The Potential Use of Non-invasive Methods in the Safety Assessment of Cosmetic Products

The Report and Recommendations of an ECVAM/EEMCO Workshop (ECVAM Workshop 36)[1–3]

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Preface

This is the report of the thirty-sixth of a series of workshops organised by the European Centre for the Validation of Alternative Methods (ECVAM). ECVAM's main goal, as defined in 1993 by its Scientific Advisory Committee, is to promote the scientific and regulatory acceptance of alternative methods which reduce, refine or replace the use of lab-

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[1]ECVAM — European Centre for the Validation of Alternative Methods.
[2]EEMCO (The European Group for Efficacy Measurements on Cosmetics and Other Topical Products) was created in 1994. It is a group of independent experts from private and public areas from different Member States of the European Union and with competence in the field of instrumental assessment of cosmetic products. EEMCO prepares overviews of existing evaluation methods and analyses the advantages and limitations of the existing techniques to provide general guidance to both the experimenter and the inspector.
[3]This document represents the agreed report of the participants as individual scientists.

oratory animals. One of the first priorities set by ECVAM was the implementation of procedures which would enable it to become better informed about the state-of-the-art of non-animal test development and validation, and the potential for the possible incorporation of alternative tests into regulatory procedures. It was decided that this would be best achieved by the organisation of ECVAM workshops on specific topics, at which small groups of invited experts would review the current status of various types of *in vitro* tests and their potential uses, and make recommendations about the best ways forward (1).

The workshop on The Potential Use of Non-Invasive Methods in the Safety Assessment of Cosmetic Products was held in Brussels, Belgium, on 10–12 March 1998, under the co-chairmanship of Michael Balls (ECVAM), Gérald Piérard (the European Group for Efficacy Measurements on Cosmetics and Other Topical Products [EEMCO]) and Vera Rogiers (EEMCO). The participants included scientists working in both academia and industry.

The current status of clinical and instrumental assessment of the efficacy of cosmetics was reviewed, together with the potential of using non-invasive techniques in safety assessment with human volunteers.

Introduction

Cosmetic products are defined as "Substances or preparations intended to be placed in contact with the various external parts of the human body or with the teeth and the mucous membranes or the oral cavity, with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition. They must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use." (2). As used by the general population in daily life, cosmetic products usually pose no problems for human health. However, side-effects induced by cosmetics can sometimes occur. The frequency of side-effects is not exactly known, but the types of ingredients and finished products involved usually are (3).

Side-effects can be both local and systemic. Mainly local skin effects occur with cosmetic products, including irritation, contact allergy, urticaria and sunlight-induced reactions. Irritation is the most frequently observed side-effect of cosmetics (3, 4). It is clear that cosmetic companies and regulatory authorities should take all reasonable measures to minimise any harmful effects.

According to the Sixth Amendment to the European Union Cosmetics Directive, a dossier must be kept ready for inspection by the Competent Authorities, and must contain a toxicological file based on the safety assessment of the ingredients and the finished products (2). An efficacy file must also be retained, in which any claims made for the product must be substantiated. These claims can be supported in a variety of ways (5, 6). One of these is to conduct studies on human volunteers and measure the effects observed, either by clinical scoring or by using quantitative or semi-quantitative non-invasive methods.

The following considerations formed the background to the workshop.

1. The developments made in bioengineering technology during recent years have been such that subtle changes in skin morphology and function can now be measured.
2. The Sixth Amendment foresees a ban on the marketing of cosmetics containing ingredients or combinations of ingredients tested on animals after 30 June 2000 (as specified in *Directive 97/18/EEC*), provided that sufficient progress has been made in developing satisfactory methods to replace animal testing (7).
3. A Seventh Amendment (in preparation) is likely to introduce an earlier ban on animal testing for finished cosmetic products. This would be based on the fact that the safety of a finished cosmetic product can be assessed from knowledge on the safety of its ingredients and by using methods that do not involve animal testing, except in very specific cases, for which an exception would be likely to be allowed.
4. The value of animal or non-animal tests in predicting exposure in the human population might be limited. Therefore, confirmatory safety tests in humans

might be scientifically and ethically necessary, provided that a safety assessment based on the ingredients had been carried out and had produced acceptable results.

5. The Scientific Committee on Cosmetology and Non-Food Products recently approved ethical guidelines for the testing of cosmetic ingredients on human volunteers (8) and discussed a draft version on human testing of the irritative capacity of finished cosmetic products (9).

Members of ECVAM and EEMCO have stated that it is pertinent to evaluate and discuss the potential use of non-invasive methodologies in the safety assessment of finished cosmetic products on human volunteers and, in particular, in assessing the occurrence of skin irritation (10–14). Skin irritation, as already mentioned, is a local side-effect which is dependent on the concentration used, the exposure time, and some other specific conditions. On the other hand, contact allergy is an immunologically based side-effect and is more difficult to predict, since it only affects predisposed individuals.

Since the terms "non-invasive" and "ethical" are open to different interpretations, some explanation is given of the sense in which they are used in this report.

Non-invasive

The term "non-invasive" carries a range of meanings in the literature, including, "without drawing blood", "without making contact", "without altering structure or function", "not penetrating the epidermis with material or with radiation", "not causing harm" and "maintaining the integrity of the organism, tissue or cell". All of these definitions can be insufficient in certain situations. Therefore, we have used "non-invasive" to mean "a procedure or instrument causing minimal and only temporary changes to structure or function, and in particular, not involving pain, incision or loss of blood".

Ethical basis

There are many ways of choosing and implementing an "ethical" basis for scientific and medical investigations on living organisms. There are also many ways of considering what should count as relevant similarities and differences between humans and other animals as subjects of such investigations, as is clear from reviews such as those by Midgley (15), Paton (16) and Gillon (17). In general, it is widely accepted that it is better, at least on scientific grounds, to conduct an investigation on humans, if the final product or procedure under investigation is intended to be used on, or applied to, human skin. This is because the structure and function of human skin and its appendages are sufficiently different to those found in other species for predictions extrapolated from tests conducted on other species to carry a significant risk of being qualitatively and/or quantitatively misleading. If such a risk of misinterpretation is unnecessary, given the available technology and other resources, then, even without other grounds, conducting an investigation on non-humans could be considered unethical. It is for this reason that the current potential of using non-invasive measurements on human volunteers in the safety assessment of cosmetics is reviewed in this report. Any such investigations should, of course, be conducted according to acceptable ethical standards, as described, for example, by Salter (18) and by Walker *et al.* (19).

The Clinical Assessment of Cosmetic Products

Normally, human volunteers with healthy, non-pathological skin are selected, and products are applied only when they are considered to be safe according to the toxicological profiles of their ingredients.

In principle, three levels of human volunteers study should be distinguished, according to the three levels of testing, i.e. safety, mildness and efficacy.

In safety testing, the ability of the test product to cause irritation is assessed in a small group of volunteers, and the data are compared with those for a similar product which has an extensive history of safe marketing. The test protocols typically involve some exaggeration of exposure, while using a regimen which involves a similar mode of application to that found during normal product use. In all circumstances, exposure to a product is stopped immediately if any signs of skin irritation appear. Evaluation of the test outcome includes a comparison of

the time (number and frequency of exposures) required to elicit mild irritation, as well as an analysis of the skin irritation scores. While non-invasive bioengineering techniques could be applied to quantify the results, to make them more objective, and even to measure some subclinical symptoms, this has not been common practice to date. Visual assessments are usually applied, and although this type of assessment is subjective, good results can be obtained by trained experimenters (19, 20).

Skin compatibility and mildness testing in human volunteers can be carried out in a similar manner to that for safety testing, but must involve exposure (normal or slightly exaggerated) which closely mimics typical consumer use of the product. Since the purpose can simply be to demonstrate an absence of effects, it is not always necessary for the test to be comparative, i.e. to involve positive and negative controls. However, to enhance the sensitivity of the null response, the use of appropriate bioengineering techniques is rather more common in this situation and is to be encouraged.

Efficacy testing, which may be used for claim substantiation, can also be carried out on human volunteers. These types of studies tend to be much more designed as normal in-use tests than those mentioned above. The need to formally substantiate the efficacy claims related to the product also requires objective measurement of the skin responses. Consequently, in addition to clinical measurements, including visual assessment and palpation, and an analysis of user questionnaires, appropriate skin bioengineering techniques are commonly employed in efficacy studies.

Signals to be Considered

When applying non-invasive methods, it is important to focus on the potential targets (stratum corneum, stratum Malpighii, dermis; Table I), to understand the theoretical risks on biological and clinical grounds. Therefore, a short description of the anatomy and physiology of the skin is given below.

Functional microanatomy of the skin

The skin is a multilayered organ, in which the stratum corneum represents the outermost layer, which covers and is produced from the living portion of the epidermis, the stratum Malpighii. Below the stratified keratinising epithelium, the dermis is formed by a richly vascularised connective tissue, where pilosebaceous follicles and sweat glands are located.

Within the epidermis, several cell types with different embryonic origins and functions are found. The keratinocyte corneocyte lineage, melanocytes and Langerhans cells will be considered in turn.

Keratinocytes and corneocytes

Keratinocytes form the bulk of the epidermis. They normally proliferate in the basal and epibasal layers and move progressively up through the stratum Malpighii toward the stratum corneum. During their ascent, they undergo differention, giving rise to the enucleated, flattened corneocytes which form the stratum corneum.

The stratum corneum is a compact, structurally heterogeneous, two-compartment system, in which layers of protein-enriched corneocytes are separated by a multilayered lipid-enriched extracellular matrix. The stratum corneum forms both a reservoir for, and a barrier to, the penetration of xenobiotics. Its thickness and molecular structure vary according to body region, and this affects its appearance and physical properties. In addition, genetic characteristics, ageing, skin diseases, and environmental hazards (for example, humidity variations, physical trauma, exposure to chemicals) alter the structure and function of the stratum corneum. Any topical product designed to be applied to the skin surface undoubtedly affects its quality and functional properties.

The most important adhesive force holding corneocytes together comes from the corneosomes, which are derived from the desmosomes that bind together the living keratinocytes. Corneosomes are normally subject to a programmed destruction after protease action. This permits the imperceptible casting off of single corneocytes from the skin surface and the continual renewal of the epidermis. Failure to degrade corneosomes correctly is the fundamental factor in most conditions where flaking is present. Such a feature results in a rough skin surface (xerosis), which is commonly called dry skin.

The maintenance of normal stratum corneum functions, including its turnover and permeability barrier homeostasis, is complex (21). It is regulated by many factors,

Table I: Tentative classification of the most commonly used non-invasive methods according to the signals to be detected

Non-invasive methods	Main location of signals
Squamometry (quantitative)	Stratum corneum
Corneosurfametry (quantitative)	Stratum corneum
Transepidermal water loss (TEWL)	Stratum corneum
Electrical methods for skin hydration	Stratum corneum + stratum Malpighii + dermis
Microrelief	Stratum corneum + stratum Malpighii + dermis
Laser-Doppler flowmetry (LDF)	Dermis
Colorimetry	Stratum corneum + stratum Malpighii + dermis
Narrow band spectroscopy	Stratum corneum + stratum Malpighii + dermis
Ultrasound	Dermis
Image analysis	Stratum corneum + stratum Malpighii + dermis
Clinical (non-instrumental) assessment	Stratum corneum + stratum Malpighii + dermis

including transepidermal water loss (TEWL) and various metabolic aspects which affect the keratinocytes. In fact, the epidermis generates a large number of biological response modifiers (BRM), which modulate the growth, maturation and apoptosis of keratinocytes. The leakage and diffusion of some BRM into the dermis can initiate vasomotor responses and inflammation.

The presentation of allergic and irritant contact dermatitis depends on intraepidermal damage. The form and extent of damage are dependent on the nature and concentration of the damaging agent, as well as on the conditions of exposure. An increasing number of chemical substances are now recognised as irritants and/or can become sensitisers.

Melanocytes

Melanocytes are regularly dispersed among the keratinocytes of the basal layer and contain melanosomes, the melanin-synthesising apparatus of the skin. Once loaded with melanin, melanosomes are transferred from the dendrites of the melanocytes to the cytoplasm of keratinocytes.

Melanosome size and degree of melanisation in epidermal melanocytes are genetically controlled, although non-genetic factors are undoubtedly important as well. Following the action of triggering factors or insults to the melanocytes, pigmentory changes can occur. Ultraviolet (UV) light, various BRM and hormones alter the skin colour related to melanin content and location. Epidermal melanosis results from increased melanin synthesis and transfer to keratinocytes. By contrast, melanoderma and certain types of ceruloderma result from melanocyte insults followed by melanin leakage into the dermis and melanosome phagocytosis by perivascular dendrocytes.

Both melanin distribution and production are altered during ageing. Chronological ageing is responsible for a progressive decrease in the density of active melanocytes. In contrast, photo-ageing induces a mottled pigmentation, with hyperactive foci adjacent to melanin-depleted areas. Such aspects are better appreciated when the skin is illuminated by long-wavelength UV light.

Langerhans cells

Langerhans cells are dendritic cells dispersed in the stratum Malpighii. They capture and process antigens which penetrate the skin, and then present them to T-lymphocytes in the initiation of a delayed-type immune response. Upon stimulation, they also release specific BRM. They represent the main resident cutaneous cell type involved in contact allergy.

Potential targets and location of signals to be detected

During the last two decades, an increasing number of non-invasive methods have been

developed for objectively determining skin properties, so that subjective, visual or tactile evaluations of skin conditions can now be supplemented with quantitative measurements. This makes comparisons between results obtained in different parts of the world more feasible. It is also important to note that these new techniques permit, in certain cases, the quantification of skin properties and subclinical symptoms that are not perceptible to the human senses. However, standardisation among instruments is at present imperfect, so measuring the same skin property with different instruments can give different results. The instruments from various companies, though based on the same principles, can use different scales. Hence, knowing that the transfer of scoring schemes between laboratories is difficult, the standardisation and calibration of the instruments is a key issue in successfully applying these methods in efficacy testing, in skin compatibility and mildness assessments and, in particular, in safety testing.

In Table I, a tentative classification of the most commonly used non-invasive techniques has been made, according to the main locations of the signals to be detected. This classification is based on the events and signals produced when surfactants are applied to the skin. A cascade of biological events takes place, resulting in clinical signs after a certain length of time and according to a specific kinetic pattern. Many authors have demonstrated that these gross clinical signs actually mask a wide range of histological and functional changes (22). The first signals are caused by stratum corneum and membrane structural damage and can be detected by techniques such as corneosurfametry and squamometry. Stratum corneum functions then undergo measurable changes, and TEWL measurements and electrical methods for skin hydration become useful. At a later stage, many kinds of BRM are liberated and have further effects. They are the basis of erythema, skin roughness, changes in pigmentation, and the development of oedema and skin thickening.

Instrumental Assessment: Available Non-invasive Methodology

As already mentioned, the standardisation of non-invasive methods is a key issue in their successful application in the safety testing of cosmetic products. This is necessary because of: a) environmental factors (for example, room temperature, relative humidity, light sources, air circulation); b) instrumental variables (for example, zero setting, calibration, probe properties, probe position); c) volunteer-linked factors (for example, age, sex, race, anatomical site, diurnal rhythm, skin type, cleansing procedures, skin diseases, medication); and d) product-linked variables (for example, galenic form, dilution, amount per surface unit, frequency and mode of application, inclusion of blanks). Only when these factors are taken into account in well-defined protocols can reproducible and relevant results be obtained.

Stratum corneum techniques: corneosurfametry and squamometry

Some minimally invasive methods have been designed to harvest the superficial part of the stratum corneum and to produce an objective and quantitative record of the tolerance of topically applied products (23).

The superficial corneocytes of the stratum disjunctum can be harvested by controlled, gentle rubbing of the skin surface. Another method consists of stripping by using adhesive tape. However, the commercially available adhesive tapes vary in their capacity to bind to the skin, so they are not usually suitable for accurate and reproducible corneocyte harvesting. A better method is to use appropriate clear self-adhesive coated discs applied to the skin under calibrated pressure for a defined period of time. Cyanoacrylate skin surface stripping is another method, which samples a thicker portion of the stratum corneum than the other procedures (24). All of these methods have their own advantages, limitations and pitfalls.

Corneocyte samples can be evaluated as they are taken or after staining with appropriate dyes. The nature of the assessments made varies according to the aim of the study. Ageing effects, xerosis, and the efficacy of cosmetic products can be assessed with a corneocyte sample by visual and microscopical observations, weight evaluation, optical measurements of light attenuation (25) and image analysis (11, 23, 26). Cyanoacrylate skin surface strippings are particularly suitable for the evaluation of the renewal dynamics of the stratum corneum (11). This type of sampling can also be used

to study the contents of follicular openings. As a result, comedogenesis, comedolysis and bacterial load can be accurately quantified (27–29).

As already mentioned, the reliability and relevance of studying the stratum corneum are best demonstrated in the assessment of the interaction between surfactants and the skin. After single or repeated short-term applications of diluted surfactants, the stratum corneum is the first structure to exhibit changes related to the aggressiveness of the product. This can be conveniently quantified by using a variant of squamometry (30–33). Briefly, a clear self-adhesive disc is applied to the test site under controlled conditions. After careful removal, the sample is stained by anionic or cationic dyes according to the nature of the reaction to be studied.

Corneosurfametry and corneoxenometry were designed as screening tools devoid of potential hazards for humans (31, 34, 35), and are performed on cyanoacrylate skin surface strippings harvested from healthy volunteers. Samples are placed in contact with the test product (surfactant or another xenobiotic) under controlled conditions (duration, temperature, dilution). The subsequent staining and evaluation procedures are identical to those of the squamometry test.

False positive and false negative data might be obtained if the test product is not sufficiently removed from the sample during the rinsing procedure, as the product could alter the binding of the stain to the stratum corneum sample.

The main advantage of corneosurfametry and corneoxenometry is the avoidance of any hazard for the human volunteers, even when neat products are tested. A negative result assures safe use in humans as far as the integrity of the stratum corneum is concerned. A strong positive result predicts a clinical problem which would present as an inflammatory irritant reaction.

Transepidermal water loss measurements

TEWL, or better, "skin surface vapour loss" (28), represents the total water loss from the viable epidermis and dermis, diffusing through the stratum corneum to the skin surface and originating from the sweat glands below the thermal theshold for sweating. In practice, with careful choice of the measurement conditions, the contribution of sweat evaporation can be made very small (36).

Numerous publications have shown that TEWL measurement is a good indicator of the integrity of the barrier function. Damage is reflected by an increase in TEWL. TEWL measurements are usually based on the measurement of the water evaporation gradient developed from skin surface hygrosensors and thermistors present in an open probe at various distances from the skin surface.

There are a range of instruments, all of which provide results in g/m^2/hour, although their calibrations might vary. It is therefore more accurate to talk of relative values than of absolute values. A number of important variables can affect TEWL measurements, including person-linked and product-linked factors, as well as environmental and instrumental variables (37–41). Of particular importance is the probe temperature, since, if this is neglected, a 200–300% variation around the actual measured value can occur (39).

TEWL measurements have many applications in the cosmetics industry; for example, for the substantiation of claims for moisturising products (42–45) and the development of new ingredients for an effective barrier function (46–49). They are also used in clinical and pharmaceutical research to provide better understanding of the characteristics of normal skin (50), the development of skin disorders (51), and the ageing process (52, 53).

In fundamental research, TEWL measurements have been particularly useful in elucidating the functional role of the stratum corneum (54, 55) and the roles of the various lipids in the intercellular matrix of the stratum corneum (56–60).

With respect to the use of TEWL measurements in safety testing, most publications have dealt with various classes of surfactants. Predictions of the degree of the irritative response to cosmetic ingredients and finished products have been performed by using TEWL measurements. It has been clearly demonstrated that the TEWL is a good indicator of barrier damage due to irritation (59, 61, 62). Subclinical measurements are possible, and it is clear that TEWL assessment is more sensitive than visual assessment (63). Characterisation of the profile of irritancy (as a result of exposure to corrosive or non-corrosive irritants) by

TEWL measurements has been proposed by Serup (64), and prediction of the percutaneous resorption of topically applied substances and products has been reported by Rougier (65).

Finally, TEWL measurements might also contribute to the development and validation of alternatives to the Draize skin irritation test, by providing data for use in *in vitro*/*in vivo* comparisons (66).

Electrical methods for estimating the moisturisation of the stratum corneum

Electrical methods for assessing the moisture content of the stratum corneum are based on measuring impedance, or conversely, conductance, as a function of one or more frequencies. Both impedance and conductance are frequency-dependent vector quantities, and the derivation of standard physical parameters such as resistance, reactance and capacitance is not simple (67–70).

Readings result in relative values, and care should be taken not to raise expectations for *in vivo* studies which cannot be justified (for example, inter-individual comparison of hydration values measured; 12).

Nevertheless, changes in stratum corneum hydration measured by electrical methods can be predictive of the irritation potential of topically applied compounds (41, 71–74). Indeed, subclinical irritant dermatitis can be detected by early changes (reduction) in stratum corneum hydration. In some experimental strategies, it has been shown that short-term surfactant application to the stratum corneum can also alter stratum corneum hydration and that this correlates well with the irritant potential of the compound tested (75). Stress tests (developed to assess stratum corneum hygroscopicity), such as the adsorption–desorption test, can be useful in evaluating subclinical changes in skin hygroscopicity and for predicting the onset of skin irritation (41, 76). Standardised procedures for hydration measurements have been published (12). The results obtained are strongly influenced by the properties of the skin surface, and the values measured decrease with increasing roughness.

Several different instruments are commercially available (12). The variables which affect the measurements are again related to the volunteers (77–79), to the environmental conditions (78, 80–82), and to the instrument used (82).

Microrelief measurements

The microrelief of skin is known to result from the three-dimensional organisation of bundles of collagen present in the superficial dermis. The appearance of the skin surface and its geometric characteristics are also dependent on the presence of living epidermis and of the stratum corneum covering it. Thus, it seems logical to assume that all changes in the thickness, composition and structure of these layers would result in changes in microrelief.

The superficial dermis also contains capillaries that provide both the nutrition and the temperature regulation of the skin. All changes in blood flow, and in capillary porosity, are likely to result in changes (at least transient changes) in the state of hydration of the affected tissue, thus also modifying the microrelief seen at the skin surface.

Besides offering the possibility of clinical evaluation, microrelief can be measured by using various non-invasive techniques (14). Most of these methods use replicas (negative) or counter-replicas (positive) of the cutaneous surface. The relief profile is then scanned by either mechanical or optical profilometrical techniques. Usually, several lines need to be scanned. Today, two-dimensional models are often complemented by three-dimensional methods, which permit the quantification of skin anisotropy.

An alternative to the profilometric methods is surface image analysis of an appropriately lit replica, which permits a global data analysis of some relief parameters. Rotation of the samples is necessary to describe skin anisotropy.

The disadvantages of these techniques are often linked with the production of replicas and counter-replicas (83–85). The length of time allowed for the silicone polymer to harden, the presence of air bubbles at the surface of the replica, the thickness of the replica, and the colour of the replica (especially when optical measurements are involved), are all critical in the success of these techniques.

Mechanical profilometric techniques can be used to measure the amplitude of skin microrelief with a high degree of accuracy, but they are slow because of their low scan-

ning speed. Optical profilometric measurements by the triangulation method with a linear sensor, measure the surface topography with a wide vertical range and involve faster scanning speeds.

Surface image analysis provides the mean density of the lines, the mean depth and the microrelief shape (86–88). This method can be automated, and the replicas can be automatically rotated. However, some data can be lost in the shadows, and the parameters obtained are not easily linked to classical standardised parameters (14, 23).

Microrelief measurements have been used to study the effects of topically applied surfactants. This is not surprising, since it is well known that the repeated application of surfactants to the skin modifies the barrier function, leaving the skin rough and dry (89). However, only a limited number of papers are available in the literature, which seems to suggest that other non-invasive methods (for example, measurement of colour, TEWL) are more advanced than quantitation of skin relief with respect to the objective determination of the effects of skin irritants.

Microscopical investigations of replicas of skin patches exposed to sodium lauryl sulphate can be used to evaluate the severity of the reactions, even with sodium lauryl sulphate concentrations as low as 0.02% (90–92).

In one study, the effects of three surfactants were distinguished by repeated patch tests and the subsequent use of cutaneous microrelief measurements (93). However, these results were not confirmed by others (94).

Some other applications of microrelief measurements after the application of irritants have been described by Agner & Serup (95). Modifications of skin relief following corticotherapy (96) and radiation (97, 98) have also been demonstrated.

It is possible that some of the methods listed above will be made redundant by new approaches made possible by the pattern projection analysers now being produced in Germany, Japan and the USA.

Skin colour measurements

Skin colour results from a combination of selective absorption and scattering of visible light wavelengths. Several major light-absorbing particles, referred to as chromophores, can be found in the skin. The colour of the epidermis is normally due to the presence of melanin (eumelanin and/or phaeomelanin) and, in rare instances, of carotenoids. Dermal chromophores can be found in the blood vessels of the skin (as oxyhaemoglobin, reduced haemoglobin and bilirubin).

In many human assays for evaluating skin response to topical products, redness is purported to be an indicator of inflammation. This concept is flawed when redness alone is used to assess all types of skin reactions. In fact, skin irritation can be non-erythematous in some instances.

Skin colour evaluations are often performed to determine the degree of physiopathological response of blood vessels to a variety of physical or chemical insults (99). Erythema is produced when exposure to irritants, allergens or short-wavelength UV light causes blood vessels close to the skin surface to dilate. Oxyhaemoglobin in the erythrocytes in the blood vessels gives the skin a red colour. The visual characterisation of erythema might be inaccurate when comparing cutaneous reactions in different subjects, because the colour of skin lesions generally depends on the colour of background normal skin. In fact, it is almost impossible to clinically detect the pink hue of discrete reactions in subjects with a dark complexion.

Traditionally, erythema has been assessed visually by trained observers according to predetermined arbitrary scales. Such assessments tend not to be consistent between studies or between observers. Additionally, the scale of increasing redness is not necessarily linear, which precludes the possibility of calculating an average value within a group of subjects.

Skin colour can be measured quantitatively by using reflectance techniques and spectrophotometry (13, 99, 100). Reflectance colorimetry takes advantage of the CIE-L*a*b* standardised system. Spectrophotometry uses either the whole light spectrum or narrow bands to quantify the levels of haemoglobin (the erythema index) and melanin (the melanin index). Instrumental measurements of erythema with modern devices result in more-objective, more-reproducible and more-quantitative data than visual scoring.

Parameter a* and the erythema index are closely correlated with the degree of redness,

and are consequently the most important measurements in the assessment of skin inflammation. In lightly pigmented subjects, the a* values and the erythema indices are linearly correlated. However, the erythema index becomes an overestimate when the melanin pigmentation increases. It is therefore advisable to measure the melanin index or the individual typology angle (101) at the same time, to better appreciate the significance of variations in recorded redness.

The major sources of variation for such colour measurements in groups of normal subjects include inter-individual differences and the test site used.

Comparative testing on the forearm should be randomised and obtained on the same longitudinal axis restricted to the mid-forearm (102). Furthermore, the position of the forearm, either horizontally or vertically oriented in an upward or downward direction, modifies the skin colour (103) and the sensitivity of the measurements. Differences in redness between an active inflammation site and a control site are likely to increase when the orthostatic blood pressure is decreased, i.e. when the forearm is held vertically upright (99).

It is important to consider the time-course of erythema when measurements are planned, as it can vary according to the nature of the test challenge. Depending on the nature and the severity of the inflammatory reaction, several distinct mediators, released by various cell types, can be involved in the erythemal response.

Some foods, medicines, neural and endocrine influences, the nycthemeral rhythm, environmental conditions and vascular diseases can considerably influence the data generated. The presence of chronic contact dermatitis, an acute allergic contact dermatitis on another part of the body, or a previous local skin challenge, can alter the skin's reaction to xenobiotics. These responses might be lessened or, conversely, boosted, according to local and systemic influences. Mast cell reactivity, identified by dermographism, and tachyphylaxia are other factors which influence the vascular response of the test site.

Laser-Doppler flowmetry

Laser-Doppler flowmetry (LDF) is a method which is able to provide continuous non-invasive measurements related to changes in microvascular perfusion, in terms of relative changes of blood volume and velocity. The method is based on the effects of the light on moving (mainly erythrocytes) and non-moving components of a limited volume of tissue.

When tissue is illuminated by a coherent, monochromatic low-powered light, such as that emitted by low-power lasers, only a small amount of light (about 3–7%) is reflected. The remaining 93–97% of the incident radiation is partly absorbed by various structures, and partly undergoes single or multiple scattering (104–106). A variable amount of this scattered light (more than 50% at 633–785nm) is then re-emitted from the surface and is collected by a photodetector. The light recaptured by the photodetector produces the LDF raw signal.

Scattering results from the collision of light photons with either static or moving components of the tissue. The collision of one photon with a static structure causes a change in the direction of the photon without Doppler frequency shifting, whereas the collision of one photon with a moving structure (typically, an erythrocyte) causes a change in the direction of the photon with Doppler frequency shifting. As a result, the use of LDF produces an output signal which is proportional to the blood cell perfusion (or flux). This represents the movement of erythrocytes through the microvasculature.

Assuming a proportionality between erythrocyte number and blood volume, the LDF signal should be linearly related to the volume–velocity product of blood in the measured volume.

In relation to dermatology, the regional complexity of the microvasculature (107), its global variability in specific regions (such as fingers; 108), and the complex nature of light scattering in tissues, make LDF measurements suitable for characterising only relative changes in blood flow. LDF data are also strictly site-specific. A probe with several integrated optical fibres is recommended for skin measurements, to minimise these variations and to provide more-relevant data (109).

LDF data have been shown to correlate well with visual scores in the assessment of patch test reactions, and to be particularly useful in detecting doubtful and non-visible responses. LDF is used in dermatology and related sciences to predict irritancy (110, 111), to evaluate patch test reactions, to

assess topical products (anti-inflammatory and vasoactive drugs, sunscreens, detergent barrier creams), to monitor skin diseases (skin irritation and allergy, scleroderma, psoriasis, atopic dermatitis), and to quantify wound healing, burns and perfusion of skin flaps. Guidelines for the proper use of the instrumentation have been published (112).

Imaging techniques

Imaging techniques are highly diverse and can be classified according to their date of appearance in the scientific literature. These techniques include the B-scan Ultrasonic Method, Magnetic Resonance Imaging, Confocal Microscopy, and Optical Coherent Tomography. These methods are based on entirely different physical principles.

To date, only the ultrasound method has been the subject of technological development to the extent that it is used in numerous laboratories for product safety studies.

This method is based on the properties of ultrasonic waves (with a frequency higher than 20KHz), which are partially reflected when they pass through the interface existing between two media with different mechanical properties. It is customary to say that an ultrasound image of the skin reproduces the echostructure of the various skin layers. In this type of image, a white point (or white line) represents the interface between a hard tissue and a soft tissue. A black zone represents homogeneous tissue, without any interface, whether it is hard or soft.

Skin images obtained by using this technique provide information related to skin morphology and the thickness of the various layers, as well as more-qualitative information with respect to tissue type. It is principally used in clinical research, in skin pharmacology and in studies on local toxicity (irritation/allergy). It can also be used to determine the depth of tumour invasion prior to excision. However, in this report, it is its application in measuring irritation which is of particular concern.

When examining an ultrasound image of the skin to which an irritant product has been applied, marked changes appear which affect both the thickness of different layers and the image contrast.

After the application of 1.5% sodium lauryl sulphate in a 24-hour patch test, the image obtained shows a very marked swelling of the dermis and epidermis. By using a high-performance system, it is even possible to obtain a clear image of epidermal acanthosis. Furthermore, dark zones distributed throughout the tissue are observed. These are very probably caused by the oedema which forms subsequent to inflammation (113–114).

Quantification of the grey levels of the image, which are indicative of the presence of water, is extremely difficult, as it is necessary to ensure that the ultrasound energy delivered to the skin has not changed between the "control" situation and the "exposed" situation. However, certain options are available, and various studies have been published with respect to the quantification of the grey levels before and after the application of weak irritants (115, 116). Nonetheless, it appears to be necessary to study a procedure for grey level quantification in detail, to ensure the reliability of this type of determination.

When determining the thickness of the various skin layers, the principal factor to be considered is epidermal hyperplasia. This is not yet possible, as the imaging systems currently available do not have sufficient resolution to enable a clear visualisation of the epidermal layer, except in certain zones such as the hands. Further development is needed to permit a more rigorous approach for the determination of various parameters, and how they change following the application of very weak irritants.

Tensile properties assessment

The assessment of the mechanical properties of the skin is complex, yet is fundamental to understanding the physiology and pathophysiology of living skin, and ultimately to objectively assessing the effectiveness of topical products applied to the skin. The wide range of scientific disciplines involved in providing this specific knowledge illustrate the underlying difficulties which still exist with the state-of-the-art technology in this field.

The tensile functions of normal skin represent an important physiological characteristic, since they provide a degree of flexibility which is essential to movement and to resistance to rupture. The biomechanical anisotropy of human skin has been thoroughly studied (117, 118). Volunteer-linked variables in healthy (119–121) and diseased (76, 117, 122–124) skin have also been studied.

From a histological point of view, it is accepted that the mechanical characteristics of human skin result from the global contributions of connective tissue, dermis and hypodermis and, at least to some degree, from the epidermis. That is, the different levels of organisation from the skin surface down to the deepest regions, including the hypodermis, determine the various mechanical characteristics of the skin (114, 125, 126).

However, knowledge of the pure mechanics of the skin has not significantly benefited the development of new techniques, despite the recent development of technological tools (some of which are now commercially available) specifically designed to assess the biomechanical characteristics of human skin (41).

These systems are based on the measurement of changes induced by the application of external forces to the skin surface and can provide quantitative indications of any changes. However, an objective appreciation on a purely mechanical basis is not yet possible, and the nature and significance of the information obtained is unclear, since there is no way of relating the quantitative data obtained to particular structures of the skin. Moreover, the available data are obtained via various techniques, applied under a range of experimental conditions, which precludes a comparison of the results.

Optimisation of the information provided by these systems should be considered essential for maximising the potential application and usefulness of this new technology. In addition, and independently of the technological advances expected in the future, the standardisation of the measurement procedures currently used would represent a significant step forward.

Conclusions and Recommendations

1. Efforts should be made to optimise the existing non-invasive methods and their use in human studies. Much variation exists in the protocols reported in the literature, and it is not always clear how they should be applied and how they could be combined with other techniques under optimal conditions.
2. There is a great need for the optimisation and standardisation of the various protocols. This is a key issue, if non-invasive methods are to be increasingly used in the future in the safety and efficacy assessment of cosmetic products. It is therefore recommended that the most promising protocols for non-invasive quantification of skin properties and responses should be optimised and standardised at the European level.
3. To minimise the risk to the consumer of adverse skin reactions caused by new cosmetic formulations, which are considered to be safe based on data on the safety of their ingredients, it is recommended that the safety of the formulation should be confirmed in human volunteer studies prior to product launch.
4. Human volunteer testing should always be subject to strict ethical controls, as agreed by the Competent Authorities. It is recommended that a standard ethical protocol is defined, in which there is a clear definition of the conditions under which the safety, compatibility, mildness and efficacy testing of cosmetics on humans is permissible.
5. Results obtained from the literature and the experiences of the workshop participants clearly indicated the possibility that some non-invasive methods for quantifying human skin irritant reactions (in particular, squamometry, corneosurfametry, TEWL measurement, electrical methods for stratum corneum hydration and LDF procedures) can be applied at a subclinical level. However, efforts are needed to better define what is meant by "a subclinical level", since human testing at this level is clearly more ethically justifiable than at the clinical level.
6. The results generated by non-invasive methods of the type described in this report cannot stand alone. Thus, whenever instrumental assessment is being considered for testing the safety, compatibility, mildness or efficacy of cosmetic products, it is recommended that the results obtained are combined with those from clinical observations and/or from *in vitro* tests. It is also recommended that strategies are defined for optimising combinations of non-animal methods with human volunteer studies for complementary and confirmatory purposes.
7. It is recommended that the general public and cosmetics companies are informed

of the potential value of such strategies and methods. This could be achieved by producing educational material for general distribution and by setting up training courses for the companies concerned, and especially for small and medium-sized enterprises.

References

1. ECVAM (1994). ECVAM News & Views. *ATLA* **22**, 7–11.
2. EEC (1993). Council Directive 93/35/EEC of 14 June 1993 amending for the sixth time Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. *Official Journal of the European Communities* **L151**, 32–36.
3. de Groot, A.C., Weyland, J.W. & Nater J.P. (1994). The spectrum of side-effects of cosmetics. In *Unwanted Effects of Cosmetics and Drugs Used in Dermatology*, 3rd edn (ed. A.C. de Groot, J.W. Weyland & J.P. Nater), pp. 437–441. Amsterdam, The Netherlands: Elsevier.
4. Berne, B., Lundin, P. & Enander Malmros, I. (1994). Side-effects of cosmetics and toiletries in relation to use: a retrospective study in a Swedish population. *European Journal of Dermatology* **4**, 189–193.
5. COLIPA (1997). *Guidelines for the Evaluation of the Efficacy of Cosmetic Products*, 10pp. Brussels, Belgium: The European Cosmetic Toiletry and Perfumery Association (COLIPA).
6. Rogiers, V. (1995). Efficacy claims of cosmetics in Europe must be scientifically substantiated from 1997. *Skin Research and Technology* **1**, 44–46.
7. EEC (1997). Council Directive 97/18/EEC of 17 April 1997 postponing the date after which animals tests are prohibited for ingredients or combinations of ingredients of cosmetic products. *Official Journal of the European Communities* **L114**, 43–44.
8. SCCNFP (1999). Guidelines on the use of human volunteers in the testing of potentially cutaneous irritant cosmetic ingredients or mixtures of ingredients. In *Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation, 2nd Draft, SSCNFP/0119/99, Revision 1,* pp. 73–77. SCCNFP.
9. SCCNFP (1999). *Position Paper Concerning Guidelines on the Use of Human Volunteers in Skin Compatibility Testing of Finished Cosmetic Products, SCCNFP/0068/98, Revision 1,* 5pp. SCCNFP.
10. Serup, J. (1995). EEMCO guidance for the assessment of dry skin (xerosis) and ichthyosis: clinical score systems. *Skin Research and Technology* **1**, 109–114.
11. Piérard, G.E. (1996). EEMCO guidance for the assessment of dry skin (xerosis) and ichthyosis: evaluation by stratum corneum strippings. *Skin Research and Technology* **2**, 3–11.
12. Berardesca, E. (1997). EEMCO guidance to assessment of the stratum corneum hydration: electrical methods. *Skin Research and Technology* **3**, 126–136
13. Piérard, G.E. (1998). EEMCO guidance to the assessment of skin colour. *Journal of the European Academy of Dermato-Venereology* **10**, 1–11.
14. Lévêque, J.L. (1999). EEMCO guidance to the assessment of skin topography. *Journal of the European Academy of Dermatology and Venereology* **12**, 103–114.
15. Midgley, M. (1983). *Animals and Why they Matter*, 158pp. Harmondsworth, UK: Penguin Books.
16. Paton, W. (1993). *Man and Mouse: Animals in Medical Research*, 2nd edn, 288pp. Oxford, UK: Oxford University Press.
17. Gillon, R. (1986). *Philosophical Medical Ethics*. Chichester, UK: John Wiley and Sons.
18. Salter, D.C. (1990). Ethics of human testing. *International Journal of Cosmetic Science* **12**, 165–173.
19. Walker, A.P., Basketter, D.A., Baverel, M., Diembeck, W., Matthies, W., Mougin, D., Paye, M., Röthlisberger, R. & Dupuis, J. (1996). Test guidelines for assessment of skin compatibility of cosmetic finished products in man. *Food and Chemical Toxicology* **34**, 651–660.
20. Basketter, D.A., Reynolds, F.S., Rowson, M., Talbot, C. & Whittle, E. (1997). Visual assessment of skin irritation: a sensitive and reproducible tool. *Contact Dermatitis* **37**, 218–220.
21. Elias, P.M. (1996). Stratum corneum architecture, metabolic activity and interactivity with subjacent cell layers. *Experimental Dermatology* **5**, 191–201.
22. Willis, C.M., Stephens, J.M. & Wilkinson, J.D. (1989). Epidermal damage induced by irritants in man: a light and electron microscopic study. *Journal of Investigative Dermatology* **93**, 695–699.
23. Piérard, G.E. & Piérard-Franchimont, C. (1996). Drugs and cosmetics evaluations with skin strippings. In *Dermatologic Research Techniques* (ed. H.I. Maibach), pp. 133–148. Boca Raton, FL, USA: CRC Press.
24. Marks, R. & Dawber, R.P.R. (1971). Skin surface biopsy: an improved technique for the examination of the horny layer. *British Journal of Dermatology* **84**, 117–123.
25. Serup, J., Winther, A. & Blichmann, C. (1989). A simple method for the study of scale pattern and effects of a moisturizer: qualitative and quantitative evaluation by D-squame tape compared with paramaters of epidermal hydration. *Clinical Experimental Dermatology* **14**, 277–282.
26. Piérard, G.E., Piérard-Franchimont, C. & Goffin, V. (1995). Digital image analysis of microcomedones. *Dermatology* **190**, 94–103.
27. Mills, O.H. & Kligman, A.M. (1982). A human model for assessing comedogenic substances. *Archives of Dermatology* **118**, 903–905.
28. Piérard, G.E. & Piérard-Franchimont, C. (1995). Squamometry in acute photodamage. *Skin Research and Technology* **1**, 137–139.
29. Letarve, C., Boone, M. & Piérard, G.E. (1998). Image analysis of the effect of topically applied linoleic acid on acne microcomedones. *Clinical and Experimental Dermatology* **23**, 56–58.
30. Piérard, G.E., Goffin, V. & Piérard-Franchimont, C. (1994). Squamometry and corneosurfametry in rating interactions of cleansing products with stratum corneum. *Journal of the Society of Cosmetic Chemists* **45**, 269–277.
31. Piérard, G.E., Goffin, V. & Piérard-Franchi-

mont, C. (1994). Corneosurfametry: a predictive assessment of the interaction of personal care cleansing products with human stratum corneum. *Dermatology* **189**, 152–156.
32. Paye, M. & Cartiaux, Y. (1999). Squamometry: a tool to move from exaggerated to more and more realistic application conditions for comparing human skin compatibility of surfactant-based products. *International Journal of Cosmetic Science*, in press.
33. Shimizu, T. & Maibach, H.I. (1999). Squamometry: an evaluation method for a barrier protectant (tannic acid). *Contact Dermatitis*, in press.
34. Goffin, V., Piérard-Franchimont, C. & Piérard, G.E. (1997). Microwave corneosurfametry: a minute assessment of the mildness of surfactant-containing products. *Skin Research and Technology* **3**, 242–244.
35. Goffin, V., Piérard-Franchimont, C. & Piérard, G.E. (1998). Shielded corneosurfametry and corneoxenometry: novel bioassays for the assessment of skin barrier products. *Dermatology* **196**, 434–437.
36. Batt, M.D. & Fairhurst, E. (1986). Hydration of the stratum corneum. *International Journal of Cosmetic Science* **8**, 253–264.
37. Pinnagoda, J., Tupker, R.A., Agner, T. & Serup, J. (1990). Guidelines for transepidermal water loss (TEWL) measurement. *Contact Dermatitis* **22**, 164–178.
38. Berardesca, E. & Maibach, H.I. (1990). Monitoring the water-holding capacity in visually non-irritated skin by plastic occlusion stress test (POST). *Clinical and Experimental Dermatology* **15**, 107–110.
39. Rogiers, V. (1995). Transepidermal water loss measurements in patch test assessment: the need for standardisation. In *Current Problems in Dermatology, Vol. 23, Irritant Dermatitis, New Clinical and Experimental Aspects* (ed. P. Elsner & H.I. Maibach), pp. 152–158. Basel, Switzerland: Karger.
40. Hildebrandt, D., Ziegler, K. & Wollina, U. (1998). Electrical impedance and transepidermal water loss of healthy human skin under different conditions. *Skin Research and Technology* **4**, 130–134.
41. Rodrigues, J.L. & Pereira, L.M. (1998). Basal transepidermal water loss: right/left forearm difference and motoric dominance. *Skin Research and Technology* **4**, 135–137.
42. Treffel, P. & Gabard, B. (1995). Stratum corneum dynamic function measurements after moisturizer or irritant application. *Archives of Dermatological Research* **287**, 474–479.
43. Grunewald, A.M., Gloor, M., Gehring, W. & Kleesz, P. (1995). Efficacy of barrier creams. In *Current Problems in Dermatology, Vol. 23, Irritant Dermatitis, New Clinical and Experimental Aspects* (ed. P. Elsner & H.I. Maibach), pp. 187–197. Basel, Switzerland: Karger.
44. Lóden, M. (1996). Urea containing moisturizers influence barrier properties of normal skin. *Archives of Dermatological Research* **288**, 103–107.
45. Lóden, M. & Andersson, A.C. (1996). Effect of topically applied lipids on surfactant irritated skin. *British Journal of Dermatology* **134**, 215–220.
46. Philippe, M., Garson, J.C., Gilard, P., Hocquaux, M., Hussler, G., Leroy, F., Mahieu, C., Semeria, D. & Vanlerberghe, G. (1995). Synthesis of 2-*N*-oleylamino-octadecane-1,3-diol: a new ceramide highly effective for the treatment of skin and hair. *International Journal of Cosmetic Science* **17**, 133–146.
47. De Paepe, K., Vandamme, P., Roseeuw, D. & Rogiers, V. (1996). Ceramides/cholesterol/free fatty acids-containing cosmetics: the effect on the barrier function. *Seifen, Öle, Fette und Wachse* **122**, 199–204.
48. De Paepe, K., Taelman, M.C., Roseeuw, D. & Rogiers, V. (1997). Ceramides in dermato-cosmetic products. In *In Cosmetics, Verlag für Chemische Industrie*, pp. 101–108. Dusseldorf, Germany: H. Ziolowsky.
49. Lintner, K., Mondon, P., Girard, F. & Gibaud, T. (1997). The effect of a synthetic ceramide-2 on transepidermal water loss after stripping or a sodium lauryl sulfate treatment: an *in vivo* study. *International Journal of Cosmetic Science* **19**, 1915–1925.
50. Conti, A., Schiavi, M.E. & Seidenari, S. (1995). Capacitance, transepidermal water loss and causal level of sebum in healthy subjects in relation to site, sex and age. *International Journal of Cosmetic Science* **17**, 77–85.
51. Okah, F.A., Wichett, R.R., Pickens, W.L. & Hoath, S.B. (1995). Surface electrical capacitance as a non-invasive bedside measure of epidermal barrier maturation in the newborn infant. *Pediatrics* **96**, 688–692.
52. Wilhelm, K.P., Cua, A.B. & Maibach, H.I. (1991). Skin aging: effect on transepidermal water loss, stratum corneum skin surface and casual sebum content. *Archives of Dermatology* **127**, 1806–1809.
53. Ghadially, R., Brown, B.E., Hanley, K., Reed, J.T., Feingold, K.R. & Elias, P.M. (1996). Decreased epidermal lipid synthesis accounts for altered barrier function in aged mice. *Journal of Investigative Dermatology* **106**, 1064–1069.
54. van der Valk, P.G.M & Maibach, H.I. (1990). A functional study of the skin barrier to evaporate water loss by means of repeated cellophane-tape stripping. *Clinical and Experimental Dermatology* **15**, 180–182.
55. Wickett, R.R., Nath, V., Tamaka, R. & Hoath, S.B. (1995). Use of continuous electrical capacitance and TEWL measurement of assessing barrier function in neonatal rat skin. *Skin Pharmacology* **8**, 179–185.
56. Feingold, K.R., Mao-Qiang, M., Proksh, E., Menon, G.K., Brown, B.E & Elias, P.M. (1991). The lovastin-treated rodent: a new model of barrier disruption and epidermal hyperplasia. *Journal of Investigative Dermatology* **96**, 201–209.
57. Shürer, N.Y. & Elias, P.M. (1991). The biochemistry and function of stratum corneum lipids. *Advanced Lipid Research* **24**, 27–56
58. Elias, P.M. & Feingold, K.R. (1992). Lipids and the epidermal water barrier: metabolism, regulation and pathophysiology. *Seminars of Dermatology* **11**, 176–182.
59. Motta, S., Monti, M., Sesana, S., Mellesi, L., Ghidoni, R. & Caputo, R. (1994). Abnormality of water barrier function in psoriasis: role of ceramide fractins. *Archives of Dermatology* **130**, 452–456.
60. Yang, L., Mao-Qiang, M., Taljebini, M., Elias,

P.M. & Feingold, K.R. (1995). Topical stratum corneum lipids accelerate barrier repair after tape stripping, solvent treatment and some but not all types of detergent treatment. *British Journal of Dermatology* **133**, 679–685.
61. Finkley, M.B. & Crowe, D.M. (1988). The use of evaporimetry to evaluate soap induced irritation on the face. *Bioengineering of the Skin* **4**, 311–321.
62. Wilhelm, K.P., Cua, A. & Maibach, H.I. (1992). Effects of short-term surfactant exposure on stratum corneum capacitance and skin surface water loss. In *The Environmental Threat to the Skin* (ed. R. Marks & G. Pewig), pp. 313–318. London, UK: Martin Dunitz.
63. Murahata, R.I., Crowe, D.M. & Roheim, J.P. (1986). The use of transepidermal water loss to measure and predict the irritation response to surfacants. *International Journal of Cosmetic Science* **8**, 225–231.
64. Serup, J. (1995). The spectrum of irritancy and application of bioengineering techniques. In *Current Problems in Dermatology, Vol. 23, Irritant Dermatitis, New Clinical and Experimental Aspects* (ed. P. Elsner & H.I. Maibach), pp. 131–133. Basel, Switzerland: Karger.
65. Rougier, A. (1991). Percutaneous absorption-transepidermal water loss relationship in man *in vivo*. In *Prediction of Percutaneous Penetration Methods, Measurements, Modelling, Vol. 2* (ed. R.C. Scott, R.H. Guy, J. Hadgraft & H.E. Boddé), pp. 60–72. London, UK: IBC Technical Services.
66. Harvell, J.D. & Maibach, H.I. (1991). Bioengineering methods to evaluate *in vivo* irritation: application to *in vitro* validation. In *Alternative Methods in Toxicology, Vol. 10, In Vitro Skin Toxicology: Irritation, Phototoxicity, Sensitization* (ed. A. Rougier, A.M. Goldberg & H.I. Maibach), pp. 9–11. New York, USA: Mary Ann Liebert.
67. Lévêque, J.L., Garson, J.C. & Boudouries (1979). L'eau dans les kératines: Etude par mesures de la conductibilité électrique. *Compte-rendu a l'Académie des Sciences* **228**, 1679–1682.
68. Lévêque, J.L. & de Rigal, J. (1983). Impedance methods for studying skin hydration. *Journal of the Society of Cosmetic Chemists* **34**, 419–428.
69. Salter, D.C. (1994). Further hardware and measurement approaches for studying water in the stratum corneum. In *Bioengineering of the Skin: Water and the Stratum Corneum* (ed. P. Elsner, E. Berardesca & H.I. Maibach), pp. 205–215. Boca Raton, FL, USA: CRC Press.
70. Salter, D.C. (1998). Examination of stratum corneum hydration state by electrical methods. In *Current Problems in Dermatology, Vol. 26, Skin Bioengineering Techniques and Applications in Dermatology and Cosmetology* (ed. P. Elsner, A. Barel, E. Berardesca, B. Gabard & J. Serup), pp. 38–47. Basel, Switzerland: Karger.
71. Blichman, C. & Serup, J. (1988). Assessment of skin moisture. *Acta Dermato-Venereologica* **68**, 284–290.
72. Effendy, I., Weltfriend, S., Patil, S. & Maibach, H.I. (1996). Differential irritant skin responses to topical retinoic acid and sodium lauryl sulphate: alone and in crossover design. *British Journal of Dermatology* **134**, 424–430.
73. Berardesca, E., Vignoli, G.P., Distante, F., Brizzi, P. & Rabbiosi, G. (1995). Effect of water temperature on surfactant-induced skin irritation. *Contact Dermatitis* **32**, 83–87.
74. Wilhelm, K.P., Freitag, G. & Wolff, H.H. (1994). Surfactant-induced skin irritation and skin repair: evaluation of a cumulative human irritation model by non-invasive techniques. *Journal of the American Academy of Dermatology* **31**, 981–987.
75. Wilhelm, K.P., Cua, A.B., Wolff, H.H. & Maibach H.I. (1993). Surfactant-induced stratum corneum hydration *in vivo*: prediction of the irritation potential of anionic surfactants. *Journal of Investigative Dermatology* **101**, 310–315.
76. Berardesca, E. & Maibach, H.I. (1993). Mechanical properties and photoaging. In *Aging Skin* (ed. J.L. Lévêque & P. Agache), pp. 29–38. New York, USA: Marcel Dekker.
77. Lévêque, J.L. (1987). Méthodes expérimentales d'étude du vieillissement cutané chez l'homme *in vivo*. *Annales de Dermatologie et de Vénéréologie* **114**, 1279–1283.
78. Rogiers, V., Derde, M.P., Verleye, G. & Roseeuw, D. (1990). Standardized conditions needed for skin surface hydration measurements. *Cosmetics and Toiletries* **105**, 73–82.
79. Clarys, P., Manou, I. & Barel, A.O. (1997). Influence of temperature on irritation in the hand/forearm immersion test. *Contact Dermatitis* **36**, 240–245.
80. Gabard, B. & Treffel, P. (1994). Hardware and measuring principle: the Nova DPM 9003. In *Bioengineering of the Skin: Water and the Stratum Corneum* (ed. P. Elsner, E. Berardesca & H.I. Maibach), pp. 177–199. Boca Raton, FL, USA: CRC Press.
81. Barel, A.O. & Clarys, P. (1995). Measurements of epidermal capacitance. In *Handbook of Non-Invasive Methods and the Skin* (ed. J. Serup & G.B.E. Jemec), pp. 165–170. Boca Raton, FL, USA: CRC Press.
82. Paquet, F., Piérard-Franchimont, C., Fumal, I., Goffin, V., Paye, M. & Piérard, G.E. (1998). Sensitive skin at menopause, dew point and electrometric properties of the stratum corneum. *Maturitas* **28**, 221–227.
83. Makki, S., Basbenel, J.C. & Agache, P. (1979). A quantitative method for assessment of microphotography of human skin. *Acta Dermato-Venereologica* **59**, 285–291.
84. Corcuff, P. & Lévêque, J.L. (1995). Skin surface replica: image analysis of furrows and wrinkles. In *Handbook of Non-Invasive Methods and the Skin* (ed. J. Serup. & G.B.E. Jemec), pp. 89–96. Boca Raton, FL, USA: CRC Press.
85. Gassmueller, J., Kecskes, A. & John, P. (1995). Stylus methods for skin surface contour measurements. In *Handbook of Non-Invasive Methods and the Skin* (ed. J. Serup. & G.B.E. Jemec), pp. 83–88. Boca Raton, FL, USA: CRC Press.
86. Grove, G.L., Grove, M.J. & Leyden, J.J. (1989). Optical profilometry: an objective method for quantification of facial wrinkles. *Journal of the American Academy of Dermatology* **21**, 631–637.
87. Grove, G.L., Grove, M.J. & Leyden, J.J. (1991). Skin replica analysis of photodamaged skin after therapy with tretinoin emollient cream. *Journal of the American Academy of Dermatology* **25**, 231–237.
88. Henry, F., Piérard-Franchimont, C., Cauwenbergh, G. & Piérard, G.E. (1997). Age-related

changes in facial skin contours and rheology. *Journal of the American Geriatric Society* **45**, 220–222.
89. Tupker, R.A., Pinnagoda, J., Coenraads, P. & Nater, J.P. (1989). The influence of repeated exposure to surfactants on the human skin as determined by transepidermal water loss and visual scoring. *Contact Dermatitis* **20**, 108–114.
90. Kawan, K. (1975). Evaluation of skin patch test using SUMP method. *Journal of Dermatology* **2**, 19–26.
91. Kawan, K., Nakagawa, M., Kawai, J. & Kawan, K. (1992). Evaluation of skin irritancy of sodium lauryl sulfate: a comparative study between the replica method and visual evaluation. *Contact Dermatitis* **27**, 174–181.
92. Sato, A., Obata, K., Ikeda, Y. & Ohkoshi, K. (1996). Evaluation of human skin irritation by carboxylic acids, alcohols, esters and aldehydes with nitrocellulose-replica method and closed patch testing. *Contact Dermatitis* **34**, 12–16.
93. Zhou, J., Mark, R., Stoudemeyer, T. & Sakr, A. (1991). The value of multiple instrumental and clinical methods, repeated patch applications and daily evaluations for assessing stratum corneum changes induced by surfactants. *Journal of the Society of Cosmetic Chemists* **42**, 105–128.
94. Lee, S.W. & Kim, H.O. (1992). Morphological change of skin surface by sodium lauryl sulfate irritation. *K'at 'ollik Tachak Vihakpu Nonmunjip* **45**, 271–281.
95. Agner, T. & Serup, J. (1987). Skin reactions to irritants assessed by polysulfide rubber replica. *Contact Dermatitis* **17**, 205–211.
96. Pelfini, C., Vignini, M. & Calligaro, A. (1993). Morphometric computerized analysis as a method to assess skin topography caused by corticosteroids. *Skin Pharmacology* **6**, 26–31.
97. Pessis, S., Grollier, J.F. & Pasero, R. (1984). Skin surface changes after UV irradiation. *International Journal of Cosmetic Science* **6**, 1–12.
98. Lefaix, J.L., Delanian, S., Dubray, B., Giraud, P., Berland E., Sahraoui S. & Mignot J. (1996). Modification of the cutaneous microrelief in the superficial radiation-induced fibrosis: qualitative study. *Bulletin du Cancer* **88**, 915–922.
99. Avila-Camacho, M., Piérard-Franchimont, C. & Piérard G.E. (1999). Skin colour assessment in safety testing of cosmetics: an overview. *International Journal of Cosmetic Science*, in press.
100. Fullerton, A., Fischer, T., Lahti, A., Wilhelm, K.P., Takiwaki, H. & Serup, J. (1996). Guidelines for measurement of skin colour and erythema. *Contact Dermatitis* **31**, 1–10.
101. Chardon, A., Cretois, I. & Hourseau, C. (1991). Skin color typology and suntanning pathways. *International Journal of Cosmetic Science* **13**, 191–208.
102. Ale, S.I., Laugier, J.P.K. & Maibach, H.I. (1996). Spatial variability of basal skin chromametry on the ventral forearm of healthy volunteers. *Archives of Dermatological Research* **228**, 774–777.
103. Takiwaki, H. & Serup, J. (1994). Variations in color and blood flow of the forearm skin during orthostatic maneuvers. *Skin Pharmacology* **7**, 226–230.
104. Anderson, R.R. & Parrish, J.A. (1981). The optics of human skin. *Journal of Investigative Dermatology* **77**, 13–19.
105. Bonner, R.F. & Nossal, R. (1990). Principles of Laser-Doppler flowmetry. In *Laser-Doppler Flowmetry* (ed. A.P. Shepherd & A.P. Oberg). Dordrecht, The Netherlands: Kluwer.
106. Nilsson, G.E., Tenland, T. & Oberg, A.P. (1980). Evaluation of a Laser-Doppler flowmetry for measurement of the tissue blood flow. *IEEE Transaction of the Biomedical Engineering* **27**, 597–604.
107. Tur, E., Tur, M., Maibach, H.I. & Guy, R.H. (1983). Basal perfusion of the cutaneous microcirculation: measurements as a function of anatomic position. *Journal of Investigative Dermatology* **81**, 442–446.
108. Oberg, A.P. (1990). Innovations and precautions. In *Laser-Doppler Flowmetry* (ed. A.P. Shepherd & A.P. Oberg). Dordrecht, The Netherlands: Kluwer.
109. Salerud, E.G. & Nilsson, G.E. (1986). Integrating probe for tissue Laser-Doppler flowmeters. *Medical and Biological Engineering and Computing* **24**, 415–419.
110. Van Neste, D. & de Brouwer, B. (1992). Monitoring of skin responses to sodium lauryl sulfate: clinical scores vs bioengineering methods. *Contact Dermatitis* **27**, 151–156.
111. Staberg, B., Klemp, P. & Serup, J. (1984). Patch test responses evaluated by cutaneous blood flow measurements. *Archives of Dermatology* **120**, 741–743.
112. Bircher, A., de Boer, E., Agner, T., Wahlberg, J.M. & Serup, J. (1994). Guidelines for measurement of cutaneous blood flow by Laser-Doppler velocimetry: a report from the standardisation group of the European Society of Contact Dermatitis. *Contact Dermatitis* **30**, 65–72.
113. Lévêque, J.L., de Rigal, J., Saint-Leger, D. & Billy, D. (1993). How does SLS alter the skin barrier function in man? *Skin Pharmacology* **6**, 111–115.
114. Seidenari, S. & di Nardo, A. (1992). Scanning evaluation of irritant reactions with binary transformations and image analysis. *Acta Dermato-Venereologica* **175**, Suppl., 9–13.
115. Pellecani, G., Francomano, M., Giusti, G. & Seidenari, S. (1997). Epidermal echogenicity as hydration parameter: the effects of moisturizers on the skin as evaluated by 20MHz B-Scanning. *Journal of the Society of Cosmetic Chemists* **48**, 243–251.
116. Pellecani, G., Beletti, B. & Seidenari, S. (1998). Evaluation of the short effects of skin care products. *Current Problems in Dermatology,* in press.
117. Piérard, G.E. (1988). A critical approach to *in vivo* mechanical testing of the skin. In *Cutaneous Investigation in Health and Disease* (ed. J.L. Lévêque), pp. 215–240. New York, USA: Marcel Dekker.
118. Salter, D.C., McArthur, H.C., Crosse, J.E. & Dickens, A.D. (1993). Skin mechanics measured *in vivo* using torsion: a new and acccurate model more sensitive to sex, age and moisturising treatment. *International Journal of Cosmetic Science* **15**, 200–218.
119. Cua, A.B., Wilhelm, K.P. & Maibach, H.I. (1990). Elastic properties of human skin: relation to age, sex and anatomical region. *Archives of Dermatological Research* **282**, 283–288.
120. Gniadecka, M., Gniadecki, R., Serup, J. & Son-

dergaard, J. (1994). Skin mechanical properties present adaptation to man's upright position: *in vivo* studies in young and aged individuals. *Archives of Dermato-Venereologica* **74**, 188–190.
121. Malm, M., Samman, M. & Serup, J. (1995). *In vivo* skin elasticity of 22 anatomical sites: the vertical gradient of skin extensibility and implications in gravitational aging. *Skin Research and Technology* **1**, 61–67.
122. Borroni, G., Vignati, G. & Vignoli, G.P. (1989). PUVA induced viscoelastic changes in the skin of psoriatic patients. *Medical and Biological Environment* **17**, 663–671.
123. Nikkels-Tassoudi, N., Henry, F., Piérard-Franchimont, C. & Piérard, G.E. (1996). Computerized evaluation of skin stiffening in scleroderma. *European Journal of Clinical Investigation* **26**, 457–460.
124. Serup, J. & Northeved, A. (1985). Skin elasticity in psoriasis. *In vivo* measurement of tensile distensibility, hysteresis and resilient distention with a new method: comparison with skin thickness as measured with high frequency ultrasound. *Journal of Dermatology* **12**, 318–324.
125. Christensen, M.S., Hargens, C.W., Nacht, S. & Gans, E.H. (1977). Viscoelastic properties of intact human skin, instrumentation, hydration, effect and the contribution of the stratum corneum. *Journal of Investigative Dermatology* **69**, 282–286.
126. de Rigal, J. & Lévêque, J.L. (1985). *In vivo* measurement of stratum corneum elasticity. *Bioengineering of the Skin* **1**, 13–23.

Appendix 1

Examples of Experience Within the Industry

Some participants in the ECVAM/EEMCO workshop presented the protocols used within their own companies. Four examples have been summarised here, but it must be emphasised that they are only intended to serve as examples, and should not be seen as guidelines.

Example 1: Evaluation Programme of Skin Mildness and Skin Safety (Irritancy Testing)

Screening tests

The formulation is first discussed with the formulator, to provide guidance on the choice of surfactants and their combination.

During or at the end of the development stage, a quick screening for skin irritation is performed, to estimate overall skin mildness. Formulations which do not meet the predefined mildness criteria are rejected.

Several *in vitro* skin irritation predictive tests, based on protein denaturation by the least-mild surfactant systems, have been selected for their rapidity and the possibility of running them during formulation. The Zein test (1), the collagen swelling test (2), and the pH-rise test (3) are currently used.

When specific ingredients are suspected to interfere with the *in vitro* tests mentioned, other tests are performed, including an *ex vivo* assessment of the effects of the product on the stratum corneum by corneosurfametry (4) and short-term patch tests (15–30 minute application) on human volunteers, with subclinical evaluation of stratum corneum alterations by squamometry (5, 6). Such methods have the advantage that the products are tested directly on their real target, the stratum corneum, and they can reveal large variations between products at various mildness levels. For this reason, small panels of human volunteers (n = 6 or 8) can be used.

It should be noted that the *in vitro*, *ex vivo* and short-term *in vivo* tests do not necessarily demonstrate the safety of the product, but only have predictive value.

Skin mildness tests

Here, the objective is to compare the mildness of the newly developed product with that of other products already present in the intended marketplace. Several types of tests can be used. Most tests exaggerate the exposure conditions, to amplify the reactions and permit a more accurate comparison between the test products.

The most commonly used tests are as follows.

1. The Frosch-Kligman soap chamber test (7): solutions of the products are applied to the volunteers (n = 25–30) under occlusion for 24 hours, followed by another 6 hours during 4 consecutive days. Erythema and dryness are evaluated visually by a trained assessor.
2. The modified soap chamber test (8): solutions of the products are applied to volunteers (n = 25–30) under occlusion, for two 24-hour periods. Visual assessment of erythema and dryness is often complemented by instrumental measurements of redness (3 hours after each of the applications), of TEWL (skin barrier damage, 3 hours after the first application), and of skin capacitance (skin surface hydration/dehydration, 3–5 days after the second application [9]).
3. The exaggerated arm wash (10), flex wash (11) and hand wash (12) tests or hand soaking (13) tests: visual assessment of erythema and dryness is usually complemented by instrumental measurement. Capacitance and TEWL measurements are the most useful. Recently, squamometry was shown to have a great potential value in such tests (14, 15): the panel of 25–30 volunteers could be reduced to 10–15 subjects.

The choice of the test to be used depends on several factors, including:

a) the number of products to be compared: for example, in a soap chamber test, up to eight products can be compared, while in an exaggerated wash/soak procedure,

usually only two products are compared ("split arm" design, however, allows four products to be tested simultaneously);

b) the intended use of the product (for example, a wash test is more appropriate for a soap bar, and a soaking test for a foam bath product);

c) the potential effect of mechanical action on the interaction between the product and the skin surface (for example, in the presence of slight abrasives or encapsulated actives, a wash test is more appropriate than a patch test); and

d) whether or not subjective, self-perceived, information on the effect of the product on the skin is needed (for example, a patch test does not provide such information).

Other factors can also be considered, such as the cost of the studies and the equipment, the experience of the testing laboratory, and the expected scale of differences between the products.

Safety tests

Safety tests are different from mildness tests and aim at checking that the products do not irritate skin. Some products can be "non-mild", but can bring other benefits to the skin. Although claims of mildness properties are not made, they are still "non-irritant" products.

For this purpose, two types of skin irritation tests are most commonly used.

1. Cumulative irritation tests: solutions of the products are applied to the backs of the volunteers (a minimum of 25 are used) under occlusion or semi-occlusion for several consecutive days (for example, the 21-day cumulative irritation test [16]). Reference products with well-known skin tolerance in the marketplace are usually included. As the objective is to check the absence of obvious skin reactions, instrumental measurement is usually unnecessary.

2. Home usage tests: volunteers apply the product at home under normal usage conditions for several consecutive weeks. Volunteers report on a regular basis to a competent assessor, who checks for the absence of unwanted effects. Before beginning such a test, a high level of confidence that the product is unlikely to cause irritation is required. The main advantage of such a procedure is the ability to check for the absence of subjective signs of skin irritation (for example, itching, stinging, burning), which are rarely detectable in patch tests. Large panels are recommended for home usage tests.

Example 1 summarises a full testing procedure for a product derived from another which was already known to have good skin compatibility. The information available on some components of the product or their interactions in mixtures is only rarely insufficient for tests on volunteers to be conducted, as described above. A more progressive testing procedure should then be used (17).

Example 2: Safety Testing (Irritancy) and Detection of Unexpected Events

Strategy

During new product development, all ingredients are scrutinised from a toxicological point of view. A critical review of the toxicological data provided by the suppliers of the ingredients is performed and other sources of information are checked (legal documents, scientific literature, case reports, own information). If the data are of good quality and indicate an absence of toxic properties, then the formulation work can begin. The next step is to approve human volunteer testing of a number of newly suggested formulations. Tests are only considered for those formulations that merely cause reversible damage to the skin (for example, irritation). The type of testing is determined on a case-by-case basis. Slight changes in an existing formulation might not require any testing, whereas formulations with new ingredients are always tested in-house for irritancy. This confirms the safety of the product with respect to irritation (sensory irritation is not covered), and confirms that nothing unexpected has happened with the product due to interactions between its ingredients or during its manufacture.

Irritancy testing on human volunteers is initiated only when the ethical committee has given its approval, and only temporary irritation in some individuals can be expected. The

new formulations are tested simultaneously with a suitable reference product, and with water as a control. The products of competitors are sometimes included. Comparative tests are considered to encourage the development toward milder products.

Procedure

The products are applied to the skin for 48 hours in large Finn chambers. Stay-on products are tested neat, and rinse-off products at a dilution of 10%. One hour after removal of the products, the areas are assessed visually for their degree of irritation, on a 4-grade scale. The following day, the test areas are also examined by using TEWL and LDF measurements. Usually, 12 volunteers are included, and up to 12 formulations are evaluated in the same study. The instrumental readings are made on one day. Statistically significant differences between formulations can usually be detected. Whether these differences are clinically relevant is another question, which needs careful consideration in the final safety assessment of the products. The visual grading of erythema correlates well with the LDF value. A fairly good correlation between TEWL and LDF can usually be found, although the instruments concerned measure completely different parameters. The absence of correlation provides important information on the mechanisms by which the products exert effects on the skin.

Advantages

Depending on the company involved, the instrumental assessment of the exposed skin areas can have major advantages, i.e.:

a) two parameters relevant for irritation are measured, namely blood flow as a component of inflammation, and barrier damage as a direct effect on the stratum corneum or an indirect effect via inflammation;

b) unexpected effects on the skin can be detected;

c) the method is objective, it is sensitive when compared to visual assessment, the values are on a continous scale and not on a categorical scale, it is inexpensive (important for small companies and firms with products with low volumes), and it is ethical (since a limited number of volunteers need to be included in the testing procedure); and

d) to date, after using this strategy, no product has been launched which has caused high complaint rates due to adverse skin reactions.

Example 3: Safety Assessment for Skin Care and Skin Cleansing Products

Product safety assurance is the result of a carefully planned and well executed safety assessment programme, a process by which the potential hazard of a product is determined. A product is considered to be safe, if no substantial hazard or significant risk of damage results under conditions of recommended use, reasonable foreseeable misuse, or accidental exposure.

The safety assessment of an ingredient or product initially involves a review of any existing data in the scientific literature or in information provided by its manufacturer or supplier. In addition, any available *in vitro* data will be evaluated at this time. A suitable alternative to animal testing for ingredients and finished products is to use a battery of *in vitro* studies to show that they are likely to be non-irritating (18, 19). If these preliminary data indicate that the item is suitable for further testing, study requirements for human clinical research are determined. In these well-defined, carefully monitored clinical studies, specific information on how the ingredient or product affects the body is generated through a series of clinical tests.

A general procedure is outlined below, to illustrate how the clinical safety testing of skin care products (stay-on category) and skin cleansing products (rinse-off category) is performed on human volunteers.

Skin care products

A distinction must be made as to whether minor alterations were made to an existing formulation, or whether new ingredients have been introduced.

Minor alterations

Examination of the formulation is followed by an in-use conditions test, in which the results are quantified by corneometry (skin hydration) and profilometry (skin rough-

ness). Twenty-five volunteers are included in this test, and the product is applied twice daily on the volar forearm as the test site of choice. Bioengineering measurements are conducted after one week of application. The application of the product is continued for an additional week. In the case of significant erythema or alterations of the skin surface, the volunteer must immediately discontinue applying the product.

New ingredients/new formulations
A single application is made in a closed 24-hour patch test on the backs of volunteers. A negative (very mild skin care formulation) and a positive control (0.1% solution of sodium dodecylsulphate) are included, to permit comparisons between tests. If the test sample shows negative results, a repeated closed patch test is performed for further characterisation of skin compatibility, in which the product is applied to the backs of human volunteers on four consecutive days, for 21 hours per day. The test is visually scored by a trained investigator. If erythema or any skin reaction occurs, the re-application of the sample is stopped immediately. Bioengineering techniques, such as LDF imaging or colorimetry, are occasionally applied to quantify the erythema. The measurement of biophysical parameters provides highly objective data on blood flow or skin redness. However, to assess the occurrence of an erythema, these latter techniques are not necessarily of higher sensitivity than the subjective visual inspection.

Skin cleansing products

Minor alterations
After examination of the formula, an in-use conditions test, such as the forearm wash test, is performed. The forearm wash test is a method for estimating the relative irritation of personal cleansers. The protocol is based on consumer washing habits, and is more useful than many other methods for evaluating personal cleanser mildness. A standard forearm wash test involves 12–16 volunteers. Two skin sites are treated with cleansing formulae and one site is treated with water only. One skin site is an untreated control. Washing occurs twice a day for 6 days. Quantification of the results is performed by using corneometry, TEWL measurement, desquamation techniques (D-squame) and visual assessment.

New ingredients/new formulations
A single application, 24-hour closed patch test is conducted on the backs of the volunteers. If negative or low positive results are found, a repeated application, closed patch test is performed on four consecutive days with a daily 6-hour application. Visual scoring is used to detect erythema. TEWL measurements and LDF imaging are occasionally conducted, to measure the damage to the skin barrier and to quantify the erythema, respectively. The results are compared with those obtained with products already on the market.

These clinical tests focus on the possible primary or cumulative irritation potentials of an ingredient or a formulation. In parallel with these investigations, the sensitising potential of an ingredient or a product is evaluated in appropriate *in vitro* studies (20, 21) and in repeated insult patch tests performed on human volunteers. In addition, for face care cosmetics, testing for phototoxicity and photoallergy must be taken into consideration.

In summary, the main parameter in safety testing on humans is the visual inspection of the skin site involved. Bioengineering methods offer an opportunity for the quantification and documentation of skin reactions. To ensure reliable conclusions in the future, a regimen of an appropriate combination of visual assessment and instrumental investigation will probably be built up as the standard approach to safety testing on human volunteers.

Example 4: The Determination of the Horny Layer Reservoir of Human Skin as a Parameter for Percutaneous Absorption

Studies on percutaneous absorption are rarely undertaken to establish a potential hazard for the skin itself. However, the major safety concern is that substances, which under in-use conditions provoke few or no apparent cutaneous side-effects, might pose systemic risks.

There is no direct approach to measuring exposure to cosmetic ingredients in humans *in vivo*. Due to intrinsic slow penetration kinetics and the low penetration rates of such materials, serum levels can only be assessed in humans *in vivo* in exceptional

cases, and then only with radiolabelled compounds. However, in almost every case, the exposure to the radiolabel would exceed the limits posed by ethical rules. The measurement of radioactivity in excreta can only be interpreted on the basis of the knowledge of the excretion kinetics after intraveneous injection of the radiolabelled substance, an approach which is unlikely to gain ethics committee approval. However, a sufficient number of medicines have been investigated *in vivo* in humans, and the results provided can serve as reference data.

A number of investigations have previously been performed on rats *in vivo*, and it has been found that rat skin is 2-fold to 5-fold more permeable than human skin. Furthermore, considerable experience has been gathered with human skin *in vitro*, and a satisfactory agreement with the *in vivo* situation has been established (22–24). However, it remains difficult to mimic in-use conditions *in vitro* for cosmetic preparations, and repeated applications are not possible with the currently available *in vitro* systems.

Therefore, a non-invasive *in vivo* method for the assessment of percutaneous absorption in humans would be welcome. Such a method would be considered suitable when a relationship between the horny layer reservoir (which builds up rapidly after topical application of any kind of material) and subsequent penetration rates could be assessed (25). In fact, such a direct relationship appears to have been well-established by the "stripping method" (26–28). There is a quantitative relationship between the material found in the horny layer of rats after 30 minutes (when the product is not yet in the dermis), as recovered by repetitive stripping with adhesive tape, and the total amount that passes into and through rat skin *in vivo* over 4 days. Furthermore, the same correlation has been shown in humans *in vivo* for some compounds, concentrations and vehicles. In fact, the limitations of this technique can be examined by using reference substances, namely, a very hydrophylic and a very lipophylic compound. Thus, the respective results can be expressed in total penetrating quantities per exposure as $\mu g/cm^2$ and mg/kg body weight and combined with data on the no adverse effect level (NOAEL), determined in toxicological studies. A safety factor for a cosmetic ingredient could be generated by using this information.

References

1. Götte, E. (1966). Synthetische tenside in medizinisch-kosmetischen badern. *Aesthetische Medizin* **10**, 313–319.
2. Blake-Haskins, J.C., Scala, D., Rhein, L.D. & Robbins, C.R. (1986). Predicting surfactant irritation from the swelling response of a collagen film. *Journal of the Society of Cosmetic Chemists* **37**, 199–210.
3. Tavss, E.A., Eigen, E. & Kligman, A.M. (1988). Anionic detergent-induced skin irritation and anionic detergent-induced pH rise of bovine serum albumin. *Journal of the Society of Cosmetic Chemists* **39**, 267–272.
4. Piérard, G.E., Goffin, V. & Piérard-Franchimont, C. (1994). Squamometry and corneosurfametry in rating interactions of cleansing products with stratum corneum. *Journal of the Society of Cosmetic Chemists* **45**, 269–277.
5. Piérard, G.E., Piérard-Franchimont, C., Saint-Léger, D. & Kligman, A.M. (1992). Squamometry: the assessment of xerosis by colorimetry of D-squame adhesive discs. *Journal of the Society of Cosmetic Chemists* **40**, 29.
6. Paye, M., Gomes, G., Zerweg, C., Piérard, G.E. & Grove, G.G. (1999). A hand immersion test in laboratory-controlled usage conditions: a need for sensitive and controlled assessment methods. *Contact Dermatitis* **40**, 133–138.
7. Frosch, P.J. & Kligman, A.M. (1979). The soap chamber test: a new method for assessing the irritancy of soaps. *Journal of the American Academy of Dermatology* **1**, 35–41.
8. Simion, F.A., Rhein, L.D., Grove, G.L., Wojtkowski, J.M., Cagan, R.H. & Scala, D.D. (1991). Sequential order of skin responses to surfactants during a soap chamber test. *Contact Dermatitis* **25**, 242–249.
9. Paye, M., Van de Gaer, D. & Morrison, B.M. Jr (1995). Corneometry measurements to evaluate skin dryness in the modified soap chamber test. *Skin Research and Technology* **1**, 123–127.
10. Sharko, P.T., Murahata, R.I., Leyden, J.J. & Grove, G.L. (1992). Arm wash evaluation with instrumental evaluation: a sensitive technique for differentiating the irritation potential of personal washing products. *Journal of the Dermatological and Clinical Evaluation Society* **43**, 187–192.
11. Strube, D.D., Koontz, S.W., Murahata, R.I. & Theiler, R.F. (1989). The flex wash test: a method for evaluating the mildness of personal washing products. *Journal of the Society of Cosmetic Chemists* **40**, 297–306.
12. Charbonnier, V., Morrison, B.M., Paye, M. & Maibach, H.I. (1999). Open application assay in investigation of subclinical irritant dermatitis induced by sodium lauryl sulfate (SLS) in man: advantage of squamometry. *Skin Research and Technology*, in press.
13. Clarys, P., Manou, I. & Barel, A.O. (1997). Influence of temperature on irritation in the hand/forearm immersion test. *Contact Dermatitis* **36**, 240–245.
14. Paye, M. & Cartiaux, Y. (1999). Squamometry: a tool to move from exaggerated to more and more realistic application conditions for comparing human skin compatibility of surfactant-based products. *International Journal of Cosmetic Science*, in press.

15. Morrison, B.M., Paye, M., Charbonnier, V. & Maibach, H.I. (1998). Demonstrating invisible (subclinical) sodium lauryl sulfate irritation with squamometry. American Academy of Dermatology Meeting, Orlando, FL, USA, 27 February–4 March, PO98.
16. Lanman, B.M., Elvers, E.B. & Howard, C.J. (1968). The role of human patch testing in a product development program. *Joint Conference on Cosmetic Science. The Toilet Goods Association*, pp. 21–23. CTFA: Washington, DC, USA: CTFA.
17. Walker, A.P., Basketter, D.A., Baverel, M., Diembeck, W., Matthies, W., Mougin, D., Paye, M., Röthlisberger, R. & Dupuis, J. (1996). Test guidelines for assessment of skin compatibility of cosmetic finished products in man. *Food and Chemical Toxicology* **34**, 651–660.
18. Pape, W.J. & Hoppe U. (1990). Standardization of an *in vitro* red blood cell test for evaluating the acute cytotoxic potential of tensides. *Arzneimittelforschung* **40**, 498–502.
19. Pape, W.J. & Hoppe U. (1991). *In vitro* methods for the assessment of primary local effects of topically applied preparations. *Skin Pharmacology* **4**, 205–212.
20. Degwert, J., Steckel, F. & Hoppe U. (1995). Antigen presenting and primary *in vitro* sensitizing capacity of CD1a+ dendritic cells generated from human blood. *Advances in Experimental Medicine and Biology* **378**, 187–189.
21. Reutter, K., Jäger, D., Degwert, J. & Hoppe, U. (1997). *In vitro* model for contact sensitization. II. Induction of IL-1 mRNA in human blood-derived dendritic cells by contact sensitizers. *Toxicology in Vitro* **11**, 619–626.
22. Pershing, L.K., Lambert, I., Shah, V.P. & Lam, S.Y. (1992). Variability and correlation of chromameter and tape stripping methods with the visual skin blanching assay in the quantitative assessment of topical 0.05% betamethasone dipropionate bioavailability in humans. *International Journal of Pharmaceutics* **86**, 201–210.
23. Pershing, L.K., Parry, G.E., Bunge, A., Krueger, G.G. & Shah, V.P. (1993). Assessment of topical corticosteroid treatment *in vivo* and *in vitro*. In *Topical Drug Bioavailability: Bioequivalence and Penetration* (ed. V.P. Shah & H.I. Maibach), pp. 351–391. New York, USA: Plenum Press.
24. Pershing, L.K., Lambert, L., Wright, E.D., Shah, V.P. & Williams, R.L (1994). Topical 0.05% betamethasone dipropionate: pharmacokinetic and pharmacodynamic dose–response studies in humans. *Archives of Dermatology* **130**, 740–747.
25. Rougier, A., Dupuis, D., Lotte, C., Roguet, R. & Schaefer, H. (1983). *In vivo* correlation between stratum corneum reservoir function and percutaneous absorption. *Journal of Investigative Dermatology* **81**, 275–278.
26. Rougier, A., Dupuis, D., Lotte, C. & Maibach, H.I. (1989). Stripping method for measuring percutaneous absorption *in vivo*. In *Percutaneous Absorption: Mechanism, Methodology, Drug Delivery* (ed. R.L. Bronaugh & H.I. Maibach), pp. 415–435. Basel, Switzerland: Marcel Dekker.
27. Rougier, A. & Lotte, C. (1993). Predictive approaches. I. The stripping technique. In *Topical Drug Bioavailability, Bioequivalence and Penetration* (ed. V.P. Shaw & H.I. Maibach), pp. 163–182. New York, USA: Plenum Press.
28. Coderch, L., Oliva, M., Pons, M., de la Maza, A., Manich, A.M. & Parra, J.L. (1996). Percutaneous penetration using the tape stripping technique. *International Journal of Pharmaceutics* **139**, 197–203.