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The TTC Approach in Practice and its Impact on Risk Assessment and Risk Management in Food Safety. A Regulatory Toxicologist's Perspective

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Abstract: There are many substances in food and drinking water from different contamination sources for which only insufficient or no toxicity data exist. In order to prioritize and preliminarily assess the human health risks, the threshold of toxicological concern (TTC) approach was developed between 1996 and 2004. This concept has since been applied increasingly by regulatory food safety authorities. In parallel, the safety of this approach has been discussed by stakeholders, primarily on a conceptual basis. However, real examples showing the practical benefits of this approach have not been discussed. In this paper, the technical feasibility, applicability, safety, and further benefits of the TTC approach are illustrated and discussed based on four real cases: 1) halogenated contaminants of unknown origin in the drinking water (polychlorinated butadienes), 2) an unwanted by-product from epoxy resin coatings in canned fish (Cyclo-di-BADGE), 3) two cyclic compounds occurring in polyamide food packaging materials and kitchen utensils, and 4) mycotoxins (from *Alternaria*). These examples from different fields of application clearly demonstrate that the results of the TTC approach are an extremely useful starting point for adequate decisions and actions (if necessary) by risk assessment and risk management in food safety.

Keywords: Cyclo-di-BADGE · *in silico* toxicology · Polychlorinated butadienes · Structural alerts · Threshold of toxicological concern

Introduction

Many of the synthetic and naturally occurring substances present in food and feed, together with their possible breakdown or reaction products, require risk assessment. In cases where no or only insufficient toxicity data are available, scientifically sound substance-specific toxicological reference values cannot be derived. In this situation, the threshold of toxicological concern (TTC) approach serves as a pragmatic tool either for priority setting or for deciding whether exposure to a substance is so low that the probability of adverse health effects is low and no further data are needed.

To understand the TTC approach in its present form, it is necessary to take a look back at its history. An earlier threshold concept, the so-called Threshold of Regulation (TOR), was introduced by the U.S. Food and Drug Administration (FDA) for indirect food additives in 1995.[1] Based on the *de minimis* principle,[2] the TOR exempts substances migrating from packaging into food at levels below a threshold value of 0.5 ppb from being listed as food additives. The TOR concept is based on a probabilistic approach to protect against untested, potentially carcinogenic substances. Carcinogenicity studies of several hundred chemical substances orally tested in rodents were analyzed. From the tumorigenic doses in rodents, doses representing an acceptable cancer risk of one in a million in humans were derived by linear extrapolation. The overall acceptable dietary exposure level of a potential carcinogenic substance was set at ≤1.5 µg/person/day. Assuming a daily intake of 1.5 kg liquid food and 1.5 kg solid food, the threshold value of 0.5 ppb in food was calculated.

Shortly after the FDA introduced the TOR, a group of scientists developed another threshold concept, the TTC approach.[3] Rather than setting a single threshold, the TTC approach further differentiates between chemical structures and presents tiered thresholds for substances with varying levels of toxicological concern. For this purpose, the Cramer decision tree established in 1978 is used which assigns substances based on their chemical structure to Cramer classes I, II, and III.[4] By applying this decision tree, Munro and colleagues classified 613 non-carcinogenic substances in the Cramer classes in 1996.[3] They analyzed the existing toxicological data of these substances and then set specific thresholds for the three Cramer classes. For each substance the most conservative no observed adverse effect level (NOAEL) was selected and all these NOAELs were plotted in three groups according to their structural class. The exposure thresholds were derived by multiplying the fifth percentile of the NOAELs of each distribution by 60 (assuming an individual weight of 60 kg) and by dividing by a safety factor 100 (factor 10 for interspecies differences and factor 10 for interindividual variability).

Two additional threshold levels were introduced into the concept by Kroes *et al*.[5,6] An exposure threshold was set at 0.15 µg/person/day for genotoxic carcinogens by applying linear extrapolation to low doses, thereby allowing the evaluation of genotoxic substances under the TTC concept. Potential genotoxic substances can be identified by genotoxic alerts. This threshold only excludes a few groups of highly potent genotoxic as well as non-genotoxic carcinogens, the so-called cohort of concern (specific substance categories see below).[6,7] Furthermore, a lower threshold was assigned to certain neurotoxic substance groups (organophosphates and carbamates) based on the conclusion that neurotoxicity was not sufficiently covered by the threshold for Cramer class III compounds (text on the scientific and historical background of the TOR and TTC concepts primarily based on ref. [8]; for more information see also ref. [9]).

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In 2012, the European Food Safety Authority (EFSA) published an opinion[9] with some refinements of the TTC approach and proposed the following human exposure thresholds: "0.15 µg/person/day for substances with a structural alert for genotoxicity, 18 µg/person/day for organophosphates and carbamates with anti-cholinesterase activity, 90 µg/person/day for Cramer Class III and Cramer Class II substances, and 1800 µg/person/day for Cramer Class I substances. For application to all groups in the population, these values should be expressed in terms of body weight, *i.e.* 0.0025, 0.3, 1.5 and 30 µg/kg bw/day, respectively. Use of the TTC approach for infants under the age of 6 months, with immature metabolic and excretory systems, should be considered on a case-by-case basis". EFSA defined a number of exclusion categories of substances for which the TTC approach would not be appropriate. The three Scientific Committees of the European Commission, SCCS, SCHER, and SCENIHR finalized an opinion on the TTC approach for human safety assessment of chemical substances focusing on cosmetics and consumer products,[10] which is essentially in line with the EFSA opinion from 2012.[9]

The TTC approach should not be applied when toxicity data allow a chemical-specific hazard assessment. According to EFSA's opinion,[9] the following categories of substances should be excluded from a TTC-based assessment: "a) high potency carcinogens (*e.g.* aflatoxin-like, azoxy- or N-nitroso-compounds, benzidines, hydrazines); b) inorganic substances; c) metals and organometallics; d) proteins; e) steroids; f) substances that are known or predicted to bioaccumulate; g) nanomaterials; h) radioactive substances; and i) mixtures of substances containing unknown chemical structures". In addition to these exclusion criteria, SCCS, SCHER, and SCENIHR recommend not to apply the TTC approach for substances displaying pharmacological effects for which no readily accessible database is available.[10]

So far, the TTC concept has been adopted in regulatory food safety management for the regulation of flavorings by the European Commission[11] and the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[12,13] In 2012, EFSA published a scientific opinion recommending the TTC approach for metabolites and degradation products of pesticides in groundwater.[14]

Over the last few years, the acceptance of the TTC approach has steadily increased among regulatory bodies, industry, and other stakeholders. Nevertheless, there is still some skepticism as to whether the TTC approach is sufficiently conservative and safe or not. The purpose of this paper is to illustrate, by means of real examples, how this tool has been applied in the risk assessment of substances in food. While the author evaluated cases 1 and 2, cases 3 and 4 were evaluated by the German Federal Institute for Risk Assessment (BfR) and by EFSA, respectively. Based on these examples and the experiences made, the applicability of this approach, certain critical steps in the evaluation process, and its impact on risk assessment and risk management in food safety are discussed.

Case 1: Polychlorinated Butadienes in Drinking Water

Tetrachlorinated butadienes (TetraCBDs), pentachlorinated butadienes (PentaCBDs) and hexachlorobutadiene (HexaCBD) were detected in groundwater wells of a drinking water supplier near Basel (Switzerland) up to levels of 157 ng/L (sum value), 15 ng/L (sum value), and <50 ng/L, respectively, in 2006. TetraCBDs and PentaCBDs are assumed to be environmental degradation products of HexaCBD (Fig. 1). Due to insufficient toxicity data, neither health-based guideline values nor maximum contaminant levels had been established for the TetraCBDs and PentaCBDs at that time.

By contrast, the toxicological profile of HexaCBD was better characterized. In a 2-year feeding study in rats with doses of 0, 0.2, 2 and 20 mg/kg bw/day, the kidney was the primary target organ. Effects included a treatment-related increase in relative and absolute kidney weights in males at 20 mg/kg bw/day, an increased incidence of multifocal or disseminated renal tubular epithelial hyperplasia in rats at 20 mg/kg bw/day and possibly at 2 mg/kg bw/day and focal adenomatous proliferation of renal tubular epithelial cells in some males at 20 mg/kg bw/day and in some females at 20 and 2 mg/kg bw/day. The NOAEL was found to be 0.2 mg/kg bw/day. In the same study, 20 mg/kg bw/day in the diet for two years caused renal tubular adenomas and adenocarcinomas, but these were not observed at 2 and 0.2 mg/kg bw/day.[15] According to the International Agency for Research on Cancer (IARC), no data were available on the genotoxic effects in humans or in rodents *in vivo*, there was weak evidence for mutagenicity in mammalian cells *in vitro*, and the findings for mutagenicity in bacteria were equivocal.[16] In 2004, the WHO derived a tolerable daily intake (TDI) of 0.2 µg/kg bw/day and a drinking-water guideline value of 0.6 µg/L for HexaCBD. This TDI was calculated with an uncertainty factor of 1000 (100 for inter- and intraspecies variation and 10 for limited evidence of carcinogenicity and the genotoxicity of some metabolites, in particular glutathione conjugation products) in relation to the NOAEL of 0.2 mg/kg bw/day. This resulted in a guideline value of 0.6 µg/L, based on an allocation of 10% of the TDI to drinking water.[17]

Since there is evidence that incompletely halogenated compounds might be more toxic than completely halogenated compounds (*e.g.* polychlorinated dioxins and biphenyls, polybrominated diphenylethers), the direct reference to the TDI of HexaCBD for TetraCBDs and PentaCBDs may not be advisable. In this situation, the TTC approach was applied as described for the evaluation of contaminants in drinking water.[18] TetraCBDs and PentaCBDs show structural alerts for genotoxicity. Based on the TTC for substances with structural alerts for genotoxicity that are not in the cohort of concern and an allocation of 100% of this TTC to drinking water, a target value of 75 ng/L was set (sum value for TetraCBDs and PentaCBDs).[19] Based on this evaluation, the Official Food Control Authority of the Canton Basel-Land issued a decision to the drinking water supplier in 2007.

Later, the genotoxicity of TetraCBDs and PentaCBDs was examined *in vitro* using the Ames test and the chromosome aberration test. All the TetraCBDs and

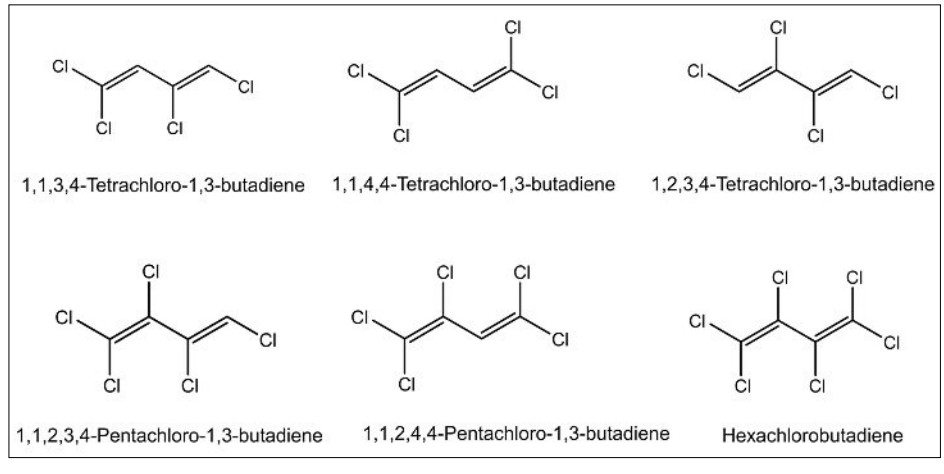

Fig. 1. Chemical structures of polychlorinated butadienes.

PentaCBDs as well as HexaCBD, were clastogenic in the chromosome aberration test. In addition, 1,1,3,4-TetraCBD and 1,2,3,4-TetraCBD were positive in the Ames test, while the other polychlorinated butadienes including HexaCBD, were negative.[20] These genotoxicity test results for TetraCBDs and PentaCBDs gave further support to the target value of 75 ng/L in drinking water (sum value of both substance groups). The Review Committee of the Stockholm Convention prepared a draft risk profile for HexaCBD in which reference was also made to these *in vitro* genotoxicity test results.[21]

Risk reduction measures had been implemented by the drinking water suppliers in Basel in April 2008 to reduce the water concentrations of polychlorinated butadienes. The entire drinking water of Basel is filtered through granular activated carbon (GAC) resulting in concentrations of polychlorinated butadienes below the corresponding detection limits (10 ng/L for 1,1,2,3-TetraCBD, 1,1,2,3,4-PentaCBD and HexaCBD; 20 ng/L for the other measured TetraCBDs and PentaCBDs).[22] The measured concentrations of TetraCBDs and PentaCBDs were clearly below the TTC-based target value of 75 ng/L (sum value of both substance groups). At one drinking water supply plant site, an activated carbon filter system with a capacity of 75'000 m^3 drinking water per day was built and put into service in December 2013. The decision issued by the Official Food Control Authority of the Canton Basel-Land in 2007 was annulled in January 2014.[23]

Case 2: Cyclo-di-BADGE in Canned Fish

Cyclo-di-BADGE (also referred to as cyclo-diBA) is a cyclic compound formed from bisphenol A (BPA) and bisphenol A diglycidyl ether (BADGE) (Fig. 2). It is a minor by-product from the manufacture of epoxy resins based on BPA for can coatings. Cyclo-di-BADGE was determined in canned food, including fish, meat and soup, in two campaigns by the Official Food Control Authority of the Canton of Zurich in 2010 and 2012. In 2012, Cyclo-di-BADGE was detectable (>25 µg/kg) in 13 of 44 fish-in-oil products. The average concentration of these 13 samples was 807 µg/kg and the maximum reached 2640 µg/kg.[24]

No experimental toxicity data on Cyclo-di-BADGE were available except for its cytotoxicity. In a liver cell line the IC_{50}-value was in the range of 10 µg/ml, which is similar to the values for BADGE and its derivatives. This *in vitro* test suggested that Cyclo-di-BADGE is bioavailable at the cellular level and may contribute approximately 18% to the total toxicity of the migrates from epoxy resin coatings.[25] In the absence of experimental toxicity data, no TDI can be derived. Cyclo-di-BADGE is a ring system and seems to be metabolically stable and with low reactivity, whereas BADGE is linear and highly reactive due to its two epoxide groups. Cyclo-di-BADGE does not fulfill two of Lipinski's rules[26] as its molecular weight is >500 D (569 D) and its logKow is >5 (7.56) and is therefore predicted to have a low oral bioavailability. By contrast, linear and non-linear regression models on intestinal absorption[27,28] predict a passive intestinal absorption of more than 50% for Cyclo-di-BADGE.[29]

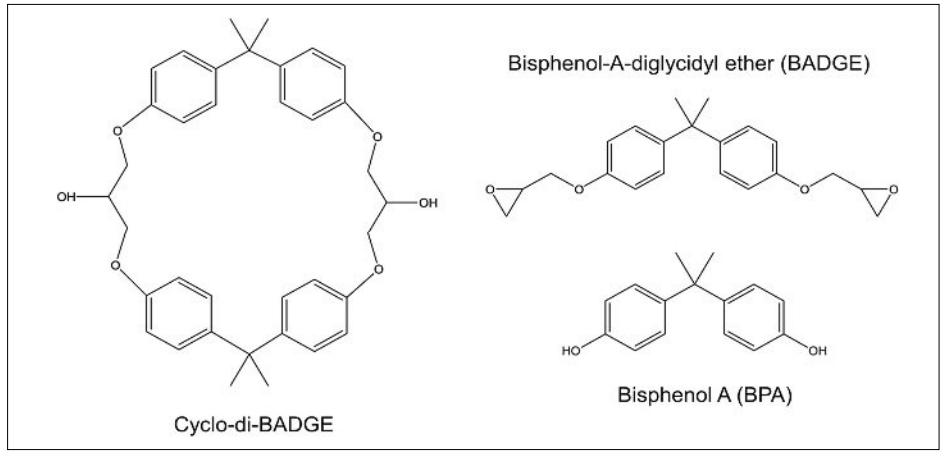

Fig. 2. Chemical structures of Cyclo-di-BADGE, BADGE and bisphenol A.

Cyclo-di-BADGE is metabolized into cyclic and acyclic metabolites. A simple read-across from BADGE to Cyclo-di-BADGE is not advisable, since the structures of the two substances are not strongly related. There is no indication of any genotoxicity of Cyclo-di-BADGE based on the structure-activity relationship (SAR) assessment. It can be assumed that acyclic Cyclo-di-BADGE metabolites, which are structurally related to BADGE derivatives, are likewise as BADGE itself not genotoxic and not carcinogenic *in vivo*.[30] *In silico* simulations predict binding affinities of Cyclo-di-BADGE to several nuclear receptors in the low µM to high nM range indicating a potential endocrine-disrupting potency.[24,31] The human relevance of these binding predictions is not yet clear and needs further investigation. On the assumption that Cyclo-di-BADGE and its metabolites are not genotoxic, it can be assigned to Cramer class III corresponding to 1.5 µg/kg bw/day or 90 µg/person/day, assuming a 60 kg body weight. In comparison, a TDI for BADGE and its derivatives BADGE.H_2O and BADGE.$2H_2O$ of 0.15 mg/kg bw/day was derived,[30] and a specific migration limit (SML) of 9 mg/kg introduced by EU Regulation 1985/2005.[32] Recently, EFSA proposed for BPA a reduction of the current TDI of 50 µg/kg bw/day[33] to a temporary TDI (t-TDI) of 5 µg/kg bw/day.[34] The current SML for BPA in food contact materials is at 0.6 mg/kg.[35]

To clarify the oral bioavailability of Cyclo-di-BADGE, the food packaging industry and epoxy resin producers were advised by relevant food safety and enforcement authorities to conduct a toxicokinetic study. Another unresolved issue was the binding of Cyclo-di-BADGE to nuclear receptors like the estrogen receptor (ER) that could have been examined by *in vitro* testing (*e.g.* ER-CALUX). It has been shown to be difficult to synthesize and/or purify Cyclo-di-BADGE in sufficient amounts for these tests. To our knowledge, neither experiment has been performed to date.

Cyclo-di-BADGE exposure from canned fish was estimated by different methods. The criterion set by the enforcement authorities for evaluating the conformity of a given product was that the consumers must be confident that they can eat as much of a given product as they like (unless it is labeled otherwise). For the same reason, brand loyalty was assumed. A scenario, proven in (at least) one case to be true, was used, namely a worker consuming 200 g of fish, corresponding to one can at lunch every working day. This corresponds to an average consumption of 130 g/day when five weeks of vacation are taken into account. The tolerable intake of 50 µg/day for non-genotoxic substances from the EFSA note for guidance[36] in 130 g fish corresponds to a concentration of 384 µg/kg; the 90 µg/day from the Cramer class III corresponds to a concentration of 692 µg/kg. Cyclo-di-BADGE exposure from other sources was disregarded.[24]

Measures were taken to implement this restriction in the Swiss market at the beginning of 2013. In the meantime, the Swiss national RASFF contact point delivered a notification to the European Commission for a specific canned fish from Morocco that had a concentration of 1900 µg/kg Cyclo-di-BADGE [37].

Case 3: Cyclic Polyamide Dimers in Food Contact Materials

The German Federal Institute for Risk Assessment (BfR) evaluated the toxicity of two cyclic polyamide dimers which are by-products in the polymerization process (Fig. 3).[38] One cyclic dimer originated from an artificial casing used for sausages and made of polyamide 6 (PA6). The other cyclic dimer was found in kitchen utensils made of polyamide 66 (PA66). Based on limited measurement data, an exposure of 0.2 to 0.4 µg/kg bw/day was estimated for cyclic PA6 dimer and an approximate exposure of 25 µg/kg bw/day for cyclic PA66 dimer and should be regarded as rudimentary estimates. Since no toxicological data were available for either substance, BfR applied the TTC approach. No structural alerts for genotoxicity were identified for either substance. Both substances were assigned to Cramer class III. As a result, it was found that the exposure did not exceed TTC for the cyclic dimer of PA6, but exceeded the TTC for the cyclic dimer of PA66 by several times. BfR recognized that more detailed analysis of the health risks for cyclic dimer of PA66 is required. BfR has begun to analyze the hydrolysis behavior of different oligomers formed from polyamide.[38,39]

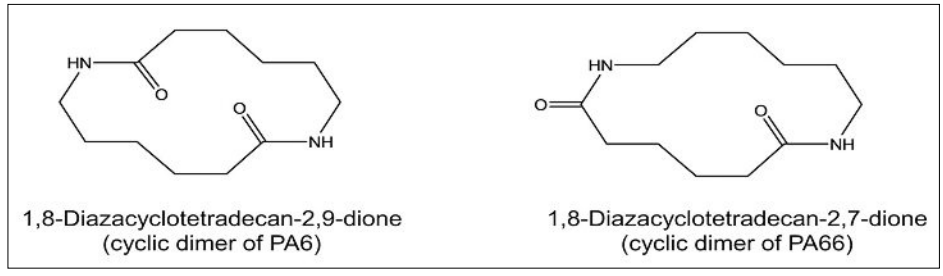

Fig. 3. Chemical structures of cyclic polyamide dimers.

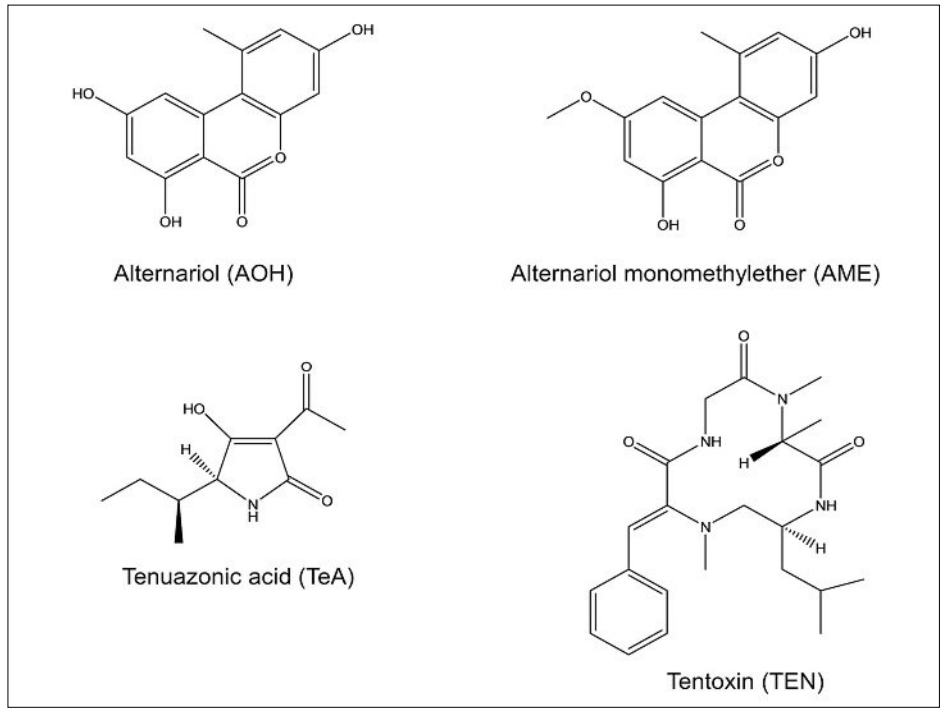

Fig. 4. Chemical structures of *Alternaria* toxins.

Case 4: *Alternaria* Toxins in Crops, Vegetables and Fruits

Alternaria toxins, including alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), and tentoxin (TEN), in food and feed were reviewed by EFSA in 2011[40] (Fig. 4). These toxins are present in grains, sunflower seeds and sunflower oil, tomatoes, fruits, beer and wine. In addition to causing plant diseases, some of these toxins are genotoxic *in vitro* and/or fetotoxic in rats. However, since little or no relevant toxicity data are available on *Alternaria* toxins, the chemical structure is known, and dietary exposure data exist for some of these toxins, EFSA used the TTC approach to assess the relative level of concern for dietary exposure of humans to these mycotoxins. *In vitro* data provided clear evidence of the genotoxicity of *Alternaria* toxins such as AME and AOH. There were no data on *in vivo* genotoxicity, and no convincing data on the carcinogenicity of these compounds. Because it is essential for the application of the TTC approach to have suitably conservative exposure estimates that take into account the high exposure scenarios, EFSA based the assessment on the mean and 95th percentile chronic dietary exposure to AOH, AME, TeA and TEN for the adult population using the lower bound (LB) and upper bound (UB). The database for other toxins was too weak for the application of the TTC. Regarding *Alternaria* toxins, there was experimental evidence of *in vitro* genotoxicity of AOH and AME in bacteria and mammalian cells. For such compounds, the TTC decision tree asks whether the estimated exposure exceeds the value of 2.5 ng/kg bw/day (0.15 µg/person/day). In the adult population, the mean chronic dietary exposure to AOH across dietary surveys ranged from 1.9 to 39 ng/kg bw/day (range represents the minimum LB to maximum UB in the various countries). The 95th percentile dietary exposure ranged from 5.9 to 82 ng/kg bw/day. These values exceeded the TTC, indicating the need for additional toxicity data to assess the potential health risk. Although the exposure estimates for AME were lower compared to those obtained for AOH (mean chronic dietary exposure ranged from 0.8 to 4.7 ng/kg bw/day; 95th percentile dietary exposure ranged from 3.1 to 15 ng/kg bw/day), both the values for high consumers and the UB values for average consumers also exceeded the TTC, indicating a need for additional compound specific toxicity data. Regarding TeA and TEN, for which there was no evidence of genotoxicity in bacteria or clear structural alerts that raise concern for potential genotoxicity, the level defined by the TTC decision tree is 1.5 µg/kg bw/day (90 µg/person/day) for compounds in Cramer class III. For TEN, the mean chronic dietary exposure ranged from 36 to 141 ng/kg bw/day and the 95th percentile dietary exposure ranged from 86 to 362 ng/kg bw/day, indicating that TEN is unlikely to be of a human health concern. Estimates of chronic dietary exposure to TeA (≤ 13 ng/kg bw/day) were much lower than the TTC value and TeA was therefore considered unlikely to be a human health concern. EFSA recommended toxicity testing for AOH and AME to enable their risk assessment. In addition EFSA recommended genotoxicity data for most of the *Alternaria* toxins (text adopted from ref. [40]).

Discussion

As analytical chemistry techniques continue to improve, more challenges can

be expected in the discovery and evaluation of the safety of very low levels of substances of unknown toxicity in food. In this situation, the TTC approach is presently the method of choice. The TTC approach offers the potential to greatly simplify the assessment and prioritization of chemical risks. Although the set of TTC values has a misleading simplicity, each value is based on widely reviewed rigorous scientific principles applied to well-regarded toxicity data. It is a low-cost and rapid method. It can help risk assessors and risk managers in the regulatory authorities and the food industry by allowing them to prioritize testing and allocation of resources to those situations where the need (*i.e.* the potential for harm to health) is greatest.[41]

It is important that the rigor, and thus the legitimacy, of the approach be maintained. In order to get transparent, non-arbitrary results, computer tools should be applied to go through the decision tree. After using of the exclusion criteria, the check for structural alerts for genotoxicity should be done by several software programs, *e.g.* Derek Nexus (Lhasa) and Benigni-Bossa rulebase (implemented in the Toxtree software; by IDEAconsult) in addition to expert judgment.[42] For the prediction of metabolites from parent compounds, software tools like Meteor Nexus (Lhasa) have proven to be very useful. Assignment to Cramer structural classes can be performed by the software ToxTree (IDEAconsult) or the OECD QSAR Toolbox (Oasis-lmc). Nevertheless, the decision tree and the TTC principle are designed as structured aids to expert judgment and should be applied only by those who have a sufficient understanding of toxicology principles and chemical risk assessment.[6]

When using the TTC approach, any available information on the compound and background information in the field of application should be considered. In addition to the TTC approach, *in silico* hazard profiling (with respect to possible metabolites of the parent compounds, binding to specific target proteins like the human estrogen receptor (ER) and androgen receptor (AR), specific organ toxicity, oral bioavailability, etc.) is strongly recommended to identify the possible most critical targets, processes and pathways.[43–45]

As illustrated in the described cases above, the outcome of the TTC approach supplemented with additional *in silico* predictions offers various options for targeted actions and decisions, *e.g.*:

- prioritization of hazards and risks (all cases);
- identification of toxicity data gaps (all cases);
- recommendations for appropriate tests to fill these data gaps (see case 1: polychlorinated butadienes; case 2: Cyclo-di-BADGE; case 3: cyclic polyamide dimers, case 4: *Alternaria* toxins);
- fixing tolerable drinking water concentrations (case 1: polychlorinated butadienes);
- determination of provisional intervention values for specific migration of substances from food packaging into food (case 2: Cyclo-di-BADGE);
- requesting risk reduction measures, as an additional drinking water purification step (case 1: polychlorinated butadienes).

More guidance on the criteria should be elaborated when read-across and/or other (quantitative) structure–activity relationship [(Q)SAR] methods should be used which might be more reliable than the TTC approach under certain conditions.

EFSA recommends that "if there are data showing that a substance has endocrine activity, but the relevance of the observation for humans is unclear, then these data should be taken into consideration, case-by-case, in deciding whether or not to apply the TTC approach. If there are data showing that a substance has endocrine-mediated adverse effects, then, as would be the case for adverse data on any other endpoint, the risk assessment should be based on the data".[9] This recommendation might lead to the challenging situation that the evaluator should know whether a substance of unknown toxicity has a potential for endocrine activity or not. *In silico* modelling of binding to several different hormone receptors (*e.g.* ER, AR, *etc.*)[44] might be an option to check for this potential as demonstrated in case 2 above (Cyclo-di-BADGE).[24,31]

"When the TTC approach is used, it is important for both risk assessors and risk managers to keep in mind that it is a probability-based screening tool and, like other risk assessment approaches, does not offer complete certainty. The various TTC values are based on frequency distributions and are not based on the lowest value in each of the distributions but on a point close to the lowest value. Thus, when using either the cancer or non-cancer TTC values, there is a chance that a substance with an exposure below the relevant TTC value may still pose a potential risk".[9] For substances in the Cramer structural classes, as well as for the organophosphates and carbamates, this probability is estimated to lie between 0 and 5%.[9]

The applicability and acceptance of the TTC approach depends on the usual data requirements in the field of application like food additives, food contact materials, flavorings, food contaminants, natural toxins, and plant protection products where great differences exist between the different fields.[9] It should not be used for regulated chemical substances deliberately added to food, where legislation requires the submission of a full toxicological package. However, the TTC approach might be a useful tool for the toxicological evaluation of degradation products, metabolites or by-products (*e.g.* metabolites of plant protection products, non-intentionally added substances in food contact materials). If it can be demonstrated that the TTC levels are not exceeded it could pre-empt for further unnecessary toxicity testing. Such 'rules' for the applicability of the TTC approach have to be elaborated separately for each field of application.

Consideration has to be given to what percentage of the TTC is allocated to the exposure from the matrix in which the substance had been detected (*e.g.* drinking water, packaged food, *etc.*) and whether other potential (as yet still unknown) exposure pathways also have to be taken into account.

It is essential that the limits of detection for potentially toxic substances in a complex mixture are lower than the corresponding TTC levels. The TTC-derived target value for substances with structural alerts for genotoxicity is at 2.5 ng/kg bw/day. The standard scenario for the consumption of 1 kg packaged food of a 60 kg person leads to a limit of detection to be reached of 0.15 µg/kg. This is an extremely low level and in many cases the limit of detection of substances will be higher. Further research and development is needed to fill this obvious gap between the concept requirement and the technical feasibility. For this purpose it was postulated to test the extracts with highly sensitive *in vitro* genotoxicity bioassays.[46–48] This might allow to rule out genotoxic effects (in the case of negative results) or to concentrate, analyze, and identify the genotoxic substances in these extracts (in the case of positive results).

The TTC approach offers the major advantage of allowing a threshold hazard value to be derived that serves as a starting point for taking decisions. There is a strong signal to all stakeholders that in urgent cases a preliminary decision can be taken even if no or only insufficient toxicity data are available. Generating additional toxicity tests can take several months to years.

In Switzerland, a guidance document from the responsible federal authorities in the field of ground and drinking water instructs risk managers how to proceed when contaminants in ground and drinking water are identified and how the possible health risks should be evaluated.[49] According to the document, the TTC approach should be applied when no or only insufficient toxicity data are available. Two maximum values (in the Swiss Regulation so-called 'tolerance values') for substances of unknown toxicity in drinking water

were set based on the TTC approach and entered into force in 2014.[50] The value for substances of unknown toxicity with structural alerts for genotoxicity was set at 100 ng/L (rounded value based on the TTC level for substances with structural alerts for genotoxicity) and for substances of unknown toxicity without structural alerts for genotoxicity at 10 µg/L (rounded value from the TTC level for organophosphates and carbamates).

Several refinements to the TTC approach have been proposed[51–54] which have not been taken into account in the EFSA opinion from 2012.[9] The subsequent updating of the approach under the auspices of an independent international organization will facilitate broad consensus and high level of acceptance.

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