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Evaluation of human skin tests for potential dermal irritant and contact sensitizing products: a position paper

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Abstract

Many new chemicals are synthesized yearly. Exposure of the skin to chemicals can lead to skin-associated diseases of which the most common are associated with allergic or possible allergic reactions. Examples are: allergic contact dermatitis, photoallergic reactions, urticaria, angioedema, atopic eczema, and certain vasculitic diseases. Prediction of human cutaneous irritation and sensitization in view of hazard identification has primarily relied on the use of laboratory animals. Such studies in laboratory animals have been very instrumental in the detection of potential contact sensitizing agents. There are however many uncertainties and assumptions when results from such studies are extrapolated to humans. In addition, there is a tendency to reduce the number of animals used for toxicity testing. For cosmetics the use of predictive animal tests is legally restricted already in some countries. For these reasons there is a further need for skin testing of specialized products in humans. For ethical reasons a test in humans should not result in sensitization of the human volunteers. In contrast to allergic patients that are being tested for diagnostic purposes, human volunteers will for themselves have no benefit from the results of the test. If testing in humans is the only possible alternative, such testing in man will be limited to the detection of the irritating activity of the unknown compounds, following the axioma that it is unlikely to get sensitization without irritation. This report deals with possibilities and restrictions of predictive testing for potential irritating and skin sensitizing activity of low molecular weight chemicals in humans.
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Samenvatting

Jaarlijks worden vele nieuw chemische producten op de markt gebracht. Blootstelling van de huid aan chemische verbindingen kan aanleiding geven tot huid aandoeningen die vaak een allergische achtergrond hebben. Voorbeelden zijn: allergische contact dermatitis, photoallergie, urticaria, angiooedeme, atopisch eczeem en verschillende vormen van vasculitis. Het voorspellen van het vermogen van verbindingen om huid irritatie en sensibilisatie te veroorzaken is voornamelijk onderzocht met behulp van dierproeven. Dergelijke studies hebben veel informatie opgeleverd betreffende de sensibiliserende activiteit van verbindingen. Echter, het is niet eenvoudig diergegevens te vertalen naar de mens. Bovendien is er sprake van een tendens het gebruik van proefdieren voor deze doelstelling te reduceren, terwijl er voor cosmetica al in een aantal landen wettelijke restricties op dit gebied bestaan. Om deze redenen is er behoefte aan predictieve huidtesten voor irritatie en sensibilisatie door bepaalde producten in de mens. Om ethische redenen dienen testen uitgevoerd in de mens niet te leiden tot sensibilisatie; in tegenstelling tot allergische patienten waarbij om diagnostische redenen testen worden uitgevoerd, hebben vrijwilligers waarbij predictieve testen worden uitgevoerd zelf geen voordeel van de uitkomst van de test. Indien testen in de mens het enige alternatief vormen, zullen dergelijke predictieve testen in het algemeen beperkt dienen te blijven tot huidirritatie testen, uitgaande van de gedachte dat over het algemeen geen sensibilisatie optreedt zonder irritatie. Dit rapport gaat in op de mogelijkheden en beperkingen van testen in de mens gericht op het voorspellen van het irriterende en sensibiliserende karakter van chemicalien.
1. Introduction

Many new chemicals are synthesized yearly. The skin can be exposed to chemicals either by direct contact or by airborne exposure; which may occur occupationally or non-occupationally. This distinction has important legal implications, but frequently the the same chemical allergens may appear both in the domestic and the occupational environment.

Exposure of the skin to chemicals can lead to skin-associated diseases of which the most common skin diseases are associated with allergic or possible allergic reactions. Examples are: allergic contact dermatitis, photoallergic reactions, urticaria, angioedema, atopic eczema, and certain vasculitic diseases (allergic vasculitis and progressive pigmentary purpura).

Especially allergic contact dermatitis is a major health problem in many industrialized countries. Contact dermatitis may have severe consequences, it starts mostly early in life and becomes chronic frequently causing years of ill health. Epidemiological studies of the general European population show an estimated prevalence of around 10% for eczema or dermatitis. For contact hypersensitivity the prevalence is 15-20% because not all sensitized individuals display dermatitis. In most countries occupational skin diseases (irritant and allergic contact dermatitis) amount to 20-40% of all occupational diseases. Working days lost because of occupational allergic contact dermatitis are significant. In addition to contact dermatitis, also atopic eczema is a serious health problem, that to a certain extent may be associated with chemical exposure.

Because exposure of (susceptible) individuals to chemicals may result in allergic contact dermatitis there is a need to prospectively evaluate the potential of chemicals to induce allergic skin diseases.

Prediction of human cutaneous irritation and sensitization in view of hazard identification has primary relied on the use of laboratory animal tests. For allergy testing several test methods are described such as the Buehler occluded patch test and the maximization test in guinea pigs, and the mouse ear swelling test and the auricular lymph node assay in the mouse and rat. Predictive tests for the evaluation of skin sensitizing effects of chemicals have existed for nearly 50 years, and guidelines have been defined for such animal studies (OECD 1981, 1992; EC 1994, 1992). Studies carried out using laboratory animals have been very instrumental in the detection of contact sensitizing agents. There are however many uncertainties and assumptions when results from such studies are extrapolated to humans. In addition, there is a tendency to reduce the number of animals used for toxicity testing. For cosmetics the use of experimental animals is legally restricted already in some countries. If human testing is the only alternative for special groups of products, then there is a further need for evaluation of skin testing in humans.

Although many skin tests were used for diagnostic purposes in hospitals, there is not much experience with predictive tests for allergy in human volunteers, due to obvious ethical reasons. In this respect most experience has been gained with human skin patch testing of detergent and cosmetic products.
In this report chemical associated skin-diseases are extensively described. Possibilities for human skin tests that might be used for predictive testing, and approaches for risk estimation based on such testing, are presented.

2. Clinical aspects of contact dermatitis

The skin is one of the first and major barriers of an individual against exogenous challenges. It is also considered an important part of the immune system, providing resistance of the body surface to microorganisms. Small molecular weight chemicals that land onto the skin are not recognized by the immune system as such, but only if they bind to skin proteins and act as a hapten. Skin contact with such small molecules tends to induce cell-mediated contact sensitization, that may lead to allergic contact dermatitis. Allergic contact dermatitis is a common disease and the prevalence at any given time varies between 2-4%. Most contact allergens are small molecules with a molecular weight below 600. Contact allergy is not inborn but is always a consequence of earlier cutaneous contact. Contact sensitization is considered to be life-long, but might become weaker if exposure is avoided. Contact sensitized individuals are at risk of developing the skin disease allergic contact dermatitis if re-exposed to the specific chemical. The term dermatitis is used synonymously with eczema and describes either an acute skin disease with redness, oedema, and vesicles (water blisters) or a more chronic type with hyperkeratosis, fissures, and scaling. The most important differential diagnosis of contact dermatitis are psoriasis, dermatophytosis, scabies, irritant contact dermatitis, and allergic contact dermatitis.

Epidemiological studies including 20,000 individuals representing the general population showed a one year prevalence of hand eczema of 10% (Meding, 1990), 20% were classified as caused by contact allergy. The average duration was 12.8 years and 22% have had periods of sick leave. Allergic contact dermatitis on the hands is therefore both a common disease but also costly for the society and can imply significant socio-economic consequences for the individual (Matthias, 1985).

Frequent causes of allergic hand eczema are nickel, chromate, rubber additives, preservatives, and fragrances (Menné & Maibach, 1993). The allergic contact dermatitis can be acute or chronic. It can be located either dorsal, volar, or only on the fingers. It can also present as a diffuse dermatitis. Spread to the face and forearms is common.

The face is second to the hands in the frequency of allergic contact dermatitis. The exposure can be direct to airborne allergens or indirect by contact with allergens born from the hands to the face. Acute allergic contact dermatitis in the face is often dramatic with severe oedema particularly of the eyelid regions. Chronic cases frequently show patchy dermatitis even if the allergen is uniformly spread on the face. Cosmetics, particularly fragrances, are the most common causes of facial dermatitis. Allergic contact dermatitis from medicaments (e.g. eye-drops) and airborne occupational dermatitis are seen. Severe oedema of the eyelids is a common pattern of plant dermatitis. Facial dermatitis causes distress to the individual because of pain, itching and disfiguration.
Stasis eczema and leg ulcers are a common disease among the elderly as complications of arterial and venous insufficiency and arteriosclerotic heart disease. Stasis eczema is a consequence of skin malnutrition and can be followed by chronic ulceration. Both entities are treated with topical medicaments such as emollients, steroids, antiseptics, and antibiotics. These compounds generally do not have a high sensitizing capacity, but because they are used on damaged skin under occlusion for prolonged periods, contact sensitivity is not uncommon. On average 50% of these patients have a positive patch test of actual or past relevance.

Intertriginous areas such as the axillae, external ear and perianal area are also frequent sites of primary sensitization from topical used medicaments and fragrances because of the natural occlusion.

Shoe dermatitis is located in the skin area in direct contact with the offending material, most frequently chromate-tanned leather, rubber, and glues (Podmore, 1995).

Allergic contact dermatitis from textiles gives a characteristic clinical pattern with dermatitis in areas where textiles are in close contact with the skin on the trunk and extremities. The offending sensitizers are textile dyes and formaldehyde releasing textile resins (Fowler et al, 1992).

Systemic contact dermatitis can be seen in primary contact sensitized individuals when they later are exposed systemically to the chemical (or drug) either orally, intravenously, by inhalation, or by transcutaneous absorption (Menné et al., 1994). The clinical symptoms can either be flare in areas with earlier contact dermatitis or a combination of symptoms including vesicular hand eczema and inflammatory skin reaction in the flexural and genital area. The explanation for the flare reaction is probably specific sensitized lymphocytes resting or homing to the site of earlier allergic contact dermatitis areas. The mechanism behind the other type of reactions is speculative. Histologically this widespread reaction does not have the picture of contact dermatitis but frequently presents the picture of a lymphocytic vasculitis. Systemic contact dermatitis is mostly seen in patients sensitized to topically used medicaments when they are systemically treated with the medicament or a cross-reacting medicament. Systemic contact dermatitis has been described for a large number of substances.

Most substances which cause photo-contact allergy are halogenated aromatic hydrocarbons or sunscreen agents (White, 1995). Only the combination of UV-radiation and the specific chemical make the complete hapten. Clinical allergic photo-contact dermatitis will therefore present a dermatitis (often severe) in sun-exposed areas. This will typically be in the face, on the forearms and dorsal aspects of the hands. In cases where photo-contact allergy is suspected, patch testing is performed in duplicate and one site is exposed to UVA. If a positive patch test only appears on the UV-exposed site, photoallergy is likely.
3. Allergic contact dermatitis as an occupational disease

Occupational skin diseases are defined as skin diseases either wholly or partly caused by the patient's occupation (Rycroft, 1995). The epidemiology of occupational skin diseases, which mostly comprises contact dermatitis of the hands, is known from population and cross-sectional studies of specific occupational groups. Skin diseases comprises between 20-40% of all occupational diseases depending on geographical area. Approximately 1/3 is caused by allergic contact dermatitis and the rest mainly by irritant dermatitis. The principal occupational contact sensitizing chemicals are listed in Table 1. Not unexpectedly there is an overlap between exposure to chemicals in the domestic, occupational, and environmental environment. The common high-risk occupations for allergic contact dermatitis, modified from Rycroft (1995), are given in Table 2. The prevalence of occupational contact dermatitis in these occupations varies between a few percent up to 15% (Rycroft, 1995).

Table 1. The principal occupational allergens
- Biocides (including formaldehyde, formaldehyde releasing substances, isothiazolinones).
- Chromate
- Cobalt
- Nickel (primary sensitization usually non-occupational)
- Rubber-chemicals
- Plastic resins
- Dyes
- Fragrances
- Plants

Table 2. High-risk occupations for allergic contact dermatitis
- Adhesives/plastics workers
- Agriculturalists
- Cement casters
- Construction workers
- Glass workers
- Graphic workers
- Hairdressers
- Horticulturalists
- Leather tanners
- Painters
- Pharmaceutical/chemical workers
- Rubber workers
- Textile workers
- Tilers
- Wood workers
4. Diagnostic methods for allergic contact dermatitis

The aim of patch testing is to diagnose contact sensitization to environmental chemicals. The patch test was introduced in 1896 by the Swiss dermatologist Jadahsson (Wahlberg, 1995). The technology is a biological test where contact allergy is proved by re-exposing the skin to the specific chemical under occlusion on a 0.5cm² large skin area on the upper back for 2 days. The extract is put in an aluminum chamber and fixed onto the skin of the patient, and the test reaction is graded after 48 and 72 h. An eczematous response is regarded as positive. It is important to establish the eliciting threshold concentration in already sensitized individuals to establish guidelines for secondary prevention. This can be done by dilution patch test series on already sensitized individuals or similar experimental exposure tests (i.e. ROAT) (Hannuksela & Salo, 1986).

A positive test is a reproduction of the clinical disease showing redness, infiltration and eventual vesicles. Patients are primarily tested with a standard series including the most frequent sensitizing chemicals such as metals, preservatives, fragrances, rubber chemicals, and topically used medicaments. Testing is frequently supplemented with substances present in patients private or occupational environments.

5. Atopic eczema (atopic dermatitis)

Atopic eczema is one of the most common skin diseases in many countries of the world with an increasing prevalence. Prevalence rates range between 10 and 20 % of school children. Due to the immense suffering caused by the skin disfigurement and the often unbearable itching, as well as the large number of people affected, it presents a major health problem. The role of allergy in this skin disease has been controversial over past decades; recent investigations, however, have shown clearly that in a majority of patients, allergic reactions - preferentially by IgE-mediated sensitization - seem to play a clinically relevant role in eliciting and maintaining eczematous skin lesions. This is in contrast to allergic contact dermatitis, that is mediated by cellular immune responses. Atopic eczema or atopic dermatitis is a chronic pruritic inflammatory skin disease characterized by a typical age-related distribution and skin morphology. The manifestation of atopic eczema is subject to a multifactorial genetic predisposition as well as to environmental provocation factors (Table 3). Low molecular weight chemicals that act as haptens in the skin have a tendency to induce cellular immune responses, and hence allergic contact dermatitis, but also IgE production can be induced by low molecular weight chemicals.
Table 3. Important exogenous provocation factors in atopic eczema

1. Unspecific provocation factors
   Irritants
   Microbial skin colonization of infection
   e.g. Staphylococcus aureus
   Pityrosporum ovale
   Herpes simplex (Eczema herpeticum)
   Psychic stress, emotional factors

2. Specific provocation factors (individual hypersensitivity)
   IgE-mediated allergy
   e.g. Food
   House dust mite
   Animal dander
   Pollen
   Microbial colonization
   Contact allergy
   Pseudo-allergy (idiosyncrasy) and intolerance
   e.g. preservatives in foods
   citrus fruits

In most patients with atopic eczema, the lesions are often found on the face, the extremities (especially extensor aspects) and finally the trunk. Oozing and crusted lesions can often be found on the scalp (cradle cap). More and more, itching becomes an essential feature; the infant may be irritable, restless and try to scratch the affected areas (after 3rd month of life). The course is chronically persistent or relapsing. Later, between 2 and 5 years, the appearance of the lesions changes. They become infiltrated, the localization changes and affects flexures of popliteal and antecubital fossae, the nape of the neck and the backs of the hands and feet. In severe cases there may be an involvement of the entire skin surface. Dry skin becomes another characteristic feature especially in the adult phase and creates itching followed by scratching. Chronic inflammation produces thickening of the skin, especially in flexural regions. In most patients, establishing the diagnosis is not too difficult. In selected cases, clinical findings, history and IgE-mediated sensitization have to be regarded critically and all important differential diagnoses have to be ruled out thoroughly. There is a genetic component favoring the manifestation of atopic eczema (Schnyder, 1960; Küster et al. 1990).
6. Diagnostic approach for atopic eczema

Skin test methods can be divided into percutaneous (skin prick, intradermal) tests and epicutaneous (patch) tests (American Medical Association, 1987). Percutaneous tests search for immediate-type IgE-mediated hypersensitivity and are especially indicated in atopic eczema. The skin prick test (prick puncture test) has found the widest acceptance, because of its high convenience and safety (Dreborg, 1989). A drop of the test extract is placed on the volar surface of the forearm and the solution is introduced into the epidermis with a disposable hypoallergenic needle. After 15 minutes the reactions are graded in relation to the erythema and wheal that are induced. In intradermal testing 0,01 to 0,05 ml of the test extract is injected intradermally with a syringe. Scratch tests (applying the extract to a superficial scratch) and rub tests (rubbing of the skin with native allergen) are other variants applied only for special indications. Because of the danger of producing anaphylactic reactions these tests should be performed only by trained allergologists with experience in emergency treatment. In patients with atopic dermatitis percutaneous tests are widely used for the detection of hypersensitivity against environmental aeroallergens and foods (Ring, 1988).

In the serum of patients with atopic eczema, IgE antibodies can be detected by laboratory methods. IgE antibodies can be determined by binding to an allergen in a solid phase and radioactive, enzymatic or fluorometric labeling (Ring, 1988). Specific antibodies against environmental allergens are detected by the RAST (Radio- Allergo-Sorbent-Test) and expressed semi-quantitatively in different classes. Because of high laboratory technical requirements and costs, cellular tests such as the histamine release (from peripheral leukocytes) and the basophil degranulation test are only rarely used.

Epicutaneous patch tests are directed to detect contact sensitivity.

7. Prediction of sensitizing potential of low molecular weight chemical in humans

The sensitizing potential of chemicals can be evaluated by structure activity relationships. Computerized comparison of new versus old chemical structures, where the contact sensitizing potential is known may indicate sensitizing potential (Benezra et al., 1985; 1989).

Presently, the only available methods of predicting contact sensitization, are by animal and human studies. Predictive testing in humans requires multiple exposures leading to sensitization (10 patches, 48 hr each, same site) followed by a 2 week rest period and then challenge (48 hr) with a patch at a new site(Marzulli and Maibach, 1973). There is a number of alterations to this scheme, including the use of a provocative material such as sodium lauryl sulfate, skin stripping or skin freezing. It is unclear how useful these variations are. It should be mentioned that for ethical reasons this type of predictive testing in humans can not be done at a routine basis as it is not the intention to sensitize people against chemical compounds. There is a possibility for subclinical sensitization to allergic compounds following such procedures. For the sensitizater
DNOCB studies in man revealed that interaction between a low sensitizing dose and the graded series of doses used in the challenge phase resulted in subclinical sensitization. Individuals showing negative responses after the first challenge showed augmented responses in a second challenge compared to control subjects (Friedman, 1990). Safety testing is done in human volunteers who in contrast to allergic patients, will for themselves have no benefit from the results of the test. Prospective tests of skin sensitization using human volunteers should therefore only be done if the aim is to confirm a negative result. For example, such studies may involve confirmation that a certain dose level of a sensitizer will remain without effect under the defined test conditions. Therefore, generally predictive testing in man has to be limited to the detection of the irritating activity of the unknown compounds, following the notion that it is unlikely to get sensitization without irritation (Grabbe et al., 1996). Irritant effects of chemicals inducing vascular changes in the skin will initiate a first local fluid extravasation. The substance will then be transported to the regional lymph node, where an immune response may be initiated. One has to be aware, however, that not all irritating compounds will indeed induce sensitization.

For testing in man basically a test compound is placed in an occlusion chamber at the skin of an human volunteer whereby the extent of the irritation is limited by a phased increase in the duration of the skin exposure and frequent observations. The exposure time is gradually increased from 15 minutes up to maximally 4 hours, while after each exposure a follow up period of at least 48 but preferably 72 hours is used. Further testing by increasing exposure time or dose after an initial exposure can only be done when there was no response in the 48 hour follow up period. A test is terminated in a person as soon as irritation is diagnosed. A skin response with a surface of 0.5cm² is taken as a positive response. As reference positive control sodium dodecyl sulphate (SDS) is used as it is widely available and there are no other adverse effects. With this positive control at a 20% w/v concentration approximately 65% of the test population will be positive. SDS is especially included as a reference to identify interindividual variability, rather than ethnic or racial variability, and can thus be used as a calibration tool for a particular human volunteer panel (Basketter et al, 1996; Judge et al, 1996). The number of test persons is recommended to be at least 30 of which at least one third belonging to either sex.

Other tests that may be used for prediction of irritant capacity are the skin prick test, the intracutaneous test, and the skin scratch test. If these tests are to be done, it is recommended that also these are performed with negative (i.e. diluent) and positive controls (i.e. histamine or SDS) (Dreborg, 1989 ; Malling,1993). The size of the wheal and erythema reaction will be recorded as the mean, the sum of, or the product of the largest diameter and its orthogonal diameter. Prick tests responses with a wheal diameter under 2 mm are negative (i.e. approximately 7 mm²). The grade of the response can be documented in relation to the wheal and/or erythema response induced by the positive control (i.e. histamine or SDS). The intracutaneous test (ICT) is mostly used for tests with extracts of low allergen concentrations or low sensitivities. 0.02-0.05 ml of test solution is injected into the skin, causing a bleb of approximately 3 mm in diameter. In this latter test a clear-cut positive reaction is greater than 5 mm in diameter (area 20mm²).
The skin prick test (SPT) is considered the best test for the clinical routine with a diagnostic purpose to establish allergy in an individual patient. The risk for systemic side effects, in particular sensitization, is considered very low. It should be mentioned however, that in the context of diagnosis, the concentration of the test compound used are low, since the concentration that is required to elicit a secondary skin response is low. For diagnosis in high risk patients a sequence of investigations was suggested: skin patch testing, followed by skin prick testing and subsequently intradermal testing, based on the results of these respective tests (Wen and Ye, 1993). For induction of skin irritation as a primary response, the concentration of the test material that is required may be higher. Since with prick testing and intradermal testing the skin barrier is broken, and consequently there will be a more intimate contact between the test chemical and immunologically relevant skin components, the risk of sensitization may be higher. It may be recommended therefore, that for predictive testing for skin irritancy in humans a stepwise approach is taken, in which skin patch testing as outlined above is carried out first. In case of positive reactions, there is no need for further testing. In case of negative findings, it may be decided to perform skin prick testing or intradermal testing, but only in those cases where in practice there will be an intimate and high exposure of a larger contingent of the population.

As mentioned above, the outcome of the tests will indicate irritant potency of chemicals, and not the capacity to sensitize. In human biopsies of skin to which test compounds were applied depletion of Langerhans cells was observed for sensitizers but not for irritants, inactive chemicals and solvents. The effect of sensitizers determined in human skin biopsies may be an approach to discriminate irritancy from sensitization capacity in those cases where positive responses are observed (Pistoore et al., 1996).

Careful test management in terms of dosages applied is needed as the combination of potentially aggressive materials and highly sensitive individuals will occasionally result in a strong response (York et al., 1996). There should be a sufficient follow up period after a positive reaction; complete repair of the skin after irritant reactions may take up to 17 days (Wilhelm et al., 1994). In case of unexpected and unintended strong skin reactions the responses may be suppressed by antihistamines and local corticosteroid treatment. In this sense it needs to be mentioned that in recently proposed OECD guidelines one of the exclusion criteria for the testing of compounds is the presence of the likelihood of skin sensitization, and that more serious damage to the skin also has to be avoided. In general terms it should be mentioned that safety and ethical considerations for studies to be done must meet any local legal requirements and conform 100% to the "Helsinki" Guidelines (World Medical Association, 1993). This implies that testing follow the principles of Good Clinical Practice and must be reviewed and considered acceptable by an independent ethical review committee. The study must be checked and allowed by the local hospital ethical committees.

Alternative assessments of the strength of skin reactions as a read out of the skin tests have been proposed. For better quantification of the responses than visual gradation of erythema or swelling, transepidermal water loss (TEWL), laser doppler flowmetry (LDF), or reflectance spectroscopy assessing the water content of the skin are promising new leads (Anderson and Maibach, 1995). These read out systems have, however, still to be validated.
8. Evaluation of results of assays in humans

For diagnostic purposes in which allergy to well known allergens is established, standard doses are tested, and establishment of dose responses curves is not considered relevant (Van Metre, 1990). In contrast, for the screening of the allergic potency of new compounds as well as for risk assessment based on such screening dose response studies are very crucial.

Since for predictive testing of chemicals in terms of sensitization mostly laboratory animals are used, such data are mostly used for risk assessment as well. Generally, the assessment of risk for chemicals that induce contact sensitivity is limited, and risk management restricted to labeling. Although not common practice for allergenic chemicals, in analogy to general toxicity testing, based on which maximal acceptable concentrations are derived from no-observed adverse effect level (NOAEL), also concentrations of test compounds that produce no skin effects may be established. In general toxicology, risk assessment then considers safety factors for the interspecies difference, and intraspecies variability. With human tests that are predictive of skin irritation, (and perhaps, as indicated earlier of sensitizing capacity) this approach may also be followed. The need to consider interspecies differences as a factor for extrapolation is absent in that case, although it should be mentioned that extrapolation from individual data to the entire population remains an important issue. In light of this latter point, it needs to be emphasized that especially in the case of compounds that induce contact sensitivity, exposure leads to an irreversible, and life long effect, which must have an impact on the safety factor for extrapolation from individual data to the entire population. The determination of concentrations that do not produce skin effects highly depends on the design of the experiment, i.e. type of dose response curve that is being evaluated, and endpoints measured. For human skin testing mainly non-objective semi quantitative estimation of either presence or absence of responses is evaluated. The number of test persons recommended for such studies is to be at least 30 of which at least one third belonging to either sex. Weak positive responses in 30 % of the individuals is often taken as the cut off point to decide on irritancy. A number of arguments need to be considered for such a decision. If 30 % of individuals in a group of 30 shows weak positive responses, taking 95 % confidence limits into account, minimally 18 and maximally 53 % of individuals in the general population might be showing such responses upon encountering similar doses of the test compound (considering a 95 % confidence limit). Obviously, if the test group is bigger, e.g. 100, with 30 % of the individual giving weak positive responses, 22 to 40 % of the individuals in the general population may show such effects after exposure. Figure 1 and 2 show the 95% upper and lower confidence limits, meaning 5% chance that the real value is outside the indicated range, using either 30 or 100 individuals in a test group. As shown, the reliability of the estimation of the incidence of a positive reaction in the population decreases when using a reduced number of individuals in the test panel. However, it has to be reminded that the actual risk for the population is not only dependent on the risk of a positive reaction to a particular compound, but also depends on the percentage of the population at risk for exposure to that particular compound. From the public health point of view a compound inducing a 60-80% positive reaction might be accepted if only a few individuals in the population are at risk for contacting such an agent. Similarly some inaccuracy of the estimation of a positive reaction, in terms of upper confidence limit, as shown by our data evaluation in figures 1 and 2 might be accepted. In contrast, if exposure is wide spread, lower percentages of individuals that will react to exposure in the general population must be accepted, and the reliability of the test results need to be more strict. If the use of a
product is not crucial and alternatives are present, while the use may potentially be population wide, perhaps no adverse effects would be expected. No effects in a test group of 30 individuals predicts that no more than 10 % of the population could develop such adverse effects. With a test group of 100 individuals it can be predicted that maximally 5 % of the population would potentially develop such effects.

Figure 1. Upper and lower 95 % confidence limits of values obtained in a test population of 30 individuals.

Figure 2. Upper and lower 95 % confidence limits of values obtained in a test population of 100 individuals.
An alternative approach for setting standards based on finding concentrations that fail to produce effects in a test group may be the benchmark approach. For this approach it is necessary to establish to what extent the effect is considered adverse. To this end, it is necessary to have at ones disposal quantitative estimates of effects in a continuous range of magnitudes. As indicated earlier, transepidermal water loss (TEWL), laser doppler flowmetry (LDF), or reflectance spectroscopy assessing the water content responses (rather than visual gradation of erythema or swelling) in the skin may serve this goal. Such data, on an individual basis compared to and expressed relative to the response with a standard histamine or SDS dose may not only fulfill the requirement for objective quantitative effect assessments, from which dose-response relationships can be deducted, but may also provide a basis for an appropriate estimation of an adverse effect, i.e. the bench mark effect. Although not enough experience has been gained using such methodologies, one position that can be taken here is that a tenth of a maximal histamine or SDS response in a positive person, which is generally considered a barely positive response, as the adverse effect, and hence the cut off point applied to dose response curves. Using advanced regression analysis, based on the bench mark effect, the critical effective dose (CED) or bench mark dose can be calculated. For this analysis it is recommendable to test 5 or 6 doses of the compound per human volunteer, since the proper description of the regression of the dose response curve will benefit from a wide range of doses, rather than few doses.

A newly developed approach for the determination of the critical effective dose is the PROAST (probabilistic approach) program at the RIVM (Slob, 1997). The standard method for deriving an acceptable exposure for humans based either on NOAEL or bench mark ignores the uncertainty in these levels as an estimate of the true no-adverse-effect dose in experimental studies. In the PROAST method uncertainties are taken into account. Instead of one estimated critical dose this newly developed approach yields a distribution which reflects the overall uncertainty based on the variability observed in the effects. The lower percentile of this distribution may be regarded as a level below which no adverse effects may be encountered.
References


Hannuksela M, Salo H. The repeated open application test (ROAT). Contact Dermatitis 1986; 14: 221.


Malling HJ. Methods of skin testing. Allergy 1993; 48 (Suppl.14): 55.


OECD. OECD guideline for testing of chemicals 404, OECD, Paris, France, 1992.


Appendix 1  Mailing list

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11. Dr.H.van Loveren, projectleider/auteur
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16. Mw.Drs.E.M.Hulzebos, CSR
17. Dr.J.W.van der Laan, LGM
18. Dr.E.J.de Waal, LGM
19. SBD/Voorlichting & Public Relations
20. Bureau Rapportenregistratie
21. Bibliotheek RIVM
22-35. Bureau Rapportenbeheer
35-40. Reserve exemplaren LPI