The *In Vitro* Percutaneous Absorption of Radiolabelled Biocide in a Single Solvent-Based Paint Formulation Through Human Skin

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Introduction

Since the introduction of OECD Guideline No. 428 in 2004[a], there has been considerable debate amongst the regulatory authorities[b][c] on how to deal with material found in the stratum corneum. Currently, the HSE[b], for example, has considered including 50% of the material associated with the stratum corneum in the dermal delivery (skin and receptor fluid) value. Clearly for test items, such as biocidal paints, that are not efficiently removed from the skin during the washing procedure, this will have a significant impact on the resultant safety assessment.

In this study, we have examined the dermal penetration of a biocide (mwt 349.5, $LogP_{ow}$ 5.85, pH 7) used in antifouling paints marketed by International Paint Ltd.

Methods

Biocidal paint, containing $[^{14}C]$-Biocide at *ca* 4.3% (w/w), was applied to 12 samples of human split-thickness skin from 5 different donors (following a tritiated water barrier with all samples having a water k_p <2.5 x 10^{-3} cm/h) at an application volume of 10 $\mu L/cm^2$. The skin remained unoccluded throughout. Absorption was assessed by collecting hourly fractions of receptor fluid, ethanol: water (1%, v/v), from 0-8 h and 2-hourly fractions from 8-24 h post dose. Exposure was terminated at 8 h by washing the skin with a dilute soap solution and drying with tissue swabs. This process did not visibly remove the paint from the skin. At 24 h post dose, i.e. after a 16 h post exposure monitoring period, the receptor chamber and lines were rinsed with receptor fluid, the skin was removed from the cell and radioactivity extracted from the donor chamber using methanol. The stratum corneum was removed with *ca* 25 successive tapes strips which were pooled in groups of 5. The paint was visibly removed within the first 5 strips. The exposed skin was separated from the unexposed skin. Representative mock dose samples, skin, pooled tapes and tissue swabs were analysed by combustion/ liquid scintillation counting. All other samples were analysed by liquid scintillation counting.
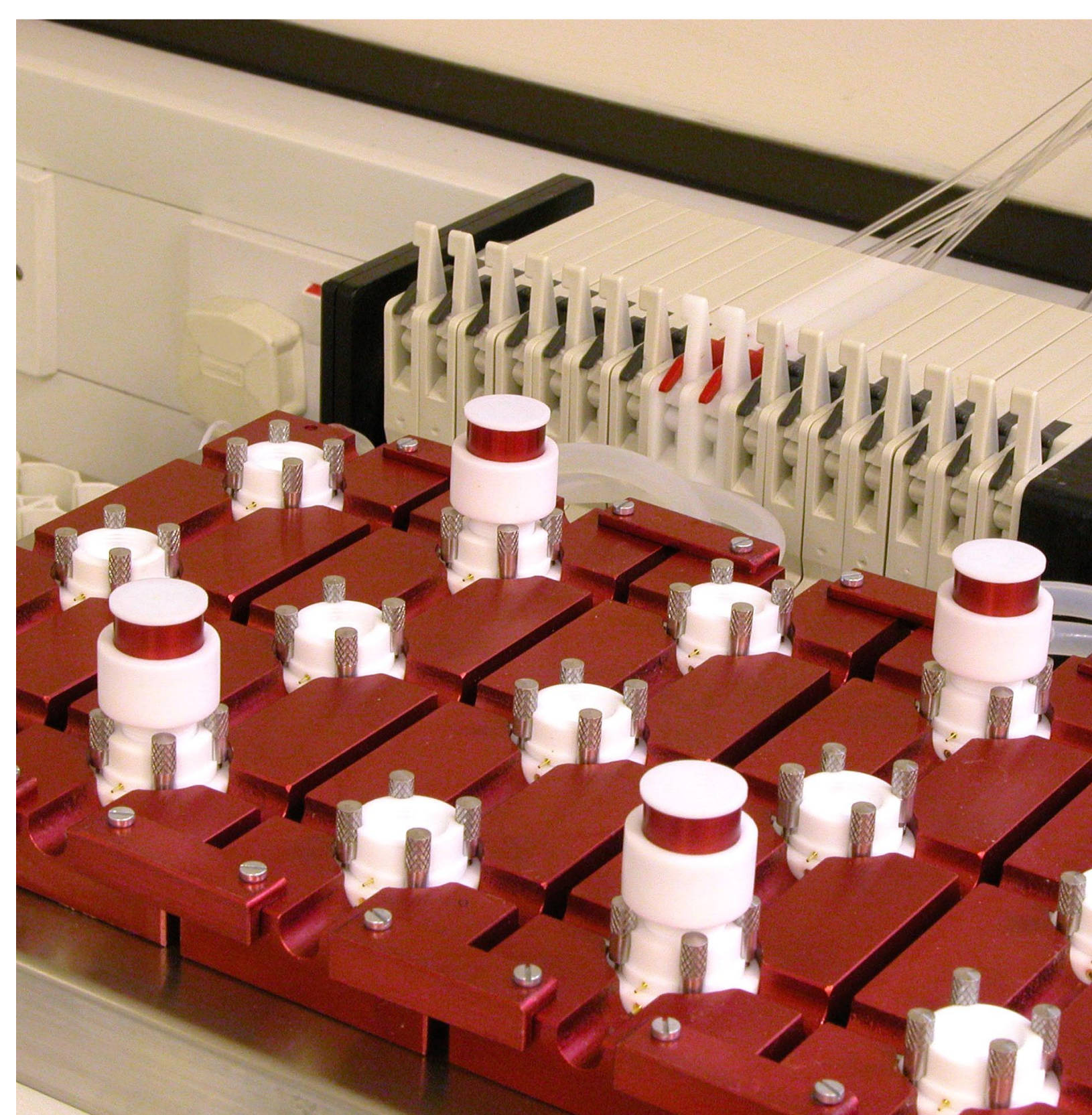
Flow-through diffusion cells

Results

Values are expressed as mean values (% applied dose). The mass balance was 101.64% (SD 3.50%). At 8 h post dose, the dislodgeable dose was 0.36% (skin wash: 0.23%, tissue swabs: 0.13%). At 24 h post dose, the donor chamber contained 4.96%, so total dislodgeable dose was 5.36%. The stratum corneum contained 95.34 (tapes 1-5, 6-10, 11-15, 16-20 and 21-25 contained 94.69%, 0.50%, 0.04%, 0.04% and 0.06%, respectively). This is shown graphically in Figure 1.

Total unabsorbed dose was 100.70%. The absorbed dose and dermal delivery were 0.78% (receptor fluid: 0.77%, receptor rinse: 0.02%) and 0.94% (absorbed dose + exposed skin: 0.15%), respectively. The lag time was *ca* 4 h. Steady state flux (0.25 μg equiv./cm²/h) was achieved from 10-24 h post dose. The absorption profile is presented in Figure 2.

The data are summarised as μg equiv./cm² in Table 1 and the absorption profiles presented in Figure 3 and Figure 4. The highest rate of absorption (0.30 μg equiv./cm²/h) was evident in the 2 h following the 8 h washing procedure.

Adding 50% of the total stratum corneum (47.67%) to dermal delivery, results in an unrealistic revised value for risk assessment of 48.61% since the rate of absorption into the epidermis/dermis and receptor fluid is so low. Taking a highly conservative approach, material in tape strips 16-25 or 11-25 may be considered as potentially absorbable, due to their closer proximity to the epidermis, resulting in revised values of 1.04% or 1.08%, respectively for dermal delivery. However, since the biocide remained within the stratum corneum after the 24 h study period, this suggests that even the biocide in the lower layers would be unlikely to pass through to the viable tissue. Operators using such antifouling products are extremely unlikely to leave paint on their skin for 24 h. Therefore, it is unrealistic to add the stratum corneum biocidal loading to the dermal delivery value.

	Mean	SD
Dislodgeable Dose	35.17	18.52
Stratum Corneum	626.02	29.73
Total Unabsorbed	661.21	23.95
Total Absorbed	5.15	2.45
Dermal Delivery	6.15	2.89
Mass Balance	667.35	23.01

Table 1. Distribution of $[^{14}C]$-Biocide (μg equiv./cm²) at 24 h Post Dose Following Application of a Solvent-Based Paint to Human Skin
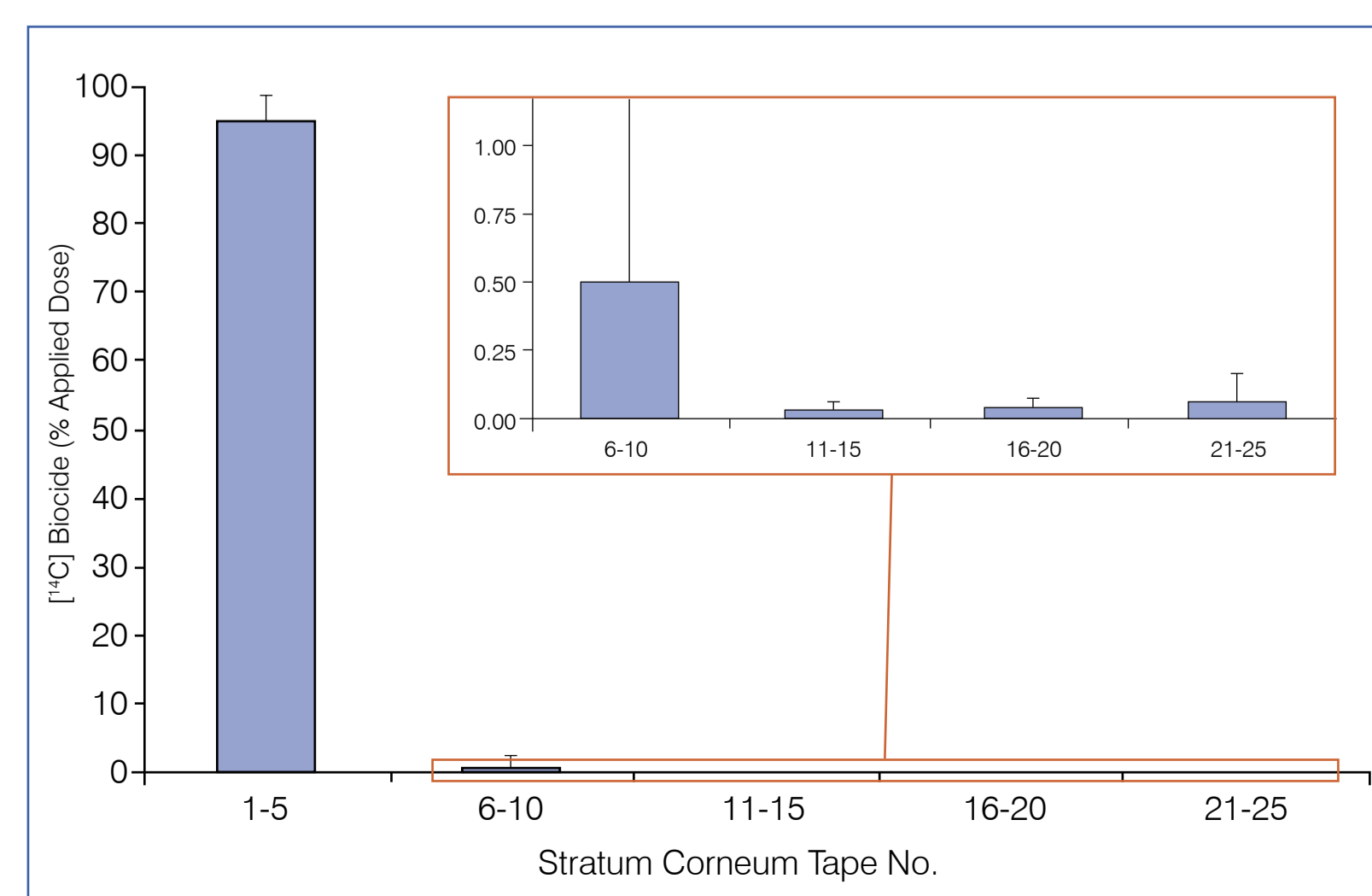

Figure 1. Distribution of $[^{14}C]$-Biocide in the Stratum Corneum (% Applied Dose)
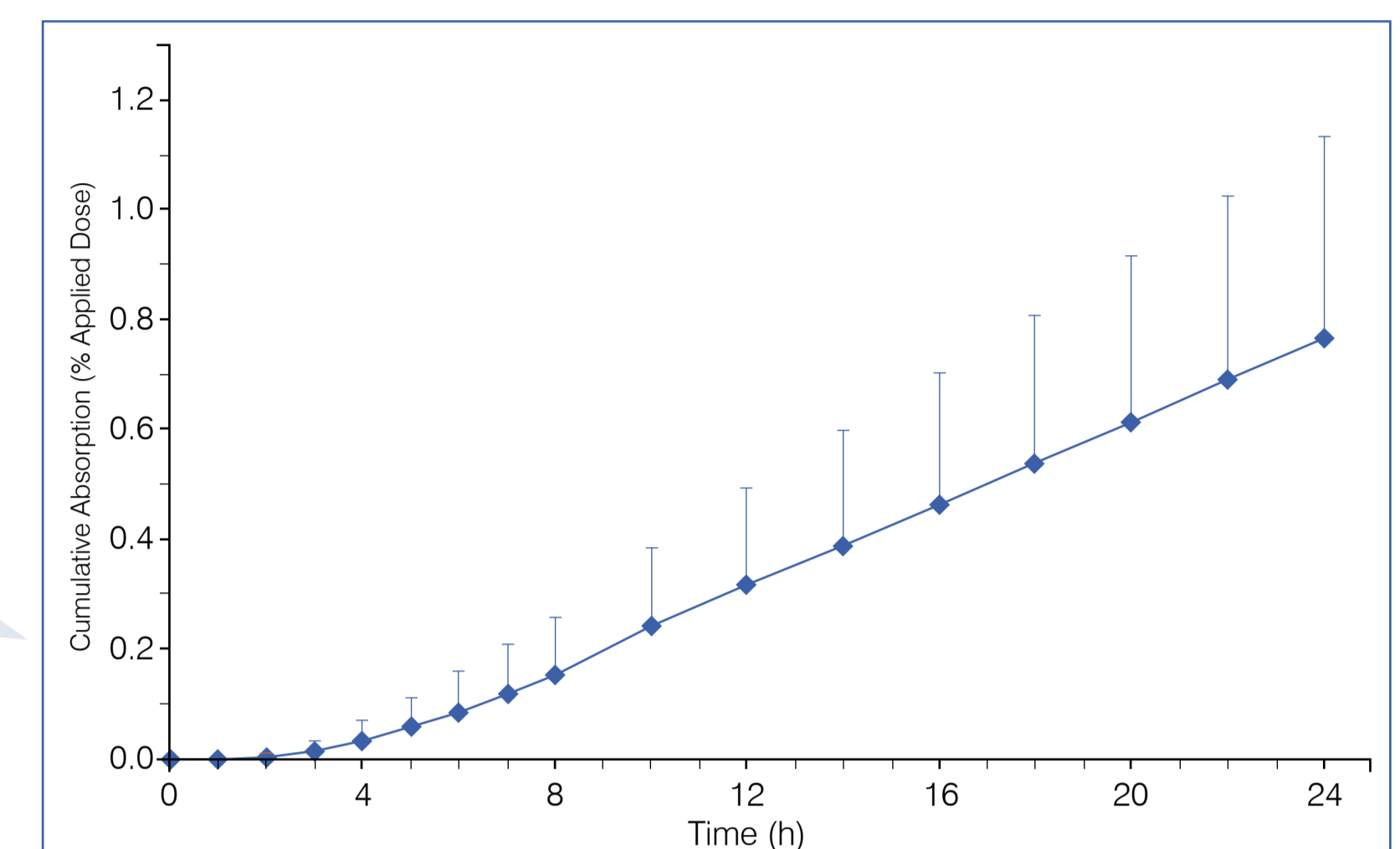

Figure 2. Absorption Profile of $[^{14}C]$-Biocide (% Applied Dose) in Receptor Fluid Following Application of a Solvent-Based Paint to Human Skin
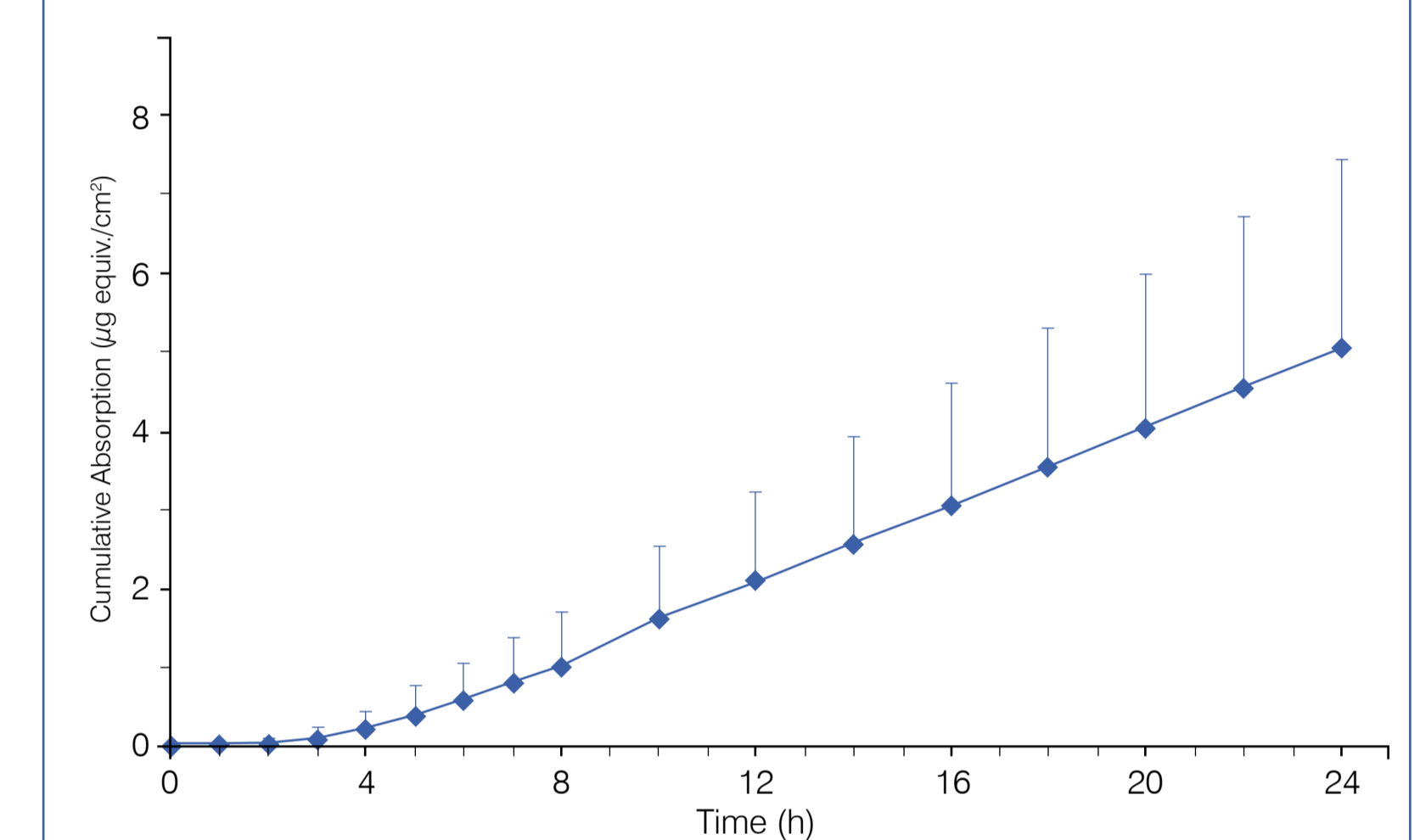

Figure 3. Absorption Profile of $[^{14}C]$-Biocide (μg equiv./cm²) in Receptor Fluid Following Application of a Solvent-Based Paint to Human Skin
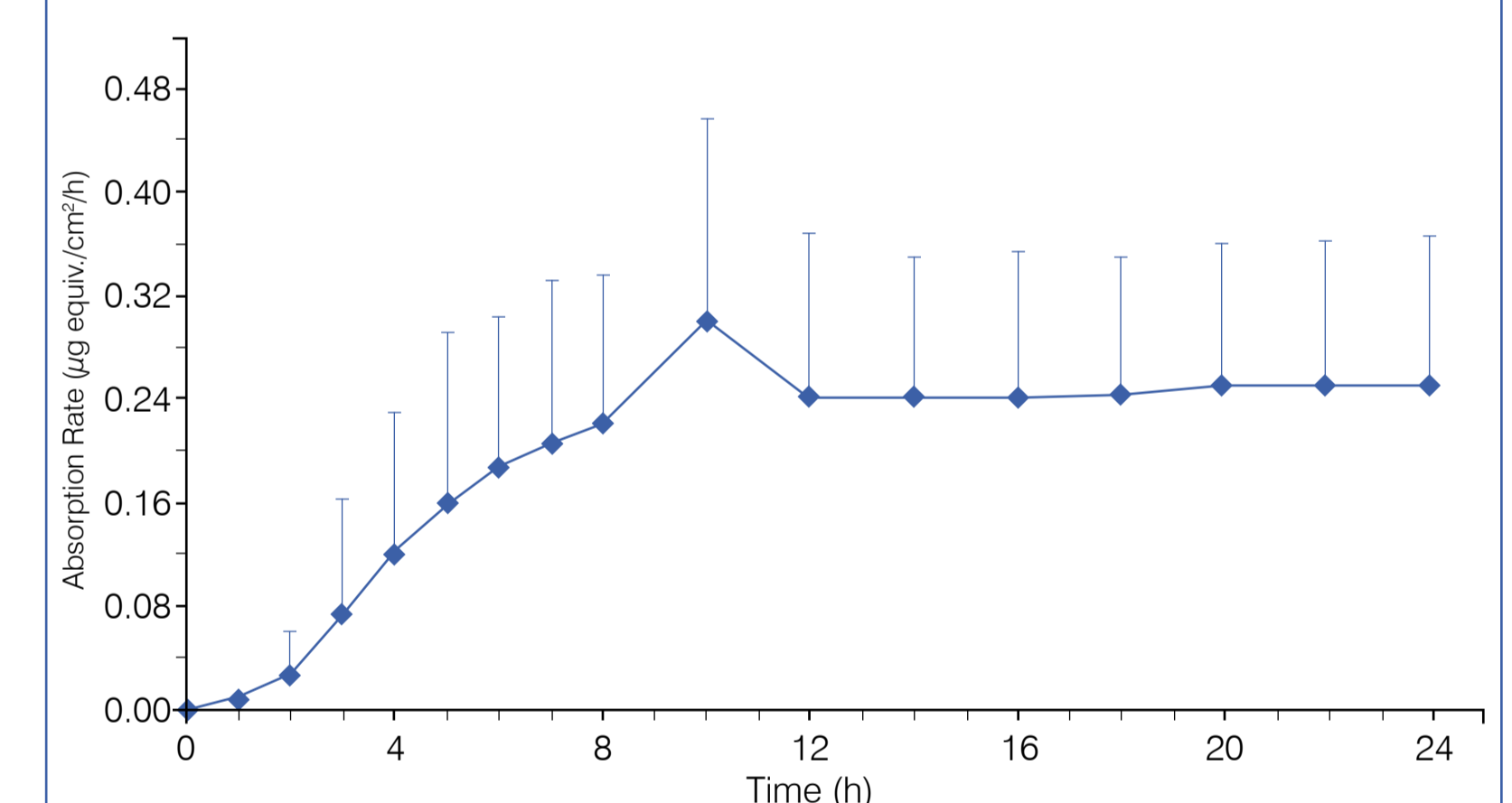

Figure 4. Absorption Rate Profile of $[^{14}C]$-Biocide (μg equiv./cm²/h) in Receptor Fluid Following Application of a Solvent-Based Paint to Human Skin

Conclusions

• The biocide did not readily penetrate through and into the skin.

• The stratum corneum acted as a good barrier to the biocide's penetration.

• Material associated with the stratum corneum should not be included in the determination of the systemic dose in a risk assessment.

• If a highly conservative approach is to be adopted and the material in the stratum corneum is considered as potentially absorbable, then only the lowest layers should be included.

References

(a) OECD (2004). OECD Guideline for testing of chemicals, guideline 428: skin absorption: *in vitro* method, OECD, Paris, France.

(b) Watch Committee (2005). Interpretation of results from *in vitro* dermal penetration studies. Watch/2005/16. HSE, Sheffield, UK. http://hse.gov.uk/aboutus/hsc/iacs/acts/watch/051005/16.pdf.

(c) KEMI, Sundbyberg, Sweden (2006). Personal communication between BO Lund and CS Roper.