*Contact Dermatitis 2002, 47, 257–266*
*Printed in Denmark . All rights reserved*



CONTACT DERMATITIS
*ISSN 0105-1873*

**Review Article**

# A review of the scientific basis for uncertainty factors for use in quantitative risk assessment for the induction of allergic contact dermatitis

Susan P. Felter[1], Michael K. Robinson[1], David A. Basketter[2] and G. Frank Gerberick[1]
[1]The Procter & Gamble Co., Miami Valley Laboratories, Cincinnati, OH, USA, [2]Safety and Environmental Assurance Centre, Unilever Colworth Laboratory, Sharnbrook, Bedford, UK

Safety evaluations for chemicals which possess the ability to cause sensitization by skin contact have traditionally been done using an ad hoc comparative risk assessment technique. Recently, several papers have been published supporting the use of an alternative, and potentially better, quantitative risk assessment approach. While they represent a relatively new approach to risk assessment for sensitizers, quantitative methods have been used for decades to support risk assessments for systemic toxicity. Historically, these methods have involved the extrapolation of toxicity data – generally from studies in laboratory animals at relatively high doses to human exposures at lower doses. For toxicity endpoints with a threshold, this process has traditionally involved the use of uncertainty factors. For example, uncertainty factors are commonly used to extrapolate from laboratory animals to humans, and from 'average' humans to sensitive subpopulations. In the absence of data to support a different value, a default factor of 10 is widely accepted for each of these areas. Recent papers have advocated the use of a similar approach to characterize the risk of the induction of skin sensitization by allergens of varying potency and potential for skin contact. As with other forms of toxicity, a quantitative assessment of risk for allergic skin reactions can be approached by identifying a NOAEL (no observed adverse effect level) and applying appropriate uncertainty factors. Three major areas of data extrapolation have been identified: inter-individual susceptibility, the influence of vehicle or product matrix, and exposure considerations. This paper provides an overview of each of these areas with an evaluation of the available scientific database to support an uncertainty factor in the range of 1–10 for each area.

*Key words:* allergic contact dermatitis; data extrapolation; sensitization risk assessment; sensitization uncertainty factors; skin. © Blackwell Munksgaard, 2002.

*Accepted for publication 25 June 2002*

The process of quantitative risk assessment (QRA) for chemicals thought to have a threshold for toxicity dates back to the early 1950s. The first publications proposing a QRA methodology were focused on the safety of food additives (1, 2). Lehman & Fitzhugh (1) of the U.S. Food and Drug Administration indicated that, 'the chemical additive should not occur in the total human diet in a quantity greater than 1/100 of the amount that is the maximum safe dosage in long-term animal experiments.' Because the database to support the safety of food additives tended to be fairly robust, the early risk assessments focused primarily on the areas of cross-species extrapolation and intraspecies extrapolation (i.e. to protect sensitive human subpopulations). It was recommended that a factor of 100 be applied to a NOAEL from a chronic (lifetime) animal study to account for these areas of extrapolation. This 100-fold factor was historically referred to as a 'safety factor', but has more recently been referred to as an 'uncertainty factor' (UF) in acknowledgement of our general lack of understanding of how the sensitivity of humans compares to that of laboratory animals and also limited information on the variability within the human population (3).

While the method has been refined over time, the basic process is still the same today: typically, a 'no observed adverse effect level' (NOAEL) or equivalent is identified (usually from a laboratory animal study), and appropriate 'uncertainty' or 'safety factors' are applied to account for areas of data extrapolation. In the decades following the advent of QRA for food additives, the method was

expanded to include evaluations of risk from various other sources – for example, contaminants in drinking water, air emissions from industrial plants or incinerators, pesticide residues on food, or environmental exposures to chemicals in hazardous waste sites. As the method grew in its application, additional areas of data extrapolation were identified. Unlike food additives, the databases upon which risk management decisions needed to be made were often less robust. Rather than having data from a chronic toxicity study, often only subchronic toxicity data were available; furthermore, these studies did not always identify a clear NOAEL. Also, the importance of including reproductive and developmental endpoints in a comprehensive risk assessment was realized. Consequently, where data were lacking, the list of 'uncertainty factors' expanded, and perhaps because the precedent was already set, each area of data extrapolation was also generally considered to have a default value of 10.

At the same time, risk assessors started challenging the use of a 10-fold factor as an appropriate default value for each of the uncertainty factors. Indeed, many investigators evaluated the scientific basis for these uncertainty factors. Numerous papers have been written on the basis for each UF, including the intraspecies UF (4, 5), the interspecies UF (6, 7), and subchronic to chronic extrapolation (8–10). More recently, there has been an increased emphasis on incorporating more data into the risk assessment process to reduce reliance on default factors (11), but it is also recognised that a default factor of 10-fold has become generally accepted as a conservative value that can be used in the absence of data (12–14).

Recent publications have demonstrated the extension of the National Academy of Sciences (NAS) paradigm and QRA methodology for use in characterizing risk of the induction of skin sensitization, or allergic contact dermatitis (ACD) (15, 16). As with traditional threshold-based risk assessments, the method involves the identification of a NOAEL, and the application of appropriate 'sensitization uncertainty factors' (SUFs) to allow us to extrapolate from one scenario to another.

### Identification of the NOAEL

The determination of the NOAEL for use in QRA for contact allergens has been described previously (16). The initial applications of this QRA method have focused primarily on perfume raw materials. Many of these chemicals have NOAELs that are based on small numbers of test subjects and are judged to be insufficiently robust for use as a definitive NOAEL. However, data from multiple patch studies in conjunction with other available data (e.g. laboratory animal data) often paint a consistent picture that supports a weight-of-evidence approach to categorizing these chemicals into 'potency classes.' For each potency class, then, a conservative default NOAEL is used which represents the low end of that potency class. The classes that have previously been described include nonsensitizers, and 5 categories of sensitizers of varying potency: extremely weak, weak, moderate, strong and potent (16). For example, allergens with a NOAEL from a human patch study in the range of 100–1000 $\mu g/cm^2$ will be assigned a default NOAEL of 100 $\mu g/cm^2$ for use in QRA. Of course, if data are available to provide a sufficiently robust NOAEL, then it would be appropriate to use these data to identify a definitive NOAEL, rather than relying on a default NOAEL from the appropriate potency class.

The use of laboratory animal data in the identification of a NOAEL or default is worth special mention here, especially as increasing emphasis is being placed on the use of data from the mouse local lymph node assay (LLNA) for both hazard and potency determinations for contact allergens. The LLNA is a recently validated and accepted method for assessing skin sensitization hazard (17). In this assay, contact allergens are identified as a function of lymphocyte proliferative responses induced in the draining lymph nodes following open, topical application of the test material. At least 3 concentrations of the test chemical are evaluated, along with a concurrent vehicle control. Under the conditions of the assay, a chemical is defined as a sensitizer if it causes a 3-fold or greater increase in proliferation relative to the vehicle control (stimulation index (SI) $\geq 3$). From the dose–response data it is then possible to derive mathematically an estimate of the concentration of test chemical required to induce an SI = 3, or a threshold positive response. This estimated concentration is called the 'EC3' value and is given as a percentage concentration (18).

Mechanistically, the biological sequelae that take place for the immune system to mount a response are the same across mammalian species, supporting the use of the mouse model to characterize human risk. Historically, for non-cancer risk assessments, a default 10-fold UF has been used to extrapolate from laboratory animal species to humans. The approach is somewhat different for using mouse data to estimate potency of contact sensitizers. In this case, much work has been done to *correlate* the dose–response data obtained in the mouse LLNA with what is known about potency in humans. It is a rare case, indeed, that this type of information would be available for systemic

toxicity endpoints. Therefore, because the mouse data have actually been correlated with human data, initial use of the LLNA data has focused on adding to the weight of evidence for *ranking* dermal allergens as to their relative potency (16). Of course, as our knowledge base expands and more quantitative experience is gained in the use of LLNA data, it is anticipated that these data may eventually be used directly, with an appropriate SUF to extrapolate from the mouse to human. An evaluation of the appropriate range of this SUF has not yet been addressed, but clearly it would have to take into account the uncertainty and variability in species' differences in both the toxicokinetics (e.g. differences in dermal penetration) and toxicodynamics related to susceptibility to the induction of ACD.

After the appropriate NOAEL or default NOAEL has been identified, the next step in the QRA for a contact allergen is to evaluate the areas of uncertainty and/or variability that need to be considered in extrapolating the NOAEL to conditions relevant to the human exposure of interest (for example, exposure to an ingredient in a personal care product). The major areas of extrapolation have been identified as:

- Inter-individual susceptibility;
- Vehicle or product matrix;
- Exposure considerations (beyond those explicitly considered in the exposure assessment)

As described above, this method has not yet evolved to the direct use of laboratory animal data in the identification of a NOAEL. As our experience grows with the use of quantitative dose–response data from the mouse LLNA, it is anticipated that these data will be used with an additional SUF to account for cross-species extrapolation.

The rest of this manuscript is devoted to an overview of the 3 SUFs that have been identified above, and previously used in QRAs for contact allergens (16). Some additional background and perspective on these areas of data extrapolation is provided, with examples of data currently available to support a 10-fold range for each SUF area.

### Inter-individual susceptibility

Quantitative risk assessments have long assumed a 10-fold default factor to account for the inherent variability in human susceptibility, which might arise from differences in age, sex, race, general health status, or genetic make-up. The use of a 10-fold uncertainty factor to account for inter-individual differences in susceptibility, then, is a natural extension for quantitative risk assessment of the induction of ACD. Robinson (1999) published an extensive review of the factors contributing to inter-individual differences in susceptibility (19). Although data are fairly limited in humans, this review provides a basis for concluding that age, sex and racial differences do not have a large influence, but rather, genetic factors and dermal integrity are likely to be more important in determining individual susceptibility. As with QRAs conducted for systemic toxicity endpoints, it is recognised that a 10-fold factor is unlikely to account for the entire range of human susceptibilities; however, these analyses support the traditional value of 10-fold as a reasonable default factor for inter-individual differences. For example:

*Age-related susceptibility:* Data exist to show that neither the elderly nor the very young are likely to be at increased risk to ACD as a function of age. In fact, some studies have suggested that infants and young children may be at lower risk of ACD. For example, Cassimos et al. (20) showed the reactivity of infants up to 9 months old to 2, 4-dinitrochlorobenzene (DNCB) to be low compared to that of adults, and also lower for younger infants, which is likely reflective of a reduced capacity for cell-mediated immunity in neonates. A total of 284 infants were evaluated in this study. Data indicated reaction rates of 6.8% in the first 15 days of life, 25.7% in infants 2–4 weeks old, 33.3% in 2-month-old infants, 62.9% in 3-month-old infants, 76.7% in 4-month-old infants, 81.5% in 5-month-old infants, 88.5% in 7-month-old infants, and 91.3% in 9-month-old infants. Similarly, in a study of children aged 1 month to 8 years, Epstein reported that the younger infants and children demonstrated less susceptibility to induction by pentadecylcatechol (Rhus, or poison ivy allergen) than the older children (21). As with the very young, there also does not appear to be a significant effect of ageing on susceptibility of the elderly to the induction of ACD (22).

*Sex:* From a biological or mechanistic perspective, sex is unlikely to be a significant factor in ACD susceptibility for an equivalent exposure, and, in fact, data suggest little difference between males and females with respect to susceptibility to ACD. There are some reports that suggest that women may be slightly more responsive than men (e.g. 23–25), but since the vast majority of patch test data are from female subjects, NOAELs determined from these studies can be considered to be protective of both sexes.

*Race:* Similarly, race is not expected to have a significant role in determining the range of inter-individual sensitivity. There are some reports suggesting that more highly pigmented skin may be

less susceptible to ACD (e.g. 26–28), but since most patch studies are conducted in subjects with lighter skin, these data can be taken to be protective of the range of human skin colours.

*Genetic factors:* While not well understood, genetic factors are clearly instrumental in determining individual susceptibility. For example, Lidén et al. (29) studied 149 patients who had positive reactions to 1 or more contact allergens, and found a relationship between specific HLA factors and allergic responses. Other investigators have also reported an increased occurrence of 1 or 2 HLA types in ACD patients (30, 31), but a consistent picture has not yet been painted providing clear evidence for an association with HLA antigens and specific allergies. Other genetically based differences may also be important, such as the relative metabolic activity of skin enzymes to metabolize absorbed xenobiotics (32). For example, skin allergy caused by poison ivy is believed to be due to the metabolic conversion of the pentadecylcatechol component of poison ivy to an orthoquinone which is known to be a strong contact allergen (33). Therefore, differences in metabolic capacities might be expected to influence susceptibility to the induction of ACD.

*Compromised skin:*. Skin that is abraded, inflamed, occluded, or otherwise altered such that the rate of penetrability is increased may be associated with a higher risk of sensitization. It is noted that while irritants may have an effect on the integrity of the skin, this alone does not generally account for the increase in susceptibility that is observed. It appears that for irritants, the effect is mediated by a combination of an effect on dermal penetrability and also an enhanced immune effect. This is discussed further in the section on 'Vehicle or Product Matrix.' Finally, caution is warranted for a risk assessment intended to apply to seriously compromised skin, as this will not necessarily fall within the range of the 10-fold SUF and should be evaluated on a case-by-case basis.

### *Studies supporting intra-individual default SUF of 10-fold*

There are few studies that provide direct quantitative evaluations of intrahuman sensitivity to the induction of allergenicity, but the available studies support the proposed 10-fold default factor. Friedmann & Moss (34) evaluated the response of 165 healthy volunteers, who were randomly assigned to 5 groups which received a sensitizing dose of 0, 62.5, 125, 250, or 500 $\mu$g DNCB. A smaller group of 8 individuals was subsequently sensitized with 1000 $\mu$g DNCB. The chemical was dissolved in a 100 $\mu$L volume of acetone, which was administered on a 3-cm diameter circle on the volar aspect of the forearm. These doses correlate to concentrations ranging from 8.8 $\mu$g/cm$^2$ (for the 62.5 $\mu$g dose) to 141 $\mu$g/cm$^2$ (for the 1000 $\mu$g dose). The area was then occluded for 48 h. Four weeks later, the volunteers were given at least 3 challenge doses, and application sites were graded for erythema, degree of induration, vesicle and bulla formation. Results showed a sigmoid relationship between the inducing dose and percent sensitized, with all subjects being sensitized to doses of 500 $\mu$g and above. The $ED_{50}$ in this study was estimated to be 116 $\mu$g, with a steep dose–response relationship: at the lowest dose of 62.5 $\mu$g (roughly half of the $ED_{50}$), the incidence of sensitization was 8%, while at the dose of 250$\mu$g (about 2$\times$ the $ED_{50}$), the incidence of sensitization was over 80%. The authors later tested the validity of their $ED_{50}$ estimate, and confirmed that, indeed, 13 out of 26 normal subjects were sensitized by a dose of 116 $\mu$g administered using the same methodology (35). In summary, then, this study indicates that $\sim$75% of the population responded to a dose within a 4-fold range (62.5–250 $\mu$g), and $\sim$95% of the population responded to a dose within an 8-fold range (62.5–500 $\mu$g), supporting a 10-fold default factor for intra-individual variability.

In addition to evaluating the range of response in the general population, it is important to consider specific subpopulations that might be expected to be at increased risk. In a study in the same laboratory (36), subjects were evaluated who had developed multiple contact allergies to environmental allergens, and thus might represent a sensitive subpopulation. For this study, subjects were chosen who had demonstrated sensitivity to 3 or more antigens in the European standard patch test series, while patients with active eczema were excluded. Using the same protocol as described above in Friedmann & Moss (34), it was found that 100% of the subjects responded to a dose of 250$\mu$g DNCB, and that at each sensitizing dose, the sensitized subjects were more responsive than 'normal' subjects. In contrast, it was reported that in subjects with multiple allergies following massive occupational exposures, these subjects had normal reactivity to DNCB such that previous allergic eczema did not enhance DNCB responsiveness (37). White et al. (38) also reported no differences in the distribution of numbers of Langerhans cells in individuals with multiple contact allergies. Finally, Moss et al. (36) reported that subjects allergic only to nickel had responses to DNCB that were intermediate between those of individuals with multiple contact allergies, and those with no history of contact allergy. In a review article, Friedmann (37) summarized these findings as indi-

cating that 'people with heightened susceptibility to contact sensitization are not a special group but are the extreme of the normal range'. In summary, there are a number of factors that are likely to contribute to inter-individual differences in susceptibility to the induction of ACD. While age, sex, and race do not appear to be major influences, certain disease states (especially those involving immune function), genetic differences and associated differences in metabolic activity, and general integrity of the skin are more likely to have a significant effect on inter-individual sensitivity. The limited quantitative data that are available suggest that a 10-fold default factor for inter-individual differences is adequate to protect the majority of the population.

### Vehicle or product matrix

The second major area for data extrapolation involves the matrix in which the allergen is present, and to which the individual is exposed. Most of the information available on the potency of various allergens comes from studies using a simple matrix in which the allergen is dissolved. Some of the more common solvents include ethanol, mineral oil, petrolatum, diethyl phthalate, or simple mixtures of these. Data from these studies, then, are extrapolated to the actual human exposure scenario, which typically involves exposure to allergens in more complex matrices. This might include, for example, a preservative in a shampoo, a dye in a laundry product, or a perfume raw material in a deodorant. Various ingredients within a product matrix may affect the permeability of the skin such that the allergen is transported across the skin more readily (39). While the mechanisms of such vehicle effects are not fully understood, several hypotheses exist, which are likely operative under different scenarios. These include effects on dermal irritation and effects on the penetration of the allergen, its uptake/processing by Langerhans cells, and the efficiency by which the Langerhans cells can migrate and accumulate in the draining lymph nodes (40). These latter factors are summarized under the heading 'penetration enhancers' below:

#### *Irritants*

Dermal irritation is known to serve as a promoter of sensitization. In fact, Smith et al. (41) proposed that one of the reasons for differences in individual responses to the same exposure to an allergen may be related to their susceptibility to skin irritation, such that those in whom the epidermal irritant response reaches a sufficient threshold level are more likely to be sensitized. Moreover, it has been demonstrated that cutaneous responses induced in mice by topical exposure to suboptimal concentrations of the allergen DNCB can be enhanced significantly by coadministration of the nonsensitizing irritant, sodium lauryl sulphate (SLS) (42). Under conditions where SLS was able to augment the stimulation of lymph node proliferative responses to DNCB, it failed to influence its ability to penetrate the skin.

In addition to the potential for an irritant present in a vehicle or matrix to affect the dermal penetration of an allergen, it may also influence the magnitude of response by affecting other steps in the process of inducing (or eliciting) an allergic response. For example, Cumberbatch et al. (43) determined that DNCB exhibited an increased potential to stimulate lymph node proliferative activity when administered in SLS. The authors interpreted their findings as an indication that the SLS served to promote the migration of the allergen-bearing Langerhans cells from the skin. Following this reasoning, it may well be that the frequently reported ability of the coadministration of SLS to augment skin sensitization (e.g. 28) is at least in part attributable to the ability of this chemical to cause local inflammation. More recently, Robinson et al. (44) provided at least circumstantial evidence that the irritant effect of an oleic acid/propylene glycol vehicle increased the allergenic potential of triprolidine, an antihistamine drug.

#### *Penetration enhancers*

Some chemicals are known specifically to effect the penetration of other chemicals through the stratum corneum. Indeed, for topical delivery of drugs, a common goal is often to formulate a medication to maximize dermal absorption by including penetration enhancers (45). For example, Azone (1-dodecylazacycloheptan-2-one) was developed as a pharmaceutical vehicle to facilitate the delivery of drugs across the stratum corneum, and is now regarded as a benchmark chemical for the enhancement of dermal penetration. Wiechers (46) provides a review of the development of Azone, as well as a summary of those chemicals regarded as having a moderate potential (up to 10-fold increase) for dermal penetration enhancement (e.g. fatty acids and alcohols, and esters of these compounds). He describes 3 main modes of actions involving

- disruption of the stratum corneum lipids;
- interaction with intracellular proteins; or;
- improved partitioning of the drug into the stratum corneum.

It is also noteworthy that penetration enhancers are associated with some specificity. For example,

Southwell & Barry (47) showed that 2-pyrrolidone (2-P) and dimethylformamide (DMF) similarly accelerated the penetration of water and polar alcohols, and retarded the penetration of non-polar alcohols. However, 2-P was found to retard the penetration of caffeine, while DMF was associated with a dramatic increase in penetration. Clearly, this is a complex area in which any assumptions must be made with caution.

*Studies supporting a SUF in the range of 1–10-fold for vehicle or matrix effects*

There are a few studies that have evaluated qualitative and quantitative differences in either dermal absorption or relative skin sensitization potency as a result of vehicle effects. In one of the earlier studies, Marzulli & Maibach (48) evaluated the effect of vehicles on the elicitation of ACD; while it is noted that this study focused on elicitation, it is reasonable to assume that similar effects might be observed for the induction phase of ACD. A modified Draize test was used to test the response of subjects to cinnamic aldehyde (cinnamal), an allergic perfume raw material, and to costus oil, administered at 2 skin sites in petrolatum or ethanol. No difference between the vehicles was reported for cinnamic aldehyde, but ethanol was more effective in eliciting a response than was petrolatum in the tests of costus oil.

Much of the quantitative data on the effect of vehicles come from the mouse local lymph node assay (LLNA), which have recently been reviewed by Basketter et al. (49). Lea et al. (50) evaluated the effect of several vehicles on the response in the LLNA to 1,4-dihydroquinone, a known sensitizer. The vehicles evaluated, and the mean EC3 values (in parentheses) obtained were reported as follows: methyl ethyl ketone (0.07%), acetone (0.08%), 80/20 mix of acetone/olive oil (0.15%), dimethylformamide (0.22%), dimethyl sulfoxide (0.4%). These results demonstrate a 5-fold difference in potency of a single chemical in the LLNA assay, depending on the solvent used. Interestingly, in this same study, propylene glycol and acetone/saline (50/50) gave negative results unless the concentration was increased, with EC3 values being reported in the range of 1–2%. The authors suggested that possible reasons for the vehicle effects include the partitioning of 1,4-dihydroquinone from the vehicle matrix to the epidermis, the effect of the vehicle on oxidation systems that activate the allergen, and the impact of the vehicle on irritancy.

Warbrick et al. (51) also evaluated the influence of several vehicles on the potency of a known allergen in the LLNA assay. In this study, the allergen was methylchloroisothiazolinone/methylisothiazolinone, the active ingredient in a commonly used biocide/preservative. The solvents tested were the same as those studied by Lea et al. (50). Results indicated a 10-fold range in the EC3 values, from a low of 0.0049% with acetone/olive oil to a high of 0.048% with propylene glycol.

Heylings et al. (42) also evaluated the influence of vehicle in the LLNA assay, testing the response to 0.5% DNCB in acetone *versus* propylene glycol. A significantly greater lymph node cell proliferation response was induced by DNCB in acetone. The authors then investigated the absorption of DNCB through the skin and found that while the flux was comparable with both vehicles after 24 h, the absorption during the first 4 h was about 3-fold higher for DNCB dissolved in acetone. This likely contributes to the increased response in the LLNA assay.

Robinson & Cruze (52) also showed that the response in the LLNA and Buehler guinea pig tests to weak allergens (antihistamines) was affected by the vehicle system. In the Buehler test, the irritating and penetration enhancing vehicle system of 1% oleic acid in propylene glycol increased the incidence and severity of sensitization responses to triprolidine relative to ethanol or propylene glycol alone. In the LLNA, positive responses to each of 4 antihistamines depended on the slight abrasion of the ears prior to allergen application or the use of the penetration enhancing vehicle, dimethylformamide.

Clearly, the vehicle or matrix in which an allergen is presented to the skin will have an influence on the potential for the induction of ACD. This effect might be mediated by an influence on dermal penetration of the allergen (either potentiating or inhibiting), and/or the magnitude of inflammatory response following penetration into the viable epidermis. Overall, the available quantitative data suggests that vehicle effects will be within an order of magnitude, so that a default SUF of 10-fold is appropriate. It is noted, however, that data available on the vehicle or matrix can be used to reduce this factor. For example, if the human exposure of interest is in a simple matrix with no penetration enhancers or irritants, a factor of 3-fold might be appropriate. Further, if the exposure of interest is in a matrix very similar to the matrix used to determine the NOAEL for risk assessment, then a reduction in the 10-fold SUF for vehicle effects is clearly justified, and may, in fact, be reduced to a 1-fold factor.

## Skin exposure considerations

Consistent with the NAS paradigm, one of the steps of a QRA for dermal sensitization is an ex-

posure assessment, which provides an estimate of dermal exposure to the allergen in units of $\mu$g allergen per square centimetre of skin. While it is recommended that appropriate conservatism be applied to the estimation of this exposure, there are other factors relating to exposure conditions that may not be explicitly – or quantitatively – evaluated as part of the exposure assessment, but which may affect the potential for the induction of ACD. For example, these include the site of the body exposed, the integrity of the skin, potential for mucosal contact, and environmental conditions such as temperature and humidity. The potential for multiple exposures (e.g. from the use of multiple products on the same area of skin) might also be considered here, although it is recognised that quantitative data in this area are generally lacking.

*Site of body exposed*

Regional differences in dermal absorption can be substantial. For example, Feldmann & Maibach (53) measured the relative regional permeability of human skin from various body sites to $^{14}$C-labelled hydrocortisone. Of 11 sites evaluated, the skin of the back (where most patch studies are conducted) was intermediate in relative permeability. The plantar foot arch was correspondingly about 12-fold less permeable than the skin of the back, while the scalp and axillae were about 2-fold higher, and the forehead was about 3-fold higher. Clearly these values will change somewhat depending on numerous factors, but this illustrates the clear differences in the potential for chemicals to be absorbed through the skin depending on the body site. With the exception of scrotal skin, which is known to be highly permeable, most sites of the human body fall well within a 10-fold range relative to the skin of the upper back and arms, where patch testing is typically conducted.

In addition to the data in humans, there is some evidence in mice that the body site exposed has some effect on susceptibility. For example, Kalish et al. (54) reported a difference in the skin permeability of contact allergens and the corresponding sensitization incidence in mice, depending on whether the study was conducted on dorsal or ventral skin for the induction exposures.

*Effect of occlusion*

The efficacy of topical drugs was first shown to be enhanced by covering the site of application by Garb (55), and has since been a common method to increase dermal penetration. As discussed in a review by Bucks et al. (56), occlusion of the skin results in multiple effects, including increases in the hydration of the stratum corneum, skin temperature, microbial count, pH, and dermal irritation. The increase in hydration state, in particular, has been associated with increased dermal penetration, although occlusion does not increase the absorption of all chemicals, and that the relative effect of occlusion is likely dependent on the lipophilicity of the chemical. It is noted that, with the exception of certain drug products, most human exposures to allergens do not generally occur under occluded conditions. In contrast, most of the human data used in the potency estimation (e.g. NOAELs and LOAELs, the non- or lowest-observed adverse effect levels, respectively) of contact allergens, have been obtained under occlusive conditions. This tends to introduce some conservatism in the sensitization risk assessment process, since effects seen under full occlusion (e.g. the LOAEL) may, in fact, have been a NOAEL in the absence of occlusion.

*Dermal integrity*

Factors influencing dermal integrity are known to have a significant effect on dermal penetration. This might include, for example, the presence of diaper rash in an infant, or dermatitis in an adult. Benfeldt et al. (57) compared the absorption of salicylic acid (SA) through healthy intact skin and barrier-perturbed skin. They found that acetone-treated skin exhibited a 2.2-fold increase in SA, mild dermatitis was associated with a 46-fold increase, and severe dermatitis with a 146-fold increase. While less dramatic, shaving has also been shown to have an influence. For example, Edman (58) estimated an odds ratio of 2.9 for the risk of perfume allergy in men who use a razor blade to shave facial hair. It is noted that there is overlap between the consideration of dermal integrity under the SUF for 'skin exposure considerations' and the consideration of compromised skin under the SUF for 'inter-individual susceptibility' and that the risk assessor needs to consider this appropriately.

*Environmental conditions*

In addition to these exposure considerations, environmental conditions may also influence susceptibility to ACD. For example, Hosoi et al. (59) have shown that environmental humidity levels influence dermal immune responses in mice, with a greater contact hypersensitivity reaction to 2,4,6-trinitrochlorobenzene being elicited in mice housed under low humidity ($\sim$10%) conditions compared with high humidity ($\sim$80%) conditions. This was found to be true both for the induction and elicitation phases of the dermal reaction. The authors

postulated 2 mechanisms whereby humidity may play a role:

1. the number of epidermal Langerhans' cells were found to be increased under low humidity conditions;
2. there may be an enhanced percutaneous penetration of the allergen under low humidity.

In humans, the data are somewhat conflicting, with some investigators suggesting fewer positive responses in the summer, but others reporting no significant seasonal influence. There are also some data to indicate that skin is more susceptible to certain irritants in the low humidity of winter *versus* summer, which may have some bearing on sensitization incidence (60). From a multifactorial analysis of about 40,000 patients who had been tested with 4 different allergens, Uter et al. (61) concluded that only formaldehyde exhibited a distinct increase in weak-positive reactions associated with dry, cold weather. Epoxy resin showed no association with climatic conditions, and methylchloroisothiazolinone/methylisothiazolinone and lanolin alcohol showed a weak association with seasonal variations.

Clearly, there are a number of factors related to human exposure that may not be explicitly factored into the exposure assessment, yet which can have a measurable impact on the potential for inducing ACD. A default SUF in the range of 1–10 is recommended to account for the uncertainty inherent in this extrapolation, and it is again emphasized that professional judgement is required in making a determination of the most appropriate SUF to account for exposure considerations. In rare circumstances, one may even determine that a factor higher than 10-fold is appropriate (e.g. for a product intended for use on mucous membranes or abraded skin). These can only be determined on a case-by-case basis.

## Conclusions

In conclusion, we have described 3 major areas of data extrapolation – each with inherent uncertainty and variability – that should be considered in a QRA for the induction of allergic contact dermatitis. Recent publications have advocated the use of the NAS paradigm and established quantitative risk assessment methodologies to estimate the risk of induction of ACD. As with quantitative risk assessments for other health endpoints, there are areas of uncertainty and/or variability associated with the extrapolation of toxicity data from one scenario (e.g. controlled human patch study) to the exposure scenario being assessed. The 3 major areas of data extrapolation identified include inter-individual susceptibility, vehicle or product matrix effects, and skin exposure considerations that are not explicitly addressed in the exposure assessment part of the risk assessment process. For each of the 3 areas, an overview of the scientific basis has been described along with a summary of the studies that are available to support a default SUF in the range of 1–10.

Finally, it is noted that while the 3 areas described above have been identified as major areas of data extrapolation with associated uncertainty, they are not entirely independent, nor can they account for 100% of the uncertainty inherent in the risk assessment process. Some degree of overlap can be seen in these areas – for example, the importance of dermal integrity might be considered to be a factor in inter-individual differences, or it may be considered to be part of the exposure considerations. Likewise, a concurrent exposure to a dermal irritant might be considered to be a vehicle/matrix effect, or it might also be considered under exposure considerations. Clearly, these areas are not sharply delineated, and nothing can replace professional judgement in performing a qualitative and quantitative evaluation of the uncertainties associated in a sensitization risk assessment.

## Acknowledgements

The authors thank Drs Harald Schlatter, Karen Kohrman and Cindy Ryan for their thoughtful review and critique of this manuscript.

## References

1. Lehman A L, Fitzhugh O G. 100-fold margin of safety. *Q Bull Assoc Food Drug Off US* 1954: 18: 33–35.
2. WHO (World Health Organization). Procedures for the testing of intentional food additives to establish their safety for use. Second Report of the Joint FAO/WHO Expert Committee on Food Additives. *Technical Report Series, no. 144.* Geneva: WHO, 1958.
3. Dourson M L, Stara J F. Regulatory history and experimental support of uncertainity (safety) factors. *Reg Toxicol Pharmacol* 1983: 3: 224–228.
4. Burin G J, Saunders D R. Addressing human variability in risk assessment. The robustness of the intraspecies uncertainty factor. *Reg Tox Pharmacol* 1999: 30: 209–216.
5. Renwick A G, Lazarus N R. Human variability and noncancer risk assessment – An analysis of the default uncertainty factor. *Reg Tox Pharmacol* 1998: 27: 3–20.
6. Chappell W R, Mordenti J. Extrapolation of toxicological and pharmacological data from animals to humans. *Adv Drug Res* 1991: 20: 1–116.
7. Travis C C, White R K. Interspecific scaling of toxicity data. *Risk Anal* 1988: 8: 116–125.
8. Kramer H J, van den Ham W A, Slob W, Pieters M N. Conversion factors estimating indicative chronic no-observed-adverse-effect levels from short-term toxicity data. *Reg Tox Pharmacol* 1996: 23: 249–255.
9. Nessel C S, Lewis S C, Stauber K L, Adgate J L. Sub-

chronic to chronic exposure extrapolation: Toxicologic evidence for a reduced uncertainty factor. *Human Ecol Risk Assess* 1995: 1: 516–526.
10. Pieters M N, Kramer H J, Slob W. Evaluation of the uncertainty factor for subchronic-to-chronic extrapolation: Statistical analysis of toxicity data. *Reg Tox Pharmacol* 1998: 27: 108–111.
11. Dourson M L, Felter S P, Robinson D. Evolution of science-based uncertainty factors for non-cancer risk assessment. *Reg Toxicol Pharmacol* 1996: 24: 108–120.
12. USEPA (US Environmental Protection Agency). Reference dose (RfD). Description and use in health risk assessments. http:http://www.epa.gov/iris/rfd.htm, 1993.
13. ECETOC (European Centre for Ecotoxicology and Toxicology of Chemials). Assessment factors in human health risk assessment. *ECETOC Technical Report no. 68.* Brussels: ECETOC, 1995.
14. WHO/IPCS (World Health Organization/International Programme for Chemical Safety). Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits. *Environmental Health Criteria 170.* Geneva: WHO/IPCS, 1994.
15. Gerberick G F, Robinson M K. A skin sensitization risk assessment approach for evaluation of new ingredients and products. *Am J Contact Dermatits* 2000: 11: 65–73.
16. Gerberick G F, Robinson M K, Felter S P, White I R, Basketter D A. Understanding fragrance allergy using an exposure-based risk assessment approach. *Contact Dermatitis* 2001: 45: 333–340.
17. ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). The murine local lymph node assay: a test method for assessing the allergic contact dermatitis potential of chemicals/compounds. *NIH publication no. 99–4494*, 1999.
18. Basketter D A, Blaikie L, Dearman R J et al. Use of the local lymph node assay for the estimation of relative contact allergenic potential. *Contact Dermatitis* 2000: 42: 344–348..
19. Robinson M K. Population differences in skin structure and physiology and the susceptibility to irritant and allergic contact dermatitis: implications for skin safety testing and risk assessment. *Contact Dermatits* 1999: 41: 65–79.
20. Cassimos C, Kanakoudi-Tsakalidis F, Spyroglou K, Ladianos M, Tzaphi R. Skin sensitization to 2,4-dinitrochlorobenzene (DNCB) in the first months of life. *J Clin Lab Immunol* 1980: 3: 111–113.
21. Epstein W L. Contact-type delayed hypersensitivity in infants and children: induction of *Rhus* sensitivity. *Pediatrics* 1961: 27: 51–53.
22. Lejman E, Stoudemayer T, Grove G, Kligman A M. Age differences in poison ivy dermatitis. *Contact Dermatitis* 1984: 11: 163–167.
23. Leyden J L, Kligman A M. Allergic contact dermatitis: sex differences. *Contact Dermatitis* 1977: 3: 333–336.
24. Jordan W P Jr, King S E. Delayed hypersensitivity in females. The development of allergic contact dermatitis in females during the comparison of two predictive patch tests. *Contact Dermatitis* 1977: 3: 19–26..
25. Rees J L, Friedmann P S, Matthews J N. Sex differences in susceptibility to development of contact hypersensitivity to dinitrochlorobenzene (DNCB). *Br J Dermatol* 1989: 120: 371–374.
26. Epstein W L. Induction of allergic contact dermatitis in patients with the lymphoma-leukemia complex. *J Invest Derm* 1958: 30: 30–53.
27. Rostenberg A, Kanof N M. Studies in eczematous sensitizations: I. A comparison between the sensitizing capacities of two allergens and between two different strengths of the same allergen and the effect of repeating the sensitizing dose. *J Invest Derm* 1941: 4: 505–516.
28. Kligman A M. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. *J Invest Derm* 1966: 47: 375–392.
29. Lidén S, Beckman L, Cedergren B, Goransson K, Nyquist H. HLA antigens in allergic contact dermatitis. *Acta Derm Venereol* 1978: 58 (Suppl): 53–56.
30. Karvonen J, Silvennoinen-Kassinen S, Ilonen J, Jakkula H, Tiilikainen A. HLA antigens in nickel allergy. *Ann Clin Res* 1984: 16: 211–212.
31. Mozzanica N, Rizzolo L, Veneroni G, Diotti R, Hepeisen S, Finzi A F. HLA-A, B, C, and DR antigens in nickel contact sensitivity. *Br J Dermatol* 1990: 122: 309–313.
32. Smith C K, Hotchkiss S A M, eds. *Allergic Contact Dermatitis: Chemical and Metabolic Mechanisms*. London: Taylor & Francis, 2001.
33. Dupuis G. Studies on poison ivy. *In vitro* lymphocyte transformation by urushiol-protein conjugates. *Br J Dermatol* 1979: 101: 617–624.
34. Friedmann P S, Moss C. Quantification of contact hypersensitivity in man. In: Maibach H I, Lowe N J, eds. *Models in Dermatology*, Vol. 2. Basel: Karger, 1985: 275–281.
35. White S I, Friedmann P S, Moss C. The effect of altering area of application and dose per unit area on sensitization by DNCB. *Br J Dermatol* 1986a: 115: 663–668.
36. Moss C, Friedmann P S, Shuster S, Simpson J M. Susceptibility and amplification of sensitivity in contact dermatitis. *Clin Exp Immunol* 1985: 61: 232–241.
37. Friedmann P S. The immunology of allergic contact dermatitis: The DNCB story. *Adv Dermatol* 1990: 5: 175–196.
38. White S I, Friedmann P S, Stratton A. HLA antigens and Langerhans' cell density in contact dermatitis. *Br J Dermatol* 1986: 115: 447–452.
39. Scheuplein R, Ross L. Effects of surfactants and solvents on the permeability of epidermis. *J Soc Cosmet Chem* 1970: 21: 853–873.
40. Dearman R J, Cumberbatch M, Hilton J et al. Influence of dibutyl phthalate on dermal sensitization to fluorescein isothiocyanate. *Fund Appl Toxicol* 1996: 33: 24–30.
41. Smith H R, Holloway D, Armstrong D K B, Basketter D A, McFadden J P. Irritant thresholds in subjects with colophony allergy. *Contact Dermatits* 2000: 42: 95–97.
42. Heylings J R, Clowes H M, Cumberbatch M et al. Sensitization to 2,4-dinitrochlorobenzene. Influence of vehicle on absorption and lymph node activation. *Toxicology* 1996: 109: 57–65.
43. Cumberbatch M, Scott R C, Basketter D A et al. Influence of sodium lauryl sulphate on 2,4-dinitrochlorobenzene-induced lymph node activation. *Toxicology:* 1993: 77: 181–191.
44. Robinson M K, Parsell K W, Breneman D L, Cruze C A. Evaluation of the primary skin irritation and allergic contact sensitization potential of transderman triprolidine. *Fund Appl Toxicol* 1991: 17: 103–119.
45. Barry B W. *Dermatological Formulations: Percutaneous Absorption*. New York: Marcel Dekker, 1983.
46. Wiechers J. Excipients in topical drug formulations. *Manufacturing Chem* 1998: July: 17–21.
47. Southwell D, Barry B W. Penetration enhancers for human skin. Mode of action of 2-pyrrolidone and dimethylformamide on partition and diffusion of model compounds water, n-alcohols, and caffeine. *J Invest Dermatol* 1983: 80: 507–514.
48. Marzulli F N, Maibach H I. Effects of vehicles and elicitation concentration in contact dermatitis testing: I. Experimental contact sensitization in humans. *Contact Dermatitis* 1976: 2: 325–329.
49. Basketter D A, Gerberick G F, Kimber I. Skin sensitization, vehicle effects and the local lymph node assay. *Food Chem Toxicol* 2001: 39: 621–627.

50. Lea L J, Warbrick V, Dearman R J, Harvey P, Kimber I, Basketter D A. The impact of vehicle on assessment of relative skin sensitization potency of the 1,4-dihydroquinone in the local lymph node assay. *Am J Contact Dermatitis* 1999: 10: 213–218.
51. Warbrick E V, Dearman R J, Basketter D A, Kimber I. Influence of application vehicle on skin sensitization to methylchloroisothiazolinone/methylisothiazolinone: an analysis using the local lymph node assay. *Contact Dermatitis* 1999: 41: 325–329.
52. Robinson M K, Cruze C A. Preclinical skin sensitization testing of antihistamines: Guinea pig and local lymph node assay responses. *Food Chem Toxicol* 1996: 34: 495–506.
53. Feldmann R J, Maibach H I. Absorption of some organic compounds through the skin in man. *J Invest Derm* 1969: 54: 339–404.
54. Kalish R, Wood J A, Wille J J, Kydonieus A. Sensitization of mice to topically applied drugs: albuterol, chlorpheniramine, clonidine and nadolol. *Contact Dermatitis* 1996: 35: 76–82.
55. Garb J. Nevus verrucosus unilateralis cured with podophyllin ointment. *Arch Dermatol* 1960: 81: 606–609.
56. Bucks D A W, Maibach H I, Guy R H. Occlusion does not uniformly enhance penetration *in vivo*. In: Bronaugh R L, Maibach H I, eds. *Percutaneous Absorption: Mechanisms − Methodology − Drug Delivery*. New York: Marcel Dekker, 1989: 77–93.
57. Benfeldt E, Serup J, Menné T. Effect of barrier perturbation on cutaneous salicylic acid penetration in human skin: *in vivo* pharmacokinetics using microdialysis and noninvasive quantification of barrier function. *Br J Dermatol* 1999: 140: 739–748.
58. Edman B. The influence of shaving method on perfume allergy. *Contact Dermatits* 1994: 31: 291–292.
59. Hosoi J, Hariya T, Denda M, Tsuchiya T. Regulation of the cutaneous allergic reaction by humidity. *Contact Dermatitis* 2000: 42: 81–84.
60. Basketter D A, Griffiths H A, Wang X M, Wilhelm K P, McFadden J. Individual, ethnic and seasonal variability in irritant susceptibility of skin: the implications for a predictive human patch test. *Contact Dermatitis* 1996: 35: 208–213.
61. Uter W, Geier J, Land M, Pfahlberg A, Gefeller O, Schnuch A. Another look at seasonal varaiation in patch test results. A multifactorial analysis of surveillance data of the IVDK (Information Network of Departments of Dermatology; University of Göttingen, Germany). *Contact Dermatitis* 2001: 44: 146–152.

Address:
*Susan P. Felter, Ph.D.*
*The Procter & Gamble Co.*
*Miami Valley Laboratories*
*Cincinnati, OH*
*USA*
Tel: +1 513 627 1958
Fax: +1 513 627 1927
e-mail: felter.sp.@pg.com