QRA 2 APPENDIX 1 B

3. SCIENTIFIC BASIS FOR THE SELECTION OF SAF VALUES

Skin Sensitization Quantitative Risk Assessment: A Review of Underlying Assumptions

David Basketter[1] and Bob Safford[2]

[1]DABMEB Consultancy Ltd, Sharnbrook, Bedfordshire, UK

[2]B-Safe Toxicology Consulting, Rushden, Northamptonshire, UK

Introduction

A substantial, divergent set of chemicals possess the intrinsic hazard of being able to induce the state of contact allergy in humans (summarised in Rietschel and Fowler, 2008; Johansen et al, 2011). Toxicologically, these chemicals are described as skin sensitizers and for decades have been identified by in vivo methods in the guinea pig or the mouse (Andersen and Maibach, 1985; Kimber and Basketter, 1992). Once an individual has become sensitized, i.e. has developed contact allergy, then given further and sufficient exposure they are inevitably at risk of the expression of the clinical disease we recognise as allergic contact dermatitis (ACD). The large majority of individuals who are exposed to skin sensitisers neither develop detectable contact allergy nor do they express allergic contact dermatitis (Krasteva et al., 2009; Basketter et al., 2011a). However, with regard to fragrance ingredients, a recent epidemiological study indicates a prevalence of 0.9-4.1% of fragrance contact allergy in the European Union (Rossi et al., 2010; Naldi et al, 2014). The frequency of positive diagnostic patch tests in dermatology clinics has remained elevated, with 1 in every 7 patients positive in a recent report (Mann et al, 2014). Thus, dermal sensitization to fragrance ingredients remains a significant issue. However, the material that follows relates to all chemical skin sensitisers and not specifically to fragrance substances.

The processes associated with the risk assessment of skin sensitizing chemicals have evolved considerably in the last two decades. In part this has arisen because of the appreciation of the large variation in the intrinsic induction potency of skin contact sensitizers, covering approximately 5 orders of magnitude (Gerberick et al, 2005; Kern et al, 2010). In 2008, a proposal was made for the dermal sensitization Quantitative Risk Assessment (QRA) for fragrance ingredients (Api et al., 2008). This has subsequently been used to establish industry guidelines, for risk assessment and as a basis for risk management of fragrance ingredients in cosmetics and household products (Api et al, 2013). The existing QRA process defines an Acceptable Exposure Level (AEL) for daily consumer exposure, expressed in $\mu g/cm^2$, which is based on a weight of evidence derived No Expected Sensitization Induction Level (NESIL), and to which various Sensitization Assessment Factors (SAFs) are applied. The use of $\mu g/cm^2$ is

based on the evidence that this measure represents the key metric governing the induction of skin sensitization (Kimber et al, 2008). The NESIL represents an exposure level which, in a Human Repeat Insult Patch Test (HRIPT) should not induce skin sensitisation, and then uses this level as the point of departure for the risk assessment. The NESIL represents the highest dose that would not induce sensitization in 100 subjects under the conditions of HRIPT exposure. However, other limitations relating to the HRIPT have to be borne in mind: for example, even when it is actually carried out, there will often only be one dose tested, and the NESIL defined as this dose (assuming that the expected outcome, no evidence of skin sensitization, was observed). Thus the measured NESIL may well be lower than the actual threshold dose which just fails to induce skin sensitization. It is understood that the number of subjects in a typical HRIPT offers limited resolving power, such that use of the HRIPT for more general prediction of human safety is inappropriate (Basketter, 2009; Gefeller et al, 2013). Human heterogeneity, the great diversity of the chemicals and wide range of uses to which they are put, introduces complexity which needs to be considered within the risk assessment of potential sensitising ingredients. For this reason, SAFs are applied to the NESIL to derive an AEL that is relevant for the whole population, and encompasses all allergens and exposure situations.

The current approach to skin sensitization quantitative risk assessment (QRA) has been fully detailed elsewhere, including documentation of the underlying assumptions used in deriving the SAFs (Gerberick et al, 2001; Felter et al, 2002, 2003; Api et al, 2008). Since those publications, a significant new body of scientific, clinical and consumer use data has become available that permits verification and possible revision of parts of the assessment process proposed in 2008. A number of reviews have appeared (e.g. Friedmann and Pickard, 2010; Thyssen et al, 2012) that provide more detail on some aspects that we touch on here. However, the present paper endeavours to summarise available data in the context of sensitising fragrance ingredients used in consumer products, although the principles outlined hereafter could be extended assessing the risk of dermal sensitization due to other allergenic materials and in other exposure scenarios. Proposals made in this paper reflect the current state of knowledge, but are not represented as the ultimate definitive risk assessment procedure. It can be anticipated

that further advances in knowledge will lead to additional improvements of this procedure. It is also recognised that while the aim of QRA is the prevention of induction of allergy, it is probably impossible to achieve this in the whole population and in all feasible exposure scenarios.

Finally, it is essential to be aware that this review does not address such aspects as the reliability of in vivo, in vitro or in silico predictions (e.g. as detailed in Thyssen et al, 2012). Neither, since the goal is to avoid the induction of contact allergy, does it consider matters concerning the elicitation of allergic contact dermatitis, except where these specifically enlighten our understanding of the variables associated with the induction of contact allergy, thus facilitating the conduct of a thorough risk assessment.

Background and Definitions

***Skin sensitizer**:* a chemical which possesses the intrinsic toxicological property (i.e. hazard) that with sufficient skin exposure in humans it can cause the induction of skin sensitization/contact allergy.

***Contact allergy**:* the asymptomatic condition which an individual has when they are sensitized to a specific chemical, and which can be detected by a diagnostic patch test.

***Diagnostic patch test**:* a clinical procedure designed to reveal whether an individual has contact allergy and who is then susceptible to the development of allergic contact dermatitis upon subsequent exposure to the allergen.

***Allergic contact dermatitis (ACD)**:* the eczema elicited following sufficient skin exposure in an individual who has contact allergy.

Frequency/prevalence: these and related terms endeavour to follow their standard usage in epidemiology

***Irritant contact dermatitis**:* an eczema clinically very similar to ACD, but of non-immunologic origin.

***Hazard identification/characterisation**:* these terms refer specifically and exclusively to the elucidation of the intrinsic skin sensitizing properties of chemicals.

***Risk assessment/characterisation**:* this term refers to the process by which skin sensitization hazard information is combined with exposure data to determine the likelihood of an exposure resulting in the induction of contact allergy and is thus the risk quotient of the induction of contact allergy and the exposure (both in µg/cm2).

***Risk management**:* this refers to the actions taken to control exposure to a skin sensitizer where the risk assessment indicates that the development of contact allergy would otherwise be likely to occur.

***Atopic**:* a genetic disposition to develop an allergic reaction (allergic rhinitis, asthma, or atopic dermatitis) associated with elevated levels of IgE to an environmental antigen and especially one inhaled or ingested. (Note that this allergy mechanism is wholly different from that associated with the development of contact allergy.)

The primary aim of safety assessment must be to avoid the induction of contact allergy by skin sensitizers and it is to this end that the quantitative risk assessment approach discussed herein is directed. In some cases, it may also be necessary to identify safe exposure levels for sensitized individuals and ensure the implementation of adequate risk management control. This latter aspect falls outside the scope of this current review, which is directed wholly to that part of the risk assessment whose aim is to establish levels of exposure which are anticipated not to cause the primary induction of contact allergy. More important has been the development of a risk assessment strategy whose aim is to predict maximum safe exposure levels (with respect to the induction of contact allergy) using a transparent quantitative approach (Api et al, 2008). In the present material, a number of these key aspects are revisited and critically reviewed and, where appropriate, proposals are made for refinement of the QRA process. As a note of caution, it nevertheless remains the case, as with risk assessment for other toxicological endpoints, that a considerable amount of expert judgement is required for the interpretation and practical application of the information. It is for that reason that three SAFs must be applied:

1. The Inter-individual SAF is applied to account for biological variability between individuals in the population at risk.
2. The Matrix SAF is applied to account for the influence of product formulation.

3. The Use SAF is applied to account for differences in normal use of the product, taking into account body areas of skin to which the product is applied and the frequency and duration of product use.

Normally, no inter-species factor is required since the NESIL is predicated on confirmatory studies in humans, or is based on an extrapolation from an in vivo murine threshold which can directly be used to predict the human NESIL (Griem et al, 2003; Basketter et al, 2005; Api et al, 2008; Safford, 2008; Safford et al, 2011; Api et al, 2014). Where there is specific knowledge of an important difference between human susceptibility and that associated with the test system used to generate a NESIL, then this should be taken into account on a case by case basis. This aspect is not addressed in this document.

Rationale for the Scientific Review

The aim of the QRA process, as with risk assessments for other toxicological endpoints, is to take data on the sensitisation potency of a chemical in an experimental situation and extrapolate this to consumer exposure in an in-use situation and thereby define a safe exposure level. The QRA is founded on the principle that induction of skin sensitization is threshold based (Kimber et al, 1999, 2008; Boukhman and Maibach, 2001; Basketter et al, 2002). That is, there is a level of dermal exposure to a skin sensitizer at, or below which, sensitization induction will not occur in an individual. This is consistent with the principles used for assessing many other non-genotoxic endpoints. Therefore, in a similar manner to other repeated dose toxicology endpoints, skin sensitisation risk assessment is conducted by applying uncertainty factors (SAFs) to the experimentally derived NESIL to account for areas of uncertainty and extrapolation from the experimental to real life conditions to derive an Acceptable Exposure Level (AEL) in the in use exposure situation. In setting the values of the SAFs, it is necessary to consider those aspects which may influence the degree of sensitisation, and also to consider how these aspects differ between the experimental situation and consumer exposure.

As previously mentioned, the NESIL is normally based on a weight of evidence decision, including a confirmatory HRIPT, thus no inter-species extrapolation is required. Whilst it is recognised that QRAs may be conducted based on results from animal experiments alone, it is appropriate here to consider interspecies factors since the key animal data, the LLNA EC3 value, has been correlated directly with human experimental induction threshold data, which therefore has any interspecies variation implicitly built into it (Ryan et al, 2000; Griem et al, 2003; Basketter et al, 2005; Basketter and McFadden, 2012; Api et al, 2014). This review is therefore concerned with other factors which may have an impact on skin sensitisation. As a starting point, a comparison is made of the conditions of the HRIPT and in use exposure to allergens. This is shown in Table 1. From this comparison a number of factors can be identified which are likely to be important in defining SAFs. These are:

1. Inter-individual variability
2. Site of exposure
3. Skin condition at site of exposure
4. Solvent/Matrix
5. Occlusion
6. Frequency and duration of exposure

The reader is reminded that in the material above, the mention of exposure variables is solely done in the context of understanding how to accommodate differences between the HRIPT exposure scenario and consumer use in order to define SAFs. Actual consumer use of products, and thus allergen exposure dose information expressed in $\mu g/cm^2$ is taken up later in the risk assessment process.

Table 1 Experimental and in use exposures compared

Experimental (HRIPT)	In use exposure
Studies are usually conducted in 100 subjects selected from a healthy adult population (including both genders)	Consumers may encompass the whole population, including a range of age, gender and ethnic origin
Subjects are chosen to exclude any with skin disorders, compromised skin (at the patch site) or any other major illness	Subjects include those with skin disorders, compromised/inflamed skin and other forms of illness that may affect skin sensitisation
Patches are normally applied to the arm or back of individuals	Products may be used all over the body, including areas of particular susceptibility
The chemical is applied in a simple solvent system (e.g. diethyl phthalate/ethanol, petrolatum)	The chemical is in a product matrix that may include ingredients that cause mild irritation/dryness or enhance skin penetration. In addition the physical state of the products will vary (liquid, cream/lotion, solid)
Exposure is under full occlusion	Exposure may be non-occluded, or at worst semi-occluded (under clothing or underarms)
Exposure is for 24/48 hours under occlusion three times per week.	Exposure is generally intermittent, especially in the case of rinse-off products. More continuous exposure may occur with some leave-on products
The exposure period is limited to 3 weeks	Exposure may be limited or occur over extended periods of time (months or years)

Review of evidence for defining inter-individual variability

As with all physiological processes, there exists a large degree of variability in the sensitization induction thresholds within the general population. So some individuals may develop contact allergy following exposure to relatively low levels of a sensitizer, whereas others tolerate much higher exposures without induction. It is also possible that the sensitization threshold of an individual will change over time, perhaps linked to their physiological and/or health status. In this respect, it is also pertinent to consider whether there exist particularly sensitive subpopulations which will require special consideration within any risk assessment and any subsequent risk management. In the context of skin sensitization therefore, two specific questions have to be addressed: what is understood regarding the spectrum of susceptibility to the induction of contact allergy arising from exposure to skin sensitizing chemicals, and whether within this spectrum there is a particular subset of individuals who are especially at risk and how best to manage this risk.

There are potentially three sources of information which can contribute to our appreciation of the spectrum of susceptibility to the induction of contact allergy. The first of these is formed by experimental studies carried out in naive subjects where there has been some investigation of induction dose response relationship. In the original development of the human maximisation test (HMT) a small number of skin sensitizers was tested at different induction doses to assess the protocol. The results, expressed as the % sensitized, showed that a 1000 fold span in concentration for several skin sensitizers was sufficient to cover the majority of the induction range – see Table 2 (adapted from Kligman, 1966a and 1966b). Note that the typical group size is 25 individuals, whose skin had been subjected to a moderate inflammatory stimulus 24 hours prior to treatment with contact allergen, which might produce a "frame shift", but is unlikely to narrow the range of susceptibility. As indicated, Penicillin G used 0.2% as the lowest test concentration and might be anticipated to be, just, positive also at 0.1%; it is a moot point whether testing at 100% would have sensitised all individuals in the HMT protocol, but the shape of the curve suggests that would be the case (graph not shown). A few weaker allergens tested produced only a modest degree of sensitization

induction, even at an induction concentration exceeding 25%, which means that they cannot make a clear contribution to the picture concerning human variability, and hence they have not been included in Table 2. Nevertheless, at least in the context of the HMT protocol, it seems likely that the spectrum of induction susceptibility in healthy humans would be accommodated within a range of approximately 3 orders of magnitude. When reviewing this data, it must be kept in mind that the extent to which the individuals in these studies had previously been exposed to these sensitizing chemicals is unknown. Of course had they already been substantially sensitized prior to the study, then their early reaction during the experiment would have become evident, but no notes to this effect appear in the publication.

Table 2 Induction dose response data from the HMT

Substance	**Induction concentration (%)**				
	0.1	**1.0**	**5.0**	**10**	**25**
Furacin	0%[1]	12%	ND[2]	28%	62%
Monobenzyl ether of hydroquinone	12%	30%	ND	64%	99%
Neomycin	ND	0%	ND	16%	31%
p-Phenylenediamine	21%	68%	ND	100%	97%
Penicillin G	9%[1]	18%	28%	44%	59%
Streptomycin	4%	36%	ND	78%	87%
Technical Malathion	4%	32%	ND	100%	100%
Tetrachlorosalicylanilide	24%	68%	86%	98%	ND[2]
Thephorin	9%	25%	ND	44%	100%

[1]Induction concentration was actually 0.2% for this substance; [2]ND = not done

In a second, smaller, series of experimental studies, the HRIPT was deployed to investigate induction dose response relationships (Marzulli and Maibach, 1974). The results generally do not cover the majority of the induction dose response range, but the most useful information available is given in Table 3. Only the results from p-phenylenediamine are really informative, covering a good portion of the induction dose response curve; they are also reasonably consistent with the conclusion from the HMT,

that a 1000 fold concentration range for induction would be likely to cover the majority of induction variability in humans. Other HRIPT dose response data have been published, but cover only a limited range of doses, used small test groups and generated very low levels of response, such that they cannot contribute meaningfully to the present analysis (e.g. Weaver, 1983).

Table 3 Induction dose response data from the HRIPT

Substance	Induction concentration (%)						
	0.01	**0.1**	**1.0**	**2.0**	**5.0**	**10**	**20**
Benzocaine	ND	ND	ND	0%	ND	1%	6%
Bronopol	ND	ND	ND	0%	12%	ND	ND
Formalin	ND	0%	5%	6%[1]	ND	8%	ND
Glutaraldehyde	ND	0%	ND	ND	23%	ND	ND
p-Phenylenediamine	7%	11%	53%	ND	ND	ND	ND

[1]Induction concentration was actually 3% for this substance

With respect to both Tables 2 and 3 above, it is worth noting that there is variability in human testing, particularly when the work is done with relatively small groups, such as in the HMT, where normally n = 25. A series of HMTs on citral tested at 5% in petrolatum gave responses ranging from 32% to 64% of test subjects positive; overall 61/124 (49%) were sensitized at this dose (Lalko and Api, 2008). With a 4% citral induction dose, positive responses ranged from 12% to 36%; overall 29/150 (20%) were sensitized. Single data points at 2% citral induction dose, where just 8% were sensitized and at 8% citral, where 33% were sensitized, completed the HMT dose response. Also, one should be aware that in some of the human testing, the challenge (elicitation) dose used remained the same as that for induction, an important error since it means that the true number sensitized may have been greater. Strictly speaking, the maximum non-irritant concentration should always be used for elicitation phase, at least for the first challenge, since the aim of the work was to examine the extent to which skin sensitization has been induced.

In addition to the above datasets, it is also appropriate to mention the work conducted by Friedmann and colleagues (reviewed in Friedmann, 2007). Of particular note is that an experimental study of induction dose response relationships using 2,4-dinitrochloro-benzene (DNCB). The evidence here has the benefit that one can be confident that the individuals had not had prior exposure to the chemical and were therefore immunologically naïve at the start of the study. With that in mind, whereas a single induction dose of 62.5 μg/cm^2 induced sensitization in only 8% of those exposed, an induction dose of 1000 μg/cm^2 sensitized 100% of those exposed, suggesting, at least for this potent allergen, that the range of susceptibility might be a little more limited than that found with weaker sensitizers. Indeed, it might be reasonable to generalise from this that for weaker allergens, the range of inter-individual susceptibility to the induction of skin sensitization is potentially wider than for the strongest of allergens, although the HMT for citral mentioned above is not entirely consistent with such a concept.

Another potential source of information concerning the spectrum of human susceptibility to the induction of contact allergy derives, in theory at least, from the more general clinical data associated with the investigation and diagnosis of allergic contact dermatitis. However this information is almost always too complex to interpret, not least since most aspects of the exposure(s) responsible for the induction of allergic contact dermatitis is (are) at best poorly defined. Consequently the data generally serve only to illustrate that humans are heterogeneous with respect to the induction of contact allergy. To give just a couple of relatively simple examples, it is well known that hair dyes are a significant cause of allergic contact dermatitis, yet at the same time it is evident that the large majority of exposed individuals develop neither contact allergy nor do they express allergic contact dermatitis (Basketter et al., 2011; Krasteva et al., 2011). Similarly, it is evident in many occupational settings that it is often only a minority of the workers exposed to a variety of skin sensitizing chemicals (e.g. epoxy resins, acrylates, rubber chemicals etc.) that develop allergic skin disease, typically hand eczema (reviewed in Kanerva et al, 2000). However, it is not possible from this information to place a quantitative figure on the individual differences, except to be aware that they exist. It should be noted that examples also exist where, when exposure

was high and involved a potent occupational allergen, then a substantial proportion of workers have be shown to be sensitised (e.g. Rasmussen et al, 2005).

A corollary to inter-individual variation in the susceptibility to the induction of contact allergy in humans is whether there exist specific sensitive subpopulations. In general terms, there is little evidence that basic factors, such as age, gender and ethnic origin play a significant role (reviewed in Rietschel and Fowler, 2008). Frequently, apparent differences, are attributable to variations in exposure patterns, e.g. use of hair dye in different groups, specific product use patterns in children versus adults and so on. A key difficulty lies in the reality that only a little controlled experimental data exists: studies using the HMT and 5 different allergens induced allergy in 62% of the Caucasian panel compared to 45% of the African American panel, a modest difference (Kligman, 1966b). In reality, the most important factor in the acquisition of contact allergy and the expression of ACD is the degree of exposure to the skin sensitizing chemical (Modjtahedi et al, 2004; Bryld et al, 2004; Schnuch et al, 2011). However, where the immune system is not yet fully functional, e.g. during the first months of life, it is much harder to induce contact allergy (Epstein, 1961; Cassimos et al, 1980). Also, in later life, there is some evidence that induction may become easier (recently reviewed in McFadden et al, 2013). However, this remains controversial. Studies using DNCB show that responsiveness does not diminish until after about 80 years of age (Friedmann and Pickard, 2010). It seems then that age, as with ethnicity and gender, is not an important source of variability.

Some experimental evidence has also suggested females are a little more susceptible than males (Rees et al, 1989), whereas in another study, a greater susceptibility in males was detected (Morrissey et al., 2008). Although data on the elicitation of previously acquired allergies is confounded by other factors such as exposure, it is interesting to note that in an extensive study on sensitivity to Peru balsam in patients there was only a small increase in the prevalence ratio to females (1.13 (95%CI: 1.06-1.20) (Uter et al., 2002). All in all, the weight of evidence supports the view that females and males react similarly to contact allergens (Robinson, 1999; Felter et al., 2002).

The aspects covered in the material previously detailed concerning inter-individual variability (age, gender, skin site/condition, the presence of inflammation etc) do not really address what is meant by specifically sensitive subpopulations, which will be tackled in the material that follows. Notably, studies designed to investigate whether there are any genotypes associated with contact allergy have failed to identify more than a few modest indicators of susceptibility (reviewed in Schnuch et al, 2011; Friedmann et al, 2015). In addition, studies on the susceptibility of individuals with atopic eczema to the development of contact allergy generally suggest that this population does not overexpress ACD; some work shows that atopics are less susceptible (Rees et al, 1990), other analysis indicates a slightly elevated susceptibility (Thyssen et al, 2012). Atopics and non-atopics have been shown to possess an almost identical frequency of contact allergy to fragrances (Buckley et al, 2008). In terms of the prevalence in patients who are allergic to Peru balsam, the preponderance of those who had present or past atopy is only at an odds ratio of 1.02 (95%CI: 0.95-1.10 (Uter et al., 2002). Generally, there is a body of conflicting data, suggesting that the effect of an atopic diathesis on the induction of contact allergy is unremarkable. A similar, but less well investigated, picture could be demonstrated for psoriatics, who may in fact be less susceptible (Barile et al, 1996; Bangsgaard et al, 2009). Indeed, generally speaking, it is evident that patients with autoimmune disease are less susceptible to sensitization (Bangsgaard et al, 2011).

Active sensitization induction experiments in humans that already have multiple contact allergies (and are therefore assumed to represent the more sensitive sub-population) have produced inconsistent data, but which, taken overall, indicate that multi-sensitized individuals appear to be somewhat more susceptible, to an extent that could be accommodated by a 3-fold safety factor (Friedmann, 1985; Bangsgaard et al, 2010). Notably, these results from this work also suggest that those with multiple unrelated sensitisations become more sensitised and so have a lower elicitation threshold. Such observations are consistent with the unreported clinical experience that it is generally only those with multiple sensitisations that are likely to be positive to the weakest contact allergens in diagnostic patch test (Klaus Andersen, personal communication).

Lastly, one other indicator that is positively associated with the development of contact allergy is an individual's susceptibility to irritant induced inflammation (Smith et al, 2000; Nagtegaal et al, 2012). Unfortunately, these studies do not quantify the effect, but are consistent with it being less than an order of magnitude.

There may be an enhanced predisposition to sensitisation caused by lesions to which fragranced medication will be applied. The classic case involves medicaments applied directly to stasis leg ulcers (e.g. Fraki et al, 1979). Rather than include such unusual cases as examples of inter-individual variability, a more appropriate approach might be to consider such products in a unique category to which are applied appropriately increased assessment factors to account for this highly specific situation.

Previously, the European Commission's Scientific Committee on Consumer Safety stated "Very little is known about susceptible groups of the population…" (SCCS, 2012). In contrast, notwithstanding the need to recognise that the information available is not altogether definitive, it does nevertheless point to a range of human susceptibility to the induction of contact allergy which spans at least 3, and more probably closer to 4, orders of magnitude. What cannot be done, however, is to identify for a specific contact allergen who the most susceptible individuals might be, and the evidence suggests that this population may well differ from allergen to allergen. Furthermore, it is important to understand that the inter-individual SAF is not intended to represent the total variability of sensitization threshold values for the entire population. As noted above, there will be extremes, such as stasis ulcer skin sites. The distribution of sensitization threshold values higher than the NESIL will have little, if any, influence on the QRA. If it were possible to conduct an HRIPT on the entire population, then the NESIL would represent a true lowest threshold value. However, since the studies are predicated on a small sample of the population, it is necessary to consider the likelihood that there are subjects in the population who have a threshold lower than the most sensitive subjects in the HRIPT. An appropriate inter-individual SAF must therefore be applied to extrapolate from the experimental situation to the wider population.

Overall, the information available suggests that, at least for practical purposes, a specific and especially sensitive subpopulation cannot be identified that exists outside of the framework of inter-individual variability already accommodated in the QRA discussions above. Hypothetically, it could be argued from a very recent study that women over the age of 34 suffering from atopic eczema and who have a history of foot/axillary dermatitis are likely to be at somewhat greater risk than the general population (Schwitulla et al, 2013). However, it is not clear whether this observation represents a true (patho)physiological difference in this gender/age group with an underlying genetic basis, or whether the result is confounded by increased opportunities for exposures and (self-)selection bias. Again, it is emphasised that these do not really represent what is meant by specifically sensitive subpopulations and that this type of susceptibility is incorporated routinely into all general consumer risk assessments for skin sensitization via the QRA SAFs.

Review of evidence on the influence of skin site

One important question is whether certain skin sites have an intrinsically lower or higher capacity for the induction of sensitization. It has been reported that the skin of the soles of the feet and the palms of the hands have a reduced number of dendritic cells (Horton et al, 1984). This observation correlates with the clinical impression that these areas are less prone to sensitization, or at least to the expression thereof (Kligman, 1966b). Interestingly, this at first sight appears to contrast with the clinical experience suggesting foot dermatitis as a risk factor (Schwitulla et al, 2013), but of course the sole of the foot is only rarely involved in the clinical expression of ACD. The limited data that exists from guinea pig studies also indicates that skin site per se does not have a dramatic effect on the induction of allergic reactions (Magnusson and Kligman, 1970).

There is certainly evidence that the frequency of ACD varies from site to site. Fifty years ago, Kligman noted that ACD was rare on the palms, soles and scalp and most common in the eyelids, axillae and scrotum. However, this may not be an indication of susceptibility in these particular sites, but rather a reflection of the locations to which

allergens are applied. Reports of site differences for elicitation/ACD include publications citing the axillae (Johansen, et al., 1997, Nardelli et al., 2008), mucosæ (Farage et al., 2003), face (Johansen et al., 1998) and hands (Heydorn et al., 2003, 2003a). Also in relation to elicitation, the upper arm has been found to be more sensitive than the forehead and lower arm in use tests (Hannuksela, 1991). Furthermore, an extensive study on patients sensitive to Peru balsam gave the following prevalence ratios compared to the trunk: hands or arms: 1.03 (95% CI: 0.94-1.12), foot or leg: 1.76 (95% CI: 1.61-1.92), head or neck: 0.94 (95% CI: 0.86-1.03) and "other" sites (including face): 0.72 (95% CI: 0.64-0.81) (Uter et al., 2002). This would seem to indicate that even for elicitation, there is not a clear and consistent difference.

Taken together, the available information suggests that skin sites may vary somewhat in terms of susceptibility for induction, but quantitative data is largely absent.

Review of evidence on the influence of skin condition

A further potential source of variation in skin sensitisation susceptibility could be the matter of a compromised skin barrier and/or the presence of dermal inflammation. However, these aspects have not to our knowledge been the subject of substantive induction dose response investigations, i.e. in pharmacological terms, measuring how much the dose response is shifted to the left. Indeed the material that exists is generally in the form of a single dose with an assessment of the impact on induction. The largest study in humans again is that of Kligman (1966a). The (rather surprising) results showed that tape stripping of skin to the glistening layer to produce a fully compromised skin barrier, or UV induced inflammation, had only a minor positive effect on induction of sensitization (8% sensitised versus 2%). In contrast, skin freezing or pre-treatment with either 5% sodium lauryl sulphate (SLS) or undiluted dimethylsulphoxide for 24 hours under occlusion produced a distinctly heightened response to all of the 4 contact allergens tested (39% versus 2%). Data from the guinea pig also suggests that physical skin damage was much less important than the presence

of irritation/inflammation as a means to enhance sensitization induction (Magnusson and Kligman, 1970).

In addition to the above, it is interesting to note that intradermal injection of skin sensitizing chemicals in humans appeared to be a particularly ***ineffective*** route to the induction of contact allergy (Kligman, 1966a), again suggesting that at least for low molecular weight substances, it is easy to overestimate the importance of barrier function, and perhaps to confound it with the influence of the often concomitant inflammation and/or disease state, which may also have a modulatory effect on sensitization induction.

Indirect information on the importance of irritant inflammation can be taken from studies on the elicitation of ACD. Studies in nickel sensitive individuals would be consistent with an order of magnitude shift lower in the elicitation dose response profile (Allenby and Basketter, 1993; Menné and Calvin, 1993). This occurs in the absence of an impact on barrier function (Agner et al, 2002). A little less than an order of magnitude shift in response to concomitant irritancy was noted in ACD elicitation studies completed with the preservative methyldibromo glutaronitrile (Pedersen et al, 2004).

Taken together, the data suggest that the effect of quite distinct skin inflammation, though hard to predict, might reasonably be accommodated within a 10 fold increase in susceptibility, but it has to be recognised that this is very much a matter of judgement.

Review of evidence on the influence of solvent/matrix

Another factor that must be considered in toxicological risk assessment, including QRA, is how the vehicle matrix in which human exposure occurs might impact the expression of the intrinsic hazard, in this case skin sensitization, of the substance under consideration. For skin sensitizing chemicals, the investigation of their properties especially their potency, is likely to have been conducted in a relatively uncomplicated

vehicle. The physicochemical properties of this vehicle and hence the delivery of the substance into the skin may differ markedly from real life human exposure to the substance in complex product formulations. Other substances that may be present in the matrix of cosmetics or other consumer products are known to affect the penetration of other chemicals through the stratum corneum (Schaefer and Redelmeier, 1996, Scheuplein and Ross, 1970, Cumberbatch et al., 1993, Heydorn et al., 2003, Hachem et al., 2006). Consequently, the consideration of matrix effects encompasses extrapolation from the vehicle used to determine the induction threshold in the experimental situation to the product formulation containing the ingredient to which the consumer is exposed. The larger the difference between the experimental situation and real life exposure, the greater the SAF might need to be. Accordingly, it is necessary in the risk assessment to take account of such differences. As originally set forth in the QRA approach for skin sensitization, it was possible to apply an uncertainty factor which ranged from 1 to 10 to account for matrix differences. However it is worth re-examining the information on which this is based to come to a judgement of whether in reality this accounts appropriately for the degree of uncertainty.

Firstly, it is necessary to recognise that the effect of vehicles on the skin penetration of substances may not be a useful, or even misleading, indicator of the impact on skin sensitization, since the former measures what goes through the skin whereas the latter depends on what remains (and reacts) within the skin. For example, barrier disruption was demonstrated to have a profound effect on the skin penetration of salicylic acid (>100 fold enhancement) (Benfeldt et al, 1999). However, as had already been demonstrated, even removal of the stratum corneum down to the glistening layer generated only a very small increase in the frequency of the induction of contact allergy (Kligman, 1966b). The impact on the nickel allergy elicitation dose response occurred in the absence of an effect on the barrier (Agner et al, 2002). What is critical here is the evidence of the impact on the induction of contact allergy. As far as the authors of this paper are aware, the most substantial investigations have been carried out using the local lymph node assay (LLNA), a tool which permits a quantitative assessment of relative potency. Reviews of the impact of a range of vehicles on expressed potency of a variety of skin sensitizing chemicals using the LLNA suggest little more than 10 fold

impact on EC3 values, the estimated concentration required to induce a threshold positive response in the LLNA, which serves as a measure of relative sensitizing potency (Lalko et al, 2004; Jowsey et al, 2008b). One key conclusion of relevance for risk assessment is that with the range of 15 different vehicles and 18 substances that were studied in the LLNA, it proved impossible to find general trends which would permit an accurate prediction of the effect of the exposure matrix on the expressed potency of a skin sensitizer, although it was noted that predominantly aqueous vehicles led to an underestimation. Such a conclusion was supported also by the earlier work in the guinea pig: "...no vehicle is optimal..." (Magnusson and Kligman, 1970).

Human data on the impact of vehicles on skin sensitizers, particularly quantitative data, are scarce and limited in scope, but are consistent with the above conclusion. For example, the vehicles petrolatum and 95% ethanol differed by a factor of about 3, with the latter the more effective, in their ability to elicit reactions in subjects sensitized either to cinnamal or costus oil (Marzulli and Maibach, 1976) (see Table 4). In contrast, Kligman had reported that across a range of 11 skin sensitizers and 6 vehicles, on balance petrolatum was the most effective for the induction of contact allergy (Kligman, 1966a). However, the vehicle differences were not particularly marked, such that the 3-4 fold range of concentrations tested was able to compensate for the impact of vehicle on the induction of sensitization.

Table 4 Impact of vehicle on the induction of skin sensitization

Allergen	**Response rate**	
	Petrolatum	***95% ethanol***
Cinnamal	0%	2%
Costus oil	8%	25%

As indicated above, the tests used to determine or confirm the NESIL are carried out on simple binary matrices of test material and vehicle which are far from the complexity of most cosmetics and consumer products. These may in fact contain numerous potentially allergenic substances. It is not known if indeed mixtures of allergenic substances will have an "auto-adjuvant" effect of enhancing induction of allergy to one

or more of these. The data we have all relate to elicitation. For instance patch testing of the standard fragrance mixes of several allergenic materials produces more positive reactions than tests carried out on their individual constituents (Schnuch et al., 2002, Frosch et al., 2005). A recent review provides further evidence for the enhancement of elicitation by mixtures of allergens (Martin, 2012). This may simply due to the elicitation-specific need to recruit sufficient numbers of T-cells to trigger the inflammatory response and may be a phenomenon that is not mirrored in the process of induction (Friedmann and Pickard, 2010). Similarly, irritants are known to enhance the elicitation of previously acquired allergies (Smith et al., 2000, 2002, Grabbe et al., 1996, McLelland et al., 1991), but in this case as indicated above, irritants such as SLS appear to immunologically enhance the process of sensitization induction (Kligman, 1966a; Cumberbatch et al., 1993). On the other hand, specific in vivo studies of synergistic effects of allergens applied in combination in the LLNA demonstrated that although induction of sensitisation tended to be more than additive, the effect was not substantially enhanced (Jowsey et al, 2008a).

In summary, it is suggested that the factor taking account of the impact of the exposure matrix should be adapted to allow for the reality that it may reduce as well as enhance expressed skin sensitization potency, when compared to the experimental vehicles used to define intrinsic potency of a skin sensitizer. This would properly accommodate the range of variation observed in the experimental mouse studies.

Review of evidence on the influence of occlusion

A further consideration here also is the extent to which the presence or absence of (semi-)occlusion can impact the induction of contact allergy. Occlusion of the skin increases the hydration of the stratum corneum, skin temperature, microbial count, pH, and can enhance dermal irritation; all of which may influence dermal penetration (Zhai and Maibach, 2001), but does it enhance sensitisation? The published works of Kligman and of Maibach associated with the development of predictive human skin sensitization tests generally did not examine the impact of open versus (semi-)occlusive

exposure on the induction of contact allergy. Studies in the guinea pig however demonstrated that full occlusion and even partial occlusion were generally more effective modes for the induction of contact allergy – see the Table 5 below (adapted from Magnusson and Kligman, 1970):

Table 5 Impact of occlusion on the induction of skin sensitization

Allergen	**Response rate**		
	Non-occlusive	***Semi-occlusive***	***Occlusive***
0.01% DNCB	17%	48%	60%
0.01% NDMA	ND[1]	36%	72%
0.1% NDMA	84%	ND	100%
0.05% PPD	30%	68%	92%

[1]ND = not done

The results in the above table for the induction of skin sensitization to DNCB in human volunteers are entirely consistent with expectations, as are those for PPD. However, those for the now less commonly used skin sensitizer para-nitrosodimethylaniline (NDMA) show no effect of full occlusion, perhaps due to the deployment of a dose level (0.1%) close the plateau of the dose response curve, which thereby produces a substantial effect also in the non-occlusive test group. Overall, it is probably reasonable to conclude that there is at least a 3 fold effect of occlusion on the induction of contact allergy.

Other information can be gleaned from clinical studies on the elicitation of allergic contact dermatitis. For example, a detailed examination of the relationship between the patch test dose response threshold and the repeated open application test (ROAT) was conducted in a group of 27 subjects with contact allergy to isoeugenol (Andersen et al, 2001). Individuals with patch test thresholds from approximately 1.0% down to 0.0005% isoeugenol all failed to respond to a single open application of 0.2% isoeugenol. The mean time to reaction in the ROAT (with twice daily applications) was 7 days. In this context, also it has to be recognised that, at least for some contact

allergens, repeated open exposures may have a lower threshold than single occlusive patch tests (Lundov et al, 2011).

In summary, the available data indicated that the presence or absence of (semi-) occlusion and the location of the skin site, of themselves, have a relatively modest impact on the induction skin sensitization. Certainly, the presence of inflammation and/or barrier damage at the skin site is likely to enhance susceptibility to induction. In combination, this set of variables may still contribute to differences in susceptibility to the induction of skin sensitization.

Review of evidence on the influence of duration and frequency of exposure

There is little scientific evidence that can be used to determine the impact of differing patterns of duration and frequency of exposure to allergens on the induction of skin sensitisation. However, valuable insights also can be gleaned from studies on the elicitation of ACD.

In one study, three separate doses of 10 $\mu g/cm^2$ DNCB applied to the same site at weekly intervals had the same effect as a single dose of 60 $\mu g/cm^2$ (Paramasivan et al, 2010). In another, weekly exposure for 5 minutes to PPD over six months sensitized 7.2% of subjects. When exposure was 30-40 minutes, but only once a month, this was reduced to 1.3% (Basketter et al., 2002). Furthermore, sensitization rates in humans in another study augmented as the number of inductions increased through 3, 5, 10 to 15 times (Kligman, 1966a, 1966b). In animals the same effect is seen. A prolonged LLNA (13 open applications over 57 days) was found to be more effective than 3 applications over 3 days producing an average of a 2.65-fold increase in stimulation indices in 8 separate studies (De Jong et al., 2007). Note that the exposure regime of the HRIPT (nine 24-hour occlusive applications over 19 days (Politano and Api, 2008) is intermediate in the time and frequency to the two extremes used in this LLNA study.

There has been some suggestion that there can be bioaccumulation in which some of the applied dose is trapped in the stratum corneum (e.g. Basketter et al, 2006; White et al, 2007). While this may prolong exposure, it is hard to see how this would increase it particularly as there is turnover of the stratum corneum every 3-4 weeks due to desquamation. Alternatively repeat exposure (as we see in the ROAT) may be priming the immunological response. Also, data from the ROAT (e.g. Lundov et al, 2002) shows that, at least on some occasions, prolonged repeated exposure (1-2 times daily, up to 3 weeks) can be more effective than a single occlusive dose, but it seems reasonable to speculate that similarly frequent occlusive exposures would be much more likely to induce contact allergy. Finally, how the fact that epidermal Langerhans cells (and presumably other relevant dendritic cells) have a half-life in skin of approximately only 2 weeks (Holt et al, 1994) impacts upon these considerations remains an unknown.

Taken together, even working from first principles, it is obvious that greater overall exposure (at a given dose per unit area) will enhance the likelihood of the induction of contact allergy. However, whether the dose accumulates upon repeated exposure to achieve a steady state level is likely to depend upon the allergen as well as the interval between exposures. Accordingly, the pragmatic approach of daily accounting (unless there is evidence to the contrary for a specific skin sensitiser) seems most appropriate.

Conclusions of the scientific review with respect to defining SAF values

From the considerations above, a number of conclusions can be drawn that allow appropriate SAF values to be determined for use in the QRA process, including specific application to fragrance ingredients. In setting these values it is recognised that, given the uncertainty in the supporting data, exact values cannot be derived, and a certain amount of expert judgment is required. Reflecting this, the values are restricted to 1, 3.16 (the half log of 10; for practical purposes the number 3 is used) and 10.

Inter-individual variability SAF

There exists in the population a considerable amount of variability in individual sensitivity to skin sensitisation. This variability is evident from the dose response curves obtained in experimental human studies described above, suggesting 3-4 orders of magnitude variability even in the small populations used in such studies. It is important in conducting a QRA to account for this variability when deriving an AEL. However, it is recognised that, since the NESIL is defined as a dose not inducing sensitisation in an HRIPT, much of this variability is already taken into account. As has already been stated, human variability at doses above the NESIL has no impact on the QRA process. It is necessary then to determine a SAF value that extrapolates from the most sensitive test subjects in the experimental population to the most sensitive subjects in the general population of consumers.

Assessment factors for inter-individual variability are of course used for estimating the risk presented by other thresholded adverse effects such as systemic, developmental and reproductive toxicity. In this area of toxicology, a 10-fold "safety factor" has been used based on a consideration of toxicokinetic and toxicodynamic factors (Renwick and Lazarus, 1998). A more recent re-examination of these has led to a proposal to reduce the safety factor to 5 for the general population and 3 for professional users (ECETOC, 2003, 2010). ECHA continues to recommend a 10-fold factor for the general population, reduced to 5-fold for professionals (ECHA, 2010). It maintains this also for local effects, although no particular justification is provided. There is however, no reason to suppose, a priori, that the immunological processes involved in the acquisition of contact allergy would necessarily demand different safety factors, except that the available human evidence suggests otherwise.

Since the available data are limited to studies involving small number of subjects, and since there are no data to indicate the real variability in the population beyond the extent that is already implicit in the human experimental studies, then it is proposed that a SAF value of 10 be used to account for inter-individual variability.

Skin site SAF

There is little evidence from the scientific literature that particular skin areas of the body are more prone to the induction of skin sensitisation than others. The incidence of ACD observed in clinics may occur to a greater extent on some skin sites compared to others, but these reflect the elicitation of skin sensitisation reactions, and may be due to the pattern of exposure to the allergen, or linked to the presence of irritant contact dermatitis (ICD).

Given the lack of evidence for significant differences in the susceptibility of different skin sites to the induction of skin sensitisation it is proposed that no SAF is required to account for this.

Skin condition SAF

One key parameter for lowering the threshold for the induction of skin sensitisation is that of compromised/inflamed skin. The HRIPT is conducted on uninflamed and intact skin, whilst consumers in the population at large may have compromised/inflamed skin due to a number of factors. There is little evidence to suggest that subjects with diseased skin (e.g. atopic eczema, psoriasis) are more sensitive to skin sensitisers. In addition, there is little evidence that compromising the skin barrier by physical or chemical means increases the potential for the induction of sensitisation. However, the generation of inflammation in skin, particularly from contact with irritant chemicals (such as SLS), may increase sensitivity to skin sensitisers.

In determining a SAF to account for skin condition, skin sites that are more prone to inflammation due to a chemical stimulus (Irritant Contact Dermatitis) or by shaving are considered to be more susceptible to the induction of skin sensitisation. The available data do not suggest that the magnitude of this increased susceptibility is 10-fold, and it is therefore proposed to include a SAF value of 3 to account for this. These sites

include hands, underarms, any shaved area, under a diaper, peri-anal and peri-ocular regions. For other skin sites a SAF value of 1 is proposed.

Matrix SAF

Experimental evidence suggests that the matrix in which the sensitiser is presented to the skin may influence the degree of induction of skin sensitisation. A 10-fold difference in induction between vehicles has been shown in studies using the LLNA, although the studies did not provide data that allow a reliable prediction of the effect of a particular exposure matrix on the expressed potency of a skin sensitizer. That said, it is clear in the study that aqueous vehicles led to an underestimation, and this has also been noted in OECD guideline 429 (OECD, 2010).

In humans, a 4-fold difference in the induction of skin sensitisation was apparent between matrices tested, with ethanol or petrolatum providing the greater degree of sensitisation depending on the study.

In considering the appropriate Matrix SAF, it must be remembered that the most common solvents used in the HRIPTs for fragrance ingredients are DEP/ethanol or petrolatum. These solvents are considered to be optimal for the induction of sensitisation in an experimental situation. Thus, for products based on these or similar solvents, a factor of 1 is considered appropriate to account for the matrix. Although it is possible that sensitisation potential will be reduced in aqueous based products based on observations in the LLNA, it is proposed to maintain a factor of 1 for these products since they are rarely purely aqueous, and will contain other ingredients such as surfactants which help the product wet the skin.

Although no data exist on the effects of applying sensitisers in a solid matrix such as talc or residues on clothing, it is considered likely that this would reduce the likelihood of sensitisation. It can be considered that the allergen itself would migrate from the solid substrate to sweat and sebum on the skin, which would then be the matrix from which

skin penetration would occur. Given the oily nature of sebum it is proposed to use a factor of 1 for such exposures. The controlling factor in the induction of sensitisation is the rate at which the allergen migrates into the sweat/sebum. This should be appropriately factored into the exposure calculation.

Further consideration should also be given to products that contain penetration enhancing ingredients. There is little evidence to suggest that the enhancement of skin bioavailability may promote the induction of skin sensitisation. However, it may be prudent to include a factor of 3 for products that included penetration enhancing ingredients. This would need to be assessed on a case by case basis.

In considering the effect that the product matrix may have on skin sensitisation it is also important to consider the irritation potential of the product. It has already been mentioned under skin condition that inflammation of the skin may increase susceptibility to skin sensitisation. A SAF of 3 is already proposed for areas of skin that may be prone to irritation from product use, and therefore a further SAF is not considered necessary.

Occlusion SAF

From the experimental data it appears that occlusion can enhance skin sensitisation, with a 3-fold difference shown in guinea pigs between non-occlusion and full-occlusion. Consumer use of products may involve a degree of occlusion such as in the axillae and under clothing, and products used under nappies may even be under full occlusion. Also, certain products containing emollients (e.g. oil-in-water or water-in-oil emulsions such as moisturising creams and lotions) may also lead to a degree of occlusion. However, it must be remembered that the HRIPT is conducted under full occlusion, and is therefore an extreme exposure compared with product use. A SAF value of >1 is therefore not appropriate to account for occlusion, and it is proposed that this value remains at 1 for all products and body sites since at some time all body parts could be occluded.

Frequency/duration of product use

With regard to the period/frequency of exposure, it is likely that many products will be used on a daily basis over extended periods of time (months, years). The experimental data from an HRIPT involves nine 24 hour exposures over a three week period, and therefore does not account for longer term use. There is limited experimental evidence to show that the induction of skin sensitisation may be increased when the normal dosing regimens of predictive tests are extended over longer times. This may be due to a "reservoir effect" or some immunological mechanism. Although the available data are limited, they do not suggest that a 10-fold increase will occur with repeated long term product use. Accordingly, a frequency/duration SAF value of 3 is proposed to account for products used routinely over extended periods of time. For some products that are used intermittently or over a limited time period, a factor of 1 should be considered.

Application of SAFs in the QRA

Based on all of the above considerations it is proposed to include five SAFs in the QRA process. The values and considerations for their use are given in Table 6 below.

Factor	Consideration	Influence	New proposed SAFs	Comments (comparison of the experimental condition with the product use condition)
Inter-Individual	There can be large inter-individual differences in response to a chemical exposure due to several different parameters	Increase of induction susceptibility	10	The inter-individual variability not accommodated in the NESIL is reflected by a SAF of 10.
Product	Role of vehicle/matrix	Delivery	0.3 or 1 or 3*	The predicted effect of product formulation versus the experimental conditions; 0.3 (inert objects with no direct contact, e.g. candles or detergent pods or no vehicle/matrix) or 1 (most products) or

				3 (penetration enhancers greater than anticipated from the experimental condition)
Frequency / duration of product use	Products may be used over extended periods resulting in bio-accumulation	Increase of induction susceptibility	1 or 3*	Products may be used frequently over extended periods of time resulting in accumulation (chemical or biological accumulation) or reservoir effect
Occlusion	Some areas of skin are semi-occluded by clothing. Products with moisturising agents may lead to semi-occlusion. Includes occlusion by body part, clothing or product.	Increase of induction susceptibility	1	To assure consistency, it was concluded that the occlusion factor should be 1 for all consumer products since at some time all body parts could be covered by clothing.
Skin condition/ site	Pre-existing inflammation, potential inflammation from product	Increase of induction susceptibility	1 or 3*	Pre-existing inflammation for body site: body areas that are specifically prone to increased level of inflammation – hands, underarms, any shaved area, under a diaper, peri-anal and peri-ocular regions

*Note: for practical purposes the number 3 is the representation of 3.16 (half log of 10)

With the exception of inter-individual variability, the SAFs are applied according to the product type (formulation and intended\reasonably foreseeable use). The proposed values are considered to be default values, and applied where other data are lacking. Thus for individual products consideration needs to be given for the formulation and the impact this may have on the induction of sensitisation (to include the potential to induce irritant contact dermatitis, influence on skin penetration and occlusive properties), and the use patterns of the product (to include the skin site of contact, likely use frequency and duration). The default values can be refined where additional data are available.

Summary

The fact that contact allergy to fragrances was perceived as too high prompted a change to risk assessment procedures used by the industry (Api et al, 2008). The absence of a significant impact in the first few years post this change has led to a re-evaluation of the science underpinning the risk assessment. The proposals contained herein are intended to assist further evolution of the assessment process, but should be seen in the context of improvements to exposure assessment (key ref), as well as requiring a pro-active monitoring of the prevalence of contact allergy to fragrances in the affected population.

References

Agner T, Johansen JD, Overgaard L, Vølund A, **Basketter** D, Menné T. Combined effects of irritants and allergens. Synergistic effects of nickel and sodium lauryl sulfate in nickel- sensitized individuals. Contact Dermatitis. 2002 47: 21-26.

Allenby CF and Basketter DA (1993) An arm immersion model of compromised skin. II. Influence on minimal eliciting patch test concentrations of nickel. Contact Dermatitis, 28, 129-133.

Andersen KE, Johansen JD, Bruze M, Frosch PJ, Goossens A, Lepoittevin J-P, Rastogi S, White I, Menné T. The time-dose response relationship for elicitation of contact dermatitis in isoeugenol allergic individuals. Toxicol Appl Pharmacol 2001: 170: 166-171.

Andersen KE and Maibach HI. Contact allergy predictive tests in guinea pigs. Karger, Basel, 1985.

Api, A.M., Basketter, D.A., Cadby, P.A., Cano, M.F., Ellis, G., Gerberick, G.F., Griem, P., McNamee, P.M., Ryan, C.A., Safford, R. (2008). Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. Regul Toxicol Pharmacol. 52: 3-23.

Api AM, Basketter DA and Lalko J (2014) Correlation between experimental human and murine skin sensitization induction thresholds. Cut Ocul Toxicol, 28, 1-5.

Api AM, Belsito D, Bickers D, Bruze M, Calow P, Greim H, Hanifin JM, McNamee PM, Rogers AE, Saurat JH, Sipes IG, Tagami H. Quantitative risk assessment of contact sensitization: clinical data to assess utility of the model. Dermatitis. 2010 21: 207-213.

Bangsgaard N, Carlsen BC, Johansen JD, Menne T, Skov L. (2010) Susceptibility and reactivity in polysensitized individual following controlled induction. Contact Dermatitis 63: 1-14.

Bangsgaard N, Engkilde K, Thyssen JP, Linneberg A, Nielsen NH, Menné T, Skov L,

Johansen JD. Inverse relationship between contact allergy and psoriasis: results from a patient- and a population-based study. Br J Dermatol. 2009 161: 1119-23.

Bangsgaard N, Engkilde K, Menné T, Løvendorf M, Jacobsen GK, Olsen J, Skov L. Impaired hapten sensitization in patients with autoimmune disease. Clin Exp Immunol. 2011 165: 310-317.

Barile M, Cozzani E, Anonide A, Usiglio D, Burroni A, Guarrera M (1996) Is contact allergy rare in psoriatics? Contact Dermatitis 35: 113-114.

Basketter DA (2009) The human repeated insult patch test in the 21st century: a commentary on ethics and validity. Cutaneous and Ocular Toxicology, 28, 49-53.

Basketter DA, Clapp C, Jefferies D, et al. Predictive identification of human skin sensitization thresholds. Contact Derm 2005; 53: 260-267.

Basketter DA, Evans PE, Gerberick GF and Kimber I (2002) Factors affecting thresholds in allergic contact dermatitis: safety and regulatory considerations. Contact Dermatitis, 47, 1-6.

Basketter D A, Jefferies D, Safford B J et al. (2006). The impact of exposure variables on the induction of skin sensitization. Contact Dermatitis 55: 178–185.

Basketter DA, Gerberick GF and Kimber I. (2007) The local lymph node assay EC3 value: status of validation. Contact Dermatitis, 57, 70 - 75.

Basketter DA, Johansen JD, McFadden J and Søsted H (2011) Hair Dyes. In "Contact Dermatitis", 5th edition, chapter 34, Springer, Berlin, Eds Johansen JD, Frosch PF and Lepoittevin J-P, pp 629-642.

Basketter DA and McFadden JP (2012) Cutaneous allergies. In "Immunotoxicity, Immune Dysfunction and Chronic Disease. Eds Dietert RR and Luebke RW., Humana Press, New York, pp 103-126.

Benfeldt E, Serup J, Menné T. Effect of barrier perturbation on cutaneous salicylic acid penetration in human skin: in vivo pharmacokinetics using microdialysis and non-invasive quantification of barrier function. Br J Dermatol. 1999 140: 739-48.

Boukhman MP, Maibach HI. Thresholds in contact sensitization: immunologic mechanisms and experimental evidence in humans--an overview. Food Chem Toxicol. 2001 39: 1125-34.

Bryld LE, Hindsberger C, Kyvik KO, Agner T, Menné T. Genetic factors in nickel allergy evaluated in a population-based female twin sample. J Invest Dermatol. 2004 123: 1025-1029.

Buckley DA, Basketter DA, Kan-King-Yu D, White IR, White JL, McFadden JP. Atopy and contact allergy to fragrance: allergic reactions to the fragrance mix I (the Larsen mix). Contact Dermatitis 2008 59: 220-225.

Cassimos C, Kanakoudi-Tsakalidis F, Spyroglou K, Ladianos M, Tzaphi R. (1980) Skin sensitization to 2, 4 dinitrochlorobenzene (DNCB) in the first months of life. J Clin Lab Immunol. 3: 111-113.

Cumberbatch, M., Scott, R.C., Basketter, D.A., Scholes, E.W., Hilton, J., Dearman, R.J., Kimber, I., 1993. Influence of sodium lauryl sulfate on 2, 4-dinitrochlorobenzene-induced lymph node activation. Toxicology 77, 181–191.

De Jong WH, De Klerk A, Beek MT, Veenman C & Van Loveren H (2007) Effect of Prolonged Repeated Exposure to Formaldehyde Donors with Doses Below the EC3 Value on Draining Lymph Node Responses. Journal of Immunotoxicology 4, 239 - 246.

Demerjian M, Hachem J P, Tschachler E et al. (2008). Acute modulations in permeability barrier function regulate epidermal cornification: role of caspase-14 and the protease-activated receptor type 2. Am J Pathol. 172: 86–97

ECHA, 2010. Guidance on information requirements and chemical safety assessment Chapter R 8 : Characterisation of dose [concentration] response for human health. Guidance on DNEL/DMEL derivation from human data for the implementation of REACH. European Chemicals Agency ECHA, 2012. Practical guide 14: How to prepare toxicological summaries in IUCLID and how to derive DNELs European Chemicals Agency

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), 2003. Derivation of Assessment Factors for Human Health Risk Assessment. Technical Report No. 86, ISSN-0773-6347-86. February, 2003

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals),, 2010. Guidance on assessment factors for deriving a DNEL, Technical Report No. 110, ISSN-0773-8072-110, 2079-1526-110, October 2010

Epstein WL (1961) Contact type delayed hypersensitivity in infants and children: induction of rhus sensitivity. Pediatrics 27: 51-53.

Farage, M.A., Bjerke, D.L., Mahony, C., Blackburn, K.L., Gerberick, G.F., 2003. Quantitative risk assessment for the induction of allergic contact dermatitis: uncertainty factors for mucosal exposures. Contact Dermatitis 49 (3), 140–147.

Felter SP, Robinson MK, Basketter DA and Gerberick GF (2002) A review of the scientific basis for default uncertainty factors for use in quantitative risk assessment of the induction of allergic contact dermatitis. Contact Dermatitis, 47, 257-266.

Felter SP, Ryan CA, Basketter DA and Gerberick GF (2003) Application of the risk assessment paradigm to the induction of allergic contact dermatitis. Regulatory Toxicol Pharmacol, 37, 1 - 10.

Fräki JE, Peltonen L, Hopsu-Havu VK. Allergy to various components of topical preparations in stasis dermatitis and leg ulcer. Contact Dermatitis. 1979 5: 97-100.

Friedmann PS. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. Br J Dermatol. 2007: 157: 1093-1102.

Friedmann PS and Pickard C. (2010). Quantifying human susceptibility to contact sensitization; risk assessments now and in the future. Contact Dermatitis 63: 237–247.

Friedmann PS, Sanchez-Elsner T, **Schnuch A**. Genetic factors in susceptibility to contact sensitivity. Contact Dermatitis. 2015 72: 263-274.

Frosch PJ, Rastogi SC, Pirker C, Andersen KE, Bruze M, Svedman C, Goosens A, White IR, Uter W, Giminez Arnau E, Lepoittevin J-P, Johansen JD and Menné T. Patch testing with a new fragrance mix - reactivity to the individual constituents and chemical detection in relevant cosmetic products. (2005). Contact Dermatitis. 52: 216-225.

Gefeller O, Pfahlberg AB, Uter W. What can be learnt from nothing? - A statistical perspective. Contact Dermatitis. 2013 69: 350-354.

Gerberick GF, Robinson MK, Felter S, White I and Basketter DA. (2001) Understanding fragrance allergy using an exposure-based risk assessment approach. Contact Dermatitis, 45, 333-340.

Gerberick, GF, Ryan CA, Kern PS, Schlatter H, Dearman RJ, Kimber I, Patlewicz G and Basketter DA (2005) Compilation of historical local lymph node assay data for the evaluation of skin sensitization alternatives. Dermatitis, 16, 157 - 202.

Griem P, Goebel C, Scheffler H. Proposal for a risk assessment methodology for skin sensitization based on sensitization potency data. Reg Toxicol Pharmacol 2003; 38: 269-290.

Hachem J P, Houben E, Crumrine D et al. (2006). Serine protease signaling of epidermal permeability barrier homeostasis. J Invest Dermatol. 126: 2074–2086.

Hannuksela M. (1991). Sensitivity of Various Skin Sites in the Repeated Open Application Test. Am J Contact Dermatitis; 2: 102-104.

Heydorn S, Johansen J D, Andersen K E, Bruze M, Svedman C, White I R, Basketter D A, Menne T. (2003). Fragrance allergy in patients with hand eczema - a clinical study. Contact Dermatitis; 48: 317-323

Heydorn S, Menne T, Johansen J D. (2003a). Fragrance allergy and hand eczema – a review. Contact Dermatitis; 48: 59-66.

Heydorn S, Andersen K E, Johansen J D, Menne T. (2003b). A stronger patch test elicitation reaction to the allergen hydroxycitronellal plus the irritant sodium lauryl sulfate. Contact Dermatitis; 49: 133-139.

Holt PG, S Haining, D J Nelson and J D Sedgwick (1994). Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. The Journal of Immunology

Horton JJ, Allen MH, MacDonald DM. An assessment of Langerhans cell quantification in tissue sections. J Am Acad Dermatol. 1984: 11: 591-593.

Hostynek JJ, Maibach HI. Thresholds of elicitation depend on induction conditions. Could low level exposure induce sub-clinical allergic states that are only elicited under the severe conditions of clinical diagnosis? Food Chem Toxicol. 2004 Nov;42(11):1859-65.

Grabbe S, Steinert M, Mahnke K, Schwartz A, Luger T A, Schwarz T. (1996). Dissection of antigenic and irritative effects of epicutaneously applied haptens in mice. Evidence that not the antigenic component but nonspecific proinflammatory effects of haptens determine the concentration-dependent elicitation of allergic contact dermatitis. J Clin Invest. 98: 1158–1164.

Johansen JD, Frosch PJ, Lepoittevin J-P. Contact Dermatitis, 5th edition. Springer-Verlag, Heidelberg, 2011.

Johansen J D, Andersen T F, Veien N, Avnstorp C, Andersen K E, Menne T. (1997). Patch testing with markers of fragrance contact allergy. Do clinical tests correspond to patients' self-reported problems? *Acta Derm Venereol.;*77: 149-153

Johansen J D, Andersen T F, Kjoller M, Veien N, Avnstorp C, Andersen K E, Menne T. (1998). Identification of risk products for fragrance contact allergy: a case-referent study based on patients' histories. Am J Contact Dermat. 9: 80-86.

Jowsey IR, Clapp CJ, Safford B, Gibbons BT and Basketter DA. (2008) The impact of vehicle on the relative potency of skin sensitizing chemicals in the local lymph node assay. Food and Chemical Toxicology, 27, 67 - 75.

Kanerva L Elsner P, Wahlberg JE and Maibach HI. Handbook of Occupational Contact Dermatitis, Springer Verlag, Berlin, 2000.

Kern PS, Gerberick GF, Ryan CA, Kimber I, Aptula A and Basketter DA (2010) Historical local lymph node data for the evaluation of skin sensitization alternatives: a second compilation. Dermatitis, 21, 8-32.

Kimber I, Dearman R J, Basketter D A, Ryan C A, Gerberick G F, Lalko J and Api A M. (2008) Dose metrics in the acquisition of skin sensitization: thresholds and

importance of dose per unit area. Regulatory Toxicology and Pharmacology, 52, 39 - 45.

Kimber I and Basketter DA (1992) The murine local lymph node assay; collaborative studies and new directions: A commentary. Food Chem Toxicol 30, 165-169.

Kimber I, Gerberick GF and Basketter DA. (1999) Thresholds in contact sensitization: Theoretical considerations and practical applications. Food and Chem Toxicol., 37, 553-560.

Kligman AM. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. J Invest Dermatol 1966a: 47: 375-392.

Kligman AM. The identification of contact allergens by human assay. II. The maximization test: a procedure for screening and rating contact sensitizers. J Invest Dermatol 1966b: 393-409.

Krasteva M, Bons B, Ryan C, Gerberick GF. Consumer allergy to oxidative hair coloring products: epidemiologic data in the literature. Dermatitis. 2009: 20: 123-141.

Lalko J and Api AM. Citral: identifying a threshold for induction of dermal sensitization. Regul Toxicol Pharmacol, 2008: 52: 62-73

Lalko J, Isola D, Api AM. Ethanol and diethylphthalate: vehicle effects in the local lymph node assay. Int J Toxicol 2004: 23; 171-177.

Lundov MD, Zachariae C, Johansen JD. Methylisothiazolinone contact allergy and dose-response relationships. Contact Dermatitis. 2011 64: 330-336.

Magnusson B and Kligman A (1970) Allergic Contact Dermatitis in the Guinea Pig. Charles C Thomas, Springfield, Illinois.

Mann J, McFadden JP, White JM, White IR and Banerjee P. (2014) Baseline series fragrance markers fail to predict contact allergy. Contact Dermatitis 70, 276-281.

Martin S. (2012) Contact dermatitis: from pathomechanisms to immunotoxicology. Experimental Dermatology, 21: 382–389

Marzulli FN, Maibach HI. The use of graded concentration in studying skin sensitizers: experimental contact sensitization in man. Food Cosmet Toxicol 1974: 12: 219-227.

Marzulli FN, Maibach HI. Effects of vehicles and elicitation concentration in contact dermatitis testing. Contact Dermatitis, 2: 325-329.

McFadden JP, White IR, Basketter DA, Puangpet P and Kimber I (2013) The cosmetic allergy conundrum. Contact Dermatitis, 69, 129-137.

McLelland J, Shuster S, Matthews J N. (1991). 'Irritants' increase the response to an allergen in allergic contact dermatitis. Arch Dermatol. 127: 1016–1019.

Menné T, Calvin G. Concentration threshold of non-occluded nickel exposure in nickel-sensitive individuals and controls with and without surfactant. Contact Dermatitis. 1993 29: 180-184.

Modjtahedi BS, Modjtahedi SP, Maibach HI. The sex of the individual as a factor in allergic contact dermatitis. Contact Dermatitis. 2004 50: 53-59.

Morrissey K, Xue W, Cooper K, Baron E. Age and gender effects on contact sensitization and photoimmune suppression in young and middle-aged adults. Photodermatol Photoimmunol Photomed 2008: 24: 46–48.

Moss C, Friedmann PS, Shuster S, Simpson JM (1985) Susceptibility and amplification of sensitivity in contact dermatitis. C;om Exp Immunol 61; 232-241.

Nagtegaal MJ, Pentinga SE, Kuik J, Kezic S, Rustemeyer T. The role of the skin

irritation response in polysensitization to fragrances. Contact Dermatitis. 2012 67: 28-35.

Naldi L, Cazzaniga S, Gonçalo M, Diepgen T, Bruze M, Elsner P, Coenraads PJ, Svensson Å, Bertuccio P, Ofenloch R; EDEN Fragrance Study Group. Prevalence of self-reported skin complaints and avoidance of common daily life consumer products in selected European Regions. JAMA Dermatol. 2014 150: 154-163.

Nardelli A, Carbonez A, Ottoy W, Drieghe J, Goossens A. (2008). Frequency of and trends in fragrance allergy over a 15-year period. Contact Dermatitis; 58: 134-141.

Newell L, Polak M, Pickard C, Owen C, Holloway J, Healy E, Ardern-Jones M R, Friedmann P S. Impaired allergic contact dermatitis response to DNCB in atopic dermatitis (AD) is enhanced by filaggrin loss of function variants. J Invest Dermatol 2009: 129: S8–S8.

OECD., (2010). Organisation for Economic Cooperation and Development. Test Guideline 429: The Local Lymph Node Assay. Paris, France.

Paramasivan P, Lai C, Pickard C, Ardern-Jones M R, Healy E, Friedmann P S. (2010). Repeated low-dose skin exposure is an effective sensitising stimulus: a factor to be taken into account in predicting sensitisation risk. Br J Dermatol. 162: 594–597.

Pedersen LK, Haslund P, Johansen JD, Held E, Vølund A, Agner T. Influence of a detergent on skin response to methyldibromo glutaronitrile in sensitized individuals. Contact Dermatitis. 2004 50: 1-5.

Politano VT and Api AM (2008). The Research Institute for Fragrance Materials human repeated insult patch test protocol. Regulatory Toxicology and Pharmacology 52: 35-38.

Rasmussen K, Carstensen O, Pontén A, Gruvberger B, Isaksson M, Bruze M. Risk of contact allergy and dermatitis at a wind turbine plant using epoxy resin-based plastics. Int Arch Occup Environ Health. 2005 Apr;78(3):211-7.

Renwick AG, Lazarus NR (1998) Human variability and non cancer risk assessment – an analysis of default uncertainty factors. Regul. Toxicol, Pharmacol. 27: 3-20.

Rees JL, Friedmann PS, Matthews JN. Sex differences in susceptibility to development of contact hypersensitivity to dinitrochlorobenzene (DNCB). Br J Dermatol. 1989 120: 371-374.

Rees J, Friedmann PS, Matthews JN. Contact sensitivity to dinitrochlorobenzene is impaired in atopic subjects. Controversy revisited. Arch Dermatol. 1990 126: 1173-1175.

Rietschel RL, Fowler JF. Contact Dermatitis, 6th edition. BC Decker, Hamilton, 2008.

Robinson, M.K., 1999. Population differences skin structure and physiology and the susceptibility to irritant and allergic contact dermatitis: implications for skin safety testing and risk assessment. Contact Dermatitis 41, 65–79

Rossi M, Coenraads PJ, Diepgen T, Svensson Å, Elsner P, Gonçalo M, Bruze M, Naldi L.(2010). Design and feasibility of an international study assessing the prevalence of contact allergy to fragrances in the general population: the European Dermato-Epidemiology Network Fragrance Study. Dermatology. 221: 267-75.

Ryan CA, Gerberick GF, Cruse LW, Basketter DA, Lea LJ, Blaikie L, Dearman RJ, Warbrick EV and Kimber I (2000) Activity of human contact allergens in the murine local lymph node assay. Contact Dermatitis, 43, 95-102.

Safford RJ. The Dermal Sensitization Threshold- a TTC approach for allergic contact dermatitis. Regul Toxicol Pharmacol. 2008 51: 195-200.

Safford RJ, Aptula AO, Gilmour N. Refinement of the Dermal Sensitization Threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regul Toxicol Pharmacol. 2011 60: 218-224.

Schaefer, H., Redelmeier, T.E., 1996. Skin barrier. Principles of percutaneous absorption. In: Skin Barrier: Principles of Percutaneous Absorption, Chapter 5, pp. 153–212,

Scheuplein, R., Ross, L., 1970. Effects of surfactants and solvents on the permeability of epidermis. Journal of Society of Cosmetic Chemists 21, 853–873.

Schnuch A, Westphal G, Mössner R, Uter W, Reich K. Genetic factors in contact allergy--review and future goals. 2011. Contact Dermatitis, 64: 2-23.

Schnuch A, Geier J, Uter W, Frosch PJ, 2002. Another look at allergies to fragrances: Frequency of sensitisation to the fragrance mix and its constituents. Exogenous Dermatology, 1: 231-237.

Schwitulla J, Gefeller O, Schnuch A, Uter W. Risk factors of polysensitization to contact allergens. Br J Dermatol. 2013; 169: 611-617.

Smith HS, Holloway DB, Armstrong DKB, Basketter DA and McFadden JP (2000) Irritant thresholds in subjects with colophony allergy. Contact Dermatitis, 42, 95-97.

Smith H R, Armstrong D K, Holloway D, Whittam L, Basketter D A, McFadden J P. (2002). Skin irritation thresholds in hairdressers: implications for the development of hand dermatitis.Br J Dermatol. 146: 849–852.

Thyssen JP, Gimenez-Arnau E, Lepoittevin J-P, Menné T, Boman A and Schnuch A (2012) Critical review of methodologies and approaches to assess the inherent skin sensitization potential (skin allergies) of chemicals. Contact Dermatitis, 66: Suppl 1.

Thyssen JP, Linneberg A, Engkilde K, Menné T, Johansen JD. Contact sensitization to common haptens is associated with atopic dermatitis: new insight. Br J Dermatol. 2012 166: 1255-61.

United Nations. 2011. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Part 3: Health Hazards. http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/03e_part3.pdf. Last accessed 22 October 2012.

Uter W, Gefeller O, Geier J, Lessmann H, Pfahlberg A, Schnuch A. Untersuchungen zur Abhängigkeit der Sensibilisierung gegen wichtige Allergene von arbeitsbedingten sowie individuellen Faktoren. Schriftenreihe der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Forschung, Fb 949. Bremerhaven: 2002.

Vocanson M, Nicolas J-F and Basketter DA (2013) In vitro approaches to the identification and characterization of skin sensitisers. Exp Rev Dermatol, 8, 395-405.

Weaver JE (1983) Dose response relationships in delayed hypersensitivity to quinolone dyes. Contact Dermatitis 9: 309-312.

White JML, Basketter D A, Pease CK, Sanders DA and McFadden JP (2007) Intermittent exposure to low concentration paraphenylenediamine can be equivalent to single higher dose exposure. Contact Dermatitis, 56, 262-265.

Zhai, H., Maibach, H.I., 2001. Effects of skin occlusion on percutaneous absorption: an overview. Skin Pharmacology and Applied Skin Physiology 14, 1–10.