



# Application of the dermal sensitization threshold concept to chemicals classified as high potency category for skin sensitization assessment of ingredients for consumer products

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## ABSTRACT

Skin sensitization evaluation is a key part of the safety assessment of ingredients in consumer products, which may have skin sensitizing potential. The dermal sensitization threshold (DST) concept, which is based on the concept of the thresholds of toxicological concern, has been proposed for the risk assessment of chemicals to which skin exposure is very low level. There is negligible risk of skin sensitization if a skin exposure level for the substance of interest was below the reactive DST which would protect against 95% of protein-reactive chemicals. For the remaining 5%, the substance with the defined knowledge of chemical structure (i.e., High Potency Category (HPC) rules) needs to be excluded from the application. However, the DST value for HPC chemicals has not yet been proposed. In this study, we calculated the 95th percentile probabilities estimate from distributions of skin sensitization potency data and derived a novel DST for HPC chemicals (HPC DST) of 1.5  $\mu\text{g}/\text{cm}^2$ . This value presents a useful default approach for unidentified substances in ingredients considering, as a worst-case scenario, that the unidentified compound may be a potent skin sensitizer. Finally, we developed a novel risk assessment workflow incorporating the HPC DST along with the previously published DSTs.

## 1. Introduction

Skin sensitization is the first step in the development of allergic contact dermatitis, which is a local skin reaction classified as a delayed-type (type IV) hypersensitivity response and characterized by redness, swelling, and itching (Murphy et al., 2012). A key part of safety assessments for consumer health is the evaluation of skin sensitization (Kimber et al., 2011). Animal testing, such as testing on guinea pig models (Buehler, 1965; Magnusson and Kligman, 1969; OECD, 1992) and the murine local lymph node assay (LLNA) (OECD, 2010), have conventionally been used to identify the skin sensitization potential of substances. However, due to increasing regulatory and ethical demands, a risk assessment approach without animal testing is desirable. Focusing on the first three key events (covalent binding to proteins, keratinocyte responses, and dendritic cell activation) of the adverse outcome

pathway for the sensitization induction phase (OECD, 2012), several non-animal test methods for skin sensitization have been developed and adopted as OECD test guidelines (OECD, 2018a, 2018b, 2019).

In addition to these non-animal test methods, the *de minimis* approach for risk assessment, which focuses on the exposure level, is important for the efficient and cost-effective risk assessment of substances with low exposure levels (e.g. impurities) for which there is little or no toxicological information. Based on the principle of establishing a human exposure threshold value for all chemicals, below which there would be a very low probability of risk to human health, the threshold of toxicological concern (TTC) is a pragmatic *de minimis* approach for risk assessment. This concept was initially proposed by Frawley (1967). Rulis (1986) performed the real estimation by applying a probabilistic assessment of a carcinogenicity potency database (Gold et al., 1984) to derive a regulatory threshold of 0.5 ppb in foods (i.e., an intake of 1.5

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$\mu\text{g/day}$  in adults) (US Food and Drug Administration, 1995). In addition, Munro et al. (1996) examined a database of systemic toxicity studies, and proposed the use of TTC related to other endpoints than carcinogenicity based on an exploration of the relationship between toxicity and chemical structures, which were divided into three structural classes developed by Cramer et al. (1978). Further studies suggested the expansion of the TTC approach to topical application of cosmetic products, including the use of default skin penetration values (Blackburn et al., 2005; Kroes et al., 2007; Yang et al., 2017).

Using the existing threshold for the induction of skin sensitization (Kimber et al., 1999; Robinson et al., 2000; Boukhman and Maibach, 2001), it is possible to conduct exposure-based a quantitative risk assessment (QRA) to determine safe use levels of skin sensitizing substances based on the evaluation of hazard (i.e., the sensitization potency, which is inversely proportional to a no effect level (No Expected Sensitization Induction Level, NESIL), expressed as dose per unit area of exposed skin ( $\mu\text{g}/\text{cm}^2$ ) and the quantification of the actual consumer exposure (Api et al., 2008). If it is possible to define a NESIL, an acceptable exposure level (AEL) for the substance of interest can be derived among consideration of sensitization assessment factors (SAF) (Api et al., 2008; Basketter and Safford, 2016). SAFs are applied for the extrapolation of NESILs based on an experimental setting—considering the actual use condition of the consumer—to a safe level. A comparison of the AEL with the consumer exposure level (CEL) enables the assessment of the risk of skin sensitization. Based on the QRA approach, the dermal sensitization threshold (DST) approach has been proposed as a concept of a skin exposure level for the substance of interest below which there is no appreciable risk for skin sensitization (Safford, 2008). Using a probabilistic analysis of potency data from a data set of chemical sensitizers, the DST applies the concept of the TTC to assess the risk of skin sensitization. A report of a similar approach based on human sensitization data for 53 fragrance allergens describes the threshold of sensitization concern concept (Keller et al., 2009). Subsequently, further reports have focused on the mechanism of the molecular initiating event in the induction of skin sensitization (Safford et al., 2011; 2015) with an analysis of LLNA data for 363 chemicals (271 sensitizers and 92 non-sensitizers) collected from four different published sources. To induce skin sensitization, the chemical needs to be reactive to endogenous proteins, or to have the potential to abiotically (through autoxidation) or through metabolic activation, be reactive in the skin (Lepoittevin, 2006). Chemicals can be categorized into reactive domains (Michael acceptors, Schiff bases,  $\text{S}_{\text{N}}2$ ,  $\text{S}_{\text{N}}\text{Ar}$  electrophiles, acylating agents, and “special cases”) or classified as non-reactive, based on their structure (Aptula and Roberts, 2006). Safford et al. (2011) established the DST of  $900 \mu\text{g}/\text{cm}^2$  for substances classified as non-reactive (non-reactive DST) and Safford et al. (2015) established the DST of  $64 \mu\text{g}/\text{cm}^2$  for substances classified as reactive (reactive DST). The non-reactive DST and the reactive DST were based on the 95th percentile-based probability estimate from distributions of skin sensitization potency data for chemicals in the non-reactive and reactive domains, respectively. Structure-based classification is similar to the structure-based filters used on TTC approaches—such as the Cramer classification (Cramer et al., 1978; Munro et al., 1996) or the cohort of concern for carcinogens (Kroes et al., 2004). However, the reactive DST would not be protective for the high potency sensitizers whose EC3—that is, the estimated concentration inducing a three-fold stimulation index (the index of lymph node cell proliferative responses induced by topical exposure of mice to the test chemical) as compared to the respective vehicle—is  $<64 \mu\text{g}/\text{cm}^2$ . To address this risk, chemical structures in the data set were examined using the HPC rule (Roberts et al., 2015). For a substance classified as reactive, evaluation as to whether the substance is classified as HPC or not is necessary. Thus, in the previously proposed process incorporating the existing DST, there were two criteria upon which to decide its applicability; 1) the non-reactive DST can be applied to the substances classified as non-reactive but not classified as reactive, and 2) the reactive DST can be

applied to the substances classified as reactive but not classified as HPC. It has been reported that the DST approach can be used for the risk assessment of skin sensitization for ingredients of consumer products such as fragrances (Api et al., 2015a). There is, however, no proposal for the DST value for chemicals classified as HPC.

Ingredients formulated into consumer products contain intentional and un-intentional (e.g. impurities, carry-overs) constituents. The quantification of the target substance using analytical standard is not possible when the chemical structure of the substance remains unidentified. However, if the concentrations (weight %) of the major components in the ingredient were quantified, the combined concentrations of any remaining components containing unidentified substances could also be calculated by the difference. There are also cases when unidentified contents can be quantified against a range of standards with suitable physicochemical characteristics.

In the majority of cases, it is likely that the skin sensitization risk of impurities in the ingredients will—due to their very low exposure levels—be low. There is, however, no guidance presently on procedures for the risk assessment of unidentified substances in the ingredients. Considering the worst-case scenario, as the default approach, it should be assumed that the entire unidentified content may consist of very strong sensitizers such as HPC chemicals. In a practical risk assessment of skin sensitization for ingredients, there is a need to develop an approach for minor amounts of unidentified substances.

To this end, here we determined a novel DST value for chemicals classified as HPC (HPC DST). Of the 363 chemicals originally analyzed in (Safford et al., 2011; 2015), 56 chemicals have already been classified as HPC chemicals. However, some of these 56 HPC sensitizers had EC3 values derived from an extrapolation based on less-than-ideal dose response data (not the linear portion) with a high SI value that did not approach 3 at the lowest dose. Thus, we tried to derive estimated EC3 values for these chemicals using the single dose probit extrapolation method derived by Roberts, 2015. In addition, analyzed EC3 values of chemicals were collected from one specific reference even though multiple data were publicly reported. To address this issue, the fixed location parameter was provided as a representative value of multiple studies that were collected from the expanded data set using the publicly available LLNA data from the OECD Toolbox database and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) database. Finally, incorporating the previously proposed DSTs and the HPC DST, a risk assessment workflow was developed.

## 2. Materials and methods

### 2.1. HPC rules

HPC rules were previously proposed based on the examination of the chemical structure of high potency sensitizers whose EC3 was  $<64 \mu\text{g}/\text{cm}^2$  of reactive DST (Roberts et al., 2015). HPC rules are summarized in Table 1.

### 2.2. Data set

For setting a novel DST value that is applicable to the chemicals classified as HPC, the following three data sources of chemicals that had LLNA data were used.

- OECD QSAR Toolbox 4.2 (freely available at [www.qsartoolbox.org](http://www.qsartoolbox.org)).
- NICEATM LLNA database (freely available at

<https://ntp.niehs.nih.gov/pubhealth/evalatm/test-meth/od-evaluations/immunotoxicity/llna/index.html#NICEATM-LLNA-Database>).

**Table 1**  
High Potency Category Chemicals (HPC) rules according to Roberts et al., 2015.

| HPC rules                         | Detailed descriptions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 Protein derivatization agents   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| 2 Direct acting Michael acceptors | <p>2a Quinones, di-imines, quinone-imines, tautomeric forms with quinone-type (ortho or para) structure or quinone-methide-type (para only) structure</p> <p>2b Electronegative substituents on the double bond, such as halogen, alkoxide (–OR), thio (–S–), or with CHO, –COR, –CO<sub>2</sub>R, –CONR<sub>2</sub> activating groups<br/>C=C–X, where X is a stronger activating group than carbonyl (e.g. NO, –NO<sub>2</sub>, SO<sub>2</sub>R, SO<sub>3</sub>R, 2,4-dinitrophenyl)<br/>Compounds with the sub-structure X–C–CH–M where X is halogen (or pseudohalogen) and M is a strong Michael activating group</p> <p>2c Michael acceptors with more than one activating group on the double bond</p> |
| 3 Pro/pre-Michael acceptors       | 3a 1,2- and 1,4- dihydroxy, di-amino, aminohydroxy benzenes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 4 Schiff base electrophiles       | 4a Schiff base electrophiles meeting the HPC criteria (Homologues of these reference compounds* if more hydrophobic)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 5 Acyl transfer agents            | <p>5a Isocyanates (–N=C=O bonded to carbon) and isothiocyanates (–N=C=S bonded to carbon)</p> <p>5b Anhydrides if the log P value is greater than 1</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 6 SN <sub>2</sub> electrophiles   | <p>6a Benzylic halides (ArCH<sub>2</sub>X) and pseudohalides (–C=C–CH<sub>2</sub>–X), halides and pseudohalides with substructures –COCH<sub>2</sub>X, –OCH<sub>2</sub>X</p> <p>6b Methylating agents (CH<sub>3</sub>–X, for X = OSO<sub>2</sub>OR (any R) and for X = OSO<sub>2</sub>R (R = aryl, R = ≥C<sub>12</sub> alkyl or alkenyl)<br/>N-methyl nitroso amido compounds (Me–N(N=O)–[C=O/N–]–)</p> <p>6c Special cases include beta-propiolactone and the diol epoxides derived from polycyclic aromatic hydrocarbons (PAHs) with 4 or more aromatic rings fused together, PAHs with carcinogenicity alerts</p>                                                                                         |
| 7 SNAr electrophiles              | <p>7a SNAr electrophiles with more than one activating group (any aromatic compound with a leaving group and two or more activating groups selected from nitro, nitroso, ring nitrogen or groups more electronegative than nitro, in ortho + ortho or ortho + para positions.<br/>The leaving group X may be considered as any covalently bonded group other than hydrocarbon, H, OH, amino (NH<sub>2</sub>, NHR, NR<sub>2</sub>), CONH<sub>2</sub>, CONHR, CONR<sub>2</sub>, CO<sub>2</sub>R (R = H, alkyl or aryl), CO<sub>2</sub>H.)</p>                                                                                                                                                                  |
| 8 Organic peroxides               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| 9 Structurally complex chemicals  | Compounds that cannot be confidently assigned to any of the other reaction mechanistic domains, and cannot be confidently classified as non-reactive (e.g. Tetrachlorosalicylanilide)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 10 Pre-electrophiles              | <p>*n-C<sub>4</sub>H<sub>9</sub>OCO.CHO (Butyl glyoxylate),<br/>CH<sub>3</sub>COCO<sub>2</sub>C<sub>6</sub>H<sub>13</sub> (Hexyl pyruvate),<br/>C<sub>2</sub>H<sub>5</sub>C<sub>6</sub>H<sub>4</sub>COCOC<sub>2</sub>H<sub>5</sub>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>COCOC<sub>2</sub>H<sub>5</sub>,<br/>PhCOCOC<sub>3</sub>H<sub>7</sub>, Any with –COCOC–</p>                                                                                                                                                                                                                                                                                                                                       |

- Original DST database which was utilized to derive the non-reactive and reactive DST (Safford et al., 2011; 2015)

LLNA studies of inorganic chemicals and of materials with no clearly defined chemical structure (e.g. plant extracts) were excluded due to being out of the domain for HPC classification (Safford et al., 2015). We included data sets in this study in the following order: (1) OECD QSAR Toolbox database, (2) NICEATM LLNA database, and (3) Original DST database. From these three data sources, we excluded the data that met the following criteria of inadequate quality:

- LLNA studies with insufficient data reliability or with a dose–response pattern that does not enable a reliable EC<sub>3</sub> to be determined by linear interpolation or log-linear extrapolation (Ryan et al., 2007)
- 4-(Bromomethyl)-benzoic acid ethyl ester (CAS: 26496-94-6), as an outlier with an extremely small reported EC<sub>3</sub> of  $1.1 \times 10^{-8}\%$  which came from an extrapolation with a high SI value and less-than-ideal dose response data
- Methyl hexadecane sulfonate (CAS: 4230-15-3) which is reported as negative in the LLNA (Gerberick et al., 2005) but meets the structural criteria for classification as HPC (Roberts et al., 2015). However, this LLNA negative result is considered anomalous due to being strongly positive in a guinea pig test (Roberts and Basketter, 1997).

After removing these studies, 705 chemicals (512 sensitizers and 193 non-sensitizers) were collected for the analysis.

EC<sub>3</sub> expressed as dose per unit area of exposed skin ( $\mu\text{g}/\text{cm}^2$ ) was calculated from EC<sub>3</sub> values as  $\text{EC}_3\% \times 250$  based on the application of 25  $\mu\text{L}$  test solutions to 1  $\text{cm}^2$  area of mouse ears. In addition, EC<sub>3</sub> [ $\mu\text{g}/\text{cm}^2$ ] was used as a sensitization potency value for humans without extrapolating from mouse to human data, based on the assumption that LLNA EC<sub>3</sub> values correlate with human NESIL (Basketter et al., 2005, 2018; Api et al., 2015b).

### 2.3. Median-like location parameter for representative EC<sub>3</sub> value

For many of the analyzed sensitizers, more than one LLNA study was available so an approach to provide a representative value for each chemical was required. For statistical analysis based on the assumption that the distribution of sensitization potencies of analyzed sensitizers is representative of all known and unknown HPC chemicals, the fixed location parameter was considered desirable. The conservative approach of using the lowest EC<sub>3</sub> value available was considered inappropriate because no fixed location parameter is estimated, the location parameter instead depending on the number of repeats (e.g. lowest value of three repeats is the 33%-quantile, while the lowest value of 10 repeats is the 10%-quantile of the underlying distribution). In addition, it was required to refrain from selecting one result from repeated studies by expert judgment because this approach not only has a high risk of bias but also disregards valuable information on variability and uncertainty.

Thus, in this study, the median-like location parameter (Hoffmann et al., 2018) was used. This parameter was defined as “the median for substances” for repeated studies with an EC<sub>3</sub> in more than half of the repeats. For substances with negative values in at least half of the repeated studies, the parameter was defined based on reviewing the negative studies as follows. When we found the maximum concentration tested in a given negative study to be lower than the median EC<sub>3</sub> of the positive studies for the same chemical, we excluded the respective negative studies, because the validity was considered limited due to too low tested concentrations. From the remaining negative and all positive studies, the median was used as a location parameter. Where half of the repeat studies were negative and half were positive, the highest EC<sub>3</sub> value was defined as the modified median. Studies testing the same chemical were considered as repeats regardless of the solvents/vehicles used.

### 2.4. Probability distribution analysis

After the definition of representative EC<sub>3</sub> values for each analyzed sensitizer, negative log(10) EC<sub>3</sub> values of the analyzed sensitizers classified as HPC were plotted in a probability distribution. In deriving the HPC DST, gamma distribution was fitted as in previous DST analysis (Safford et al., 2011; 2015).

## 2.5. Single-dose probit extrapolation method (SDPEM)

The dose–response relationship, as with many other toxicological endpoints, is fundamentally fitted to the probit function for skin sensitization as “Pr (Response) = a Log (Dose) + b” (Roberts and Williams, 1982). Roberts, 2015 proposed a probit function to estimate an EC3 value from a single dose of LLNA. While SDPEM was originally developed to estimate potency using a reduced version of the LLNA (rLLNA) (Kimber et al., 2006), it can also be used to recalculate the EC3 value of a chemical whose EC3 value came from an extrapolation using less-than-ideal dose response data with a high SI value. The formula was derived based on the assumption that the highest SI reported in the LLNA database (Gerberick et al., 2005) is close to the  $SI_{max}$  value and performing the analysis using an arbitrary selection of compounds for which EC3 values had been derived by interpolation with dose response data from Ashby et al. (1995).

$$\text{Log EC3} = (\text{Pr}_{78.5}(2) - \text{Pr}_{78.5}(\text{SI}-1) + 0.87 \times \text{Log (Dose)}) / 0.87 \cdots (*)$$

## 3. Results

### 3.1. HPC chemicals in the data set

Of the 705 chemicals in the database, 116 chemicals were classified as HPC (Table 2) and listed in the Supplementary Table. Distributions of the potencies as defined in the LLNA of 116 sensitizers classified as HPC are shown in Fig. 1.

### 3.2. DST for HPC chemicals

The DST for HPC chemicals (HPC DST) value was derived such that 95% of HPC chemicals in the DST database are either non-sensitizers or have potencies less than this value, as with previously reported DST approaches (Safford et al., 2011; 2015). To derive this value, we fitted gamma probability distributions of “gamma (4.3808, 0.4605), shift (−3.987).”

Next, the 95th percentile from the distribution in Fig. 1 was taken for the derivation of the HPC DST. This value is −0.168 (negative log (10) EC3)—equivalent to 1.5  $\mu\text{g}/\text{cm}^2$ . This value was based on the 95% probability of analyzed HPC chemicals. It is expected that the remaining 5% of sensitizers classified as HPC have EC3 values which are lower than this value. In Table 3, among the 116 analyzed sensitizers, five sensitizers had EC3 values lower than 1.5  $\mu\text{g}/\text{cm}^2$ .

### 3.3. Calculation of EC3 values using SDPEM for chemicals with EC3 values lower than HPC DST

Five of the 116 analyzed sensitizers had reported EC3 values lower than 1.5  $\mu\text{g}/\text{cm}^2$ . Two sensitizers—oxazolone (CAS: 15646-46-5) and chorothalonil (CAS: 1897-45-6)—had EC3 values derived by linear interpolation. However, the EC3 values of the remaining three chemicals were estimated by log-linear extrapolation from less-than-ideal dose-response data (not the linear portion) with a high SI value that, at the lowest dose, did not approach 3 (Table 4), contrary to the recommendation of Ryan et al. (2007) for application of the log-linear extrapolation method. Here we calculated the EC3 values of these three chemicals using the  $\text{Pr}_{78.5}(\text{SI}-1)$  function of formula (\*) in section 2.5 for the lowest dose data, which derived the most conservative EC3 values in all doses

**Table 2**

Numbers of chemicals classified as High Potency Category Chemicals (HPC) in the analyzed dataset.

|         | Sensitizers | Non-sensitizers | Total |
|---------|-------------|-----------------|-------|
| HPC     | 116         | 0               | 116   |
| Non-HPC | 396         | 193             | 589   |
| Total   | 512         | 193             | 705   |

(data not shown). It should be noted that the novel HPC DST was derived based on the originally published data of section 2.2 and not the recalculated EC value. The EC3 values derived by the SDPEM were used only for discussion of the practical risk of the remaining 5%.

The resulting, calculated EC3 values of 4'-hydroxychalcone (CAS: 2657-25-2) and (4R,5S)-1,5-Dimethyl-3-(1-oxo-2-propenyl)-4-phenyl-2-imidazo-lidinone (CAS: 139109-23-2) were greater than 1.5  $\mu\text{g}/\text{cm}^2$ . For 1-chloromethylpyrene (CAS: 1086-00-6), even when we applied the SDPEM, the EC3 value was less than the HPC DST value.

### 3.4. Risk assessment workflow using the DST approaches

Based on the HPC DST concept described above and the previously reported DST approaches for chemicals in the non-reactive/reactive domain (Safford et al., 2011; 2015), a proposed workflow for overall risk assessment is shown in Fig. 2.

In the overall process, there are two decision points based on chemical structure information. The first one is the decision as to whether the chemical belongs to a reactive domain based on chemical structure rules (Aptula and Roberts, 2006). This step is necessary to determine whether the non-reactive DST of 900  $\mu\text{g}/\text{cm}^2$  can be applied. The second one, for chemicals that are classified as reactive, is the decision as to whether the chemical falls into the HPC classification of Table 1 (Roberts et al., 2015). This step is necessary to determine whether the reactive DST of 64  $\mu\text{g}/\text{cm}^2$  can be applied. The reactive DST cannot be protective to chemicals classified as HPC. Here, for chemicals classified as HPC, the HPC DST of 1.5  $\mu\text{g}/\text{cm}^2$  is applied.

If the target substance does not have chemical structure information, it is considered that the HPC DST can be applied as the default approach because the HPC DST covers the risk of the most potent sensitizers. Thus, in the absence of structural information for a substance of interest, a risk assessor would use the HPC DST until further information can be obtained. In addition, to determine which DST value (the non-reactive, reactive or HPC DST) to be applied, the appropriate background information for the substance of interest could be useful. The assumed chemical structure of hydrolysis products or reaction by-products is good examples for the information to determine which DST value to be applied.

In cases where the CEL of a chemical does not exceed the AEL based on each DST value, the proposed exposure to the chemical is considered acceptable in terms of the risk of skin sensitization. In contrast, a substance of interest must be further investigated if the CEL exceeds the AEL based on each DST value.

## 4. Discussion

The concept of the DST approach has been proposed based on the principles of the TTC concept, as a level below which—even when the sensitization potency of a chemical has not been identified—there is no appreciable risk of skin sensitization (Safford, 2008). It has been reported that the reactive DST value of 64  $\mu\text{g}/\text{cm}^2$  would be protective against 95% of reactive chemicals (Safford et al., 2015). To address the risk of the remaining 5% for which the reactive DST would not be protective, chemical structures in the data set were examined using the HPC approach (Roberts et al., 2015) and applying the reactive DST to the substances classified as reactive but not classified as HPC. Here, we proposed a novel DST value for HPC chemicals based on the data set of 705 chemicals expanded using the OECD QSAR Toolbox database and the NICEATM LLNA database. For chemicals with repeated LLNA studies, we used the median-like location parameter to define the representative EC3 value. Based on this representative EC3 value, the HPC DST value has been derived to be 1.5  $\mu\text{g}/\text{cm}^2$  based on a 95% probability of analyzed HPC chemicals.

A remaining problem for assessing the risk of skin sensitization from impurities found in ingredients was the lack of a proposed threshold value for HPC chemicals. When considering the worst-case scenario, as

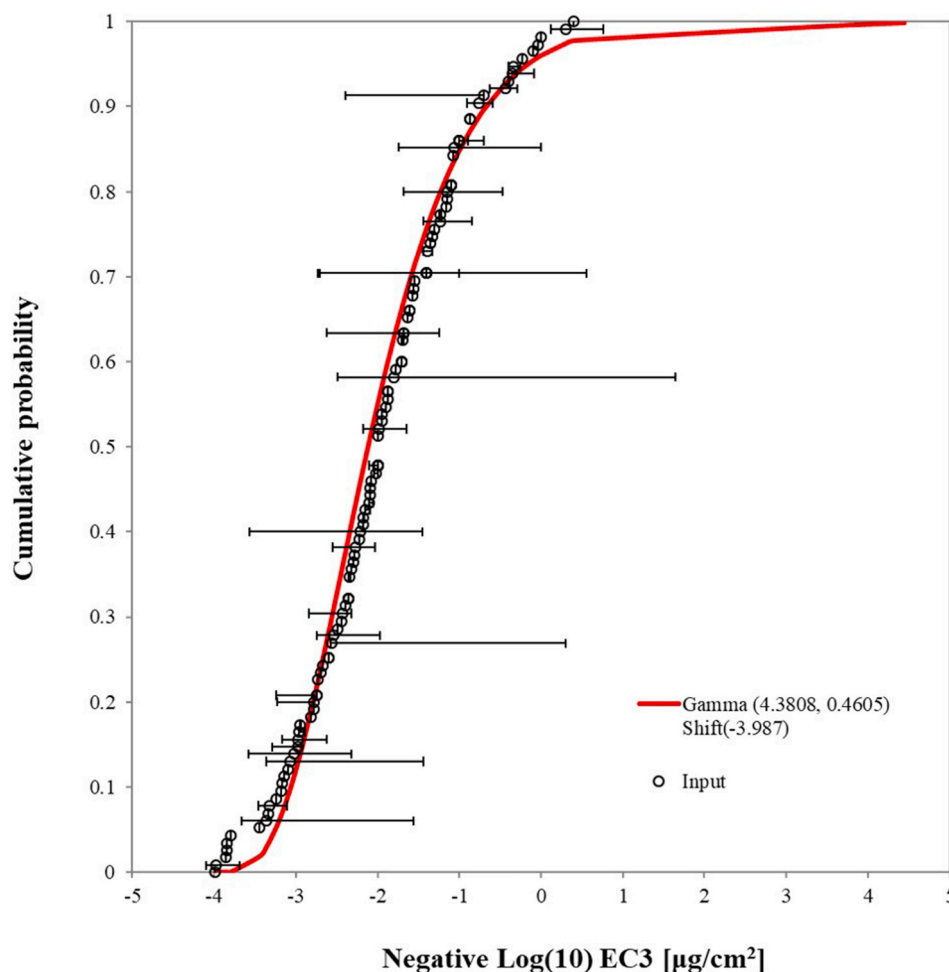


**Fig. 1.** Cumulative probability distribution of negative log(10) EC3 [ $\mu\text{g}/\text{cm}^2$ ] for HPC chemicals (Each end of bar: maximum and minimum EC3 of repeated studies, respectively).

**Table 3**

Five sensitizers had lower EC3 values than the HPC DST of  $1.5 \mu\text{g}/\text{cm}^2$  based on 95th percentiles in the gamma distributions shown in Fig. 1.

| Chemicals                                                             | CAS         | LLNA                                     |
|-----------------------------------------------------------------------|-------------|------------------------------------------|
|                                                                       |             | Median EC3 [ $\mu\text{g}/\text{cm}^2$ ] |
| 4'-Hydroxychalcone                                                    | 2657-25-2   | 0.4                                      |
| Oxazolone                                                             | 15646-46-5  | 0.5                                      |
| Chorothalonil                                                         | 1897-45-6   | 1.0                                      |
| (4R,5S)-1,5-Dimethyl-3-(1-oxo-2-propenyl)-4-phenyl-2-imidazo-lidinone | 139109-23-2 | 1.1                                      |
| 1-Chloromethylpyrene                                                  | 1086-00-6   | 1.3                                      |

the default approach, it should be assumed, if necessary, that the entire unidentified content in the ingredient might consist of HPC chemicals. HPC DST could cover the risk of the majority of potent sensitizers. Using HPC DST, therefore, the risk of skin sensitization is considered to be very low for unidentified substances even when the unidentified content is assumed to be HPC chemicals. It was suggested that the workflow using DST approaches in Fig. 2 could be a novel risk assessment strategy for substances using the probabilistic approach.

Derivation of the HPC DST value in this study was based on the assumptions (1) that the distribution of sensitization potencies of the chemicals analyzed in this study are representative of all known and unknown HPC sensitizers, and (2) that a substance that is not included in

the data set is very unlikely to have intrinsic sensitization potency much stronger than the analyzed HPC chemicals.

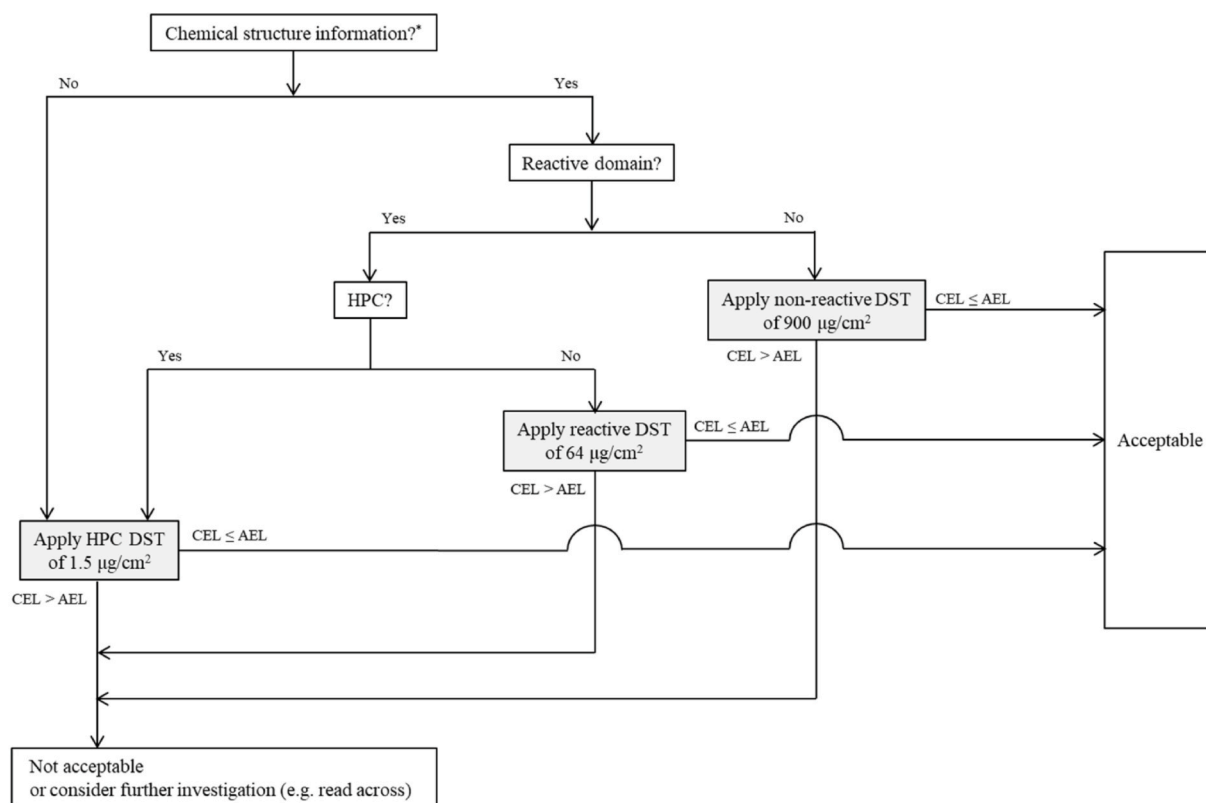
To evaluate the importance of considering the LLNA variability, Hoffmann (2015) previously analyzed data for a set of chemicals with repeated studies. This report suggested the importance of considering variability in reference data. To address this point, we used the median-like location parameter for a representative EC3 value with the assumption that the variability of EC3 values derived from repeated studies would converge to the median. This led to a minimization of the uncertainty of data variability in our study because the DST approach used the distribution of the data sets comprised of fixed location parameters.

Moreover, when estimating the potencies for each analyzed sensitizer, we did not extrapolate from mouse data to human data, because LLNAs have been shown to correlate well with human skin sensitization threshold data (Basketter et al., 2005, 2018; Api et al., 2015b). Thus, it is unnecessary to include additional safety factors for interspecies differences when using the DST approach. It may be necessary to conduct a case-by-case evaluation, taking account of specific chemical property (e. g. aromatic Schiff base electrophiles, chemicals readily susceptible to autoxidation under LLNA conditions) that can lead to over/under-prediction (Roberts and Api, 2018). The specific circumstances and chemical properties associated with over/under prediction of sensitization potency were not observed for the five chemicals which had EC3 values lower than  $1.5 \mu\text{g}/\text{cm}^2$ . In addition, the DST concept is a probability-based approach with the assumption that the analyzed chemicals are representative of all known and unknown sensitizers.

**Table 4**

Quoted EC3 value vs calculated EC3 value using single dose probit extrapolation method (SDPEM), and dose response data for 3 chemicals where the original estimated EC3 value came from an extrapolation with less-than-ideal dose response data.

| Chemicals                                                             | CAS         | EC3 [%] ([ $\mu\text{g}/\text{cm}^2$ ]) | EC3 [%] ([ $\mu\text{g}/\text{cm}^2$ ]) | Raw data |       |      |      | Reference                    |
|-----------------------------------------------------------------------|-------------|-----------------------------------------|-----------------------------------------|----------|-------|------|------|------------------------------|
|                                                                       |             | in reference                            | by SDPEM                                |          |       |      |      |                              |
| 4'-Hydroxychalcone                                                    | 2657-25-2   | 0.002                                   | 0.178                                   | Dose [%] | 1     | 10   | 20   | Kern et al. (2010)           |
|                                                                       |             | (0.5)                                   | (44.5)                                  | SI       | 8.6   | 10.6 | 10.8 |                              |
| (4R,5S)-1,5-Dimethyl-3-(1-oxo-2-propenyl)-4-phenyl-2-imidazo-lidinone | 139109-23-2 | 0.004                                   | 0.037                                   | Dose [%] | 0.5   | 5    | 50   | Submitted by GlaxoSmithKline |
|                                                                       |             | (1.0)                                   | (9.3)                                   | SI       | 14.1  | 19.5 | 16.8 |                              |
| 1-Chloromethylpyrene                                                  | 1086-00-6   | 0.005                                   | 0.003                                   | Dose [%] | 0.025 | 0.05 | 0.1  | Gerberick et al. (2005)      |
|                                                                       |             | (1.3)                                   | (0.8)                                   | SI       | 11.6  | 15.4 | 18.6 |                              |



\* Not only completely determined chemical structure information but also estimated or assumed one based on the background information could be useful (e.g. assumed hydrolysis products or reaction by-products).

**Fig. 2.** Proposed risk assessment workflow using the DST approaches.

The DST values, rather than being based on the lowest value in each of the distributions, are instead based on a point close to the lowest value because the derivation of the HPC DST values is a probability-based approach derived from frequency distributions. Thus, there is a probability that a substance with an exposure below the relevant DST value may still have a potential risk using DST values and the probability can be estimated to lie between 0% and 5%. Like the TTC approach, the DST approach relies on probabilistic methods. As such it must be understood that, as with risk assessments based on similar approaches, the DST approach does not offer complete certainty.

It has been shown that there was good agreement ( $R^2 = 0.940$  ( $n = 33$ )) between logEC3 value from the interpolation and that extrapolated from the single-dose data using SDPEM (Roberts, 2015). Thus,

recalculation of EC3 values using SDPEM was performed for three chemicals whose EC3 value came from an extrapolation with less-than-ideal dose response data, and recalculated EC3 values were greater than  $1.5 \mu\text{g}/\text{cm}^2$  for two chemicals. EC3 value of 1-chloromethylpyrene was less than  $1.5 \mu\text{g}/\text{cm}^2$  even when we applied SDPEM; however, it is necessary to consider that the unreliability of the literature EC3 value of this chemical came from an extrapolation with a high SI value ( $>10$ ), contrary to the recommendations of Ryan et al. (2007). The remaining two sensitizers of oxazolone and chlorothalonil in the analyzed sensitizers might present a risk of skin sensitization. However, it should be noted that the incidence of these sensitizers in the world of chemicals is very low (approximately 0.08% [ $= 20\% \times 2/513$ ]) considering the number of analyzed chemicals and the 20% incidence of

skin sensitizers in all chemicals, which is based on an examination of the European list of notified chemical substances database (Safford, 2008). This value was also validated in an examination of the database of Annex I of Directive 67/548 EEC, the European Flavor and Fragrance Association database, and the International Fragrance Association/Research Institute for Fragrance Materials database (Keller et al., 2009).

One final note is that, because the QRA approach is based on exposure levels that do not induce skin sensitization, the HPC DST would be protective for the induction phase of skin sensitization but not for the elicitation phase. As is generally known, elicitation reactions in subjects already sensitized to chemicals, occur at lower levels of exposure than those required to induce skin sensitization.

In summary, the novel DST approach for HPC chemicals or structure unidentified substances was developed. A probabilistic assessment using 95th percentiles of the representative EC3 values for HPC sensitizers collected was performed and the HPC DST value of 1.5 µg/cm<sup>2</sup> was derived. However, the probability that a sensitizing constituent will cause skin sensitization remains to lie between 0% and 5%, and the HPC DST would not be protective for elicitation. To minimize these remaining uncertainties, it is necessary to consider clinical and epidemiological studies, post-marketing surveillance data, and case reports.

## Declaration of competing interest

G. Frank Gerberick receives support from the Kao Corporation and the Research Institute for Fragrance Materials as a consultant. David W. Roberts and Robert J. Safford receive support from the Research Institute for Fragrance Materials as consultants. The remaining authors are not aware of any conflicts of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yrtph.2020.104732>.

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