Toxicology in Vitro 32 (2016) 198–204

Contents lists available at ScienceDirect

Toxicology in Vitro

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

In vitro human skin penetration model for organophosphorus compounds with different physicochemical properties

L. Thors [a,*], B. Koch [a], M. Koch [a], L. Hägglund [a], A. Bucht [a,b]

[a] Swedish Defence Research Agency, Division of CBRN Defence and Security, Umeå, Sweden
[b] Department of Public Health and Clinical Medicine, Unit of Respiratory Medicine, Umeå University, Umeå, Sweden

ARTICLE INFO

Article history:
Received 11 September 2015
Received in revised form 14 December 2015
Accepted 6 January 2016
Available online 9 January 2016

Keywords:
In vitro skin penetration
Human skin
Epidermal membrane
Organophosphorus compounds

ABSTRACT

A flow-through diffusion cell was validated for *in vitro* human epidermal penetration studies of organophosphorus compounds (OPCs) applied by infinite dosing. By testing OPCs with similar molecular weight but different physicochemical properties, it was shown that hydrophilic and lipophilic properties are major determinants for the penetration rate. Lipophilic OPCs displayed maximum cumulative penetration in the 20–75% agent concentration range whereas the hydrophilic OPCs displayed maximum cumulative penetration at 10 or 20% agent concentration. Low penetration was observed for all agents at 1% agent concentration or when applied as neat agents. The impact of the receptor solution composition was evaluated by comparing the penetration using receptor solutions of different ratios of ethanol and water. For diluted OPCs, a high concentration of ethanol in the receptor solution significantly increased the penetration compared to lower concentrations. When OPCs were applied as neat agents, the composition of the receptor solution only affected the penetration for one of four tested compounds. In conclusion, the flow-through diffusion cell was useful for examining the penetration of OPCs through the epidermal membrane. It was also demonstrated that the penetration rates of OPCs are strongly influenced by dilution in water and the receptor fluid composition.

1. Introduction

Organophosphorus compounds (OPCs) are produced for a variety of industrial applications, for example as petroleum additives and stabilizers for plastics and industrial oils. Some OPCs are highly toxic, primarily due to inhibition of the enzyme acetylcholinesterase. The enzyme inhibition results in an accumulation of acetylcholine, which causes hyper stimulation of the cholinergic receptors in the central nervous system and in the neuromuscular junctions. In worst cases, the intoxication results in life-threatening cholinergic crisis and respiratory failure (Moretto, 1998). Highly toxic OPCs were initially used as insecticides and were later developed to chemical warfare nerve agents (CWNA), such as tabun, sarin, soman and VX. Following exposure to OPCs, the most likely routes of entry into the body are *via* inhalation through the respiratory system, and *via* skin penetration upon dermal exposure. The dermal route is especially important for low-volatile agents, such as VX, malathion, and chloropyrifos (Marrs et al., 2006). Due to the high toxicity of OPCs, evaluation of skin penetration properties and toxicokinetic measurements cannot be performed in humans (Craig et al., 1977). Therefore, *in vivo* models in experimental animals and *in vitro* models on isolated skin have been developed for the evaluation of OPC skin penetration (Chilcott et al., 2003; Dalton et al., 2006; Godin and Touitou, 2007; Joosen et al., 2008; Mircioiu et al., 2013; van der Schans et al., 2003). Despite the various benefits of *in vivo* studies, regulatory authorities have considered *in vitro* test systems advantageous over *in vivo* models (OECD, 2004). Because of the limited access of human skin, *in vitro* skin penetration experiments are frequently performed utilizing animal skin, preferably from pigs (Barbero and Frasch, 2009; Marcarian and Calhoun, 1966; Simon and Maibach, 2000). Due to the similarities between pig and human skin, it is recognized that dermal exposure on pig skin *in vivo* is a relevant model for human exposures, although the relevance of this model has been questioned (Barbero and Frasch, 2009; Boudry et al., 2008; Chilcott et al., 2001; Dalton et al., 2006).

In *in vitro* studies the skin is normally mounted on top of a diffusion cell, typically the Franz cell chamber which is a static cell with a self-contained receiver compartment (Bartosova and Bajgar, 2012; Franz, 1975). Flow-through diffusion cells offer advantages over static cells concerning automatic sampling and maintenance of the sink condition throughout the experiment (Bronaugh and Stewart, 1985; Clowes et al., 1994). Depending on the addressed parameters, full-thickness skin (epidermis and the entire dermis), dermatomed skin (epidermis and part of dermis), or epidermal membranes can be studied in the *in vitro* experimental setup. The epidermal membrane, resting on the extracellular matrix of the dermis layer underneath, is considered the first barrier against chemical exposures. Within the epidermal

* Corresponding author at: Division of CBRN Defence and Security, Swedish Defence Research Agency, Cementvägen 20, SE-901 82 Umeå, Sweden.
E-mail address: lina.thors@foi.se (L. Thors).

http://dx.doi.org/10.1016/j.tiv.2016.01.003
0887-2333/

membrane, *i.e.* the stratum corneum, the external cornified layer of the epidermal membrane has proven to be the primary rate-limiting barrier to skin absorption of OPCs (Archibald et al., 1994; Benford et al., 1999; Craig et al., 1977; Yanagisawa et al., 2006).

In previous *in vitro* skin penetration studies, OPCs have been studied using a variety of experimental approaches. The skin penetration of CWNA has been studied using skin samples from different parts of the human body, with the abdominal skin being the most commonly used. Comparisons of OPC penetration through skin from different areas have demonstrated that hairy skin, taken from the scalp, is more permeable to OPCs than abdominal skin (Rolland et al., 2011; Rolland et al., 2013). Parameters possibly influencing the skin penetration of OPC have been examined and it has been shown that the physicochemical properties of the agents, the agent concentration, the hydrolysis of the agent, and the thickness of the skin samples all have an impact on the penetration rates of OPC. In addition, the choice of the experimental system may also influence the results (Griffin et al., 2000; Hawkins and Reifenrath, 1984; Lodén, 1985; Mircioiu et al., 2013; Nielsen et al., 2007; Sartorelli et al., 1997; van Hooidonk et al., 1980). The ability to predict the outcome of an *in vivo* skin penetration in humans has been examined with the conclusions that *in vitro* models are able to closely mimic the skin penetration of OPC (Boudry et al., 2008; Capt et al., 2007; Hawkins and Reifenrath, 1984). Due to the high toxicity of CWNAs, low-toxic OPCs have been used as simulants for CWNA in skin penetration experiments (Vallet et al., 2007; Vallet et al., 2008).

The aim of the present study was to examine the epidermal penetration of OPCs using an *in vitro* flow-through skin penetration compartment, designed according to the OECD guidelines (OECD, 2004). The evaluation was performed considering a number of parameters that influence the skin penetration of OPCs, such as the hydrophilic/lipophilic properties of the OPC, the agent concentration, and the composition of the receptor fluid beneath the epidermal membrane. Infinite dosing of the OPCs was used throughout all experiments. Human epidermal membranes were used in all experiments to avoid the extrapolation of animal data to human conditions. This approach enables a more precise understanding of parameters influencing *in vivo* penetration of OPCs through human skin.

2. Material and methods

2.1. Chemicals

O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX; >97% pure measured by NMR; CAS No. 50782-69-9) was synthesized at the Swedish Defence Research Agency (Umeå, Sweden). Triethyl phosphate (TEP; ≥99.8% pure; CAS No. 78-40-0), tripropyl phosphate (TPP; 99% pure; CAS No. 513-08-6), and triethyl phosphonoacetate (TEPA; 98% pure; CAS No. 867-13-0) and triisopropyl phosphate (TIPP; 95% pure; CAS No. 513-02-0) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl 2-acetoxyethylphosphonate (DAEP; 98% pure; CAS No. 39118-50-8) was purchased from Epsilon chime (Brest-Guipavas, France). The OPCs were prepared in a series of dilutions with deionized water: 1, 10, 20, 50, 75, 90, and 100% agent. Dilutions were freshly prepared prior to each experiment.

A mixture of ethanol and deionized water was used for the receptor solution. When investigating the impact of the receptor solution on skin penetration, four different receptor solutions were used: 1:1, 1:3 and 1:9 ethanol in deionized water (v:v) and one solution with only deionized water.

2.2. Skin preparation

Full-thickness human Caucasian skin, from the breast or the abdominal region, was obtained from plastic surgery after informed consent from the patients. The full-thickness skin was cropped from subcutaneous fat and connective tissue and then stored at −80 °C. Following acclimatization at −20 °C for 3–4 days, the skin was thawed at room temperature and small disks (0.6 cm^2) were punched out from the skin. The epidermis was excised from the underlying dermis by heat separation; after immersion of the skin disks in 60 °C water for 2.5 min, the epidermis was gently torn off with forceps and the epidermal disks were subsequently hydrated for at least 24 h at +4 °C before use. The amount of appendages in the diffusion area of the epidermis disk was determined; all epidermal disks exceeding one appendage in the diffusion area were discarded to avoid excessive penetration beyond the principal route of percutaneous diffusion. The average thickness of the excised epidermis was 104 ± 36 μm and was measured with bright field microscopy (results not shown). The separate experimental set-ups were performed with skin from the same donor patient.

This study was approved by the Research Ethics Committee, Faculty of Medicine and Odontology, Umeå University, Umeå, Sweden (No. 03–161).

2.3. Skin penetration experiments

The skin penetration experiments were performed using an *in vitro* skin penetration model, consisting of a flow-through receptor solution compartment, designed in-house according to the OECD guidelines (OECD, 2004). A schematic drawing of the diffusion chamber is shown in Fig. 1. The temperature of the diffusion chamber (AccuBlock Digital Dry Bath; Labnet Int. Inc., Edison, NJ, USA) was maintained at 32 °C throughout the experiments. Prior to all experiments the system was allowed to reach equilibrium in temperature and water balance. The receptor solution was continuously pumped through the diffusion cell at a flow rate of 20 μL min^{-1}, using a syringe pump (CMA 400; CMA Microdialysis, Kista, Sweden). The epidermis disk was mounted on the diffusion cell and a Teflon seal was positioned on top to achieve a closed system. The exposed area of the epidermal membrane and equally the diffusion area was 12.6 mm^2. The donor solution containing an infinite dose of OPC was added (25 μL) and samples from the receptor solution were collected every 10 min and kept at 10 °C in a fraction collector (CMA 470 Refrigerated Fraction Collector; CMA Microdialysis, Kista, Sweden) until the analysis. Before adding the donor solution, a zero sample of the receptor solution was collected. In order to ensure maximum recovery, a coverslip was placed on top of the Teflon seal following the agent application. The experiment was terminated after 300 min and all vials were sealed immediately after the experiment was completed. Each experiment was repeated six times. If the analysis was not performed instantly, samples were stored at −20 °C. The skin integrity was confirmed by post-experimental assessment of the data for all experiments. Experiments with disintegrated skin, identified from excessive flux through- the skin within the first 20 min of the experiment, were disregarded.

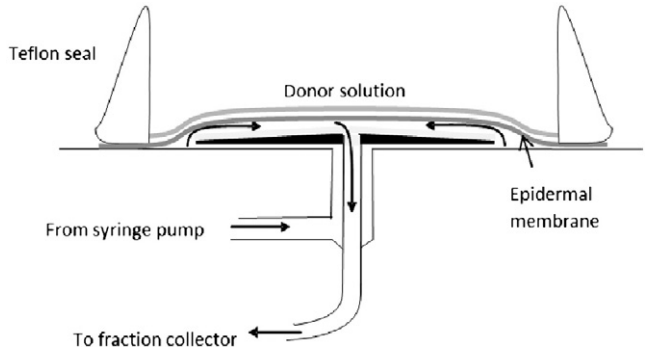

Fig. 1. A schematic drawing of the flow through the diffusion chamber. The epidermal membrane is mounted in the centre of the chamber. The receptor solution continuously flows from the outside edges of the exposed epidermal membrane towards the centre of the chamber carrying the penetrated sample to the fraction collector.