The *in vitro* Assessment of Different Methods for Removal of Radiolabelled Biocide in a Solvent-Based Paint Formulation from Human Skin

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Introduction

In vitro absorption studies are used to estimate worker exposure to chemicals in biocidal paint formulations[1]. However, for an *in vitro* assessment, washing the skin surface with soap solution does not effectively remove the paint. This does not reflect the removal *in vivo* (i.e. in the field) as flecks of paint can be removed by a more mechanical action such as picking or thorough washing with industrial hand cleansers. In order to develop a more realistic operator exposure scenario *in vitro*, different decontamination methods were assessed in human skin[2].

Methods

Split-thickness human skin membranes were mounted into flow-through diffusion cells (Figure 1). A tritiated water barrier integrity assessment was performed[3]. For phase 1, the effect of the decontamination methods on the integrity of the skin was assessed. The paint containing non radiolabelled biocide (4%, w/w) was applied to skin surface at 10 μL/cm^2 and allowed to dry. At 8 h post dose, the paint was removed from the skin by one of the four methods below and the skin barrier integrity was measured again:

(A) Application of Simple® antibacterial soap and gentle rubbing (in accordance with the standard method)

(B) Picking off paint from skin using a micro spatula and tissue swab

(C) Application of Swarfega® solution and gentle rubbing

(D) Tape stripping using two or three successive 3M Scotch™ tapes.

For phase 2, the same commercial paint containing radiolabelled [^{14}C]-biocide (4%, w/w) was applied as in phase 1 to 16 human split-thickness skin membranes mounted into flow-through diffusion cells. At 8 h post dose, four skin samples were decontaminated with one of the four methods. Absorption was assessed over a 24 h period. At 24 h post dose, stratum corneum was removed by tape stripping and epidermis was separated from dermis by heated block. Samples were analysed by liquid scintillation counting.

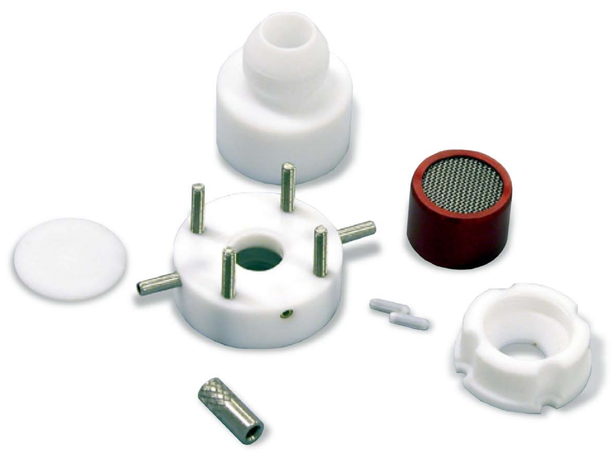
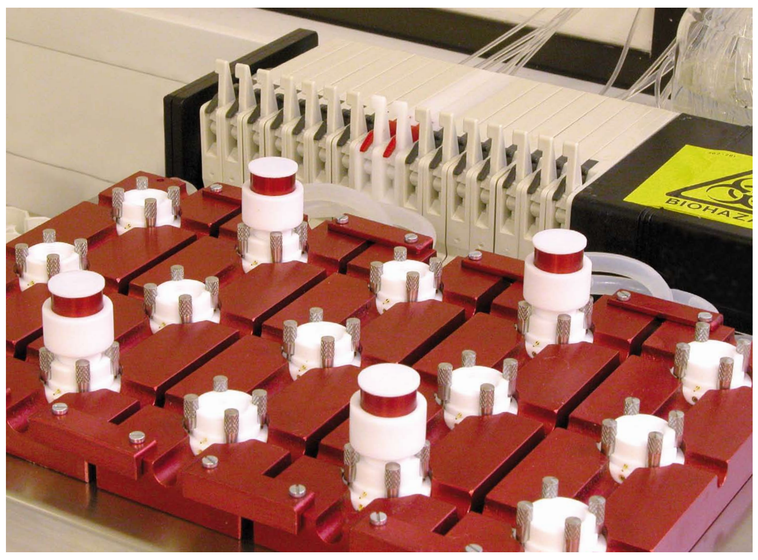


Figure 1. Flow-through diffusion cell



Results

For phase 1, the predose and 8 h barrier integrity test results were relatively similar for methods (A), (C) and (D). There was no reduction in barrier function for methods (A) and (C). For method (D), there was a small decrease (1.3-fold) in barrier function. Therefore, this indicated that these methods did not damage skin. Method (B) resulted in a 24-fold decrease in barrier function indicating that this method significantly ($P = 0.02$) damaged the skin.

The distribution results for phase 2 are presented in Table 1. The rank order of effectiveness of [^{14}C]-biocide removal as dislodgeable dose was D (538 μg equiv./cm^2) > B (386 μg equiv./cm^2) > C (89.4 μg equiv./cm^2) > A (78.6 μg equiv./cm^2). The cumulative absorption was relatively similar for the four test groups (Figure 2).

The rank order for dermal delivery and potentially absorbable dose was (D)<(A)<(C)<(B) (Figure 3). The individual components that contribute to dermal delivery and potentially absorbable dose (i.e. stratum corneum tape strips 6-25, epidermis, dermis and receptor fluid) are presented in Figure 4.

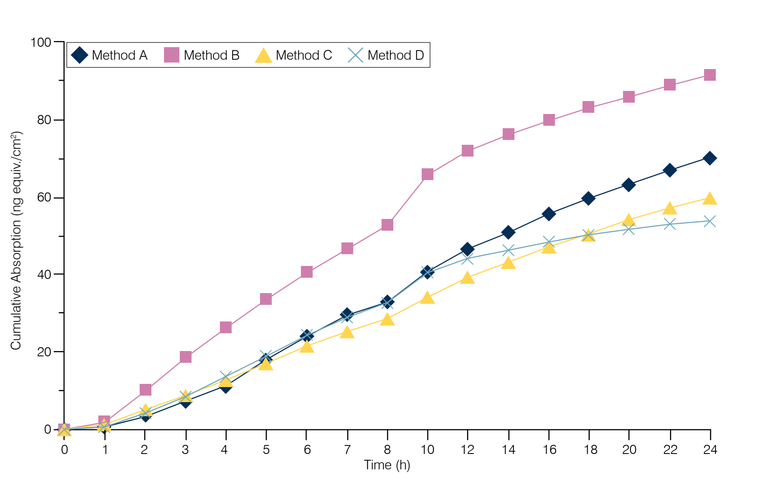


Figure 2. Cumulative absorption of biocide after topical application of [^{14}C]-biocide in paint to human skin in vitro. Decontamination method was performed at 8 h post dose. Each point is the mean of 4 samples.

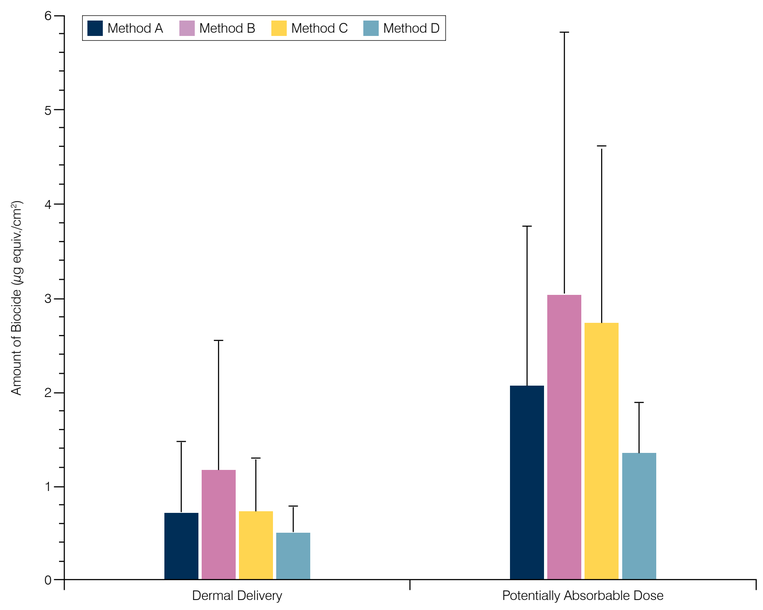


Figure 3. Dermal delivery and potentially absorbable dose of biocide after topical application of [^{14}C]-biocide in paint to human skin in vitro (Mean+SD, n = 4)

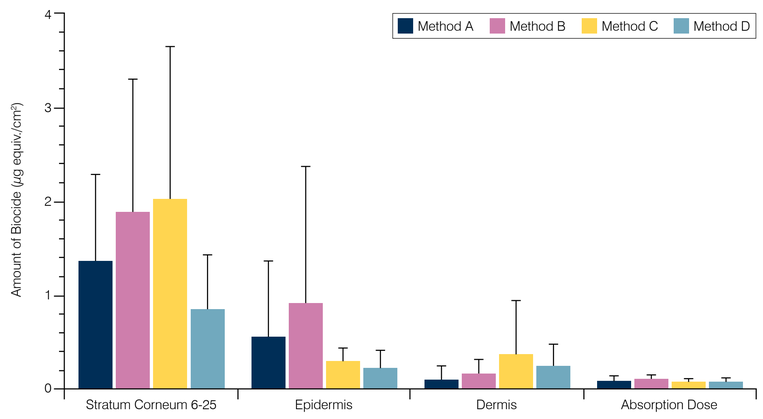


Figure 4. Amount of biocide in skin and receptor fluid after topical application of [^{14}C]-biocide in paint to human skin in vitro (Mean+SD, n = 4)

The paint picking method (B) *in vitro* was not considered to truly reflect picking *in vivo*. Picking paint from the skin *in vivo* was expected to be less harsh as paint would be much easier to access with fingers than paint on skin *in vitro*. Conversely, the tape stripping method *in vitro* was considered to be a more reflective model of paint removal by picking *in vivo* as this method had improved access to the paint on the skin in the flow through cells.

Removal Procedures	A Simple Soap	B Picking	C Swarfega	D Tape Strip
Dislodgeable Dose 8 h (% Applied Dose)	13.42	65.97	15.25	91.91
Stratum Corneum (% Applied Dose)	70.70	3.06	78.92	1.77
Unabsorbed Dose (% Applied Dose)	95.02	93.10	97.78	100.75
Absorbed Dose (% Applied Dose)	0.01	0.02	0.02	0.01
Dermal Delivery (% Applied Dose)	0.12	0.20	0.12	0.08
Potentially Absorbable Dose (% Applied Dose)*	0.35	0.52	0.47	0.23
Mass Balance (% Applied Dose)	95.14	93.29	97.90	100.84
Dislodgeable Dose 8 h (µg equiv./cm²)	78.63	386.41	89.35	538.39
Stratum Corneum (µg equiv./cm²)	414.16	17.90	462.29	10.36
Unabsorbed Dose (µg equiv./cm²)	556.59	545.34	572.77	590.19
Absorbed Dose (µg equiv./cm²)	0.07	0.09	0.06	0.06
Dermal Delivery (µg equiv./cm²)	0.70	1.15	0.71	0.49
Potentially Absorbable Dose (µg equiv./cm²)*	2.05	3.02	2.73	1.33
Mass Balance (µg equiv./cm²)	557.29	546.49	573.48	590.69

** Potentially Absorbable Dose = Stratum Corneum Tape Strips 6-25 + Dermal Delivery*

Table 1: Distribution of biocide (% applied dose and µg equiv./cm²) at 24 h post dose following topical application of [^{14}C]-biocide in paint to human split-thickness skin (n=4).

Conclusion

In conclusion, the tape stripping method was the most effective method in removing the paint. This is considered to be a representative model for skin decontamination for *in vivo* operator exposure.

References

1 OECD Test Guideline 428 (2004). Skin Absorption: *In Vitro* Method.

2 *Toxicological Risk Assessment Using In Vitro Dermal Models: Importance of Washing Procedures.* Stephen Madden, Clive Roper, John Biesemeier, James McBriarty and Klaus Rothenbacher. Conference Poster, ISSX, Sendai (2007).

3 Toner F, Madden S, Roper C. Maintenance of Human Skin Barrier Function for 1 Week in an *In Vitro* Test System. ISSX, Puerto Rico (2006).

Figure 5. Workmen painting the hull of a large ship

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