Dermal Exposures to Organophosphorus Pesticides for Ambulance Workers - Permeation Through Disposable Gloves: Findings for Omethoate

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Abstract
Ambulance personnel may be exposed to organophosphorus pesticides during patient handling in scenarios involving accidental or intentional poisonings. While disposable gloves are routinely worn, there appears to be a significant knowledge gap with respect to the potential for dermal exposure, and mediating this, the level of protection afforded by disposable gloves. Research was conducted to investigate the influence of organophosphorus pesticide concentration, exposure duration and temperature on the amount of chemical permeating the gloves.

Omethoate, an organophosphorus pesticide typically used in horticulture and home gardens, was investigated. American Society for Testing and Materials (ASTM) permeation cells were used to test gloves used by South Australian ambulance workers; neoprene gloves (0.07 mm thickness) and nitrile gloves (0.11 mm thickness), individually and in combination. Chemical analysis was via High Performance Liquid Chromatography (HPLC-UV (220 nm)).

It was found that gloves provided limited protection against the full strength formulated omethoate at an elevated temperature (45°C). Maximum flux was 1093 (±154.1) µg/cm²/min (nitrile) and 132 (±27.9) µg/cm²/min (neoprene) over a 4-hour exposure period, and 52 (±6.7) µg/cm²/min for combined gloves. It is recommended that the current SAAS practice of combining nitrile and neoprene gloves continue, but considerations should be given on more frequent changing of gloves, especially when working in warmer conditions.

Introduction
Accidental chemical exposures and poisonings may have significant public health impacts, as well as placing logistic demands on response agencies (Edwards et al. 2003). In South Australia, it is estimated that there are 4 to 5 poisoning cases every month attended by ambulance workers, with an unknown proportion of organophosphorus pesticides (OP) cases (Casey, 2014, pers. comm.). The health risks associated with handling OPs are well identified and include inhibition of cholinesterase enzymes (Bey, Sullivan & Walter 2001; Eskenazi, Bradman & Castorina 1999; Holmggaard & Nielsen 2009). Skin contact with the OPs when ambulance workers attend to victims of OP accidents and poisoning cases may expose them to potential health risks (Figure 1). Therefore a policy to protect South Australia Ambulance Service (SAAS) ambulance workers with particular gloves for skin protection has been developed.
The Australian Defence and US Paramedical/Fire Services recommended double gloving method of neoprene gloves to prevent skin contact with chemicals whilst handling casualties (Stevenson, 2013, pers. comm.). In practice, however, due to cost factors, the South Australia Ambulance Service (SAAS) opted for nitrile gloves worn over neoprene gloves for this purpose (Figure 2).

There does not appear to be a detailed appraisal of the effectiveness of the gloves in resisting permeation, and the decision to use the double gloving method was most likely based on glove selection guides, which are based on glove tests conducted by manufacturers at room temperature only. Moreover, barrier protection may vary depending on the types of OPs and the concentrations on the glove surface. This is especially so if the formulated products come with co-solvents and additives that behave differently (Ehntholt, D.J, Bodek & Valentine 1989; Klingner & Boeniger 2002; Que-Hee 1989; Schwope & Goydan 1992).

This study was conducted to examine the resistance of nitrile and neoprene gloves (individually and in combination) to permeation against a typical OP in various conditions. It was assumed that; ambulance workers could be dealing with undiluted products; exposure time may vary (realistic maximum duration is 4 hours (Casey, 2014, pers. comm.); and exposure could occur at various temperature (normal ambient temperature or hot summer days).

**Methodology**

**Experimental design**

Nitrile and neoprene gloves used by SAAS workers were tested individually and in combination, on formulated omethoate. Omethoate is one of the OPs commonly used in Australia in both commercial and home garden situations, and it is classified as Chemicals of Security Concern in Australia by the Council of Australian Government (COAG) (Commonwealth of Australia 2013; Immig 2010). In order to mimic a real-life scenario, the gloves were tested with two concentrations of OP solution; full strength (814g/L) and application strength (0.6g/L). Temperatures selected for the tests were 23 (+2)°C for exposure in normal room temperature and 45°C to resemble the worst case scenario; extreme hot conditions that may be experienced in South Australia. For each concentration and temperature conditions, experiments were repeated four times to ensure reproducibility and reliability of the data.
Glove performance (permeation resistance) tests were conducted using American Society for Testing and Materials (ASTM) permeation test cells and disposable gloves as used by SAAS. The first type of glove was Sterling® Nitrile (powder-free exam gloves) (KC300, Ref 13941, Lot SM204230CLXX) produced by Kimberly-Clark. The second type was Micro-touch Affinity® Neoprene (non-sterile examination gloves) (C/No 0362, Ref 3772, Lot 12082134EQ) manufactured by Ansell.

In this study, glove swatches were cut out of the palm area of the gloves and the thickness was measured at several points. For this purpose, a digital thickness gauge (547-301, 0.01mm-10mm, Mitutoyo) was used. Thickness of nitrile gloves and neoprene gloves were 0.07 and 0.11 mm respectively.

A simple two-compartment cell (ASTM permeation test cell) was used to determine OP permeation, by sandwiching the glove swatches between the donor chamber and the receptor chamber of the cell. Cells exhibited a diffusion-available surface area of 5.31 cm$^2$ and a receptor compartment volume of 16.4 ml. Based on suitability and chemical solubility, pure MilliQ water was used as receptor fluid. Receptor fluid was continuously stirred with a modified stirrer connected to two 1.5V batteries at 350 rpm. Samples (200 µL) were taken from the receptor chamber at appropriate intervals during the experimental period and replaced with fresh receptor fluid. The experiments were either set-up in ambient conditions (23 (±2)°C ) or in an oven for 45°C.

**Chemicals**

Formulated omethoate with the brand name Folimat® from Ospray Pty Ltd (Queensland, Australia) containing 814 g/L omethoate and 400 g/L propylene glycol methyl ether acetate (PGMEA) as co-solvent was tested. This concentrated omethoate is hereto referred as ‘full strength’. Application strength omethoate (0.6 g/L) was prepared by diluting the full strength omethoate with pure MilliQ water. Analytical grade compounds (Fluka-36181 for omethoate, and 82300 for PGMEA) from Sigma-Aldrich was used for preparing standard solutions. Stock solution (25 mg/ml) was prepared in pure MilliQ water and stored in the fridge at 4°C.

**Apparatus**

All samples were analysed by High Performance Liquid Chromatography (HPLC) with the operating conditions adapted from Sartorelli et al. (1998). Omethoate concentration was determined by a GBC LC 1120 HPLC Pump connected to a PE Nelson 900 Series Interface and Shimadzu SPD-20A Prominence UV/Vis detector. A volume of 20 µl sample was directly injected into the Alttech Altitima (C18, 5 micron, length 150 mm, I.D 4.6 mm) separation column. The retention time for omethoate was 5.4 minutes. The software program used for peak integration was Perkin Elmer TotalChrom Navigator. The mobile phase was aqueous methanol 30:70 v/v (flowrate 0.5 ml/min) with the UV detector set to a wavelength of 220 nm.

**Calibration curve**

A calibration graph for determination of omethoate was obtained by diluting the stock solution with pure MilliQ water to 10 working standards. Their concentrations ranged from 0.01 – 100 µg/ml. To obtain a linear calibration curve, peak areas were plotted as a function of the concentration and the curve was used to determine the concentrations in the experimental samples. Good linear fits were obtained daily for accuracy, with correlation coefficient ranging from 0.9959 to 0.9999. The Limit of Detection (LOD) (3:1 signal to noise ratio) was found to be 0.01 µg/ml.

**Descriptors of permeation**

The following variables were used as descriptors for the ability of omethoate to permeate through the gloves into the receptor fluid: maximum flux, breakthrough time and cumulative permeation. Flux (µg/cm$^2$/min) refers to the speed or permeation rate of omethoate (µg) crossing a defined glove area (cm$^2$) in a set time (min). Breakthrough time (minutes) is based on the AS/NZS 2161 standard which defines that breakthrough only occurs when the flux reaches 1 µg/cm$^2$/min. Cumulative permeation (µg) is the amount of omethoate recovered in the receptor chamber after the 4-hour test duration. Calculation of these descriptors was based upon measurement of omethoate in the receptor chamber during the experimental period.

**Data processing**

As a dilution factor is introduced every time a 100 µL sample is removed from the receptor chamber and replaced by 100 µL fresh receptor fluid, all data on total permeation were corrected for these dilutions to avoid underestimating total permeation.
Results

Comparison of omethoate permeation through gloves under variable conditions.

Table 1 summarises the results of the glove performance (permeation resistance) tests under various temperature and application concentrations with the descriptors by glove permeation by omethoate.

**TABLE 1:**

Summary of glove test outcomes for omethoate under various temperatures and concentrations

<table>
<thead>
<tr>
<th>Type of gloves</th>
<th>Results</th>
<th>45°C/814 g/L</th>
<th>23 (±2)°C/814 g/L</th>
<th>45°C/0.6 g/L</th>
<th>23 (±2)°C/0.6 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrile</td>
<td>Breakthrough time (BT)</td>
<td>20 min</td>
<td>15 min</td>
<td>not achieved</td>
<td>not achieved</td>
</tr>
<tr>
<td></td>
<td>Average flux (µg/cm²/min) at BT</td>
<td>3.5 (±0.5)</td>
<td>1.3 (±0.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average concentration (µg/ml) at BT</td>
<td>6.8 (±0.7)</td>
<td>3.4 (±0.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average maximum flux (µg/cm²/min)</td>
<td>1093 (±154.1)</td>
<td>21 (±3.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average cumulative permeation after 4hrs (mg)</td>
<td>865 (±98.4)</td>
<td>23 (±3.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoprene</td>
<td>Breakthrough time (BT)</td>
<td>20 min</td>
<td>120 min</td>
<td>not achieved</td>
<td>not achieved</td>
</tr>
<tr>
<td></td>
<td>Average flux (µg/cm²/min) at BT</td>
<td>1.6 (±0.2)</td>
<td>2.5 (±0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average concentration (µg/ml) at BT</td>
<td>2.8 (±0.3)</td>
<td>54 (±10.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average maximum flux (µg/cm²/min)</td>
<td>132 (±27.9)</td>
<td>5.4 (±0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average cumulative permeation after 4hrs (mg)</td>
<td>115 (±16.3)</td>
<td>5 (±0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>Breakthrough time (BT)</td>
<td>50 min</td>
<td>not achieved</td>
<td>not achieved</td>
<td>not achieved</td>
</tr>
<tr>
<td>(nitrile on neoprene)</td>
<td>Average flux (µg/cm²/min) at BT</td>
<td>1.1 (±0.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average concentration (µg/ml) at BT</td>
<td>5.6 (±0.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average maximum flux (µg/cm²/min)</td>
<td>52 (±6.7)</td>
<td>0.8 (±0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average cumulative permeation after 4hrs (mg)</td>
<td>38 (±4.3)</td>
<td>0.5 (±0.04)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No breakthrough was achieved for application strength omethoate tested at both ambient and high temperatures for all gloves. However, for full strength formulated omethoate, the protection afforded by the gloves varied.

In general, higher temperature decreased the breakthrough time and permeation flux was higher. At ambient temperatures, nitrile recorded breakthrough after 15 minutes, neoprene 2 hrs and no breakthrough was observed for the combined gloves. Where breakthrough was achieved at higher temperatures, the average maximum flux was greatest with the thinner nitrile gloves (1,093 (±154.1) µg/cm²/min), followed by neoprene gloves and combined gloves (132 (±27.9) and 52 (±6.7) µg/cm²/min, respectively). Reduced flux rates of 21 (±3.6) µg/cm²/min for nitrile gloves and 5.4 (±0.5) µg/cm²/min for neoprene were evident at ambient temperatures. Similarly, flux for combined gloves was least at both temperatures. Cumulative permeation showed a similar pattern with values greatest for nitrile gloves (865 (±98.4 mg) and least for combined gloves (38 (±4.3) mg).

Although the breakthrough time for 23 (±2)°C/full strength formulated omethoate for nitrile gloves is shorter than the breakthrough time at higher temperature (45°C/full strength formulated omethoate), the permeated amount at the specified breakthrough time of the former (3.4 (±0.4) µg/ml) was only half of the latter (6.8 (±0.7) µg/ml). The flux for neoprene gloves were similar under both temperature conditions (1.6 (±0.2) µg/cm²/min for 45°C, 2.5 (±0.5) µg/cm²/min for 23 (±2)°C) (Table 1). When handling the full strength formulated omethoate, combined gloves may be suitable but only under ambient temperature conditions (Table 1 and Figure 3).
Permeation of full strength formulated omethoate through the gloves at ambient temperature, 23 (±2)°C over the 4-hour exposure period. Note: Breakthrough was not achieved based on AS/NZS 2161 standard.

It is also shown that wearing individual gloves (either nitrile or neoprene gloves) at ambient temperature provided less protection because permeation was observed as early as 15 minutes for nitrile and 120 minutes for neoprene (with an average maximum flux of 21(±3.6) and 5.4 (±0.5) µg/cm²/min respectively).

Figure 4 shows that at the higher temperature, protection afforded by combined gloves began to decrease after 50 minutes. In other words, elevated temperature resulted in shorter breakthrough times and enhanced permeation of pesticides through the gloves. Similar to the permeation pattern observed for ambient temperature, the average cumulative permeation of omethoate at the end of the 4-hour tests was more for nitrile gloves, followed by neoprene gloves and combined gloves.

**Independent behaviour of co-solvent**

Figure 5 displays the comparison of permeation of omethoate (the active ingredient) and PGMEA (the solvent used to mix the formulated product) using full strength formulated omethoate at high temperature.
FIGURE 5
Comparison of omethoate and PGMEA permeation through the gloves (individually and in combination) using full strength formulated omethoate at 45°C over the 4-hour exposure period.

Although the concentration of PGMEA is lower than omethoate in the formulated product, PGMEA permeated through the gloves into the receptor chamber much faster and in a greater amount for all conditions. Breakthrough times of PGMEA were 5 minutes (for nitrile gloves), and 10 minutes (for neoprene gloves). With combined gloves, the average cumulative permeation of PGMEA was six times more than omethoate at the end of the test (Figure 6).

FIGURE 6
Comparison of omethoate and PGMEA permeation through combined gloves (nitrile on top of neoprene) using full strength formulated omethoate at 45°C.
Discussion

There appear to be no comparable glove permeation studies of omethoate. While the selection of most gloves is based on general glove selection guides that generally test gloves in restricted conditions at room temperature, this study shows that glove effectiveness may vary depending on the type (material) and combination of gloves, exposure duration and environmental conditions.

Elevated temperatures decreased breakthrough time and resulted in higher cumulative concentrations of omethoate at the end of the 4-hour test. The lower toxicity co-solvent, PGMEA permeated through the gloves much faster and in greater amounts and may accelerate the permeation of omethoate. A previous study concluded that the concentration of the solvent in the formulations strongly affected the breakthrough and the total mass of material permeating the glove materials (Ehntholt, D.J et al. 1990). This suggests that co-solvents need to be considered when selecting an appropriate type of glove for skin protection.

Nitrile and neoprene gloves (used individually or combined) provide good protection at higher temperature conditions only for up to 4 hours when handling application strength omethoate. Although the use of full strength formulated chemicals could be an unlikely scenario in poisoning/suicide cases, the speed, severity, and extent of dermal absorption are relative to the formulation of the OPs (te Brake et al. 2012).

Average maximum flux for combined gloves was 65 times higher when the temperature increased from 23(±2)°C (0.8 (±0.1) µg/cm²/min) to 45°C (52 (±6.7) µg/cm²/min). With those rates, it was found that the average cumulative permeation of omethoate through the combined gloves after a 4-hour exposure were 0.5 (±0.04) mg and 38 (±4.3) mg respectively. For occupational risk assessment, the flux is often used to estimate the risk. Nevertheless, flux itself is insufficient to evaluate the toxicity profile of a pesticide after dermal exposure (Holmgård & Nielsen 2009).

Once a pesticide permeates the gloves, the next stage will be skin absorption of the OPs which may result in local effects on the skin or systemic effects. This, however, will be influenced by many factors. Under normal conditions, the rate of diffusion through the skin will depend on hydrophilicity (‘water-liking’) or lipophilicity (‘fat-liking’) as well as solubility, molecular weight and size of the compounds (Holmgård & Nielsen 2009). Besides, it also depends on the thickness of the skin membrane that the pesticide has to pass through, skin pores, anatomical location and skin condition (Semple 2004). Temperature may also affect solubility and diffusion coefficients of compounds, shorten the breakthrough time and consequently contribute to dermal absorption (Vahdat & Bush 1993; Zellers & Sulewski 1993).

In the worst case scenario, the cumulative permeated omethoate (865 (±98.4) mg) could potentially be absorbed through the skin at different rates and result in sensitisation or inhibition of cholinesterase enzyme. Although this is unlikely because of the limited contact time with the full strength formulated omethoate when wearing only one layer of gloves, extra precautions should be taken and the duration of wearing gloves in contact with pesticides should be minimized especially when working on hot days.

Although the thicker neoprene gloves provided better protection than the thinner nitrile gloves, the permeation resistance of individual gloves may appear different when tested with other OPs. Physicochemical properties (e.g. solubility, octanol-water partition coefficient (Kₕₒ), molecular weight) of the pesticides and the properties of the gloves (e.g polarity, quality) may all affect the permeation of OPs and the effectiveness of the different glove types. Composition, molecular size, and partitioning behaviour have been listed as further variables for consideration in permeation of this complex mixture (Lin & Que-Hee 1998).

Demonstrated breakthrough times do not necessarily represent safe limits for handling the pesticides. All findings and conclusions presented are based on the AS/NZS 2161 standard which defines that breakthrough occurs when the flux reaches 1 µg/cm²/min. However, observations have found that the pesticides have permeated the gloves earlier than this (based on Limit of Detection/Limit of Quantification 0.05). Although permeation under normal working conditions while wearing combined gloves is unlikely to be significant, extra precautions (e.g good personal hygiene practices) should be taken while handling high concentration pesticides in hot conditions.

There is a possibility of large discrepancies between products, even if they are produced by the same manufacturers, therefore it is reasonable to assume that each product may perform differently. It is worth noting that the tests conducted in the laboratory may not be directly applicable to the workplace environment because of the difference in conditions (Schneider et al. 1999). In this study, the gloves are tested out-of-box and only exposed to the test conditions, whereas gloves used by the ambulance workers in the real scenario might have decreased performance due to movement or flexing effects, as reported by Phalen and Wong (2012).
Conclusions & Recommendations
At ambient temperature, combined gloving seemed to provide adequate protection (breakthrough not reached) while handling full strength formulated omethoate for up to 4 hours. It is therefore recommended that the SAAS practice of wearing combined nitrile and neoprene gloves continue.

However, at elevated temperature (45°C), gloves provided limited protection against full strength formulated omethoate, even when worn in combination. Given the outcomes of enhanced permeation at elevated temperature, in combination with AS/NZS 2161 standard, consideration should be given to more frequent changing of gloves when working in warmer conditions. In practice, it is unlikely that gloves are in contact with the full strength formulated omethoate for long periods of time e.g. 4 hours. To better clarify the influence of varying temperature conditions on glove permeation of omethoate, further work is recommended at other moderate temperatures e.g. 30-35°C. Tests should also be conducted on other types and brands of gloves that might be used by ambulance workers in other states and countries, to establish a better understanding of these variables. Exploring the effects of movement on the permeation resistance may also provide more accurate information under worker-use conditions.

In addition, for future studies, glove permeation of other OPs of different physicochemical properties should be conducted. Work is currently underway assessing the ability of omethoate to penetrate human skin in vitro in order to better understand the potential risks.

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