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Triclosan: release from transdermal adhesive formulations and in vitro permeation across human epidermal membranes

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Abstract

Malarial resistance is an escalating global problem and consequently new and more efficacious treatments to combat malaria are urgently needed. The transdermal delivery of anti-malarials may provide an effective alternative or adjunct to conventional regimens. Triclosan is widely used as an anti-bacterial agent and it has recently been demonstrated that this compound has anti-malarial properties. Its high lipophilicity makes it a potential candidate for delivery across the skin and this paper examines in vitro the potential for the transdermal delivery of triclosan from 'drug-in-glue' formulations. Model patches were prepared using DuroTak® 2287, 2516 and 2051 acrylic polymer adhesives loaded with 0, 30 and 50 mg per 0.785 cm^2 triclosan and dissolution was measured over a 12-h period. There was no apparent difference between the adhesives at the 30 mg patch loading, but at 50 mg, the trend for increased release was 2051 > 2516 > 2287. No significant burst effect was apparent. Patches of 50 mg per 0.785 cm^2 were then used to determine the permeation of triclosan across heat-separated human epidermal membranes in Franz diffusion cells, over a period of 48 h. The above general trend was reflected in the steady state flux values obtained: 2051:16.91 μg cm^{-2} h^{-1} (S.E.M. 1.29), 2516:15.05 μg cm^{-2} h^{-1} (S.E.M. 1.00), 2287 12.83 μg cm^{-2} h^{-1} (S.E.M. 2.81). Although pharmacokinetic data are not currently available to permit calculation of an efficacious patch size, the transdermal delivery of triclosan is feasible. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Triclosan; Trandermal delivery; Malaria; Transdermal adhesive; Skin

1. Introduction

The protozoan disease, malaria, is an escalating world wide problem. The WHO estimates that 40% of the global population inhabit the 101 countries and territories where malaria is endemic. The prevalence of the disease is in the order of 300–500 million clinical cases per annum with 90% of these being in sub-Saharan Africa. It is predicted that 2.7 million people die from the disease each year (WHO, 1998). The development of multi-drug resistant strains of the parasite has made adequate disease control increasingly difficult. For example, in many areas of Africa and Asia relatively inexpensive drugs such as chloroquine and quinine are now ineffective. Mefloquine is an effective treatment and prophylactic but has been associated with severe adverse effects (British National Formulary, 2001). The

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high cost of other anti-malarials such as malarone (atovaquone and proguanil HCl) limits their use in the third world. These factors are necessitating the development of novel drugs and new treatment regimens in order to control the spread of the disease.

The synthetic 2-hydroxydiphenylether, triclosan, is a widely-used antibacterial and is a component of many consumer products such as toothpastes, facial washes, mouth-washes and detergents. Recently, it has been shown to inhibit the biosynthesis of Type II fatty acids by targeting the *Fab*I enzyme (enoyl-acyl carrier protein reductase) in bacteria (Hoang and Schweizer, 1999). Due to the presence of the *Fab*I enzyme in the plamodium parasite, triclosan can inhibit the growth of *P. falciparum* in vitro (Surolia and Surolia, 2001) and in a mouse model, *P. berghei* in vivo (Namita et al., 2001) by inhibiting the parasitic growth of trophozoites in the erythrocytic phase. In the mouse model, a single injection of 38 mg kg^{-1} triclosan completely cleared the parasite from circulation after 4 days and no side effects were seen up to doses of 40 mg kg^{-1}. Studies involving the incorporation of radiolabelled substrates into fatty acids suggest that triclosan may reduce the oxidation of NADH and inhibit fatty acid synthesis by promoting the binding of NAD^+ to the enzyme (Beeson et al., 2001).

Although no such data is in the literature pertaining to triclosan, many anti-malarial drugs have complex oral administration regimens and can cause gastrointestinal upsets. This has contributed to poor compliance and resistance of the parasite to the drug. Alternative methods of delivery could reduce this poor compliance and maximise the effectiveness of the drugs. Transdermal delivery has the advantages of reducing the systemic toxicity and side effect profiles, whilst also minimising the loss of drug due to first pass metabolism in the liver. A particular advantage of transdermal administration in respect of some anti-malarials (Perkins and Heard, 1999) is that gastrointestinal adverse effects can be avoided. Also, delivery of drugs via patches is useful if systemic toxicity develops as the patches are readily removed and therefore the effects seen are reversible. Release of the drug is controllable and zero-order absorption kinetics are obtainable (Florence and Attwood, 1998), enabling the systemic concentration of drug to be held within the therapeutic range. Overall, the ease of use would allow for a greater patient compliance especially where antimalarial prophylaxis is concerned. However, not all compounds make good candidates for transdermal delivery and only those of high potency and lipophilicity are usually suitable. Although the potency of triclosan against Plasmodium microorganisms is not known precisely, it has a high calculated $\log P$ 4.89 (http://www.lnh.unil.ch/~itetko/logP) which could limit its oral bioavailability yet make it a potential candidate for transdermal delivery.

In this paper we describe in vitro investigations into the plausibility of drug-in-glue patch-based systems for the transdermal administration of triclosan as a potentially useful new anti-malarial regimen.

2. Materials and methods

2.1. Materials

Triclosan was a gift from Reckitt Benckiser. Duro-tak® 387-2051, 387-2287 and 387-2516 transdermal adhesives were gifts from National Starch and Chemical, Holland. Buffer salts were obtained from Fisher Scientific, UK, as were HPLC-grade ethanol and methanol. Female breast and abdomen skin specimens (age range 32–38) were obtained post cosmetic surgery procedures and stored at −20 °C until use.

2.2. Preparation of model patches

Moulds for the model patches (Allender et al., 1998) were prepared by covering small plastic weighing boats with aluminium foil, the total area of the patches being approximately 6.25 cm^2. It was found that approximately 1.5 g of triclosan and adhesive mixture was required to produce a patch thickness of approximately 1 mm after evaporation of the solvent. In order to determine the maximum triclosan loading, masses of 1, 2 or 3 g were weighed on an electric pan balance and

added to 3 ml methanol in screw-cap vials then shaken until dissolved. The equivalent mass of 4.5 ml of the adhesives Duro-tak® 387-2051, 387-2287 or 387-2051 were added to the vials and repeatedly inverted to ensure a homogenous mixture was formed, but without the introduction of air bubbles into the formulation. The preformed moulds were tared on an electric pan balance and 1.5 g of the formulation was poured into the mould. The patches were covered and left overnight to allow the solvent to evaporate.

Preliminary experiments performed to estimate the maximum loading of triclosan in the patches showed that adding in excess of 3 g to 4.5 ml of the adhesives produced a matrix was inferior, being excessively tacky and fluid. A loading of 2.4 g was found to be optimal.

2.3. Dissolution of triclosan from model patches

The aim of this experiment was to observe the differences in the release rates of triclosan from the three different adhesives Durotak® 387-2051, 387-2287 and 387-2516. Patches were prepared at two different loading doses, approximately 50 mg per 0.785 cm^2 (1 cm diameter, total triclosan loading for each 6.25 cm^2 patch approximately 380 mg), and 30 mg per 0.785 cm^2 (1 cm diameter, total triclosan loading for each 6.25 cm^2 patch approximately 235 mg). Table 1 shows the methods used in order to make to two different strength solutions with the three different adhesives. The differing density of each adhesive was taken into account. Control patches were also prepared by adding 3 ml methanol to 4.5 ml adhesives but without the addition of triclosan. To each of the pre-prepared aluminium foil covered moulds, 1.5 g of the solutions were added. The patches were covered and left for 48 h to allow the solvent to evaporate. The depth of the resulting film was 1 mm.

The patches were then removed from the polystyrene moulds and inserted into individual screw cap vials containing 30 ml ethanol:PBS 25:75, filtered and degassed prior to use. The vials were placed on a Stuart Scientific gyro-rocker (Fisher, UK) set at 35 rpm to ensure adequate mixing of the dissolution medium. At time intervals of 5, 30, 60,120, 240, 350, 440 and 720 min, 0.5 ml of dissolution medium was sampled and placed into glass vials. The receptor phase was replenished with 0.5 ml fresh dissolution medium after each sample was taken. Samples were analysed by UV spectrophotometry (Section 2.6) and two replicates of each patch were used in this experiment.

2.4. Preparation of human epidermal membranes

The skin samples were thawed to room temperature and the subcutaneous fat removed by blunt dissection using a combination of razor blades and scissors. The skin was cut into approximately 2.5 cm^2 pieces and immersed in de-ionised water at 60 °C for 60 s. The epidermis was then carefully peeled away from the dermis using surgical forceps. With the stratum corneum uppermost, the epidermis was floated onto aluminium foil and stored in a crease-free state at −20 °C until required.

Table 1
Formulae used to prepare model patches

	387-2051	387-2051	387-2287	387-2287	387-2516	387-2516
	50 mg per 0.785 cm^2	30 mg per 0.785 cm^2	50 mg per 0.785 cm^2	30 mg per 0.785 cm^2	50 mg per 0.785 cm^2	30 mg per 0.785 cm^2
Triclosan (g)	2.4	1.3	2.3	1.2	2.3	1.2
Methanol ml (g)	3 (2.36)	3 (2.36)	3 (2.36)	3 (2.36)	3 (2.36)	3 (2.36)
Adhesive ml (g)	4.32 ($\approx$4.5)	4.32 ($\approx$4.5)	4.05 ($\approx$4.5)	4.05 ($\approx$4.5)	3.96 ($\approx$4.5)	3.96 ($\approx$4.5)

2.5. Permeation of triclosan across human epidermal membranes from model patches

The epidermal membranes were thawed to room temperature, refloated on de-ionised water and taken up onto a filter paper support. Excess water was removed from the surface of the skin by gently dabbing with lint-free tissue paper. About 1 cm diameter patches were excised using a cork borer, firmly affixed to the centre of the skin specimen then mounted in individually calibrated Franz-type diffusion cells. The donor chamber, with pre-greased flange, was placed over the patch onto the membrane (nominal diffusion area 1 cm diameter) and the entire assembly inverted. The filter paper was then carefully removed and lower surface of the skin wiped dry before attachment of the receptor compartment. Receptor compartments (nominal volume 2.4 ml) were filled with receptor phase (filtered and degassed ethanol:PBS 25:75) and a small magnetic follower added. The cells were then placed on a magnetic stirring plate (Camlab, UK) immersed in a water bath maintained at 37 °C. At pre-determined time intervals aliquots of 200 μl were removed from each receptor compartment and placed in glass autosampler vials, sealed and stored at −20 °C prior to analysis by HPLC (Section 2.7). The receptor compartments were replenished with the receptor solution, which was maintained at 37 °C. Eight replicates from three different donors were used for each patch loading. Steady state flux, J, was determined from the gradient of a linear regression of the resulting permeation profiles, and permeability coefficient, K_p obtained by dividing J by C (concentration per cm^3 patch). Lag times were determined by extrapolating the linear portion of the graph, representing steady state flux, to the x-axis.

2.6. UV spectrophotometry

Samples were analysed at 281 nm using a PU8620 UV/VIS/NIR Spectrophotometer and Hellma Precision Quartz Glass Suprasil 6100-Blue cells (10 mm light path). The reference cell was filled with 2 ml ethanol:PBS 25:75 and the absorbance set at zero. Each 0.5 ml sample was diluted to 2 ml with ethanol:PBS 25:75 and the absorbance read. A set of standard solutions of triclosan in ethanol:PBS 25:75 (30, 20, 10, 5, 1, 0.5 μg ml^{-1} and 50 ng ml^{-1}) was also prepared and determined and used to determine the amount and percentage triclosan released and the data plotted as a function of time.

2.7. Reverse-phase HPLC analysis

Analysis was performed using a Thermo Separation Product Spectra System HPLC chromatograph (AS3000 pump, UV3000 detector, AS3000) fitted with a Sphericlone 3 μm ODS column (100 × 3.2 mm). The UV detection was at 281 nm and data was acquired using TSP PC1000 software on an Aztec Pentium 133 PC. The mobile phase composition was 25% water:acetic acid (98:2)/75% acetonitrile:acetic acid (98:2). Before use the mobile phase was filtered and degassed under vacuum using 0.2 μm nylon filter. The injection volume was 50 μl and the flow rate was set at 1.0 ml min^{-1}. Under these conditions, a retention time of approximately 7.5 min was obtained. Standard solutions of triclosan were prepared in the range of 0.04–10 μg ml^{-1} and analysed to produce a calibration curve.

3. Results and discussion

3.1. Dissolution from model patches

Cumulative release profiles of triclosan from the three different adhesives at 0, 30, and 50 mg triclosan 0.785 cm^{-2} are shown in Fig. 1a–c (normalised for receptor volume and patch area). No major initial burst of triclosan release was observed suggesting a uniform distribution of triclosan within the patch matrix. In some cases, eg for 2287/30 mg loading, the release profile was approximately linear indicating the near zero-order kinetics. The release from 50 mg patches was, as expected almost twice that from the 30 mg patches. Inter-patch variability was low as indicated by the small error bars. The release in most cases began to plateaux due to depletion effects, rather than receptor saturation (solubility of triclosan 245.2 ± 3 μg ml^{-1} at 32 °C).

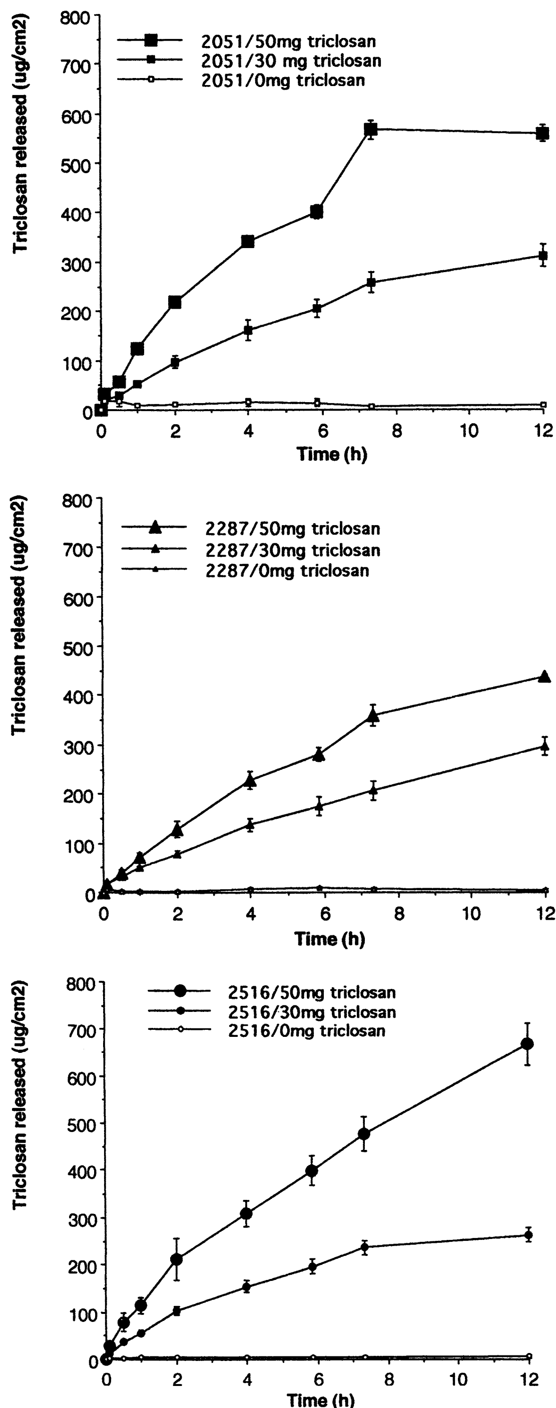

Fig. 1. Release of triclosan from three transdermal adhesives (upper 2051, middle 2516, lower 2287) at loading: 50 (triangle), 30 diamond and 0 (square) mg 0.785 cm^{-2}, $n=2$, $\pm$S.E.

The percentage of the loading dose released from each patch was determined to allow for slight variation associated with the patch preparation and to facilitate comparison of the data (Fig. 2a–b). For the 30 mg loaded patches there was no significant difference in the percentages of the loading doses released from the three adhesives. However, Fig. 2b shows a clear numerical difference between the patches and the greatest release was from 2051 (50 mg patches 1.029% after 12 h) and the lowest from 2287 (50 mg patches 0.728% after 12 h). These differences are probably attributable to differential affinities of triclosan for the adhesive matrices. Adhesives 2287 and 2516 both have OH functionality, whereas 2051 has COOH—the greater release from the latter suggests triclosan is less strongly bound to the COOH functionalised matrix.

3.2. In vitro permeation across heat separated epidermis from patch formulations

Permeation profiles are presented as cumulative percentage of the initial dose of triclosan from the three adhesives (Fig. 3). Linear profiles indicated sink conditions during the experiment and generally small error bars indicated good reproducibility between donors and maintenance of the barrier integrity. The profiles show behaviour representative of permeation across the skin. The lag times were determined by extrapolating the linear portion of the plot, representing steady state flux, to the x-axis. The lag times seen are relatively short indicating that steady state flux is achieved rapidly and the rapid uptake from transdermal patches followed by rapid permeation is a highly desirable characteristic to ensure rapid attainment of therapeutic plasma levels. Zero-order kinetics occurs when the rate of flux is constant and independent of the concentration of the drug (steady state) and is evident that after 6 h. Steady state was maintained to the completion of the experiment (24 h).

Table 2 shows the steady state flux and permeability coefficients along with the standard deviations (S.D.) and standard error (S.E.) of the mean for each formulation. In each case, steady state flux was achieved by 6 h. Analysis of variance

(ANOVA) statistics performed on the flux values gave $P = 0.052$, demonstrating statistically significant differences but just outside the 5% level. Fig. 4 is a histogram of the steady state fluxes and it can be seen that the rank order of the skin permeation was the same observed in the release experiment: 2051 > 2516 > 2287. This indicates that the efficiency of the release from the patch is an important determinant of permeation in such systems, especially as the high lipophilicity of triclosan enables it to readily partition into the stratum corneum (Guy and Hadgraft, 1989). The greater the release from the patch the larger is the concentration gradient across the skin and the more rapid the permeation.

By 24 h, only some 0.7% of the total applied dose had permeated the skin, which is a feature of this type of delivery system. That such a high proportion of triclosan is effectively ballast, may have implications in terms of the economic viability of delivering triclosan transdermally.

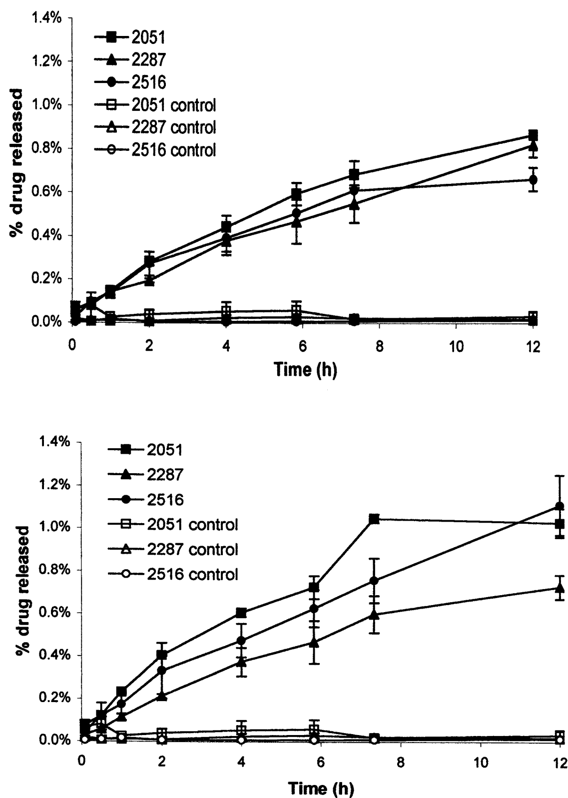

Fig. 2. Percentage of total dose of triclosan released from the three adhesives loaded with 30 mg 0.785 cm^{-2} (upper) and 50 mg 0.785 cm^{-2} (lower).

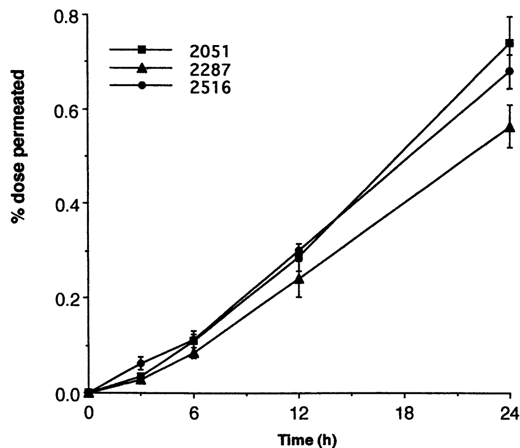

Fig. 3. Cumulative permeation of triclosan across excised human epidermal membranes $n = 7$, $\pm$S.E.

Triclosan has been used topically for some 30 years (Savage, 1971) and having been subjected to toxicological examination it has been found to be practically non toxic. The LD_{50} in mice, rats and dogs is approximately 5000 mg kg^{-1} and no local irritant or systemic toxic effects were noted when it was applied to rat skin over 4 weeks (Bhargava and Leonard, 1996). In human studies, no cases of sensitisation or phototoxicity were reported. The authors also reported that when acute, sub-acute/sub chronic, and chronic toxicity profiles were conducted triclosan proved to be neither carcinogenic, mutagenic nor teratogenic.

Surolia and Surolia found that 38 mg kg^{-1} triclosan completely cleared the parasite, *P. bergei* from the mouse model after 4 days (Surolia and Surolia, 2001). If this dose was equated to man (average weight 70 kg), doses of 2.66 g could be effective. Although this amount would be too high for delivery by the transdermal route, the figure is based on data from a bolus dose and is therefore likely to be a gross over-estimation.

The multiple kinetic events involved in skin delivery, particularly when transdermal devices are used, are complicated and still not fully understood. Fig. 5 shows a histogram of the difference between the amount released from the patches

Table 2
Steady state flux and permeability coefficient for the permeation of triclosan across heat separated human epidermal membranes from 50 mg triclosan-loaded patches

Formulation	Lag time (h)	Steady state flux (μg cm^{-2}h^{-1})	S.D.	S.E.M.	Permeability coefficient k_p (cm h^{-1})	S.D.	S.E.M.
50 mg 2051	1.60	16.91	3.66	1.29	3.40×10^{-5}	7.36×10^{-6}	2.60×10^{-6}
50 mg 2287	2.37	12.83	2.81	0.99	2.58×10^{-5}	5.65×10^{-6}	2.00×10^{-6}
50 mg 2516	2.62	15.05	2.83	1.00	3.00×10^{-5}	5.64×10^{-6}	1.99×10^{-6}

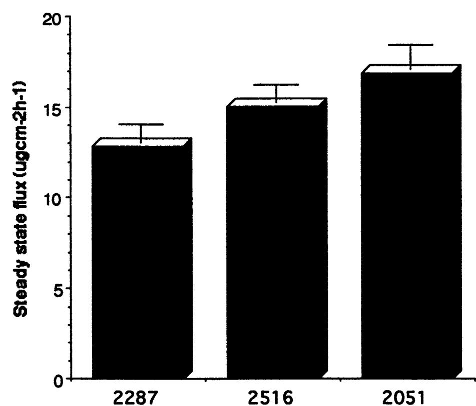

Fig. 4. Histogram of steady state flux of triclosan across human epidermal membranes from the three adhesives.

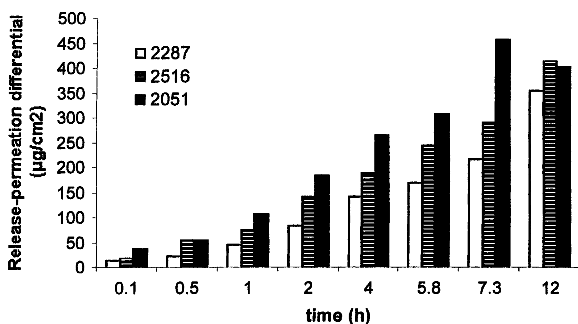

Fig. 5. Plot of the differences between triclosan from patch and permeated across skin at different timepoints.

and the amount permeated over the first 12 h. The percentage permeated across the skin in the first 12 h is much smaller compared with that released. This may be due to the substantial difference between the dissolution medium and the stratum corneum or reflect reservoir formation within the skin. It was reported previously that triclosan can accumulate in the skin and that it is also subject substantial dermal metabolism, principally to the sulphate and glucuronide forms (Moss et al., 2000). Such effects may undermine the efficacy of transdermally administered triclosan and the relative kinetics of conversion and permeation would need to be established in vivo, possibly using microdialysis.

4. Conclusions

In conclusion, this study has shown that triclosan can be delivered at a constant dose transdermally from simple drug-in-glue matrices. As therapeutic plasma levels are not yet established, prediction of clinically effective patch dimensions is not possible at the present time. However, given the current urgency surrounding the global menace caused by malaria, further investigations including field trials are warranted.

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