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Assessment 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 an integral part of non-dietary human safety risk assessments for agrochemicals. Typically, dermal absorption data for agrochemical active substances are generated from the undiluted formulation concentrate and its spray dilutions. European Food Safety Authority (EFSA) guidance, which combines highly conservative default values, very limited opportunities for read-across from existing data and other overly conservative conclusions, was the driver for this assessment. To investigate the reliability of the EFSA guidance, a homogeneous data-set of 190 GLP and OECD guideline compliant *in vitro* human skin studies, chosen to match the test method preferred by EU data requirements, was evaluated. These studies represented a wide range of active substances, formulation types, and concentrations. In alignment with EFSA guidance on human exposure assessment, a conservative estimate of absorption (95th percentile) was chosen to define defaults, which were also based on the EFSA worst-case assumption that all material in skin, excluding the first two tape strips, is absorbed. The analysis supports dermal absorption defaults of 6% for liquid concentrates, 2% for solid concentrates, and 30% for all spray dilutions, irrespective of the active substance concentration. Relatively high dermal absorption values for organic solvent-based formulations, compared to water-based or solid concentrates, support their use as worst-case surrogate data for read-across to other formulation types. The current review also shows that dermal absorption of sprays does not increase linearly with increasing dilution, and provides a novel, science-based option for extrapolation from existing data.

1. Introduction

1.1. Skin is a barrier to systemic exposure

Skin is a multilayered organ comprising epidermis, dermis and hypodermis from outer to inner layers, respectively (Fig. 1). The epidermis is nonvascular having a general protective role, including as a barrier to penetration of chemicals to the vascular dermis. It has four layers - stratum corneum, stratum granulosum, stratum spinosum, and stratum basale - and is divided from the dermis by the basement membrane, which itself also functions as a permeability barrier (Breitkreutz et al., 2013; Ko and Marinkovich, 2010). On average, human stratum corneum has about 16 layers and takes about two weeks to completely desquamate and renew (Hoath and Leahy, 2003). Chemicals in contact with skin must penetrate the entire epidermis, including the basement membrane, to reach capillaries in the underlying dermis; only then do they have the potential for absorption, systemic circulation, and opportunity to cause systemic effects. This penetration and absorption occurs by passive diffusion but they may also occur via sweat glands

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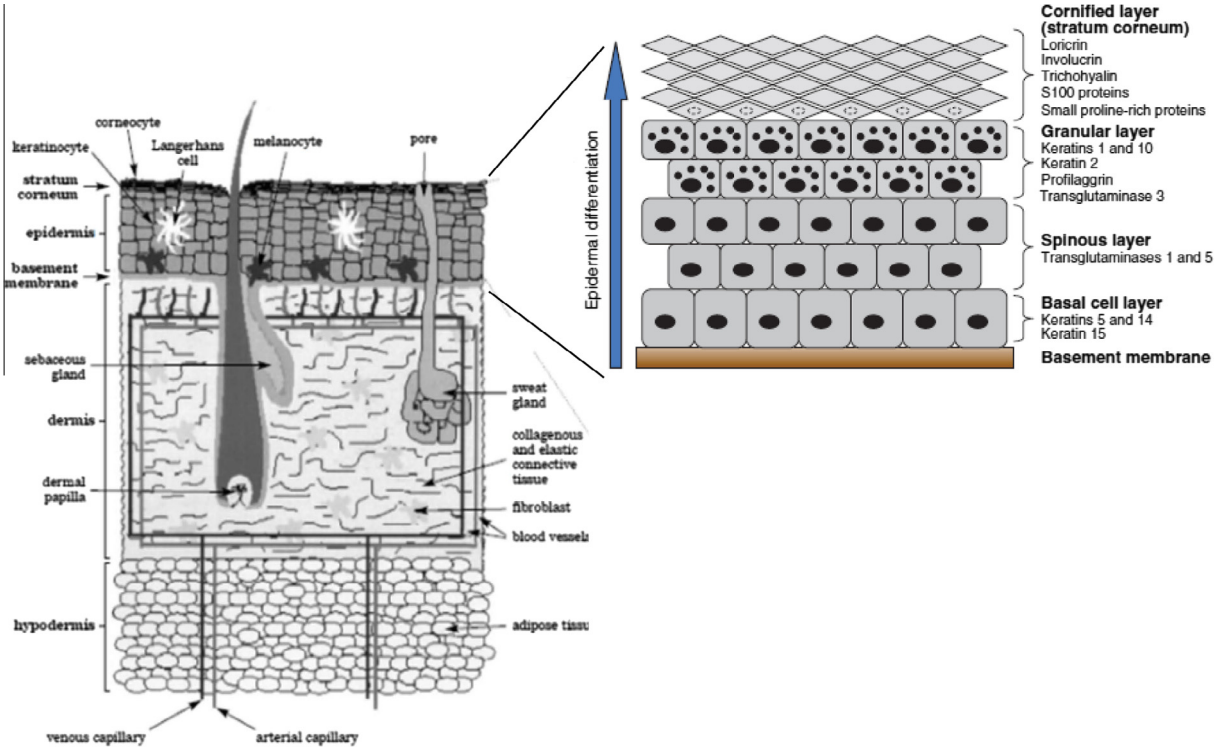

Fig. 1. A typical structure of the skin. Figure is modified from Basketter et al. (2007) and Sandilands et al. (2009).

and hair follicles directly to the dermis (Ngo et al., 2010). Chemical residues limited to the epidermis will be eliminated from the exposed skin by desquamation (Sundberg et al., 2012) and never be available for absorption into the bloodstream by the dermal route.

1.2. Dermal exposure scenarios to plant protection products

Skin is the main route of human exposure to a plant protection product (PPP) during mixing, loading and application in the field. In the European Union (EU), human-health risk assessments for operators, workers, bystanders, and residents to an active substance in a PPP are based on a systemic reference dose, the Acceptable Operator Exposure Level (AOEL). Since the AOEL is a systemic reference value, the risk assessment is conducted by comparing systemic human exposure values with the AOEL.

Dermal absorption data for an active substance from a PPP is crucial for assessing systemic exposure to these chemicals via the dermal route. Dermal absorption of any given active substance depends on many factors, including physico-chemical properties and structure of the active substance, the matrix (PPP type) in which it is formulated and delivered to skin and its concentration and solubility in that matrix (Baynes and Riviere, 2010; WHO, 2006b). The presence of organic solvents or surfactants (*i.e.,* formulation composition) can alter the dermal absorption of the active substance (Baynes and Riviere, 2010; Brand and Mueller, 2012; OECD 156, 2011; Sartorelli et al., 1997; van der Merwe and Riviere, 2008; WHO, 2006b).

Most commercial PPPs are applied by spraying and are typically sold as concentrated formulations that are diluted with water prior to application. For operators, dermal exposure to a concentrated PPP can occur during mixing and loading into the spray tank; exposure to the spray dilution of the PPP results from deposition of spray on skin or from contact with contaminated surfaces (*e.g.,* spray nozzles). In modern agriculture, exposure to spray is usually very limited due to use of tractors with cabins, personal protective equipment and product stewardship/training of professional operators. Bystanders and residents may have accidental, brief exposures with spray drift on isolated occasions. Workers re-entering treated crops may be exposed to dry foliar residues of the spray as label instructions stipulate that re-entry is not permitted before spray deposits have dried - although the default approach for risk assessment is to assume exposure is to a liquid residue and hence dermal absorption data for the spray dilution are used, giving a highly conservative outcome.

Dermal exposure to a concentrate or a spray dilution differs both in the chemical nature of the matrix and the concentration of the active substance. As both factors affect dermal absorption, agrochemical registrants typically generated dermal absorption data for a formulation concentrate and spray dilutions based on proposed or established good agricultural practices.

1.3. Dermal absorption studies

Dermal absorption studies can be performed on live animals *in vivo* (OECD 427, 2004) or with skins from animals or human *in vitro* (OECD 428, 2004). Various 'official' guidance are published on the review of the dermal absorption conduct or interpretation of the data generated (OECD 28, 2004; OECD 156, 2011; WHO, 2006b).

Agrochemical registrants are no longer allowed to generate dermal absorption data in human volunteers (EC, 2013). *In vivo* dermal absorption data from rats over-predict absorption in humans: rat skin displays a much higher permeability to chemicals than human skin (typically in the 2- to 10-fold range) due to a relatively thin *stratum corneum* and a much higher hair follicle density (Ngo et al., 2010; Ross et al., 2000; van Ravenzwaay and Leibold, 2004). Therefore, alternative methods have been developed (OECD 428, 2004) and in the EU, an *in vitro* study using human skin is now the preferred choice (EC, 2009, 2013). In a typical study, a PPP and its spray dilutions containing radiolabelled active substance are applied to the skin to represent a typical work