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Bolstering the existing database supporting the non-cancer Threshold of Toxicological Concern values with toxicity data on fragrance-related materials

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ABSTRACT

The use of threshold of toxicological concern (TTC) supports the safety assessment of exposure to low levels of chemicals when toxicity data are limited. The Research Institute for Fragrance Materials (RIFM) delivers safety assessments for fragrance materials that result in safe products for consumer use. A major goal for the RIFM safety assessment program is to invest in alternative methods to animal testing for use in assessment of fragrance materials. This includes use of TTC, which provides a pragmatic approach for safety evaluation of fragrance materials in the absence of chemical-specific toxicity data and reduces the need to generate new animal data. To bolster the TTC approach for support of fragrance materials and specifically to strengthen the Cramer class II threshold, the RIFM database was reviewed with a goal of identifying fragrance materials with data that can be added to the existing TTC databases. The RIFM database identified a total of 476 chemicals that were added to the existing TTC databases. The chemicals were then individually assigned a Cramer class and 238, 76 and 162 chemicals in Cramer class I, II and III respectively were identified. The RIFM-TTC dataset was then combined with the COSMOS-Federated TTC dataset for a total of 421, 111 and 795 chemicals in Cramer class I, II and III respectively. The combined dataset further expands the chemical space thereby providing more robust 5th percentile thresholds. Moreover, the combined dataset bolsters the threshold for Cramer class II to include a total of 111 chemicals which is an improvement over the original (Munro) TTC dataset which only included 28 chemicals in Cramer Class II and the COSMOS Federated dataset which had 40 chemicals. This allows for a more reliable and robust 5th percentile NOAEL value for Cramer class II chemicals of 1.27 mg/kg bw/day. The 5th percentile NOAELs for Cramer class I, II and III from the combined dataset are 4.91, 1.27 and 0.29 mg/kg bw/day, which supports the threshold values derived from the original Munro dataset. This work confirms the adequacy of the existing TTC values and provides further support for the use of TTC as a tool to conduct safety assessments for fragrance materials. It further opens the future possibility of updating the existing values with more robust TTC values for fragrance and cosmetic materials.

1. Introduction

The Research Institute for Fragrance Materials (RIFM) independently assesses the safety of fragrance materials in consumer products using a quantitative, exposure-based safety assessment approach that follows the RIFM criteria document (Api et al., 2015). For systemic toxicity, when no data are available on a fragrance material or its appropriate read-across analog and, if exposure falls below the relevant Threshold of Toxicological Concern (TTC) limit, the safety assessment is considered to be complete with no further data requirements following a thorough genotoxicity assessment. Thus, TTC is a strategic part of the safety assessment of fragrance materials and a critical animal alternatives methodology.

The application of TTC is a safety assessment approach that defines exposure thresholds for chemicals with limited or no toxicity data below which there are no concerns for adverse effects. The TTC thresholds have

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been established from repeat-dose toxicity data for cancer and non-cancer endpoints of many hundreds of chemicals. The first TTC-like approach, introduced as the Threshold of Regulation (ToR), was developed by the US FDA as a single value from carcinogenicity data and was adopted in the 1990s to expedite the assessment of low-level migrants from food contact materials (US-FDA, 1993; US-FDA, 1995). Since then, the TTC approach has been expanded to be used as one of the animal alternative methods for safety assessment to support low-level exposure to materials including consumer products, food, flavor ingredients, drinking water, and pharmaceuticals (EFSA, 2016).

The original non-cancer TTC limits proposed by Kroes et al. (2004) were supported by a reference dataset of 613 chemicals from Munro et al. (1996) (Munro et al., 1996; Kroes et al., 2004). The chemicals were binned into three classes (Class I, II and III) according to chemical structure via a series of 33 questions following the Cramer et al. (1978) decision tree (Cramer et al., 1978). The 5th percentile NOEL values were derived for each structural Cramer class from cumulative frequency distribution curves of all the NOELs in each respective Cramer class. Recently Yang et al., 2017 expanded the Munro et al., 1996 dataset to include cosmetic-related chemicals and merged them with the Munro dataset to form a COSMOS-Federated dataset with963[2] total chemicals (Yang et al., 2017). The COSMOS-Federated dataset further expanded the already broad chemical space and derived thresholds that were similar to the Munro TTC thresholds.

For both the Munro and COSMOS-Federated datasets, the number of chemicals in Cramer classes I and III are considerably higher compared to the number of chemicals in Cramer class II. Specifically, the Munro Cramer classes I and III contained 137 and 448 chemicals respectively and the COSMOS-Federated Cramer class I and III contained 243 and 671 chemicals, respectively. Chemicals designated Cramer class II were present at much lower numbers in both Munro- (28) and COSMOS- (49) TTC datasets. While Munro derived a 5th percentile NOEL and threshold for Cramer class II, Yang et al. (2017) concluded that size of the Cramer class II dataset was inadequate to derive a threshold (Yang et al., 2017). More recently Nelms et al. derived TTC values for chemicals contained within the EPA's ToxVal database of over 4500 chemicals (Mark et al., 2019). They screened and eliminated chemicals with genotoxic structural alerts as well as organophosphates and carbamates and used the minimum NOAEL for each chemical to derive TTC values from a final dataset of 1300 chemicals. However, there still remained very few chemicals in Cramer class II (39) for which there was no statistically significance difference in the cumulative distribution frequency curve when compared to either Cramer class I or III. Overall, Nelms et al. concluded that the original Munro et al. (1996) TTC values remain consistently lower than the thresholds they derived. (Mark et al., 2019).

In the 2012 joint opinion on TTC issued by the European Commission's three non-food Scientific Committees, it was concluded that "the TTC value for Cramer class II is not supported by the currently available databases and these substances should be treated as Class III substances" (SCCS, 2012). This was on the basis of there being too few chemicals in Cramer class II to support the derivation of the 5th percentile threshold value. This opinion was also carried forward by the Scientific Committee on Consumer Safety in its most recent Notes of Guidance for cosmetics (SCCS, 2018). However, in the same time frame, the recommendation from an expert group convened by EFSA/WHO to evaluate the state of the science for TTC was that Cramer class II should continue to be used (EFSA, 2016). Thus, there are different opinions among scientific authorities in considering the use and applicability of Cramer class II for assessing the safety of chemicals with known structure and unknown toxicity.

[2] In the abstract of the Yang et al. (2017) paper it states that there are 966 chemicals in the Federated dataset, however, the paper lists 963 materials in the tables. Of the 552 cosmetic-related chemicals, there was an overlap of 190 chemicals that were already in the Munro database.

As previously reported and confirmed from an internal review, the RIFM database contains many fragrance materials that are in Cramer class II (Bhatia et al., 2015). Thus, the RIFM database was reviewed with a goal of identifying fragrance materials with data that could be added to the existing TTC datasets. The RIFM database is the largest available inventory of toxicity studies on over 6000 unique entries (including fragrances, flavours, extracts, naturals, UVCBs and others). The RIFM database was used to identify a total of 476 unique materials having repeated dose and/or reproductive and developmental toxicity studies. These studies comprise the RIFM-TTC dataset for integration into the most recently developed COSMOS-Federated TTC dataset (Yang et al., 2017). The goal was to integrate the RIFM-TTC dataset with the COSMOS-Federated dataset to enhance the Cramer class II dataset and increase confidence in the resulting TTC values. Additionally, because TTC has been recognized as an important safety assessment tool to support the relatively low level exposures to fragrance materials, available data on Cramer class I and III chemicals from the RIFM database were also considered in this analysis.

2. Materials and methods

2.1. The RIFM database

The RIFM database was queried for chemicals with repeated dose, reproductive, and developmental toxicity data following oral administration. This resulted in a total of 516 chemicals with a total of 625 studies in mammalian species that met the study inclusion criteria. These went through a rigorous peer-review process that resulted in 476 chemicals for inclusion in the final RIFM-TTC dataset (Fig. 1). Detailed information on the process is provided in sections below.

2.2. Toxicology information assembly criteria

The RIFM database provided the most comprehensive source of toxicology data available for the generation of the RIFM-TTC dataset. Where complete toxicity information was not available in the RIFM database, it was sourced from peer-reviewed scientific literature and regulatory authoritative sources. databases to source toxicity information included PubMed, published Opinions of the European Food Safety Authority (EFSA), information obtained from substances in the European Chemicals Agency (ECHA) database, the National Toxicology Program (NTP), the US EPA ChemView database (IRIS, HPVIS and Robust Summaries), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluations, the International Agency for Research on Cancer (IARC) monographs, Japan Existing Chemical Data Base (JECDB), published OECD assessments, the German Federal Institute for Risk Assessment (BfR), Cosmetic Ingredient Reviews, RIFM-fragrance material reviews or group summaries, the Opinions of the European Commission's Scientific Committee on Consumer Safety (SCCS), and the National Industrial Chemicals Notification and Assessment Scheme, Australia (NICNAS).

For the purposes of this project, criteria similar to those described by Yang et al., 2017 for the development of the COSMOS-Federated -TTC dataset were followed when characterizing a chemical in terms of test-chemical or chemical structure (Yang et al., 2017). Similar inclusion, exclusion and study evaluation criteria along with adjustment factors as those of the COSMOS-Federated-TTC dataset were used (Yang et al., 2017). Each test-chemical included in the RIFM-TTC dataset was associated with a unique chemical abstract service number (CAS#) and a corresponding Simplified Molecular-Input Line-Entry System (SMILES) identification code. Each sourced study for the individual test-chemical was detailed with information outlined in Appendix A.

2.3. The COSMOS-Federated TTC dataset

A recent development in the area of TTC included expanding the

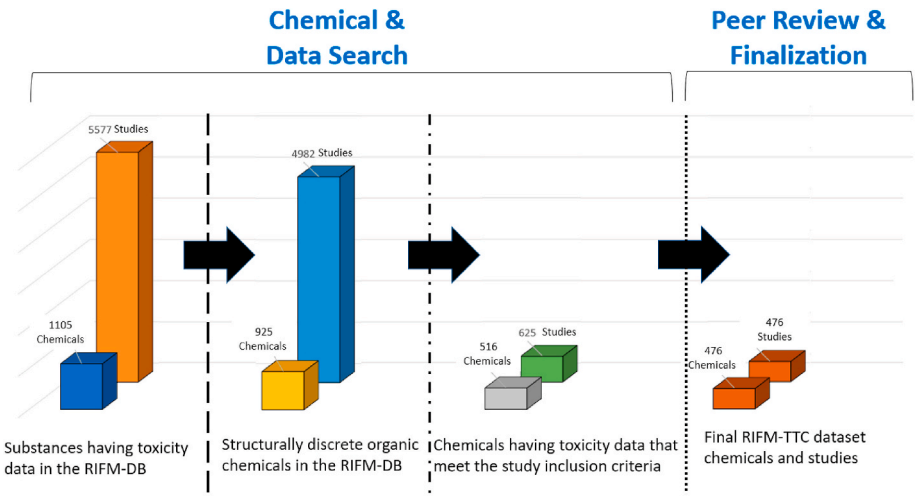

Fig. 1. Initiation, curation and finalization of the RIFM-TTC dataset.

original Munro dataset (Munro et al., 1996) by the inclusion of cosmetic ingredients resulting in the COSMOS-Federated TTC dataset with a total of 966 chemicals ((Yang et al., 2017). Yang et al. identified 190 chemicals that overlapped between the Munro dataset and the COSMOS-TTC dataset. These were carefully reviewed by the authors for final inclusion and completion of the COSMOS-Federated TTC dataset which was downloaded from the COSMOS-Federated dataset v2.0 (Molecular-Networks, 2017) for further federation with the RIFM-TTC dataset.

2.4. Study inclusion and point-of-departure (POD) selection criteria

Chemicals identified in this project were screened for toxicology data that met the study selection criteria listed in Table 1. Overall, this resulted in a total of 516 chemicals with a total of 625 studies. In order to identify the point-of-departures (POD) for each chemical entry, the criteria listed below were applied. These criteria were similar to those used by Yang et al., 2017 (Yang et al., 2017):

1) For each chemical, a search was conducted within the RIFM database for studies that met the criteria listed in Table 1. A data matrix was created to include all toxicology data available on the chemical within the RIFM database that included filling each criterion listed in Appendix A, Table A.1. Each study listed in the data-matrix was carefully peer-reviewed by and marked for consideration into the RIFM-TTC dataset.

Table 1
Study inclusion criteria for RIFM-TTC chemicals.

Parameter	Criteria
Study Type	Short term, Sub-acute, sub-chronic, developmental, reproductive, carcinogenicity, chronic, immunotoxicity, or neurotoxicity.
Species	Rodents (rat, mouse, hamster), primates, humans, dogs or rabbits.
Treatment duration	For repeated dose toxicity studies, a minimum of 28 days repeated dose toxicity studies. For developmental toxicity (10–14 days for pregnant rats, mice or rabbits) covering duration of organogenesis among treated animals. For reproductive studies treatment duration was not defined.
Route of exposure	Gavage, intubation, drinking water or diet/feed
Reference	RIFM-Study reference number or publicly available regulatory evaluations or manuscripts.

2) Following compilation of the data from the RIFM database, a wider search was conducted on each chemical for relevant toxicity data and evaluations in publicly available data sources listed above. It should be noted that most of the studies referenced in publicly available toxicity databases were already included in the RIFM database as original study reports or published manuscripts. Thus, the RIFM database typically provided the most comprehensive source of toxicity data on fragrance materials. In cases where publicly available data sources provided the most relevant information, the NOAELs cited therein were used with appropriate citation. Any discrepancies in NOAELs from multiple publicly available sources citing different NOAELs for the same study were resolved by peer-review.
3) Once clear NOAELs/LOAELs were identified, appropriate adjustment factors were included to derive the POD that would be used for a study to be included into the RIFM-TTC dataset. These adjustment factors are the same as those used by Yang et al. (2017) in developing the COSMOS-Federated TTC dataset (Yang et al., 2017):
   a. For sub-chronic studies (studies with duration of 90–180 days), an adjustment factor of 3 was applied to extrapolate POD for chronic effects.
   b. For short-term studies (28 days to <90 days), an adjustment factor of 6 was applied to extrapolate POD for chronic effects.
   c. For reproductive and developmental studies, no duration adjustment factor was applied.
   d. Benchmark Dose analysis was used in cases where a clear dose response was observed for the critical effect driving the NOAEL and appropriate duration adjustment factors outlined above were applied.
   e. An adjustment factor of 3 was applied to the LOAEL for POD derivation in cases where a clear NOAEL could not be identified.

The adjustment factors listed above were applied to studies on chemicals included in the RIFM-TTC project to derive appropriate PODs for finalization of the RIFM-TTC dataset and compilation of chemicals. Criteria for study and chemical inclusion to derive the POD included the following:

1) Where chronic studies were available, NOAELs from these studies were favored, with the intent to cover lifetime exposures. Where shorter duration studies were used over the chronic, an explanation is provided within the dataset.
2) Where multiple studies were conducted on the same chemical, a thorough review was conducted to derive an appropriate POD. Criteria for review in such cases included:

a. Studies that provided a clear NOAEL and associated LOAEL were given preference.
b. Cases where a study with the highest tested dose provided the NOAEL, an alternate study providing a clear NOAEL and associated LOAEL value were given preference.

3) Studies conducted according to good laboratory practice (GLP) and/or conducted in accordance with standardized protocols (e.g. OECD or EPA) were given preference over non-guideline non-GLP studies in cases where both such studies were made available at the time of review.

Finalization of studies selected for respective chemicals resulted in a total of 476 chemicals into the final generation of the RIFM-TTC dataset.

2.4.1. Single dose studies

Repeat dose toxicity studies are most commonly conducted by administering multiple doses and often at doses high enough to establish a dose-response for identifying test material related toxicity, thereby defining a NOAEL and/or LOAEL. In some cases, however, such studies may be conducted using only a single dose level. This might be the case where a study is conducted on a test chemical known to have very low toxicity (e.g., tested at a limit dose), or to support the safety of the test chemical at an estimated human exposure level at the time the study was conducted, thereby providing a free-standing NOAEL. While these studies might be adequate for assuring safety for known human intakes, they may not be ideally suited for inclusion in a TTC dataset and are generally excluded (e.g. (Yang et al., 2017)).

A total of 93 RIFM chemicals were identified to have single dose studies (with appropriate controls) during the dataset finalization. Of these chemicals, 48 were in Cramer class I, 8 in Cramer class II, and 37 in Cramer class III. Before excluding these chemicals from the dataset, the studies were evaluated to confirm there were no adverse effects observed at the dose level tested. If an adverse effect was identified in the study, it was included but, with appropriate adjustment factors listed above to extrapolate the NOAEL from a LOAEL. For chemicals with free-standing NOAELS, the PODs were compared to the respective TTC thresholds to determine if any single dose studies would potentially impact the 5th percentile threshold. Single dose studies with PODs less than 3X the threshold for the respective Cramer class were removed to prevent any inappropriate lowering of the thresholds where only a single, very low dose was tested in the toxicity study and no adverse effects were identified. This pragmatic decision was made to include the single dose studies where the PODs were 3X greater than the respective 5th percentile thresholds to expand the database without inappropriately impacting the 5th percentile. A total of 15 single dose studies were excluded from the dataset – 8 from Cramer class I and 7 from Cramer class III.

2.5. Cramer class assignments

The original Cramer decision tree consists of 33 sequential questions with binary results providing "yes" or "no" answers that ultimately result in classifying chemicals into 3 potency tiers based on chemical structure (Cramer et al., 1978):

- Class I: Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity.
- Class II: Substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III.
- Class III: Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.

Although the Cramer decision tree serves to improve consistency between toxicological evaluation made by different experts, its reliance on knowledge of organic chemistry, chemical reactivity, toxicity and metabolism invariably included some degree of subjectivity. Thus, several software-based platforms were introduced to minimize subjectivity and consistently apply the Cramer decision tree for any chemical requiring evaluation. Software-based platforms such as, ToxTree and OECD QSAR toolbox, that provide Cramer classifications following the original Cramer decision tree or their own modification of the Cramer decision tree, have raised the potential for differential Cramer classes for an individual chemical (Bhatia et al., 2015). To maintain consistency with the COSMOS-Federated dataset, ToxTree v2.6.13 was used for assigning Cramer classes for all chemicals within the RIFM-TTC dataset although, Cramer classifications from OECD QSAR Toolbox were also included to compare the resulting outcome. Several discrepancies resulting from the different software programs used were attributed to differences in source databases for characterizing a chemical as a normal constituent of the body (Q1) or a common component of food (Q22). These 2 questions are critical and often debated for their importance towards classifying chemicals in their individual Cramer classes. This is due to the fact that a "yes" answer to Q1 or Q22 invariably results in classifying the chemical as Cramer class I or II, respectively. Additional information regarding the discrepancy arising from Q1 and Q22 is discussed in the following section. The RIFM-TTC dataset was carefully peer-reviewed for each Cramer Decision Tree question (ToxTree and OECD toolbox).

2.5.1. Cramer Decision Tree, Q1 and Q22

From the Cramer decision tree, two questions cannot be answered solely based on evaluation of chemical structure (Cramer et al., 1978):

- Q1 asks "Is the substance a *normal constituent of the body* or an optical isomer of such?" This is further clarified as meaning any systemic constituent present at a normal physiological level, whether free or combined, except hormones. This includes essential nutrients and major food constituents, as well as their normal physiological metabolites. However, it excludes transitory chemicals present only as a result of (a) trace constituents of food, (b) gut contents or (c) products of the actions of the gut flora. If the answer to Q1 is "yes" then, that results in the chemical being assigned to Cramer class I.
- Q22 asks, "Is the substance a *common component of food* or *structurally closely related* to a *common component of food*?" This question refers to the natural and nearly natural-identical substances that were not put into Cramer class I by answering "yes" to Q1 or other structural criteria. In fact, Cramer et al. (1978) did acknowledge that the definition of common component of food is diverse, changing, and occasionally uncertain (Cramer et al., 1978). Hence, they offer the following guideline: a chemical that has been reported in the recognized literature as occurring in significant quantity (approximately 50 ppm or more) in at least one major food, or in trace quantities at the ppm level or less in several foods, including minor or less frequently consumed foods. The latter include spices, herbs and ethnic specialties. This definition *excludes* natural or man-made contaminants, and hormones. An answer of "yes" to Q22 results in the chemical being assigned to Cramer class II.

The 1978 Cramer Decision Tree was employed as a tool to support the tiered TTC approach described initially by Munro et al. (1996) (Munro et al., 1996). As the application of TTC has expanded, the decision tree continues to be considered fit-for-purpose, but it was also recognized that the decision tree needed to be re-evaluated and potentially updated. During a state-of-the-science EFSA/WHO workshop convened in December 2014, a group of experts focused on evaluating the Cramer classification scheme; and they recognized that several groups are working towards updating the Cramer decision tree. But the general consensus was that in the interim, the decision tree "is well suited for its intended purpose and when used in conjunction with the

associated TTC values is protective." The group generally agreed that it would be preferable to delete Q22, but this is not a simple modification because the question is linked to many other questions in the decision tree and would have several other consequences that would need to be carefully evaluated. In the meantime, they recommended the development of "clear and harmonized criteria of what '*common component of food*' means" (EFSA, 2016).

As described above, an answer of "yes" to Q1 or Q22 results in assignment of that chemical to Cramer class I or II; thus, this question has importance for establishing the dataset of chemicals to support the 5th percentile NOAEL underlying the TTC for the respective Cramer classes. For *in silico* tools such as ToxTree and the OECD toolbox used to assign the appropriate Cramer class, Q1 or Q22 will be answered "yes" only if that chemical is found in the respective reference list. In fact, ToxTree continues to evolve as more chemicals are added to the reference lists against which the program can check for answering Q1 and Q22. Notably, the program specifically remarks that, "the answer to these questions relies on an incomplete list of compounds, identified by an expert as a normal body constituent. If you believe a query compound is wrongly identified as such, or not recognized, please consult and/or update the list." This allows the end user to apply expert judgment and override the ToxTree answer.

During peer review of the RIFM-TTC dataset, several discrepancies in Cramer classifications were found, resulting from the different software programs used. As described above, these discrepancies were primarily due to differences in reference lists used to identify a chemical as a normal body constituent (Q1) or a common food component (Q22). This discrepancy in Cramer class assignments was also noted for chemicals in the COSMOS-Federated dataset. Thus, a peer review was conducted for all chemicals within the RIFM-TTC and COSMOS-Federated datasets in relation to Q1 and Q22. Following peer review, evidence based expert judgement was accepted for Cramer classification based on Q1 and Q22 for 46 chemicals where the originally assigned Cramer class was overridden. These resulted in 18 Cramer class I and 28 Cramer class II chemicals. In addition, 34 Cramer Class I and 3 Cramer class II chemicals were derived by using ToxTree by answering "yes" to Q1 and Q22 respectively for a total of 52 Cramer class I and 31 Cramer class II chemicals based on Q1 and Q22 respectively. For any chemical assigned evidence based expert judgement for the answer to Q1 or Q22, reliable sources of references were included, following the criteria defined by Cramer for Q1 and Q22, while differing from the software-based platforms. Only sources citing the exact chemical and quantified values of the respective chemical's presence in the human body or as a normal constituent of food were considered evidence for differing from the software-based platforms. It should be noted that several food and flavor chemicals occur naturally or are synthetic forms of naturally occurring chemistries. In order to avoid their misclassification, Cramer classification of these chemicals was done based on evidence based expert judgement of Q1 or Q22. As a result, fragrance chemicals in Cramer class I or Cramer class II present in the RIFM database contribute towards bolstering the resulting TTC dataset (Table 2).

2.6. Curation of a combined RIFM-COSMOS-Federated dataset

The RIFM-TTC dataset was combined with the COSMOS-Federated dataset to curate a complete master TTC dataset, referred to here as, RIFM-COSMOS-Federated Dataset. This master TTC dataset offers a holistic evaluation of the most current database of the original Munro dataset, cosmetics and fragrance materials together expanding the dataset that supports the TTC values for each Cramer class. As outlined by Yang et al. (2017), similar challenges in combining the RIFM-TTC and COSMOS-Federated datasets were encountered here, including instances where overlapping chemicals among the RIFM-TTC and COSMOS-Federated datasets were encountered (Yang et al., 2017). In order to avoid conflicting decisions towards deriving PODs for such overlapping chemicals, the PODs derived by Yang et al., 2017 were included in the final combined dataset (Yang et al., 2017). However, before consolidating the final PODs, all chemicals were subjected to a thorough data search for any new toxicity data. In doing so there were two chemicals for which new data were found: 2-ethyl butyric acid (CAS# 88-09-5) and allyl heptanoate (CAS# 142-19-8). In addition, although data on canthaxanthin (CAS# 514-78-3) was included in the COSMOS-Federated dataset and due to the fact that toxicity data was also available in humans, a more relevant human study was considered here for inclusion into the dataset for POD derivation. Additional details on the POD derivation for these three chemicals is made available in Appendix B of the manuscript. Details on RIFM-COSMOS-Federated dataset chemicals are outlined in Supplementary Table 1 (S1).

Table 2
Distribution of Cramer class for discrete RIFM-fragrance materials within the RIFM-database.

Cramer class	Number of RIFM Fragrance Materials in the TTC Dataset
I	238
II	76
III	162
Total	**476**

3. Results

A total of 476 RIFM Chemicals with repeat dose, developmental, and/or reproductive toxicity data were identified from the RIFM database and considered adequate for addition to the RIFM-TTC dataset based on the inclusion criteria described previously. The 5th percentile PODs for each Cramer class was determined from the cumulative distribution of NOAELs for the RIFM-TTC dataset (Fig. 2). The RIFM-TTC dataset was then combined with the COSMOS-Federated dataset and the combined dataset was analyzed to determine the 5th percentile PODs (Fig. 3). The 5th percentiles were derived from parametric fitting of the lognormal distribution of NOAELs using the "survival" package from the R-Project for Statistical Computing (Therneau, 2015; Team, 2019). This approach is consistent with what has become standard in deriving human exposure thresholds for TTC purposes (Munro et al., 1996; Yang et al., 2017).

For the RIFM-TTC dataset, the 5th percentile PODs were 5.39, 1.97, and 1.17 mg/kg bw/day for Cramer classes I, II, and III, respectively. The RIFM-COSMOS-Federated dataset provides 5th percentile PODs of 4.91, 1.27, and 0.29 mg/kg bw/day. Comparing the RIFM-COSMOS-Federated dataset and existing Munro PODs shows a significant increase in the number of chemicals analyzed and yields slightly higher 5th percentile PODs as compared to the original values derived by Munro et al. (1996) (Table 3). Particularly significant is the increase of the number of Cramer class II chemicals from 28 in the original Munro dataset to 111 in the RIFM-COSMOS-Federated dataset. The RIFM-TTC dataset adds 53 new Cramer class II chemicals to the dataset, increasing the statistical weight of the analysis.

From these 5th percentile PODs, human exposure thresholds for each Cramer class were calculated using an adjustment factor of 100x (10x for interspecies differences and 10x for human variation), consistent with the approach used by Munro et al. (1996). The human exposure thresholds derived for the RIFM-TTC and RIFM-COSMOS-Federated datasets are shown in Table 4 with the corresponding values calculated by Munro et al. (1996) and Yang et al. (2017).

As described earlier, we generally agreed with the values used by COSMOS for both the Cramer class designation and the NOAEL after examination for chemicals in the COSMOS-Federated dataset. There are three exceptions to this: 2-ethylbutyric acid, canthaxanthin, and allyl heptanoate. In the case of 2-ethylbutyric acid, our data search revealed a newer repeated dose/developmental and reproductive toxicity screening study which provided a more robust basis for the assessment. For allyl heptanoate, we identified an error in the dose conversion from

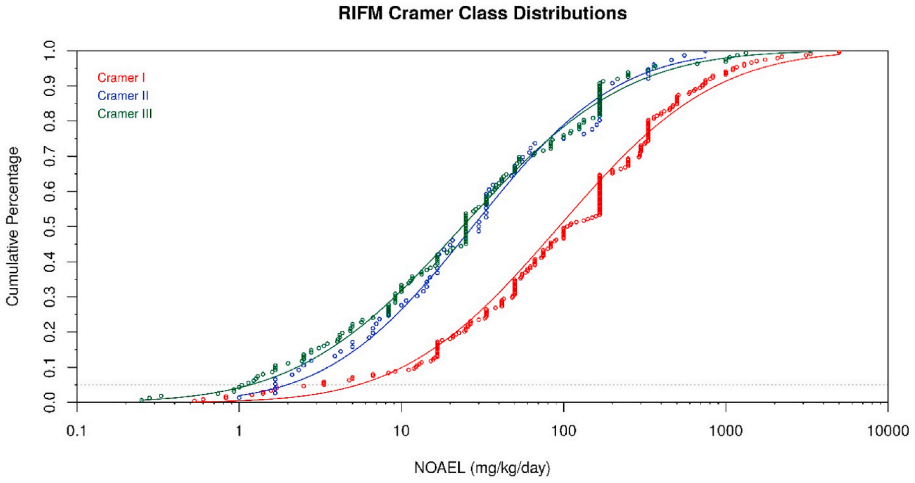

Fig. 2. Cumulative Distribution of NOAELs from the RIFM-TTC dataset.

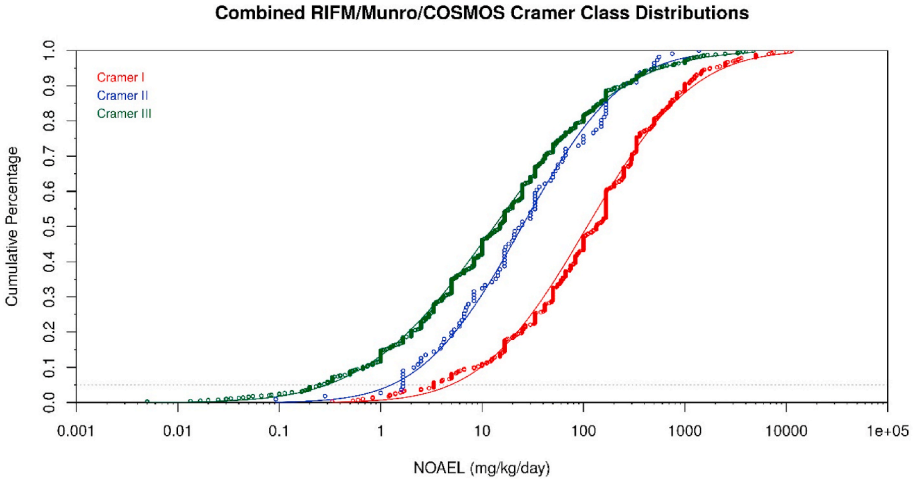

Fig. 3. Cumulative Distribution of NOAELs from the RIFM-COSMOS-Federated dataset.

Table 3
5th percentile PODs (mg/kg bw/day).

Cramer class	Munro et al. (1996) [b]	COSMOS (2017) [b]	RIFM	RIFM-Munro-COSMOS Combined Set
I	3.0 (N = 137)	4.20 (N = 219)	5.39 (N = 238)	4.91 (N = 421)
II	0.91 (N = 28)	0.58 (N = 40)[a]	1.97 (N = 76)	1.27 (N = 111)
III	0.15 (N = 448)	0.79 (N = 293)	1.17 (N = 162)	0.29 (N = 795)

[a] This 5th percentile value is driven by the choice of NOAEL of Allyl Heptanoate and Canthaxanthin, which is discussed in detail in Appendix B.

[b] Represents the 5th percentile PODs from the as derived by Munro et al. (1996) and Yang et al. (2017).

Table 4
Human exposure threshold (μg/kg bw/day).

Cramer class	Munro	COSMOS	RIFM	RIFM/Munro/COSMOS Combined Set
I	30	42	53.9	49.1
II	9	NA[a]	19.7	12.7
III	1.5	7.9	11.7	2.9

[a] While COSMOS reported a 5th percentile for Cramer class II, a human exposure threshold was not proposed because of the small number of chemicals and overlap of Cramer class II and III in the COSMOS-Federated dataset.

the original study cited in COSMOS and also found a recent 90-day GLP/OECD guideline study that provides a much more robust basis for the assessment. For canthaxanthin, we modified the NOAEL entered into the RIFM-COSMOS-Federated dataset because it was based on human data (and therefore should not have 10X UF applied for interspecies extrapolation). It is noted that allyl heptanoate and canthaxanthin were two of the chemicals in the COSMOS-Federated dataset that were driving the 5th percentile POD for Cramer class II, so it is particularly important that these chemicals are reviewed in detail. More details on each of these chemicals, and the basis for our decision to update the information from Yang et al. (2017) is provided in Appendix B.

4. Discussion

4.1. Chemical space

As is the case with any chemistry-based safety assessment tool, it is crucial to determine the chemical space distribution of the related dataset in order to ascertain if the chemical of interest falls within the domain of applicability. Our analysis revealed that the chemical space defined by the RIFM-COSMOS-Federated dataset is representative of all

the fragrance related chemicals in current use, thereby confirming the applicability of TTC for their safety assessments. Chemical space for any dataset can be defined by several methods (such as, principle component analysis, partial least squares, atom centered fragments) and using a range of parameters (including but not limited to chemical structure, physico-chemical properties and chemical functional groups). At present there is no agreement on a single approach to define the chemical space. The Munro and COSMOS Federated datasets evaluated chemical space using various approaches as outlined above and concluded that the chemical space covered by either datasets is representative of the 'world of chemicals'. Thus, the datasets can be used to identify systemic toxicity threshold limits for any chemical with unknown toxicity, considering the typical TTC excluded certain chemical groups (e.g. inorganics, metals, etc.) (EFSA, 2016). Here, inclusion of fragrance related chemicals to the TTC datasets aims to bolster the combined dataset and its chemical space to further represent fragrance chemicals and the applicability of TTC for their safety assessments. The WHO/EFSA review of the TTC approach recommends that any new combined dataset should be tested using chemical domain analysis methods to ensure coverage of a wide range of chemical structures. The chemical space for the combined RIFM-COSMOS-Federated dataset was assessed using principle component analysis performed on molecular descriptors (physico--chemical properties) as outlined in Yang et al., 2017 (Yang et al., 2017) along with assessment of organic functional groups (OECD QSAR Toolbox v3.4) as outlined in Richard et al. (2016) for all chemicals within the dataset. Overall, our analysis confirms that fragrance materials are well represented in the chemical space of the RIFM-COSMOS-Federated dataset chemicals when queried for organic functional groups and molecular descriptors used to define the chemical space.

In Europe, the Scientific Committees (SC) on Consumer Safety, Health and Environmental Risks and Emerging and Newly Identified Health Risks published a joint opinion on use of TTC for the evaluation of chemicals contained in cosmetics and other consumer products for human health risk assessment (SCCP/1171/08 2012). The SC concluded there were an insufficient number of chemicals in Cramer class II in the databases available at the time of the review and therefore could not support the threshold value for this class. The SC instead recommended that these chemicals should be conservatively considered as Cramer class III. More recently, Yang et al. (2017) re-examined the Munro and COSMOS-Federated datasets and concluded that (a) using the pair-wise Kolmogorov-Smirnov (K-S) test, there were no differences between Class II and III distributions for both datasets and (b) because of the low number of chemicals in Cramer class II, the 5th percentile thresholds are driven by 1–2 chemicals. Others have noted challenges in the number of chemicals in the Cramer class II dataset which prevents a meaningful analysis of the threshold (Munro et al., 1996; Escher et al., 2010; Batke et al., 2011; Pinalli et al., 2011; Tluczkiewicz et al., 2011; Committee, 2012; Feigenbaum et al., 2015). Nonetheless, there continues to be support for Cramer class II, with recognition that if additional chemicals are added then a reanalysis of the 5th percentile threshold should be performed (EFSA, 2016). Here we demonstrated that addition of 53 new fragrance chemicals to the COSMOS Cramer class II dataset results in a clear separation of the Cramer class II and III distribution curves. Specifically, in the RIFM-COSMOS-Federated dataset, the Class II and III POD distributions were statistically significantly different (p-value = 0.02 N = 906) when the non-parametric pair-wise K-S test was performed (Conover, 1998). In addition, reanalysis of Cramer class II chemicals, canthaxanthin and allyl heptanoate (chemicals that drove the 5th percentile thresholds for Cramer class II) using human-relevant information and more robust data, respectively, resulted in clear separation of the Cramer class II and III POD distribution curves and clear distinction between 5th percentile thresholds for Cramer class II and III. The work presented here helps to address the concerns from the EU SC on Cosmetic and Consumer Product Safety as well as others who have pointed to lack of confidence in the Cramer class II TTC threshold.

4.2. Cramer Class III

The Munro et al., 1996 Cramer class III threshold is 90 μg/day (1.5 μg/kg bw/day) based on the original TTC decision tree (Munro et al., 1996; Kroes et al., 2004). Because several publications have suggested that the level for Cramer class III should be increased, it is worth a short discussion here. The TTC level derived by Munro et al. (1996) was derived from the entire dataset of Cramer class III chemicals which included a series of neurotoxic organophosphate and carbamate pesticides of which the organophosphates were found to be more potent. These chemicals were found to consistently have PODs below the 5th percentile, so when Kroes et al. (2004) published their tiered TTC decision tree in 2004, a separate TTC for neurotoxicity of 18 μg/day (0.3 μg/kg bw/day) was derived for these chemicals. Following this, Munro reanalyzed the Cramer class III distribution *without* the organophosphates and this analysis increased the Cramer class III TTC to 180 μg/day (3 μg/kg bw/day) (Munro et al., 2008). Since then, additional analyses have been published that support using a Cramer class III TTC lower than 180 μg/day (e.g., (Feigenbaum et al., 2015)), but these are based on datasets of pesticide actives that are generally not relevant to fragrance materials and/or they would be assigned to the organophosphate/carbamate tier of 18 μg/day (0.3 μg/kg bw/day). It is also noteworthy that there are other published analyses of databases of Cramer class III chemicals (Kalkhof et al., 2012; Leeman et al., 2014; Mark et al., 2019) which support the use of a higher Cramer class III threshold. Specifically, for cosmetics-related chemicals, the 5th percentile for Cramer class III published by Yang et al. (2017) was equivalent to a TTC of 470 μg/day (7.9 μg/kg bw/day). While the basis for Cramer class III has been considered in regulatory opinions on TTC, the publications recommending a higher level TTC for Cramer class III have not been widely accepted. This remains an opportunity for potential refinement of TTC, and in the meantime should be recognized as offering significant conservatism for chemicals assigned to this tier.

4.3. Use of TTC for safety assessment of fragrance materials

The use of TTC is a strategic component of the RIFM safety assessment program. Following the criteria outlined in Api et al. (2015), application of TTC is the third step in the safety evaluation of a fragrance material (Api et al., 2015). If no data are available on the material itself or suitable read-across material, then TTC is explored as a potential safety assessment tool. This third step involves comparing exposure of a fragrance material to the appropriate TTC threshold. Many fragrance ingredients have a low total aggregated systemic exposure. As such, since these criteria have been implemented in 2013, it has been demonstrated that not only is TTC an important safety assessment approach, but it is also a significant animal alternatives methodology. TTC was employed 537 times for the repeated dose toxicity, 688 times for developmental toxicity and 732 times for reproductive toxicity endpoints in safety assessments on fragrance materials. To date, this has resulted in more than 146,000 animals saved.

5. Conclusion

In this study we integrated a dataset of RIFM fragrance chemicals into the COSMOS-Federated dataset with the aim of enhancing the Cramer class II dataset and increasing confidence in the resulting TTC threshold. Available data on Cramer class I and III chemicals from the RIFM database were also added to bolster the overall TTC datasets with fragrance materials. The RIFM database added 53 new Cramer class II chemicals, for a total of 111 chemicals in the combined dataset, increasing the number of chemicals in this class. In addition, Cramer class I and III chemicals were increased in the combined RIFM-COSMOS-Federated dataset to 421 and 795, respectively. The TTC thresholds derived from the combined datasets for each Cramer class support the original thresholds proposed by Munro and further verifies the

robustness of the TTC approach.

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Jane Rose, Michael Laufersweiler, and Susan P. Felter are full-time employees of Procter & Gamble, a consumer products company that manufactures and formulates consumer products with perfume and flavor substances. They received no funding in cash or kind for their contributions to this manuscript. This manuscript provides the scientific basis to further substantiate the use of the Threshold of Toxicological Concern as a tool in the human safety assessment process, which is important for many perfume and flavor substances.

Atish Patel was a full-time employee of the Research Institute for Fragrance Materials, for a major part of the project.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Atish Patel was a full-time employee of the Research Institute for Fragrance Materials, a nonprofit organization that evaluated the safety of fragrance materials. Jane Rose, Michael Laufersweiler and Susan P. Felter are full-time employees of Procter & Gamble, a consumer products company that manufactures and formulates consumer products with perfume and flavor substances. They received no funding in cash or kind for their contributions to this manuscript. This manuscript provides the scientific basis to further substantiate the use of the Threshold of Toxicological concern as a tool in the human safety assessment process, which is important for many perfume and flavor substances. Kaushal Joshi and Anne Marie Api are full-time employees of the Research Institute for Fragrance Materials, a nonprofit organization that evaluated the safety of fragrance materials. This manuscript provides the scientific basis to further substantiate the use of the Threshold of Toxicological Concern as a tool in the human safety assessment process, which is important for many fragrance and flavor substances. This work was funded by RIFM, Inc.

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Appendix C. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yrtph.2020.104718.

Appendix A

Table A.1
Data-matrix used for entry into the RIFM-TTC dataset.

RIFM-database	Identifies if a test-chemical was listed in the RIFM-DB
RIFM-database-Fragrance	Identifies if a test-chemical is currently being used as a fragrance material
Dataset	Identifies test-chemical and study source (RIFM, RIFM + COSMOS or COSMOS)
Single dose	Identifies if the included test-substance has single dose study.
CAS#	CAS# associated with the test-substance
SMILES	SMILES identifier for structural definition
Name	Common or chemical name associated with the test-substance
Overall Cramer class assignment	Assigned Cramer class by evidence based expert judgement, COSMOS or ToxTree.
Cramer class decision path	Expert judgement path for Cramer classification determination
Evidence based Expert Judgement Comments	Additional comments to supplement Expert Judgement
Study Type	Identifies the study type according to criteria listed in Table 1
OECD Protocol	OECD guideline followed for toxicity study performed
Route	Route of administration of test-substance
Exposure Period (as reported)	Exposure duration reported in source reference
Exposure Period (converted to weeks)	Exposure duration converted into weeks for database uniformity
Male(M)/Female(F)	Identifies Sex of animal tested
Species	Identifies species of animal tested
Doses (mg/kg bw/day)	Doses administered
NOAEL (mg/kg bw/day)	No-observed adverse effect level
LOAEL (mg/kg bw/day)	Lowest-observed adverse effect level
Value for TTC calculation (mg/kg bw/day)	POD used for TTC calculation
Effect at LOAEL	Test-substance related toxic effects reported at the LOAEL
Inter-individual UF	Adjustment factors for inter-individual differences (1–10)
Inter-species UF	Adjustment factors for inter-species differences (1–10)
Duration AF	Adjustment factors for duration extrapolation (2–6)
Other AF (e.g., LOAEL)	Adjustment factors for LOAEL to NOAEL conversion (3–10)
Composite AF	Combined adjustment factors used for TTC derivation
POD	Point of departure used for TTC derivation
TTC value (mg/kg bw/day w/AF applied)	Human Reference dose or accepted daily intake equivalent
Reference	Study reference
Comments	Additional study details or toxicity information for selection of NOAEL/LOAEL
QA stamp/comment	Quality control checks

(*continued on next page*)

Table A.1 (*continued*)

RIFM-database	Identifies if a test-chemical was listed in the RIFM-DB
ToxTree Extension (v2.6.13)	Cramer classification according to ToxTree extension (v2.6.13)
ToxTree Extension (v2.6.13) Description	Path description for Cramer classification according to ToxTree extension (v2.6.13)
ToxTree Cramer (v2.6.13)	Cramer classification according to ToxTree Cramer (v2.6.13)
ToxTree Cramer (v2.6.13-Description)	Path description for Cramer classification according to ToxTree Cramer (v2.6.13)
OECD Toolbox (Toxic hazard classification by Cramer (extension))-v3.4	OECD toolbox Cramer classification according to extended version (v3.4)
OECD Toolbox (Toxic hazard classification by Cramer (original))-v3.4	OECD toolbox Cramer classification according to Cramer (v3.4)

Appendix B

2-ethylbutyric acid (CAS RN 88-09-5)

The COSMOS POD for 2-ethylbutyric acid (CAS RN 88-09-5; Cramer class I) is 50 mg/kg bw/d calculated by dividing the LOAEL of 150 mg/kg bw/d by a default adjustment factor of 3. The LOAEL is based on a 10-day oral gavage developmental toxicity study in pregnant female rat with doses of 0, 150 or 200 mg/kg bw/d with maternal toxicity including body weight changes and clinical toxicity and decrease in fetal survival at the low dose, 150 mg/kg bw/d (Narotsky et al., 1994).

A more recent study on 2-ethylbutyric acid, from a repeat dose and developmental and reproductive screening study in rats by gavage at 0, 10, 50 or 250 mg/kg bw/d is available (SIDS, 2006). Males were dosed for 42 days beginning 14 days pre-mating and females were dosed 14 days pre-mating to day 4 of lactation throughout mating and pregnancy period. There was no mortality in the study and no effects on body weight gain and food consumption in both sexes. White blood cell counts were decreased at 50 mg/kg bw/d and higher and platelet count decreases at 250 mg/kg bw/d in males only, with no effects in female rats. Kidney weight increases were observed in males and females at 250 mg/kg bw/d but no histological changes were noted. Regarding the developmental and reproductive evaluations, there were no adverse findings reported for any reproductive parameters. The number of live pups on lactation days 0 and 4, birth index and live birth index were all decreased at 250 mg/kg bw/d. Therefore, based on these effects the repeat dose NOAEL for males is considered 10 mg/kg bw/d and for females 50 mg/kg bw/d. The developmental NOAEL is considered 50 mg/kg bw/d and the reproductive NOAEL is considered to be 250 mg/kg bw/d.

Taken together, it was considered appropriate to use the more recently conducted repeat dose and developmental and reproductive study that included both sexes, evaluation of multiple endpoints and a defined NOAEL to be included in the TTC dataset, and therefore a POD of 10 mg/kg bw/d is proposed. This is considered protective for repeat dose and reproductive toxicity in males and females and developmental toxicity in the pups.

Canthaxanthin (514-78-3)

Canthaxantin is a naturally-occurring keto-carotenoid pigment that is approved for use as a coloring agent at low levels in many countries, including the EU and the United States (FDA, 2018). Canthaxantin also has been historically used in ingested self-tanning products (banned by the US FDA) as its deposition in the epidermis and subcutaneous fat leads to the formation of an orange-brown appearance of the skin. A number of adverse effects have been noted following ingestion of large amounts of canthaxanthin (Garone et al., 2015). The EFSA Panel on Food Additives and Nutrient sources added to Food (EFSA, 2010) re-confirmed an earlier-established ADI of 0.03 mg/kg bw/day (JECFA, 1995; EC, 1997). The ADI is based on a NOAEL of 0.25 mg/kg bw/day in humans for canthaxanthin-induced retinopathy, manifested as scotopic b-wave changes in a electroretinogram (without impairment of vision), and benchmark doses ranging from 0.2 to 0.33 mg/kg bw/day based on findings of crystals in the retina of exposed humans. Because the NOAEL and BMDs were established in humans, a total UF of 10-fold was applied to establish the ADI of 0.03 mg/kg bw/day.

The COSMOS derived NOAEL for canthaxanthin is 0.2 mg/kg bw/day based on a 3-year study conducted in monkeys that identified the formation of crystalline deposits in the retina as the critical effect seen at levels of 0.6 mg/kg bw/day and higher. It was noted by COSMOS that the monkey study was used by EFSA and JECFA along with a human study (which similarly derived a NOAEL of 0.25 mg/kg bw/day for scotopic b-wave changes without impairment of vision) to establish the Acceptable Daily Intake (ADI) for canthaxanthin. The inclusion of the NOAEL for canthaxanthin in the TTC database introduces a unique challenge because the other NOAELs are all from laboratory animal studies and the 100-fold UF applied to the 5th percentile NOAEL includes a 10X factor to extrapolate from laboratory animals to humans and a 10X factor to account for intra-human variability. In the case of canthaxanthin, human data with a reliable NOAEL are available, and are preferable to use. To include the human data in the TTC database of NOAELs requires an artificial raising of the human NOAEL for canthaxanthin by 10X (from 0.25 to 2.5 mg/kg bw/day), since a default 100-fold UF is subsequently applied to the 5th percentile NOAEL in the derivation of the TTC.

Allyl heptanoate (142-19-8)

The COSMOS NOAEL for allyl heptanoate is 0.125 mg/kg bw/day, which is one of the lowest in Cramer class II, and hence a driver for the 5th percentile NOAEL. The NOAEL is based on a chronic dog study (Hagan et al., 1965), with exposure levels of 0, 5, 25 and 75 and mg/kg. COSMOS incorrectly described these as levels in the diet, so that the lowest dose (5 mg/kg bw/day), which was identified as the NOAEL, was converted to 0.125 mg/kg bw/day by applying a 2.5% food conversion factor. In fact, the doses of allyl heptanoate were delivered by capsule, so the NOAEL from this study was actually 5 mg/kg bw/day.

Subsequent to the COSMOS evaluation of allyl heptanoate, results of a 90-day OECD guideline feeding study in Wistar rats conducted in 2016 were reported in the ECHA database (ECHA, 2018). Male and female rats (10/sex/group for the main study and an additional 5/sex in the control and high-dose groups for evaluating recovery) were administered allyl heptanoate in feed at levels of 0, 100, 400 or 1500 ppm. These doses were equivalent in males and females to 6.37 and 6.85 mg/kg bw/day (100 ppm); 24.43 and 27.05 mg/kg bw/day (400 ppm) and 84.25 and 93.08 mg/kg bw/day (1500 ppm). Decreases in body weight and body weight gain corresponding with reduced food consumption were seen in male and females in the mid- and high dose groups, with corresponding effects on several organ weights. There were no gross or histopathological lesions and no hematological, clinical biochemistry or urinalysis findings. There were also no behavioral changes as assessed by functional tests. It was concluded that the highest dose (1500 ppm; 84.25–93.08 mg/kg bw/day) was a free-standing NOAEL. Although not stated as such in the ECHA summary, this implies that the reduced food consumption, decreased body weight and organ weight changes were associated with taste aversion as opposed to an effect of

generalized toxicity. This is consistent with the changes in food consumption and body weight gain being noted from the first week of the study.

For the purpose of including allyl heptanoate in the TTC database, a conservative approach is taken here to treat the body and organ weight effects in the subchronic rat study as potentially adverse, such that the lowest dose (100 ppm; 6.37–6.85 mg/kg bw/day) is assigned as the NOAEL (this is in contrast to the ECHA summary which assigns the highest dose as the NOAEL). After applying a 3-fold UF for extrapolation from subchronic to chronic, it is recommended that a NOAEL of 2.1 mg/kg bw/day be used in the TTC database. It is recognized that a higher NOAEL can likely be supported for a chemical-specific assessment.

References

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler, D.C., Miyachi, Y., Politano, V.T., Renskers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the Research Institute for fragrance materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82 (Suppl. l), S1–S19.

Batke, M., Escher, S., Hoffmann-Doerr, S., Melber, C., Messinger, H., Mangelsdorf, I., 2011. Evaluation of time extrapolation factors based on the database RepDose. Toxicol. Lett. 205 (2), 122–129.

Bhatia, S., Schultz, T., Roberts, D., Shen, J., Kromidas, L., Marie Api, A., 2015. Comparison of Cramer classification between toxtree, the OECD QSAR toolbox and expert judgment. Regul. Toxicol. Pharmacol. 71 (1), 52–62.

Committee, E.S., 2012. Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA J. 10 (7), 2750.

Conover, W.J., 1998. Practical Nonparametric Statistics, third ed.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard–a decision tree approach. Food Chem. Toxicol. 16 (3), 255–276.

EC, 1997. Opinion on Canthaxanthin (Expressed on 13 June 1997).

ECHA, 2018. https://echa.europa.eu/registration-dossier/-/registered-dossier/12576/7/3/2. (Accessed 7 May 2018).

EFSA, 2010. Scientific Opinion on the re-evaluation of canthaxanthin (E 161 g) as a food additive. EFSA J. 8 (10), 1852.

EFSA, 2016. Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree. EFSA Support. Publ. 13 (3), 1006E.

Escher, S.E., Tluczkiewicz, I., Batke, M., Bitsch, A., Melber, C., Kroese, E.D., Buist, H.E., Mangelsdorf, I., 2010. Evaluation of inhalation TTC values with the database RepDose. Regul. Toxicol. Pharmacol. 58 (2), 259–274.

FDA, U.S., 2018. Electronic Code of Federal Regulations Title 21: Food and Drugs, PART 73—Listing of Color Additives Exempt from Certification. Subpart A—Foods, §73.75: Canthaxanthin. (Accessed 18 April 2018).

Feigenbaum, A., Pinalli, R., Giannetto, M., Barlow, S., 2015. Reliability of the TTC approach: learning from inclusion of pesticide active substances in the supporting database. Food Chem. Toxicol. 75, 24–38.

Garone, M., Howard, J., Fabrikant, J., 2015. A review of common tanning methods. J. Clin. Aesthetic Dermatol. 8 (2), 43–47.

Hagan, E.C., Jenner, P.M., Jones, W.I., Fitzhugh, O.G., Long, E.L., Brouwer, J.G., Webb, W.K., 1965. Toxic properties of compounds related to safrole. Toxicol. Appl. Pharmacol. 7, 18–24.

JECFA, 1995. Forty-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants. World Health Organization. Technical Report Series, No 859.

Kalkhof, H., Herzler, M., Stahlmann, R., Gundert-Remy, U., 2012. Threshold of toxicological concern values for non-genotoxic effects in industrial chemicals: re-evaluation of the Cramer classification. Arch. Toxicol. 86 (1), 17–25.

Kroes, R., Renwick, A.G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., Schlatter, J., van Schothorst, F., Vos, J.G., Wurtzen, G., I. European branch of the International Life Sciences, 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem. Toxicol. 42 (1), 65–83.

Leeman, W.R., Krul, L., Houben, G.F., 2014. Reevaluation of the Munro dataset to derive more specific TTC thresholds. Regul. Toxicol. Pharmacol. 69 (2), 273–278.

Mark, D.N., Prachi, P., Grace, P., 2019. Evaluating Potential Refinements to Existing Threshold of Toxicological Concern Values for Environmentally Relevant Chemicals. NEED COMPLETE CITATION.

Molecular-Networks, 2017. COSMOS.

Munro, I.C., Ford, R.A., Kennepohl, E., Sprenger, J.G., 1996. Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food Chem. Toxicol. 34 (9), 829–867.

Munro, I.C., Renwick, A.G., Danielewska-Nikiel, B., 2008. The threshold of toxicological concern (TTC) in risk assessment. Toxicol. Lett. 180 (2), 151–156.

Narotsky, M.G., Francis, E.Z., Kavlock, R.J., 1994. Developmental toxicity and structure-activity relationships of aliphatic acids, including dose-response assessment of valproic acid in mice and rats. Fund. Appl. Toxicol. 22 (2), 251–265.

Pinalli, R., Croera, C., Theobald, A., Feigenbaum, A., 2011. Threshold of toxicological concern approach for the risk assessment of substances used for the manufacture of plastic food contact materials. Trends Food Sci. Technol. 22 (9), 523–534.

Richard, A.M., Judson, R.S., Houck, K.A., et al., 2016. ToxCast chemical landscape: paving the road to 21st century toxicology. Chem. Res. Toxicol. 29 (8), 1225–1251. https://doi.org/10.1021/acs.chemrestox.6b00135.

SCCP/1171/08, 2012. Scientific committee on consumer safety (SCCS) scientific committee on health and environmental risks (SCHER) scientific committee on emerging and newly identified health risks (SCENIHR) OPINION ON use of the threshold of toxicological concern (TTC) approach for human safety assessment of chemical substances with focus on cosmetics and consumer products. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf.

SCCS, S.a.S., 2012. Opinion on the Use of the Threshold of Toxicological Concern (TTC) Approach for Human Safety Assessment of Chemical Substances with Focus on Cosmetics and Consumer Products. SCCP/1171/08.

SCCS, 2018. The SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation 10th Revision. SCCS/1602/18.

SIDS, O., 2006. SIDS Initial Assessment Profile for 2-ethyl Butyric Acid.

Team, R.C., 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Version 3.5.3. https://www.r-project.org/.

Therneau, T., 2015. A Package for Survival Analysis in S, version 2.43.3. https://CRAN.R-project.org/package=survival.

Tluczkiewicz, I., Buist, H.E., Martin, M.T., Mangelsdorf, I., Escher, S.E., 2011. Improvement of the Cramer classification for oral exposure using the database TTC RepDose – a strategy description. Regul. Toxicol. Pharmacol. 61 (3), 340–350.

US-FDA, 1993. United States food and drug administration. Food additives: threshold of regulation for substances used in food-contact articles. Fed. Regist. 58, 52719–52729.

US-FDA, 1995. Food Additives: threshold of regulation for substances in food contact articles (Final Rule). Fed. Regist. 60 (136), 36582–36596.

Yang, C., Barlow, S.M., Muldoon Jacobs, K.L., Vitcheva, V., Boobis, A.R., Felter, S.P., Arvidson, K.B., Keller, D., Cronin, M.T.D., Enoch, S., Worth, A., Hollnagel, H.M., 2017. Thresholds of Toxicological Concern for cosmetics-related substances: new database, thresholds, and enrichment of chemical space. Food Chem. Toxicol. 109 (Pt 1), 170–193.