# Chapter 1
# Sunscreen Cosmetics: Advantages and Drawbacks

## 1.1 The Protective Skin Barrier

The skin is the largest organ in the human body. Among its main functions, the skin is able to convey feelings, regulate body temperature, produce vitamin D and protect against external aggression. This protection is not based on the repulsion of noxious agents, but in the interaction with them through various defensive mechanisms which prevent the extension of possible skin lesions (Cohen and Rice 2008).

Regarding the histology of the skin, there are two main parts which are separated by a wavy basal membrane called papillary plexus. The outer part is called epidermis, while the innermost is dermis. In turn, the epidermal appendages, mainly the hair follicles, penetrate the epidermis and are embedded in the dermis (see Fig. 1.1).

The dermis is about 90 % of the thickness of the skin and acts as a cushion to support the epidermis. It consists mainly of collagen and elastin fibers, and contains nerves, blood and lymph vessels, and both sweat and sebaceous glands. Between the dermis and underlying tissues, there is a layer of adipocytes, also called hypodermis, where the fat is accumulated in the form of triglycerides.

Blood flow of the epidermis comes from the capillaries located in the papillary plexus. The epidermis consists of four distinct layers, which are called in order of increasing depth: corneum, granulosum, spinosum and basal or germinative layer. The outermost layer, the stratum corneum, consists mainly of keratinocytes, which are squamous cells strongly bonded together, and have no nucleus, then being biologically inactive. In the epidermis, there are also melanocytes, which produce melanin granulation under the stimulus of ultraviolet (UV) light. These granulations, once ejected, are incorporated into the surrounding epidermal cells that are then pigmented. Moreover, many Langerhans cells can be found in the epidermis, which have major importance in the immune reactions of the skin against foreign agents.

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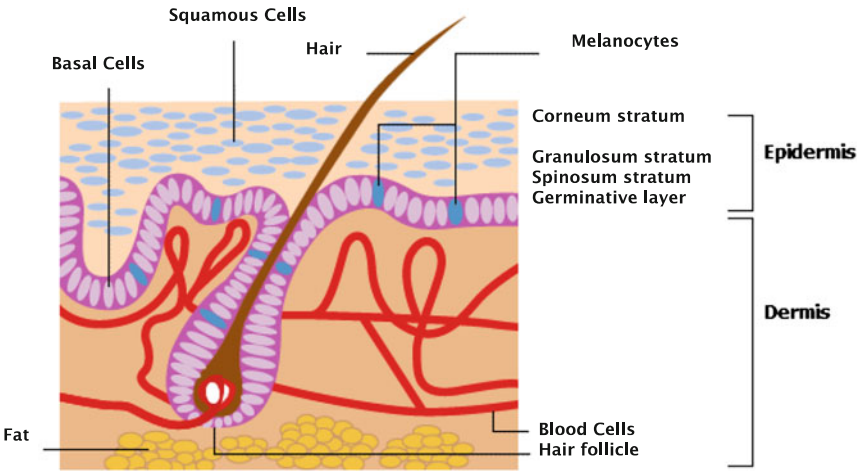


**Fig. 1.1** Schematic drawing of a human skin section

## 1.2 Interaction Between Electromagnetic Radiation and Skin: Phototoxicology

Throughout life, the skin is exposed to different wavelengths of the electromagnetic spectrum that reach the Earth's surface, including UV, visible light and infrared (IR) radiation from the sun, artificial light and sources of heat. These radiations have different levels of penetration when they impact the human skin, as can be seen in Fig. 1.2.

It is interesting to compare the penetration ability of the UV-A (320–340 nm) and UV-B (290–320 nm) radiations through the skin. While the UV-A radiation gets through the dermis, the more energetic UV-B radiation only penetrates the

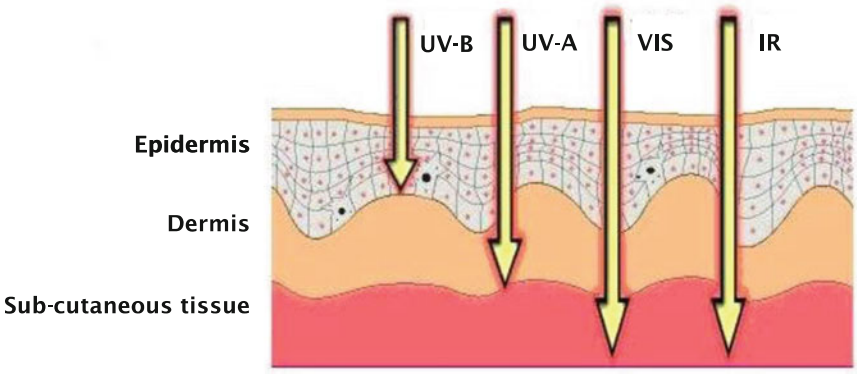


**Fig. 1.2** Skin penetration of the different radiation achieving the Earth's surface

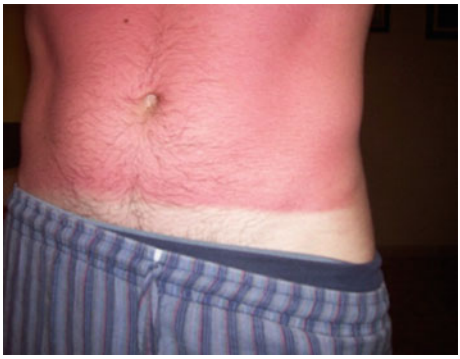

**Fig. 1.3** Sunburn effects

epidermis. The visible and IR radiations have greater penetrating ability but, fortunately, they are less energetic.

Taking into account the effects induced by the UV radiation on the skin, it is important to consider the length of exposure, distinguishing between acute and chronic exposures, as well as the influence of environmental conditions, such as season, latitude, deterioration of the ozone layer, the exposed body region, skin pigmentation and the previous exposures (Herman et al. 1996).

The most obvious manifestation of acute exposure to UV radiation is sunburn (redness or sunburn, see Fig. 1.3). UV-B radiation has the greater capacity to cause sunburn in human skin.

Another typical sign of acute exposure to UV radiation is the dark pigmentation of the skin. This may be due to the increased production of melanin by melanocytes or to the photo-oxidation of melanin. The tanning or increased pigmentation usually occurs after 3 days of exposure to UV light, while the photo-oxidation is observed immediately.

The pigment darkening that occurs immediately after exposure to UV-A and visible light does not increase the capacity of photo-protection. However, tan, which appears more readily after exposures to UV-B, enhances the protective effects of melanin on skin. Thus, acute exposure to UV-B radiation brings the thickening of the stratum corneum, which means, in turn, a greater protective effect against subsequent attacks caused by UV radiation.

Within this framework favourable, it is considered that the natural and environmental exposure to light is essential for the development of life. UV radiation is essential to promote the blood circulation (Barth et al. 1994) and the action of certain neurotransmitters in the brain that are responsible for good mood and feeling of well-being (Grant and Gruijl 2003; Lowry et al. 2009). In addition, the conversion of 7-dehydrocholesterol to provitamin D3, a necessary precursor for the endogenous formation of vitamin D, is enhanced (Chapuy et al. 1997). The toxic action of UV light emitted by artificial sources has also been used for decades to treat processes causing excessive proliferation of skin, as psoriasis and seborrheic dermatitis (Parrish and Jaenicke 1981).

In contrast, chronic exposure to solar radiation can stimulate some skin lesions that depend on the degree of basal pigmentation of the individual. Thus, freckles and skin spots, caused by inadequate distribution of pigments, wrinkles and actinic keratosis (pre-cancerous lesion) are direct consequences of prolonged exposure to UV light (Lim and Cooper 1999). Also, excessive exposure to the sun can lead to premature aging caused by the destruction of elastin and collagen fibers of the dermis, and skin cancer, which is the most common cancer malignancy in humans (Naylor and Farmer 1997). Even, it can be said that the main cause of skin cancer is sunlight. UV radiation can cause pyrimidine dimers in epidermal cells, thus triggering a series of mutations in genes. UV light has also immunosuppressive effects that can promote the persistence of some skin tumours. It should be noted that the incidence of skin cancer is higher in the tropics and in Caucasians and pale skin people. For this reason, there are many public health programs aimed at establishing measures for the sunscreen to reduce the risk of this cancer (ICNIRP 2007). Changes in social behaviour, including increased leisure activities outdoors (water sports, winter sports, etc.) require the consideration of prevention efforts on those risks (Hiom 2006).

The most effective photo-protective measure to suppress harmful effects of the UV radiation is to avoid sun exposure. However, this is not practical or desirable as indicated above. Therefore, it is recommended as far as possible, to minimize sun exposure during the hours when UV radiation is most harmful, to use suitable clothing and sunglasses, and to apply sunscreen cosmetics to obtain optimal protection (Nohynek and Schaefer 2001; Kullavanijaya and Lim 2005; Nash 2006; Gaspar and Fields 2007).

## 1.3 The Cosmetics for Sun Protection in the European Union

Cosmetic products for sun protection are classified in different categories by the different countries, which in turn depend on the relevant legislation. The three major regulatory systems of cosmetic products in the world are the Cosmetic Products Regulations of the European Union (EU), the rules of the food and drug administration (FDA) and Japanese legislation. Both the EU and Japan consider the sun protection products as cosmetics, while the United States considers them as over-the-counter products. Cosmetic products for sun protection include various chemicals in the composition, commonly known as UV filters, which act as active ingredients absorbing or reflecting solar radiation.

### 1.3.1 UV Filters. Classification and Properties

As indicated previously, cosmetics for sun protection are regulated in the EU by the Cosmetic Products Regulation (Regulation (EC) No 1223/2009), which replaces the previous Cosmetics Directive (Council Directive 76/768/ECC). Annex VI of this Regulation contains a list of chemicals that can be used as UV filters in cosmetic products, indicating their maximum allowed levels. UV filters can be defined as *substances which, contained in cosmetic sunscreen products, are specifically intended to filter certain radiation to protect skin from certain harmful effects of these rays* (Cosmetics Directive (76/768/ECC)). The allowed compounds are reviewed periodically by the Scientific Committee on Consumer Products (SCCP), which includes scientists from different member countries that study the experimental data obtained from the compounds of interest. After considering the relevant reports, the European Commission takes the appropriate actions. Currently, there are 26 substances that make up the Annex VI. Currently allowed UV filters and their authorized contents are shown in Table 1.1.

Note that *p*-amino benzoic acid (PABA) is the UV filter whose ban to be used as a cosmetic ingredient has been more recently applied (Directive 2008/123/EC). Nevertheless, no official analytical methods exist for the determination of these compounds in cosmetic products for sun protection (Salvador and Chisvert 2005).

UV filters can be divided into two groups, according to a classification based on chemical nature. On the one hand, the inorganic or physical UV filters, which act mainly reflecting or scattering the incident UV radiation and, on the other hand, the organic or chemical UV filters, which absorb UV light.

In general, physical UV filters are metal oxides. Although providing increased protection compared to chemical UV filters, they have a lower acceptance due to their low solubility in water that allows the formation of a protective film on the skin that is not pleasing to the user. Currently, only titanium dioxide is authorized as physical UV filter for Cosmetic Products Regulation in the EU, as shown in Table 1.1.

Meanwhile, the chemical UV filters are defined as organic compounds with a high molar absorptivity in the UV wavelength range. These compounds usually have one or more aromatic rings, sometimes conjugated with carbon–carbon double bonds and/or carbonyl groups. Cosmetic products containing these compounds normally have better acceptance than those formulated with physical UV filters due to their more convenient form of application. In turn, the chemical UV filters can be classified according to their chemical structures in different families, such as benzophenone derivatives (BZ3, BZ4, DHH), *p*-aminobenzoic acid derivatives (EDP, P25), salicylates (ES, HS), methoxycinnamates (EMC, BMI), camphor derivatives (3BC, MBC, BCS, CBM, TDS, PBC), triazine derivatives (ET, DBT, EMT), benzotriazole derivatives (DRT, MBT), benzimidazole derivatives (PBS, PDT) and others (BDM, OCR, P15). Some of these UV filters have a structure with ionizable functional groups (e.g., sulphonic) that confers water solubility.

**Table 1.1** Updated list (April 2011) of the UV filters that can be used in sunscreen cosmetics according to the current legislation in the EU

| Key[a] | INCI Nomenclature[b] | Maximum concentration[c] |
|---|---|---|
| 3BC | 3-benzylidene camphor | 2 |
| BCS | Benzylidene camphor sulphonic acid | 6[d] |
| BDM | Butyl methoxydibenzoylmethane | 5 |
| BZ3 | Benzophenone-3 | 10 |
| BZ4 | Benzophenone-4 | 5[d] |
| CBM | Camphor benzalkonium methosulphate | 6 |
| DBT | Diethylhexyl butamido triazone | 10 |
| DHH | Diethylamino hydroxybenzoil hexyl benzoate | 10 |
| DRT | Drometrizole trisiloxane | 15 |
| EDP | Ethylhexyl dimethyl PABA | 8 |
| EMC | Ethyhexyl methoxycinnamate | 10 |
| EMT | Bis-ethylhexyloxiphenol methoxyphenyl triazine | 10 |
| ES | Ethylhexyl salicylate | 5 |
| ET | Ethylhexyl triazone | 5 |
| HS | Homosalate | 10 |
| IMC | Isoamyl *p*-methoxycinnamate | 10 |
| MBC | 4-methylbenzilidene camphor | 4 |
| MBT | Methylene bis-benzotriazolyl tetramethylbutylphenol | 10 |
| OCR | Octocrylene | 10[d] |
| P15 | Polysilicone-15 | 10 |
| P25 | PEG-25 PABA | 10 |
| PBC | Poliacrilamidomethyl benzilidene camphor | 6 |
| PBS | Phenylbenzimidazole sulphonic acid | 8[d] |
| PDT | Phenyl dibenzimidazole tetrasulphonatoe disodium | 10[d] |
| TDS | Tereftalidene dicamphor sulphonic acid | 10[d] |
| $TiO_2$ | Titanium dioxide | 25 |

[a] Key used along this Ph.D. Thesis
[b] International Nomenclature of Cosmetic Ingredients
[c] Expressed as weight percentage in the final product (*m/m*)
[d] Expressed as acid

UV filters can also be classified into UV-A or UV-B, depending on which UV radiation that is attenuated. Generally, all absorb UV-B radiation, except BZ3, BZ4 and DHH that also absorb partially UV-A, and TDS and BDM, which only absorb UV-A radiation.

Given the potential of these compounds to protect the skin, the trend of the cosmetic industry is the inclusion of UV filters in the composition of daily-used cosmetic products, apart from their use in specific sunscreen products.

However, UV filters can produce undesirable side effects on the skin. Thus, cases of contact dermatitis and photo-allergies have been reported (Berne and Ros 1998; Alanko et al. 2001; Darvay et al. 2001; Maier and Korting 2005). Moreover, although cosmetic products for sun protection are designed to be applied externally and remain on the surface layers of the skin (Benson 2000; Nohynek and Schaefer 2001),

some UV filters can penetrate into the body through the skin (Arancibia et al. 1981; El Dareer et al. 1986; Hagedorn-Leweke and Lippold 1995; Marginean-Lazar et al. 1996). A number of effects that can be attributed to these processes of percutaneous absorption are the appearance of endocrine disruption effects, oestrogenic activity, the generation of free radicals and the induced toxicity in keratinocytes (Schlumpf et al. 2001; Heneweer et al. 2005; Sayre et al. 2005; Damiani et al. 2006; Kunz and Fent 2006; Soeborg et al. 2006). By contrast, other studies soften the extent that these side-effects can cause to humans (Janjua et al. 2004).

## 1.4 Disposition of the UV Filters in the Human Body

The toxicity of a substance, defined as the response to cause harmful effects to the body, depends on the concentration that reaches to the organ or tissue, which in turn depends on both the dose that the substance is administered and the kinetics of accumulation (Rozman and Klaasen 2008).

The disposition of the substance goes from absorption, ingestion or inhalation, until their excretion, taking into account the processes of distribution and biotransformation. Thus, the toxicokinetics and disposition of xenobiotics are closely related.

Considering the case of percutaneous absorption, if the absorbed amount or velocity of absorption is high, the xenobiotic can achieve a high enough concentration to cause toxicity in a particular place. Likewise, the more slowly the chemical is excreted outside the body, the higher is its concentration and, therefore, its toxicity in the corresponding tissue.

Furthermore, the distribution of the chemical cause that it reaches other tissues at lower concentration levels, then decreasing the toxicity.

In the particular case of the UV filters percutaneous absorption, their elimination from the general circulation of the body passes through biotransformation processes, the reservoir in the different parts of the body and excretion.

### *1.4.1 Percutaneous Absorption*

The percutaneous absorption of the UV filters is defined as the process by which these compounds pass through the skin and are incorporated into the bloodstream. To be absorbed through the skin, UV filters must cross by diffusion the stratum corneum, which is the main barrier of the process. Then, they must come into contact with the deeper layers of the epidermis, the dermis and, finally, incorporated into the general circulation to reach the subcutaneous fatty tissue (see Fig. 1.4). During this way, cutaneous metabolism processes may occur, especially based on hydrolysis reactions catalyzed by enzymes present in the pilosebaceous system (Howes et al. 1996).

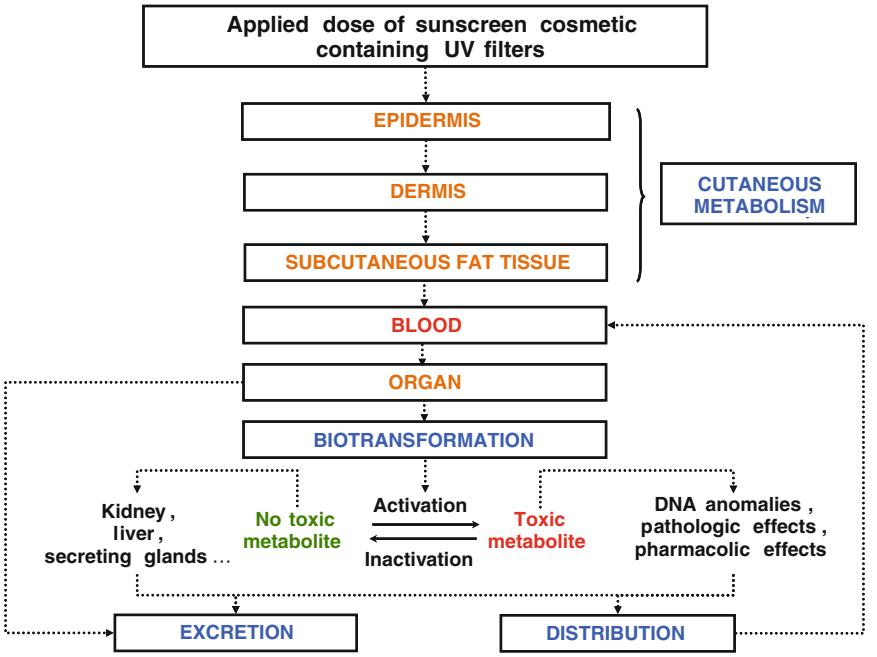


**Fig. 1.4** Schematic representation of the body disposition and toxicity associated with percutaneous absorption of UV filters

Percutaneous absorption of UV filters depends on different aspects. First, the diffusion capacity of the UV filter to pass through the stratum corneum, which is the mechanism of conveyance and which is an inverse function of the molecular weight or volume. Second, the lipophilic nature of the UV filter, which affects their ability to disintegrate in the epidermal lipids and can be estimated using the partition coefficient octanol/water ($K_{ow}$). Third, the morphology of the stratum corneum, since the thickness of this skin barrier differs greatly depending on the area of the body. Finally, the nature of the dermatological carrier which the cosmetic preparation is formulated (Walters and Roberts 2002). In the latter case, it is important to discuss some details to understand the importance of this aspect.

Generally, the UV filters are not directly applied to the skin as pure chemical compounds, but they are usually incorporated via a suitable carrier, known as vehicle, which is defined as the set of accompanying ingredients to the UV filter in the formulation and represents the existence of a physically structured matrix (Smith et al. 2002). As indicated above, the vehicle type or the nature of the employed excipients can markedly affect the percutaneous absorption of a specific UV filter. Thus, formulations prepared with a particular UV filter at the same concentration but using different vehicles can cause different levels of percutaneous absorption for the target compound. Normally, the highest kinetic percutaneous absorption of UV filters is obtained with alcohol-based lotions and lipid ointments.

The sunscreen cosmetic formulations differ from pharmaceutical preparations for topical use in purpose, since the sunscreens should ideally remain on the skin surface without causing percutaneous absorption processes to carry out their protective function. Thus, during formulations development, the stability and compatibility of UV filters and excipients are taken into account, apart from the cosmetic acceptance of the vehicle itself. Therefore, the sunscreen cosmetic formulations can be defined as a situation of continuous and dynamic equilibrium, where the constituents interact with each other and with the skin, once it has been applied.

In this context, other important factors that determine the extent of the cutaneous absorption are the exposure conditions, mainly the UV filter concentration, the area of exposed skin surface and the application frequency of the cosmetic product.

### *1.4.2 Distribution*

Once in the blood, the UV filters, like any other xenobiotic, can be distributed and/or move around the human body (see Fig. 1.4). Distribution to the various organs or tissues relies mainly on the blood flow and the rate of diffusion from the capillary bed into the corresponding cells. The final distribution depends largely on the affinity of the UV filter to the different tissues.

### *1.4.3 Biotransformation*

The UV filters, similar to some endogenous substances, can undergo biotransformation processes, consisting of the metabolic conversion of parent compounds in other more soluble compounds (see Fig. 1.4). In general terms, the properties of lipophilicity, which promote the percutaneous absorption of the UV filters through the skin, are replaced by hydrophilic properties that facilitate their excretion, mainly via the urine or feces.

The chemical modification induced by the biotransformation can vary the biological effects of compound. In most cases, the biotransformation of the xenobiotic toxicity decreases. However, the formation of active metabolites can sometimes occur and then, they can exert more toxic effects than the own parent compounds (Koda et al. 2005; Jeon et al. 2008) and may cause various diseases and abnormalities.

The biotransformation of xenobiotics is competence of a small number of enzymes that have broad substrate specificity. Some of these enzymes are synthesized in response to the xenobiotic through enzyme induction process, but in most cases they are constitutive enzymes, and thus their synthesis is carried out in the absence of an external stimulus discernible. Moreover, the structure or amino

**Table 1.2** General biotransformation mechanisms of xenobiotics and major subcellular localizations

| Reaction | Enzyme | Subcellular localizations |
|---|---|---|
| Phase I | | |
| Hydrolysis | Esterase | Microsomes, cytosol |
| | Peptidase | Lysosomes |
| | Epoxide hydrolase | Microsomes, cytosol |
| Reduction | Azo and nitro reductase | Microsomes, cytosol |
| | Carbonyl reductase | Microsomes, cytosol |
| | Disulfide reductase | Cytosol |
| | Sulfoxide reductase | Cytosol |
| | Quinone reductase | Microsomes, cytosol |
| | Dehalogen reductase | Microsomes |
| Oxidation | Alcohol dehydrogenase | Cytosol |
| | Aldehyde dehydrogenase | Mitochondria, cytosol |
| | Aldehyde oxidase | Cytosol |
| | Xanthine oxidase | Cytosol |
| | Monoamine oxidase | Mitochondria |
| | Diamine oxidase | Cytosol |
| | H Prostaglandin synthase | Microsomes |
| | P450 Cytochrome | Microsomes |
| Phase II | | |
| Glucuronide conjugation | Uridine diphosphate-glucuronosyltransferase | Microsomes |
| Sulphate conjugation | Sulfotransferase | Cytosol |
| Glutathione conjugation | Glutathione S-transferase | Cytosol, microsomes |
| Aminoacids conjugation | Aminoacyl-tRNA synthetase | Mitochondria, microsomes |
| Acylation | N-acyltransferase | Mitochondria, cytosol |
| Methylation | Phenol-O-methyltransferase | Cytosol, microsomes |

acid sequence of a biotransformer enzyme can display interindividual variations that can cause differences in the rate of biotransformation of the xenobiotic (Parkinson and Olgivie 2008).

The reactions catalyzed by enzymes that biotransform xenobiotics are divided into two groups and they are commonly known as phase I and phase II reactions, as can be seen in Table 1.2.

Phase I reactions are limited to hydrolysis and oxidation–reduction reactions. These reactions display or introduce a functional group, mainly hydroxyl, amino, carboxyl and sulphide, and typically result in a small increase in the hydrophilicity of the xenobiotic.

Phase II biotransformation covers glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis) and conjugation with amino acids such as glycine, glutamic acid and taurine. With the exception of methylation and acylation, the rest of these reactions increase

significantly the hydrophilicity of the xenobiotic, which greatly enhances its excretion (Parkinson and Olgivie 2008).

Xenobiotics biotransformation enzymes are widely distributed throughout the body and are located in various subcellular compartments. In humans, the most abundant source of enzymes that catalyze the biotransformation reactions is the liver. These enzymes are also found in skin, lung, nasal mucosa, kidney, eye and gastrointestinal tract as well as in various tissues. At a subcellular level, biotransformation enzymes are mainly located in the endoplasmic reticulum (microsomes) and the soluble fraction of the cytoplasm (cytosol), and they appear in smaller amounts in the mitochondria, nucleus and lysosomes.

Cofactors for phase II reactions react with the functional groups that are present in the xenobiotic or have been introduced in the phase I biotransformation. Glucuronidation, sulfation, acylation and methylation reactions require activated or high-energy cofactors, such as ATP (adenosine triphosphate), while the amino acid or glutathione conjugation is carried out with previously activated xenobiotics.

Most phase II biotransformation enzymes are mainly located in the cytosol. In general, phase II reactions are much faster than phase I reactions, and therefore, the removal rate of excretion of xenobiotics that depends on a phase I reaction followed by a phase II conjugation, will generally be defined by the first reaction.

### *1.4.4 Excretion*

UV filters having a high lipophilicity, and thus a high coefficient $K_{ow}$, can efficiently be absorbed into the bloodstream and may undergo biotransformation process, while more polar compounds may be directly removed from the body by various means, including usually the urinary via (Okereke et al. 1993). However, apart from urine, it seems that all corporal secretions are able to excrete chemical substances administered externally to the human body. Thus, UV filters have been also found in faeces (Volkel et al. 2006), milk (Hany and Nagel 1995) and, as will be seen in this Thesis, in semen (Chaps. 5, 8).

## 1.5 Methodology for the Study of Processes Derived from the Percutaneous Absorption of UV Filters Contained in Sunscreen Cosmetics

As discussed above, there is a clear evidence to show that the human body can absorb through the skin some of the organic UV filters contained in sunscreen cosmetic formulations. These observations have prompted the researchers to study the disposition of these organic compounds in humans.

Despite the many complex interactions that can occur during transport of the UV filter from the outermost surface of the skin to the circulatory system or excretion, various systems have been proposed to study the series of processes that includes the UV filter body disposition. In this context, it should be noted the importance to develop analytical methods to obtain sensitive, selective and accurate estimations of the percutaneous absorption levels of UV filters by using *in vitro* systems or by means of the non-invasive determination of UV filters and/or their metabolites in biological fluids.

Researches found in the literature that employ *in vitro* and *in vivo* systems to consider processes derived from the body disposition of UV filters contained in sunscreen cosmetic products are detailed in **Part II** and **Part III** of this Ph.D. Thesis, respectively. Likewise, the main objectives that address these investigations and the instrumental techniques employed are also discussed.

## *1.5.1 In Vitro Methodology*

The percutaneous absorption may be studied using *in vitro* methods, by means of membranes that simulate artificial skin or excised skin itself from animals or humans. This is possible because the excised skin maintains the barrier properties of the stratum corneum.

Among the advantages of using these alternatives, it must be highlighted not only the fact that the human skin can be used, but also that no procedures on live animals are carried out, allowing reduction and substitution of animal tests to estimate the percutaneous absorption of chemicals. The failure to obtain pharmacokinetic parameters and the possible difficulty of getting enough necessary human skin are the main drawbacks. Moreover, artificial membrane systems or animal skin have only limited use in predicting human absorption due to the large differences in skin permeability properties between human skin and animal skin or artificial membrane (Howes et al. 1996).

The primary methodology to estimate the *in vitro* percutaneous absorption of the UV filters is based on the use of diffusion cells. Furthermore, the distribution of UV filters can also be estimated from *in vitro* form using the tape-stripping technique on excised skin, which due to its non-invasive nature can also be applied directly on human volunteers and animals.

### 1.5.1.1 Diffusion Cells

The required number of physical and working conditions to carry out a general *in vitro* procedure based on the use of diffusion cells to assess the percutaneous absorption of UV filters contained in sunscreen products is described below.

Figure 1.5 shows the outline of the diffusion cells used in this Ph.D. thesis, which are based on Franz-type cells (Franz 1975) and designed by professors Dr.

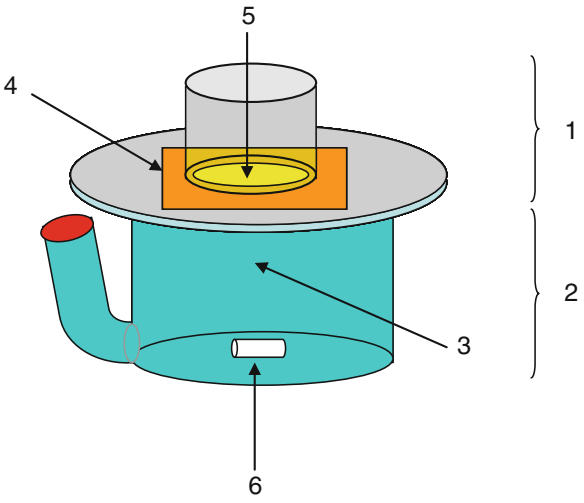


**Fig. 1.5** Diffusion cell used in our laboratory for the *in vitro* procedure. Donor compartment (*1*), receptor compartment (*2*), receiver solution (*3*), epidermis (*4*) sunscreen (*5*) and magnetic stirrer (*6*)

M. Herráez and Dr. O. Díez, from the Department of Pharmaceutical Technology of the University of Valencia. They have been part of our research group for several projects.

The used diffusion cells consisted mainly of glass and two compartments, called donor and acceptor compartments. The receptor compartment contained a receiver fluid and a magnetic stirrer. A piece of skin and the donor compartment were subsequently placed over the receptor compartment with care, sealing the entire cell with forceps and placing a plug in the hole on the receptor compartment that allowed the sampling. Next, the sunscreen cosmetic product containing the UV filter of interest was added on the skin surface available.

As previously discussed, to correctly predict the percutaneous absorption in humans, it is recommended the use of split human skin samples, which can be obtained from the surgeries remains or directly from corpses, strictly applying ethic work practices. In order to minimize variability in the permeability properties of the skin between the various anatomical parts, it is recommended to take the skin from a single specific zone. Then, depending on the study, the skin can be used either as a whole or only considering the epidermal membrane. In the last case, the subcutaneous fat can be removed by applying a heat source to promote the separation of the epidermal layer from the rest of the skin using tweezers (see Fig. 1.6).

Typically, the skin portion is subjected to a thermostated water bath at 60 °C for one minute. This separation process is carried out with extreme caution in order to obtain the thin layer of epidermis without breaks. Next, the epidermal layer is usually placed on absorbent paper to get greater rigidity when preparing the diffusion cell.

To prepare the receptor fluid in the procedures followed in this Thesis, a solution of phosphate salts was used under salinity and pH experimental conditions

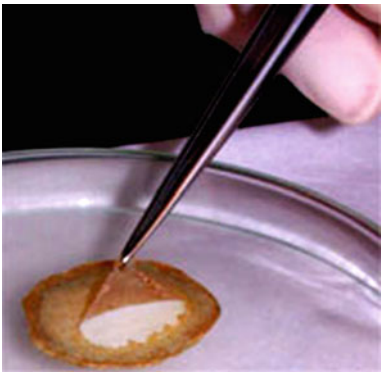

**Fig. 1.6** Process of separation of the epidermis layer

close to those found in body fluids. It must also be taken into account that the receptor fluid must not alter the barrier properties of the skin. In this regard, the physicochemical properties of the UV filter and the requirements for the final analysis must be also considered

The cell must provide adequate clamping of the skin and enable a good temperature control of the receptor compartment, which should be maintained constant and close to normal skin temperature. Optimal homogenisation and easy sampling of the receptor fluid should be also allowed.

The study of percutaneous absorption can be made by applying the cosmetic formulation containing, besides the UV filter, excipients which may have their own intrinsic effects on the skin. Hence, the choice of vehicle is one of the key considerations in the study. Typically, the concentration of UV filter is selected according to the authorized conditions of use. After the application of the cosmetic product, the target UV filter remains in contact with the skin in the upper donor compartment for a period of time. The average length of the experiments ranges from 24 to 48 h. The absorbed amount of UV filter is collected in the receptor fluid that is usually sampled at the end of the experiment or at intermediate times.

Although the state of the human skin before the initial preparation can be assessed at a glance, it is recommended to check the integrity of the skin barrier used before (Howes et al. 1996) or after (Balaguer et al. 2006) the application of the sunscreen cosmetic product by conducting tests with coloured markers. Thus, the viability of the assay can be confirmed (see Fig. 1.7), thus avoiding the consideration of samples that are defective or skin that is damaged during handling and, therefore, is abnormally permeable.

Finally, both the receptor fluid and skin itself can be analysed to verify the results through the determination of the amount remaining on the surface, using appropriate methods.

**Fig. 1.7** Image obtained in our laboratory after a test of skin integrity with a marker (Balaguer et al. 2006) on a diffusion cell which proved to be discarded (*left*) and one that proved to be valid (*right*) after the corresponding analytical determinations of the marker in the receptor fluid

### 1.5.1.2 Tape-Stripping Technique

It is based on the application of a cosmetic formulation containing the UV filter on a limited surface of stratum corneum during a certain time, normally set to 30 min (see Fig. 1.8). Then, after removing the rest of the formulation and successive washings, the estimation is made from the amount recovered from the stratum corneum by application of various adhesive tapes at different times (Weigmann et al. 2001). The determination of absorbed UV filter is carried out using a suitable analytical method.

The methodology to predict *in vivo* topical bioavailability is largely based on the correlation between the application of the UV filter at a short-term and its permeability in the steady state. Thus, given a limited exposure period, the fraction of the dose which penetrates into the stratum corneum from the rest that cannot be removed by simple washing will be equal to the fraction which has reached the circulatory system.

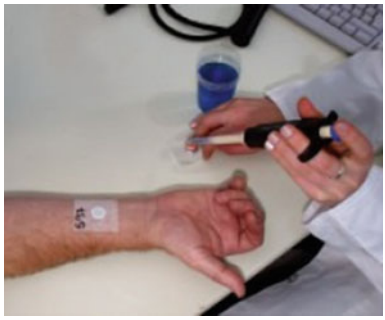

**Fig. 1.8** Application of tape-stripping technique on a volunteer

The procedure is non-invasive, direct and low cost although the skin preparation, regarding anatomical selection, shaving and cleaning is critical for the UV filter recovery.

### *1.5.2 In Vivo Methodology*

The methodologies described in the literature to show the process of percutaneous absorption of UV filters using *in vivo* systems are based mainly on the application of a cosmetic formulation containing a UV filter of interest on the individual skin under certain conditions, in terms of applied amount of product, studied skin area and dose repetition. Subsequently, an analytical determination is performed to quantify the content of the UV filters and/or its metabolites in biological fluids and tissues at established time intervals after the product application.

The predominant biological matrix in these studies is urine, followed by plasma or serum, and quite far from biological tissues and breast milk, which have been only analyzed occasionally, and semen, whose analysis has been first proposed by our research group (see Chaps. 5, 8). Exceptionally, the tape-stripping technique have also been applied to human volunteers, especially to evaluate the effect of the vehicle on the percutaneous absorption of the UV filter.

## 1.6 Effect of the UV Filters in the Male Reproductive System

As the processes of percutaneous absorption of UV filters contained in sunscreen cosmetic products are evident, it is necessary to take a series of measures to establish adequate security for the user. First, the development of new products that protect and minimize the harmful effects of the sun through the use of UV filters that are non-toxic and maintain a minimum percutaneous absorption kinetics is essential. On the other hand, however, as a result of these processes, the expression of estrogen activity and the appearance of effects of endocrine dysfunction (Schlumpf et al. 2001), both associated with the use of such cosmetic products, have increased alarm on possible implications that UV filters can cause on the human reproductive system.

### 1.6.1 Effects of Toxics on the Reproductive System

The endocrine function of germ cells consists mainly in the perpetuation of the species. The genes located on the chromosomes of these cells and the transmitted genetic information modulate cell differentiation and organogenesis.

Contact with chemicals that disrupt the endocrine function has been linked to lower fertility in birds, fish, shellfish and mammals, with the loss of attributes of masculinity and the feminization of fish, birds and gastropods (Vos et al. 2000). In general, endocrine disorder mechanisms caused by chemicals except heavy metals, is based on competition with receptors or inhibition of the synthesis of steroids.

In humans, it is estimated that one in five couples are infertile unwillingly, that more than one-third of the embryos die early and that about 15 % of diagnosed pregnancies are aborted spontaneously. Of foetuses that survive and reach the birth, about 3 % has developmental defects (not always anatomical), from which more than twice of that number is detected during growth. Even under normal conditions, the reproductive system is not working fully. Therefore, if the presence of xenobiotics is added to these problems, it is not surprising that the interference of various processes or phenomena of reproduction increases markedly (Thomas and Thomas 2008).

### 1.6.2 Evaluation of Reproductive Capacity

There are several tests to assess endocrine function in humans (Thomas and Thomas 2001). The fact that there are chemicals capable of altering the reproductive system is an added difficulty when attempting to evaluate the harmful effects of toxic products in general. Apart from taking into account the considerable structural diversity of xenobiotics, the areas of the body where they may act and their very different mechanisms of action must be considered.

For men, the two basic methods to check if a chemical can be harmful to the generation of sperm are the testicular morphology evaluation and the functional assessment of spermatogenesis (Sharpe 1998). The finding of impaired spermatogenesis/testicular morphology, the degeneration of germ cells depending on their stage of development, and the insufficient delivery of normal sperm are included in these methods. At the molecular level, hormonally active androgens, primarily dihydrotestosterone and testosterone, stimulate anabolic and reproductive functions, which are mediated by its interaction with the nuclear receptors of steroid-androgen, also known as androgen receptors (AR). The action of the AR is very specific, despite the homology between them and other steroid receptors. The androgen target cells contain enzymes that can activate, deactivate and change the specificity of AR. However, different xenobiotics have been described to inhibit the binding of androgens to their receptors (Donovan et al. 1980) and to act as potent AR antagonists that can affect the reproduction of man (Kelce et al. 1995).

The evaluation of the processes of reproduction in mammals is much more complex in women than in men. Among females, those processes are ovulation, development of sexual receptivity, the transport of gametes and zygote, fertilization and implantation of the conception. All these phenomena may be disturbed by the action of external chemical agents.

It should be noted that estrogen, that are steroid hormones, have influence on growth, differentiation and function of several reproductive organs, such as mammary gland, uterus, vagina, ovary, as well as in some organs of male reproductive (testes, prostate). Estrogens can be located outside or inside the cells, but certain tissues can retain them with high avidity and specificity by action of intranuclear binding proteins known as estrogen receptor (ER).

Serum levels of estrogens, such as estradiol, and estrogenic effects on target tissues constitute a normal sign of follicular function. Using cell culture techniques, the chemicals ability to inhibit cell proliferation and estrogens production can be detected selectively (Zeleznik et al. 1979). The ratio of estrogen and progesterone in the nucleus and cytoplasm can have important applications in toxicology. Estradiol receptors and progesterone are particularly important because certain chemicals compete for these receptors and may alter its molecular conformation (Thomas 1975).

### *1.6.3 Risk Factors for Human Fertility*

Most humans are exposed to a vast number of chemicals that can be dangerous to their reproductive capacity. Through laboratory studies, it is known that numerous chemicals are harmful to reproduction. Although the data obtained using laboratory animals may lose validity when extrapolated to the human species, it has also been shown that these chemicals exert harmful effects on human reproductive function.

It has been suggested that men are more vulnerable to environmental and occupational toxic than other mammals (Overstreet et al. 1988). The dangers and risks to reproduction have led to the formulation of policies for protection because certain professional occupations are related to semen quality. Thus, prolonged sitting, working with high heat sources, or exposure to X-rays can be causes of poor sperm quality.

In the early 1990, Carlsen and colleagues conducted an analysis of sperm density on average about 15,000 men over 50 years and it was revealed an alarming gradual decrease in semen quality (Carlsen et al. 1992). Although the application of mathematical models to describe the same set of data could allow the obtaining of other conclusions (Bromwich et al. 1994), there is a consistent evidence to suspect that changing lifestyles and increased exposure to estrogenic agents and other endocrine disruptors may correlate with the increased incidence of reproductive health problems of men, including testicular carcinomas, poor semen quality and other male reproductive system disorders (Skakkebaek et al. 2006).

Meanwhile, epidemiology has increased its importance in establishing cause-effect relationships, since it is inherently related to the risk control. The design of epidemiological studies may involve the use of both retrospective and prospective data, as well as statistical aspects such as the level of significance and effect size. Therefore, the importance of developing analytical methods to determine selectively and sensitively potential toxic compounds in biofluids related to human reproductive ability is unquestionable and can relate, for example, the concentration of a particular analyte to the ease binding with different hormone receptors.

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