Toxicology Letters 314 (2019) 117–123

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Testing the thresholds of toxicological concern values using a new database for food-related substances

Linda Reilly[a,b], Rositsa Serafimova[a], Falko Partosch[c], Ursula Gundert-Remy[d], José Cortiñas Abrahantes[a], Jean-Lou M.C. Dorne[a], George E.N. Kass[a,*]

[a] European Food Safety Authority, Parma, Italy
[b] Toxicology Masters Programme, Pharmacology and Therapeutics, School of Medicine, NUI Galway, Galway, Ireland
[c] Institute for Occupational, Social and Environmental Medicine, Medical School, Göttingen, Germany
[d] Charité - Institute of Clinical Pharmacology and Toxicology, Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

ARTICLE INFO

Keywords:
Threshold of toxicological concern
TTC
Food ingredients
Cramer classification
Globally harmonized system

ABSTRACT

The Threshold of Toxicological Concern (TTC) concept integrates data on exposure, chemical structure, toxicity and metabolism to identify a safe exposure threshold value for chemicals with insufficient toxicity data for risk assessment. The TTC values were originally derived from a non-cancer dataset of 613 compounds with a potentially small domain of applicability. There is interest to test whether the TTC values are applicable to a broader range of substances, particularly relevant to food safety using EFSA's new OpenFoodTox database. After exclusion of genotoxic compounds, organophosphates or carbamates or those belonging to the TTC exclusion categories, the remaining 329 substances in the EFSA OpenFoodTox database were categorized under the Cramer decision tree, into low (Class I), moderate (II), or high (III) toxicity profile. For Cramer Classes I and III the threshold values were 1000 μg/person per day (90% confidence interval: 187–2190) and 87 μg/person per day (90% confidence interval: 60–153), respectively, compared to the corresponding original threshold values of 1800 and 90 μg/person per day. This confirms the applicability of the TTC values to substances relevant to food safety. Cramer Class II was excluded from our analysis because of containing too few compounds. Comparison with the Globally Harmonized System of classification confirmed that the Cramer classification scheme in the TTC approach is conservative for substances relevant to food safety.

1. Introduction

The threshold of toxicological concern (TTC) is a pragmatic prioritization and risk assessment tool used for compounds of known structure with insufficient compound-specific toxicity data to enable risk assessment (Munro et al., 1996). First proposed by Munro and co-workers in 1996, it estimates a threshold of exposure level below which negligible risk to human health is assumed. The TTC approach uses the Cramer classification of compounds (Cramer et al., 1978) which places compounds into one of three structural classes based on their structural complexity (Munro et al., 1996). For each class, the 5th percentile of the lognormal cumulative distribution of the No-Observed-Effect-Levels (NOELs) was used to derive the human exposure threshold values, known as TTC values. The TTC approach was originally aimed at addressing substances that are present at low levels in the diet (Barlow, 2005). As such it has been used first by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and subsequently by the European Food Safety Authority (EFSA) for evaluating flavoring substances (EFSA Scientific Committee, 2012a).

Following on from Munro's original work, Kroes et al. (2004) refined the TTC approach by creating two additional structural classes, one for substances with a structural alert for genotoxicity and another one for organophosphate and carbamate substances to cover substances with anti-acetylcholinesterase activity and by recommending the exclusion of certain types of substances (Kroes et al., 2004). The latter include among others, proteins, polyhalogenated-dibenzodioxins, -dibenzofurans and -biphenyls, non-essential metals in elemental, ionic or organic forms and

Abbreviations: BMD, benchmark dose; BMDL, benchmark dose lower 95% confidence limit; bw, body weight; EFSA, European Food Safety Authority; ELINCS, European list of notified chemical substances; GHS, globally harmonized system; JECFA, joint FAO/WHO expert committee on food additives; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; TTC, threshold of toxicological concern

* Corresponding author at: European Food Safety Authority, Via Carlo Magno 1/a, I-43126 Parma, Italy.
E-mail address: georges.kass@efsa.europa.eu (G.E.N. Kass).

https://doi.org/10.1016/j.toxlet.2019.07.019
Received 22 March 2019; Received in revised form 6 July 2019; Accepted 15 July 2019
Available online 17 July 2019
0378-4274/

substances with structural alerts for high potency carcinogenicity (Kroes et al., 2004). However, Munro's original dataset of 613 substances tested for non-cancer endpoints can be criticized for not representing the 'world' of chemicals and hence to have a limited domain of applicability. Since the original publication by Munro, other chemical databases have been checked for non-cancer endpoints, for example the COSMOS database an inventory of cosmetic-related substances co-funded by the European commission and Cosmetics Europe and the RepDose database a commercial chemicals database developed by the Fraunhofer Institute of Toxicology & Experimental Medicine (ITEM), Germany, both of which use publicly available repeated dose toxicity data. Overall, the threshold values derived for Cramer Classes I and III across four databases (Munro, RepDose, ELINCS and Cosmos) are overall very similar (Kalkhof et al., 2012; Munro et al., 1996; Tluczkiewicz et al., 2011; Yang et al., 2017). Cramer Class II, much like other databases is underrepresented and lacked enough compounds to be analyzed.

The OpenFoodTox chemical hazards database was published by EFSA and consists of compounds for which EFSA is responsible for chemical risk assessment in the context of food and feed safety. These include pesticides, food additives, flavorings and nutrient sources, feed additives and both natural and man-made contaminants. The EFSA OpenFoodTox database is an open source of information on all the chemicals regarding compound characterization, background regulations, toxicological summaries used for human health but also animal health and ecological hazard assessments and links to the relevant EFSA scientific opinions. Given that EFSA's OpenFoodTox database consists of food and feed compounds for which toxicity studies have been collected, this makes it a candidate for testing the applicability of TTC values to substances relevant to food safety. Here, we analyzed the OpenFoodTox database for the protectiveness of the TTC values within the three Cramer classes after elimination of the substances with a structural alert or empirical evidence for genotoxicity, substances belonging to the organophosphate or carbamate groups and substances belonging to the exclusion categories for the TTC approach (EFSA and WHO, 2016; EFSA Scientific Committee, 2012a). This work therefore complements and extends earlier publications that have examined the applicability of the TTC approach to food contact materials (Pinalli et al., 2011) and pesticides (Feigenbaum et al., 2015).

This study also looked at the Cramer classification scheme as a toxicity prediction tool for food-related substances compared to guidance values set by the Globally Harmonized System of classification and labelling of the United Nations (GHS) for repeated dose toxicity testing. Furthermore, the study computed the internal TTC values using bioavailability predictions and compared them with those previously predicted from the combined Munro, ELINCS and food contact materials databases (Partosch et al., 2015).

2. Materials and methods

2.1. Munro TTC dataset

Since Munro's dataset was used to derive the current TTC values, it was used as a comparator when deriving TTC threshold values from our dataset. The Munro dataset consisted of non-cancer endpoints from 613 diverse compounds (609 unique compounds (Yang et al., 2017)), and included 200 chronic, 233 subchronic, 91 teratogenicity and 89 reproductive studies. Chemical and toxicological information of Munro's dataset (including chemical names, SMILES, study design, reference values and toxicity endpoints assessed) was obtained from EFSA's electronic file version (Bassan et al., 2011). However, chronic and subchronic studies were the only durations considered during our curation of a TTC dataset.

2.2. OpenFoodTox database and its curation

Compound specific information was obtained from EFSA's OpenFoodTox database published as an open source repository. Curation of the TTC dataset required application of a number of inclusion and exclusion criteria that were based on Munro's publication (Munro et al., 1996), along with recommendations from more recent publications (EFSA and WHO, 2016; EFSA Scientific Committee, 2012a; Kroes et al., 2004). Inclusion criteria specified oral studies using rat and mouse species. Where multiple studies were available for the same compound, the most sensitive/lowest reference value was chosen. NOAEL/BMDL/LOAEL reference values in the EFSA OpenFoodTox database were accepted and an extrapolation factor of 3 was used to derive NOAEL values from LOAEL values (Dourson et al., 1996; ECHA, 2008, 2010; Kalberlah et al., 2003; Tluczkiewicz et al., 2011). Although LOAEL values were not used in the Munro et al. (1996) analysis, they were utilized here to include as many compounds as possible given the study quality and dose spacing of studies included in the OpenFoodTox database. Subchronic and chronic study duration were accepted with the use of an extrapolation factor of 2 for 90-day subchronic studies (EFSA Scientific Committee, 2012b).

Organophosphates and carbamates were removed from the dataset following the recommendation that they should be assigned to a separate class that are distinct from Cramer Classes I, II and III (EFSA and WHO, 2016; EFSA Scientific Committee, 2012a; Kroes et al., 2004). Compounds belonging to this class were identified using the OECD QSAR toolbox (Version 4.2). Genotoxic compounds were also removed from the dataset based on their reporting as positive for genotoxicity in the OpenFoodTox database. Furthermore, compounds in the OpenFoodTox database reported as ambiguous, no data, not applicable, not determined, or other; were analyzed on a case-by-case basis using EFSA and JECFA publications where applicable. Finally, compounds that fell outside the applicability of the TTC approach (EFSA Scientific Committee et al., 2019) (Table 1) were also excluded from the dataset (188 compounds, of which 184 were inorganic or metals in elemental, ionic or organic form and 4 were proteins).

2.3. Cramer classification of compounds

Once the final dataset was curated, all compounds were classed using Cramer's classification scheme (Cramer et al., 1978). This was performed by profiling the compounds according to 'Toxic hazard classification by Cramer' (original) in the OECD QSAR toolbox (Version

Table 1
Exclusion categories for substances falling outside the TTC approach (EFSA Scientific Committee et al., 2019).

Substances outside of the domain of applicability	Substances with special properties
Inorganic substances	High potency carcinogens: aflatoxin-like, azoxy- or *N*-nitroso-substances and benzidines
Proteins	Steroids
Nanomaterials	Substances with a potential for bioaccumulation. This includes substances like polyhalogenated-dibenzodioxins, -dibenzofurans and -biphenyls
Radioactive substances	
Organo-silicon substances	
Metals in elemental, ionic or organic form[1]	

[1] For salts where the counter ion is a nutritionally essential metal, the organic ions is not excluded from the TTC approach.

4.2, February 2018), (Source: http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.html). In the OECD QSAR toolbox, categorization of compounds into either class of toxicological concern was based on the original decision tree comprising of 33 questions.

2.4. Distribution analysis of Cramer classes and derivation of human exposure thresholds

Compounds were categorized into one of the three Cramer classes and a cumulative distribution of compound reference values (NOAEL/BMDL/LOAEL) were plotted using R (version 3.3.2). The cumulative distribution of compounds was plotted for each Cramer class against the log of their reference values. To derive the exposure threshold values, the 5th percentile reference value (μg/kg bw per day) was calculated from the distribution of compounds for each class. Additionally, a 100-fold uncertainty factor was applied to account for interspecies differences (x10) and population variability (x10). The resulting value was then multiplied by a factor of 60 to account for the average human body weight (kg) as used by Munro et al. (1996) to give a threshold value for each Cramer class, as follows.

$$TTC\ threshold\ value = \frac{5\text{th percentile Reference value}}{100\ [uncertainty\ factor]}\ x\ 60\ [average\ human\ weight\ ]$$

2.5. Bioavailability and calculation of internal TTC

The same dataset was used additionally to calculate the internal TTC as similarly performed by Partosch et al. (2015). The commercially available ACD/Percepta (ACD/Labs, Advanced Chemistry Development, Inc., Toronto, ON, Canada, version 2017.2, www.acdlabs.com/Percepta, 2017) was used to calculate the fraction absorbed and bioavailable for all chemicals as previously reported (Partosch et al., 2015). The ACD/Percepta tool allows prediction of physicochemical properties as well as absorption, distribution, metabolism, excretion (ADME). The module for oral bioavailability uses a combination of probabilistic and mechanistic models for a quantitative prediction of human bioavailability after oral administration it was used here because of the high predictive power (Reynolds et al., 2009). Compounds reference values were multiplied by the fraction of their bioavailability and used to derive an internal TTC metric value. In the case where bioavailability was zero, a value of 0.001 was used to avoid a zero value being used for the Internal TTC. The empirical distribution of the internal reference values was plotted for each Cramer class to derive internal TTC values. The internal TTC value was calculated from 5th percentile of the cumulative distribution of NOAEL values for each Cramer class using a reduced uncertainty factor of 25 (Partosch et al., 2015). The rationale for reducing the total uncertainty factor of 100 to 25 is that the default uncertainty factor for the interspecies toxicokinetic sub-factor uncertainty factor (with a default value of 4) is already taken into consideration.

Calculation:

$$internal\ TTC\ threshold\ value = \frac{5\text{th percentile Reference value}}{25\ [\text{uncertainty factor}]}\ x\ 60\ [average\ human\ weight\ ]$$

2.6. GHS and Cramer classification

Compounds were analyzed for concordance of the Cramer classification obtained with the GHS classification STOT RE (Single Target Organ Toxicity- Repeated Dose) guidance values. This was accomplished by comparing the allocation of Cramer Class I, II, and III with the GHS STOT RE guidance values below (United Nations, 2017). In our dataset, BMDL values were also used with NOAEL values to compare against GHS guidance values.

1 GHS STOT RE 1: NOAEL $\leq$ 10 mg/kg bw per day
2 GHS STOT RE 2: NOAEL $>$ 10 $\leq$ 100 mg/kg bw per day
3 GHS not classified for STOT RE: NOAEL $>$ 100 mg/kg bw per day

Subchronic studies which had a NOAEL below 10 mg/kg bw per day were classified as GHS STOT RE 1, NOAELs between 10 mg/kg bw per day and 100 mg/kg bw per day were classified as GHS STOT RE 2; and NOAELs above 100 mg/kg bw per day were not classified for GHS STOT RE.

2.7. Statistical analysis

The cumulative distribution function of the reference values was calculated. Additionally, the 5th percentiles and their 90% confidence intervals for each Cramer class were determined for both the external TTC and internal TTC analysis using R (version 3.3.3). Using R, the cox model was applied to the cumulative distribution of the NOAEL values to determine if Cramer classes were proportionally distinct from each other.

3. Results

The TTC dataset was collated by applying a set of inclusion and exclusion criteria to the OpenFoodTox dataset described above. Using EFSA's OpenFoodTox database, 1855 studies assessing human health were extracted, of which 938 were unique organic compounds not falling within the exclusion criteria listed in Table 1. Only chronic and subchronic studies were considered reducing the dataset to 586 compounds, out of which 423 were tested in rat or mouse species while 395 had either BMDL, LOAEL or NOAEL reference values reported (see Supplementary Material, Table 1a and b). A total of 38 compounds were additionally excluded as they were either organophosphates or carbamates, the remainder (35 compounds) were excluded for their genotoxicity potential on a case-by-case basis using EFSA and JECFA opinions (see Supplementary Material, Table 1c; the table also contains compounds with ambiguous or undetermined genotoxic potential) to give a final TTC dataset of 329 compounds. Out of these, over half were pesticides (n = 180), with flavorings being the second largest class of substances (n = 69), the composition of the dataset is shown in Table 2. See Supplementary Material, Tables 1a and 1b, for the full list.

3.1. Cramer classification

The Cramer classification was applied to this dataset (OpenFoodTox-derived) to classify each compound into one of the three classes. The results for the OpenFoodTox-derived (n = 329) and Munro's dataset (n = 613), are shown in Table 3. In Munro's dataset, Class I and Class II account for 22% and 5% of the data, while Class III

Table 2
Compound class of use for each Cramer Class (I, II & III) in the OpenFoodTox dataset.

	Cramer Classes (n)				
Class of use	**I**	**II**	**III**	**Sum**	**%**
Pesticides	3	2	175	180	54.7
Flavorings	19	4	46	69	21.0
Additives	15	1	19	35	10.6
Food contact materials	5		8	13	4.0
Nutrient sources	4		6	10	3.0
Contaminants	2		3	5	1.5
Other	5		12	17	5.2
Sum	53	7	269	329	100

Table 3
Cramer classification of the OpenFoodTox and Munro's datasets.

	OpenFoodTox		Munro	
Cramer classification	N	%	N	%
Class I	53	16	137	22
Class II	7	2	28	5
Class III	269	82	448	73
Total	329	100	613	100

accounts for the majority of data at 73%. In comparison, the OpenFoodTox TTC dataset also has most compounds categorized as Class III at 82%, while Class I and II account for only 16% and 2% of the data, respectively. In terms of class of use, nearly all pesticides fell into Cramer Class III (97%), whereas for flavorings and additives, the proportion in Cramer Class III decreased to 67% and 54%, respectively. Because Cramer Class II was not well represented ($n=7$) and lacked any statistical weight, this class was excluded from further analysis in this paper. The classification of the individual substances in the OpenFoodTox dataset is shown Table 1S.

3.2. Cumulative distribution analysis of the TTC dataset

To derive TTC values from the OpenFoodTox-derived dataset and to compare them with Munro's current threshold values, the cumulative distribution of compounds log NOAEL values was plotted for both Cramer Classes I and III. The 5th percentile was calculated for each class and used to derive TTC values (Fig. 1). Fig. 1 shows that the two distributions are proportionally distinct ($p < 0.001$***) until the cut-off value 5.5 μg/kg bw per day after which both distributions overlap.

The 5th percentile calculated from the cumulative distribution of the OpenFoodTox-derived and Munro's dataset for Cramer Class I were 1667 μg/kg bw per day and 3000 μg/kg bw per day, respectively. For Cramer Class III, the 5th percentiles for the OpenFoodTox-derived and Munro's Cramer Class III were 145 μg/kg bw per day and 150 μg/kg bw per day, respectively. The 5th percentiles were used to derive TTC values for Cramer Classes I and III. The derived threshold values for Cramer Class I from the OpenFoodTox dataset was 1000 μg/person per day (90% confidence interval: 187–2190) as compared to the original TTC value of 1800 μg/person per day as calculated by Munro. This is equivalent to 17 μg/kg bw per day (90% confidence interval: 3–37). Similarly, for Cramer Class III the OpenFoodTox-derived TTC value was 87 μg/person per day (90% confidence interval: 60–153) whereas Munro's TTC value was 90 μg/person per day. This is equivalent to 1.5 μg/kg bw per day (90% confidence interval: 1.0–2.6).

In Table 4 the TTC values expressed as μg/kg bw per day are given from different data bases.

3.3. Internal TTC

Calculating internal TTC values could be a way to make the information from oral toxicological studies available and usable for non-oral route of administration. Using ACD/Percepta, the compound specific bioavailability data (expressed as fraction 0.001 to 0.995) were derived and the cumulative distributions of the computed internal dose reference values were plotted for both Cramer Class I and II/III. The 5th percentile was calculated for each class and used to derive internal TTC values. Fig. 2 shows both Cramer classifications when plotted are proportionally distinct ($p < 0.001$***) from each other.

The 5th percentile and the 90th confidence intervals of Cramer Class I for the OpenFoodTox ($n = 53$) was 0.5 μg/kg bw per day (90% confidence interval: 0.25–37.8). For Cramer Class III, the 5th percentiles for the OpenFoodTox ($n = 269$) was 0.4 μg/kg bw per day (90% confidence interval: 0.15-0.88). Table 5 shows the comparison with the internal TTC values derived by Partosch et al. (2015) which were 6.9 μg/kg bw per day (90% confidence interval: 3.8–11.5) for Cramer Class I ($n = 287$) and 0.1 μg/kg bw per day (90% confidence interval: 0.08-0.14) for Cramer Class III (n = 1289), respectively.

3.4. GHS and Cramer classification scheme

The prediction of the Cramer classification was compared to guidance values according to the Globally Harmonized System of classification and labelling of the United Nations (GHS) using reference values from 90-day repeated dose toxicity studies ($n = 124$) (Table 6). The Cramer classification for these compounds from the OpenFoodTox database was found to be concordant with the GHS classification in 43% of compounds ($n = 60$). The Cramer classification scheme underestimated the toxicity in 15% of compounds ($n = 19$), and overestimated the toxicity compared with the GHS cut-off value in 36% of compounds ($n = 45$).

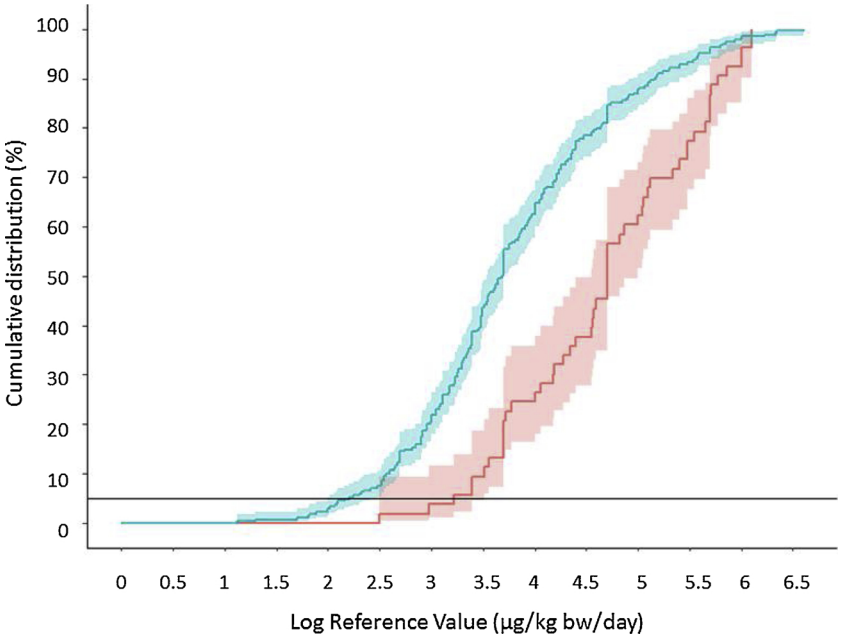

Fig. 1. Cumulative distribution of Log Reference Values for Cramer Classes I (red) and III (blue). Data are expressed as log Reference Value (μg/kg bw per day) value for each compound with 90th percentile confidence bands. The cumulative distribution of compounds from the OpenFoodTox database for Cramer Classes I and III are expressed as a percentage and the 5th percentile is indicated by the black horizontal line.

Table 4
TTC values (in μg/kg bw per day) across different databases.

	Munro database (Munro et al., 1996) *n = 613*	Munro database (Leeman et al., 2014)*	RepDose (Tluczkiewicz et al., 2011) *n = 554*	ELINCS (Kalkhof et al., 2012) *n = 824*	COSMOS (Yang et al., 2017) *n = 552*	OpenFoodTox (this paper) *n = 329*
Substances	chemicals mixed origin	chemicals mixed origin	chemicals mixed origin	industrial chemicals	cosmetics-related chemicals	chemicals in food and feed
Cramer Class						
I	30	30	32	25	42	17
III	1.5	4.0	1.0	13	8	1.5

*Only Class III compounds were re-analyzed.

4. Discussion

The TTC approach has repeatedly been demonstrated to be a conservative approach to identify exposure levels below which no toxicity is expected to occur. The TTC approach has been found applicable to different chemicals subject to different applications or uses. However, there was a need to check whether the TTC approach is protective enough for the substances found in the food/feed chain. This study gathered data from EFSA's OpenFoodTox database with the aim of specifically testing the TTC application with chemicals relevant to food safety. This work therefore complements and extends earlier publications that have examined the applicability of the TTC approach to food contact materials (Pinalli et al., 2011) and pesticides (Feigenbaum et al., 2015). The latter publication used the EU-Pesticides database that includes pesticides also evaluated by EFSA. Curation of the dataset resulted in a total of 329 compounds. However, unlike Munro's dataset, organophosphates, carbamates and compounds with alerts for genotoxicity were not included in our dataset, as was done by (Tluczkiewicz et al., 2011) and (Leeman et al., 2014). We also compared the two databases for potential overlap in the coverage of substances and found that there were 69 substances that were common to both databases. This corresponds to an overall overlap between the two databases of 21%. Of these substances, 8 belonged to Class I, 2 were in Class II and 59 in Class III.

Comparison of the Cramer distribution of the compounds from EFSA's OpenFoodTox-derived database with those from the Munro database shows that the OpenFoodTox-derived database has higher percentage of compounds falling in Class III whereas the percentage in Classes I and II is approximately only half of the percentage found in the respective classes in the Munro database (Table 3). The reasons for this difference are not clear but this has an impact on the total number of compounds from the OpenFoodTox database available for analysis in Classes I and II.

Table 5
Internal TTC values derived from Partosch et al. (2015) and OpenFoodTox datasets.

	Partosch et al., 2015	*OpenFoodTox*
Cramer Class	TTC value	TTC value
I	6.9 (3.8-11.5)	0.5 (0.25-37.8)
II/III	0.1 (0.08-0.14)	0.4 (0.15-0.88)

Data are expressed as μg/kg bw per day. The external reference values corrected for compound specific bioavailability, the 5th percentile of the cumulative distribution was divided by an uncertainty factor of 25. Data are expressed as μg/kg bw per day for Cramer Class I & II/III.

Table 4 compares the threshold values of published TTC values. Overall, the threshold values derived range from 17 to 42 μg/kg bw per day in Cramer Class I and from 1.0 to 13 μg/kg bw per day in Cramer Class III across databases with substances related to food and feed (OpenFoodTox), chemicals (RepDose, ELINCS, Cosmos) and mixed (Munro) (Kalkhof et al., 2012; Leeman et al., 2014; Munro et al., 1996; Tluczkiewicz et al., 2011; Yang et al., 2017). Similar to other studies, Cramer Class II lacked enough compounds to be included in our analysis. Overall, given the overlap in the 90th percentile confidence intervals with the TTC values for Classes I and III from the other databases, the Munro TTC values are interpreted as being sufficiently protective against potential health hazards over a wide range of

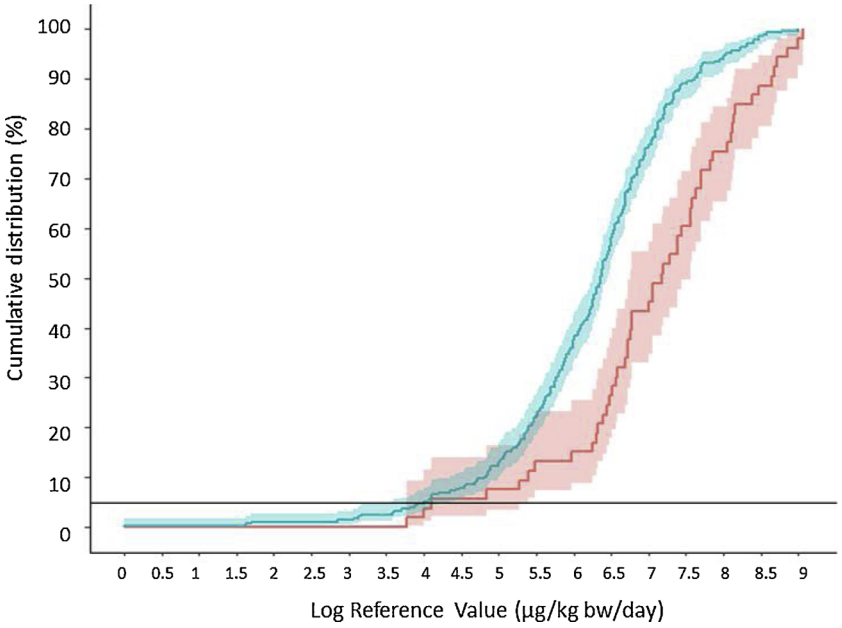

Fig. 2. Cumulative distribution of internal NOAEL values for Cramer Classes I (red) and II/III (blue) derived using predicted bioavailability. Data are expressed as **log** Reference Value (μg/kg bw per day) for each compound from the OpenFoodTox database with 90th percentile confidence bands. The cumulative distribution of compounds for Cramer Class I and II/III, expressed as a percentage and the 5th percentile is indicated by the black horizontal line. Both Cramer classifications are proportionally distinct ($p < 0.001$***) therefore, cox's proportional hazard assumption is not violated (see Supplementary Fig. 1S).

Table 6
Comparison of predicted toxicity by Cramer classification and GHS STOT RE guidance values.

	90-day repeated dose toxicity			
	GHS STOT RE 1 (NOAEL < 10mg/kg bw per day)	GHS STOT RE 2 (NOAEL > 10 < 100mg/kg bw per day)	GHS not classified for STOT RE (NOAEL > 100mg/kg bw per day)	Sum
Cramer Class I	**6**	**11**	*17*	34
Cramer Class II	**2**	*3*	1	6
Cramer Class III	*40*	22	22	84
Sum	48	36	40	124

A total of 124 reference values from 90-day repeated toxicity studies was used. Concordant classification is underlined while under-predicted classification by Cramer is recorded in bold numbers.

chemical sectors. Therefore, the Munro TTC values are robust enough for use in risk assessment, including in the case of substances pertinent to food safety.

The internal TTC was also calculated using the conversion of external oral reference point values using compound specific bioavailability data predicted using an in-silico approach. The derived values may be used for the assessment of the exposure from all routes of exposure after conversion to internal exposure. The simple approach we have taken here to correct the external dose for absorption/bioavailability has been criticized and a more appropriate procedure for deriving values representing internal dose may be required to perform a full physiologically based pharmacokinetic (PBPK) modelling (see for example (Blackburn et al., 2019; Chebekoue and Krishnan, 2019)). However, given the information available for most of the data sets e.g. ELINCS, Food Contact Materials, the lack of data which can be used to calculate the clearance of the substance from the body would prevent a more ambitious approach. The use of prediction tools would introduce additional uncertainty in the derived values.

The data we have derived show that the internal TTC values are considerably lower in comparison to the external TTC values, in agreement with previous findings (Partosch et al., 2015). In the case of the OpenFoodTox-derived dataset, there was no clear difference in threshold value between Classes I and III. Whereas for Class III the threshold value is similar to that reported previously using Munro, the ELINCS and the food contact materials databases (Partosch et al., 2015), the Class I value is considerably lower. However, the Class I value had a wide 90% confidence interval that indicates that there is considerable uncertainty about the precision of this value presumably because of the overall low number of substances in that class ($n = 53$). For comparison, Partosch et al. (2015) had 287 substances in their Class I. Three of the 53 internal class I compounds fell below the 5th percentile, and their predicted bioavailabilities were very low. These compounds were lacto-*N*-neotetraose, and beta-cyclodextrin, which were applied a predicted default bioavailability value of 0.001 (bioavailability predicted to be zero), and 1,2-cyclohexanedicarboxylic acid, 1,2-diisononyl ester which was applied a predicted bioavailability value of 0.02. These drive down the internal TTC value.

The Cramer classification scheme is based on the metabolic and toxicological information available nearly four decades ago. Although, calls for revisions and refinements of the scheme have been made to incorporate advances in scientific knowledge, the analysis carried out by EFSA in 2012 has shown that the application of the Cramer classification scheme in the TTC approach is conservative and therefore protective of human health (EFSA Scientific Committee, 2012a).

Kalkhof and co-workers came to a similar conclusion when analyzing the ELINCS database for concordance between Cramer classification and the Global Harmonized System (GHS) for classification and labelling (Kalkhof et al., 2012). Their analysis, which used 813 NOAEL values from both subacute and subchronic studies converted to chronic NOAELs figured out that less than 5% of the Cramer classifications underestimated toxicity, whereas the toxicity was overestimated by nearly 70%. Here, the Cramer scheme using the OECD QSAR toolbox on 124 reference values from 90-day repeated toxicity studies was shown to be a poor predictor for toxicity according to GHS. The Cramer classification was found to be concordant with the GHS classification in 43% of compounds while it underestimated the toxicity in 15% of compounds and overestimated the toxicity compared with the GHS cut-off value in 36% of compounds. From a regulatory perspective the Cramer classification scheme in the TTC approach is conservative and therefore, protective of human health in situations of chemical hazard assessment where little or no safety information is available.

5. Conclusion

The present study aimed to test the Threshold of Toxicological Concern values proposed by Munro et al. (1996) for their conservatism

using EFSA's new OpenFoodTox database and comparing the results with the TTC values derived from other databases. Although the number of chemicals of the OpenFoodTox database is relatively low and therefore vulnerable to variability, the threshold values were similar to those obtained from the Munro and other databases. The Cramer classification scheme results in an overestimation of toxicity and hence it is conservative. Therefore, attempts should be made to improve the classification scheme because overpredicting toxicity would lead to unnecessary testing/actions. The internal TTC might be an additional tool when assessing combined exposure from different routes. Finally, comparison with the GHS classification confirmed that the Cramer classification scheme in the TTC approach is conservative for substances relevant to food safety.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclaimer

The opinions expressed here are those of the authors and do not reflect the views of EFSA.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxlet.2019.07.019.

References

Barlow, S., 2005. Threshold of Toxicological Concern (TTC): a tool for assessing substances of unknown toxicity present at low levels in the diet. ILSI Europe pp. 37.

Bassan, A., Fioravanzo, E., Pavan, M., Stocchero, M., 2011. Applicability of physicochemical data, QSARs and read-across in Threshold of Toxicological Concern assessment. EFSA Supp Pub 8 159E.

Blackburn, K.L., Ellison, C.A., Stuard, S.B., Wu, S., 2019. Dosimetry considerations for in vivo and in vitro test data and a novel surrogate iTTC approach for read-across based on metabolites. Comp Toxicol (in the press).

Chebekoue, S.F., Krishnan, K., 2019. Derivation of internal dose-based thresholds of toxicological concern for occupational inhalation exposure to systemically acting organic chemicals. J. Occup. Environ. Hyg. 1–12.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - Decision tree approach. Food Cosmet Tox 16, 255–276.

Dourson, M.L., Felter, S.P., Robinson, D., 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. Regul. Toxicol. Pharmacol. 24, 108–120.

ECHA, 2008. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.5: Adaptation of Information Requirements. European Chemicals Agency. May 2008 Available at: https://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r5_en.pdf?vers=20_08_08. European Chemicals Agency.

ECHA, 2010. Guidance on Information Requirements and Chemical Safety Assessment. Part B: Hazard Assessment. Chapter R.5: Adaptation of Information Requirements.

EFSA and WHO, 2016. Review of the Threshold of Toxicological Concern (TTC) Approach and Development of New TTC Decision Tree. EFSA Supporting Publication, pp. 1–50.

EFSA Scientific Committee, 2012a. Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA J 10, 103.

EFSA Scientific Committee, 2012b. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA J 10, 2579.

EFSA Scientific Committee, More, S.J., Bampidis, V., Benford, D., Boesten, J., Bragard, C., Halldorsson, T.I., Hernández-Jerez, A.F., Hougaard Bennekou, S., Koutsoumanis, K.P., Naegeli, H., Nielsen, S.S., Schlatter, J.R., Schrenk, D., Silano, V., Turck, D., Younes, M., Gundert-Remy, U., Kass, G.E.N., Kleiner, J., Rossi, A.-M., Serafimova, R., Reilly, L., Wallace, H.M., 2019. Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA J (in preparation).

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 and chemical toxicology: an international journal published for the British Industrial Biological Research Association 75, 24–38.

Kalberlah, F., Schneider, K., Schuhmacher-Wolz, U., 2003. Uncertainty in toxicological risk assessment for non-carcinogenic health effects. Regul. Toxicol. Pharmacol. 37, 92–104.

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, 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., 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem. Toxicol. 42, 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, 273–278.

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, 829–867.

Partosch, F., Mielke, H., Stahlmann, R., Kleuser, B., Barlow, S., Gundert-Remy, U., 2015. Internal threshold of toxicological concern values: enabling route-to-route extrapolation. Arch. Toxicol. 89, 941–948.

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, 523–534.

Reynolds, D.P., Lanevskij, K., Japertas, P., Didziapetris, R., Petrauskas, A., 2009. Ionization-specific analysis of human intestinal absorption. J. Pharm. Sci. 98, 4039–4054.

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, 340–350.

United Nations, 2017. Globally Harmonized System of Classificationand Labelling of Chemicals (GHS). United Nations, New York and Geneva pp. 534.

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, 170–193.