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Assessment of an extended dataset of *in vitro* human dermal absorption studies on pesticides to determine default values, opportunities for read-across and influence of dilution on absorption

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ABSTRACT

Dermal absorption is a key parameter in non-dietary human safety assessments for agrochemicals. Conservative default values and other criteria in the EFSA guidance have substantially increased generation of product-specific *in vitro* data and in some cases, *in vivo* data. Therefore, data from 190 GLP- and OECD guideline-compliant human *in vitro* dermal absorption studies were published, suggesting EFSA defaults and criteria should be revised (Aggarwal et al., 2014). This follow-up article presents data from an additional 171 studies and also the combined dataset. Collectively, the data provide consistent and compelling evidence for revision of EFSA's guidance. This assessment covers 152 agrochemicals, 19 formulation types and representative ranges of spray concentrations. The analysis used EFSA's worst-case dermal absorption definition (*i.e.*, an entire skin residue, except for surface layers of *stratum corneum*, is absorbed). It confirmed previously proposed default values of 6% for liquid and 2% for solid concentrates, irrespective of active substance loading, and 30% for all spray dilutions, irrespective of formulation type. For concentrates, absorption from solvent-based formulations provided reliable read-across for other formulation types, as did water-based products for solid concentrates. The combined dataset confirmed that absorption does not increase linearly beyond a 5-fold increase in dilution. Finally, despite using EFSA's worst-case definition for absorption, a rationale for routinely excluding the entire *stratum corneum* residue, and ideally the entire epidermal residue in *in vitro* studies, is presented.

1. Introduction

The current authors published a paper based on an *in vitro* human dermal absorption dataset collected up to 2012 (Aggarwal et al., 2014). This paper provides a new dataset of studies performed mainly between 2012 and February 2014; the combined dataset is also presented and evaluated.

1.1. Background

Skin is a multi-layered organ that forms a natural barrier to absorption of exogenous substances, including chemicals. Its upper layer is the non-vascular epidermis, from which chemicals cannot be absorbed *per se*. In addition, the epidermis divides and grows in an outward direction whereby retained residues are eliminated via normal epidermal desquamation (Aggarwal et al., 2014; Maibach and Patrick, 2001; WHO, 2006a).

Dermal absorption is a key parameter used in human (operators, bystanders, residents and re-entry workers) exposure risk assessments for agrochemicals in Europe. Exposure models are used to estimate the external skin dose and dermal absorption values are used to convert them into systemic (internal) exposures, which are then compared to reference doses (*e.g.*, Acceptable Operator Exposure Level – AOEL).

In a typical OECD test guideline-compliant dermal absorption study (OECD 428) at least 4 skin replicates (in the case of current datasets, from at least two donors) are exposed to the undiluted formulation (concentrate) and one or more spray dilutions for

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6–8 h to match a representative working day. Dermal absorption is measured for 24 h to match with reference dose and estimated exposure units. Dermal absorption studies preferentially use human skin, *in vitro*; rat skin can also be used, notably to allow bridging to *in vivo* studies in rats. Rat skin is anatomically significantly different (thinner *stratum corneum*, more hair follicles, *etc.*) to human skin and typically much more permeable to chemicals, so it represents a worst-case model for human skin (Bronaugh et al., 1982; Chan et al., 2010; van Ravenzwaay and Leibold, 2004). When all three studies are performed the differences between human and rat skin can be mitigated by correcting the penetration observed in rats *in vivo* by a factor experimentally determined from human and rat skin *in vitro* in a so-called 'triple pack' approach.

More recently, EFSA published a scientific opinion (EFSA, 2011) and guidance (EFSA GD, 2012) on dermal absorption. Similar guidance has also been published by OECD (OECD guidance note 156). While OECD (Guidelines 427, 428 and Guidance 28) explain how to conduct these studies, the subsequent EFSA (and OECD) guidance introduced secondary standards and different criteria for calculation of dermal absorption, including:

- high default values:
  - o concentrate 25% (75% if active substance content is <5%)
  - o spray dilution 75%
- no bridging from one formulation to another containing the same active substance if:
  - o any formulation constituent differs by ±25%
  - o skin irritation and skin sensitisation properties differ
- linear extrapolation, or use of a default value, if a tested concentration is higher than a label spray dilution
- assuming the skin residue (except some or all of the *stratum corneum*) at the end of a study has been absorbed
- normalising a mean if recovery is <95% (*versus* <90% in all other OECD studies)
- adding a standard deviation to a mean when it's > 25% of a mean (unique for OECD-compliant studies).

The EFSA GD triggers the requirement for dermal absorption studies for almost all formulations (including virtually identical formulations of the same active substance) even when expert judgement indicates that reliable surrogate data are already available.

To investigate the reliability of EFSA guidance, the authors initiated a project to collect, collate and systematically analyse all available dermal absorption data derived from *in vitro* studies using human skin, which is the preferred method in EU data requirements (EC 1107/2009; EC 284/2013EC).

Dermal absorption values in this analysis were calculated using EFSA's worst-case assumption that the whole skin residue (except tape strips 1 and 2) is absorbed. Previously, a dataset of 190 OECD GLP and test guideline-compliant *in vitro* human skin studies were published (Aggarwal et al., 2014). In this assessment, a further 171 studies (referred to as the 'new dataset') were collected and evaluated. The new dataset was then combined with the previous dataset (referred to as the 'combined dataset') and assessments were made on both the new and the combined dataset.

1.2. Summary of the previous data evaluation

The previous assessment (Aggarwal et al., 2014) of 190 *in vitro* human-skin dermal absorption studies using EFSA's worst-case definition of dermal absorption and 95th percentile absorption values – to match EFSA's proposal to use 75th or 95th percentile exposure data distributions for chronic or acute risk assessments, respectively – supported:

- dermal absorption default values of 6% for liquid and 2% for solid concentrates, irrespective of the active substance concentration
- dermal absorption default values of 30% for all spray dilutions, irrespective of the formulation type
- dermal absorption values for solvent-based formulations to be conservative read-across data for water-based and solid formulations
- dermal absorption does not increase linearly with dilution; a proposal was made for no adjustment when dilution increased by less that 2-fold, and a linear increase for a 2- to 5-fold increase in dilution up to a default value of 30%.

1.3. Objectives

The current publication investigates the validity of earlier conclusions (Aggarwal et al., 2014) by providing a new dataset of 171 studies and a combined dataset of 295 *in vitro* human dermal absorption studies.

2. Methods

2.1. Selection of studies

All available GLP and OECD 428-compliant *in vitro* human dermal absorption studies, mostly performed between 2012 and 2014, were collected (OECD, 1997; OECD 428, 2004). This comprised 171 studies and provided 182 dermal absorption values for concentrates and 277 for spray dilutions. The resulting dataset was then combined with the published dataset (Aggarwal et al., 2014). To ensure homogeneity of the dataset, the following type of studies were excluded from the new dataset, and also from the combined dataset:

- studies with exposure duration exceeding 10 h
- studies with an experimental duration exceeding 24 h
- studies where values for tape strips 1 and 2 were not available
- studies with added surfactants or adjuvants (*i.e.*, that were not components of the agrochemical formulation).

Since these studies are OECD test guideline and GLP compliant, adjustments for recovery (where <95%) or additions of standard deviations (where >25% of mean) required by the EFSA GD were not made as they are considered to represent unacceptable regulatory conservatism as opposed to scientifically substantiated and defined criteria.

Further, upon assessing the previous dataset two errors were identified: (1) one dermal absorption estimate was mistakenly excluded from the evaluation because of a presumably negative dermal absorption value; this value was re-calculated and incorporated into the combined assessment, and (2) four values derived using an added external surfactant were included in the previous evaluation; these values were excluded from the combined assessment. However, these changes do not affect the overall conclusions previous published by Aggarwal et al. (2014).

This assessment comprises studies covering 19 different formulation types (Table 1) and 152 different active substances. The concentration range of the active substances in the formulations was 0.061 to 853.0 g/L for the concentrates and 0.00075 g/L to 187.5 g/L for dilutions. The active substances had a wide range of octanol–water partition coefficients ($\mathrm{Log}P_{ow}$; −3.2 to 9.085) and molecular weights (169 to 1632.525 g/mol).

2.2. Data analysis

EFSA Guidance (2012) provides decision logic for assessors to determine the extent to which pesticide residue in the *stratum*