the compartments were covered with a glass slide. Samples of the receptor fluid (pumped at *ca.* 1.8 mL/h) were collected hourly up to 3 h after application. Subsequently, the tritiated water remaining at the application site was removed and the skin surface carefully dried with cotton swabs. Radioactivity was measured using liquid scintillation counting (LSC) and Kp values were calculated over the 1–3 h interval (*i.e.* the flux in DPM/cm^2/h, divided by the concentration in DPM/cm^3). Skin discs with a Kp value over 2.5×10^{-3} cm/h were rejected. The skin membranes were left in the diffusion cells overnight with continuous flow of receptor fluid to allow full hydration of skin and to wash-out of the tritiated water, similar as done in the previous studies conducted with concentrate and spray dilution.

2.8. Receptor fluid

On the day of application, the receptor fluid was replaced with test item-specific receptor fluids as was used in the previously available studies and did not limit the dermal uptake. The receptor fluid consisted of a) phosphate buffered saline (PBS) containing 0.01% sodium azide (w/v), supplemented with 6% polyoxyethylene 20-oleyl ether (w/v) at pH *ca.* 7.4 (for Myclobutanil, Fenbuconazole, Metazachlor, Triclopyr-BEE and Propyzamide); b) saline (0.9% sodium chloride (w/v) containing 0.01% sodium azide (w/v)), supplemented with 5% bovine serum albumin (BSA, w/v) (for Ethofumesate, Acetamiprid, Folpet and Propiconazole); or c) saline (0.9% sodium chloride (w/v) containing 0.01% sodium azide (w/v) (for Tebuconazole). Receptor fluid composition was identical between the experiments performed on the concentrate and spray dilutions (data already available) *vs* dry residues (generated in this study). The flow-rate was kept at *ca.* 1.8 mL/h to ensure adequate sink conditions. The temperature was recorded in one or two diffusion cells without test item exposure.

2.9. Dose application

Concentrate and Spray dilution absorption experiment (previously available studies): prior to dose application, the skin surface was carefully blotted dry using cotton swabs. The liquid test preparation was applied with a pipette and subsequently spread evenly on the skin surface within the donor compartment using a disposable glass rod (dose volume *ca* 10 μL/cm^2). A slightly higher volume than 6.4 μL (*i.e.* 6.7 μL) was applied to account for the expected loss of material during the distribution over the skin surface. Thus, ensuring that a net volume of approximately 6.4 μL was applied.

Dry residue absorption experiment: before dose transfer, each skin membrane was wetted using *ca.* 6.4 μL of tap water that was distributed over the skin surface (*i.e.* 10 μL/cm^2) for 5 ± 1 min (e.g. to represent sweaty worker conditions). Just prior to application, excess water was removed by gently blotting the skin surface using blotting paper. The PTFE septum was then carefully placed on the skin surface and kept on the surface for 30 s. A further transfer of the dried residue was accomplished by clockwise rotating the septum at the handle around its axis three times on the skin surface without applying (any manual) pressure. After dose transfer the septum was removed from the Franz diffusion cell and analysed for left over ^{14}C radioactivity. Actual dose transferred (or applied) was calculated as difference in ^{14}C available as mean dose of 6 parallel treated mock septa applied the same dose as the application septa subtracted by the remaining dose of the individual application septa after application. Based on the transfer efficiency (difference in ^{14}C available on the septa before and after dose application), dose on septa was adjusted for the dermal absorption experiment.

2.10. Recovery assessment

Except the dose application methodology, rest of the protocol was very similar between testing dry residue, spray dilution or concentrate.

Eight and 24 h after dose application, the skin surfaces were washed as described below.

Skin wash: after an exposure period of 8 h, the unabsorbed test substance was removed from the application site; a volume of 40 μL of a warm (*ca.* 37 °C) mild soap solution (*i.e.* 3% (w/v) Dove or Teepol soap in demineralised water) was applied on the skin surface and removed using a cotton swab. This procedure was repeated four to six times. The skin was then washed twice with 40 μL of demineralised water and cotton swab after which the skin was dried with two subsequent dry cotton swabs.

Twenty-four hours after application, the mass balance was determined, considering receptor fluid samples, skin wash, receptor and donor chamber wash, tape strips, and skin (epidermis and dermis separately).

Receptor fluid: receptor fluid samples were collected 0–1 h, 1–2 h followed by 2-h intervals until 24 h after application.

Chamber wash: at 24 h after dosing, the diffusion cell was dismantled, and the donor and receptor compartments washed separately.

Tape stripping: each skin disc was tape-stripped 15 times where possible using Stripping Discs (CuDerm Corporation, USA) and a D-Squame® pressure instrument (D500; CuDerm Corporation, USA), a spring-loaded device providing uniform pressure. Tape strips were collected and individually analysed for radioactivity. Tape-stripping was discontinued in case the epidermis was ruptured.

Skin fractions: the remaining skin was either directly dissolved in a 1.5 M KOH solution containing 20% (v/v) ethanol or was split into epidermis and dermis before digestion of the different skin fractions for at least 24 h.

2.11. Radioactivity measurement

Scintillation liquid was added to the test preparations (Hionic Fluor™ for epidermis and dermis samples, Ultima Gold™ for any other sample). Radioactivity in the samples was measured by liquid scintillation counting (LSC) on a Tri-Carb 3100 TR and/or Tri-Carb 3110 TR liquid scintillation counter using QuantaSmart™ software. All counts were converted to DPM using tSIE/AEC (transformed Spectral Index of external standards coupled to Automatic Efficiency Correction).

2.12. Radio-HPLC analysis

Radiochemical purity of the radiolabels in the test preparations was determined by radio-HPLC (Agilent 1100 series) using an Inertsil ODS-2, 250 × 4.6 mm, 5 μm C-18 column. Radiochemical purity was determined both before and after the air-drying procedure. Demineralised water (A) and acetonitrile (B, analytical grade) both supplemented with +0.1% (v/v) trifluoroacetic acid (TFA, added if required to improve the chromatography) were used as mobile phases, applying a linear gradient from 20% to 100% B in 25 min, followed by a 5 min elution at 100% B. The temperature of the column compartment was set at 25 °C, mobile phase flow-rate as 1 mL/min and the UV detector at 220 nm or a more specific wavelength if required. Radioactivity was determined using a β-ram detector with Laura software. Scintillant flow (Ultima flow, PerkinElmer) was *ca.* 3 mL/min.

2.13. Calculation of flux and dermal absorption

The cumulative absorption of test substance equivalents was calculated from the receptor fluid samples by the following equation:

$$\text{Cumulative } DPM_T = DPM_T + \Sigma(DPM_{T-1} \ldots DPM_1)$$

- DPM_T: radioactivity at sampling time T
- DPM_{T-1}: radioactivity at the sampling time preceding T
- DPM_1: radioactivity at the first sampling time

For each receptor fluid sample, background values, i.e. the radioactive background of the naïve receptor fluid, were subtracted.

According to OECD TG 428 (2014), absorbed dose is defined as radioactivity observed in the receptor fluid only. However, potential dermal absorption was calculated assuming that the residue in dead *Stratum Corneum* could become systemically available, a very conservative approach. Therefore, potential dermal absorption was calculated as sum of the per cent recovered amounts of radioactivity in receptor fluid and whole skin (dermis, live epidermis and *Stratum Corneum*) except tape strips 1 and 2.

No further adjustment of that value was made suggested by EFSA guidance (EFSA, 2014A), *e.g.* due to “variation”, because such adjustments skew the dermal absorption estimate and obscure any inference that can be derived from the data.

Some of the replicate were excluded from the calculation, as detailed in the results tables, where significant differences in their absorption profile were seen compared to other concurrently-tested replicates.

Mean of flux values from individual replicates were also calculated from the steepest part of the cumulative receptor fluid values.

3. Results

An important aspect in the development of this novel application method was that it should result in a robust methodology (i.e. adequate dose transfer to the skin without damaging it and a relatively low variation therein), which should be easily transferrable between testing laboratories. Complex or less definable procedures would surely be introducing additional variation in the data. Variation is already well known in dermal absorption *in vitro* testing and subject to considerable debate (EFSA, 2017a; EFSA, 2017b; Hothorn, 2017). The materials used for the transfer device are commercially available and the device itself

is easy to assemble.

3.1. Dose transfer method development

Primary objective of the study to develop methodology to create a dry residue dose of an agrochemical spray in laboratory setting and then to transfer the dry residue dose on the surface of the human skin in *in vitro* conditions.

Initially, various type of septa was tried to transfer the dry residue dose on the skin surface. Single-faced PTFE-coated silicone septa were used with the non-coated, silicone side glued to the plastic stick. Transfer of the dry residue was accomplished by rotating the septum around over the skin surface without applying any manual pressure. However, these were found to have a slightly concaved surface (on the coating side) upon gluing the septum to the disposable plastic rod. This decreased the degree of contact between the septum and the membrane and resulted in reduced dose transfer and/or increased the variation in the transfer between replicates.

The choice of material used for the septum could play an important role as was demonstrated by the evaluation of two metal alternatives to the PTFE-coated septa, *i.e.* stainless steel and titanium septa, that in initial experiments (without applying any manual pressure other than weight of the septum) showed only a transfer efficiency of *ca.* 30%.

Furthermore, it was hypothesized that increasing the number of rotations over the skin surface or the pressure applied to the septum would increase the transfer efficiency. However, transfer results were not notably improved with increasing the number of rotations (data not shown). Applying firm pressure tended towards lower variation in initial tests (data not shown) but was not consistent when three compounds were tested (Experiment 1, Table 2a–c). At the same time, it resulted in substantial skin surface damage of several replicates which would require the inclusion of additional skin membranes in the study to account for the loss of damaged membranes and would potentially be a further factor of unwanted variability. More importantly, defining the applied pressure appeared a less controllable factor when using the transfer device as described in this paper.

The outcome of the preliminary tests can be summarized as follows:

- Double-faced PTFE coated septa gave best results with respect to transfer efficiency and the variability therein, compared to single-faced PTFE coated septa and two metal alternatives (i.e. stainless steel and titanium septa).
- Ten times rotation did not increase transfer efficiency significantly and (too) firm pressure resulted in substantial skin surface damage of several replicates without substantial higher dose transfer.
- Three times rotation using double-faced PTFE coated septa with no additional pressure (i.e. only the weight of the rod and septa providing the downward force) applied resulted in comparable transfer efficiencies between formulations (*ca.* 60–70%) without notable damage to the skin membranes.
- Based on transfer efficiently, loading dose volume on the septa were adjusted accordingly (i.e. increased 30–40%).

Although transfer appeared rather consistent in this study, Authors recommend that the actual transfer efficiency be evaluated for each study before the main study, as it may vary not only between formulation types but also depending on the actual loading on the PTFE septum. Transfer dose should in all cases be properly quantified in the main experiment to have a good understanding about true skin loading of each replicate.

3.2. Comparison of dry residue dermal absorption to that of the respective spray dilution

Second key objective was to compare the dermal absorption from the dry residue to that from its respective spray dilution at similar dose. Previously available studies have already tested concentrate and the spray dilution (taken from Aggarwal et al., 2015). To this end, the concentration of the spray dilution tested earlier was used to prepare the dry residue for each compound.

Table 2a–c (Experiment 2 and 3) and Table 3a–g shows the target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions. The tables also show the potential dermal absorption values, *i.e.* the sum of the recovery in the receptor fluid, the skin (epidermis + dermis) and the tape-strips, except tape strips 1 + 2, which assumes that the residue in the *stratum corneum* will become systemically available.

All tested compounds showed lower mean absorption from the dry residue compared to their respective spray dilutions (Fig. 2). Except for Folpet, absorption from dry residue was higher than the corresponding concentrate (Fig. 2). The Folpet potential dermal absorption for the concentrate is higher (due to the high amount of residue retained in

Table 2a
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Myclobutanil, Experiment 1 was conducted with "firm" rotation and not included in the mean.

Myclobutanil (47 g/L, EW)	Concentrate (47 g/L)	Spray dilution (0.023 g/L)	Dry dislodgeable residue			
			Exp. 1[1]	Exp. 2	Exp. 3	Mean of Exp. 2 and Exp. 3
Target dose (μg/cm^2)	470	0.22	0.22	0.22	0.22	0.22
Actual dose (μg/cm^2)	419	0.22	0.18 ± 0.05	0.24 ± 0.01	0.20 ± 0.04	–
Transfer efficiency (%)	NA	NA	53.6 ± 13.4	72.8 ± 3.7	62.3 ± 13.9	67.5 ± 11.2
Flux (μg/cm^2/h)	1.048 ± 0.462	0.0065 ± 0.004	0.0009 ± 0.0008	0.0036 ± 0.0023	0.0014 ± 0.0014	0.0025 ± 0.0022
Group size	6[2]	6[2]	8[3]	8[3]	8[3]	–
Recovery (% of applied dose)						
Receptor fluid (0–24 h)	3.19 ± 0.88	24.7 ± 12.4	6.8 ± 3.9	14.4 ± 6.8	9.9 ± 8.1	12.2 ± 7.6
Tape strips 1 + 2	0.26 ± 0.19	0.36 ± 0.17	0.19 ± 0.09	0.07 ± 0.04	0.17 ± 0.11	0.12 ± 0.10
Tape strips 3 + rest of all	0.45 ± 0.24	1.66 ± 0.31	0.25 ± 0.08	0.17 ± 0.04	0.43 ± 0.32	0.30 ± 0.26
Epidermis except tape strips	–	–	0.28 ± 0.39	–	0.71 ± 0.73	0.71 ± 0.73
Dermis	–	–	0.42 ± 0.34	–	0.47 ± 0.63	0.47 ± 0.63
Whole skin except all tape strips	0.57 ± 0.27	2.33 ± 0.74	0.70 ± 0.71	1.39 ± 1.15	1.18 ± 1.03	1.28 ± 1.06
Total recovery	94.8 ± 1.7	99.0 ± 2.1	98.8 ± 2.7	100.2 ± 1.8	100.3 ± 3.5	100.2 ± 2.7
Potential dermal absorption[4]	3.94 ± 1.53	29.0 ± 11.6	7.9 ± 4.7	16.1 ± 7.6	11.7 ± 8.4	13.9 ± 8.1

EW: Emulsion, oil in water.
[1] Three times 'firm' rotation.
[2] Two skin membranes from each of three donors.
[3] Two skin membranes from each of four donors.
[4] Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

Table 2b
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Ethofumesate, Experiment 1 was conducted with "firm" rotation and not included in the mean.

Ethofumesate (200 g/L, SE)	Concentrate (200 g/L)	Spray dilution (0.14 g/L)	Dry dislodgeable residue			
			Exp. 1[1]	Exp. 2	Exp. 3	Mean of Exp. 2 and Exp. 3
Target dose (μg/cm^2)	2000	1.39	1.39	1.39	1.36	1.38
Actual dose (μg/cm^2)	2122	1.40	1.16 ± 0.14	0.96 ± 0.15	1.26 ± 0.11	–
Transfer efficiency (%)	–	–	60.1 ± 7.2[1]	55.4 ± 8.4	67.9 ± 5.8	61.5 ± 9.8
Flux (μg/cm^2/h)	1.80 ± 0.85	0.032 ± 0.016	0.013 ± 0.009	0.016 ± 0.006	0.018 ± 0.014	0.017 ± 0.010
Group size	8[2]	8[2]	8[2]	7[3]	8[2]	–
Recovery (% of applied dose)						
Receptor fluid (0–24 h)	0.82 ± 0.36	26.0 ± 9.3	11.5 ± 7.1	16.7 ± 5.1	11.5 ± 8.6	13.9 ± 7.4
Tape strips 1 + 2	0.32 ± 0.14	1.15 ± 0.68	0.18 ± 0.14	0.05 ± 0.03	0.031 ± 0.031	0.04 ± 0.03
Tape strips 3 + rest of all	0.45 ± 0.27	1.35 ± 0.73	0.21 ± 0.14	0.12 ± 0.06	0.10 ± 0.07	0.11 ± 0.06
Epidermis except tape strips	–	–	0.13 ± 0.15	–	0.074 ± 0.068	0.074 ± 0.068
Dermis	–	–	0.77 ± 0.46	–	0.51 ± 0.38	0.51 ± 0.38
Whole skin except all tape strips	0.36 ± 0.23	4.4 ± 2.3	0.89 ± 0.59	1.11 ± 0.34	0.59 ± 0.38	0.83 ± 0.44
Total recovery	99.0 ± 0.7	99.2 ± 6.6	99 ± 9.6	98.7 ± 10.3	101.7 ± 3.1	100.3 ± 7.3
Potential dermal absorption[4]	1.69 ± 0.61	32.4 ± 10.2	12.7 ± 7.8	18.0 ± 5.2	12.3 ± 8.7	15.0 ± 7.6

SE: Suspo-emulsion.
[1] Three times 'firm' rotation.
[2] Two skin membranes from each of four donors.
[3] Two skin membranes from each of three donors and one skin membrane from one donor.
[4] Potentialdermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

Stratum Corneum), however, the receptor fluid value was lower (Fig. 2). Spray dilution and dry residue pronouncedly differed with respect to flux, *i.e.* the rate of uptake into the receptor fluid (Fig. 3). With the exception Acetamiprid, Fenbuconazole and Myclobutanil, flux was higher from spray dilution as compared to from dry residue (Fig. 3 B and C).

As can be seen from Fig. 4, while the numerical ratios of spray dilution to dry residue dermal absorption estimates change between the test compounds, e.g. the ratio is greater for Tebuconazole than for Acetamiprid, the spray dilutions consistently result in higher dermal absorption estimates than the dried residues. Conversely, the concentrates, which have a significantly different target dose than dry residue, resulted in lower values (Fig. 2).

The extent of overestimation when using dermal absorption from spray dilution instead of dry residue values can be estimated from the overall averages; namely, range from 2.4 (median) to 3.2 (mean) based on potential absorption as percentage of applied dose, and 3.0 (median) to 5.4 (mean) based on flux ratio (Fig. 4).

Tables 3a–3c additionally show the results from the first experiment (Exp. 1) performed using Myclobutanil, Ethofumesate and Acetamiprid that applied firm pressure instead of no pressure during dose transfer. In this experiment, several replicates were replaced after dose transfer as these were visibly damaged. In addition, dermal absorption values obtained for several replicates were very high compared to the other replicates of the same group indicating damage during dose transfer. These observations were the reason for switching to the 'no pressure'-regime. Nevertheless, mean dermal absorption data generated on the remaining skin membranes were comparable to the data obtained in the other experiments (*i.e.* with no pressure applied during dose transfer), especially compared to Experiment 3. Overall, Experiment 2 showed

Table 2c
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Acetamiprid, Experiment 1 was conducted with "firm" rotation and not included in the mean.

Acetamiprid (200 g/L, SL)	Concentrate (200 g/L)	Spray dilution (0.036 g/L)	Dry dislodgeable residue			
			Exp. 1[1]	Exp. 2	Exp. 3	Mean of Exp. 2 and Exp. 3
Target dose (μg/cm^2)	2000	0.37	0.37	0.37	0.37	0.37
Actual dose (μg/cm^2)	2077	0.37	0.36 ± 0.08	0.44 ± 0.04	0.38 ± 0.03	–
Transfer efficiency (%)	–	–	64 ± 14.9[2]	77.4 ± 6.6	72.1 ± 6.9	75.2 ± 7.5
Flux (μg/cm^2/h)	6.38 ± 3.12	0.0041 ± 0.0034	0.0022 ± 0.0013	0.0124 ± 0.0060	0.0030 ± 0.0020	0.0080 ± 0.0066
Group size	8[3]	7[4]	7[4]	7[4]	8[3]	–
Recovery (% of applied dose)						
Receptor fluid (0–24 h)	2.75 ± 0.69	14.2 ± 7.3	7.1 ± 2.8	20.9 ± 7.8	9.7 ± 5.6	15.7 ± 8.8
Tape strips 1 + 2	0.10 ± 0.03	0.79 ± 0.36	0.06 ± 0.01	0.05 ± 0.04	0.05 ± 0.04	0.05 ± 0.04
Tape strips 3 + rest of all	0.31 ± 0.09	1.92 ± 1.00	0.22 ± 0.08	0.14 ± 0.07	0.17 ± 0.03	0.15 ± 0.05
Epidermis except tape strips	–	–	0.88 ± 0.56	–	0.98 ± 0.66	0.98 ± 0.6
Dermis	–	–	0.38 ± 0.18	–	0.36 ± 0.19	0.36 ± 0.19
Whole skin except all tape strips	0.36 ± 0.14	8.73 ± 3.62	1.26 ± 0.73	0.88 ± 0.37	1.34 ± 0.60	1.09 ± 0.53
Total recovery	99.7 ± 2.5	98.8 ± 4.8	94.8 ± 3.1	98.3 ± 1.5	102.0 ± 2.1	100.0 ± 2.6
Potential dermal absorption[5]	3.4 ± 0.6	25.1 ± 5.9	8.7 ± 3.2	22.2 ± 7.6	11.4 ± 5.2	17.1 ± 8.5

SL: Soluble concentrate.
[1] Three times 'firm' rotation.
[2] Data are from 5 replicates only; one replicate was damaged upon dose transfer and was lost; two replicates gave very high absorption compared to the other replicates and were therefore considered damaged as well and rejected.
[3] Two skin membranes from each of four donors.
[4] Two skin membranes from each of three donors and one skin membrane from one donor; [5]Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

Table 3a
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Tebuconazole.

Tebuconazole (250 g/kg, WG)	Concentrate (250 g/kg)	Spray dilution (0.24 g/L)	Dry dislodgeable residue
Target dose (μg/cm^2)	1250	2.4	2.42
Actual dose (μg/cm^2)	1470	2.42	1.78 ± 0.26
Transfer efficiency (%)	–	–	50.1 ± 7.4
Flux (μg/cm^2/h)	0.22 ± 0.10	0.038 ± 0.010	0.0027 ± 0.0009
Group size	8[1]	8[2]	7[3]
	Recovery (% of applied dose)		
Receptor fluid (0–24 h)	0.21 ± 0.08	18.1 ± 3.7	2.1 ± 0.7
Tape strips 1 + 2	0.27 ± 0.17	2.1 ± 0.9	0.07 ± 0.04
Tape strips 3 + rest of all	0.25 ± 0.18	3.5 ± 0.9	0.13 ± 0.08
Epidermis except tape strips	–	–	0.40 ± 0.35
Dermis	–	–	0.34 ± 0.15
Whole skin except all tape strips	0.27 ± 0.19	2.9 ± 0.9	0.74 ± 0.43
Total recovery	97.2 ± 2.9	99.9 ± 4.8	101.5 ± 5.4
Potential dermal absorption[4]	0.74 ± 0.29	24.7 ± 4.1	3.0 ± 0.5

WG: Water dispersible granulate.
[1] Five skin membranes from one donor and three membranes from each of three donors.
[2] Four skin membranes from one donor and two membranes from one donor and two membranes of each of two donors.
[3] Two skin membranes from each of three donors and one skin membrane from one donor.
[4] Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

somewhat higher absorption despite identical procedures were followed between Experiments 2 and 3. The difference was mainly caused by two out of four donors showing relatively high absorption values compared to the other two donors. Nevertheless, absorption values for all replicates used in Experiment 2 were lower than values measured for the spray dilution, with exceptions for Acetamiprid (*i.e.* two out of eight replicates showed a higher absorption compared to the mean potential dermal absorption for the spray dilution (data not shown)).

When the mean potential dermal absorption was calculated using the data from all three experiments, values were as follows: Myclobutanil 11.9 ± 7.6% (vs 13.9 ± 8.1% means of Experiments 2 and 3), Ethofumesate 14.2 ± 7.6% (15.0 ± 7.6% mean of Experiments 2 and 3) and Acetamiprid 15.0 ± 8.3% (17.1 ± 8.5% mean of Experiments 2 and 3). Thus, the mean dermal absorption values did not notably change.

4. Discussion

This manuscript presents a novel and simple dose application method on the skin surface to test dry dislodgeable residues from agrochemical spray dilutions for dermal absorption within the well-established OECD TG 428 study design. This procedure can be used to generate relevant dermal absorption values for re-entry worker and - to some extent – bystander/resident risk assessments. Radiolabelled dry residues from agrochemical spray dilutions were generated on PTFE-coated septa by air-drying. These septa were used to transfer the dry residue to pre-wetted human skin membranes mounted in flow-through diffusion cells. Another objective of this study was to compare the dermal absorption from the dry residue to that from its respective spray dilution at a similar dose level. Therefore, the concentration of the spray dilution tested earlier was used to prepare the dry residue.

Absorption through the skin is a complex process, known to be thermodynamically driven by the concentration gradient, and influenced by various factors related to the compound itself, *e.g.* molecular weight, water solubility, lipophilicity etc. (Nielsen et al., 2004), the form in which it is applied to the skin (Aggarwal et al., 2014, 2015) and also relates to the properties of the skin itself such as its physical condition or the part of the body being exposed, including the hair density present on the skin (Hueber et al., 1992; Ngo et al., 2010; Otberg et al., 2008; Trauer et al., 2009). The dead *stratum corneum*, being the outermost layer of the epidermis, is considered to be the barrier to the absorption (WHO, 2006; Yourick et al., 2004). Absorption through this 10–20 μm layer and the viable epidermis below occurs *via* the intercellular route, the transcellular route or a combination thereof, again depending on the physico-chemical properties of the compound but also on the composition of the vehicle the compound is applied in.

Since good agricultural practice (GAP or product label) dictates that the worker should not re-enter the crop until the spray is completely

Table 3b
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Fenbuconazole.

Fenbuconazole (25 g/L, EW)	Concentrate (25 g/L)	Spray dilution (0.025 g/L)	Dry dislodgeable residue
Target dose (μg/cm^2)	250	0.25	0.26
Actual dose (μg/cm^2)	262	0.26	0.25 ± 0.03
Transfer efficiency (%)	–	–	66.4 ± 6.9
Flux (μg/cm^2/h)	0.23 ± 0.16	0.0016 ± 0.0006	0.0011 ± 0.0010
Group size	8[1]	8[1]	8[1]
	Recovery (% of applied dose)		
Receptor fluid (0–24 h)	1.53 ± 0.63	11.5 ± 4.0	5.3 ± 3.2
Tape strips 1 + 2	0.45 ± 0.24	0.29 ± 0.19	0.30 ± 0.06
Tape strips 3 + rest of all	0.83 ± 0.52	0.44 ± 0.27	0.65 ± 0.34
Epidermis except tape strips	–	–	0.56 ± 0.34
Dermis	–	–	0.72 ± 0.41
Whole skin except all tape strips	2.24 ± 1.07	7.1 ± 2.5	1.28 ± 0.69
Total recovery	95.6 ± 4.8	98.3 ± 5.6	103.6 ± 4.1
Potential dermal absorption[2]	4.8 ± 0.9	19.9 ± 5.2	7.7 ± 3.3

EW: Emulsion, oil in water.
[1] Two skin membranes from each of four donors.
[2] Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

Table 3c
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Folpet.

Folpet (450 g/L, SC)	Concentrate (450 g/L)	Spray dilution (0.93 g/L)	Dry dislodgeable residue
Target dose (μg/cm^2)	4500	9.84	9.7
Actual dose (μg/cm^2)	4671 ± 211	9.86 ± 0.06	9.38 ± 1.89
Transfer efficiency (%)	–	–	62.8 ± 12.6
Flux (μg/cm^2/h)	2.1 ± 1.1	0.17 ± 0.05	0.015 ± 0.006
Group size	8[1]	8[1]	8[1]
	Recovery (% of applied dose)		
Receptor fluid (0–24 h)	0.46 ± 0.21	18.3 ± 2.2	2.41 ± 0.62
Tape strips 1 + 2	14.8 ± 10.8	3.5 ± 1.6	0.41 ± 0.31
Tape strips 3 + rest of all	5.5 ± 3.5	3.6 ± 1.4	0.39 ± 0.24
Epidermis except tape strips	–	–	1.00 ± 1.20
Dermis	–	–	0.49 ± 0.47
Whole skin except all tape strips	3.1 ± 3.0	2.9 ± 0.6	1.48 ± 1.66
Total recovery	96.6 ± 5.1	117.3 ± 11.7	100.9 ± 9.8
Potential dermal absorption[2]	9.1 ± 5.7	25.0 ± 1.7	4.45 ± 2.15

SC: Suspension concentrate.
[1] Two skin membranes from each of four donors.
[2] Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

dry, the deposits to which they are exposed may be significantly different to the physical form tested in the current dermal absorption study (i.e. dry *vs* liquid). The analysis of a relatively large data set of absorption from agrochemicals indicates that the absorption from solid form was significantly lower than the liquid form (Aggarwal et al., 2014, 2015). The dermal absorption from a dry residue may thus notably deviate from dermal absorption from the spray dilution, since dermal absorption is affected by the physical nature of the test material and absorption from a completely dry powder (solid) will not occur. Although, GAP labels suggest workers not to enter in the treated field until the spray has dried, moisture may be available based on other conditions, e.g., human sweating, dew drops on the plant surface, etc., which potentially influence the dermal absorption (Gordon and Leon, 2005). Therefore, in the current studies, skin, in addition to full hydration, was wetted with water to simulate sweating conditions. Immediately prior to dose application the skin surface was gently blotted dry to remove excess water.

All compounds tested showed lower mean potential dermal absorption levels from the dry residue compared to their respective spray dilutions. However, dry residue potential dermal absorption values were higher when compared to the data generated for their respective concentrate formulations. Based on the data (Fig. 4), one can formulate a rule of thumb (within the limitation that n = 10) that using the absorption from spray dilution as a surrogate for dry residue over-predicts absorption (as percentage of applied dose) or flux by about 2–5 times.

At the relatively low dose levels tested in this study, any substantial limitation of the absorption due to saturation of the available diffusion routes seems irrelevant. This is demonstrated by the fact that for five out of ten compounds tested (*i.e.* Myclobutanil, Acetamiprid, Tebuconazole, Folpet and Propyzamide) a tendency towards higher absorption with higher transfer efficiency (and thus a higher dose), was seen while for four, a decrease was observed. For one compound (Triclopyr BEE) no clear relationship was found. Since the dilution rates were relatively high (1:130 for Metazachlor to 1:5700 for Acetamiprid), an effect of any remaining formulation ingredients on the absorption is expected to be very low if not negligible. Thus, water solubility, logP and/or the molecular weight of the test compound are likely to play the most predominant roles in determining the absorption. In that respect, it is interesting to see that Acetamiprid, having the highest water solubility and a logP < 1, showed the lowest difference in absorption between dry residues *vs* liquid spray dilution. Although a clear relationship between absorption *vs* logP or molecular weight were not observed in the previous analysis (Aggarwal et al., 2015).

It is noteworthy that the variation in the data obtained for the dry

Table 3d
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Metazachlor.

Metazachlor (500 g/L, SC)	Concentrate (500 g/L)	Spray dilution (3.5 g/L)	Dry dislodgeable residue[1]
Target dose (μg/cm^2)	5000	35	37.9
Actual dose (μg/cm^2)	4610 ± 146	37.9 ± 0.5	23.5 ± 9.3
Transfer efficiency (%)	–	–	47.4 ± 18.7[2]
Flux (μg/cm^2/h)	1.79 ± 1.25	0.71 ± 0.24	0.060 ± 0.034
Group size	7[2]	7[2]	7[2]
	Recovery (% of applied dose)		
Receptor fluid (0–24 h)	0.19 ± 0.09	7.29 ± 2.17	3.17 ± 2.00
Tape strips 1 + 2	0.037 ± 0.027	0.09 ± 0.05	0.62 ± 1.56
Tape strips 3 + rest of all	0.15 ± 0.10	0.23 ± 0.06	0.07 ± 0.06
Epidermis except tape strips	–	–	0.23 ± 0.38
Dermis	–	–	0.09 ± 0.15
Whole skin except all tape strips	0.04 ± 0.02	0.58 ± 0.16	0.32 ± 0.52
Total recovery	103.8 ± 9.6	96.8 ± 3.6	102.8 ± 6.9
Potential dermal absorption[3]	0.38 ± 0.16	8.2 ± 2.2	3.6 ± 2.5

SC: Suspension concentrate.
[1] Due to solubility issues, methanol was added to the dose solution to achieve a homogenous preparation. The applied volume was increased accordingly to correct for the dilution. Most likely due to 'solvent drag', distribution over the PTFE septum was less adequate, *i.e.* more towards the middle of the septum which could be visually noticed. This explains the relatively low transfer efficiency and high variation therein. N.B: exposure to Metazachlor 500 g/L SC (both concentrate and spray dilution) was 10 h instead of 8 h.
[2] Two skin membranes from each of three donors and one skin membrane from one donor.
[3] Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

Table 3e
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Triclopyr BEE.

Triclopyr BEE (90 g/L, EC)	Concentrate (90 g/L)	Spray dilution (0.3 g/L)	Dry dislodgeable residue
Target dose (μg a.e./cm^2)	900	3.0	3.04
Actual dose (μg a.e./cm^2)	915 ± 10	3.04 ± 0.27	2.90 ± 0.38
Transfer efficiency (%)	–	–	64.2 ± 8.4
Flux (μg a.e./cm^2/h)	3.36 ± 0.42	0.016 ± 0.015	0.010 ± 0.004
Group size	8[1]	8[2]	7[3]
	Recovery (% of applied dose)		
Receptor fluid (0–24 h)	3.50 ± 0.76	7.84 ± 2.82	4.5 ± 2.2
Tape strips 1 + 2	0.65 ± 1.57	3.86 ± 1.03	2.3 ± 1.7
Tape strips 3 + rest of all	0.18 ± 0.06	6.28 ± 1.89	4.3 ± 4.2
Epidermis except tape strips	–	–	3.7 ± 2.0
Dermis	–	–	1.9 ± 1.2
Whole skin except all tape strips	0.43 ± 0.09	5.4 ± 1.7	5.6 ± 2.9
Total recovery	95.7 ± 2.2	95.0 ± 2.5	104.5 ± 5.6
Potential dermal absorption[4]	4.1 ± 0.8	19.8 ± 2.9	14.6 ± 6.6

EC: Emulsifiable concentrate; a.e. = acid equivalent.
[1] Two skin membranes from each of four donors.
[2] Two skin membranes from each of two donors, one membrane from one donor and three membranes from one donor.
[3] Two skin membranes from each of three donors and one skin membrane from one donor.
[4] Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

Table 3f
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Propyzamide.

Propyzamide (400 g/L, SC)	Concentrate (400 g/L)	Spray dilution (0.3 g/L)	Dry dislodgeable residue
Target dose (μg/cm^2)	4000	3.0	2.94
Actual dose (μg/cm^2)	3921 ± 49	2.94 ± 0.03	2.22 ± 0.60
Transfer efficiency (%)	–	–	57.2 ± 15.5
Flux (μg/cm^2/h)	0.17 ± 0.10	0.025 ± 0.008	0.0049 ± 0.0031
Group size	8[1]	8[1]	6[2]
	Recovery (% of applied dose)		
Receptor fluid (0–24 h)	0.05 ± 0.02	7.42 ± 3.29	3.1 ± 1.3
Tape strips 1 + 2	0.04 ± 0.03	0.26 ± 0.25	0.053 ± 0.023
Tape strips 3 + rest of all	0.10 ± 0.09	1.07 ± 0.62	0.23 ± 0.22
Epidermis except tape strips	–	–	0.11 ± 0.08
Dermis	–	–	0.25 ± 0.08
Whole skin except all tape strips	0.18 ± 0.33	1.40 ± 0.86	0.36 ± 0.15
Total recovery	97.0 ± 1.3	102.5 ± 14.7	100.5 ± 1.5
Potential dermal absorption[3]	0.33 ± 0.40	10.0 ± 3.9	3.8 ± 1.3

SC: Suspension concentrate.
[1] Two skin membranes from each of four donors.
[2] Two skin membranes from each of two donors and one skin membrane from each of two donors.
[3] Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

Table 3g
Target and actual concentrations tested, the transfer efficiency, the flux values and the recovery (as % of applied dose) in the various fractions for Propiconazole.

Propiconazole (90 g/L, EC)	Concentrate (90 g/L)	Spray dilution (0.15 g/L)	Dry dislodgeable residue
Target dose (μg/cm^2)	900	1.5	1.60
Actual dose (μg/cm^2)	879 ± 25	1.60 ± 0.02	1.55 ± 0.08
Transfer efficiency (%)	–	–	64.2 ± 3.5
Flux (μg/cm^2/h)	0.16 ± 0.12	0.014 ± 0.009	0.0042 ± 0.0006
Group size	8[1]	8[1]	7[2]
	Recovery (% of applied dose)		
Receptor fluid (0–24 h)	0.36 ± 0.24	11.6 ± 6.6	3.6 ± 0.4
Tape strips 1 + 2	0.15 ± 0.09	2.5 ± 0.7	0.17 ± 0.11
Tape strips 3 + rest of all	0.16 ± 0.10	3.5 ± 1.4	0.28 ± 0.11
Epidermis except tape strips	–	–	0.6 ± 0.3
Dermis	–	–	0.99 ± 0.49
Whole skin except all tape strips	0.19 ± 0.16	2.1 ± 2.3	1.56 ± 0.80
Total recovery	104.5 ± 1.8	94.7 ± 2.9	102.2 ± 3.7
Potential dermal absorption[3]	0.73 ± 0.43	21.0 ± 7.9	5.6 ± 1.1

EC: Emulsifiable concentrate.
[1] Two skin membranes from each of four donors.
[2] Two skin membranes from each of three donors and one skin membrane from one donor.
[3] Potential dermal absorption = Receptor fluid + whole skin except tape strips 1 + 2.

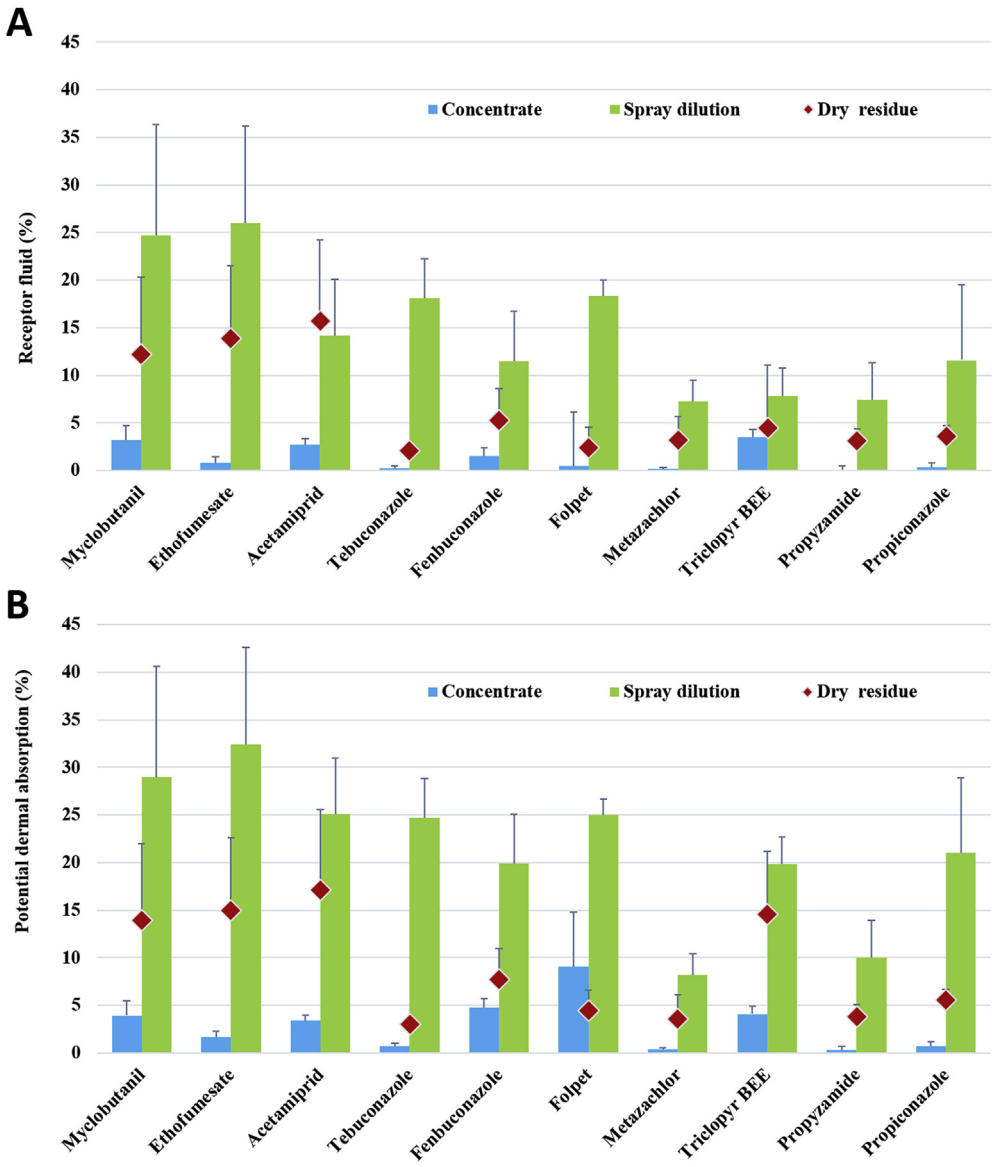

Fig. 2. Dermal absorption [in percentage of the applied dose] for individual compound from concentrate, spray dilution and dry residue as defined by (A) Potential dermal absorption = receptor fluid + whole skin except tape strip 1 + 2, and (B) receptor fluid. Bar plots and diamonds represent the mean with associated standard deviation as an error whisker. Note: for the first 3 compounds, absorption from dry residue is the mean of Exp 2 and Exp 3 (Table 2 a-c).

residue tended to be higher compared to the variation in the data obtained for the spray dilution. The variation coefficient (% CV) in the spray dilution data, ranges from 7 to 40% (mean 26 ± 11), while for the dry residues it ranges from 17 to 69%. Since donor variation was less for the spray dilutions, there seemed to be another factor causing the variation to increase. However, the most likely explanation for higher variability of results this is probably that more steps are needed to conduct the presented method, which of course increases the amount of variation.

Variation in the transfer efficiency (here standard deviation) seems to inversely correlate with actual transfer efficiency (plot not shown), e.g. Metazachlor 47.4% (± 18.7) *vs* Acetamiprid 75.2% (± 7.5). Hence, keeping the variation in transfer efficiency to a minimum contributes to lowering the overall variation in the data.

A recent publication (Clarke et al., 2018), described an experiment very similar to that being described in this manuscript. The authors investigated whether dry residue results in lower absorption than the respective spray dilution of the active ingredients from formulations with different physical-chemical properties. The results strongly support our conclusions even if the methodology of dose transfer to the skin surface was slightly different. The key differences were that porcine skin of a single animal dermatomed to ~750 µm was used in static Franz cells with 2 cm^2 surface in an incubator (32 °C) applying unlabelled "cold" test item, whereas the studies described in here used human skin dermatomed to 200–400 µm from multiple donors in standard flow-through cells with a water-jacket system to achieve physiological skin temperature of 32 °C and applying radiolabelled test item that allows the determination of a robust mass balance. The study conditions applied in this manuscript are thus conform with the methodology in place for routine testing of pesticidal product concentrates and their spray dilutes. The application device differed in that it was a steel disk attached to a HPLC vial and the method in the current manuscript used a double coated PTFE-septum to which a disposable plastic rod was glued. Further, whereas the authors of the current manuscript used the level of transfer efficiency as the quality criterion, the Clarke et al. working group based their choice of transfer device on SEM image anlaysis as described in Belsey et al. (2011). The differences in transfer efficiency criteria may have led to the use of different

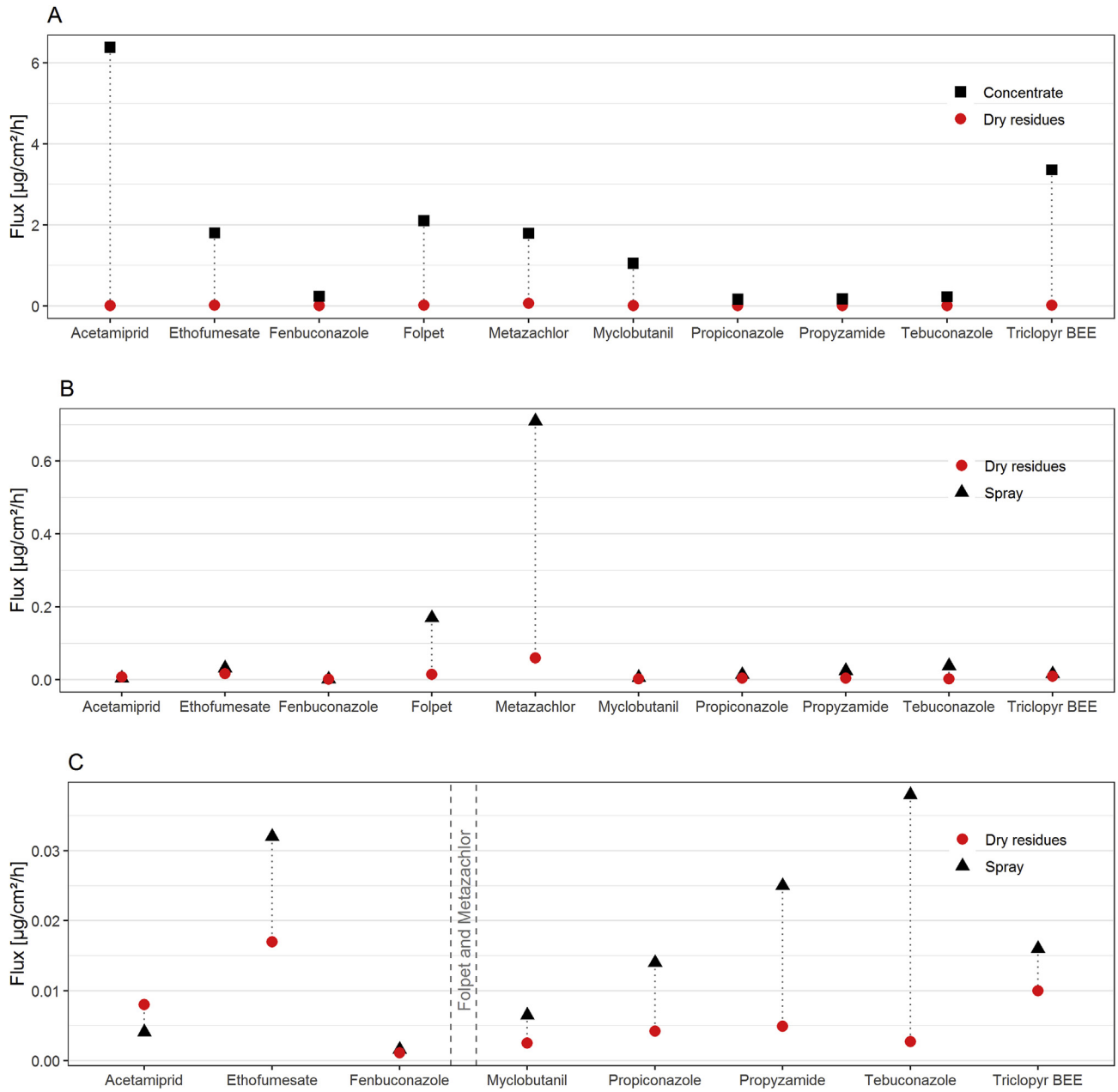

Fig. 3. Mean flux [µg/cm^2/h] for individual compounds. Triangle, square and dot symbols represent the means for concentrate, dry residue and spray dilution, respectively. (A) Concentrate versus dry residue (B) spray dilution versus dry residue and (C) a higher resolution of B excluding the actives Folpet and Metazachlor.

application devices. The dose transfer device described in this manuscript has some clear advantages, namely being simpler, cost-effective, and possibly easily transferable between testing laboratories. Additionally, dose transfer methodology described herein strictly defined as "three rotations" without applying any manual pressure, whereas the Clarke et al. used a random movement approach, which was however convincingly replicated. The authors' recommendation is therefore to assess the actual transfer efficiency within each study and to apply measures aiming to reduce variation in transfer efficiency between replicates.

Although only a few publications are available on this subject, overall, the conclusions from the different methodologies are congruent, supplementary and in the same effect size range. It is demonstrated that testing of dry residues is feasible by inclusion of an appropriate application technology into the methodology in place for routine dermal absorption testing of pesticidal products and their spray dilutions within OECD TG 428.

5. Conclusion

The work described here introduces a novel and simple application method to generate dry residue of the spray in laboratory setting and transfer of the same on to the surface of the human skin *in vitro* conditions within OECD TG 428. The methodology uses equipment/materials that are commercially available and is likely to be easily transferable between laboratories.

Data generated with ten compounds showed notable differences between dermal absorption values obtained for the spray dilution *vs* for the dry residue at a similar dose level. The use of dermal absorption data obtained for the spray dilution, in the dermal risk assessment for re-entry workers (and/or bystander/resident) results in an overestimation of the risk and introduce additional conservatism.

The present data support the importance of introducing an extra group to test the dry residue of the spray with the described application technique into the standard *in vitro* dermal absorption study (OECD TG

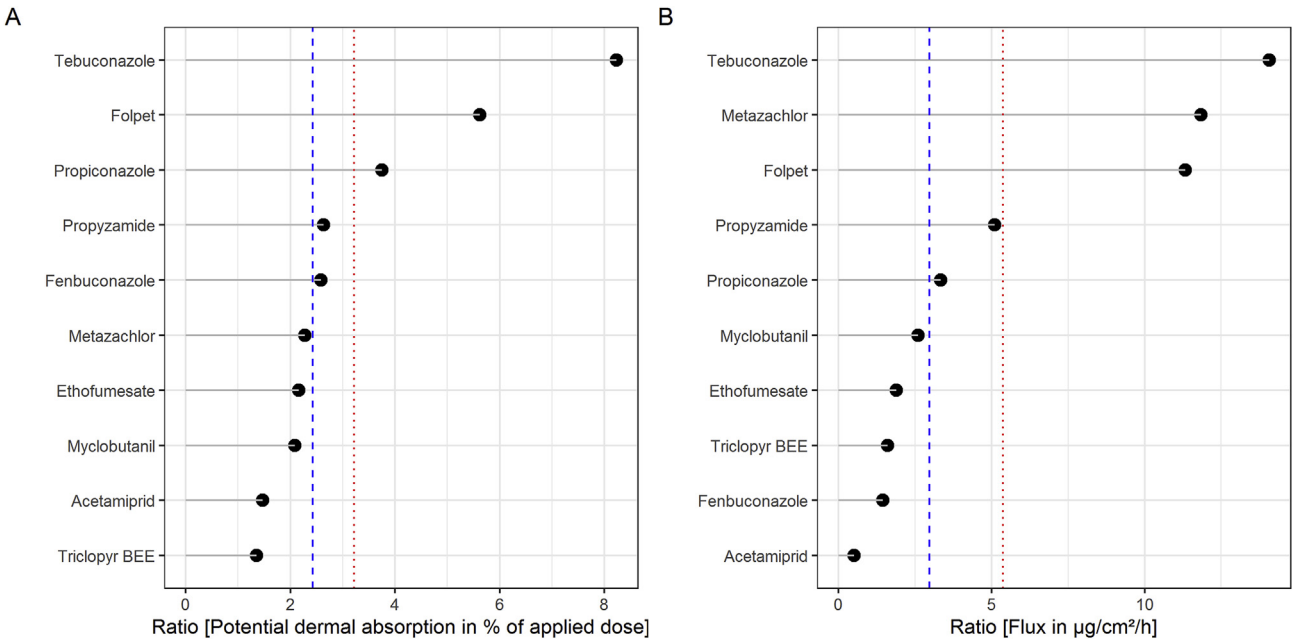

Fig. 4. The ratio of the spray dilution to the dry residue absorption show that the spray dilution overestimates absorption based on A) the percentages of the applied dose, i.e. potential dermal absorption, and B) the flux values. Overall mean (red dotted line) and median (blue dashed line) indicate the extent of overestimation, i.e. 2–3 times based on the percentage of the applied dose and 2.5–5 times based on flux. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

428) with agrochemicals that are presently performed on the concentrated product and representative spray dilutions.

Conflict of interest

WM was the study director for the dermal absorption experiments with dry residues at TNO Triskelion, Netherlands (during 2016), who is now (2019) at Charles River Laboratories, Netherlands. The European Crop Protection Association (ECPA) owns the dataset and the other authors are representatives at ECPA for the indicated companies. The authors are scientists and experts in the field of dermal absorption or regulatory toxicology. They actively participated in the data evaluation and preparation and review of this article.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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