Toxicology in Vitro 30 (2015) 454–461

Contents lists available at ScienceDirect

Toxicology in Vitro

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

# Effect of exposure area on nerve agent absorption through skin *in vitro*

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ARTICLE INFO

*Article history:*
Received 9 June 2015
Received in revised form 14 September 2015
Accepted 17 September 2015
Available online 25 September 2015

*Keywords:*
Percutaneous penetration
Percutaneous absorption
Diffusion cell
Skin surface spread
Chemical warfare agent

ABSTRACT

Diffusion cells are used to determine the penetration of chemicals through skin *in vitro*. The cells have a limited surface area defined by the edge of the donor chamber. Should the penetrant spread rapidly to this containment limit the penetration rate can be accurately quantified. For the hazard assessment of small droplets of toxic chemicals, such as cholinesterase inhibitors, limiting skin surface spread *in vitro* could lead to underestimation of percutaneous penetration and hence underestimation of systemic toxicity *in vivo*. The current study investigated the dependency of the percutaneous penetration of undiluted radiolabelled nerve agents (VX and soman (GD), 10 μl) on skin surface spread (pig and guinea pig) using Franz-type glass diffusion cells with an area available for diffusion of either 2.54 $cm^2$ or 14.87 $cm^2$. Both VX and GD spread to the edge of the 2.54 $cm^2$ cells, but, not the 14.87 $cm^2$ cells over the study duration. Amounts of VX and GD penetrating pig and guinea pig skin in the 2.54 $cm^2$ cells were less than in the 14.87 $cm^2$ cells (except for GD under unoccluded conditions); however, penetration rates expressed per unit area were similar. Artificial limitation of skin surface spread *in vitro* does not impact percutaneous penetration *in vitro* as long as penetration is expressed in terms of mass per unit area.

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## 1. Introduction

Absorption through the skin is one of the major routes by which chemical warfare agents enter the body (Chilcott, 2007; Rice, 2007). The rate of diffusion through the skin and into the blood determines the length of time agent remains on the surface and nature and order of onset of toxic effects. The dosage of agent absorbed is dependent on the concentration of the agent on the skin, the area exposed and the duration that it remains on the surface (Wester and Maibach, 1993).

Static diffusion cells are used to determine the penetration of chemicals through skin *in vitro*. These chemicals may be chemical warfare agents or constituent parts of topically applied pharmaceutical formulations, being evaluated for hazard assessment purposes or for countermeasure development (e.g. decontaminants, protective clothing or medical countermeasures). The diffusion cell consists of a donor and receptor chamber in between which a skin is secured. The chemical or formulation of interest is applied to the surface of the skin in the donor chamber and percutaneous penetration quantified by measurement of chemical diffusing into the receptor fluid in the receptor chamber (Pendlington, 2008). Best practice for the type of skin preparation, receptor fluid and other parameters are outlined in OECD guidelines (OECD).

Static diffusion cells traditionally have a limited surface area defined by the edge of the donor chamber over which a penetrant may spread during a diffusion cell study. Should the penetrant spread rapidly to this containment limit the penetration rate can be accurately quantified. If chemicals are applied in vehicles, creams or ointments, these products are applied over the entire skin surface area within the diffusion cell, and spread to the containment limit before the experiment begins. However, for the hazard assessment of small droplets of toxic chemicals, such as cholinesterase inhibitors, limiting skin surface spread *in vitro* could lead to underestimation of percutaneous penetration and hence underestimation of systemic toxicity *in vivo*.

It is essential that any potential underestimation of percutaneous penetration by the *in vitro* diffusion cell be elucidated and quantified to ensure that hazard assessment and medical countermeasure development against percutaneous insult using this data be as accurate as possible.

The current study investigated the dependency of the percutaneous penetration of VX and GD on skin surface spread using both pig and guinea pig skin *in vitro*. The link between evaporation and skin surface spread was also investigated. It was hypothesised that for volatile chemicals, increasing skin surface spread would increase evaporation.

## 2. Materials and methods

The synthesis, use and destruction of chemical warfare agents in this study were conducted in accordance with the Chemical Weapons Convention (1996). $^{14}C$ radiolabelled pinacoylmethylfluorophosphonate (GD) and S-[2-(diisopropylamino)ethyl]-O-ethyl methylphosphonothioate (VX) were synthesised by Dstl (Dstl, UK) and had a radiochemical purity >97% (as determined by radiometric HPLC analysis). The chemical purity of unlabelled GD and VX was reported to be >97% (as measured by NMR). Both

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http://dx.doi.org/10.1016/j.tiv.2015.09.016
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radiolabelled and cold agents were mixed in appropriate proportions to give a nominal activity of approximately 0.5 μCi μl$^{-1}$.

Liquid scintillation counting (LSC) materials (soluene-350, Ultima Gold and opaque plastic vials) were purchased from PerkinElmer (Chandler's Ford, Hampshire, UK). All other chemicals were analytical grade and were purchased from Sigma-Aldrich (Dorset, UK).

The use of animals was conducted in accordance with the Animals (Scientific Procedures) Act 1986. Eight pigs (large white strain, weight range 50–60 kg) were purchased from a local supplier. Animals were group housed and given 24 h access to food and water. Each animal was sedated with Hypnovel® (Midazolam, 6 ml i.m., 5 mg ml$^{-1}$), anaesthetised with inhaled isofluorane (5%) and culled with an overdose of intravenous Euthatal™ (sodium pentobarbitol, 20 ml i.v., 200 mg ml$^{-1}$). The whole abdominal skin flank (approximately 40 × 30 cm) was excised from each animal. Sixteen guinea pigs (Dunkin–Hartley, weight range 350–450 g) were purchased from Harlan Interfauna, UK. Animals were culled by cervical dislocation. The whole skin (approximately 15 × 10 cm) was excised from each animal. Skins were stored flat between sheets of aluminium foil at −20 °C for up to 6 months prior to use. Prior to commencement of the study, skin

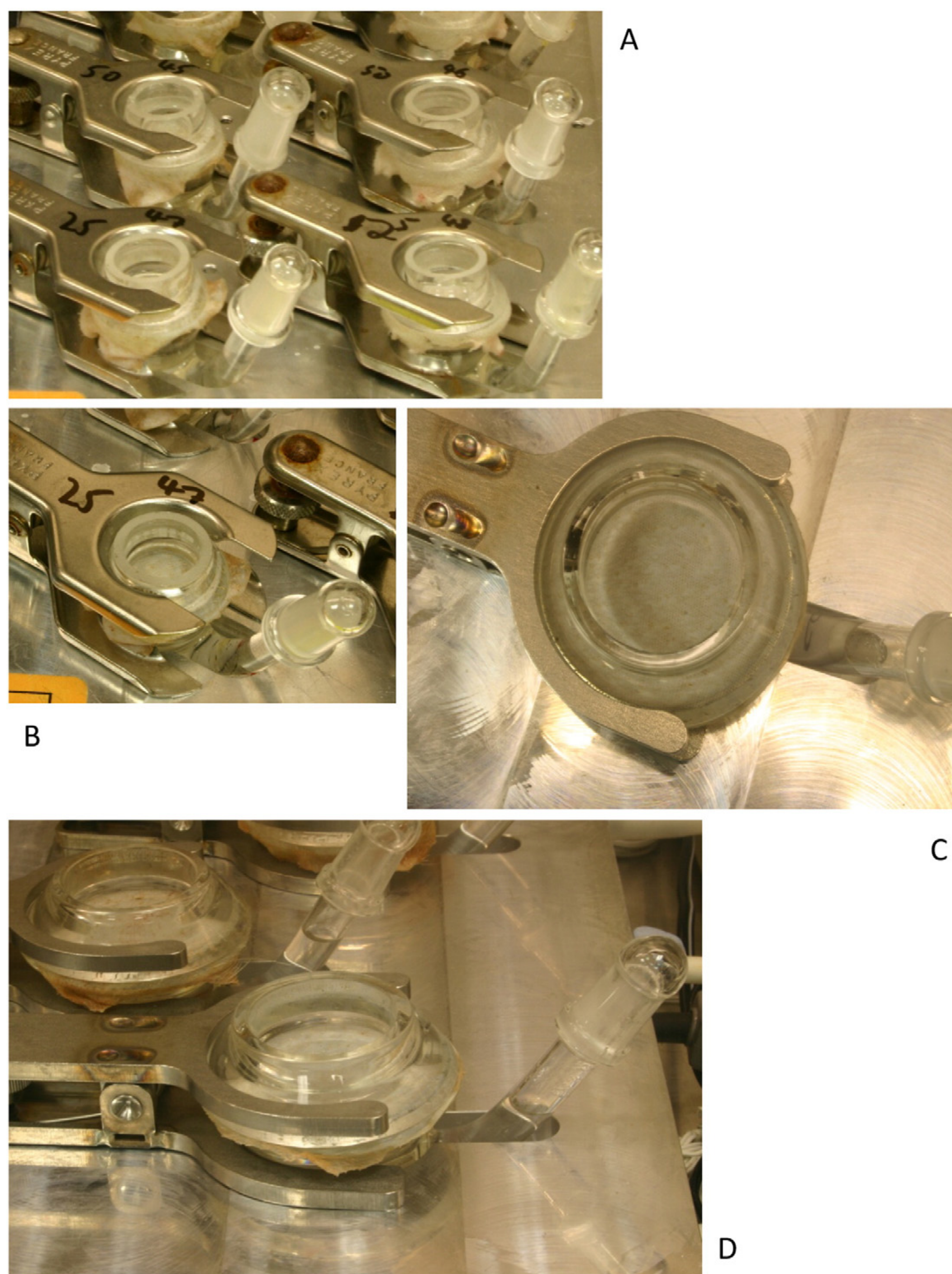


**Fig. 1.** "Small" (Panel A & B) and "large" (Panel C & D) type static diffusion cells. "Small" diffusion cells had a maximum area available for diffusion of 2.54 cm$^2$, "large" diffusion cells had a maximum area available for diffusion of 14.87 cm$^2$.

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