Threshold of Toxicological Concern Approach:

Conclusions and Recommendations of the EFSA/WHO Expert Workshop
DRAFT for public consultation

These are the conclusions and recommendations as agreed by the experts.
A full workshop report will be published together with the final conclusions and recommendations after the public comment period.

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1. Background

In light of ever improving methods in analytical chemistry, it is to be expected that many more unintended chemicals will be detected in our environment, including food and drinking water, as well as in our bodies. To allow for a health risk assessment of these exposures when there are insufficient chemical-specific data, other methods need to be applied to estimate the potential human health impact and to make informed risk management decisions. The Threshold of Toxicological Concern (TTC) is a methodology that may be used to assess potential human health concerns for a chemical based on its chemical characteristics and estimated exposure when chemical-specific toxicity data are scarce or absent.

Overall, the TTC approach integrates data on exposure, chemical structure, metabolism, and toxicity consistent with standard risk assessment principles. It has been proven to be a pragmatic, scientifically-valid approach for the safety evaluation of chemicals with relatively low oral exposure and for which limited chemical-specific data are available. Application of a science-based systematic approach will allow risk managers to prioritize actions and target further testing and evaluation strategies. It is important that scientific research continues to provide refinement of and improvement to the TTC approach in order to continue to assure its adequacy, appropriate application, and usefulness for public health protection.

1.1 Objectives of the workshop

EFSA and WHO initiated a project that intends to provide recommendations as to how the existing TTC framework may be improved and expanded by updating/revising the Cramer classification scheme (Cramer et al., 1978; hereafter referred to as the Cramer scheme or Cramer decision tree) and extending the TTC approach, thereby building on existing and ongoing work in this area. A call for data was issued by WHO in 2013 and information was collected regarding new proposals and on-going scientific work in the area.

The overall goal of the project is to develop a globally harmonised decision tree for a tiered approach on the application of the TTC in the risk assessment of chemicals.

To gain the broadest possible input for this project, a stakeholder public hearing was organised on 2 December 2014 in Brussels, where around 100 people representing NGOs, industry, government, academia and consumer organisations participated (list of participants is included in annex 1). Stakeholders who had submitted a written request, were given the opportunity to express their views with short presentations that have been published on EFSA's website (link here). The points raised by the stakeholders were considered in the subsequent expert meeting held on 3-5 December for which an open call for experts was published on WHO's website in August 2014, deadline for submission of expression of interest on 30 September 2014.

From the 50 applications received, 26 participants were selected to participate in the workshop according to the expertise needed and published in the call, taking regional and gender aspects into account. A list of participants is included in annex 2. Most of the participants in the expert workshop also participated in the stakeholder hearing held the day before. The experts completed a declaration of interests and a declaration of confidentiality that were evaluated by WHO according to the organisations' rules. WHO concluded that the interests declared did not warrant experts to be excluded from the discussion at the meeting. Five experts, who were found to have potential conflicts of interests, did not take part in the development of conclusions and recommendations. Dr Diane Benford served as Chairperson of the workshop and Dr Gordon Barrett and Dr Kristi Jacobs served as rapporteurs. The agenda as adopted is included in annex 3.

The outcome of the workshop is a series of conclusions and recommendations agreed by the expert group. The document, in the present form, is published for public consultation for a period of six (6) weeks on the WHO and EFSA websites. Comments received will be considered and addressed by the expert group. A final workshop report will then be published by mid-2015.

During the workshop, experts were divided in two breakout groups: the first group addressed questions in relation to the Cramer classification scheme and underlying scientific concepts; the second group addressed questions in relation to the TTC values and an overall TTC decision tree. Discussions from the breakout groups were presented to and discussed by the whole expert group. The main questions addressed by the experts are summarised below:

Cramer classification Scheme

- Is the framework in the Cramer classification scheme for sorting chemicals into structural classes sufficient and representative of the most up-to-date scientific knowledge?
- Does the scientific evidence support replacing or expanding the potency classes of the Cramer Scheme with a larger number of structural sub-categories? Are there other revisions to the Cramer classification scheme that are supported by the available scientific database?
- How should Class II be treated (eliminated, strengthened etc.)?
- Are there classes of chemicals, other than those already excluded, that the TTC approach should not be used to evaluate?
- Can the Cramer Classification Scheme be redesigned in order to avoid the high degree of overlap in NOEL/NOAEL values between Classes I and III?
- Should phenols and primary amines be reassigned to Class II, based on outlier analysis of their NOAEL/NOEL distributions, as proposed in Tluczkiewicz et al. (2011)?
- How can genotoxicity, ADME, and mechanism of action data be used to refine the classification scheme and/or class toxicity thresholds?

Background/Scientific Principles/Criticisms

- Is the TTC concept based on scientific risk assessment principles and sufficiently conservative for public health protection?
- What is the TTC approach intended for and when should it not be used?
- Can the TTC framework be modified to take possible effects of low-dose mixtures into account?
- Can the TTC approach take into account non-monotonic/low-dose only effects?

2. Preamble

The TTC approach is a screening and prioritizing tool for the safety-assessment of chemicals when hazard data are incomplete. Exposures exceeding the relevant TTC value are not necessarily associated with any health concerns but rather are flagged as warranting further evaluation. This may lead to a decision that for some chemicals further work and risk mitigation steps are necessary while for others exposure is so low that the probability of adverse health effects is also low and no further data are necessary. In principle, the TTC approach can be applied in any area of chemical risk assessment for which human exposures are low, whether exposure is from deliberate addition or due to contamination.

TTC provides a health protective approach in situations where it is not feasible to acquire chemical-specific data (e.g. impurities and breakdown/reaction products in food additives, trace contaminants in food and water), where evaluation of a large number of compounds with low exposure is required (such as flavouring substances), in prioritization of large numbers of compounds where resources are limited (e.g. contaminants in surface water), or when a rapid safety assessment (chemical food safety incidents) is needed.

However, TTC is not applicable when compound-specific assessment and toxicity data are required under existing regulations, and available compound-specific toxicity data should be examined except in certain priority-setting or screening cases (see section 3.1). Moreover, specific classes of chemicals are excluded from the TTC approach, either because of toxicological considerations or for lack of representation in the underlying database (see section 3.4).

There are generic questions in the risk assessment of chemicals that are under discussion in the scientific community, sometimes for decades (e.g. the existence of a toxicological threshold dose below which no adverse effect is produced, low-dose effects due to non-monotonic dose-response relationships, mixtures, interspecies extrapolation, adequacy of endpoints tested, fetal origin of adult disease, epigenetics, dose-metric, extrapolation from subchronic to chronic studies, endocrine disruption). Such questions apply also to the TTC approach but are not specific to it and discussion on such generic risk assessment considerations are not in the scope of this report. The present report is also not intended to be a review of all publications on the development and application of the TTC approach and therefore only a few references are included. For recent, comprehensive reviews of the TTC approach the reader is referred to EFSA (2012) and Dewhurst and Renwick (2013).

3. Conclusions

3.1 General conclusions

The TTC approach as currently applied is a valid, science-based screening tool useful for the prioritisation of chemicals and for more general applications in chemical risk assessment. The TTC approach was developed for chemicals where human exposure is estimated to be very low and chemical-specific toxicological data are lacking. As such, conservatism was built into the approach to establish sufficiently protective TTC values. It should be noted that the TTC approach is not appropriate to assess the safety of chemicals for which a toxicological data-package is required. In any risk assessment all data available on the chemical under consideration should be evaluated, and application of the TTC approach is no different in this aspect, although in certain circumstances (e.g. prioritisation of a large number of chemicals) it could be acceptable to perform a preliminary screening assessment based on the TTC without evaluating all the data on each chemical as a first step. The TTC approach is not intended to supersede evaluation of available toxicological data, as compound-specific data are generally preferred for the purposes of a robust risk assessment. Moreover, when a class of structurally similar chemicals are to be assessed and a well-studied lead chemical is available, this lead chemical can be used for the assessment of the structural analogues by means of read-across, however, depending on the context a TTC approach could also be used as a first step. This issue had already been raised by Kroes et al. (2004): "*Prior to application of the TTC approach, all available toxicity data on the compound should be collected and evaluated (Renwick et al., 2003). The TTC approach should be used only in cases where the available chemical-specific data are inadequate for normal risk characterisation. Any available information on the compound should be considered at the same time as the decision tree is applied, to ensure that any decision is compatible with the available data. The TTC is not designed to replace conventional approaches to risk characterisation for established and well-studied chemicals, such as food additives and pesticides."* It was further recognised in that publication that in-depth expert knowledge is needed to reaching a conclusion to some of the questions in the Kroes et al. (2004) decision tree: *"The decision tree and the TTC principle are designed as structured aids to expert judgement and should be applied only by those who have a sufficient understanding of toxicology principles and chemical risk assessment."* The expert group concurred with this assessment.

3.2 The Cramer scheme is fit for purpose

The expert group concluded that major revisions to the tree are not warranted, as the Cramer decision tree is well suited for its intended purpose and when used in conjunction with the associated TTC values is sufficiently protective. The group acknowledged that the sorting process of the Cramer decision tree does work, is reproducible and has been validated by *post hoc* comparison with numerous newer databases. None of the alternative classification schemes developed in various published analyses have turned out to be significantly better than the Cramer scheme. In consequence, the expert group concluded that there is no scientifically-based justification for major restructuring of the decision tree.

The Expert group recommended minor suggestions to modify the Cramer decision tree to remove ambiguity, improve its clarity and to harmonize with the electronic tool Toxtree. The expert group

recognised that there are a number of efforts underway, including those of the US Food and Drug Administration (FDA) and the International Organization of the Flavour Industry (IOFI), that propose significant modifications to the Cramer decision tree, indicating that the developers interpret a need for revision of the scheme. Major modification to and restructuring of the Cramer decision tree could result in a situation in which the original TTC values derived by Munro *et al.* (1996) and subsequently validated using different databases may be altered, and the implications for existing safety assessments need to be evaluated. Because the Cramer decision tree has been applied for the evaluation of flavouring agents for over 15 years, there is a need for broad acceptance of any future changes.

The expert group noted that the reasoning underlying the development of individual nodes in the Cramer decision tree in the 1970s is not transparent. Any revisions to the existing decision tree or the creation of any new decision tree(s) should be thoroughly documented by capturing the scientific rationale for creating branch points, and the questions associated with those branch points. This process would ensure transparency of the development process and provide a strong foundation for peer review and validation. In addition, any revisions to the current decision tree or the development of a new decision tree should be discussed and agreed upon widely at an international level and the resulting output freely available as an expert system.

The Cramer et al. (1978) decision tree has been computerised in the Toxtree computer program and is described as the Cramer tree with extensions (version 2.6.0). The modified Cramer decision tree proposed by this expert group incorporates some, but not all aspects of the Toxtree extensions (please refer to Appendix I for additional details).

In the Joint EFSA/WHO stakeholder meeting on TTC on 02 December, 2014, stakeholders emphasized that there would be great value in having a publically available database underlying the TTC approach that could be consistently peer reviewed. The expert group concurred but noted in this regard that all of the original data collected by Munro in support of the original TTC were peer-reviewed and publically available at that time. However, some of these original studies may no longer be available.

Because there are a relatively small number of compounds that are classified in Cramer Class II, it has been previously proposed to evaluate under the Class III TTC threshold all the chemicals categorized as Class II (EFSA, 2012).

Kroes *et al.* (2004) proposed removing organophosphates (OPs) and carbamates from Cramer Class III and assigning them their own TTC value of 18 μg/person per day, which is considered sufficiently conservative to cover the anti-cholinesterase activity of these substances. However, the expert group is aware of NOAELs for carbamates derived from studies involving humans that indicate these substances are less potent in humans than in rodents. As such, the group concluded that they should remain in Cramer Class III.

The group supported a separate class threshold for OPs. However, the group concluded that the current Class III threshold value should be maintained and should not be recalculated by excluding the OP chemicals from Class III. The rationale for not recalculating the threshold for Class III at this time is twofold: (i) to maintain the current level of health protectiveness; and (ii) the evaluation of another database (RepDose) that does not contains OPs or carbamates yielded a TTC value for Class III that is similar to the Munro value. Therefore, it was considered premature to change the threshold at this time.

Some modifications to the Cramer decision tree were suggested by Tluczkiewicz et al. (2011) based on a combined assessment of four databases of repeated-dose toxicity studies (RepDose, Munro, ToxRef, and Toxbase). Analyses of the tails of the Cramer Class I, II and III distributions for the presence of different functional groups showed that phenolic compounds and primary amines had higher ratios of outliers to non-outliers (i.e. a larger proportion were in the tail of the distribution). However, interpretation of these observations is complicated by the fact that some of the outlier phenols and primary amines contained other structural characteristics, which could have resulted in assignment to Class III before the phenol or amino function would have been considered. In consequence, it is premature to reassign these functional groups to Cramer Class II.

The expert group concluded that additional consideration of Question 22 of the Cramer scheme, which asks if a substance is "a common component of food or structurally closely related to a common component of food" is required. It was generally agreed that it would be preferable to delete this question, since it is not well defined what 'common component of food' means, nor is the question related to specific structural considerations that can be linked to toxicological properties. However, since Q22 is linked with many other questions (12, 14, 15, 20, 26, 32), the consequences of removing this question from the decision tree need to be carefully evaluated, and the implication for Class II considered when this is re-evaluated in the future with an expanded database.

3.3 Metabolism is an inherent part of the TTC values

Analysis of the Cramer classification scheme and the TTC values used in the Kroes et al. (2004) decision tree shows that metabolism (metabolic bioactivation/metabolic detoxication and hindered metabolism as well as the potential for rapid elimination) is an inherent critical component of the TTC approach that contributes to the assignment of chemicals to a particular structural class.

The experts also discussed whether the Cramer decision tree could be used in the evaluation of plant-metabolites of pesticide. It was concluded by the expert group that plant metabolites of pesticide can be evaluated by the TTC concept including the Cramer decision tree. For pesticide plant metabolites of unknown structure, the group recommended these substances be placed directly in Cramer Class III, provided it can be reasonably argued that there is no concern for genotoxicity based on knowledge of the parent compound.

3.4 TTC Domain of applicability is sufficiently broad.

The expert group considered the available chemical domain assessments conducted on the TTC dataset sufficient to conclude that the domain of applicability of the chemicals in the TTC is sufficiently robust; but acknowledged that some known categories are not in the TTC database. The TTC approach should be limited to the evaluation of the structure(s) that are represented by the chemicals in the database used to derive the respective TTC value; therefore, the TTC approach should not be used for the following categories of chemicals: inorganic chemicals, metals and organometallics, proteins, steroids, organo-silicon compounds, chemicals that are predicted to bio-accumulate, nanomaterials, radioactive substances. The TTC value for chemicals with certain structural alerts for genotoxic carcinogenicity may not be sufficiently protective for high potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds and benzidines) and therefore, these classes of compounds should also be excluded from the current TTC approach.

The current database has been evaluated and found to sufficiently cover a wide range of chemicals. Testing the databases with alternative methods available for performing chemical domain analyses would add additional evidence for concluding that the structure of a chemical under consideration is represented by the chemicals in the database used to derive the respective TTC value.

3.5 The TTC for Genotoxic compounds is sufficiently protective.

The TTC value for substances with certain structural alerts for genotoxicity and carcinogenicity in the Kroes et al. (2004) decision tree is considered adequate and fit for purpose since it was derived from the largest available rodent carcinogenicity database, and was calculated by deriving the exposure at which the vast majority of chemicals with TD_{50} values would not exceed the level of 1 in 10^6 risk for carcinogenesis. The values in the CPDB database are derived assuming linearity of the dose response curve by extrapolation from the lowest TD_{50} for each chemical. In addition, it was assumed that any chemical with a relevant structural alert for genotoxicity could be a human carcinogen, irrespective of the human relevance of the tumour observed in the rodent database or a possible threshold mode of action. Although further expansion of the CPDB is desirable, it is not considered a priority as it is not expected that the overall distribution of the TD_{50} would significantly change.

The expert group considered whether alternative approaches to establishing TTC values for carcinogens such as using BMD/MOE values or using the geometric mean of the TD_{50} in cases where several studies are available are warranted. The group concluded that the current approach is reasonable since it relies on linear low dose extrapolation to a 1 in 10^6 risk which is generally regarded as a conservative approach to evaluating carcinogenic risk.

High potency carcinogens should be evaluated case-by-case. For high potency carcinogens, any TTC value(s) derived to adequately ensure low concern for health would be extremely low and possibly impracticable due to difficulties in obtaining reliable exposure data.

3.6 TTC Tiers are sufficient for non-DNA reactive carcinogens and non-cancer endpoints.

Carcinogens which are not directly DNA reactive can be considered to have a threshold mode of action and, in general, NOAELs for these are in the same range or higher than NOAELs for other types of toxicity. Thus, EFSA (2012) concluded that TTC values that are higher than the value of 0.15 µg/person/day are appropriate for any chemical where the weight of evidence for DNA reactivity is negative. The expert group concurred with this statement.

For non-cancer endpoints the Munro *et al*. (1996) database covers a range of chemical classes and end-points relevant to the vast majority of chemicals. This database is considered adequate and fit for purpose, and is additionally supported by TTC values derived from several subsequent analyses of different chemical datasets which result in TTC values similar to those derived using the Munro database.. Classification by the Cramer decision tree is based on the single functional group with the greatest potential toxicity present in the molecule. Most complex chemicals are assigned to Class III, the class with the lowest TTC value of the 3 Cramer classes. The group acknowledges that there are very few chemicals in Class II and therefore the TTC value for this class is not well supported within the current TTC approach. Merging the different non-cancer databases would increase the power of the calculated respective TTC values.

3.7 Conclusions on other considerations

3.7.1 Point of Departure (POD) and database

The current TTC database for non-cancer effects is based on the lowest NOELs or NOAELs in mg/kg bw/day identified in repeated dose animal studies. Newer approaches to dose-response analysis, such as BMD derivations, determining doses on a molar basis, or using allometric assessment factors, can be seen as having greater scientific rigour but the expert group concluded that they would not significantly affect the approach or add real benefit.

A merge of the different non-cancer databases is desirable as it would increase the statistical power and improve transparency in the database. If a new non-cancer database is generated, then the "overall TTC's" should be recalculated. It remains to be seen whether after merging the different databases the number of chemicals in the Cramer Class II increases to a more representative number of compounds as seen for the other classes. Should the recalculated TTC values for the respective classes increase, it needs to be determined if the new TTC values can still be considered sufficiently protective for adverse effects on specific endpoints, such as reproductive or developmental toxicity, as has been demonstrated for current TTC values.

In order to keep the future use of TTC contemporary with evolving databases, it would be ideal to have a centralized dataset that would be continually maintained to allow inclusion of new data as they become available. This would keep the chemical domain of the TTC dataset from stagnating as well as ensuring the current data are available for use and that any TTC values derived are representative of the current state of science. There would be a need for an organisation or group (preferably independent) to manage the overall database to ensure consistency, quality, public access and maintenance.

Combining different databases would be facilitated by agreement on the method of dose-response analysis and the appropriate dose-metric should be harmonised. Application of the TTC approach would benefit from the development of a standardised approach to defining chemical domain and agreed method(s) to identify structural alerts for DNA reactivity.

To determine the applicability of TTC to a certain chemical or group of chemicals, it is important to identify the key functional groups of interest and determine if those groups are within the TTC database. The ability to readily interrogate the databases supporting TTC would be of considerable benefit in this area.

3.7.2 Exposure considerations

As with any risk assessment, when using the TTC approach, exposure to a chemical from all sources (i.e. exposure from all relevant pathways and routes) should be considered, if possible. In a tiered approach to assessment of combined exposures to multiple chemicals, proposed by the International Programme on Chemical Safety (Meek et al., 2011), a case study on lower tier assessment demonstrated how TTC values could be used as the hazard point of departure for groups of substances belonging to specific Cramer classes and combined with their exposure potential used to evaluate the need for further combined exposure assessment.

Pending the outcome of the EFSA project on low-dose effects and non-monotonic dose-response, it is premature to make conclusions on this issue, and the TTC is not different from other methods of risk assessment in this respect.

3.7.3 Expression of TTC values

TTC values should be expressed in terms of µg/kg body weight/day to allow for application of the TTC approach to the whole population, including infants and children. Since the µg/person values were initially derived by multiplying by a default adult body weight of 60kg, the TTC values in µg/kg body weight are obtained by dividing the µg/person values by 60. In cases where the estimated exposure is in the range of the TTC value, additional consideration needs to be given on a case-by-case basis.

3.8 Overall conclusion

The TTC approach is a valid screening tool, based on scientific risk assessment principles, to assess low dose chemical exposures and to distinguish those for which further data are required to assess the human health risk from those with no appreciable risk. The scope of this meeting was to provide recommendations on how the method can be refined considering the toxicological databases and the Cramer *et al*. (1978) and Kroes *et al*. (2004) decision trees.

4. Recommendations

4.1 Cramer decision tree

- The expert group concluded that the sorting process of the Cramer decision tree does work; it is reproducible and has been validated by applying it to numerous newer databases, and therefore no major restructuring is recommended. However, the group acknowledges that the toxicological rationale underlying the development of each Cramer decision tree question is not provided and, therefore, it is not possible to address whether the decision tree reflects the most up-to-date scientific knowledge. Should the Cramer scheme be modified in the future, the scientific rationale for each question should be made explicit for increased transparency.

- The expert group is aware of efforts, including those of the US FDA and the International Organization of the Flavour Industry (IOFI), that propose significant modifications to the Cramer

decision tree. It is the recommendation of the expert group that once these revisions are peer-reviewed and validated, any new schemes need to be discussed and agreed upon widely at the international level before implementation.

- The group recommended only minor changes to a small number of Cramer questions to clarify and remove ambiguity (see Appendix I). It is recommended that the proposed minor changes to the Cramer et al. (1978) decision tree as outlined in Appendix I are implemented in Toxtree.

- After review of Question 22 of the current Cramer scheme, the expert group recommended further consideration of this question as the term "common component of food' is not sufficiently defined, nor is the question related to specific structural considerations that can be linked to toxicological properties. The expert group recommended that the implications of deletion of Q22 be evaluated and if minor the question should be deleted. However, if this is not feasible at this point, clear and harmonised criteria of what 'common component of food' means need to be developed, and lookup tables within Toxtree updated in accordance with the criteria.

- It is recommended that phenols and primary amines not be reassigned to Class II, based on an outlier analysis of their NO(A)EL distributions, at this time. The group recommended that for such reassignments an enlarged and consolidated database is needed to assess the toxicity data for these structural groups. This will allow for consideration of sorting into different toxicity tiers based on appropriate modification of the decision tree.

- The working group recommended that Cramer Class II continue to be used and applied to the TTC approach. The working group recommends that the applicability of Class II be reviewed once the different non-cancer databases have been merged as this may enrich and increase the confidence in the class. It was also recommended that this review include an evaluation of the distributions to determine if there is a need to modify the decision tree to strengthen the specificity of sorting to Class II.

- The expert group recommended caution in developing additional classes to ensure that the process does not introduce too much granularity into the decision tree such that the end product becomes a "read-across" tool rather than a screening tool.

- The Cramer scheme has been criticized as lacking specificity due to the high degree of overlap in NO(A)EL values between Classes I and III. However, the expert group emphasized that overlap per se is not a deficiency of the scheme but in fact contributes to its overall conservatism, and that only clear differentiation at the 5^{th} percentiles is critical. The group recommended that the distributions be re-evaluated following the development of any new or consolidated/merged databases.

4.2 Metabolism

- The experts considered that mammalian metabolism is an inherent and critical component of the current TTC approach and no additional measures to incorporate metabolism were recommended.

- The group also recommended that plant metabolites of pesticides of known structure could proceed down the decision tree, and that metabolites of unknown structure be placed directly in Class III provided there are no concerns for genotoxicity based on knowledge of the parent structure.

4.3 Expand/modify the overall database and derivation of class thresholds

- The expert group recommended that a permanent repository for data supporting TTC and the Cramer decision tree should be created and a body responsible for holding the data should be

identified. In addition to the development of a centralized database for supporting TTC values, it is recommended that minimum criteria for inclusion of data in the TTC databases should be developed and published. At a minimum, it is recommended that supporting data should be of sufficient detail to recreate decisions on NO(A)ELs and LO(A)ELs.

- It is recommended that the different non-cancer databases should be merged and made public as it would increase the power, transparency, and the confidence in the TTC values. Once the databases are merged:
  - It is recommended that any new combined databases select the lowest appropriate NOAEL per compound as a starting point for deriving TTC levels as this approach will provide the most reasonably conservative values.
  - BMD levels could be considered for inclusion in cases where a study has not identified a NOAEL.
  - Future combined datasets should consider the most up to date science, such as subchronic to chronic extrapolation factors, allometric scaling, *etc*, when selecting NOAEL levels for the derivation of TTC values.
  - Recalculating the "overall TTC's" for the respective classes is necessary:
    - OPs should be analysed separately and the consequence for the threshold value for class III evaluated,
    - the impact on specific endpoints (e.g. developmental toxicity) needs to be checked,
    - it should be checked whether the number of chemicals in the Cramer Class II is sufficient to provide a robust TTC value.

- Expanding the CPDB (e.g with the TOXREF database) would enhance the power and range of chemical structures covered. However, this is not considered a priority as it would be resource demanding and is not expected to significantly affect the approach.

- If a revision of the carcinogenicity/genotoxicity based TTC were to be envisaged, it is recommended considering approaches other than TD_{50}-based linear extrapolation, which may be overly conservative.

4.4 Chemical domain analyses

- The current database has been evaluated and found to sufficiently cover a wide range of chemicals, and although additional analyses could be performed, this is not considered a high priority. However, it is recommended that any new combined database be tested using chemical domain analyses methods. These analyses are considered to be informative and would provide further reassurance that the databases cover a wide range of chemical structures, but are not considered to be a high priority.

- In addition, it is recommended that a tool for evaluating whether a chemical, or group of chemicals, is represented in the underlying TTC databases be developed once the databases have been consolidated. This is likewise not considered a high priority.

4.5 Point of departure

- A reanalysis of all toxicological studies present in the current TTC databases using BMD analysis is not recommended as it would be very resource intensive and not all studies have sufficient datasets to allow for BMD analysis. A reanalysis using allometric scaling is also not recommended for the current databases, as the current approach already incorporates a factor for interspecies extrapolation that is appropriate for a screening tool.

- The inclusion of sub-chronic studies in the non-cancer database is supported, and when extrapolating from subchronic to chronic study duration in rodents the group finds the current extrapolation factor of 3 is appropriate for a screening tool.

- The expert group acknowledged that expressing TTC values on a molar basis may have greater scientific rigour, but recommended maintaining the units in µg/kg bw/day for greater consistency with other health-based guidance values.

4.6 Exclusion of chemical categories

- The application of the TTC approach is not recommended for the following categories of chemicals: High potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds, benzidines), compounds not adequately covered in the database, inorganic chemicals, metals and organometallics, proteins, steroids, nanomaterials, radioactive substances and organo-silicon compounds or chemicals that are known or predicted to bioaccumulate.

- The applicability of the TTC as a tool for the evaluation of mixtures that are not fully characterised is only endorsed if sufficient information or analysis is available to confirm that the mixture does not contain compounds from the exclusion classes, in which case the unknown component could be treated as potentially genotoxic and the TTC of 0.0025 µg/kg bw would apply. However, if it can also be determined that there are no concerns for genotoxicity, the substance may be placed directly in Cramer Class III.

- TTC values for Cramer Classes are considered sufficiently protective for adverse effects on reproduction and development and no changes are recommended. However, the TTC values would need reconsideration should the point of departure or the overall database be changed.

- Specific consideration was given to pyrrolizidine alkaloids (PAs), since they have been suggested as an exclusion class. The group considered the available information as insufficient at this point and recommended the issue to be reconsidered once potency estimates for additional PAs are available[1].

4.7 Specific TTC Values

- The expert group recommended organophosphates be treated as a separate class within the TTC approach, with a threshold value of 0.3 µg/kg body weight per day. It is also recommended not to group carbamates with organophosphates and to address them within the standard decision tree (Class III).

- Despite comprising a distinct class with a specific TTC value, the group recommends that prior to consolidation and review of the non-cancer databases, the organophosphate NO(A)ELs should remain in Cramer Class III in order that the current threshold for this class be maintained.

- The group did not recommend the setting of an additional generic TTC value(s) to cover high potency carcinogens and recommended evaluation on a case-by-case basis.

- The group recommended that the Threshold of Regulation value of 1.5 µg/person/d in the Kroes et al. (2004) decision tree should be removed as although it is of historical importance it is of little practical application.

[1] JECFA will evaluate the health risk of PAs in the 80th meeting in June 2015.

4.8 Combined oral exposure to multiple chemicals and from multiple sources

Accounting for combined oral exposure is not specific to the TTC approach, but applies to all approaches of risk characterisation. Therefore, case-by-case considerations were recommended in most circumstances:

- Applying the TTC approach to mixtures of known composition is possible. A tiered approach is recommended beginning with the assumption of dose addition. In the case of more complex mixtures containing compounds with dissimilar structures or in the event of known or anticipated interactions among components of the mixture, additional methodological refinements are needed.

4.9 Acute and other less than lifetime exposures

- If acute, or other less than lifetime TTCs were to be generated, it is recommended that a database for acute or other less than lifetime toxicity should be produced and methodology for the analysis determined. When performing these assessments there is a need to ensure that developmental toxicity endpoints are covered.

- Until such databases and analyses are developed, it is recommended considering less than lifetime or intermittent exposure on a case-by-case basis.

4.10 Infants and children, potentially sensitive life-stages

- The TTC approach can be used to evaluate the safety of exposures in infants in the same way as would be done with any risk assessment. For infants under 3 months of age, case-by-case considerations are needed if the estimated exposure approaches the TTC value.

- There is a need to ensure that exposure data are suitable for an infant assessment, an adult exposure assessment is not appropriate.

- There is no need to derive specific TTC values for infants and children. A number of analyses have shown that the NOAELs in the current TTC approach cover reproductive and developmental studies.

- It is recommended to express TTC values as µg/kg body weight (rather than on a per person basis) to facilitate the application of the TTC approach to the whole population including infants and children.

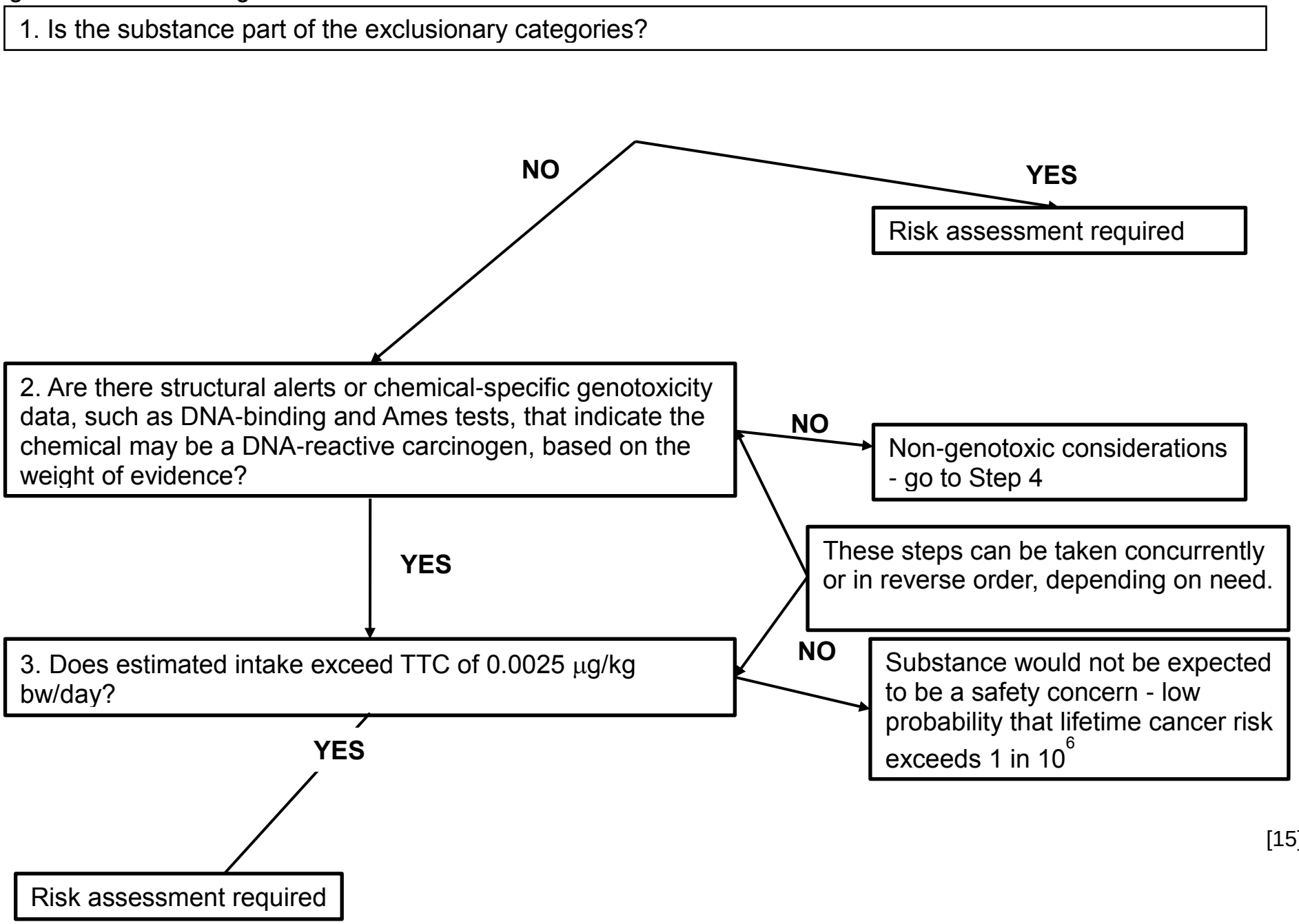
4.11 Additional recommendations

A leaflet explaining the TTC approach in accessible language should be produced

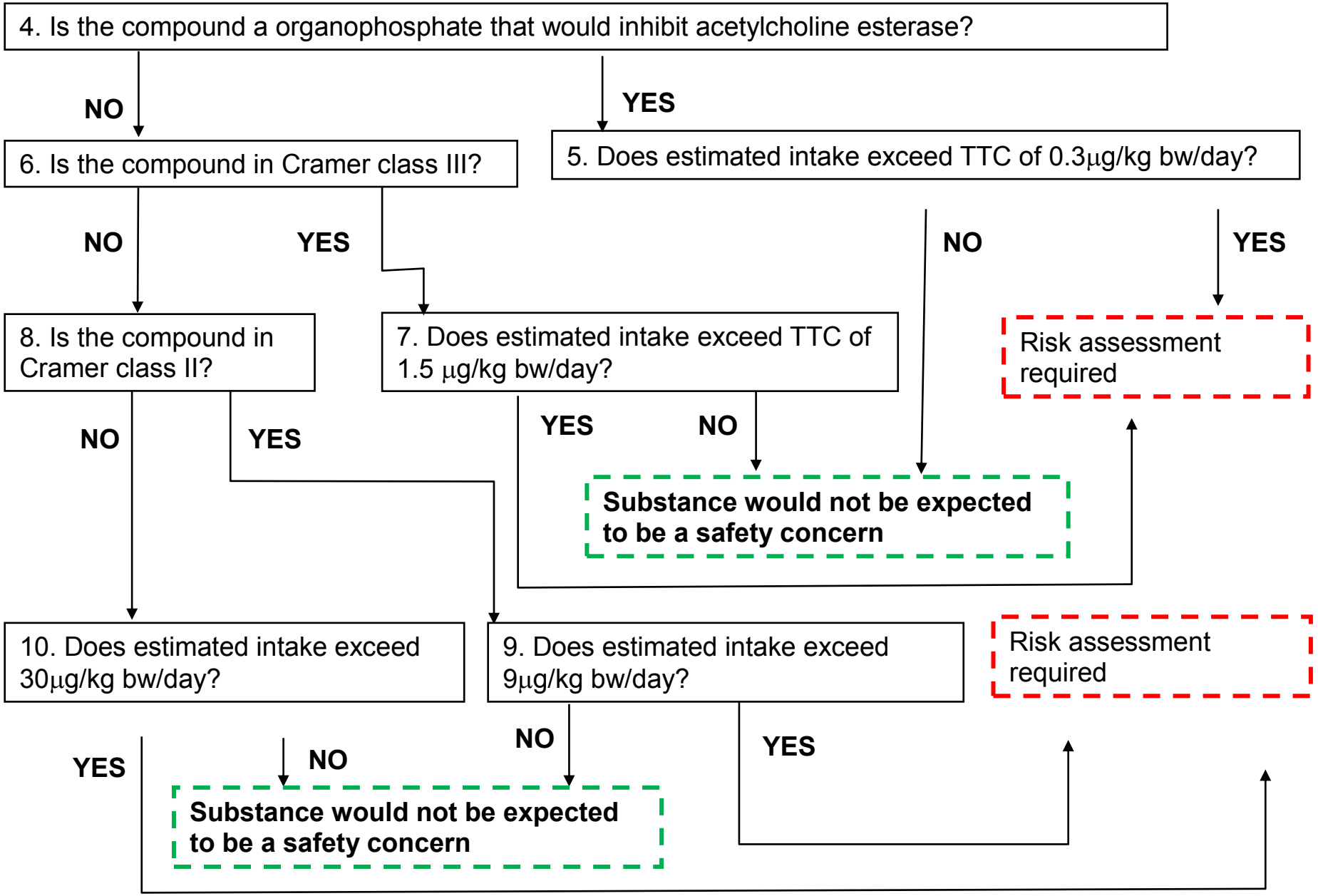
5. TTC Decision Tree

Taking the above considerations into account the expert group reviewed the overall TTC decision tree proposed by Kroes et al. (2004), and recommended a revised decision tree as proposed in Figure 1. An explanation of decision tree steps is given below the scheme.

Figure 1. Schematic diagram of the revised TTC decision tree



[15]


4. Is the compound a organophosphate that would inhibit acetylcholine esterase?
NO
YES
6. Is the compound in Cramer class III?
5. Does estimated intake exceed TTC of 0.3μg/kg bw/day?
NO
YES
NO
YES
8. Is the compound in Cramer class II?
7. Does estimated intake exceed TTC of 1.5 μg/kg bw/day?
Risk assessment required
NO
YES
YES
NO
Substance would not be expected to be a safety concern
10. Does estimated intake exceed 30μg/kg bw/day?
9. Does estimated intake exceed 9μg/kg bw/day?
Risk assessment required
YES
NO
NO
YES
Substance would not be expected to be a safety concern

[16]

Decision Tree Explanations

A literature search should be undertaken on the chemical to be evaluated, prior to applying the TTC decision tree. The TTC approach should be used only for chemicals of known structure (or those that are sufficiently characterised to confirm they are not in the exclusion groups) that lack adequate chemical-specific toxicity data and with low predicted human exposures.

Prior to applying the TTC - The TTC approach should not be used if the chemical is a member of a group that has well-established toxicity data. The TTC approach should also not be used if the structural characteristics of the chemical are not adequately represented in the TTC database. Therefore, proteins; steroids; chemicals that are known or predicted to bioaccumulate; nanomaterials; radioactive chemicals were also added to the list of chemicals for which the TTC approach is not appropriate.

Step 1 – The TTC approach should not be used for compounds that are part of the Cohort of Concern (CoC) proposed by Kroes et al. (2004) because more than 10% of chemicals with this structural alert would give a risk >1 in 10^6 at an exposure at the TTC value given in Step 3. The CoC includes: aflatoxin-like compounds, N-nitroso-compounds, azoxy-compounds, steroids, benzidines and polyhalogenateddibenzo-p-dioxins and-dibenzofurans. Step 2 - The weight of evidence for genotoxicity should be evaluated to indicate if the chemical is likely to be a DNA-reactive carcinogen. This should include an analysis of the structure by considering the presence of structural alerts (identified using the Benigni / Bossa rulebase as implemented in Toxtree) as well as any available genotoxicity tests for DNA reactivity, such as the Ames test.

Step 3 – The TTC value (expressed per kg body weight) is based on the TD_{50} data for chemicals with positive carcinogenicity data in the CPDB and with structural alerts given in Table 1 of Kroes et al. (2004).

Step 4 – Identifies whether the chemical has the potential to act as an OP, such as trialkyl-phosphates, phosphorothionates and phosphonates.

Step 5 – Gives the TTC value for organophosphates expressed per kg body weight.

Step 6 – Identifies chemicals in Cramer Class III.

Step 7 – Gives the Cramer Class III TTC value expressed per kg body weight.

Step 8 – Identifies chemicals in Cramer Class II.

Step 9 – Gives the Cramer Class II TTC value expressed per kg body weight.

Step 10 – Gives the Cramer Class I TTC value expressed per kg body weight.

6. References

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. Food and Cosmetic Toxicology 16, 255-276.

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Appendix 1 – Explanations to Cramer scheme

Q	Question	NO	YES	Additional explanation
1	Is the substance a normal endogenous constituent of the body that undergoes metabolism to CO_2 and water?	2	**I**	*Endogenous substances* are intermediary metabolites of normal biological processes present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included.
2	Does the substance contain any of the following functional groups: an aliphatic secondary amine or a salt thereof, cyano,N-nitroso, diazo (e.g. CH_2N_2), triazeno ($RN=NNH_2$) or quaternary nitrogen, except in any of the following forms: $>CN^+=R_2$, $>CN^+=H_2$ or the organic anion salts thereof?	3A	**III**	Classifies chemicals that have functional groups associated with enhanced toxicity early in the decision tree.
3A	Does the structure contain elements other than carbon, hydrogen, oxygen, nitrogen, or divalent sulphur?	5	3B	
3B	*Is any phosphorus atom present as a simple ionic phosphate ester R-O-PO_3^{2-}, either as the free acid or as a Na, K, Ca, Mg or NH_4 salt (if so proceed based on the hydrolysis product R-OH)?*	***III***	*4*	
4	Do all elements not listed in Q3A occur only as a Na, K, Ca, Mg or NH_4 salt of a. carboxylic acid, or as a SO_4 or HCl salt of an amine, or a Na, K, Ca, sulphonate, sulphamate or sulphate?	**III**	7	
5	Is it a simply branched acyclic aliphatic hydrocarbon or a common carbohydrate?	6A	**I**	
6A	Is the substance a benzene derivative bearing substituents consisting only of (a) hydrocarbon chains or l'-hydroxy or hydroxyl ester-substituted hydrocarbon chains and (b) one or more alkoxy groups, one of which must be para to the hydrocarbon chain in (a)?	6B	**III**	In Toxtree the answer NO goes to Q 42 *Does the compound consist of one aromatic ring, with at most one heavy atom connected to each aromatic atom?* which aims to assign "possibly harmful analogue of benzene" to Class III.
6B	*Does the compound consist of one benzene ring, with at most one heavy atom (oxygen, nitrogen or sulphur) connected to one or more of the aromatic carbon atoms?*	*7*	***III***	
7	Is the substance heterocyclic?	16	8	
8	Is it a lactone or cyclic diester?	10	9	

Q	Question	NO	YES	Additional explanation
9	Is it a lactone fused to another ring, or a 5- or 6-membered α,β-unsaturated lactone?	*	**III**	* If it is a lactone treat the structure as if it were the hydroxy acid in the form of its more stable tautomer and proceed to Q20 if it is open chain, to Q10 if it heterocyclic, and to Q23 if it is carbocyclic; if it is a cyclic diester treat as the separate components (i.e. the predicted hydrolysis products).
10	Is it a 3-membered heterocycle?	11	**III**	
11	Disregarding only the heteroatoms in any one ring, does that heterocyclie ring contain or bear substituents other than simply branched hydrocarbons (including bridged chains and monocyclic aryl or alkyl structures), alkyl alcohols, aldehydes, acetals, ketones, ketals, acids, esters (including cyclic esters other than lactones), mercaptans, sulphides, *thioesters*, methyl ethers, hydroxy or single rings (hetero or aryl) with no substituents other than those just listed?	12	33	Under Q11, do not consider the atom(s), usually O, N or S making the ring heterocyclic. If there is more than one hetero ring, regard each ring separately, with the remainder of the structure as substituents of that hetero ring. Addition of "thioesters" accounts for their rapid hydrolysis.
12	Is it heteroaromatic?	22	13	This question separates the aromatic heterocyclics for the purpose of considering whether they are polynuclear (Q14) or unsubstituted (Q13).
13	Does the ring bear any substituents?	**III**	14	
14	Does the structure contain more than one aromatic ring?	22	15	
15	Is it readily hydrolysed to mononuclear residues? (If yes, treat the mononuclear heterocyclic residues by Q22 and any carbocyclic residue by Q16.)	33	22	
16	Is it a common terpene (D)-hydrocarbon, -alcohol –aldehyde or -carboxylic acid (not a ketone)?	17	**I**	
17	Is the substance readily hydrolysed (H) to a common terpene (D), -alcohol, -aldehyde or -carboxylic acid? (If yes, treat the hydrolysed residues separately and proceed to Q18 for the terpene moiety and to Q19 for any non-terpenoid moiety.)	19	18	
18	Is the substance one of the following? i. a vicinal diketone; or a ketone or ketal of a ketone attached to a terminal vinyl group or, ii. a secondary alcohol, ester *or thioester*	**I**	**II**	Addition of "thioester" accounts for their rapid hydrolysis.

Q	Question	NO	YES	Additional explanation
	of a secondary alcohol attached to a terminal vinyl group or, iii. allyl alcohol or its acetaL ketal or ester derivative or, iv. allyl mercaptan, an allyl sulphide, an allyl thioester or allyl amine or, v. acrolein, a methacrolein or their acetals or, vi. acrylic or methacrylic acid or, vii. an acetylenic compound or, viii. an acyclic aliphatic ketone, ketal or ketoalcohol with no other functional groups and with four or more carbons on either side of the keto group or, ix. a substance in which the functional groups are all sterically hindered.			
19	Is the substance open chain?	23	20	
20	Is the structure a linear or simply branched aliphatic compound containing any one or combination of only the following functional groups: i. four or less, each, of alcohol, aldehyde, carboxylic acid or esters and/or ii. one each of one or more of the following: acetal, either ketone or ketal but not both, mercaptan, sulphide (mono- or poly-), thioester, polyoxyethylene [(-OCH_2CH_2-)x with x no greater than 4], or primary or tertiary amine *iii. a readily reducible disulphide group (if so continue the assessment for each resulting thiol or dithiol separately)?*	22	21	The rapid reduction of disulphides to the corresponding thiols by thioltransferases and exchange reactions with glutathione, cysteine and other endogenous thiols has been taken into account.
21	Does the structure contain three or more different types of functional groups (exclude methoxy and consider acids and esters as one functional type)?	18	**III**	Aliphatic (A) compounds with three or more different functional groups (excluding methoxy) are too complex to permit prediction of toxicity.
22	Is the substance a common component of food or structurally closely related to a common component of food *and is the ratio between natural occurrence and the amounts added >10*?	33	**II**	*For flavouring agents and other chemicals added to food, the ratio between natural occurrence and the amounts added should be >10.*
23	Is the substance aromatic?	24	27	
24	Is the substance monocarbocyclic (excluding cyclopropane or cyclobutane and their derivatives) with ring or aliphatic side chains, unsubstituted or containing only alcohol, aldehyde, side-chain ketone,	25	18	

Q	Question	NO	YES	Additional explanation
	acid, ester, or Na, K or Ca sulphonate or sulphamate, or acyclic acetal or ketal?			
25	Is the substance either i. a cyclopropane or cyclobutane with only the substituents mentioned in question 24 or ii. a mono- or bicyclic sulphide or mercaptan?	26	**II**	
26	Does the structure contain no functional groups other than those listed in Q24 and is it either a monocyloalkanone or a bicyclic compound with or without a ring ketone?	22	**II**	
27	Does (do) the ring(s) have any substituents?	**III**	28	
28	Does the structure contain more than one aromatic ring?	30	29	
29	Is it readily hydrolysed *or reduced* to mononuclear residues? (If yes treat the individual aromatic mononuclear residues by Q30 and any other residue by Q19.)	33	30	
30	Disregarding ring hydroxy or methoxy does the ring bear substituents other than 1-5 -carbon aliphatic groups, either hydrocarbon or containing alcohol, ketone, aldehyde, carboxyl or simple esters that may be hydrolysed to ring substituents of five or less carbons? (If a simple ester that may be hydrolysed, treat the aromatic portion by Q18 and the residue by Q19.)	18	31	
31	Is the substance an acyclic acetal, -ketal or -ester *or an alkylaryl disulphide* of any of the above substances (see Q30)? (If yes, assume hydrolysis *or reduction* and treat the aromatic residue by Q18 and the non-aromatic residues by Q19).	32	18	
32	Does the substance contain only the functional groups listed in Q30, or their derivatives listed in Q31, but with any or all of the following: i. a single fused non-aromatic carbocyclic ring or, ii. aliphatic substituent chains longer than five carbon atoms or, iii. a polyoxyethylene [(-OCH_2CH_2-)x with x no greater than 4] chain either "on the aromatic ring or on an aliphatic side chain?	22	**II**	Part (i) is intended to allow simple derivatives of tetralin into Class II while putting polycyclic compounds such as the steroids ultimately into Class III (except those that may be normal food), Part (ii) allows compounds with permitted functional groups but longer side chains into Class II instead of Class III. Part (iii) puts short-chain polyoxyethylene derivatives of aryl compounds into Class II

Q	Question	NO	YES	Additional explanation
				rather than Class III.
33	Does the substance bear on every major structural component at least one sodium, potassium, or calcium sulphonate or sulphamate for every 20 or fewer carbon atoms without any free primary amines except those adjacent to the sulphonate or sulphamate.	**III**	**I**	

Extensions to the Cramer decision tree

Toxtree Rule ID 4 and Rule ID 40 – adds phosphate to list of elements that do not automatically go to Class III (Q4 in scheme above) and then under Rule ID 40 questions whether it is a possibly harmful organophosphate type of chemical. If the answer to this is yes then it is assigned to Class III, but if the answer is no and it is a simple phosphate ester it is assigned to Class I; but this assumes that the non-phosphate hydrolysis product would be Class I – which may not always be correct. This Rule *has not* been incorporated into the modified decision tree given above.

Toxtree Rule ID 42 – assigns to Cramer Class III possibly harmful analogues of benzene that "consist of one aromatic ring with at most one heavy atom connected to each aromatic atom". The examples shown in the explanation for this step under Toxtree version 2.6.0 are phenol and benzamide. Benzene with one or more hydroxyl-, amino- or thiol- group, or a combination of these groups, with or without an aromatic methyl group, but without further substitution are assigned using this step; more complex benzene derivatives such as benzoic acid, benzamide, acetamido-benzene, phenylhydrazine, and ethyl-substituted phenol, ethyl-substituted aniline etc are not. This Rule *has* been incorporated in a simplified form into the modified decision tree given above.

Toxtree Rule ID 43 – assigns "possibly harmful divalent sulphur" to Class III. The explanation to this rule given in the program is "*Does the compound [*contain*] a non-natural divalent sulphur*?" It appears that this is a question about natural occurrence, which is not related to the potential for toxicity. Interestingly, none of the different types of structures in the 10 sub-groups of sulphur-containing flavouring agents evaluated by the JECFA (WHO, 2000) would have been assigned to Class III using Rule ID 43. This Rule *has not* been incorporated into the modified decision tree given above.

Toxtree Rule ID 44 – assigns any free α,β-unsaturated heteroatom, such as an α,β-unsaturated alcohol or ketone to Class III. In its evaluations of flavouring agents the JECFA has considered the extent of detoxication processes for such chemicals and concluded that "metabolic processes such as oxidation and conjugation effectively eliminate reactive aldehyde functional groups from such substances when they are consumed in the amounts that would arise from their use as flavouring agents" (WHO, 2002). Therefore, as the TTC approach is only applicable at low levels of exposure, this Rule *has not* been incorporated into the modified decision tree given above.

Annex 1: List of participants to the Stakeholder hearing

Stakeholder hearing on Threshold of Toxicological Concern 2 December 2014 | Brussels, Belgium			
Last name	**First name**	**Affiliation**	**Country**
ARNAUD	Ludovic	FEFANA	BEL
ARNAUTS	Jan	DSM Ahead	NLD
ARNICH	Nathalie	ANSES	FRA
ARVIDSON	Kirk	Food and Drug Administration	USA
BAKEN	Kirsten	KWR Watercycle Research Institute	NLD
BARRETT	Gordon	Health Canada	CAN
BARTOLO	Ivan	Seafood Importers and Processors Alliance	GBR
BENFORD	Diane	Food Standards Agency	GBR
BLUM	Rene	Lonza Ltd	CHE
BRÜSCHWEILER	Beat	Federal Food Safety and Veterinary Office	CHE
BURNETT	Thomas	Elanco Animal Health	USA
CACHET	Thierry	IOFI	BEL
CAVALLINI	Eugenio	CEPI aisbl	BEL
CHEESEMAN	Mitchell	Steptoe & Johnson LLP	USA
CIMMARUSTI	Floriana	Healthy Food Europe	BEL
COREA	Namali	SC Johnson	GBR
CREANGA	Adina	Bunge	BEL
DE LUCA	Lucia	European Food Safety Authority	
DEMPE	Julia	Dr. Knoell Consult GmbH	DEU

DETKEN	Dirk	European Food Safety Authority	
DEWHURST	Ian	Health and Safety Executive	
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EARL	Lesley	LSR Associates	GBR
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FATTORI	Vittorio	Food and Agriculture Organization of the United Nations	
FEELEY	Mark	Health Canada	CAN
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FEIGENBAUM	Alexandre	Technopole Alimentec	FRA
FELTER	Susan	Procter & Gamble	USA
FLETCHER	Samuel	Veterinary Medicines Dire torate	GBR
FRUTH	Lothar	ATC GmbH	DEU
FUART-GATNIK	Mojca	National Institute of Public Health	SVN
GEUEKE	Birgit	Food Packaging Forum	CHE
GRANERO-ROSELL	Miguel Angel	European Commission	(EC)
GUNDERT-REMY	Ursula	Federal Institute for Risk Assessment	DEU
HÜSER	Anja	Knoell Consult GmbH	DEU
HYNES	Geoffrey	Givaudan	GBR
JACOBS	Kristi	Food and Drug Administration	USA
JEONG	Sang-Hee	Hoseo University	KOR
JIA	Xudong	World Health Organization	
JUNGHANS	Angelika	Clariant Produkte GmbH	DEU

KANUNGO	Debabrata	Ministry of Agriculture	IND
KRUL	Lisette	TNO	NLD
LEINALA	Eeva	OECD	FRA
LEYDECKER	Matthias	FEICA	DEU
LIEM	Djien	European Food Safety Authority	
LIU	Zhaoping	China National Centre for Food Safety Risk Assessment	CHN
LUPTON-BOWERS	Pamela	Moderator of the event	UK
LYSSIMACHOU	Angeliki	PAN Europe	BEL
MAURICI	Daniela	European Food Safety Authority	
MEROLLA	Luciano	Dow AgroSciences Ltd	GBR
MILLSTONE	Erik	University of Sussex	GBR
MORTENSEN	Alicja	Technical University of Denmark	DNK
MUELLER	Utz	Food Standards Australia New Zealand	AUS
MUILERMAN	Hans	PAN Europe	BEL
ORISAKWE	Orish Ebere	University of Port Harcourt	NGA
PLATZEK	Thomas	Federal Institute for Risk Assessment	DEU
POLITANO	Valerie	Research Institute for Fragrance Materials Inc.	USA
PRIETO ARRANZ	Miguel Angel	Cefic	BEL
RENWICK	Andrew G.	University of Southampton	GBR
REYNDERS	Hans	Flemish Government	BEL
RICHERT	Susann	Evonik Industries AG	DEU
ROBINSON	Tobin	European Food Safety Authority	
RONGA-PEZERET	Sylvaine	EDF – DRH Groupe - Direction Emploi et Développement des Salariés	FR

ROSSI	Annamaria	European Food Safety Authority	
ROVIDA	Costanza	University of Konstanz	DE
SCHLATTER	Josef		CHE
SCHNABEL	Juergen	Givaudan International AG	CHE
SHAH	Prakashchandra	US Environmental Protection Agency	USA
SHEN	Jie	Research Institute for Fragrance Materials Inc.	USA
STIENON	Sarah	ISK Biosciences Europe NV	BEL
STROHEKER	Thomas	Nestlé	CHE
SUSIN	Carolina	European Chemical Industry Council	BEL
TAYLOR	Sean	International Organization of the Flavor Industry	USA
TERRON	Andrea	European Food Safety Authority	
TRITSCHER	Angelika	World Health Organization	
TROISFONTAINES	Paul	Scientific Institute for Public Health	BEL
TWEEDALE	Anthony C.		BEL
UMEMURA	Takashi	National Institute of Health Sciences	JPN
VAN BOSSUYT	Melissa	Scientific Institute for Public Health	BEL
VANSTHERTEM	David	Japan Agro Services S.A.	BEL

Annex 2: List of participants of expert workshop

Joint EFSA/WHO Expert Workshop on Threshold of Toxicological Concern 3-5 December 2014 | Brussels, Belgium			
Last name	**First name**	**Affiliation**	**Country**
ARVIDSON	Kirk	Food and Drug Administration	USA
BARRETT	Gordon	Health Canada	CAN
BENFORD	Diane	Food Standards Agency	GBR
BOOBIS*	Alan	Imperial College London	GBR
BRÜSCHWEILER	Beat	Federal Food Safety and Veterinary Office	CHE
CHEESEMAN*	Mitchell	Steptoe & Johnson LLP	USA
DEWHURST	Ian	Health and Safety Executive	GBR
DORNE	Jean-Lou	European Food Safety Authority	
DOURSON	Michael	Toxicology Excellence in Risk Assessment	USA
ESCHER	Sylvia	Fraunhofer Institute for Toxicology and Experimental Medicine	DEU
FATTORI	Vittorio	Food and Agriculture Organization of the United Nations	
FEELEY	Mark	Health Canada	CAN
FELTER*	Susan	Procter & Gamble	USA
GUNDERT-REMY	Ursula	Federal Institute for Risk Assessment	DEU
JACOBS	Kristi	Food and Drug Administration	USA
JEONG	Sang-Hee	Hoseo University	KOR
JIA	Xudong	World Health Organization	

KANUNGO	Debabrata	Ministry of Agriculture	IND
KRUL*	Lisette	TNO	NLD
LEINALA	Eeva	OECD	FRA
LIEM	Djien	European Food Safety Authority	
LIU	Zhaoping	China National Centre for Food Safety Risk Assessment	CHN
MAURICI	Daniela	European Food Safety Authority	
MENNES	Wim	National Institute for Public Health and the Environment	NLD
MÜLLER	Utz	Food Standards Australia New Zealand	AUS
ORISAKWE	Orish Ebere	University of Port Harcourt	NGA
RENWICK*	Andrew G.	University of Southampton	GBR
ROSSI	Annamaria	European Food Safety Authority	
SCHLATTER	Josef		CHE
SHAH	Prakashchandra	US Environmental Protection Agency	USA
TRITSCHER	Angelika	World Health Organization	
UMEMURA	Takashi	National Institute of Health Sciences	JPN
YANG	Chihae	Molecular Networks GmbH	DEU

Experts taking part in the workshop were selected following a public call for expert published on the WHO website (http://www.who.int/foodsafety/call-data-expert/en/).Experts were selected according to the criteria indicated in the call for expert. The screening of their DOIs was performed by WHO according to the organisation's rules. *Following this screening, experts who have been found to have a potential conflict of interests, did not attend on the last day of the workshop where the group agreed on conclusions and recommendations.

Annex 3: Agenda of expert workshop

EFSA/WHO Expert workshop on Threshold of Toxicological Concern (TTC)

Brussels, 3-5 Dec 2014

Management Centre Europe

Rue de l'Acqueduc 118, Brussels, Belgium

Day 1 – Wednesday, 3 December 2014	
08.30-09.00	Registration
09.00- 09.10	Welcome and Opening
Session 1:	**Introduction: setting the stage**
09.10 – 09.30	Background to the WHO project
09.30 – 09.50	EFSA's work on TTC
9.50 – 10.10	Stakeholder meeting summary
10.10 – 10.30	**Coffee break**
10.30-11.30	Report on TTC approach
11.30-12.00	Discussion
12.00-13.00	**Lunch**
Session 2:	**Introduction to work in the breakout groups**
13.00- 13.20	Breakout Group1: Cramer Decision Tree
13.20-13.40	Breakout Group2: TTC threshold levels & TTC decision tree
13.40-14.00	Discussion
Session 3:	**Breakout groups**
BOG1	**Cramer Decision Tree**
BOG2	**TTC threshold levels & TTC decision tree**
14.00-15.30	Breakout group discussions
15.30-16.00	**Coffee break**
16.00-17.00	Continuation of breakout groups

17.00-18.00	Summary and report back to plenary
	End of the first day
Day 2 – Thursday, 4 December 2014	
Session 3 (continued):	**Breakout groups**
BOG1	**Cramer Decision Tree**
BOG2	**TTC threshold levels & TTC decision tree**
09.00-10.30	Breakout group discussions
10.30-11.00	**Coffee break**
11.00-12.30	Continuation of breakout groups
12.30-13.00	Brief report back from breakout groups to plenary
13.00-14.00	**Lunch**
14.00-15.00	Continuation of breakout groups
15.00-15.30	**Coffee break**
15.30-17.30	Continuation of breakout groups
17.30-18.30	Report back form breakout groups to plenary
	End of the second day
Day 3 – Friday, 5 December 2014	
Session 4:	**Report back from breakout groups**
09.00 – 09.30	Summary of the two-day discussion and report back from Cramer Decision Tree (BOG1)
09.30 – 10.00	Summary of the two-day discussion and report back from TTC threshold levels & TTC decision tree (BOG2)
10.00-10.30	Discussion on the outcomes
10.30-11.00	**Coffee break**
Session 5:	**Summing up**
11.00- 12.20	Discussion and agreement on recommendations
12.20-12.30	Closing remarks and end of the workshop