Toxicology Letters 219 (2013) 99–106

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

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

Evaluation of *in vitro* absorption, decontamination and desorption of organophosphorous compounds from skin and synthetic membranes

Constantin Mircioiu[a,b], Victor A. Voicu[a,c], Mihaela Ionescu[a], Dalia S. Miron[b], Flavian S. Radulescu[b], Adrian C. Nicolescu[d,*]

[a] *Army Center for Medical Research, Bucharest, Romania*
[b] *University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, Bucharest, Romania*
[c] *University of Medicine and Pharmacy "Carol Davila", Faculty of Medicine, Department of Clinical Pharmacology, Toxicology and Psychopharmacology, Bucharest, Romania*
[d] *Queen's University, Faculty of Health Sciences, Kingston, Ontario, Canada*

HIGHLIGHTS

- Organophosphorous compound membrane transfer was found to be a first-order process.
- The transfer depended on the toxicants' partition coefficient and the membrane type.
- Synthetic membranes may represent a model for the skin without the stratum corneum.
- Adsorptive powders inhibited the organophosphorous compound membrane transfer.

ARTICLE INFO

Article history:
Received 18 December 2012
Received in revised form 5 March 2013
Accepted 9 March 2013
Available online 18 March 2013

Keywords:
Adsorptive powders
Organophosphorous compounds
Skin protection
Stratum corneum

ABSTRACT

Chemical warfare agents, such as soman, and pesticides, such as chlorpyrifos, dichlorvos or malathion, are toxic organophosphorous compounds (OPCs) that are readily absorbed by the skin. Decontamination using solvents or surfactants may modify the cornified layer – the skin's main barrier against xenobiotic penetration. Thus, effective skin decontamination with fewer side effects is desired. We determined the membrane absorption, decontamination and desorption of toxic OPCs using human skin and synthetic membrane (cuprophane, cellulose acetate, methyl ethyl cellulose, acetophane and nylon) models, and estimated the efficacy of adsorptive powders (bentonite and magnesium trisilicate) at inhibiting this transfer. Using validated flow-through and static diffusion cell and HPLC methods, we found that the transfer of OPCs depends on their membrane affinity. The chlorpyrifos transfer decreased with a decrease in the membrane hydrophilicity, and that of malathion across hydrophilic membranes was less than half of that across hydrophobic membranes. We reliably modeled the toxicant transfer through the skin and synthetic membranes as first-order kinetic and/or square root law transfer processes, suggesting a potential application of synthetic membranes for predicting percutaneous absorption of OPCs. All tested adsorptive powders, applied either alone or as mixtures, significantly reduced the toxicant amount transferred across all membrane models, suggesting a potential therapeutic application with fewer later undesired effects on intact skin.

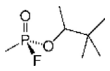
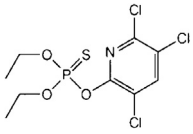
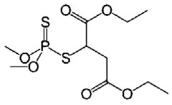
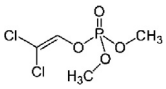
1. Introduction

Organophosphorous compounds (OPCs), such as soman, are used as chemical warfare agents (CWAs) despite many international conventions interdicting the chemical war. Therefore, it is important to understand the mechanisms of their percutaneous absorption and clinical effects, as well as to identify effective protective treatments (Maynard and Chilcott, 2009). There is a large consensus in the military non-medical community that showering represents a panacea for mass-casualty decontamination (Amlot et al., 2010). However, various reports show that lipophilic and other toxic compounds can accumulate in the stratum corneum, which becomes a reservoir for later undesired systemic effects (Hattersley et al., 2008). Decontamination with apolar solvents or surfactants may damage the corneous layer of the skin – the main barrier against skin penetration of all xenobiotics. Many novel active substances and pharmaceutical formulations, including perfluorinated polymers (Hobson and Braue, 2001), nanomaterials (Braue and Hobson, 2005), enzymes

* Corresponding author. Tel.: +1 6134499619.
E-mail addresses: nicolesc@queensu.ca, a.c.nicolescu@gmail.com (A.C. Nicolescu).

0378-4274/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.03.005

Table 1
Physico-chemical properties of the tested organophosphorous compounds.

	Soman	Chlorpyrifos	Malathion	Dichlorvos
Chemical structure				
Chemical name	O-Pinacolyl-methylphosphono-fluoridate	O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothionate	O,O-dimethyl-phosphorodithioate of diethyl-mercaptosuccinate	2,2-dichlorovinyl dimethyl phosphate
Appearance	Oily liquid	Solid	Yellow oil	Liquid
Water solubility (g/l)	21	0.002	0.13	10
log $P_{o/w}$	1.8	4.8	2.9	1.2
Density (g/ml)	1.02	1.51	1.23	1.42
Vapor pressure (mmHg)	0.4	1.8×10^{-5}	1.8×10^{-4}	1.6×10^{-3}

(Braue et al., 2002a), polyoxometallates (Braue et al., 2002b), metal complexes (Braue et al., 2002c), polymer coated metal alloys (Hobson et al., 2002a), reactive nanoparticles (Hobson et al., 2002b) or metal salts (Hobson et al., 2002c) have been tested with various outcomes for skin decontamination.

There are two major problems with studying percutaneous penetration of CWAs and testing novel antidote methods against CWAs. Firstly, the extremely high toxicity associated with these compounds and, thus, the high risk of intoxication for researchers. Secondly, the animal tests have been restricted following an international decision urging to find alternative tests to laboratory animals (ATLA). Although apparently a good correlation between *in vitro* and *in vivo* skin absorption models could be seen when the experimental conditions are similar (Vallet et al., 2007), the search for *in vitro* models for percutaneous absorption and the use of synthetic membranes become unavoidable. In addition, due to high toxicity of CWAs, related OPCs used as pesticides have been suggested as suitable surrogates for predicting the behavior of CWAs at the skin level (Vallet et al., 2007).

The present study characterized the transfer and accumulation of OPCs dichlorvos through skin, and chlorpyrifos, dichlorvos, malathion and the highly toxic soman through five synthetic membranes. The efficacy of adsorptive powders, as non-specific antidotes, at retention and desorption of these toxicants from membranes in order to reduce the risks of later undesired effects (Ionescu et al., 1997) has been also investigated. To our knowledge this is the first study that characterizes the transfer of chlorpyrifos through synthetic membranes. We found that the kinetics of the toxicant transfer followed a square-root law for both the synthetic membranes and the skin without stratum corneum. Overall, we found that the amount of the toxicant transferred depended on the nature of the toxicant and the membrane, and that both the hydrophilic and lipophilic adsorptive powders effectively reduced the transfer of all toxicants across membranes.

2. Materials and methods

2.1. Chemicals

The physico-chemical properties (Benschop and Wesselman, 1989; Boure and Vanholder, 2004; de Lara et al., 2006; Ionescu et al., 1996; Moffat et al., 2004) of the OPCs used in this study are presented in Table 1. Acetonitrile, methanol and magnesium trisilicate were purchased from Fluka, 98% sulphuric acid from Merck, clorpyrifos (Pestanal) from Sigma Aldrich, malathion from Carlo Erba, bentonite from Riedel-deHäen. The purified water had conductivity above 2.3 μS/cm. All reagents and solvents were of analytical grade.

2.2. Characteristics of the synthetic membranes and adsorptive powders

The characteristics of the membranes used in this study are presented in Table 2. The OPCs were applied on the membranes as such (*e.g.*, dichlorvos, malathion) or as aqueous solution (*e.g.*, chlorpyrifos, soman). The receptor fluid was aqueous solution (1 ml of 2.5 M H_2SO_4 in one liter of ultrapure water, pH of 3.2) and it was under the membrane. The composition and characteristics of the adsorptive powders used in this study are presented in Table 3. The previously weighed adsorptive powders were sprayed on the surface of the membranes 2 min after applying the toxicant (considered as time 0) resulting in a suspension in the donor compartment, as well as in a sediment of the powder onto the membrane surface. The donor compartment was then sealed to prevent the loss of volatile OPCs.

2.3. In vitro skin membrane studies

Fresh, viable human skin samples were obtained after femoral amputation in an orthopedic clinical unit with the full consent of each patient. All human donors were anonymous Caucasian males (age range 17–64). Skin specimens were kept at 4 °C until experimentation. On the day of the experiment, skin samples were warmed at room temperature and mounted on Franz-type static glass diffusion cells maintained at 39 ± 1 °C in a water bath. This assured that the temperature of the skin membrane was 32 ± 1 °C. The integrity of dermis, epidermis and stratum corneum was microscopically inspected before and after each experiment. Dichlorvos (0.3 ml) was applied to the surface of the isolated human skin and retained in the flow-through diffusion cells (receptor volume 12.6 ml, flow rate 2 ml/min). The evaluation of the percutaneous transfer included two phases: (i) skin permeation, measured over a 10 h period under flow-through conditions, and (ii) measurement of the concentration of the toxicant in the receptor compartment at 12 and 36 h, respectively, under static conditions. The skin was subsequently washed with water and stored in isotonic saline solution. The amount of dichlorvos released from the skin was determined at 24, 36 and 48 h.

2.4. In vitro synthetic membrane transfer studies

The *in vitro* system consisted of four to five Franz-type static, vertical glass diffusion cells with a capacity of 12 ml (Hanson Research) that were equipped with a thermostatic bath, an injection system and a magnetic stirrer with a helix in the receptor compartment. The temperature was maintained at 32 ± 1 °C with a circulating water jacket. Chlorpyriphos and malathion were applied onto the synthetic membranes as an aqueous solution (1.25 mg/ml) or as a liquid (0.3 ml), respectively. The donor compartment was adapted for the study of each toxic compound. In the case of the highly toxic soman, the experimental model consisted of four Franz diffusion flow-through cells, and the experiments were performed with special precautions under the hood, using diluted solution of soman (0.2 mg/ml) in the donor compartment. The receptor fluid was continuously supplied with a flow rate of 2 ml/min using a peristaltic pump. The volume of the receptor compartment was 12.6 ml, and the flow rate assured a total change of fluid in 6 min (Mircioiu et al., 1995; Ionescu et al., 2008). The transfer of water-insoluble compounds was studied using a flow-through cell. All membranes were equilibrated in the receptor fluid for 30 min, after which they were mounted on each cell according to the standard testing procedure.

Table 2
Characteristics of the tested membranes.

Membrane	Composition	Characteristics
Cuprophane	Type of hydroxicellulose	Hydrophilic
Cellulose acetate	Regenerated cellulose	Hydrophilic
ME Cellulose	Mixed esterified cellulose	Hydrophilic
Acetophane	Type of cellulose acetate	Hydrophobic
Nylon (Teknokroma)	Polyamide	Hydrophobic; thickness