Regulatory Toxicology and Pharmacology 90 (2017) 197–205

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Dermal absorption for pesticide health risk assessment: Harmonization of study design and data reporting for North American Regulatory submissions

Kristie M. Sullivan [a, *], Manoj Aggarwal [b], Jonathan M. Akins [c], Eric Fabian [d], Jon R. Heylings [e], Hans Raabe [f], Prakashchandra (P.V.) Shah [g], Christiane Wiemann [h], Richard Peffer [i]

[a] Physicians Committee for Responsible Medicine, 5100 Wisconsin Ave NW, Suite 400, Washington, DC 20016, United States
[b] Dow AgroSciences Ltd, 3B Park Square, Milton Park, Abingdon, Oxfordshire OX14 4RN, United Kingdom
[c] Tessenderlo Kerley, Inc., 2255 North 44th Street, Suite 300, Phoenix, AZ 85008, United States
[d] BASF SE, GB/TB - Z470, 67056 Ludwigshafen, Germany
[e] Dermal Technology Laboratory, Ltd, Med IC4, Keele University, Science and Business Park, Keele, Staffordshire ST5 5NL, United Kingdom
[f] Institute for In Vitro Sciences, 30 W. Watkins Mill Road, Suite 100, Gaithersburg, MD 20878, United States
[g] US EPA Headquarters, Ariel Rios Building, 1200 Pennsylvania Avenue, N.W., Mail Code: 7505P, Washington, DC 20460, United States
[h] BASF Österreich GmbH, Millennium Tower, 25. OG, Handelskai 94-96, A-1200 Wien, Austria
[i] Syngenta Crop Protection, LLC, P.O. Box 18300, 410 Swing Road, Greensboro, NC 27419-8300, United States

ARTICLE INFO

Article history:
Received 24 April 2017
Received in revised form
4 August 2017
Accepted 10 September 2017
Available online 12 September 2017

Keywords:
In vitro
Dermal absorption
Pesticide
Risk assessment
North America
Triple-pack
Human

ABSTRACT

Although an internationally-adopted *in vitro* dermal absorption test guideline is available (OECD Test Guideline 428), the replacement of the *in vivo* approach in North America for pesticide formulations has not occurred due to concern over the reliability and consistency of the *in vitro* results. A 2012 workshop convened a panel of experts in the conduct of *in vitro* studies used for pesticide risk assessment, together with North American regulators, to examine techniques for *in vitro* dermal absorption testing. Discussions led to the recommended "best practices" for the conduct of *in vitro* dermal absorption studies provided herein. The workshop participants also developed recommendations for reporting study results in order to improve the quality and consistency of the data submitted to regulatory agencies in North America. Finally, a case study is presented that illustrates the use of the "triple-pack" approach; the studies, conducted for the registration of sulfoxaflor, follow the standardized recommendations provided at the workshop. In addressing the concerns of these regulators and of the regulated community, and providing harmonized recommendations to facilitate comparative data analyses, it is anticipated that wider acceptance of *in vitro* dermal absorption studies alone can be achieved for pesticide risk assessment.

1. Introduction

The assessment of exposures and the dermal absorption potential of regulated products, including, for example, pharmaceuticals, personal care products, and pesticides, is an important consideration for toxicologists and risk assessors. For scientific and animal welfare reasons, the assessment of dermal absorption *in vitro,* using human or animal skin sources, has become more common, and in some sectors, such as for cosmetics and personal care, *in vivo* dermal absorption studies have been completely replaced by *in vitro* methods. However, due to the potential toxic effects of some pesticides (e.g., cholinesterase inhibitors) and the fact that exposure can occur unintentionally (e.g., drift), there is compelling interest in ensuring that pesticide dermal absorption

Abbreviations: DAF, Dermal Absorption Factor; CDPR, California Department of Pesticide Regulation; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; NAFTA, North American Free Trade Agreement; OECD, Organization for Economic Cooperation and Development; PMRA, Health Canada Pest Management Regulatory Agency; SD, standard deviation; TEER, trans-epithelial electrical resistance; TEWL, trans-epidermal water loss.

* Corresponding author.
E-mail address: ksullivan@pcrm.org (K.M. Sullivan).

http://dx.doi.org/10.1016/j.yrtph.2017.09.012
0273-2300/

values are not underestimated, in order to be protective of human health.

The use of excised skin preparations for the purpose of estimating dermal absorption via *in vitro* methods capitalizes on the understanding that the process of passive diffusion of substances through the *stratum corneum* does not require a metabolically active *in vitro* test system. It has been established that human *stratum corneum* is a much more effective barrier to absorption compared to rat skin for most chemicals examined in these models (Aggarwal et al., 2014, 2015; Dumont et al., 2015; Fasano et al., 2005). Indeed, literature reviews and prospective studies found that the dermal absorption potential of pesticides, industrial chemicals, and cosmetic ingredients was higher in rat skin than human skin, usually by factors ranging from 5 to 100-fold and in some cases up to 500-fold (Bartek et al., 1972; Jung and Maibach, 2015; Ross et al., 2001; van Ravenzwaay and Leibold, 2004). OECD Test Guideline 428 (OECD, 2004c) provides a protocol for using excised human or rat skin for the purpose of assessing the dermal absorption of chemicals and formulated products and/or dilutions. However, as is the case with most OECD test guidelines, this test guideline and its accompanying guidance, Guidance Document No. 28 (OECD, 2004a), were written for general use, and generally do not include sector- or chemical property-specific protocol recommendations.

Since that time, a number of organizations, including the OECD, have published guidance documents on the conduct and interpretation of *in vitro* dermal absorption studies (OECD, 2011). The WHO International Programme on Chemical Safety recommendations state that human skin should be the "gold standard" in human health risk assessment for all chemical classes (WHO, 2006), and recommends the development of "consistent and well controlled studies with human skin in order to predict dermal absorption in humans." More recently, the EFSA has published guidance for the conduct and interpretation of dermal absorption studies and also state that *in vitro* studies performed with human skin are preferred (EFSA, 2012). This EFSA guidance document is used for the registration of pesticide-containing products in the European Union, and is currently under revision. A recent review by Dumont and coworker summarizes some of the similarities and differences in these guidance documents (Dumont et al., 2015).

While data from *in vitro* studies alone are fully accepted by European pesticide regulators, in North America an *in vivo* study, usually conducted using rats, continues to be required by the US EPA, CDPR, and PMRA to determine dermal absorption values for pesticides. In 2008, the NAFTA Dermal Absorption Working Group (EPA, PMRA and CDPR) (NAFTA, 2008) issued a policy statement outlining the triple pack approach, which was recommended as the preferred testing method(s) for new pesticidal active ingredients that are submitted for registration to regulatory authorities in North America. In this approach, three studies—an *in vitro* rat, an *in vivo* rat, and an *in vitro* human—may be submitted together in order to set a DAF for use in human health risk assessments for pesticides (NAFTA, 2008). The purposes of this recommended approach were: 1) to help improve/standardize the quality of *in vitro* studies both in terms of conduct and reporting to the NAFTA regulatory agencies and 2) to allow the assembly of a comparative database (with existing and new data), which is critical to determining whether the *in vitro* human skin method is predictive of *in vivo* dermal absorption, with the ultimate goal being the acceptance of *in vitro* studies alone.

A workshop held on May 1–2, 2012 in Gaithersburg, Maryland, USA, convened a small international panel of academic and industry experts in the field of dermal absorption, together with North American pesticide regulators and non-governmental representatives, to determine the barriers to acceptance of stand-alone *in vitro* dermal absorption studies. An expected outcome of the workshop was to build consensus around best practices for the conduct and reporting of *in vitro* dermal absorption studies for pesticide risk assessment and to increase comparability of *in vitro* studies across different laboratories. Steps outlined as part of this Workshop are captured herein with the aim of evaluating the predictive power of the *in vitro* method in typical in-use conditions, and to help North American regulatory agencies define the criteria by which *in vitro* study values can be used in future risk assessments. To help illustrate the type of data that is contained within a triple pack and its use in deriving values for risk assessments in North America, a brief review of a triple pack of studies with the pesticidal active ingredient sulfoxaflor is provided as a case study.

2. Regulatory agency considerations

For pesticides to be approved in North America, regulatory agencies are required to ensure there are no human health (or environmental) risks of concern when used according to the label directions. Dermal absorption values are used in estimating systemic exposure via the dermal route in order to facilitate comparison with critical effect levels derived from oral toxicological studies. Chemical-specific dermal absorption studies are used to determine the DAF, where possible.

Currently, *in vitro* dermal absorption studies on pesticides are not accepted in the absence of an *in vivo* dermal absorption study in North America for regulatory decisions, but can be accepted as part of a triple pack, as mentioned previously. North American regulators state that differences in test protocols have led to variable results such that a range of *in vitro* dermal absorption values would be obtained for the same test substance depending on the study methodology. This results in a lack of confidence in using *in vitro* data as standalone. Incomplete test reports or data dossiers also contribute to this uncertainty.

During the workshop all three North American regulatory agencies reported that a major barrier to acceptance of the *in vitro* dermal absorption method is the high degree of flexibility in the test protocol parameters that are described in established test guidelines and guidance (OECD, 2004a; OECD, 2004c; OECD, 2011). This prevents comparison between laboratories and studies, and creates uncertainty when reviewing individual study submissions, including the extent to which such variations may affect the study outcome. Some of the variable protocol elements identified include:

- Skin used, source, thickness, separation procedures
- Receptor fluid choice
- Barrier function testing method and criteria for exclusion of a skin sample
- Tape stripping methods and other post-exposure activities
- Numbers of individual donors, and samples per donor per test

US EPA described the *in vivo* dermal absorption rat study protocol, which uses four male rats per dose per time point (EPA, 1998). Studies usually contain at least three dose levels and four to six exposure times, using 80–120 rats, though reduced protocols are also accepted. The OECD *in vivo* dermal absorption test guideline (TG 427) is also accepted (OECD, 2004b). As the conditions for *in vivo* dermal absorption studies are somewhat standardized and reproducible, confidence in the use of *in vivo* dermal absorption studies for pesticide regulatory purposes is higher. However, *in vitro* dermal absorption studies using human skin offer several advantages, such as the use of skin from the relevant species of interest (i.e., human vs. rat) while avoiding human testing, the ability to better capture volatile chemicals, enhanced control over