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The dermal sensitisation threshold—A TTC approach for allergic contact dermatitis

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ABSTRACT

The Threshold of Toxicological Concern (TTC) is a useful concept that is becoming of increasing interest as an addition to the arsenal of tools used for characterising the toxicological risk of human exposure to chemicals. Traditionally used for low level indirect additives, flavours and contaminants in foods, the TTC obviates the need for toxicological testing of chemicals where human exposure is low. Proposals have recently been made for the use of the TTC for low level ingredients in cosmetic and personal care products. However, use of the TTC is only protective for systemic toxicity endpoints, and cannot be used for local endpoints such as contact sensitisation. In this paper a probabilistic analysis of available sensitisation data, similar to that used in the development of the TTC, is presented. The incidence of sensitisers in the world of chemicals was estimated using the ELINCS (European List of Notified Chemical Substances) data set, and a distribution for sensitisation potency was established using a recently published compilation of Local Lymph Node Assay data. From the analysis of these data sets it is concluded that a Dermal Sensitisation Threshold (DST) can be established below which there is no appreciable risk of sensitisation, even for an untested ingredient. Use of a DST would preclude the need for sensitisation testing of ingredients where dermal exposure is sufficiently low.

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1. Introduction

The Threshold of Toxicological Concern (TTC) is a useful concept that is becoming of increasing interest as an addition to the arsenal of tools used for characterising the toxicological risk of human exposure to chemicals. Initially proposed by Frawley (1967), the TTC acknowledges that a human exposure threshold can be determined, below which there is no appreciable risk to health, even when the toxicological profile of a chemical is not known. Although Frawley's estimation of the TTC was based on limited systemic toxicity data, he did establish a principle that has since been developed over many years, resulting in the current approach. The first real estimation of the TTC (then termed the Threshold of Regulation, ToR) was made by Rulis (1986), who used a probabilistic assessment of carcinogenicity potency data from lifetime rodent studies to estimate a ToR of 0.5 ppb in foods (equivalent to an intake of 1.5 µg/day in an adult). Since this original proposal, the TTC process has been the subject of a number of modifications by different authors (Cramer et al., 1978; Munro, 1990; Munro et al., 1996; Cheeseman et al., 1999; Kroes et al., 2000, 2004), particularly with respect to the use of screens based on chemical structure to provide a tiered TTC approach. In the most recent proposal put forward by Kroes et al. (2004), a decision tree process is used

to place the chemical of interest into one of five levels of TTC ranging from 0.15 µg/day for potential genotoxic carcinogens to 1800 µg/day for chemicals considered to be of low toxicity. In each case, the levels of TTC have been established using probabilistic analysis of existing animal toxicity data. An overview of the history of the TTC, and the current Kroes decision tree can be found in an ILSI Europe (2005).

Since its inception, the TTC process has been used successfully in assessing the safety of certain chemicals at low levels in foods. This includes food packaging migrants (US Food and Drug Administration, 1995; Munro et al., 2002; Cheeseman, 2005) and flavouring agents (JECFA, 1997; Munro et al., 1999; Renwick, 2004). More recently the European Medicines Agency has adopted the TTC concept to assess genotoxic impurities in pharmaceutical preparations (EMA, 2006; Muller et al., 2006). Use of the TTC has also been proposed for ingredients used in personal care products (Kroes et al., 2007). The latter proposal marks a deviation from other uses of the TTC in that it considers systemic exposure via the dermal route as opposed to the oral route. In that publication, the authors consider the relevance of the TTC to exposure from the dermal route, and also propose a Quantitative Structure Activity Relationship (QSAR) based methodology for establishing the likely dermal penetration. However, it was stressed that the TTC approach applies only to systemic toxicity, and does not apply to local effects such as dermal sensitisation. This is implicit since the databases used to develop the TTC included only those studies investigating systemic effects.

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A vital part of assessing the safety of ingredients and contaminants in personal care products is the assessment of the risk of delayed contact sensitisation in consumers. At the present time, best practice for establishing safety for this hazard endpoint involves determining the sensitisation potential of an ingredient using animal assays. Although a variety of animal assays are available, the current assay of choice is the mouse Local Lymph Node Assay (LLNA, Kimber et al., 2002). For those materials identified as having skin sensitisation potential, sensitising potency can also be derived from the animal assay. In the case of the LLNA, interpolation of the dose–response data can be used to estimate the concentration of a chemical required to produce a threshold positive response (a 3-fold increase in activity compared with concurrent vehicle controls, the EC₃ value). In some cases, confirmation of the absence of induction of sensitisation at threshold doses may then be obtained in humans, typically using a Human Repeat Insult Patch Test (HRIPT, McNamee et al., 2008). Based on the results of the animal and human assays, the sensitisation threshold for humans (the No Expected Sensitisation Induction Level, or NESIL) can be established using a weight of evidence approach. This NESIL, expressed as dose per unit area of skin ($\mu\text{g}/\text{cm}^2$), can then be used to conduct a Quantitative Risk Assessment (QRA). In the QRA process, Sensitisation Assessment Factors (SAFs) are applied to the NESIL to take account of interindividual variability, variations in vehicle (product formulation) and use conditions (area of body, skin condition etc.), and an Acceptable Exposure Level (AEL) for humans determined. This AEL can then be compared to the expected Consumer Exposure Level (CEL), thus completing the risk assessment (see Api et al., 2008 for a full explanation of this QRA, approach). An AEL:CEL ratio of 1 or higher is generally considered to be acceptable.

Not only is this a time consuming and costly exercise, but the requirement to use animals, and in some cases humans in the evaluation raises ethical issues that need to be weighed against the potential risk of sensitisation in consumers. In some cases where the skin exposure to a material can be predicted to be very low, it may not be fully justified to conduct this test programme. Some ingredients, such as colours, may be used at low levels in products, leading to very low dermal exposure, particularly from a rinse-off product. For example, an ingredient used in a shampoo at 0.01% would lead to a dermal exposure of around 0.005 $\mu\text{g}/\text{cm}^2$ (calculation based on SCCP guidelines, 2006). The question then becomes where does the cut-off point lie? It is possible to benchmark exposure of an untested chemical based on the assumption that its sensitising potency may be similar to that of well known potent sensitisers such as 1-chloro-2,4-dinitrobenzene (DNCB) or 5-chloro-2-methyl-4-isothiazolin-3-one (MCI). Use of such an approach is undoubtedly very protective, but is over-conservative since the calculated AELs for these chemicals are extremely low. A more useful approach would be to develop a Dermal Sensitisation Threshold (DST) that could be applied in exactly the same way as the TTC.

In this publication, skin sensitisation data from freely available published sources has been evaluated using the same probabilistic principles as those used by Rulis (1986) in his determination of the TTC. The aim is to show that such an approach can be used to develop a scientifically valid and defensible Dermal Sensitisation Threshold.

2. Methods and results

The main steps in the analysis were as follows:

1. Estimate the proportion of skin sensitisers in the world of chemicals
2. Investigate the distribution of sensitisation potencies for known skin sensitisers
3. Calculate the distribution of acceptable risk to humans based on potency estimates
4. Determine the overall probability that an untested chemical may exceed an acceptable risk for a given DST

Details of these steps are as follows:

2.1. Estimating the proportion of sensitisers in the world of chemicals

The ELINCS (European List of Notified Chemical Substances) provides classification data on chemicals notified in accordance with the Commission Decision 85/71/EEC. As part of the notification, any chemical produced at >100 kg p.a. requires data on sensitisation testing. The current version (6th version) was downloaded from the European Chemicals Bureau (ECB) web site at <http://ecb.jrc.it/elincs/>, and contained classification data on all chemicals notified from 18th September 1981 to 30th June 2005.

A total of 4381 chemicals (including mixtures and reaction products) are included on the database, of which 2390 have received formal classification. From statistics obtained from the ECB web site, it is estimated that 12.9% of chemicals fail to reach the 100 kg p.a. threshold, and may not therefore have been tested for sensitisation. For the purposes of this exercise it was assumed that 87.1% of those chemicals that have received formal classification (i.e. 2082 out of 2390) have been tested for sensitisation. Those chemicals that have been shown to be skin sensitisers are classified as R43 (may cause sensitisation by skin contact), sometimes in conjunction with R42 (may cause sensitisation by inhalation). Four hundred and nine chemicals are classified as R43 or R42/43; that is 19.6% of the 2082 chemicals classified.

For the purposes of establishing a DST it is proposed that a figure of 20% incidence of sensitisers in the world of chemicals be used.

2.2. Establishing the potency distribution for known sensitisers

Identification of the sensitisation potential for chemicals has long been conducted using animal tests, historically guinea pig assays of various kinds, and more recently using the LLNA. As part of these assays, the sensitisation potency has also been established. In guinea pig assays this has mostly been limited to a categorisation of potency (extreme, strong, moderate, weak). Such data were not considered suitable for establishing a distribution of sensitisation potencies. The LLNA, on the other hand, provides a continuous readout of potency in the form of an EC₃ value. In a recent publication by Gerberick et al. (2005), data from peer reviewed LLNAs on 211 chemicals were presented (subsequently referred to here as the “Master Table”). Of these, 167 chemicals gave positive results, and EC₃ data were presented for these.

A plot of the distribution of the EC₃s from the Master Table (as a percentage applied—Fig. 1) shows a very highly skewed distribution, apparently towards the most potent category. However, it must be remembered that sensitisation potency is more usually described on a pseudo log scale (Kimber et al., 2003). For example, sensitisers are often classified according to their EC₃ value as follows:

EC ₃ (%)	Categorisation
<0.1	Extreme
0.1–<1	Strong
1–<10	Moderate
10–100	Weak

Using this scale, it can immediately be seen that the leftmost bar in Fig. 1, which has an arbitrary cut-off value of around 3.5%, would contain not only all of the extreme and strong sensitisers, but a good proportion of the moderate sensitisers.

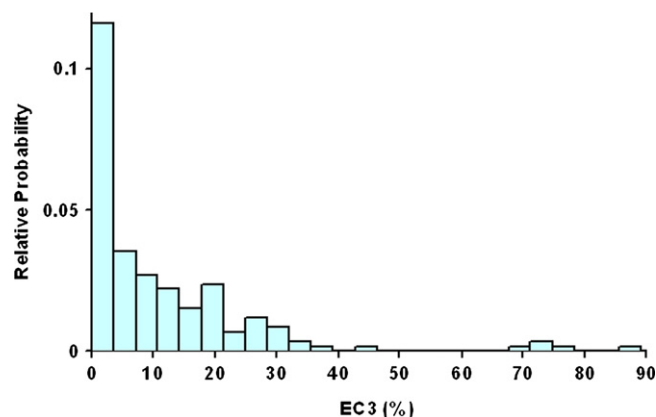


Fig. 1. Distribution of EC₃s for 167 sensitising chemicals from the Master Table (Gerberick et al., 2005).

Plotting the distribution as a negative log(10) of the EC3 values provides a much more intuitive description of the distribution (Fig. 2). In this it can be seen that the bulk of the chemicals fall into the weak and moderate categories (66 and 69 chemicals, respectively), with a tail consisting of the strong and extreme sensitisers (21 and 13 chemicals, respectively).

In the graph, two distributions are fitted, lognormal and gamma. Both can be bounded at the lower end to –2 (i.e. 100%), and appear to fit the data well. Comparing the actual data points to the fitted quantiles in a Q–Q plot (a graphical technique to determine the goodness of fit of the data set to a specific distribution) shows that the gamma distribution fits better at the top end (i.e. the more potent sensitisers). For this reason a gamma distribution has been used in the analysis.

2.3. Calculating risk of sensitisation in humans based on potency estimates

In order to establish a DST, the EC3 values need to be converted to a predicted human sensitisation potency, and from there to doses (expressed as $\mu\text{g}/\text{cm}^2$) giving an acceptable human risk. Again, this process mirrors that used by Rulis (1986), who established a virtually safe dose (VSD) for humans by linear extrapolation from TD50 values (i.e. the doses calculated from rodent bio-assay dose response curves to give an increase in tumour incidence of 50%).

EC3 values were used to establish a NESIL in humans for each of the 167 sensitisers. Evidence that support the use of EC3s for predicting human sensitisation potency of chemicals can be found in a number of publications (Schneider and Akkan, 2004; Griem et al., 2003; Basketter et al., 2005). The latter publication concludes that “The results from each assay, expressed as dose per unit area ($\mu\text{g}/\text{cm}^2$) revealed a clear linear relationship between the two values, thereby substantiating further the utility of LLNA EC3 values for prediction of the relative sensitising potency of newly identified skin sensitisers.”

In this analysis, human NESILs have been estimated by applying a mouse to human factor derived from the correlation determined by Basketter et al. (2005). Although the formula of the linear regression line was not shown in that publication, this has been calculated in Microsoft Excel using the data quoted in the publication. Based on the 18 chemicals for which NOELs in the HRIPT were determined, the regression equation is as follows:

$$\text{Log}(10) \text{ NESIL } \mu\text{g}/\text{cm}^2 = 1.16 \text{ Log}(10) \text{ EC3 } \mu\text{g}/\text{cm}^2 - 0.64$$

AELs were then calculated by applying the appropriate SAF.

The overall procedure is detailed as follows:

EC3 values for the 167 chemicals were firstly converted to dose per unit area. The formula is based on application of 25 μl of test solution to a mouse ear of 1 cm^2 , giving the following equation:

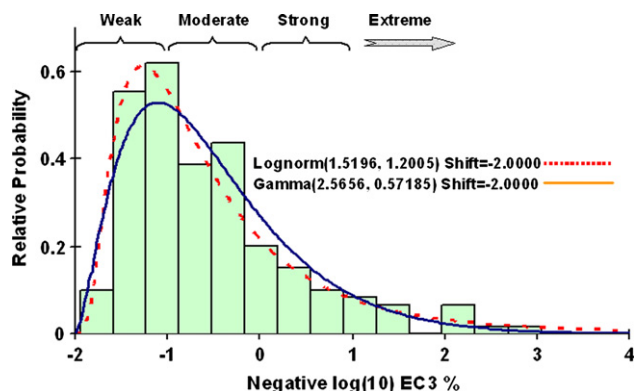


Fig. 2. Distribution of EC3s for 167 sensitising chemicals from the Master Table (Gerberick et al., 2005) using a negative log(10) scale.

Table 1

Rationale for determining Sensitisation Assessment Factors for the example products, shampoo and deodorant

SAF	Shampoo		Deodorants	
	Value	Rationale	Value	Rationale
Human variability	10	Standard value used in QRAs	10	Standard value used in QRAs
Matrix variability	3	Product is a complex matrix which may contain irritating ingredients and may enhance penetration	3	Product is a complex matrix that may enhance penetration
Use variability	3	Scalp skin is highly follicular and generally considered to be more permeable	10	Axilla skin is easily irritated, highly follicular and an area that is shaved.
Overall SAF	100*		300	Product is applied under semi-occlusion

* Rounded value.

$$\text{EC3 } \mu\text{g}/\text{cm}^2 = \frac{\text{EC3\%} \times 0.025 \times 1000000 \text{ (g to } \mu\text{g)}}{100 \times 1}$$

NESILs were then estimated from these EC3 values by applying the correlation factor for mouse to human as follows:

$$\text{Log}(10) \text{ human NESIL } \mu\text{g}/\text{cm}^2 = \text{Log}(10) \text{ EC3 } \mu\text{g}/\text{cm}^2 \times 1.16 - 0.64$$

Finally, the AEL for each chemical was determined by applying an appropriate SAF:

$$\text{AEL } \mu\text{g}/\text{cm}^2 = \text{NESIL } \mu\text{g}/\text{cm}^2 / \text{SAF}$$

Since the SAF used for an individual risk assessment varies according to the nature of the dermal exposure (in terms of product type and usage, Api et al., 2008), the AELs for ingredients used in different products will vary. In this analysis, and for the purposes of demonstrating the calculation of the DST, two example product types have been chosen, shampoo and deodorants. Shampoo provides an example of a widely used rinse-off product, where exposure to a given ingredient would be expected to be low, and deodorant is a widely used leave on product, providing a higher level of exposure.

The SAFs for these product types were determined according to the rationale laid out in Table 1. It should be noted that, whilst a value of 3 has been used for the matrix SAF in both cases, this is a generalisation since, in a standard sensitisation QRA, this figure is applied to compensate for differences between vehicle/matrix in the experimental situation and the consumer product formulation (Api et al., 2008).

Fig. 3 shows the fitted gamma distributions of the AELs for shampoo and deodorant, again using a negative log(10) scale.

2.4. Establishing an acceptable risk

In order to set a DST it is first necessary to define what level of risk is acceptable. In other words, if an untested chemical were to be used in a product delivering a defined skin dose level, what is the probability that the AEL for that chemical would be exceeded?

In his analysis of carcinogens, Rulis (1986) concluded that

Assuming 1:5 chemicals to be carcinogens, 95 out of 100 Threshold of Regulation decisions will lead to a risk of less than 1 in 10^6 .

It is proposed that a similar figure could be adopted for determining a DST.

2.5. Determining DST values

Based on the distributions shown in Fig. 3, probabilities that an untested chemical would exceed the AELs at a given dose can be established for our example products (shampoo and deodorants). These are shown in Table 2.

From the table it can be seen that, assuming 20% of chemicals to be sensitisers, use of DSTs of 1.64 and 0.55 $\mu\text{g}/\text{cm}^2$ for shampoo and deodorants, respectively, would give a 95% probability of not exceeding the AEL. These values would, of course, apply to any products where SAFs of 100 or 300 can be applied.

3. Discussion

The aim of this paper is to present a probabilistic method that may be used to establish a DST applicable to low level exposure to cosmetic and personal care product ingredients. In doing so, it is not the intention to establish precisely what this DST should be. This will require a wider discussion of what is considered to be an acceptable risk. As has been previously stated, Rulis (1986) considered that a 5% probability that the carcinogenic risk for an untested chemical would exceed $1:10^6$ was acceptable. It is sug-

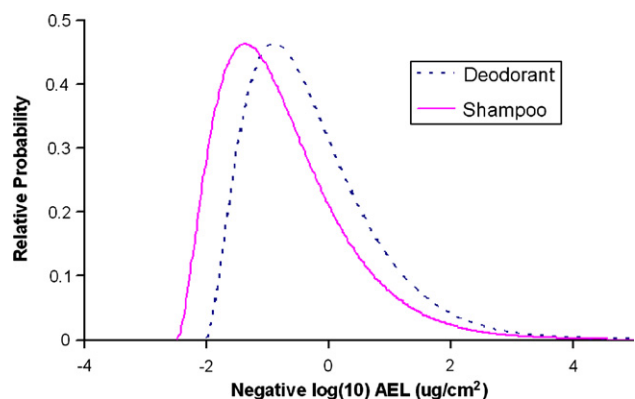


Fig. 3. Distribution of AELs for 167 chemicals in the Master Table for the example products, shampoo and deodorant, plotted on a negative log(10) scale.

gested that this yardstick could be used to establish DSTs from the described analysis.

An analysis has been carried out using the principles established by Rulis (1986) in setting a systemic TTC based on the carcinogenicity endpoint (see Table 3 for a comparison of the two approaches). One fundamental difference in the approaches is that the DST is not a single value, but will vary from one product type to another where different SAFs are applied. Also, since the DST is expressed as $\mu\text{g}/\text{cm}^2$, conversion of the DST to a concentration in the product will vary depending on how the product used (ie. amount of product used per surface area of skin, rinse-off vs. leave-on, etc.). So, for example, skin exposure (dose per unit area) to an ingredient included in a hand cream will be higher than for the same level of ingredient in a shampoo. As a result, the acceptable concentration determined from the DST will be considerably lower for a hand cream than for a shampoo, even though the same SAF is applied.

Whilst it is concluded from this analysis that such methodology can provide a robust scientific basis for setting a DST, it is recognised that shortcomings in the data set used will provide a certain amount of uncertainty in the process, and in the results obtained. The main sources of uncertainty are discussed below.

In establishing the incidence of sensitising chemicals in the world of chemicals the ELINCS database published by the ECB has been used. This database was chosen as it provides a large pool of chemicals that have been tested for sensitisation for regulatory purposes (i.e. they were not tested because they were suspected of being sensitisers), and therefore more likely to provide an unbiased indication of the true incidence of sensitisation. Other data sets investigated, including the published Master Table (Gerberick et al., 2005) and unpublished proprietary data did not provide unbiased numbers since, in the most part, chemicals were tested because they were suspected of being sensitisers, or only positive results were reported. Although the ELINCS database is unbiased in this respect, it could, of course, be argued that it is not generally

Table 3

Comparison of the main aspects of the DST approach to the TTC approach of Rulis (1986)

	DST approach	TTC approach
Endpoint	Sensitisation	Carcinogenicity
% of chemicals with hazard	20	20
Bioassay	Mouse LLNA	Rat/mouse long term bioassays
Hazard metric	EC3	TD50
Exposure units	$\mu\text{g}/\text{cm}^2$	$\text{mg}/\text{kg bw}/\text{day}$
Data set	167 sensitisers	343 carcinogens
Distribution plot	Negative log(10)	Negative log(10)
Fitted distribution	Gamma	Normal
Extrapolation to man	Apply correlation formula and SAF	Linear extrapolation
Risk metric	AEL/CEL ratio = 1	Virtually Safe Dose
Probability of acceptable risk	TBD*	95%

* To be decided.

representative of the world of chemicals or of chemicals used in cosmetic and personal care products. Indeed, looking at the statistics published on the ECB website, it can be seen that only 6% of the new chemicals were registered as cosmetic and personal care product ingredients. A much larger proportion of the chemicals (25%) were intermediates (i.e. chemicals used for the synthesis of other chemicals) which might be expected to be more chemically reactive than cosmetic and personal care product ingredients, and therefore more likely to be sensitisers. Other chemicals include colouring agents, photochemicals and process regulators, making up a further 27% of the chemicals. However, in the database it is not possible to identify which chemicals were registered for each function, and a more detailed analysis is therefore not possible. However, use of a dataset that contains such a diverse range of chemicals, including many reactive intermediates, will, if anything, provide a high estimate of the percentage of sensitisers in the world of chemicals. This only serves to make the setting of the DST more conservative.

It is also recognised that the data set used to establish the distribution of sensitisation potency is relatively small, including just under half of the number of chemicals used by Rulis (1986). Use of such a limited data set could undoubtedly introduce uncertainty in setting a DST, particularly if the chemicals represented are biased towards the lower end of the sensitisation potency scale, and the more potent chemicals are not fully represented in the data set. However, at this moment in time the Master Table is the largest publicly available data set of results for chemicals tested in the LLNA, and the data appear to fit a gamma distribution very well. It is hoped that additional information can be made available in the future that will increase the size of this data set, and help to reduce uncertainties in setting DSTs. The Master Table is currently being updated with a view to establishing a larger data set of

Table 2

Probabilities that an untested chemical would exceed the AELs at given DSTs estimated from the distributions shown in Fig. 3

Percentile from distribution in Fig. 3	DST shampoo ($\mu\text{g}/\text{cm}^2$)	DST deodorants ($\mu\text{g}/\text{cm}^2$)	Probability of AEL < 1 given the following percentage of sensitisers			
			50	30	20	10
95	0.06	0.02	97.5	98.5	99	99.5
90	0.24	0.08	95	97	98	99
80	1.00	0.33	90	94	96	98
75	1.64	0.55	87.5	92.5	95	97.5
70	2.50	0.83	85	91	94	97
60	5.04	1.68	80	88	92	96
50	9.10	3.03	75	85	90	95

Table 4Chemicals from the Master Table where the AEL would be exceeded if a DST based on the 95th percentile from Table 2 were used to determine a maximum CEL

Chemical	EC3 [*]	AEL/CEL	Chemical	EC3 [*]	AEL/CEL
Benzo[a]pyrene	0.23	0.0003	1,4-Phenylenediamine	40.0	0.11
Oxazolone	0.75	0.001	Dimethyl sulfate	47.5	0.13
1-Chloromethylpyrene	1.25	0.002	2,5-Diamino-toluene	50.0	0.14
7,12-Dimethylbenz[a]anthracene	1.5	0.003	Benzyl bromide	50.0	0.14
5-Chloro-2-methyl-4-isothiazolin-3-one	2.25	0.004	Lauryl gallate (dodecyl gallate)	75.0	0.22
p-Benzoquinone	2.48	0.004	Methyl dodecane sulphonate	97.5	0.3
1-Methyl-3-nitro-1-nitrosoguanidine	7.5	0.016	2-Aminophenol	100.0	0.3
Tetrachlorosalicylanilide	10.0	0.02	2-Methyl-5-hydroxyethylaminophenol	100.0	0.3
4-Nitrobenzyl bromide	12.5	0.03	2-Nitro-p-phenylenediamine	100.0	0.3
N-Methyl-N-nitrosourea	12.5	0.03	3-Phenylenediamine	122.5	0.4
1-Chloro-2,4-dinitrobenzene	15.0	0.03	4-(N-Ethyl-N-2-methan-sulphonamido-ethyl)-2-methyl-1,4-phenylenediamine	150.0	0.5
Potassium dichromate	20.0	0.05	Isopropyl isoeugenol	150.0	0.5
2,4,6-Trichloro-1,3,5-triazine (cyanuric chloride)	22.5	0.06	Formaldehyde	152.5	0.5
Glutaraldehyde	25.0	0.06	2-Methyl-4H,3,1-benzoxazin-4-one	175.0	0.6
Hydroquinone	27.5	0.07	Methyl hexadecane sulphonate	200.0	0.7
Fluorescein-5-isothiocyanate	35.0	0.09	4-Methylaminophenol sulfate	200.0	0.7
Propiolactone	37.5	0.1	1,2-Dibromo-2,4-dicyanobutane	225.0	0.8

^{*} EC3 expressed as µg/cm².

chemicals. On publication, this larger dataset can be used to determine the impact of adding more sensitisers on the distribution of EC3 values, and to help reduce uncertainty in the analysis.

Finally, the analysis used is based on setting a human threshold of sensitisation (NESIL) estimated from animal assay data (mouse LLNA). Again, the database used to provide the value used to extrapolate from mouse to humans is limited, and is based mainly on historical human assays. However, from the data available it is reasonable to assume that, for most chemicals, the LLNA EC3 is a good predictor of potency in humans. In this case, however, it is unlikely that any significant amount of additional data will be generated in human studies in the future that might reduce the level of uncertainty. So, although it is considered acceptable in the most part to use the HRIPT to confirm the lack of sensitisation of an ingredient, there are ethical considerations that rule out the use of the assay to generate positive sensitisation data (and therefore to determine a genuine NOEL).

Acknowledging that there are uncertainties in the analysis due to limited data sets, it is proposed that such an approach can be used to set a pragmatic DST for cosmetic and personal care ingredients where the consumer dermal exposure will be limited. That said, it should be remembered that this is a probabilistic approach, and the DST will not be protective for every single chemical. This is no different from the approach that Rulis took when looking at carcinogenicity. If the DST were set at the 95th percentile level, then it follows that there is a 5% probability that an untested chemical would give an undue risk. Table 4 shows the list of chemicals from the Master Table that would give an AEL:CEL ratio of less than 1 if the DST were to be set at the 95th percentile. It can be seen that many of these chemicals are known to be reactive, and would not be considered as ingredients in cosmetic and personal care products. The one material that stands out is 5-chloro-2-methyl-4-isothiazolin-3-one, CMI, which is a component of a preservative mixture currently used in cosmetic and personal care products. The sensitisation potential of this ingredient is well characterised, and levels of its use in products are restricted in order to avoid adverse effects in consumers. Other chemicals in the list that also are used in cosmetic products are a number of oxidative hair dyes, including 1,4-phenylenediamine and 2,5-diamino-toluene, and formaldehyde, which was, until recently, used as a preservative. Again, the sensitisation risk of these materials is well characterised, and consumer use controlled accordingly.

From this simple examination of “at risk” chemicals, it can be seen that even the inclusion of a simple step such as a rule base that precluded the use of the DST for chemically reactive chemicals

such as preservatives and oxidative dyes would make the DST even more protective. It may also be possible to refine the DST, or create a tiered DST (similar to that of Cramer et al., 1978) by making use of expert systems to identify sensitisation alerts in molecules (e.g. DEREK, Barratt et al., 1994), mechanistic chemistry approaches such as those described by Aptula and Roberts (2006) or by using in vitro tests for sensitisation as and when these become available.

One final note is that the DST will be protective for the induction of sensitisation, and not necessarily for the elicitation of sensitisation. This is because it is based on the AELs, which are the exposure levels estimated not to cause sensitisation induction in humans. It is generally accepted that, in subjects already sensitised to a chemical, elicitation of reactions will occur at a lower level of exposure than that required to induce the sensitisation.

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