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Feasibility study to support a threshold of sensitization concern concept in risk assessment based on human data

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Abstract In analogy to the Threshold of Toxicological Concern concept, a Threshold of Sensitization Concern (TSC) concept is proposed for chemicals with respect to their ability to induce an allergic contact dermatitis. Recently, the derivation of a dermal sensitization threshold was suggested based on an evaluation of animal data. In order to establish the concept with human data, we conducted a meta-analysis taking into account No Expected Sensitization Induction Levels for fragrance ingredients from the IFRA/RIFM dataset. Based on a statistical analysis by applying Sensitization Assessment Factors that account for interindividual variability and different exposure conditions, TSC values of 0.91 or 0.30 μg/cm^2 can be derived in terms of amount per skin area. TSC values are compared with typical exposure levels of cosmetic products. A substance can be considered to be virtually safe if the quotient of exposure level and TSC is <1. The findings derived from human data include several conservative assumptions and largely support the dermal sensitization thresholds previously derived from animal data. The TSC concept might in principle be used for any untested chemical and therefore help in some cases to waive animal testing.

Keywords Dermal sensitization · Quantitative risk assessment · Fragrance ingredients · Threshold · TTC

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Abbreviations

AEL	Acceptable Exposure Level
Deo/AP	Deodorants/antiperspirants
EC3	Effective concentration inducing a threefold increase of lymph node responses compared to controls
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ELINCS	European list of notified substances
HRIPT	Human repeated insult patch test
IFRA	International Fragrance Association
LLNA	Local lymph node assay
NESIL	No Expected Sensitization Induction Level
NO(A)EL	No Observed (Adverse) Effect Level
QRA	Quantitative risk assessment
(Q)SAR	(Quantitative) Structure Activity Relationship
REACH	Registration, Evaluation and Authorisation of Chemicals
RIFM	Research Institute for Fragrance Materials, Inc.
SAF	Sensitization Assessment Factor
TSC	Threshold of Sensitization Concern
TTC	Threshold of Toxicological Concern

Introduction

The determination of safe human exposure levels for compounds with comprehensive toxicological data sets is well established in risk assessment. In cases where no or insufficient data is available for a chemical substance, pragmatic approaches to determine acceptable threshold limits have been developed over more than four decades. In

particular, the Threshold of Toxicological Concern (TTC) is a concept that refers to the establishment of a level of exposure for all chemicals, whether or not there are chemical-specific toxicity data, below which there would be no appreciable risk to human health (Kroes et al. 2005). Historically, a "Threshold of Regulation" was first established in the US for the food sector (Frawley 1967; Rulis 1986), and subsequently evolved into the "Threshold of Toxicological Concern" concept (Munro et al. 1996; Kroes et al. 2000, 2004, 2005; Renwick 2004). The approach compares the estimated oral daily intake with a threshold value derived from chronic oral toxicity data, and has also been applied to other areas, such as ingredients in personal and household care products (Blackburn et al. 2005) and cosmetic ingredients (Kroes et al. 2007).

Whereas the TTC approach was originally developed to cover aspects related to systemic effects, a Threshold of Sensitization Concern (TSC) procedure aims to provide an assessment for an effect at the site of application. Consequently, the units for the threshold values are provided as amount per skin area (μg/cm^2), unlike those of the TTC concept (μg/kg bw/day or μg/person/day). In analogy to the TTC concept, a TSC concept aims to establish a human exposure threshold value below which the risk of inducing a new skin allergy is considered to be acceptable.

Only recently, a concept to derive a dermal sensitization threshold was suggested, based on a probabilistic analysis of published sensitization animal data (Gerberick et al. 2005; Safford 2008), referred to in the following as "the LLNA (local lymph node assay) database".

In the current study, we evaluate the feasibility of a TSC approach by conducting a meta-analysis of human data, taking into account the available NESILs (no expected sensitization induction level) published by the RIFM (Research Institute for Fragrance Materials, Inc.) Expert Panel (Api et al. 2006) and/or accessible through the International Fragrance Association (IFRA) amendments to the IFRA code (www.ifraorg.org), referred to in the following as "the IFRA/RIFM dataset".

Reducing the number of animal tests is a highly relevant topic as reflected, e.g., by the European Cosmetics Directive (2003/15/EC, 7th Amendment) and chemicals legislation (REACH). A TSC concept might allow the avoidance of extensive animal testing in those cases where exposure of the human skin is below the established threshold of concern without compromising human health.

Relationship between a TSC and the dermal sensitization quantitative risk assessment (QRA)

The QRA concept for fragrance ingredients lays the basis for the establishment of thresholds for specific skin sensitizers in humans. Adopted by RIFM and IFRA in May 2006, the QRA methodology is used, in particular, to establish standards for potentially sensitizing fragrance ingredients on an ongoing basis. As hazard identification should never be conducted with human subjects, predictive testing for the induction of sensitization in humans is not performed. The current practice of RIFM involves a hazard assessment using an animal model (usually the LLNA), followed by a human repeated insult patch test (HRIPT) with interval exposure to confirm the absence of an allergenic potential at a concentration below or at the no-effect level identified in the animal test (Api 2002). The concept consists of the following elements (Api et al. 2008):

- The dose–response relationship for induction of skin sensitization is determined using animal assays.
- HRIPTs are performed to confirm the lack of sensitization at an exposure level which was identified as a NOEL in the LLNA model (McNamee et al. 2008; Politano and Api 2008). The NO(A)EL from a HRIPT is the dose at which no sensitization in the exposed subjects has occurred and is calculated from the concentration of the substance tested, the patch size, and the application volume.
- A NESIL is determined by applying a weight-of evidence approach with the highest preference on good quality HRIPT data. Acceptable Exposure Levels (AELs) are obtained by dividing the NESILs by a set of safety factors that are called Sensitization Assessment Factors (SAFs). SAFs range from 10 to 1,000, taking into account interindividual variability, matrix effects, as well as use considerations like disturbed barrier function or partial occlusion.
- A risk assessment is performed by comparing the AEL with the different consumer exposure levels. If the AEL exceeds the exposure, it can be assumed that at that specific exposure no induction would occur in a nonsensitized person.

Conclusions from animal data

Based on entries in ELINCS (European List of Notified Substances), it has been estimated that approximately 20% of notified chemicals are classified as skin and/or respiratory sensitizers (Safford 2008). Following an analysis of 3,366 substances officially classified in the EU and listed in Annex I of directive 67/548 EEC, (including 30th and 31st ATP), we found that 694 substances (20.6%) are classified as skin sensitizers. Moreover, our evaluation of 1,487 fragrance chemicals and natural complex substances (e.g., essential oils or extracts from botanical sources) published on the website of the European Flavour & Fragrance

Association (EFFA 2008) revealed a comparable result: 347 substances (23.3%) are classified as skin sensitizers. These classifications are usually based on information derived from animal studies and to a much lesser extent on human evidence. As a result, in all three cases the percentage of sensitizers is very similar.

The LLNA is the test system which in general is recommended by the regulations to assess skin sensitization (e.g., EC regulation 440/2008; OECD guideline No. 429). Whereas previously established guinea pig maximization tests (GPMT) or Buehler tests are mainly used for hazard classification and only allow for a crude estimate of potency, the LLNA is especially suited to provide a quantitative measure for the potency of a chemical to induce skin sensitization, expressed in EC3 values. The LLNA database, a compilation of historical LLNA data covering 211 individual substances is currently the most comprehensive database in the field (Gerberick et al. 2005).

According to the ECETOC criteria (Kimber et al. 2003) >90% of the evaluated sensitizers fall in the categories weak, moderate, or strong, and <10% belong to the extreme sensitizers (cf. Table 3). In a recent publication, dermal sensitization thresholds have been proposed for low exposure of otherwise not tested ingredients (Safford 2008). Based on a probabilistic approach that takes into account the percentage of nonsensitizers in the ELINCS database as well as the potency distribution among the allergens in the LLNA database, the study demonstrated that threshold values of 1.64 μg/cm^2 [as exemplified for a cosmetic application (shampoo)] or 0.55 μg/cm^2 (for deodorant) would provide a 95% probability of not exceeding an acceptable exposure level with regard to the induction of sensitization.

Rationale for assessing human data

Based on the conclusions drawn from animal data, we have assessed the validity of the findings by performing an evaluation of human data. It is widely agreed that the use of a properly determined human NOEL (No Observed Effect Level) has precedence over NOELs from preclinical tests since humans are the target species and the extrapolation of test results from one species to another can be avoided. However, human data that are suitable for risk quantification are rather scarce. When selecting the cases for our study, we therefore used an information-oriented approach: The meta-analysis is based on data of a group of 53 fragrance ingredients from the IFRA/RIFM dataset which are either known or strongly suspected to have a skin sensitizing potential and for which human data is available to complement information from animal studies (see Table 1). Regarding their structure, the tested chemicals cover a relatively wide range of different classes of organic molecules. The list includes the most relevant allergens in the field of fragrances, namely the 26 fragrance ingredients to be labeled on cosmetics products in Europe.

Fragrance ingredients have long attracted the interest of risk assessors. A standard fragrance mix, composed of eight relevant sensitizers, forms part of routine clinical allergy testing (Schnuch et al. 1997, 2007; Oppel and Schnuch 2006). A main reason why fragrance ingredients are among the most frequent causes of allergic contact dermatitis is their widespread use in many different product types, including cosmetic products which intentionally come into contact with human skin.

In contrast to this focused selection of fragrance ingredients with sensitizing potential which can be seen as an information-oriented spot check, substances in the ELINCS database fulfill the criteria of a random sample because testing is mainly triggered by reaching certain production volume limits. As animal tests usually provide sufficient hazard information for the purpose of classification and labeling under chemicals law, mostly no further investigations are initiated. By contrast, in the case of the fragrance ingredients under consideration, additional human data have been collected and made available in the context of risk assessment.

Establishing a TSC concept based on the IFRA/RIFM dataset

To evaluate whether the available human data allows for the establishment of TSC values we used the following route, taking into account the previous analysis of animal data (Safford 2008):

1. Analysis of the distribution of chemicals according to their sensitizing potency in the IFRA/RIFM dataset.
2. Comparison of the distribution of sensitizers in the IFRA/RIFM dataset and the LLNA database
3. Analysis of the risk following application of the threshold values derived from animal data to the IFRA/RIFM dataset
4. Determination of potential TSC values based on the distribution of AELs in the IFRA/RIFM dataset
5. Calculation of practical examples in the field of cosmetics

Analysis of the potency distribution of sensitizers in the IFRA/RIFM dataset

An analysis of the distribution of the potency of 53 sensitizers in the IFRA/RIFM dataset based on a semiquantitative definition (Gerberick et al. 2001) was performed (Table 2, column 3). This classification system was chosen

Table 1 NESIL and AEL values for fragrance allergens from the IFRA/RIFM dataset

No.	Fragrance ingredient	CAS no.	NESIL ($\mu g/cm^2$)	IFRA standard	AEL (SAF 100)	AEL (SAF 300)
1	Methyl ionone, mixture of isomers/alpha-iso-methylionone	1335-46-2/ 127-42-4	71,000	International Fragrance Association (2008w)	710.00	236.67
2	Benzyl benzoate	120-51-4	59,000	International Fragrance Association (2007g)	590.00	196.67
3	Amberonne (OTNE)	54464-57-2	47,200	International Fragrance Association (2008a)	472.00	157.33
4	Hexyl salicylate	6259-76-3	35,400	International Fragrance Association (2007l)	354.00	118.00
5	*dl*-Citronellol	106-22-9	30,000	International Fragrance Association (2007j)	300.00	100.00
6	α-Amylcinnamaldehyde	122-40-7	24,000	International Fragrance Association (2007b)	240.00	80.00
7	α-Hexyl-cinnamaldehyde	101-86-0	24,000	International Fragrance Association (2007c)	240.00	80.00
8	Benzyl salicylate	118-58-1	18,000	International Fragrance Association (2007i)	180.00	60.00
9	Linalool	78-70-6	15,000	International Fragrance Association (2003)	150.00	50.00
10	Geraniol	106-24-1	12,000	International Fragrance Association (2007k)	120.00	40.00
11	*d*-Limonene	5989-27-5	10,000	Api et al. (2008)	100.00	33.33
12	Majantol	103694-68-4	9,900	International Fragrance Association (2008e)	99.00	33.00
13	*Jasmin sambac* extract	91770-14-8	8,850	International Fragrance Association (2008s)	88.50	29.50
14	Isocyclocitral	1335-66-6, 1423-46-7, 67634-07-5	7,000	International Fragrance Association (2007m)	70.00	23.33
15	Benzyl alcohol	100-51-6	5,900	International Fragrance Association (2007f)	59.00	19.67
16	Eugenol	97-53-0	5,900	International Fragrance Association (2008n)	59.00	19.67
17	Hydroxycitronellal	107-75-5	5,000	International Fragrance Association (2008o)	50.00	16.67
18	Methoxy dicyclopentadiene carboxaldehyde (scentenal)	86803-90-9	5,000	International Fragrance Association (2008u)	50.00	16.67
19	Benzyl cinnamate	103-41-3	4,700	International Fragrance Association (2007h)	47.00	15.67
20	p-t-Butyl-α-methylhydrocinnamic aldehyde (BMHCA)	80-54-6	4,100	International Fragrance Association (2008α)	41.00	13.67
21	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC)	51414-25-6 31906-04-4	4,000	International Fragrance Association (2008g)	40.00	13.33
22	Isocyclogeraniol	68527-77-5	3,800	International Fragrance Association (2008p)	38.00	12.67
23	Alpha-methyl cinnamic aldehyde	101-39-3	3,500	International Fragrance Association (2007d)	35.00	11.67
24	α-Amylcinnamyl alcohol	101-85-9	3,500	International Fragrance Association (2007a)	35.00	11.67
25	Coumarin	91-64-5	3,500	International Fragrance Association (2008m)	35.00	11.67
26	1-Octen-3-yl acetate	2442-10-6	3,500	International Fragrance Association (2008b)	35.00	11.67
27	Cinnamic alcohol (cinnamyl alcohol)	104-54-1	3,000	International Fragrance Association (2008j)	30.00	10.00
28	Farnesol	4602-84-0	2,700	International Fragrance Association (2006b)	27.00	9.00
29	Carvone	99-49-0	2,650	International Fragrance Association (2008i)	26.50	8.83
30	Ylang ylang extracts	8006-81-3	1,770	International Fragrance Association (2008η	17.70	5.90
31	Anisyl alcohol	105-13-5	1,500	International Fragrance Association (2007e)	15.00	5.00
32	Jasmine absolute (grandiflorum)	8022-96-6	1,470	International Fragrance Association (2008r)	14.70	4.90
33	Citral	5392-40-5	1,400	International Fragrance Association (2006a)	14.00	4.67
34	p-tert-Butyldihydrocinnamaldehyde (bourgeonal)	18127-01-0	1,100	International Fragrance Association (2008β)	11.00	3.67
35	Cinnamyl nitrile	1885-38-7	1,060	International Fragrance Association (2008l)	10.60	3.53
36	Menthadiene-7-methyl formate	68683-20-5	1,060	International Fragrance Association (2008t)	10.60	3.53
37	*trans*-β-Damascone	23726-91-2	1,000	International Fragrance Association (2008δ)	10.00	3.33
38	Balsam oil, peru (myroxylon pereirae)	8007-00-9	950	International Fragrance Association (2008γ)	9.50	3.17

Table 1 continued

No.	Fragrance ingredient	CAS no.	NESIL (μg/cm^2)	IFRA standard	AEL (SAF 100)	AEL (SAF 300)
39	3-Propylidenephthalide	17369-59-4	920	International Fragrance Association (2008f)	9.20	3.07
40	Oakmoss	90028-68-5	700	International Fragrance Association (2008y)	7.00	2.33
41	Treemoss	90028-67-4	700	International Fragrance Association (2008ζ)	7.00	2.33
42	Perilla aldehyde	2111-75-3	700	International Fragrance Association (2008z)	7.00	2.33
43	Cinnamic aldehyde (cinnamal)	104-55-2	590	International Fragrance Association (2008k)	5.90	1.97
44	Phenylacetaldehyde	122-78-1	590	International Fragrance Association (2006c)	5.90	1.97
45	*trans*-α-Damascone	24720-09-0	500	International Fragrance Association (2008δ)	5.00	1.67
46	Hexylidene cyclopentanone	17373-89-6	300	International Fragrance Association (2008h)	3.00	1.00
47	Isoeugenol	97-54-1	250	International Fragrance Association (2008q)	2.50	0.83
48	2-Ethoxy-4-methylphenol	2563-07-7	230	International Fragrance Association (2008c)	2.30	0.77
49	Methyl 2-octynoate (methyl heptine carbonate)	111-12-6	120	International Fragrance Association (2008v)	1.20	0.40
50	2-Methoxy-4-methylphenol (creosol)	93-51-6	118	International Fragrance Association (2008d)	1.18	0.39
51	Rose ketones (e.g. damascenone)	23696-85-7	100	International Fragrance Association (2008δ)	1.00	0.33
52	Methyl 2-nonynoate (methyl octine carbonate)	111-80-8	24	International Fragrance Association (2008x)	0.24	0.08
53	*trans*-2-Hexenal	6728-26-3	24	International Fragrance Association (2008ε)	0.24	0.08

Table 2 Potency distribution of sensitizers in the IFRA/RIFM dataset in comparison with the LLNA database following the criteria of (Gerberick et al. 2001)

Sensitization potential	NESIL[a] (μg/cm^2)	Total no./% of 53	Total no./% of substances in the LLNA database[b]
Non-sensitizing	Not calculated	Category not used	42 (not considered)
Extremely weak	≥10,000	11/20.7%	5/2.9%
Weak	≥1,000 to <10,000	26/49.1%	89/53.3%
Moderate	≥100 to <1,000	14/26.4%	50/29.6%
Strong	≥10 to <100	2/3.8%	16/9.5%
Potent	<10	None	7/4.1%

[a] Regarded here as equivalent to experimental human NOELs, classification according to Gerberick et al. (2001), with some precisions at the borders of each class added

[b] The total number of sensitizing substances in the database is 169; two false positives were excluded from the evaluation (Gerberick et al. 2005)

since it is the only one that directly refers to amount per skin area. It can be observed that NESIL values cover about three orders of magnitude. The dataset includes mostly weak and moderate sensitizers.

As a next step, a suitable distribution curve for the AEL values was identified. AELs were calculated following transformation of NESIL values to $\log_{10}$ (AEL) values. In accordance with the QRA concept, we applied SAFs of 100 for typical modest exposure conditions of a cosmetic product (exemplified in the following by "shampoo") and 300 for leave-on products with enhanced exposure conditions (exemplified in the following by "Deo/AP" = deodorants/antiperspirants). The data was initially fitted with a normal distribution in analogy to the procedure outlined by Rulis (1986). We identified that a skew normal distribution (Azzalini 1985) renders the dataset better, as determined by quantile-quantile-plots. Skew normal distributions were calculated for shampoo and Deo/AP products (Fig. 1a, b). The optimal parameters for these distributions are the maximum-likelihood estimators, as analytically computed and as derived by an expectation-maximization algorithm. Calculations were performed in R (R-Development-Core-Team 2008) with the package "sn" (Azzalini 2008). In the case of the LLNA database where a fixed upper limit of the applied dose of 25,000 μg/cm^2 is assumed and the EC3 values do not exceed 100%, the author applied a Gamma distribution to the corresponding AEL values for which the existence of such boundaries is a prerequisite (see also under 2). More flexible testing procedures like the HRIPT, however, do not give rise to upper limits, although in

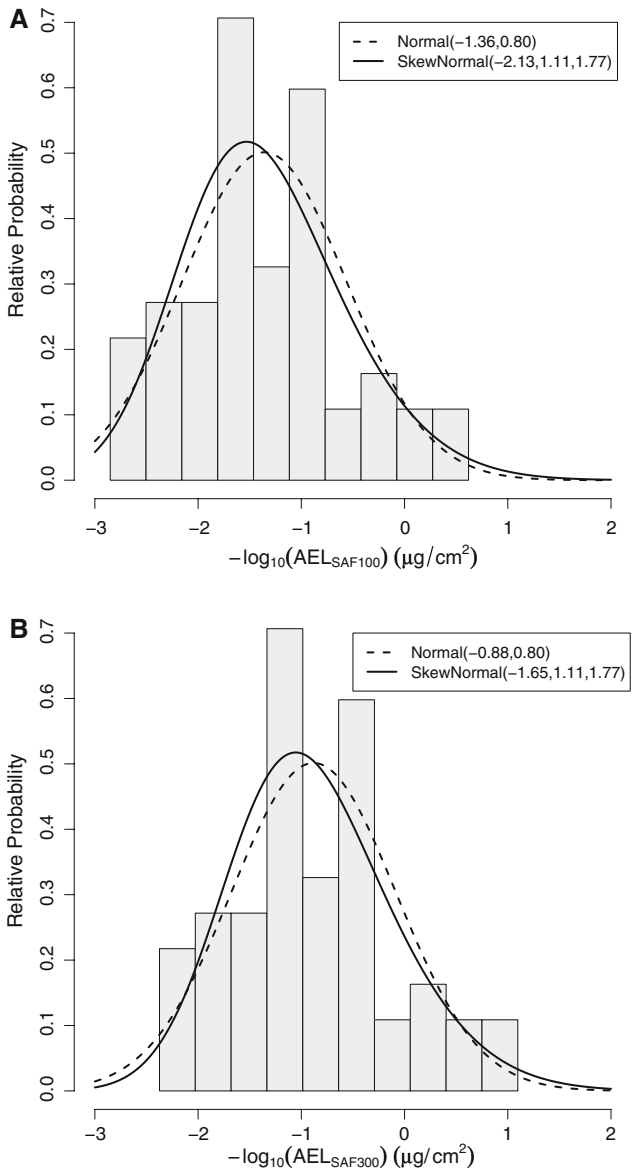

Fig. 1 Histogram of AELs of fragrance allergens from the IFRA/RIFM dataset. The *dashed line* depicts the density function of a fitted normal distribution; for the *solid line*, a skew normal distribution has been applied. **a** histogram and distribution curves for shampoo (SAF 100), **b** the values for Deo/AP (SAF 300) have been calculated. This corresponds to a shift of $\log_{10}$ (3) to the right compared to (**a**)

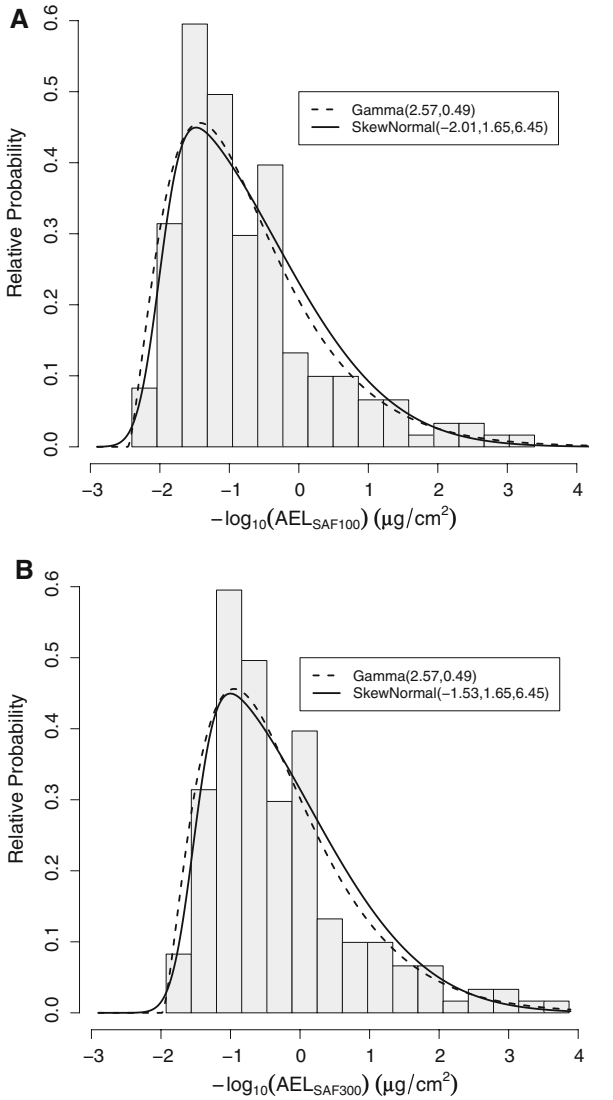

Fig. 2 Histogram of the AELs of the LLNA database. The *dashed line* shows the probability density function of a Gamma distribution with parameters chosen by maximum-likelihood, after the application of the appropriate shift (modified after Safford 2008). The *solid line* shows the density function of a skew normal distribution, with optimally adapted parameters. These calculations have been performed for **a** shampoo (SAF of 100) and **b** Deo/AP (SAF 300)

practice the highest values in Table 1 are seldom exceeded. As a consequence, a Gamma distribution is not suited to fit the IFRA/RIFM dataset.

Comparing the distribution of sensitizers in the IFRA/RIFM dataset and the LLNA database

A comparison of the two datasets needs to be performed via common denominators, i.e., NESIL or AEL values. Data in the LLNA database are initially presented in terms of the concentration required to reach a threefold increase of activity as compared to vehicle control (EC3). In order to derive human NESIL values, the previously described procedure was followed (Safford 2008), which is based on evidence that the logarithm of human NESIL can be related to the logarithm of EC3 values of the same substance via linear regression (Basketter et al. 2005b).

Figure 2a, b show histograms of the LLNA database after transformation to the negative $\log_{10}$ (AEL)-scale. Since the upper boundary for the EC3-values is 100%, a lower boundary for the negative logarithmic AEL-values

Table 3 Distribution of sensitizers in the LLNA database following the criteria of ECETOC (Kimber et al. 2003)

Sensitization potential (according to Kimber et al. 2003)	EC3 value (%)[a]	Total no./% of substances in the LLNA database[b]
Non-sensitizing	>100	42/19.9%[c]
Weak	≥10 (2,500 μg/cm^2) to ≤100 (25,000 μg/cm^2)	64/30.3%/38.3%
Moderate	≥1 (250 μg/cm^2) to <10 (2,500 μg/cm^2)	69/32.7%/40.8%
Strong	≥0.1 (25 μg/cm^2) to <1 (250 μg/cm^2)	21/10.0%/12.4%
Extreme	<0.1 (25 μg/cm^2)	13/6.2%/7.7%

[a] Amount per square centimeter is calculated in addition by assuming an standard application volume of 25 μl is used in the LLNA and that the area of the exposed mouse ear is 1 cm^2

[b] Gerberick et al. (2005); class according to EC3 values. Percent are calculated for the total database, then for the 167 sensitizers only

[c] Category not considered by Safford (2008)

can be computed and fitted with a Gamma distribution (cf. Step 1.).

When comparing the distribution curves from the different datasets, the Gamma curve for the LLNA database is more skewed to the right than the symmetric curve that results from the Gaussian normal fitting and skewed normal fitting for the IFRA/RIFM dataset. Fitting of the LLNA data seems to be smoother than with RIFM data, which may partly be due to the larger amount of data in this collection. In order to be able to perform a direct comparison between distribution curves for the two datasets we fitted the LLNA database with a skew normal distribution, as was done for the IFRA/RIFM dataset and found that the initial impression was confirmed; the third parameter, reflecting the amount of skew in the distribution, is much lower for the RIFM dataset (Fig. 1a, b).

Assuming that NESILs derived from LLNA and HRIPT data are quantitatively comparable in terms of percentage, the IFRA/RIFM dataset contains less strong and extreme sensitizers than the LLNA database but a similar percentage of weak and moderate sensitizers (Table 2). Since different classification systems are in use, we also display in Table 3 the results for a classification system proposed by ECETOC (Kimber et al. 2003) as previously applied to the LLNA database (Safford 2008).

The NESIL values for the subgroup of fragrance ingredients span about three orders of magnitude and their distribution is less inhomogeneous than the EC3 values for the broader range of chemicals in the LLNA database that span some five orders of magnitude.

Analysis of the risk following application of the thresholds derived from animal data to the IFRA/RIFM dataset

We applied the previously derived potential thresholds of 1.64 μg/cm^2 for shampoos (SAF 100; corresponding to the 75% percentile of the Gamma distribution) and 0.55 μg/cm^2 for Deo/AP to the IFRA/RIFM dataset. These thresholds would cover 48 of the 53 fragrance ingredients (90.6%), while five substances would be missed (i.e., damascenone, 2-methoxy-4-methylphenol, methyl 2-octynoate, *trans*-2-hexenal and methyl 2-nonynoate), compared to the 133 substances (79.6%) that are covered in the LLNA database and where 34 substances are not covered.

Derivation of potential TSC values based on the distribution of AELs in the IFRA/RIFM dataset

The probability that an untested substance would fall below specific AELs can be estimated from the fitted distribution curves. Table 4 lists the AEL values corresponding to the percentiles from the distributions depicted in Figs. 1 and 2. The percentile values have been calculated for the respective distributions in the negative $\log_{10}$ (AEL) domain, and have been transformed by exponentiation.

We suggest using the 95% percentile of the skew normal distribution for the NESIL-derived AEL values to set up the TSC. As a consequence, threshold values of 0.91 μg/cm^2 for shampoos (SAF 100) and of 0.30 μg/cm^2 for Deo/APs (SAF 300) are proposed which would cover 51 of the 53 substances, resulting in safety factors >100 for about 96% of the substances in the IFRA/RIFM dataset. The two substances of the investigated sensitizers that are not covered by the TSC, i.e., *trans*-2-hexenal and methyl 2-nonynoate, both have a NESIL of 24 μg/cm^2, which is still over 20 times higher than the proposed TSC value. This provides a lower, but still relevant safety factor.

In general, as a TSC based on AEL values already includes safety factors, a substance can be considered to be virtually safe if the quotient of the exposure level and TSC is <1.

We used the 95% percentile of the distribution of sensitizers to derive a potential threshold value, while Safford used the 75% percentile of the distribution of sensitizers in the LLNA database, finally reaching a 95% likelihood not

Table 4 Probability estimate for untested chemicals not to exceed the AELs at given TSC values from the distributions shown in Fig. 1

Percentile	LLNA database Gamma Dist. (SAF 100/300)	LLNA database Skew Normal Dist. (SAF 100/300)	IFRA/RIFM Normal Dist. (SAF 100/300)	IFRA/RIFM Skew Normal Dist. (SAF 100/300)
95	0.06/0.02	0.06/0.02	1.12/0.37	**0.91/0.30**
90	0.24/0.08	0.20/0.07	2.18/0.73	2.03/0.68
85	–	0.43/0.14	3.42/1.14	3.43/1.14
80	1.00/0.33	0.79/0.26	4.88/1.63	5.14/1.71
75	1.64/0.55	1.30/0.43	6.63/2.21	7.22/2.41
70	2.50/0.83	2.01/0.67	8.73/2.91	9.72/3.24
60	5.04/1.68	4.22/1.41	14.35/4.78	16.32/5.44

Potential TSC values correspond to the AELs that are assigned to different percentiles. The suggested TSC values based on the IFRA/RIFM dataset is marked in bold type

falling below the threshold assuming that only 20% of all substances are sensitizers.

It would also be possible to apply an analogous approach to the human data. We have identified that approximately 20% of the fragrance ingredients in the IFRA/RIFM dataset are sensitizers, which supports the Safford's assumption based on the listed chemicals The resulting threshold values at the 75% percentile would be clearly higher (7.22 μg/cm^2 (SAF 100)/2.41 μg/cm^2 (SAF 300), but still in a similar range to the threshold values derived from animal data (Table 4). Either of these values might be applied and are not expected to lead to a considerable underestimation of risk. Furthermore, it should be noted that if the categorization scheme proposed by ECETOC (Kimber et al. 2003) is used, the proposed values are at least one order of magnitude below the amount per unit area (μg/cm^2) that has been so far associated with the category "extreme" (cf. Table 3). The same holds true for the category "extreme" based on LLNA data that has been proposed by the expert group on skin sensitization nominated by the Technical Committee on Classification and Labelling on behalf of the European Commission (Basketter et al. 2005a).

Calculation of practical examples in the field of cosmetics

As the TSC concept is primarily applicable for chemicals where the human exposure is low, estimation of the skin contact is of critical importance.

The threshold value needs to be converted for the corresponding concentrations in products by taking into account specific product types and exposure conditions. Examples of concentrations in typical cosmetic products not exceeding the TSC values when compared with exposure levels as summarized by the SCCP are provided in Table 5. In general, exposure might occur more than once per day or repeatedly during a longer period of time and may lead to accumulation of the substance on the same skin site. However, no such behavior has been reported so far for the fragrance ingredients under consideration and therefore this has not been taken into account.

As expected, the main potential application areas of the TSC concept in the field of cosmetics are the rinse-off products (e.g., shampoos, shower gels, soap) where virtually safe concentrations in the lower percentage range are achievable. In addition, chemicals that are present in very low concentrations in stay-in (e.g., hair spray or styling gel) or classical leave-on products (e.g., face cream, body lotion) may be below the thresholds defined by a TSC concept.

Discussion

We have assessed the applicability of a TSC approach with the IFRA/RIFM human dataset for sensitizing fragrance ingredients. The derived potential threshold values are in the same order of magnitude as in the previous probabilistic analysis of LLNA animal data. The IFRA/RIFM dataset is currently still limited in size. A larger sample size will in the future lead to an increased precision in the statistical estimates. Our analysis relies on an estimation of the probability distribution generated by the data collection.

Many distribution classes are conceivable, the normal distribution being used ubiquitously in biologic and chemical research (Healy 1979; Rulis 1986; Bailey 1995; Varmuza 1998). However, normal distributions cannot account for a possible skew of the sample. The LLNA database as well as to a lesser extent the IFRA/RIFM dataset reveal a strong right skew, which prohibits the use of the normal distribution. A normal distribution would underestimate the weight of the right tail which includes the most potent sensitizers.

The potency distribution of sensitizers, both in the LLNA and in the IFRA/RIFM database, is likely to be

Table 5 Example for a practical application: TSC-derived acceptable concentrations in different cosmetic product types

Product type	Chemical in product (%)[a]	Amount per use (g)[b]	Retention factor skin[b]	Contact area skin (cm^2)[b]	SAF[c]	Dose per unit area ($\mu g/cm^2$)
Eye make-up	0.07	0.01	1	24	300	0.292
Mascara	0.0019	0.025	1	1.6	300	0.297
Eyeliner	0.019	0.005	1	3.2	300	0.297
Lipstick	0.035	0.01	1	12	300	0.292
Body lotion	0.058	8	1	15,670	300	0.296
Deo/antiperspirant	0.006	0.5	1	100	300	0.300
Face cream	0.064	0.8	1	565	100	0.906
Hair styling products	0.18	5	0.1	1.010	100	0.891
Oxidation hair dye	0.005	100	0.1	580	100	0.862
Semi perman. hair dye	0.015	35	0.1	580	100	0.905
Make-up remover	0.2	2.5	0.1	565	100	0.885
Shampoo	1.63	8	0.01	1,440	100	0.906
Hair conditioner	0.93	14	0.01	1,440	100	0.904

[a] Virtually safe concentration of chemical in product resulting in doses of 0.91 or 0.30 $\mu g/cm^2$, respectively

[b] SCCNFP/0690/03 Final

[c] QRA Technical Dossier, Revised June 22, 2006 pp. 30–34, Migration through stratum corneum is assumed to be 100% (worst case scenario)

biased in relation to a hypothetical potency distribution of all skin sensitizing substances (selection bias). The different relative coverage of sensitizers (probability estimates) when applying the same threshold values in the LLNA and IFRA/RIFM datasets can be explained by the different sensitization potency spectra of the substances in these datasets. Therefore, as long as only relatively small sample sizes are available for analysis, the derived threshold values are expected to vary related according to individual data distribution differences.

Future developments of the concept might include an initial analysis of structural alerts leading to more differentiated TSC values. There is evidence that the sensitization potential is related to a combination of reactivity and easily computable parameters like hydrophobicity and molecular weight. Chemicals with relevant structural similarities to known allergens could be a priori allocated to separate classes, in analogy (but not identical) to the Cramer classes in the TTC approach (Kroes et al. 2004). (Q)SAR tools for skin sensitization have been developed (Barratt and Langowski 1999; Langton et al. 2006) and might assist in such an exercise. Also, the predictive capacity of (Q)SAR tools should further be optimized.

The process of safety assessment implies a number of underlying assumptions and determinations that are the result of a balance between a high protection level, practicability, and the aim to minimize animal testing. The outcome of this process depends on ethical considerations and the acceptance by safety assessors, regulators, and the general public. There is a remaining risk that very strong sensitizers (e.g., the preservative 2-methyl-3-(2H)-isothiazolinone) can induce a new allergy even if the amount applied to skin under rinse off conditions is ≤ 0.91 $\mu g/cm^2$. However, it should be kept in mind that the derivation of suggested TSC values was based on a number of conservative assumptions:

(a) A subgroup exclusively composed of sensitizing substances was used to derive threshold values based on a 95% percentile; we did not take into account that the real incidence of sensitizers is much lower.
(b) NESILS from the IFRA/RIFM dataset reflect the highest concentration tested in humans without sensitization—not the highest achievable NOEL,
(c) The chosen skewed normal distribution fits the part with lower AEL values better than the normal distribution and provides lower TSC values
(d) The safety assessment factor concept is conservative, because it adds assessment factors of 100 and 300 to human data.

In contrast to the TTC, TSC is more specific for individual exposure conditions. Penetration through the stratum corneum limits the biologic availability of the effector molecules and depends on a number of factors like contact time, partition/retention, and penetration-enhancing conditions like partial occlusion.

Validated and accepted alternative approaches to replace animal testing are not available for many toxicological effects. Several in vitro assays to detect sensitizing properties of a chemical are currently under development for the assessment of chemical reactivity and cell-based assays, but currently no officially accepted in vitro tests for

skin sensitization are available. At the same time, the European REACH legislation requires animal testing to assess skin sensitizing properties of chemicals produced or imported in quantities >1 ton per year in the EU. Aside from waiving criteria such as technical feasibility, exposure plays a decisive role in the waiving process, as stated in the REACH documents. Although threshold of toxicological concern values are not explicitly defined, the stipulation of lower limits of consideration (0.1% or 1%) from European chemicals law imply that below this value the risk can be considered negligible. Up to now scientific criteria hardly exist which precisely define such thresholds. The evaluation and application of threshold concepts might help to fill these gaps. A dialogue with experts/regulators will be needed to further assess the applicability in various regulatory contexts.

Conclusions

This investigation provides a preliminary analysis of the reliability and validity of the TSC concept for a convenience sample drawn from well investigated fragrance ingredients. Our analyses indicate that our human data-derived findings support conclusions drawn from the LLNA data, thereby suggesting plausibility of the previous findings.

The small survey shows that a TSC approach might be feasible, and that a slight decrease in previously proposed threshold values can improve the margin of safety without losing the practicability of the concept. The absolute differences between TSC based on human data (0.91 and 0.30 $\mu g/cm^2$) and the dermal sensitization thresholds derived from animal data (1.64 and 0.55 $\mu g/cm^2$) values are small, especially when considering the different underlying data and assumptions.

The TSC concept can be used to estimate the risk of low exposures without the need for chemical-specific animal toxicity data. A consensus with regulators will be needed to agree on specific thresholds to be used in risk assessment for various purposes.

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