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Synthetic membranes as an alternative to animal skin to investigate dermal permeation of chlorpyrifos †

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Abstract: Chlorpyrifos is a pesticide revised as dangerous for human health. While dermal permeation of chlorpyrifos is still poorly investigated, alternatives to animal and/or human skin are demanded. In this work, the suitability of synthetic membranes as alternative models to study dermal permeation of chlorpyrifos was investigated. Silicone and STRAT-M® membranes were tested on Franz cells using different receptor compositions. By adapting the concentration of ethanol in the receptor fluid, the results of chlorpyrifos permeation through both membranes were close to those found in human skin studies, supporting the use of those membranes as non-animal skin-equivalent models.

Keywords: Organophosphorus pesticide; skin permeation; polymeric membranes; alternative methods; environmental and occupational toxicology.

1. Introduction

The human body is exposed to air pollutants not only by inhalation but also by the dermal route. This exposure route is gaining increasing interest with some works reporting it as a relevant carcinogenic route [1].

Chlorpyrifos is a broad-spectrum pesticide revised by the European Food Safety Authority and by the Environmental Protection Agency as representing a risk for human health [2]. This pesticide is a lipophilic compound and we recently showed that the (aqueous) skin permeability coefficient is higher than previously reported [3]. In addition, there are also great differences in the experimental flux (J) of chlorpyrifos through *ex vivo* human skin, depending on the receptor fluid employed in the diffusion cell [3].

A few studies investigated the permeation of chlorpyrifos through the skin by either using *ex vivo* animal skin or human skin, but alternatives to animal and human skin urge for a more ethical mode of action in scientific research.

The Organization for Economic Cooperation and Development (OECD) provides guidelines defining the experimental conditions to be used when assessing the skin permeation of compounds [4,5]. The J and lag time (Tlag) are important permeation parameters defined in the OECD guidelines [4,5].

The purpose of this study was to test the suitability of two synthetic membranes as non-animal alternatives to study the dermal permeation of chlorpyrifos in human health risk assessment.

2. Materials and Methods

All chemicals were from Sigma-Aldrich, Fisher Chemical or Chem-Lab/Honeywell. STRAT-M® and silicone membrane were from Millipore and Lintec, respectively.

The permeation of chlorpyrifos through synthetic membranes was performed in static diffusion Franz cells [5]. The membranes – silicone and STRAT-M® - were mounted

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between the donor and receptor compartments with a permeation area of 0.64 cm². After membranes' conditioning, chlorpyrifos was applied in acetone at a dose of 400 μg/cm² (1 μmol/cm²), representing a similar dose to the one tested for the permeation of this pesticide in human skin of volunteers [6]. During the assay, the Franz cells were kept at 32 °C with an agitation of 600 rpm. Samples were collected for the pesticide analysis. Chlorpyrifos was quantified by reverse-phase HPLC (Agilent 1100) with a C18 column and detection at 225 nm. The mobile phase was acetonitrile and water (85:15) and the flux 1 mL/min.

3. Results and Discussion

One of the experimental conditions recommended by the OECD guidelines for lipophilic compounds, such as chlorpyrifos, is the use of 50% (v/v) ethanol in the receptor fluid [4]. However, there is no clear evidence that this percentage of ethanol is appropriate to reproduce human skin absorption of chlorpyrifos, so we decided to test different ratios of ethanol:saline in the receptor fluid of the Franz cells. The results are presented below.

3.1. The composition of the receptor fluid affects the permeation of chlorpyrifos

The permeation of chlorpyrifos through both the synthetic membranes was studied with different percentages of ethanol in the receptor fluid (10, 30, 40 and 50%). As shown in figure 1, the receptor composition influenced the permeation kinetics. Higher ethanol percentages in the receptor contributed to a faster permeation of the pesticide either through the silicone membrane (Fig. 1a) or through the STRAT-M® membrane (Fig. 1b). Consequently, at 8h – a time point simulating a work-shift - the quantity of chlorpyrifos that crossed the membranes was also higher for receptors richer in ethanol (Table 1).

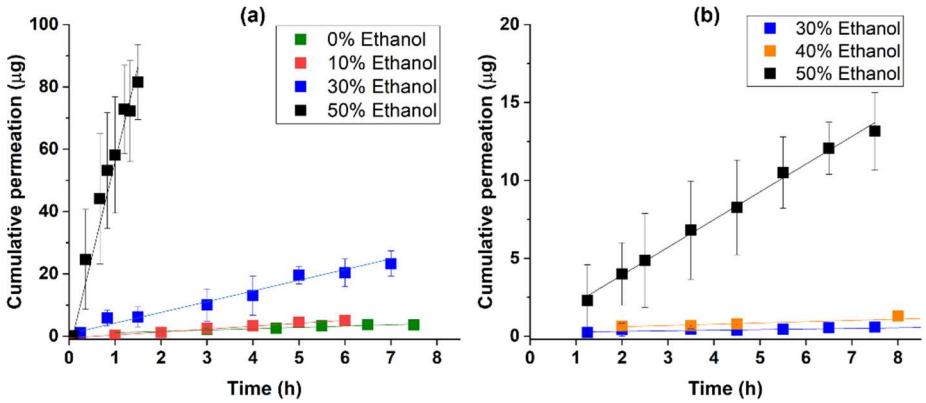

Figure 1. Cumulative permeation of chlorpyrifos through silicone (a) and STRAT-M® (b) membranes, using different concentrations of ethanol in the receptor fluid.

Table 1. Quantity (μg) of chlorpyrifos that permeated through the silicone and STRAT-M® membranes at 8h using different percentages of ethanol in the Franz cell receptor fluid.

Receptor Fluid	Chlorpyrifos permeating Silicone membrane (μg)	Chlorpyrifos permeating STRAT-M® (μg)
0% Ethanol	3.8 ± 0.4	ND
10% Ethanol	6.8 ± 0.8	ND
30% Ethanol	24.3 ± 6.8	0.6 ± 0.1
40% Ethanol	NA	1.3 ± 0.2
50% Ethanol	112.3 ± 4.3	13 ± 2.5

[ND] Not detected; [NA] Not assayed.

3.2 Flux and Tlag obtained for the chlorpyrifos' permeation through the membranes

The kinetics in figure 1 were used to calculate the parameters *J* and Tlag of the pesticide permeation. Flux values are represented in figure 2 for the different receptors

tested, showing their variation with the percentage of ethanol present in the receptor fluid. This effect is more pronounced for the silicone membrane. Regarding Tlag (Table 2), all the values obtained were inferior to 1h, including when the silicone membrane was tested with saline fluid (no ethanol) in the receptor. These results indicate that Tlag with the synthetic membranes is not influenced by the percentage of ethanol present in the receptor fluid.

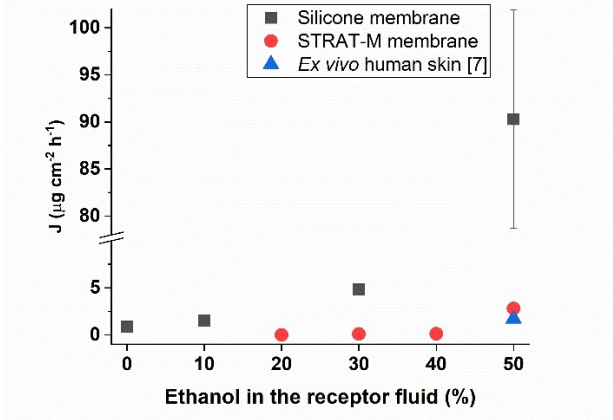

Figure 2. Graphical representation of the flux of chlorpyrifos' permeation through the synthetic membranes measured using different concentrations of ethanol in the Franz cell receptor fluid.

Table 2. Tlag of chlorpyrifos' permeation through the silicone and STRAT-M® membranes using different Franz cell receptor fluids.

Receptor Fluid	Tlag (h) for Silicone	Tlag (h) for STRAT-M®
0% Ethanol	0.2 ± 0.2	ND
10% Ethanol	0.6 ± 0.3	ND
30% Ethanol	0.3 ± 0.2	0 ± 0
40% Ethanol	NA	0 ± 0
50% Ethanol	0.05 ± 0.02	0.7 ± 0.5

[ND] Not detected; [NA] Not assayed.

3.3. Comparison of study results with chlorpyrifos permeation through human skin

To understand how synthetic membranes can be useful as alternative skin models, we compared the values of permeation parameters obtained in this work with those reported in [7] for the permeation of the pesticide through *ex vivo* human skin (Table 3).

Since the experimental conditions (ethanol in the receptor fluid) influence the kinetics of chlorpyrifos permeation through the membranes, we selected the flux and corresponding Tlag that best approximates the *ex vivo* human skin data [7]. In the case of the silicone membrane, this was achieved by using 10% of ethanol in the receptor fluid, while for STRAT-M® the closer values were obtained for 50% of ethanol in the receptor (Table 3).

Table 3. Comparison of the closer chlorpyrifos permeation parameters obtained in this work to the values obtained with *ex vivo* human skin.

Skin Membrane	Permeated chlorpyrifos at 8h (µg/cm²)	*J* (µg cm⁻² h⁻¹)	Tlag (h)	Reference
Ex vivo Human skin	13.4[1]	1.7 (range 0.98-2.45)	0	[7][2]
Silicone	10.6 ± 1.3	1.5 ± 0.1	0.6 ± 0.3	Our study[3]
STRAT-M®	20.3 ± 3.9	0.12 ± 0.02	0.7 ± 0.5	Our study[2]

[1]Value obtained from [7] after converting moles to grams and dividing per permeation area; [2]50% ethanol in the receptor fluid; [3]10% ethanol in the receptor fluid.

Althougth not identical, the results obtained in the selected conditions with each membrane afforded permeation parameters close to the values measured with ex vivo human skin (Table 3).

4. Conclusion

In this work, we have explored different experimental conditions using synthetic membranes as possible alternatives to animal and human skin when investigating the permeation of an organophosphorus pesticide. Both membranes in selected conditions could provide results close to *ex vivo* human skin. However, having in mind the goal of this study, the results achieved by the silicone membrane are more attractive in terms of the quantity of permeated pesticide and flux obtained when compared to *ex vivo* human skin.

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Data Availability Statement: Data is contained within the article or supplementary material.

Conflicts of Interest: The authors declare no conflict of interest.

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