CC/2017/21

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Recent developments on the Threshold of Toxicological Concern (TTC) approach to support the update of Guidance Statement G05

Introduction

1. The Threshold of Toxicological Concern (TTC) is a *de minimus* approach for chemical risk assessment that is based on the analysis of large toxicological databases from which exposure levels likely to be of negligible risk are determined. The approach has been reviewed by the Committee on a number of occasions, most recently in 2012 (CC/2012/18) and has been incorporated into the Guidance Statement G05: Defining a Point of Departure and Potency Estimates in Carcinogenic Dose Response (2014). The Guidance outlines the historical developments of the approach, based on Cramer classification classes (Munro et al 1996; Kroes et al 2004; Felter et al 2009) and the decision tree formulated by the European Food Safety Authority (EFSA) for their Guidance in 2012 (EFSA 2012). The Committee adopted this decision tree and broadly endorsed the views of EFSA and EU non-food expert Committees (at the time SCHER, SCENIHR, SCCS). This paper presents some recent evaluations of the TTC methodologies, developments of the analyses and opinions, with reference to different regulatory scenarios with the aim of updating the Committee to facilitate the decision on whether to revise their current opinions and Guidance with regards to the TTC approach.

2. Overall, the Committee recognised the TTC approach as a pragmatic means of assessing whether exposure to a chemical, for which no or limited toxicity data is available, is of low concern or whether further testing is required. However, it was advised that the TTC approach should not be used to replace data for any chemical under consideration, but should be used in a scenario where data are lacking or insufficient, to help in reaching informed risk management decisions.

3. Since the COC Guidance was published in 2014, there have been a number of reviews, updates and examinations of the approach from different perspectives. An International Life Sciences Institute (ILSI) workshop on the topic was held in 2011 (reported by Dewhurst and Renwick 2013) and EFSA have updated their guidance in conjunction with the World Health Organisation (EFSA/WHO 2016). Some evaluations in relation to regulatory frameworks have also been undertaken e.g. impurities in human pharmaceuticals, food contact materials (FCM) and developments of the applicability of the approach for inhalation and dermal exposure have been published. Attention has also been drawn to the fact that a re-examination

of both the original cancer potency databases used to derive the threshold values, and the assumptions for concluding that a substance is DNA reactive, has not been undertaken.

ILSI workshop

4. A workshop organised by ILSI in June 2011 and attended by delegates from a wide variety of industries and regulatory organisations was reported by Dewhurst and Renwick (2013). The goals of the workshop were to broaden awareness of the application and practical experiences of TTC; characterise the key challenges associated with the TTC; explore the scope and limitations of the methodology; and identify and understand the scientific barriers that limit its application (see also paragraph 5). Amongst the points discussed at the workshop were:

- the databases from which the Cramer classifications were derived were based upon no observed adverse effect levels (NOAELs) - updated approaches such as benchmark dose (BMD) modelling are now available, which may be more appropriate for deriving points of departure (POD);
- the cancer databases required refinement to ensure all potentially genotoxic functional groups were captured;
- developments in route to route extrapolation.

5. Modifications of the current thresholds were also reviewed. In the current COC Guidance, the threshold value for a chemical which has structural alert for genotoxicity is 0.15 µg/person/day. It was considered by the ILSI workshop that a move to increase this value from 0.15 to 1.5 µg/person/day, based on absence of alerts for genotoxicity, was sufficiently robust but that inclusion of mode of action (MOA) information would be useful in examining whether there were threshold mechanisms for effect. However, it is noteworthy that the TTC is generally applied when there is insufficient data so it is not clear how MOA information would be available. Overall it was concluded that the TTC approach is valid for first tier risk characterisation but that further refinement of the approach, for example by expanding the database, was recommended.

EFSA update

6. In their previous review, the Committee examined the EFSA Opinion published in 2012, endorsed their views and used the decision tree in the current COC Guidance Statement G05. An updated EFSA Opinion was published in 2016 in conjunction with World Health Organization (WHO) following a joint workshop that had the goals of improving and updating the Cramer classification and TTC decision tree (EFSA/WHO 2016). It was noted that the TTC approach is continually being scrutinised in terms of its origins, developments and applicability. The following aspects were the focus of the workshop and underpin the discussions in the EFSA review:

- *The range of chemicals in the supporting databases and the 'applicability domain',*
- *The suitability of the Cramer scheme for dividing chemicals into different classes of toxic potential,*
- *Whether certain chemicals should be excluded a priori from the TTC approach (e.g. the cohort of concern – see Kroes et al 2004),*
- *The tools used to identify structural alerts to allocate chemicals into the 'genotoxic cancer' tier of 0.15 µg/person/day,*
- *Whether the production of extra TTC values for specific end-points or chemical classes (for example using Quantitative Structure–Activity Relationship models (QSARs)) would be of value,*
- *Additional criteria necessary for application to non-oral routes of human exposure or for short-term or intermittent human exposures.*

7. Much of the 2016 document references the original 2012 document, the publications that preceded it and what the original framework derivation was based on. The key points documented in 2016 were that:

- The current Cramer classification scheme is conservative and therefore protective of human health,
- The current Cramer questions adequately incorporate steps to predict metabolism for all classes (including potentially DNA reactive carcinogens and organophosphates (OPs)),
- The validity of the databases used to derive the TTC values (including the use of carcinogenic potency (TD_{50}) vs BMD) was examined and it was concluded that the Munro (1996) and the Carcinogen Potency Database (CPDB) were broadly representative of the world of chemicals,
- Transforming doses from mg/kg to molar concentrations did not change the results or facilitate separation of chemicals into different levels of toxicity or reduce the overlap in the Cramer classes,
- A default value of chemicals with genotoxic alerts of 0.15 µg/person/day was appropriate and sufficiently protective but that its scientific rationale should be examined and strengthened (for example, by extending the database using BMD methodology),
- Question 22 of the Cramer classification process- 'is the substance a common component of food or structurally related to a common component of food' should remain for the time being despite the term 'common' not being well defined.

The following table, converting µg/person/day to µg/kg bw /day was provided;

Type of TTC value	TTC value (µg/person/day)	TTC value (µg/kg bw/ day)
With structural alert for genotoxicity	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

8. A number of changes to the decision tree were made in the 2016 update that include:

- The first step in the 2012 version '*does the substance have a known structure and are exposure data available*' (for which the answer 'no' meant that TTC could not be applied) is omitted in the 2016 version,
- The step-wise process is now numbered,
- Recommending a '*risk assessment is required*' rather than the '*substance requires a non-TTC approach (toxicity data, read-across etc.)*' when a chemical exceeds its Cramer classification exposure limit or is deemed part of the exclusion categories,
- Development of the '*are there structural alerts*' step by recommending inclusion of chemical-specific genotoxicity data to identify potentially DNA-reactive carcinogens,
- Recommending '*substance would not be expected to be a safety concern*' if a substance does not exceed the exposure threshold for its Cramer class instead of the advice '*low probability of health effect* (with the proviso; '*if exposure of infants < 6 months is in the range of TTC, consider if TTC is applicable*')
- Moving the question asking whether the compound is an OP or carbamate to precede the question regarding whether the exposure exceeds 0.3 µg/kg bw/day
- Inclusion of a step for identifying Cramer class II, giving an acceptable exposure of 9 µg/kg bw/day

9. Key recommendations of the 2016 report were that:

- Cramer class II should be used and applied but that phenols and primary amines are not reassigned to this class as they were outliers in the analysis,
- The distributions between Cramer classes should be re-evaluated,

- Plant metabolites of pesticides of unknown structure should be placed directly into class III,
- A permanent, centralised repository for data supporting the TTC and Cramer scheme should be created,
- Databases for different, non-cancer endpoints should be merged and made public,
- The TTC cancer database should be expanded (e.g. with Toxref),
- OPs and carbamates should be treated separately with their own threshold value of 0.3 µg/kg bw/day,
- TTC is appropriate for mixtures of chemicals, starting with dose addition assumptions,
- Less than lifetime exposures should be considered on a case-by-case basis [see also paper CC/2017/19 for discussion at this COC meeting],
- TTC approach can be used for children. Those under 3 months of age should be considered on a case-by-case basis.

Development of applications of TTC for different routes of exposure

TTC approach for inhalation exposure:

10. There are a number of publications that describe the development of the TTC approach for exposure to chemicals via the inhalation route and that provide background to its application and extrapolation from the oral route (considered for the previous COC paper, CC/2012/18).

11. Drew and Frangos (2007) introduced the 'Concentration of No Toxicological Concern (CoNTC)' as a risk assessment tool for inhaled substances. The CoNTC of 0.03 µg/m^3 was calculated from inhalation data and by applying the (now mostly historical) TTC value of 0.02 µg/kg bw/day derived by the Food and Drug Administration (FDA) for genotoxins/carcinogens.

12. Escher et al (2010) provided a detailed analysis of TTC values for inhalation exposure based on Cramer classifications from the RepDose database. By calculating the 5th percentile no observed effect concentrations (NOECs) for Cramer classes I-III resulted in thresholds of 1.5 x 10^{-3} ppm for chemicals assigned as Cramer class I and 2.2 x 10^{-5} ppm for those in Cramer class III. However, when calculated as µg/person/day, the inhalation thresholds for classes I and III (71 and 4 µg/person/day, respectively) are considerably lower than the oral thresholds derived by Munro (1800 and 90 µg/person/day). Based on further refinement (including omission of OPs and compounds with structural alerts for genotoxicity), two inhalation TTCs for non-genotoxic compounds are proposed: 3.6 × 10^{-3} ppm (180 µg/person/day) for Cramer class I; 2.4 × 10^{-5} ppm (4 µg/person/day) for Cramer class III. Threshold values are thought to be much lower than the oral thresholds

derived by Munro due to the high sensitivity of the respiratory tract to local effects. A threshold for Cramer class II is not proposed, as only a few substances are classified into this class.

13. More recently, a number of publications describe different analyses of chemicals from the RepDose database, all of which have repeated-dose toxicity data from 28-, 90-day or 1-year exposure assays.

14. Schüürmann et al (2016) aimed to derive structural alerts that discriminated between high and low toxic chemicals following inhalation exposure with the eventual aim to derive more specific thresholds for identifying levels of exposure that are unlikely to cause harm. Using the RepDose database, 296 chemicals with subacute, subchronic and chronic inhalation NOEC data were classified as having high, medium or low potency based on their NOEC values. Structural alerts were then identified that discriminated between high and low toxic chemicals. 110 chemicals were classified as high-toxic (NOEC < 0.75 ppm), 92 as medium-toxic (0.75 ppm ≤ NOEC ≤ 12 ppm) and 95 as low-toxic (NOEC > 12 ppm). 14 high-toxicity and 7 low-toxicity structural alerts were identified that only moderately discriminated between the three groups. Unlike Escher et al (2010) thresholds for each group of chemicals were not calculated.

15. In a companion publication, Tluczkiewicz et al (2016) provide a detailed description of methods and the outcome of a programme of work that utilised the RepDose data set to derive classifications and threshold values from NOECs following inhalation exposure. The goal of the project was to generate an integrative grouping of chemicals for inhalation exposure classification. NOEC values were standardised; structural feature groups were assigned alerts to those predicting high and low inhalation toxicity and chemicals allocated to Cramer classes. Overall 28 different chemical groups were derived; 19 with high toxicity and 9 with low toxicity with thresholds of 2 µg/person/day and 4260 µg/person/day, respectively. The chemical evaluation of phosphoric acid esters (most commonly used as pesticides), aliphatic and aromatic amines were detailed, providing examples of how structurally similar chemicals group together with regard to absorption characteristics, systemic effects and target organ toxicities. Chemicals with structural alerts for genotoxicity were found in 11 classes from both high and low toxicity groups. However, it was noted that the RepDose database does not contain information on genotoxic potential structural alerts.

TTC approach for dermal exposure:

16. To date, examination of thresholds following dermal exposure focuses principally on skin sensitising potential and the derivation of a Dermal Sensitisation Threshold (DST), historically derived using data derived from the local lymph node assay (Safford 2008). There are two more recent publications that provide extensions of the DST approach to identify High Potency Category Chemicals and incorporation of chemicals considered to be ‘reactive’. However, these frameworks are based only on sensitising potential (Roberts et al 2015; Safford et al 2015).

17. An application considering systemic toxicity following dermal exposure is included below under 'cosmetics' (Williams et al 2016).

Application of TTC approach in different regulatory settings

Mutagenic impurities in pharmaceuticals:

18. Discovery of potentially mutagenic impurities, e.g. remnants from the synthetic process or as drug degradation products, is not uncommon and there have been significant developments in the application of TTC in human pharmaceuticals to facilitate these risk management scenarios. Controlling the level of these impurities, and thus potential exposure and risk is of importance in development of drug products. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guideline M7 entitled 'Assessment and control of DNA reactive (mutagenic impurities) in pharmaceuticals to limit potential carcinogenic risk' (ICH 2017) provides guidance on impurity evaluation and the application of the TTC. Two negative results from independent QSAR approaches are required to establish that the impurity is not a mutagenic concern. Positive results should be followed up with a bacterial mutagenicity test and if this is positive, then further follow up tests, including *in vivo* evaluation are required. A chemical that demonstrates a practical threshold or has positive carcinogenicity data should be evaluated, on a case-by-case basis, to derive appropriate acceptable daily intakes (ADIs).

19. Within the pharmaceutical industry, for an impurity with structural alerts or positive mutagenicity data, the TTC value of 1.5 µg/person/day is considered acceptable for a pharmaceutical used for long-term/lifetime daily exposure (> 10 years), assuming a body weight of 60 kg. The ICH Guideline addresses less than lifetime exposures using an acceptable cumulative lifetime dose (1.5 µg/day x 25,550 days = 38.2 mg) and suggests acceptable threshold values for different durations of exposure as follows:

Duration of treatment	**< 1 month**	**> 1 - 12 months**	**> 1 - 10 years**	**> 10 years to lifetime**
Maximum Daily intake (µg/person/day)	120	60	30	1.5

The Guideline also provides examples of different exposure duration scenarios and medical conditions.

20. An addendum to ICH M7 (ICH 2015 – included as appendix 3 in ICH 2017) provides analyses of 15 chemicals considered to be mutagenic carcinogens that have data from well conducted carcinogenicity studies. They were selected because they are commonly used in pharmaceutical manufacturing, and are useful to illustrate the principles for deriving compound-specific intakes described in ICH M7.

The paper provides calculations of compound specific acceptable intakes (AI) which can be applied in place of TTC values. Some calculations were based on linear extrapolation from the $TD_{50,}$ some were based on derivation of a threshold. A sizeable range of figures was derived – the lifetime AI for bis-(chloromethyl)ether (BCME) was lower than the TTC (0.004 µg/day), whilst many were greater than TTC value (e.g. 1-chloro-4-nitrobenzene, 115 µg/day; ethyl chloride 1810 µg/day). In the calculations, weight was assumed to be 50 kg, providing an additional safety factor.

21. Snodin and McCrossen (2013) critiqued the derivation of the one default cancer TTC value of 1.5 µg/person/day used in the pharmaceutical industry and argued that EFSA (2012) did not formally re-evaluate and update their cancer database based on the updated Cheeseman et al dataset, whilst they had examined other critical compound lists for non-cancer endpoints. They attempted to ascertain the concordance of conventional structural alerts with mutagenicity and carcinogenicity data and the relative carcinogenicity potency of various categories of structural alerts, using the extended Cheeseman database; and provided an extensive evaluation of the derivation of the TTC by Kroes et al (2004) by using other carcinogenicity databases [the Cheeseman dataset (Cheeseman et al 1999), the Gold et al database (CPDB) and the 1999 supplement to the CPDB (Gold et al 1999)]. Overall, the authors concluded that the current cancer TTC derivation was overly conservative in relation to the application of TTC approach for pharmaceuticals. They considered that a single cancer TTC value could be replaced by a series of structure-based limits similar to the three Cramer classes.

22. Galloway et al (2013) provides a comprehensive evaluation of chemicals commonly used in drug synthesis with DEREK structural alerts. A comparison was made with the TD_{50} of mutagenic carcinogens in the Gold database and examination of the cohort of concern. The authors concluded that the TTC is based on more potent chemicals than those used in drug synthesis. It was also demonstrated that of the 361 chemicals examined, only 54% were Ames positive. The authors highlight that this emphasises the conservative nature of the ICH M7 approach. Lifetime limits for mutagenic carcinogens of various potencies were also examined in relation to TTC for some mutagenic impurities (e.g. alkyl halides, aromatic amines).

Veterinary medicines:

23. In a draft Guideline on assessment and control of DNA reactive (mutagenic) impurities in veterinary medicinal products (CVMP 2017), impurities were classified with respect to mutagenic and carcinogenic potential. A TTC approach was applied to known mutagens with unknown carcinogenic potential and to chemicals with structural alerts for mutagenicity, which are unrelated to the drug substance. An acceptable intake of 0.025 µg/kg bw/day, with regards to the target animal's safety was given.

Food and food contact materials:

24. Consistent with EFSA's application of the TTC to substances in food, there have been several publications that evaluate its application in the context of unknown substances in food or non-intentionally added substances (NIAS). NIAS can be present as complex mixtures, identified using chromatographic analysis, described by some as a 'forest of peaks', and are detected in a wide variety of food matrices, for example, from extracts of FCM or the degradation of chemicals residues.

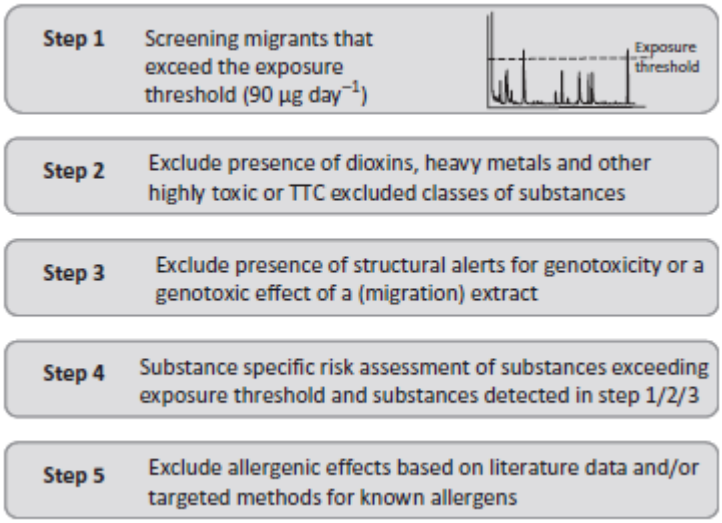
25. A report from EFSA's Contact materials, Enzymes, Flavourings (CEF) unit describes an investigation of whether the TTC approach can be applied to substances present in FCM (Pinalli et al 2011). The authors evaluated 232 materials used in the manufacture of FCM for which tolerable daily intakes (TDIs) or ADIs are known. They were categorised according to Cramer classifications and compared to the Munro dataset. It was concluded that the TTC was more conservative than a routine risk assessment process (using known no observed effect levels (NOELs)) and it was suggested that it can also be used to prioritise testing for chemicals without data.

26. Rennen et al (2011) provide a framework to investigate chemically complex food matrices, many of which contain unidentified substances, using the TTC approach. An analytical screening threshold of 540 µg/person/day is applied by estimating intake using the size of chromatographic peak and the anticipated exposure scenario. Firstly, those that exceed 540 µg/person/day (Cramer class I) are identified. Step 2 applies targeted analysis to identify metals, cohort of concern chemicals, OPs and organohalogen (OH) compounds. Step 3 involves identifying other alerts for genotoxicity which are excluded. It is noted that it would be of value to develop a screening process that could identify genotoxic chemicals present at low levels but that exceed the 18 µg/person/day threshold. Overall it was concluded that the framework could be applied but that analytical tools and genotoxicity testing methods were required to identify genotoxic chemicals and those in the cohort of concern.

27. An ILSI project describes the application of the TTC to unknown substances in foods from sources such as food processing, FCM and reaction by-products (Koster et al 2011). Again, a step-wise approach is described focusing on quantification of unknowns which exceed TTC thresholds, including identifying the potential sources, the use of chromatographical methods and consumption estimates. Methods to aid identification of peaks and the prediction of whether unknown chemicals in food will be part of the cohort of concern and/or be genotoxic are suggested. A number of examples are given with a view to examining the applicability of the approach (e.g. pesticide residues on cucumber; plastic food contact material extract). It is concluded that it is possible to apply a TTC threshold of 90 µg/person/day. However it is acknowledged that there are limitations to the

approach given that it is possible that exposures exceeding 0.15 µg/person/day will occur and thus evaluation of genotoxic potential is required.

28. The Complex Mixture Safety Assessment Strategy (CoMSAS), is described by Koster et al (2014), which examines the risk posed by NIAS migrating from carton FCM. The approach consists of five steps based on the exclusion of the presence of groups of substances, following the Kroes et al (2004) decision tree, modifications proposed by Munro et al (2008) and conclusions from EFSA (2012). Presented as follows:



29. Specific migration tests were performed on a sample of food carton, the Bluescreen assay was used to assess genotoxicity and the presence of NIAS found in the screening was assessed using the CoMSAS approach. From the data obtained the authors concluded that the NIAS detected in the carton samples are not considered of toxicological relevance. For the five substances assigned as Cramer class III, quantitative migration testing was recommended. If, following exposure evaluation, levels of NIAS were deemed to be below the TTC 90 µg/person/day exposure threshold, there was no concern with regards to toxicity of the sample. It was also highlighted that expert judgement is required to assess whether any of substances within the mixture require actual analytical screening to identify chemicals in the cohort of concern.

30. More recently, Bolognesi et al (2017) examined the genotoxicity testing approaches for evaluating FCMs. Their evaluation led them to conclude that, because of the potential for number of *in vitro* positives, that it was not appropriate to use the TTC approach for the evaluation of FCM. This was in accordance with the EFSA CEF panel for the evaluation of NIAS (also mentioned in EFSA 2016).

Pesticides:

31. Feigenbaum et al (2015) examined the reliability of the TTC approach by examining pesticides which had been previously evaluated by an EU regulatory body (see paragraph 40 below).

Drinking water:

32. A programme of work undertaken by Dutch water utility companies implementing a water quality initiative, namely the Drinking Water Quality in the 21st century, that used the TTC approach was described by Mons et al (2013). Target concentrations for individual genotoxic chemicals and for the total sum of all other organic chemicals were set at 0.01 and 1 µg/L, based on consumption average of 2L/day and a 60kg adult.

Cosmetics:

33. The Scientific Committee on Consumer Safety (SCCS), Scientific Committee on Health and Environmental Risks (SCHER) and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (2012) examined the application of the TTC approach to cosmetics. It was concluded that oral TTC values for Cramer Class III compounds would likely overestimate the potential toxicity of the same chemical following topical exposure, even if 100% of the topical dose entered the systemic circulation as the parent compound. Furthermore, the TTC concept can only be used for those compounds which belong to a sufficiently represented structural class in the TTC database and where appropriate exposure data are available.

34. As part of an ILSI project, Williams et al (2016) provided a detailed consideration of the application of TTC for cosmetics applied to skin. Two approaches were undertaken; derivation of dermal-specific TTC's and extrapolation from oral data, predicting systemic availability. A decision tree was proposed for the latter. It was concluded that oral TTC values are appropriate for where toxicity data are not available. The model was considered to overpredict bioavailability in most circumstances but underprediction was also observed and therefore the process needs to be applied on a case-by-case basis.

35. Yang et al (2017) compiled a new dataset of cosmetics-related chemicals for the TTC approach. The COSMOS dataset comprised of 552 chemicals (219, 40, and 293 chemicals in Cramer Classes I, II, and III, respectively) which were rigorously evaluated and human exposure thresholds of 42 mg/kg bw/day for Cramer Class I and 7.9 mg/kg bw/day for Class III were derived. A value for Cramer Class II was not derived due to insufficient data. This new dataset was amalgamated with the Munro dataset (1996). The 966 substances in the combined database comprise 245, 49 and 672 chemicals in Cramer Classes I, II and III, respectively and TTC values of 46, 6.2 and 2.3 mg/kg bw/day were generated which are broadly similar to those of the original Munro dataset.

Re-evaluation of the databases used to derive the original TTC values:

36. The EFSA review published in 2016 did not attempt to re-evaluate the data which underpins the original derivation of the TTC values. This was subsequently examined by Boobis et al (2017) attached at Annex A, alongside providing a brief description of the history and the existing TTC values. It is noted that the Cramer classification using the decision tree for chemicals that are non-genotoxic is based upon the 5^{th} percentile of NOAEL frequency distributions and assumes a lifetime exposure. An evaluation of the background to the database that underpins the derivation of the current 0.15 µg/person/day threshold is provided as it was noted that this value has not been reviewed or updated since Munro (1990).

37. From the original evaluation of CPDB (Gold) database (343 carcinogens tested via the oral route) Boobis et al (2017) estimated that about half of carcinogens would exceed the 1×10^{-6} tumour risk when individuals were exposed over a lifetime, if they were if exposed at 0.15 µg/person/day (0.0025 µg/kg bw/day). A re-examination of the data set indicates a bias in the National Toxicology Program (NTP) evaluation of chemicals that were suspected carcinogens, as only 29 of those that were deemed positive had been selected based on the possibility of high [human] exposure potential. Additionally some chemicals had exceeded the maximum tolerated doses (MTDs), giving rise to queries of the tumour induction being secondary to other toxicities.

38. The paper also queries the approach that has been used to derive the lowest TTC value. For example, whether: the dataset of chemicals evaluated is representative of carcinogens *per se*; the number of chemicals evaluated is sufficient; the carcinogens evaluated have a linear dose response; and that chemicals that are carcinogenic in animals are also carcinogenic to humans.

39. Their commentary on genotoxic carcinogens draws attention to the fact that 20-30% of chemicals that are positive in an Ames test are not carcinogenic and they considered that those that are positive in multiple *in vivo* tests are more likely to be true genotoxic carcinogens. The authors also considered that *in vivo* positive results found only in the Comet assay may not be robust indicators of carcinogenic potential. The authors state that there is a clear case to be made for re-assessment of the values used in EFSA's 2016 opinion, based on the belief that they are currently overly conservative and that the original derivation has been superseded by recent advances in evaluation and knowledge base.

40. It was also noted that an analysis of the potency of non-genotoxic carcinogens in regard to TTC has not been undertaken. It is suggested that chemicals that are classified under some well-defined non-genotoxic mechanisms (e.g. peroxisome proliferation, aryl hydrocarbon receptor (AhR) activation, growth stimulation, immunosuppression, endocrine perturbation) could be used to identify a priority list of human relevant non-genotoxic carcinogens for inclusion in database. The authors propose an approach to re-assess TTC's for carcinogens which includes: general criteria for acceptability of study design; what constitutes an

acceptable positive or negative study; and study exclusion criteria (see boxes 1-3 in attached paper).

41. It is also proposed that each carcinogenic response is evaluated using the International Programme on Chemical Safety (IPCS) MOA relevancy framework (Boobis et al 2006, Meek et al 2014), and that all should be assessed using the same POD. The use of identifying structural alerts using software approaches is also considered.

42. Feigenbaum et al (2015) investigated the reliability of the TTC approach by examining pesticides which had previously been evaluated by an EU regulatory body. The ADIs were then compared to the threshold value obtained by following the TTC/Cramer approach. One substance was allocated to the excluded category; 43 gave alerts for neurotoxicity (acetylcholinesterase (AChE) inhibitors); 279 were Cramer Class III; 3 were Class II and 2 were Class I. The ADI values for the Cramer III substances ranged from 0.2×10^{-3} to 10 mg/kg bw/day and were calculated to be 0.13 to 6000-fold the threshold values for Class III according to Munro. This indicates that for the majority of these chemicals (96%), the TTC value provides a conservative risk estimate. The substances with a lower ADI than the TTC were examined and it was concluded that the derived ADIs were either cautious or justified. An overview of the structural classes within the Cramer decision tree is given and an updated decision tree is proposed.

43. A re-evaluation of the original database was also proposed by Leeman et al (2014). Firstly the authors verified the previous thresholds using the Munro database available on the EFSA website. The NOELs were subjected to lognormal conversion and the 5th percentile NOEL calculated for each respective class for which TTC thresholds are calculated. Some small differences were noted when compared to the original calculations but these were attributed to rounding up/down and were not significant. New thresholds for Cramer class III chemicals were derived using these values, including subgrouping for OP, carbamates and organohalogens (OHs). The following results were derived, and threshold values proposed.

	All Cramer class III	**OP**	**Cramer class III w/o OP**	**OH**	**Cramer class III w/o OH**	**Cramer class III w/o OP or OH**
No of substances	448	40	408	166	282	242
Calculated 5th percentile NOEL mg/kg bw/day	0.15	0.032	0.22	0.15	0.18	0.40
Proposed threshold µg/kg bw/day	1.5	0.30	2.2	1.5	1.8	4.0

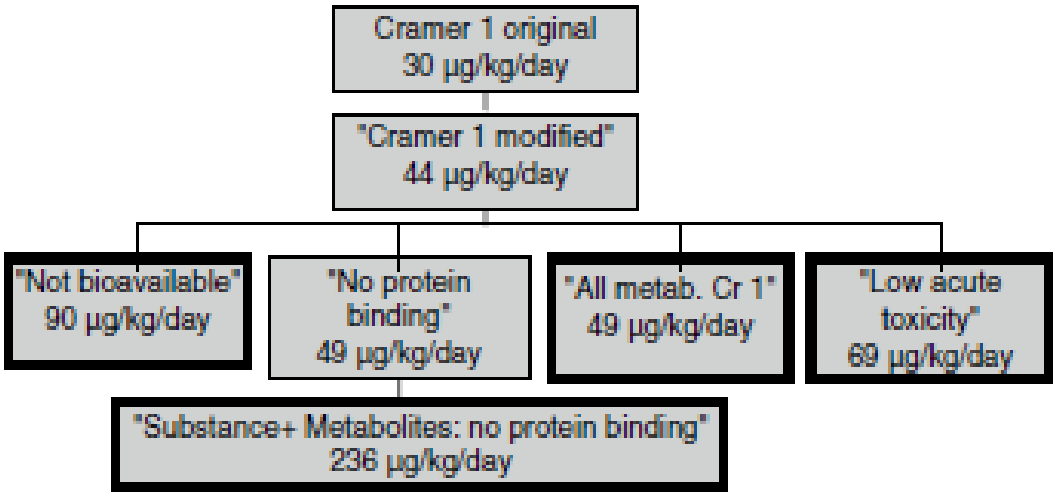
44. Leeman et al (2016) examined how the bioaccumulation of substances can be taken into account within the TTC framework, in particular in relation to physical chemical properties using the octanol/water partition coefficient (Log Po/W), and H-bond acceptor value. The relative toxicities of bioaccumulating and non-bioaccumulating substances were also taken into account. A list of substances from the Munro database with the potential to bioaccumulate was provided by calculating the 5th percentile of the NOEL. 59 chemicals of 448 Cramer class III substances examined showed the potential to bioaccumulate and 389 did not bioaccumulate. Nine Cramer class I substances that bioaccumulate were also identified. Only 1 Cramer class III substance was below the 5th percentile of the NOEL. In conclusion, a threshold of 0.59 µg/kg bw/day was calculated for class III bioaccumulating chemicals. It was pointed out that there is no harmonised definition of 'bioaccumulation' based on structure alone, therefore the EFSA edict '*that the TTC approach should not be used for bioaccumulating substances*' is difficult to interpret. Taking these aspects together, the authors concluded that it was unnecessary to exclude potential bioaccumulating substances from the database.

The use of QSAR in Cramer classification/TTC derivation:

45. Cramer classifications were derived for 1016 fragrance ingredients using Toxtree, the OECD toolbox and expert judgment and examined with regard to TTC (Bhatia et al 2015). The overall concordance was that 602 chemicals were assigned to Cramer class I, 94 were assigned to Class II and 155 to Class III by both Toxtree and the OECD toolbox; 165 of the 1016 chemicals assessed had differing outcomes generated by the different models, with the majority discordant between Class I and II. When compared with expert judgment, there were 171 chemicals that were assigned to different classes compared with Toxtree, 68 of which also differed from OECD toolbox classification. Overall there was discordant classification of 20% of the substances examined. It was concluded that *in silico* evaluations are chemical class dependent and checkpoints were recommended for future use, which aim to reduce the disparities uncovered.

46. Contrera (2011) investigated *in silico* QSAR methods to predict TD_{50} potency of genotoxic impurities in pharmaceuticals. Calculation of a risk specific dose (RSD), which takes into account carcinogenic potency, was proposed as an extension of the TTC in these scenarios. The authors claim that the results demonstrate the general applicability of SciQSAR (a commercially available software used by the US FDA, Center for Drug Evaluation and Research (CDER)) to predict TD_{50} values from which RSDs for genotoxic impurities can be determined.

47. Hauge-Nilsen and Keller (2015) used computer modelling in the OECD Toolbox with a view to refining the TTC by introducing new examination criteria. This included aspects such as bioavailability, protein binding and predicted hepatic metabolism. This generated new sub-groups and from this the highest TTC level was set at 236 µg/person/day. The following overview was presented.



Summary:

48. The TTC approach is constantly being scrutinised and updated. A detailed evaluation undertaken by EFSA/WHO and published in 2016 included a number of changes to the decision tree. However, no substantial re-evaluation of the databases used to derive the Cramer classifications was undertaken. It was recommended that a permanent, centralised repository for data supporting the TTC and Cramer scheme should be created and that the databases should be expanded by using, for example, the Toxrep database. A number of publications have suggested ways in which the databases could be updated. The usefulness of QSAR modelling in examining Cramer classifications has also been addressed. Another publication has examined the impact of bioaccumulation on TTC thresholds.

49. Exploration of the use of TTC in a wide variety of different regulatory settings is ongoing. Its use in evaluating impurities in human pharmaceuticals is well established, although there are some publications in which it is suggested that the current guidelines, according to ICH M7, are overly conservative. Application of TTC in the risk assessment of food matrices and FCM is more problematic given that many of the chemicals are unidentified.

50. There have been some developments in deriving threshold values for inhalation exposure. To date, dermal exposure assessments are concerned mainly with sensitising potential.

Questions for the Committee

51. Members are asked to provide general comments on the paper and in particular:

i. Do Members have a view on what the Committee should say about the use of the TTC in Guidance Statement G05, in light of the developments in the area since 2012/2014?

ii. Do Members think specific advice could be given on an inhalation or dermal administration TTC, given most of the frameworks are for TTC via the oral route?

NCET at WRc/IEH-C under contract supporting the PHE Secretariat
October 2017

Abbreviations:

AChE	Acetylcholinesterase
ADI	Acceptable Daily Intake
AhR	Aryl Hydrocarbon Receptor
AI	Acceptable Intake
BMD	Benchmark Dose
CCFM	Chemically Complex Food Matrices
CDER	Center for Drug Evaluation and Research
CEF	EFSA's Contact Materials, Enzymes, Flavourings unit
CoMSAS	Complex Mixture Safety Assessment Strategy
CoNTC	Concentration of No Toxicological Concern
CPDB	Carcinogen Potency Database
DST	Dermal Sensitisation Threshold
EFSA	European Food Safety Authority
FCM	Food Contact Materials
FDA	Food and Drug Administration
ICH	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
Log Po/W	Octanol/water Partition Coefficient
MOA	Mode of Action
MTD	Maximum Tolerated Dose
NIAS	Non-Intentionally Added Substance
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration

NOEL	No Observed Effect Level
NTP	National Toxicology Program
OH	Organohalogen
OP	Organophosphate
POD	Point of Departure
Q21	Drinking Water Quality in the 21st century
QSAR	Quantitative Structure–Activity Relationship models
RSD	Risk Specific Dose
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
TD_{50}	Carcinogenic Potency
TDI	Tolerable Daily Intake
TTC	Threshold of Toxicological Concern
WHO	World Health Organisation

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COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Recent developments on the Threshold of Toxicological Concern (TTC) approach to support the update of Guidance Statement G05

The following publication is attached:

Boobis A, Brown P, Cronin MTD, et al (2017) Origin of the TTC values for compounds that are genotoxic and/or carcinogenic and an approach for their re-evaluation. Crit Rev Toxicol.16:1-23.

These papers are attached; they are not being made publicly available for copyright reasons

Secretariat

November 2017

The following publications are available online:

EFSA (European Food Safety Authority) and WHO (World Health Organization) (2016) Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree. EFSA Supporting Publication. 13*(3):EN-1006, 50 pp. doi:*10.2903/sp.efsa.2016.EN-1006

ICH (2017) ICH M7 - Assessment and Control of DNA Reactive (Mutagenic Impurities) in Pharmaceuticals to Limit Potential Carcinogenic Risk. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_R1_Addendum_Step_4_31Mar2017.pdf

SCCS/SCHER/SCENIHR (2012) Joint Opinion on the Use of the Threshold of Toxicological Concern (TTC) Approach for Human Safety Assessment of Chemical Substances with focus on Cosmetics and Consumer Products. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf