In order to achieve the stated SPF (2 mg/cm²) of a sunscreen preparation, about 35 ml are needed for a whole-body application for adults. If it is applied at the recommended frequency, at least twice this amount could be applied during a day at the beach. During other periods, sunscreens may be applied to sun-exposed sites such as the face, hands and arms for extended periods or used daily in cosmetic preparations. Ideally, sunscreens should remain on the skin surface, so that they can be washed off, or should be bound only to the non-viable epidermis (stratum corneum). Relatively little is known about the percutaneous absorption and metabolism of sunscreens. Since they might be used daily throughout life, it is clearly important that any interactions that sunscreens may have within viable skin and systemically should be assessed. The percutaneous absorption of sunscreens has been studied in humans and animals in vivo. Ex-vivo techniques, in which viable excised skin is used as a membrane in a diffusion chamber and test material is collected in a receptor fluid, have also been used.

Humans

Organic sunscreen ingredients

para-Aminobenzoic acid

Arancibia et al. (1981) studied the percutaneous absorption and pharmacokinetics of three topical sunscreen preparations containing 5% PABA in six male volunteers. A dose of 20 g of sunscreen was applied to the face, neck, trunk, and upper extremities (at approximately the recommended level of application) in the early morning. Urine samples were taken before application and at periods 2–48 h after application and were analysed for PABA and its acetylated derivative. The cumulative urinary excretion of total PABA at 48 h ranged from 16 to 96 mg, which represented 1.6–9.6% of the applied dose. No difference was found between the three preparations. Most of the recovered PABA (70–90%) was in the acetylated form. When the volunteers received a 500-mg oral dose of PABA, acetylated PABA represented 50–82% of the recovered compound (it is not stated at which time this analysis was carried out, but elimination after oral administration was rapid, with a half-time of 56 min). The authors stated that significantly (p < 0.05) more acetylation, a common metabolic pathway for many drugs, occurred after topical application than after oral administration, perhaps because of slow, sustained presentation to metabolizing enzymes.

Wester et al. (1998) compared the percutaneous absorption of [14C]PABA in human skin in vivo and in an isolated, perfused porcine skin flap. In each case, 10 cm² of skin were treated with PABA delivered at a dose of 21.5 μg/cm² in 50 μL ethanol. In the five volunteers, the site was washed 24 h after application and tape-stripped on day 7; the volunteers collected their urine over 7 days. With the pig skin, venous effluent was collected every 30 min for 8 h, and then the skin surface was washed and tape-stripped, and the remainder of the tissue was digested. The urine of the volunteers contained 12% ± 6.3% (SD) of the applied dose; 30% ± 13% was recovered from the skin surface wash and 0.56% ± 0.47% from the stratum corneum by tape-stripping. In a comparison with urinary excretion after intravenous injection in rhesus monkeys, in which the systemic availability is assumed to be 100%, it was estimated that 15% ± 8.4% PABA had been absorbed percutaneously. In the pig system, 5.9% ± 3.7% (perfusate + skin) had penetrated viable tissue. These results show that absorption of PABA through human skin is under-estimated in the perfused porcine skin flap. Determination of the percutaneous absorption of other compounds in the two systems gave more comparable results.

Ethylhexyl salicylate

The penetration of 5% (w/w) [14C]ethylhexyl salicylate in an oil-in-water emulsion and a hydroalcoholic formulation through human epidermis was evaluated in vitro after application of a finite dose, a target of 5 mg/cm², and an infinite dose, a target of 100 mg/cm². [1H]Sucrose was added to the formulations to allow monitoring and confirmation of the integrity of the membrane. Samples were taken from the receptor fluid between 2 and 48 h and assessed by 14C-scintillation counting. The results (Table 15) show that < 1% of the applied dose penetrated the epidermis, and the amount remaining bound to the epidermis represented 11–33%, depending on the experimental conditions. In a similar study with a finite dose of [14C]salicylic acid in an oil-in-water vehicle, the total penetration (1.6 μg/cm²) was similar to that of ethylhexyl...
The epidermal penetration of the active 'chemical' ingredients of sunscreen products was evaluated in vitro over 8 h. In the system used, heat-separated epidermis acts as a membrane in a Franz diffusion cell in which there is a donor and a receptor chamber. Of five 'chemical' absorbers assessed, only benzophenone-3 was found in the receptor fluid at concentrations representing up to 10% of the applied dose. Up to 14% of the applied dose of other sunscreen ingredients remained in the epidermis (Jiang et al., 1999).

The percutaneous penetration of five sunscreens was assessed in fresh skin discarded at surgery from women aged 17–65 years. The samples were 344-μm dermatome slices which were placed on static diffusion cells containing only receptor fluid. Sunscreens containing ethylhexyl methoxycinnamate (5%), benzophenone-3 (4.9%), benzophenone-4 (6.9%), ethylhexyl triazone (4%) and octocrylene (8%) were applied at 3 mg/cm² and left for 16 h. They were then washed off, the stratum corneum was tape-stripped 16 times, and the viable epidermis was heat separated from the dermis. The sunscreen content of the tape (stratum corneum), epidermis, dermis, receptor fluid and washing solution was determined by high-performance liquid chromatography (HPLC), which resulted in recovery rates of 93–97%, depending on the ingredient. With the exception of benzophenone-3, the quantity in the receptor fluid was low or below the limit of detection. The largest amounts of all ingredients were found in the stratum corneum, with very little in the viable epidermis or the dermis (Potard et al., 1999).

The percutaneous absorption of benzophenone-3 in a sunscreen containing 6% benzophenone-3, 7.5% ethylhexyl methoxycinnamate, 5% ethylhexyl salicylate and 7% octocrylene (all v/v) was studied in nine healthy volunteers with a mean age of 29 years. The product was applied to the forearms at six times (12 mg/cm²) the application density for SPF assessment (2 mg/cm²) and left for 12 h, after which it was washed off. Urine samples were taken just before application and for 48 h after application. Analysis for benzophenone-3 and its metabolites in urine (the authors do not mention analysis for the other ingredients) showed a steady increase over the 48-h period in all volunteers, and the authors estimated that 1–2% of the applied benzophenone-3 had been absorbed over 10 h (Hayden et al., 1997). This study has been criticized on the grounds of the very high application density of the sunscreen (Agin et al., 1998). Furthermore, the urinary concentrations may be an underestimate of skin penetration, as tissue-bound sunscreen and metabolites, unknown urinary metabolites and excretion via other routes cannot be assessed.

Various organic ingredients
The epidermal penetration of the active 'chemical' ingredients of six sunscreen products was evaluated in vitro over 8 h. In the system used, heat-separated epidermis acts as a membrane in a Franz diffusion cell in which there is a donor and a receptor chamber. Of five 'chemical' absorbers assessed, only benzophenone-3 was found in the receptor fluid at concentrations representing up to 10% of the applied dose. Up to 14% of the applied dose of other sunscreen ingredients remained in the epidermis (Jiang et al., 1999).

The transdermal absorption of sunscreens was assessed in human skin in vivo. Saturated solutions of sunscreens in a glycol–water mixture were placed in a glass chamber attached to the skin of the arm for 1 h, and percutaneous absorption was modelled from decreases in the sunscreen concentration in the vehicle. The authors estimated the amount of sunscreen absorbed over the whole skin surface (1.8 m²) within 1 h. Benzophenone-3 and isoamyl-para-methoxycinnamate were absorbed to the greatest degree, at rates of 80 and 89 mg/h, respectively. This approach does not yield information on the degree of accumulation in tissue compartments nor on systemic accumulation (Hagedorn-Leweke & Lippold, 1995).

The penetration of 5% benzophenone-3, 7.5% ethylhexyl methoxycinnamate and 3% ethylhexyl salicylate in two vehicles (an emulsion gel and petroleum jelly) was evaluated in vitro in 600-μm slices of skin in static Franz diffusion chambers, and the concentrations of the ingredients were determined in a skin

<table>
<thead>
<tr>
<th>Medium</th>
<th>Finite dose</th>
<th></th>
<th>Infinite dose</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oil-in-water</td>
<td>Hydro/coholic</td>
<td>Oil-in-water</td>
<td>Hydro/coholic</td>
</tr>
<tr>
<td>Receptor fluid</td>
<td>0.65 ± 0.16</td>
<td>0.59 ± 0.09</td>
<td>0.47 ± 0.22</td>
<td>0.23 ± 0.05</td>
</tr>
<tr>
<td>Wash</td>
<td>37 ± 5.3</td>
<td>36 ± 6.0</td>
<td>34 ± 3.1</td>
<td>44 ± 7.4</td>
</tr>
<tr>
<td>Epidermis</td>
<td>17 ± 1.3</td>
<td>33 ± 4.7</td>
<td>12 ± 1.4</td>
<td>14 ± 2.4</td>
</tr>
<tr>
<td>Improved recovery technique</td>
<td>54 ± 5.5</td>
<td>70 ± 6.8</td>
<td>46 ± 2.2</td>
<td>83 ± 6.4</td>
</tr>
<tr>
<td>Total</td>
<td>54 ± 5.5</td>
<td>70 ± 6.8</td>
<td>46 ± 2.2</td>
<td>83 ± 6.4</td>
</tr>
</tbody>
</table>

Modified from Walters et al. (1997)
surface wash, the whole epidermis including stratum corneum, the dermis and the receptor fluid 2 min, 30 min, 2 h and 6 h after application. Benzophenone-3 was the only ingredient detected in the receptor fluid, a maximum of about 5% of the dose being found at 2 and 6 h when it was applied in petroleum jelly. Benzophenone-3 was also found in the dermis at all times, with a maximum of about 6% in petroleum jelly at 6 h. The concentrations of the ingredients were also evaluated in vivo in the stratum corneum at various depths. The highest concentrations were found 30 min after application, with slightly lower values at 2 and 6 h (data not given). The initial one to five tape strips contained the highest concentrations of all ingredients, with much higher values in the emulsion gel vehicle (35% of the applied dose) than in petroleum jelly (10% of the applied dose). The values for the three sunscreen ingredients were about the same in a given vehicle at a given depth of stratum corneum (Treffel & Gabard, 1996).

Gas chromatography with mass spectrometry was used to assess the presence of five sunscreen ingredients — isomyyl-para-methoxycinnamate, benzophenone-3, 4-methylbenzylidene camphor, ethylhexyl dimethyl PABA and ethylhexyl methoxycinnamate — in the breast milk of six women who had used sunscreens or skin-care products or used public swimming pools. Benzophenone-3 was detected in four of six samples at concentrations of 16–420 ng/g of fat. A concentration of 20 ng/g was reported in the milk of one woman who had not used sunscreens during the summer of the study. Two samples contained ethylhexyl methoxycinnamate at concentrations of 28 and 47 ng/g of fat. No other sunscreen ingredient was detected (Hany & Nagel, 1995).

Inorganic sunscreens

TiO₂ and ZnO are generally considered to be harmless pigments that cannot enter the skin and are largely unaffected by optical radiation. TiO₂ is, however, a semiconductor which can absorb light and, under certain conditions, generate free radicals which can cause cell damage (Warner et al., 1997). Therefore, TiO₂ particles used in sunscreen preparations are often coated with other materials, such as aluminium and silicon, to reduce any potential photoreactivity. This coating has been shown to be efficient, remaining stable even after application to the skin and subsequent UV irradiation (Van der Molen et al., 1999). The literature on the potential of inorganic sunscreens to penetrate the skin shows some confusion, as in most cases the metal, Ti or Zn, and not the metal oxide was identified, whereas the metals themselves are not photoactive.

Titanium dioxide

As Ti has been found in biopsy samples, it has been inferred that TiO₂ can penetrate the skin (Dupre et al., 1985; Dundas & Laing, 1988; Moran et al., 1991; Tan et al., 1996). In a pilot study, Tan et al. (1996) assessed the percutaneous absorption of microfine TiO₂ in a sunscreen containing 8% of this agent through 16 samples of skin from 13 patients (aged 59–62 years) who were due to undergo skin surgery. The product was applied twice a day for 2–6 weeks before surgery. The recovery of TiO₂ from tissue, excluding the stratum corneum (removed by stripping), was 0.0 to about 4.5 μg/g of wet weight, with a mean value of about 1.6 μg. The values in skin from nine untreated cadavers of unspecified age were equal to (n = 1) or lower than (n = 7) this mean value in eight of the samples, but the other showed a value close to the maximum of the treated group. When this outlier was excluded, the treated group had higher (p = 0.0006) values than the controls, but the metal oxide removed this difference (p = 0.14). These data, although inconclusive, suggest that TiO₂ penetrates the stratum corneum. It should be noted that the mean age of the study population was 71 years; furthermore, the samples were taken from skin at a site destined for surgery. In a study of the percutaneous absorption of TiO₂ particles by X-ray microanalysis in combination with scanning electron microscopy, no Ti was found in deeper layers of the skin (Van der Molen et al., 1997).

TiO₂ and ZnO in a sunscreen formulation applied to human skin removed during plastic surgery was found to be restricted to the surface, with no intercellular or intracellular penetration (Dussert et al., 1977). The skin was prepared for examination immediately after application, however, and studies of the time-course of penetration were not performed.

Zinc oxide

No evidence for percutaneous absorption of Zn was found from a topically applied sunscreen product containing 40% ZnO in a controlled cross-over study carried out with six normal volunteers aged 21–24 years, who received ZnO in a white petrolatum base over a large surface area. The serum concentration of Zn was assessed at 1, 2 and 3 h (Derry et al., 1983). In contrast, evidence of percutaneous absorption of Zn was found 48 h after topical application of a sunscreen containing 25% ZnO to five healthy volunteers aged 22–54 years (Agren, 1990). Zn was assessed in epidermis and blister fluid after the raising of suction blisters, a process that takes 2–3 h and is likely to compromise the integrity of barrier function. Analysis of epidermis per se yields no information on the barrier function of the stratum corneum.

Non-sunscreen chemicals present in sunscreens

Citropen and bergapten

Sunscreens containing bergamot oil were applied to human volunteers at a concentration of 3.2 mg/cm² in an oil-in-water emulsion and at 1.4 mg/cm² in an oil vehicle. Suction blisters were raised 100–220 min after application, and the
fluid was assessed by HPLC for 5,7-dimethoxycoumarin and 5-methoxy-Vitamin E.

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neous absorption; however, it can be for analysis at the end of the 3-month period to provide baseline data. Similarly, blood samples were taken. Analysis of plasma from all subjects showed no difference between the baseline and treatment periods in the concentrations of free \( \alpha \)-tocopherol (13 ± 6.3 (SD) and 13 ± 6.1 \( \mu \)g/mL, respectively) or \( \alpha \)-tocopherol acetate (2.1 ± 0.9 and 2.5 ± 1.3 \( \mu \)g/mL, respectively). The analysis of four lots of randomly pooled biopsy samples showed a substantial increase in the concentration of \( \alpha \)-tocopherol acetate (baseline, 5.9 ± 12 (SD) \( \mu \)g); all values 0 except one outlier; treated, 260 ± 200 \( \mu \)g/g) but no difference in the concentration of \( \alpha \)-tocopherol or \( \gamma \)-tocopherol. These data indicate that \( \alpha \)-tocopherol acetate is not metabolized to the free form of \( \alpha \)-tocopherol in plasma or skin (Alberts et al., 1996).

Experimental models

Animal models are widely used to study percutaneous absorption. The pig in particular is considered to be suitable because its skin is similar to that of humans.

Micro-Yucatan pig

Gupta et al. (1999) studied the percutaneous absorption of radio-labelled ethylhexyl methoxycinnamate and benzophenone-3 through excised micro-Yucatan pig skin sliced at 250–300 \( \mu \)m and placed in diffusion cells. The sunscreens were dissolved in either a hydroalcoholic or an oil-based (diisopropyl adipate) vehicle. Analyses were conducted on the receptor fluid to assess penetration, washes of skin to assess remaining sunscreen, stratum corneum (from tape stripplings) and the viable tissue, which was digested. Analyses of stratum corneum showed that, in each vehicle, it retained more ethylhexyl methoxycinnamate than benzophenone-3. The maximum concentrations in the stratum corneum were reached at 1 h and remained fairly constant for the 10-h duration of the experiment. Removal of the stratum corneum before sunscreen application resulted in much greater total penetration of both sunscreens in both vehicles. When the ratios of retained: penetrated dose of sunscreens alone and in combination were compared (Table 16), the higher the ratio, the more sunscreen remained in the stratum corneum. In both vehicles, the ratio was higher for ethylhexyl methoxycinnamate than benzophenone-3, and for both sunscreens the ratio was higher when they were applied in combination than when applied alone. The ratios were significantly different for each sunscreen alone or in combination in the hydroalcoholic vehicle but not in the oil vehicle. The formulation can thus markedly affect the pharmacokinetics of sunscreens.

Rat

Fischer 344 rats were treated topically on shaved skin with up to 800 \( \mu \)g of benzophenone-3 in ethanol or 50 \( \mu \)g in a lanolin/white petrolatum base. Up to 39% of the compound was recovered from the urine 72 h after application. Benzophenone-3 was also detected in internal organs. The results were similar with the two bases (El Dareer et al., 1996).

In a study of the distribution and metabolism of benzophenone-3, rats were given a single topical application on a limited area of shaved skin. As the animals were not restrained, however, intake may have occurred during grooming. Plasma samples from one group were analysed at various times between 5 min and 48 h. Animals in another group were killed 6 h after administration and the content of various tissues was analysed. For a third group, urine and faeces were analysed for periods up to 168 h after administration. The parent compound and its metabolites were detected in plasma within 5 min, with peak absorption at 2.5 h. The plasma time-course was biphasic, with half-times of 1.3 and 15 h (Okereke et al., 1994).

In a study of the effect of two daily applications of benzophenone-3 at 100 mg/kg bw in a petroleum jelly base for 4 weeks, blood samples were taken on day 16, and reduced glutathione concentrations were assessed. The treated rats had a significantly lower concentration than those given the vehicle only, suggesting that reduced glutathione is involved in the metabolism of benzophenone-3 in rats. However, the contribution of unintended intake during grooming in these studies is not known (Okereke et al., 1995).

A study of the metabolism of benzophenone-3 after oral administration to rats is described even though sunscreens are not taken orally. The animals were given 100 mg/kg bw, and three metabolites, 2,4-dihydroxybenzophenone (the major metabolite), 2,2'-dihydroxy-4-methoxybenzophenone and 2,3,4-trihydroxybenzophenone, were identified in free and conjugated forms by HPLC. The parent compound and its metabolites (free and bound) were detected after 6 h in most tissues, and all were detected in plasma 5 min after administration. Both benzophenone-3 and its metabolites were excreted primarily in urine, and faeces were a
Table 16. Distribution of benzophenone-3 and ethylhexyl methoxycinnamate in a hydroalcoholic vehicle when applied individually or in combination to micro-Yucatan pig skin in a diffusion chamber

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Benzophenone-3</th>
<th>Ethylhexyl methoxycinnamate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone</td>
<td>With ethylhexyl methoxycinnamate</td>
</tr>
<tr>
<td>Receptor (% applied dose)</td>
<td>1.8</td>
<td>0.32</td>
</tr>
<tr>
<td>Viable skin (% applied dose)</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Penetrated (receptor + viable skin) (% applied dose)</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Retained (in stratum corneum) (% applied dose)</td>
<td>24</td>
<td>34</td>
</tr>
<tr>
<td>Retained:penetrated</td>
<td>1.7</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Modified from Gupta et al. (1999)

secondary route of excretion. The authors suggested that O-dealkylation is the major pathway of metabolism of this compound (Okereke et al., 1993).

The pharmacokinetics of benzo-phenone-3 was investigated in blood samples taken at various times after oral administration to male rats at a dose of 100 mg/kg bw. Some free benzophenone-3 was detected, but the majority was bound to plasma protein and could be detected only after acid hydrolysis. The compound was absorbed rapidly from the gastrointestinal tract and was detected in the blood 5 min later. The peak plasma concentration (26 μg/mL) was found at 3 h. Elimination was biphasic, with half-times of elimination of 0.88 and 16 h. This two-compartment model of elimination has been associated with distribution to the tissues. In some studies, animals were killed 6 h after administration and the tissue distribution of benzophenone-3 was assessed. The highest concentrations were found in the liver and kidneys, which accounted for 6.5% and 0.97% of the initial dose, respectively. When excretion in urine and faeces was assessed for up to 96 h, urine was found to be the major route of excretion, with a peak at 6–12 h. Hydrolysis of urine samples with β-glucuronidase showed that the main form was a conjugate with glucuronic acid; however, acid hydrolysis revealed other forms of conjugation. Faecal excretion was largely complete within 24 h, and about 50% of the benzophenone-3 was in a conjugated form (Kadry et al., 1995).

Zinc oxide administered as a suspension or mixed into the adhesive layer of tape was shown to penetrate the skin into the blood within 1 h of application to intact skin of Sprague-Dawley rats (Hallmans & Liden, 1979).

Hairless guinea-pig
In a study of the dermal absorption and metabolism of [14C]PABA in vitro, 200-μm sections of skin were obtained by microtome and placed in a diffusion cell with HEPES-buffered Hanks balanced salt solution, which ensured their viability for 48 h. Experiments were also carried out with distilled water, which made the skin non-viable. PABA was applied in ethanol at a dose of about 2 μg/cm². The skin surface was washed 24 h later and left for a further 24 h to allow any remaining absorbed compound to enter the receptor fluid. Analysis 48 h after application showed that 5% of the total dose was in the receptor fluid and 21% in skin maintained under physiological conditions. Significantly more absorption occurred with water as the receptor fluid, 19% of the total dose being found. When skin was maintained under physiological conditions, the acetyl derivative accounted for most (61% of absorbed dose) of the PABA in the receptor fluid, but the parent molecule predominated in the skin (86% of absorbed dose). In both compartments, only small amounts of the acetyl derivative were recovered when water was used as the receptor fluid. These studies show that the skin readily metabolizes PABA (Nathan et al., 1990).

Rabbit
The percutaneous uptake of 65ZnO was estimated by γ-radiation counting in a small study: 20–25% of the total zinc applied remained in the skin 6 or 24 h after a single or double application. Autoradiography indicated that little 65Zn was present in the epidermis, but large amounts were present in the subdermal muscle layer. Only trace amounts were observed in the dermis, but there was evidence of Zn in hair follicles (Kapur et al., 1974).