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Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment

EFSA Scientific Committee

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Abstract

The Scientific Committee confirms that the Threshold of Toxicological Concern (TTC) is a pragmatic screening and prioritisation tool for use in food safety assessment. This guidance provides clear step-by-step instruction for use of the TTC approach. The inclusion and exclusion criteria are defined and the use of the TTC decision tree is explained. The approach can be used when the chemical structure of the substance is known, there is limited chemical–specific toxicity data and the exposure can be estimated. The TTC approach should not be used for substances for which EU legislation requires the submission of toxicity data or when data are available that allow for a risk assessment or if the substance under consideration falls into one of the exclusion categories. Substances with exposures below the TTC values are considered of low probability of adverse health effects. For substances that have the potential to be DNA-reactive mutagens and/or carcinogens based on the weight of evidence, the relevant TTC value is 0.0025 µg/kg bw per day. For organophosphates or carbamates, the relevant TTC value is 0.3 µg/kg bw per day. All other substances are grouped according to the Cramer classification. The TTC values for Cramer classes I, II and III are 30 µg/kg bw per day, 9 µg/kg bw per day and 1.5 µg/kg bw per day, respectively. If the estimated exposure to a substance is higher than the relevant TTC value, a non-TTC approach is required to conclude on potential adverse health effects.

Keywords: Threshold of toxicological concern, risk assessment, Cramer classification scheme.

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1. Introduction

The threshold of toxicological concern (TTC) approach is a pragmatic, scientifically valid methodology to assess the safety of substances of unknown toxicity found in food (EFSA and WHO, 2016a). It has been developed to screen and prioritise the safety assessment of substances when the chemical structure of the substance is known and where human oral exposure can be estimated to be relatively low. The TTC approach is used when there is limited chemical–specific toxicity data and can be used for substances with or without structural alerts for genotoxicity and for cancer and non-cancer endpoints.

The TTC approach should not be used for substances for which EU legislation requires the submission of toxicity data. Furthermore, when data are available that allow for a risk assessment, these data should be used and not the TTC approach.

TTC values are numbers describing generic human chronic exposure thresholds that have been established by grouping experimental toxicity data from animal bioassays. TTC values are derived by applying a probabilistic methodology such that the chance of adverse effects at exposures below these threshold values are considered to be low (Kroes et al., 2004).

This guidance has been developed to provide practical help in the appropriate use of the TTC approach taking into account updated scientific information, new methodologies and recommendations from the EFSA and WHO report (2016a).

1.1. Background and Terms of Reference as provided by EFSA

The Threshold of Toxicological Concern (TTC) approach is a screening and prioritization tool for the safety assessment of chemicals when hazard data are incomplete and human exposure can be estimated. In 2012, the Scientific Committee published a scientific opinion on the TTC approach (EFSA Scientific Committee, 2012b). In 2013, WHO and EFSA initiated a project to provide recommendations for improving the existing TTC approach and update/revise the methodology. A call for data and review of publicly available information led to development of a background paper by WHO that was discussed at an expert EFSA/WHO workshop in December 2014 (EFSA and WHO, 2016a). The key topics of discussion at the workshop related to the Cramer classification scheme and its underlying concepts, and to the TTC values and decision tree. In the expert workshop, it was concluded that the TTC approach is based on scientific risk assessment principles and is fit for purpose as a screening tool to assess low dose chemical exposures and to identify those chemicals for which further data are necessary to assess the human health risk. The expert group made recommendations to improve and expand the TTC concept and update the methodology, considering the state-of-the-science and available toxicological databases. Following the workshop, the conclusions and recommendations were published for consultation, and responses to the consultation were addressed by the expert group prior to publication of the final workshop report (EFSA and WHO, 2016b).

The conclusions and recommendations of the expert group related to the following topics:

- The Cramer classification scheme
- Consideration of metabolism in the TTC values
- The TTC domain of applicability
- The TTC approach and value for genotoxic substances
- TTC values for non-DNA reactive carcinogens and non-cancer endpoints
- The points of departure and available databases
- Chemical categories excluded from the TTC approach
- Specific TTC values
- Combined exposure to multiple chemicals and from multiple sources
- Acute and other less than lifetime exposures

- Potentially sensitive life-stages
- A revised TTC decision tree

Terms of Reference

To update of the 2012 EFSA Scientific Opinion on exploring options for providing advice on possible human health risks based on the concept of Threshold of Toxicological Concern (TTC) by preparing a guidance document on the use of the TTC approach in food safety. The Guidance should take into consideration particular recommendations from the EFSA/WHO workshop (i.e. Cramer classification scheme, the exclusion of chemical categories and the TTC Decision Tree), as well as the latest scientific developments in the field. The Guidance will be subject to a public consultation prior to adoption by the EFSA Scientific Committee.

1.2. Approach taken to develop this Guidance

In this document, the TTC approach is summarised and updated. The 2012 EFSA opinion remains available as a comprehensive review of the methodology but guidance on how to apply the TTC approach within EFSA is developed here. The Guidance covers only the application of the TTC approach to human exposure via the oral route; it does not address the applicability of the TTC approach to target animal species or ecotoxicological risk assessment. The recommendation of the workshop to combine existing databases is not addressed in this Guidance.

The Guidance also takes into account the literature on the TTC approach published since the EFSA/WHO report (EFSA and WHO, 2016a). The periods covered ranged from January 2012 to November 2017 and the searches were performed in Web of Science (http://wok.mimas.ac.uk/). No search limits for document type or language were used, and the search strings were 'threshold' and 'toxicological concern' (topic). The number of hits was 262. Following application of the exclusion criteria (when TTC only appeared as keyword and was not further used or described in title or abstract or was mentioned only as a general method for risk assessment with no further description or analysis), the number of papers selected for further evaluation was 70.

1.3. Audience and degree of obligation

This Guidance is aimed specifically at all those contributing to EFSA chemical risk assessments but is broadly applicable for general use of the TTC approach. If using the TTC approach within EFSA, the application of this Guidance is unconditional.

2. The Cramer classification scheme

2.1. Development of the Cramer classification scheme

The application of the TTC concept utilises the classification scheme[1] which was originally proposed by Cramer, Ford and Hall (Cramer et al., 1978) as a priority setting tool and as a means of making expert judgements in food chemical safety assessment more transparent and reproducible. These authors drew upon their experience in classifying food flavouring substances (Oser and Hall, 1977) and in evaluating pesticides and industrial chemicals. The criteria they proposed for the three structural classes are shown in Table 1.

[1] To avoid confusion between the Cramer classification scheme for the structural classes (originally referred to as decision tree by Cramer et al. (1978) and the TTC decision tree, the term decision tree is exclusively used in this Guidance to make reference to the TTC decision tree.

Table 1: Structural classes for chemicals proposed in the Cramer scheme (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. This class would include normal constituents of the body (excluding hormones); simply-branched, acyclic aliphatic hydrocarbons; common carbohydrates; common terpenes; substances that are sulphonate or sulphamate salts, without any free primary amines.
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. This class would include common components of food; substances containing no functional groups other than alcohol, aldehyde, side-chain ketone, acid, ester, or sodium, potassium or calcium sulphonate or sulphamate, or acyclic acetal or ketal and are either a monocycloalkanone or a bicyclic substance with or without a ring ketone.
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. This class would include structures that contain elements other than carbon, hydrogen, oxygen, nitrogen or divalent sulphur; certain benzene derivatives; certain heterocyclic substances; aliphatic substances containing more than three types of functional groups.

Cramer et al. (1978) based their classification on a series of 33 questions. These were mostly related to chemical structure, but also to natural occurrence in food and in the body. The set of 33 questions were intended as a compromise between discrimination (into the three classes) and complexity (of the questions and their ordering). The logic of the sequential questions was based on the available knowledge on toxicity available at the time and on how chemical structures are metabolised by mammalian metabolic pathways. The Scientific Committee concurs with EFSA (2012b) that the application of the Cramer classification scheme in the TTC approach is conservative and therefore protective of human health.

Cramer et al. (1978) predicted that the majority of substances would fall into either Class I or Class III, and that is indeed borne out by the database established by Munro et al. (1996) and by subsequent experience with the TTC approach. Cramer et al. (1978) tested the validity of their classification scheme by classifying 81 chemicals (used as food additives, drugs, industrial chemicals or pesticides), on which toxicity data from short-term or chronic studies were available, into the three structural classes and by tabulating the NOAELs.[2] There was overlap in the range of magnitudes of the NOAELs between the three structural classes, but it was clear that the NOAELs of Class I substances were generally higher than those of Class III, with those of Class II being in between.

2.2. Computer-based implementation of the Cramer classification

Following a recommendation made in a workshop (Patlewicz et al., 2007), the JRC commissioned the development of a Toxtree rule base to facilitate the consistent application of the Cramer scheme. Toxtree is freely downloadable from the JRC website (https://ec.europa.eu/jrc/en/scientific-tool/toxtree-tool) and from Sourceforge (http://toxtree.sourceforge.net/).

[2] The term NOAEL (No-Observed-Adverse-Effect Level) is used throughout this Guidance. It is noted that Munro *et al.*(1996) used the term NOEL with the same meaning.

Toxtree (current version v3.1.0, May 2018) includes both the original Cramer rule base with the 33 structural rules and an extended rule base with 5 additional rules which were introduced to overcome misclassification (in Class I or Class II) of several substances with low NOAELs. In both versions of the Cramer rule base, two predefined 'look-up' lists of normal body constituents (around 100 substances) and common food components are used (more than 400 substances).

Cramer rule bases (original and extended) are also implemented in OECD QSAR Toolbox (current version v4.2, February 2018) (http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm). In the manual of the software it is mentioned that the current versions of the Cramer rule bases implemented are comparable with those in Toxtree v.2.6.6.

It should be noted that the computer-based implementation of the Cramer classification scheme in Toxtree and the OECD QSAR Toolbox has inevitably involved some decisions by the programmer, such as the chemically based interpretation of the original rules, and the establishment of pre-defined 'look-up lists' of normal body constituents and common food components. Therefore, the use of different software tools and also their application by individual experts might lead to different classifications (Bhatia et al., 2015; Roberts et al., 2015), and, therefore, the process used should be clearly documented. Both software platforms provide a decision tool for classification and list the rules that lead to the classification of the chemical. This allows for assessment of the classification as part of the weight of evidence.

3. The TTC approach

The original concept of the TTC approach and the databases that support that concept have been reviewed many times and will not be reiterated here (Munro et al., 1996; Cheeseman et al., 1999; Gold et al., 1999; Barlow et al., 2001; Kroes et al., 2004; 2007; 2008; SCCP, 2008; Brown et al., 2010; Boobis et al., 2017). As the validity of the TTC values is critically dependent on the quality of the databases used to derive them, a critical evaluation of the existing databases was performed and is detailed extensively in the EFSA opinion (EFSA Scientific Committee, 2012b).

A TTC value was calculated from the distribution of NOAELs for each of the three Cramer structural classes, using a database of 613 chemicals with 2941 NOAELs (Munro et al., 1996). This represented a broad range of chemicals: industrial, food, environmental, agricultural, pharmaceuticals and consumer product chemicals likely to be found commercially and with good supporting toxicological data, yielding 137, 28 and 448 chemicals in Cramer class I, II and III, respectively. For each of the 613 chemicals, the most conservative NOAEL was selected, based on the most sensitive species, sex and endpoint. Subchronic NOAELs were divided by a factor of three to extrapolate to a chronic NOAEL. (EFSA Scientific Committee, 2012a) recommended a factor of two for extrapolating from subchronic to chronic study duration in rodents, which means that the factor of three used by Munro et al. (1996) is more conservative. The fifth percentile NOAEL (in mg/kg bw per day) was calculated for each structural class and this was converted to the intake for a 60 kg person following the application of an uncertainty factor to calculate the TTC value. A 100-fold uncertainty factor was used, which is the default factor used for establishing health-based guidance values for chemicals using toxicity data from animal studies. This procedure resulted in TTC values of 30, 9.0, 1.5 µg/kg bw per day for Cramer classes I, II and III, respectively (Table 2).

In 2012, the Scientific Committee recommended that substances that would be classified in Cramer Class II under the Cramer classification should be treated as if they were Cramer Class III substances (EFSA Scientific Committee, 2012b). The rationale was that Cramer Class II was based on very few substances. However, the subsequent EFSA and WHO workshop recommended that Cramer Class II continue to be used and applied to the TTC approach for the time being (EFSA and WHO, 2016a).

Kroes et al. (2004) explored whether particular neurotoxicants should be considered as a separate class. They noted that the 5th percentile NOAEL for organophosphates (OP) was lower, by around an order of magnitude, than the corresponding 5th percentile NOAEL for other neurotoxicants. The other neurotoxicants resulted in a plot comparable to the Cramer Class III substances examined by Munro et al. (1996). By applying an uncertainty factor of 100 to the 5th percentile NOAEL for OPs, Kroes et al. (2004) derived a human exposure threshold of 0.3 µg/kg bw per day (18 µg/person per day) (EFSA Scientific Committee, 2012b). The Scientific Committee conducted a further analysis of OPs and carbamates. It recommended that a TTC value of 0.3 µg/kg bw per day (18 µg/person per day) (Table 2) should be used for both these groups of substances rather than the value of 1.5 µg/kg bw per day used for other substances in structural Class III. The rationale and validity of this value is discussed in detail in EFSA Scientific Committee (2012b).

For potentially genotoxic substances, Kroes et al. (2004) derived a TTC value of 0.0025 µg/kg bw per day (0.15 µg/person per day) from the Carcinogenic Potency Database (CPDB) (Cheeseman et al., 1999; Gold et al., 1999) (Table 2). The rationale and validity of this value is discussed in detail in EFSA (2012b). Recently, Boobis et al. (2017) reviewed the origin of the TTC values for genotoxic or carcinogenic substances and recommended an approach for updating the database on the basis of current knowledge, including mode of action.

Table 2. TTC values – classification of substances

Classification	TTC value in µg/person per day	TTC value in µg/kg bw per day[3]
Potential DNA-reactive mutagens and/or carcinogens	0.15	0.0025
OPs and carbamates	18	0.3
Cramer Class III	90	1.5
Cramer Class II	540	9.0
Cramer Class I	1800	30

The Scientific Committee agrees with these TTC values. The Scientific Committee notes the recommendations from the EFSA/WHO 2016 report that review of the existing non-cancer databases is needed. In the light of the review by Boobis et al. (2017) this should include relevant cancer databases. This requires an international agreement on the format and curation of all existing databases. The Scientific Committee is aware of the ongoing CEFIC-LRI-sponsored project[4] to generate a curated and quality-controlled database on genotoxic and non-genotoxic carcinogens.

To facilitate the application of the TTC approach, Kroes et al (2004) proposed a decision tree which has since been modified. The TTC decision tree presented in this Guidance (Fig. 1) is based on the EFSA and WHO (2016a) version.

The TTC approach is currently used by several international and European bodies (e.g. JECFA, ECHA, SCP, EMA, the non-food Scientific Committees of the European Commission). Adaptation of the TTC concept has been considered with respect to other routes of human exposure such as inhalation

[3] Note that there is no conflict with EFSA's recent recommendation to use a default value of 70 kg, when appropriate, for adult body weight (EFSA Scientific Committee, 2012a). In the case of the TTC approach, a body weight value of 60kg was used by Munro et al. (1996) to derive the generic human exposure threshold values. Therefore, to convert these values back from a per person basis to a body weight basis, 60 kg must also be used.
[4] http://cefic-lri.org/projects/b18-carcinogen-dose-response-database-for-threshold-of-toxicological-concern-cdrd-ttc/

(Drew and Frangos, 2007; Carthew et al., 2009; Escher et al., 2010; Barle et al., 2016; Schuurmann et al., 2016; Tluczkiewicz et al., 2016; Chebekoue and Krishnan, 2017) and dermal exposure (Safford, 2008; Safford et al., 2011; Williams et al., 2016). Similar principles to those underlying the TTC approach have also been considered for use in screening of chemicals for effects on environmental species (De Wolf et al., 2005; Belanger et al., 2015).

Within EFSA, examples of the use of the TTC approach include the evaluation of:

- Flavouring substances in food (EFSA CEF Panel, 2010)
- Impurities, metabolites and degradation products of food additives (EFSA ANS Panel, 2012)
- Pharmacologically active substances present in food of animal origin (EFSA CONTAM Panel, 2018)
- Some metabolites and degradation products of plant protection products in the context of residue definition for risk assessment (EFSA PPR Panel, 2016)
- The derivation of 'maximum acceptable feed concentrations' for flavouring additives based on default values for feed consumption (EFSA FEEDAP Panel, 2017)
- The development of the criteria for the safety evaluation of mechanical processes to produce recycled poly(ethylene terephthalate) (PET) intended to be used for manufacture of materials and articles in contact with food (EFSA CEF Panel, 2011)

3.1. Considerations of TTC values for less-than-lifetime exposure

Exposure to substances in food or feed will generally be of a chronic nature, and the TTC values are calculated based on chronic exposure studies. However, there may be situations where a short-term or intermittent exposure period may be considered, such as incidents or presence of a substance during time-limited production period. The TTC approach may be applicable in these situations. Some authors have proposed methods for applying the TTC approach to short-term and less than life time exposures in the area of pharmaceutical impurities (EMEA, 2006; Muller et al., 2006), cosmetics (Kroes et al., 2007) and trace chemicals with structural alerts for genotoxicity (Felter et al., 2009; 2011).

Less than lifetime exposure was also considered at the EFSA and WHO expert workshop, which recommended that such TTC values would require development of a database for acute or other less than lifetime toxicity (EFSA and WHO, 2016a). The Scientific Committee confirms that there are currently insufficient data to establish TTC values specifically for short-term exposure. Furthermore, robust data on the duration of short term exposure to substances in food are rarely available. The Scientific Committee therefore confirms its previous conclusion that the issue of less than chronic exposure should be addressed case-by-case.

3.2. Developments

Several initiatives have confirmed the original TTC values of Munro et al. (1996) using additional data sources, e.g. (Pinalli et al., 2011; Tluczkiewicz et al., 2011; Laufersweiler et al., 2012; Leeman et al., 2014; Feigenbaum et al., 2015; Zarn et al., 2015; Yang et al., 2017; Baken et al., 2018). Within the framework of COSMOS (http://www.cosmostox.eu), a collaborative EU 7th framework project that was conducted 2011-2015, one task force considered approaches to developing TTCs values for cosmetic-related substances (Yang et al., 2017). The TTC values derived in these studies were generally in agreement with those of Munro et al. (1996).

Additional work addressed the derivation of internal TTC values as a more accurate approach that would also allow for route-to-route extrapolation (Partosch et al., 2015). In that approach, NOAEL values for each chemical in the three Cramer classes as described by Munro were multiplied by their own bioavailability. The Scientific Committee is also aware of ongoing project entitled 'The Expanded Decision Tree (EDT) Project' by the US-FDA. More than 18,000 scientific studies were reviewed to determine the influence of species, strain, sex and target organ on toxicity (US FDA, personal communication). These studies provided NOAELs for approximately 2,000 substances that will be

organized according to their structure, metabolic fate and toxic potential. Publications on the concept and approach are expected in the near future. In addition, FDA is in the process of developing an EDT software.

3.3. Substances currently not suitable for the TTC approach

As outlined in the opinion of the Scientific Committee on the TTC approach (EFSA Scientific Committee, 2012b) and reiterated from Section 1, the TTC approach should not be used for substances for which EU legislation requires the submission of toxicity data. Furthermore, when data are available that allow for a risk assessment, these data should be used and not the TTC approach.

It is necessary to consider whether the substance under consideration belongs to one of the categories of substances for which it is not appropriate to apply the TTC approach. Several categories for exclusion have been identified by Cramer et al. (1978), Kroes et al. (2004), EFSA (2012b) and in (EFSA and WHO, 2016a).

The TTC approach should be limited to the evaluation of structure(s) that is represented by the chemicals in the database used to derive the respective TTC value. Substances which are not represented in the database are therefore outside of the domain of applicability. Furthermore, some substances with special properties were also excluded. The rationale for these exclusions from the TTC approach can be found in the publications by Cramer et al. (1978), Kroes et al. (2004) and EFSA (EFSA Scientific Committee, 2012b). For the current list of exclusions, see Section 4.1.

However, the Scientific Committee has made modifications to the exclusion list presented in EFSA (2012b). Hydrazines are no longer excluded from the TTC approach because only 4% of the hydrazines (2 out of 57 hydrazines) exceed a cancer risk of 1 in 10^6 at an intake of 0.0025 µg/kg bw (i.e. the TTC value for potential DNA-reactive mutagens and/or carcinogens).

The 2014 EFSA/WHO workshop recommended excluding organo-silicon substances from the TTC approach because organo-silicon substances are not represented in the toxicity database of Munro et al. (1996) (EFSA and WHO, 2016a). The Scientific Committee concludes, therefore, that they should also be excluded from the TTC approach.

3.4. Applicability of the TTC approach to chemical mixtures

For mixtures of fully defined chemical composition, a tiered approach is recommended beginning with the assumption of dose addition (EFSA Scientific Committee, 2012a; EFSA and WHO, 2016a), in line with the EFSA guidance on risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2018b).

EFSA and WHO (2016a) recommended that for mixtures that are not fully defined, the application of the TTC approach may be acceptable if sufficient information or analysis is available to confirm that the mixture does not contain substances from the exclusion categories. In this case, the unknown components could be treated as potentially genotoxic and the TTC value of 0.0025 µg/kg bw would apply to the sum of these mixture components. If it were determined that there are no concerns for genotoxicity and the mixture does not contain OPs or carbamates, the mixture may be placed directly in Cramer Class III. Use of the lowest applicable TTC value to the sum of the components in a mixture is a conservative approach if some components are of lower toxicity.

The applicability of the TTC approach as a tool for the evaluation of mixtures depends on the nature and the level of characterisation of the mixture and should, therefore, be considered on a case–by-case basis.

3.5. Applicability of the TTC values for infants and children

Infants and children have a higher food intake per kg bw than adults, and also have other dietary habits and food preferences, and therefore it is important to take these into consideration when making exposure estimates for the TTC approach. In addition, infants and children are often assumed to be potentially more sensitive to (some) toxicological insults than adults.

Potential differences between infants or children and adults in dietary exposure and susceptibility to chemicals are addressed in the recent guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017) and in the scientific opinion on pesticides in foods for infants and young children (EFSA PPR Panel, 2018). Generally, the major physiological differences between infants as compared to adults are observed in the first weeks after birth, in particular in preterm neonates (WHO, 2006; EFSA Scientific Committee, 2017; EFSA PPR Panel, 2018). The capacity of enzymes involved in phases I and II metabolism is generally up to 2-(full-term) to 3-fold (preterm) lower when comparing infants with healthy adults (Hines, 2008; van den Anker et al., 2011; de Wildt et al., 2014). Likewise, renal function is also reduced at birth but reaches 50% of the activity of adults within the first 2 months of age. A number of analyses showed overall 1.5- to 3-fold differences between neonates, infants and healthy adults for renal clearances and total clearances and half-lives of substances that are cleared by the kidney.

In infants older than 16 weeks generally the expression of metabolizing enzymes and the renal excretion approach adult levels (Hattis et al., 2003; Dorne et al., 2005; Valcke and Krishnan, 2013) (see also the scientific opinion on pesticides in foods for infants and young children (EFSA PPR Panel, 2018). For this reason, EFSA Scientific Committee (2017) concluded that the TTC values were sufficiently conservative in the case of infants over the age of 16 weeks. However, for infants below 16 weeks of age, the difference in toxicokinetics between this population and the older infant and adult population may be greater than the default toxicokinetic factor of 3.2 embedded in the UF of 10, typically used to account for the inter-individual human variability within the general population. Therefore, EFSA Scientific Committee (2017) recommended that, in the case where no data are available on the excretion or metabolism of a substance in infants below the age of 16 weeks, an additional uncertainty factor of 3 should be applied to give an aggregate human uncertainty factor of 30 to account for the inter-individual human variability. Using the same rationale, the TTC approach can also be applied to infants below 16 weeks of age but the relevant TTC values should be divided by 3 to allow for the differences in toxicokinetics between infants below 16 weeks of age and older infants or adults.

3.6. Genotoxicity prediction tools

In applying the TTC approach, it is necessary to assess the potential for DNA-reactive mutagenicity or carcinogenicity often based on few or no experimental data. Evidence may come from read across from structurally similar chemicals, use of structural alerts or (Q)SAR models. Modelling of genotoxicity is one of the most extensively developed fields in computational toxicology (Serafimova et al., 2010; Worth et al., 2010; 2013; Mombelli et al., 2016; Patlewicz and Fitzpatrick, 2016). This has been facilitated by our understanding of the underlying biological mechanisms, well established experimental protocols, and availability of a large amount of experimental data in the public domain. Some of the software packages implementing these models are freely available (e.g. Toxtree, T.E.S.T, VEGA, LAZAR).

Prediction of genotoxicity should not be based on the use of a single model alone. In order to optimise sensitivity/specificity when using prediction tools, it is recommended to apply at least two independent (Q)SAR models which are suitable for the structure under consideration to maximise the sensitivity and specificity of the prediction (EFSA PPR Panel, 2016). The independence of the models is based on different training sets or algorithms (e.g. knowledge-based and statistically based models) used for developing the models (EFSA PPR Panel, 2016). Each prediction should be evaluated, based on expert

judgement, for relevance and reliability following internationally agreed standards (OECD, 2007; ECHA, 2008, 2016).

3.7. Exposure

It is essential for application of the TTC approach to have fit-for-purpose estimates of dietary exposure at the upper end of the distribution. These should be calculated using the methods commonly applied for dietary exposure assessment, for example high percentile food consumption (e.g. 95th percentile) and average measured chemical concentration values to estimate chronic dietary exposure for high consumers. It is also important to consider exposure in specific population subgroups, for example infants and children for whom dietary exposure is often higher when expressed on a bodyweight basis. Where the structure of the substance indicates a potential for acute toxicity, it might be necessary to consider acute exposure (24 hours or less), using high percentile concentration values as well as high percentile food consumption. If there are insufficient data to calculate a high percentile, then the maximum reported level could be used in order to be conservative. In the absence of TTC values for acute exposure, the chronic TTC values should be applied, which is conservative for acute exposure.

The estimates of exposure for substances to which the TTC approach is applied should, ideally, take into account not only exposure via the diet but also any systemic exposure resulting from non-oral routes and sources. However, this is often difficult to achieve in practice due to lack of data. If this is the case, it adds further uncertainty to the estimates of exposure, which should be described [see also the EFSA Guidance on uncertainty assessment (EFSA Scientific Committee, 2018a) .

4. Guidance

The threshold of toxicological concern (TTC) approach is a pragmatic, scientifically valid methodology to assess the safety of substances of unknown toxicity found in food and the environment. From a scientific perspective, the TTC approach could, in principle, be applied to any substances with known structure and that do not belong to the chemical exclusion categories, for which oral exposures can be estimated and toxicity data are sparse. In the EU, there are legislative requirements to submit toxicity data in several areas (e.g. the technically active substances in pesticides, food and feed additives, etc.). Therefore, the TTC approach should not be used for substances for which EU legislation requires the submission of toxicity data.

For the work of EFSA in the area of food and feed, the TTC approach is recommended as a useful screening tool. It can be used either for the setting of priorities for data needed to allow a chemical-specific risk assessment or for deciding whether exposure is so low that adverse health effects are unlikely. Consequently, the substance has a low priority for risk assessment.

This Guidance uses the TTC decision tree in Figure 1, which is based on the EFSA and WHO (2016a) version.

Sections 4.1 and 4.2 give guidance on what considerations are needed before applying the TTC decision tree and section 4.3 describes the application of the TTC decision tree.

4.1. Initial considerations

Before applying the TTC decision tree:

1. Perform a literature search for toxicity data for the substance under consideration (or a structural analogue) and decide whether there are sufficient data available to allow for a substance specific risk assessment (including read-across considerations). If the substance is a member of a group that has well-established toxicity data, the TTC approach is not applicable.
2. Check whether the substance under consideration falls under any EU legislation which requires submission of toxicity data. If so, the TTC approach is not applicable.

3. Check whether the substance under consideration falls into one of the exclusion categories (see section 3.3). If so, the TTC approach is not applicable. The exclusion categories are:

Substances which are not represented in the database or are outside of the domain of applicability:

- Inorganic substances
- Proteins
- Nanomaterials
- Radioactive substances
- Organo-silicon substances
- Metals in elemental, ionic or organic form. However, in the case of organic salts, where the counter ion is an essential metal (e.g. sodium), the Scientific Committee recommends that the TTC approach could be applied to the organic ion.

Substances with special properties:

- High potency carcinogens: aflatoxin-like, azoxy- or N-nitroso-substances and benzidines
- Steroids
- Substances with a potential for bioaccumulation: polyhalogenated-dibenzodioxins, -dibenzofurans and –biphenyls

4.2. Exposure considerations

1. Estimate chronic exposure using the methods commonly applied for dietary exposure assessments and take the resulting exposure at the upper end of the distribution. It is also important to consider exposure in specific population subgroups, for example infants and children for whom dietary exposure is often higher when expressed on a bodyweight basis. Where the structure of the substance indicates a potential for acute toxicity, it might be necessary to consider acute exposure (24 hours or less), using high percentile concentration values as well as high percentile food consumption. If there are insufficient data to calculate a high percentile, then the maximum reported level could be used in order to be conservative.
2. Decide on what the exposure duration will be. If less than chronic exposure does not exceed the relevant TTC value, there is a low probability of adverse health effect. If the relevant TTC value is exceeded, expert judgement is necessary to consider if a non-TTC approach is required.

4.3. Applying the TTC decision tree

Step 1: Check whether the TTC approach is applicable (see 4.1 Initial Considerations).

If the TTC approach is applicable proceed either to Step 2 or Step 3:

Step 2: Decide whether the substance raises concern for potential DNA-reactive mutagenicity or carcinogenicity. The decision should not be based on a single piece of evidence. Evidence may come from experimental data, read across from structurally similar chemicals, use of structural alerts or (Q)SAR models. A 'weight of evidence' approach should be followed, based on expert judgment on all available information (see section 3.6). If the weight of evidence does not indicate that the substance has the potential for DNA-reactive mutagenicity or carcinogenicity, proceed to Step 4. Otherwise proceed to Step 3.

Step 3: If the estimated exposure is below the TTC value for DNA-reactive mutagenic or carcinogenic substances of 0.0025 µg/kg bw per day, it can be concluded that there is a low probability of adverse health effects.

If Step 2 is considered first and a concern regarding genotoxicity was identified together with an estimated exposure higher than this TTC value, then a non-TTC approach (e.g. substance specific risk assessment) is required to conclude on potential adverse health effects[5].

If Step 3 is considered before Step 2 and the estimated exposure is higher than the TTC value for DNA-reactive mutagenic or carcinogenic substances, go to Step 2.

Steps 4/5: If the substance is an organophosphate or carbamate (*Step 4*) and the estimated exposure is below the TTC value of 0.3 µg/kg bw per day (*Step 5*), it can be concluded that there is a low probability of adverse health effects. If the estimated exposure is higher than this TTC value, a non-TTC approach (e.g. substance specific risk assessment) is required to conclude on potential adverse health effects.

If the substance is not an organophosphate or carbamate, proceed to Step 6.

Steps 6/7: Identify the appropriate Cramer class of the substance (see sections 2.1 and 2.2). If the substance belongs to the Cramer class III (*Step 6*) and the estimated exposure is below the TTC value of 1.5 µg/kg bw per day (*Step 7*), it can be concluded that there is a low probability of adverse health effects. If the estimated exposure is higher than this TTC value, a non-TTC approach (e.g. substance specific risk assessment) is required to conclude on potential adverse health effects.

If the substance does not belong to the Cramer class III, proceed to Step 8.

Steps 8/9: If the substance belongs to the Cramer class II (*Step 8*) and the estimated exposure is below the TTC value of 9 µg/kg bw per day (*Step 9*), it can be concluded that there is a low probability of adverse health effects. If the estimated exposure is higher than this TTC value, a non-TTC approach (e.g. substance specific risk assessment) is required to conclude on potential adverse health effects.

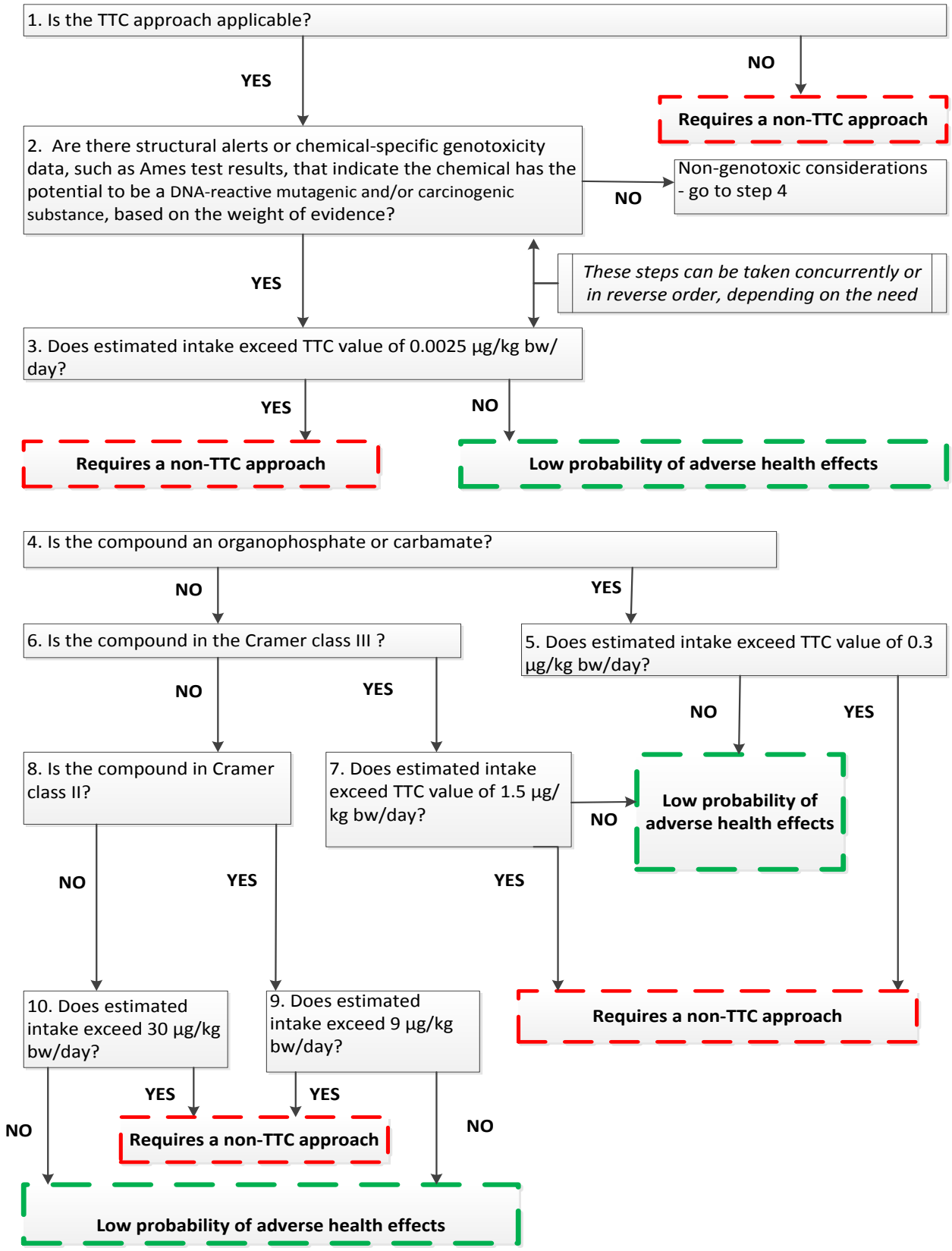
If the substance does not belong to the Cramer class II, proceed to Step 10.

Step 10: The substance belongs to the Cramer class I. If the estimated exposure is below the TTC value of 30 µg/kg bw per day, it can be concluded that there is a low probability of adverse health effects. If the estimated exposure is higher than this TTC value, a non-TTC approach (e.g. substance specific risk assessment) is required to conclude on potential adverse health effects.

If exposure of infants below the age of 16 weeks is likely, the TTC approach can be applied but the relevant TTC values should be divided by 3 to allow for the differences in toxicokinetics between infants below 16 weeks of age and older infants or adults (see section 3.5).

[5] A more refined approach for exposure assessment may be considered (EFSA Scientific Committee, 2012b). It is likely that there will be insufficient data for such refinement, and therefore exceedance of the TTC value generally indicates the need for chemical-specific toxicity data.

Figure 1: The TTC decision tree (This decision tree is intended for use only in conjunction with the guidance provided in Section 4.)



5. Recommendations

There are generic issues noted in this guidance, such as improved methods to assess aggregate exposure to chemicals from multiple routes and sources,that are not specific to the TTC approach. The following are the main TTC-specific recommendations from the current guidance, which should be carried out in the order given:

1. International agreement on the format and curation of all existing databases, including the inclusion/exclusion criteria to be used should be sought.

2. An overall non-cancer database should be created by an international collaboration using these criteria.

3. A review of the existing cancer databases should be carried out through an international collaboration effort.

4. An assessment of the impact of these curated databases on the TTC values should be carried out through an international collaboration effort.

5. EFSA should review this guidance if the TTC values change.

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