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FORUM

The Threshold of Toxicological Concern Concept in Risk Assessment

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The concept that "safe levels of exposure" for humans can be identified for individual chemicals is central to the risk assessment of compounds with known toxicological profiles. The Threshold of Toxicological Concern (TTC) is a concept that refers to the establishment of a level of exposure for all chemicals, whether or not there are chemical-specific toxicity data, below which there would be no appreciable risk to human health. The concept proposes that a low level of exposure with a negligible risk can be identified for many chemicals, including those of unknown toxicity, based on knowledge of their chemical structures. The present paper aims to describe the history of the TTC principle, its use to date, its potential future applications and the incorporation of the TTC principle in the Risk Assessment paradigm.

Key Words: **Threshold of Toxicological Concern, Risk Assessment.**

The concept that ''safe levels of exposure'' for humans can be identified for individual chemicals is central to the risk assessment of compounds with known toxicological profiles. The traditional approach to risk assessment is separated into hazard identification, hazard characterization, exposure assessment, and risk characterization, and data from toxicity studies on the specific chemical under evaluation are necessary for hazard identification and characterization.

In previous publications (Kroes *et al.*, 2000, 2004) the TTC principle was examined for general toxicity end points as well as for specific end points including carcinogenicity, teratogenicity, reproductive toxicity, and immunotoxicity. In addition, consideration was given to structural alerts for high potency carcinogens, endocrine disrupting chemicals, and food allergens and to the potential for metabolism and accumulation. A decision-tree approach, which incorporates a tiered approach for applying the TTC principle, has been proposed as a preliminary step in food safety evaluation (Kroes *et al.*, 2004).

The present paper aims to describe the history of the TTC principle, its use to date, its potential future applications and the incorporation of the TTC principle in the Risk Assessment paradigm.

HISTORY

The TTC concept was the scientific basis of the U.S. Food and Drug Administration Threshold of Regulation for indirect food additives (Federal Register, 1993; U.S. FDA, 1982, 1993). The concept of a TTC evolved from the review Munro (1990) of the Threshold of Regulation as applied by the Food and Drug Administration in the United States for food contact chemicals.

Further developments (Munro *et al.*, 1996, 1999) were based on an extensive analysis of available chronic toxicity data of substances, which were divided into three chemical classes on the basis of their structure using the Cramer (Cramer *et al.*, 1978) decision tree. In principle, the TTC principle can be applied for low concentrations in food of chemicals that lack toxicity data, provided that their structure is known and that there exist valid estimates of intake. The concept was adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to evaluate flavoring substances (JECFA, 1993, 1995, 1999; Munro *et al.*, 1999), and since 1997 a tiered system, incorporating different TTCs for the Cramer *et al.* (1978) structural classes, has been used for the safety evaluation of over 1250 flavoring substances (Renwick, 2004). It was also used by the former EC Scientific Committee on Food and now is used by the European Food Safety Authority to evaluate flavoring substances (EFSA, 2004).

Subsequent work by an ILSI Europe Expert Group (Kroes *et al.*, 2000, 2004) extended the approach adopted by the JECFA to cover compounds with certain structural alerts for possible activity as genotoxic carcinogens. In the TTC decision tree proposed by Kroes *et al.* (2004), proteins, heavy metals, and polyhalogenated dibenzo-*p*-dioxins and related compounds were excluded because the databases used to derive the TTC values did not include toxicity data on proteins and heavy

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metals such as cadmium, lead, and mercury, and because the uncertainty factors used to derive the TTC values would not allow for extreme species differences in elimination seen with polyhalogenated-dibenzo-*p*-dioxins, polyhalogenated-dibenzofurans, and polyhalogenated-biphenyls. In addition, there is a well-established risk assessment approach for such polyhalogenated compounds based on toxicity equivalence factors, and the TTC approach would not be required or appropriate.

The use of the decision tree provides a systematic structured approach for consistent application of the TTC principle to chemicals in food at low exposure. When chemicals are assessed using the TTC approach, a review of prior knowledge and use should always be performed preceding application of the decision tree.

The first step in the decision tree approach is the identification and evaluation of possible structural alerts for genotoxicity and high-potency carcinogenicity. This step excludes high-potency genotoxic substances (aflatoxin-like compounds, N-nitroso-compounds, azoxy-compounds) from consideration and applies a generic threshold for all other structural alerts of 0.15 µg/person/day (0.0025 µg/kg bw/day) (Fig. 1), which is a highly conservative approach [see Kroes *et al.* (2004) for the derivation of this value]. The next step considers nongenotoxic substances (compounds lacking structural alerts) in a sequence of steps related to their structure and the estimates of intake. For organic phosphates a TTC of 18 µg/kg bw/day was proposed, whereas for substances belonging to Cramer *et al.* (1978) structural classes III, II, and I, TTC levels of 90, 540, and 1800 µg/person/day were proposed (1.5, 9, and 30 µg/kg bw/day, see Fig. 2). For more details on the assignment of substances to structural classes I, II, and III, see Cramer *et al.* (1978) and Kroes *et al.* (2004). The thresholds of toxicological concern for Cramer *et al.* (1978) structural classes were derived by Munro *et al.* (1996, 1999), based on an analysis of data from chronic toxicity studies on 137, 28, and 448 compounds in classes I, II, and III, respectively. The no observed effect level (NOEL) from a chronic toxicity study in rodents is usually divided by an uncertainty factor of 100 (WHO, 1987) to derive an acceptable daily intake. The cumulative distributions of NOELs for the compounds in each Cramer *et al.* (1978) structural class were plotted, and a log-normal distribution was fitted (Munro *et al.*, 1996). The fifth percentile values for the class I, II, and III NOEL distributions were calculated to be 3.0, 0.91, and 0.15 mg/kg body weight/day. Therefore, there is a 95% probability that the NOEL from a chronic animal bioassay on an unstudied compound would be below the relevant fifth percentile value. The fifth percentile NOEL values were converted to corresponding human intakes by dividing by the usual 100-fold uncertainty factor and then multiplied by 60 to scale to the adult human body weight. These analyses gave thresholds of toxicological concern of 1800, 540, and 90 µg per person per day for structural classes I, II, and III.

APPLICATIONS OTHER THAN FOOD

The TTC principle may be more broadly applicable than simply to chemicals in food and has potential value in the assessment of risks in other exposure scenarios.

For example, there is an ongoing concern that humans are exposed to a diverse array of chemicals, and there exists a demand to evaluate large numbers of chemicals. At the same time, there exists a strong pressure to reduce our reliance on

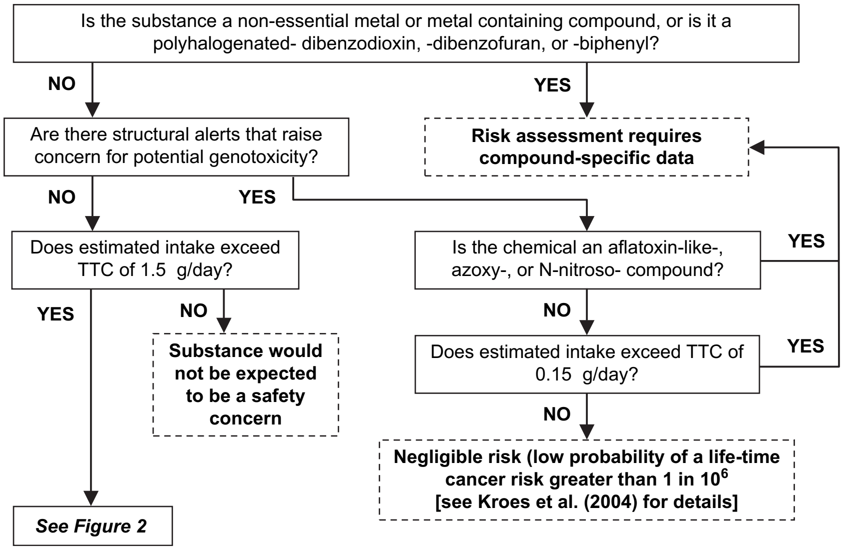

FIG. 1. Consideration of potential genotoxicity using the TTC approach developed by Kroes *et al.* (2004). Estimated intake refers to chronic oral exposure, since the TTC values are derived from a database were chronic oral exposure as evaluated. The required compound specific data depend on the predicted duration and extend/magnitude of the population exposed.

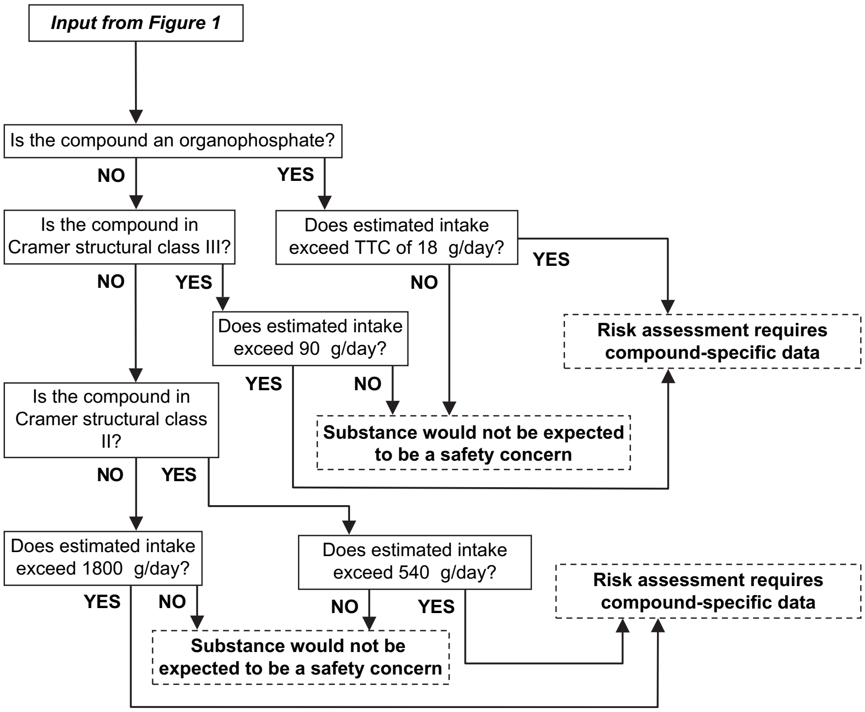

FIG. 2. Consideration of potential nongenotoxic effects using the TTC approach developed by Kroes *et al.* (2004). Estimated intake refers to chronic oral exposure, since the TTC values are derived from a database were chronic oral exposure as evaluated. The required compound specific data depend on the predicted duration and extend/magnitude of the population exposed.

animal experimentation and to rely increasingly on *in vitro* and in silico data (see the recent Report of the Royal Commission on Environmental Pollution in the UK; Royal Commission, 2003).

Evaluation and prioritization of chemicals should not be based solely on hazard identification; it is imperative that potential exposure is taken into account and that predictive tools are used to relate their chemical structure to their potential toxicity. Without a scientifically based predictive approach, an unnecessarily large number of chemicals have to be investigated at high costs with a considerable use of experimental animals.

The application of the TTC principle provides a method to assess the potential risk to human health based on the following:

- already available data (including chemical structure plus any *in vitro, in vivo* and/or in silico information),
- information on potential exposure and intake,
- the predicted *in vivo* toxicity based on chronic toxicity data for compounds that have similar chemical structures.

The TTC principle is used by the European Medicines Evaluation Agency (EMEA) to assess genotoxic impurities in pharmaceutical preparations (EMEA, 2004). The TTC principle has also been endorsed by the WHO International Program on Chemical Safety for the risk assessment of chemicals (IPCS, 1998) and by the EU Scientific Committee on Toxicology, Ecotoxicology and the Environment (Bridges, 2003).

Application of the TTC principle could be extended to other categories of chemical use, such as constituents of cosmetics and consumer products, as well as trace impurities present in regulated compounds/substances, such as food additives, pesticides, and pharmaceuticals. To extend the TTC method to nonoral exposures, appropriate methodologies should be developed to allow for route-to-route extrapolation and to assess combined multiroute exposure. Application of the TTC approach could be used to indicate analytical data needs, based on the levels of an impurity in a product that would result in an intake that was not a health concern (as for example for indirect food additives, as it is used in the United States).

In addition, since the principle is based on safety evaluations relating to daily intake throughout life, the approach could be used to define exposure levels that would be of concern for contaminants and naturally occurring plant constituents, or as a science-based alternative to define concentrations of chemicals in nature as part of the application of the precautionary principle.

BENEFITS OF THE USE OF THE TTC APPROACH

The establishment of more widely accepted TTC values would benefit consumers, industry, and regulators. By avoiding unnecessary extensive toxicity testing and safety evaluations when human intakes are below the relevant TTC value, it will allow limited resources of time, animal use, cost, and expertise to be devoted to the testing and evaluation of those substances with greater potential to pose risks to human health. In consequence, its application will contribute to a considerable reduction in the number of animals used for safety testing.

THE USE OF THE TTC PRINCIPLE IN RISK ASSESSMENT

Risk characterization is the important stage of risk assessment that brings together hazard characterization and exposure assessment. In the hazard identification and hazard characterization steps, chemicals are evaluated based on the available chemical-specific knowledge leading to a qualitative assessment. In safety evaluation it provides a quantitative estimate of an intake that would not give a significant risk of adverse effects in humans. In the absence of chemical-specific data, risk assessors may rely on structurally related substances to assess qualitatively the nature of potential hazards but seldom quantitatively. Application of the TTC principle as a preliminary risk characterization approach for comparing potential exposure and the TTC threshold value, based on the substance's structure, could avoid the need for further detailed assessment of chemicals to which humans are exposed at low levels.

The place of the TTC principle in the established risk assessment paradigm is shown in Figure 3. It is clear from this scheme that the assessment of (chronic) exposure is an extremely important step and should be carried out in the most appropriate way in order to produce sound exposure/intake estimates. If such figures are unavailable, science-based methods (Kroes *et al.*, 2002) should be used to estimate potential exposure. As described by Kroes *et al.* (2002), a tiered approach can be helpful to optimize the use of available resources. If relatively crude tools—designed to provide a "worst case" estimate—do not predict an intake above the relevant TTC level, the use of more sophisticated tools may not be necessary.

In relation to the application of the TTC principle, it is important that the chronic exposure evaluated is relevant to the exposed population; in certain cases combined multiroute exposures should be assessed, whereas in other cases, when a substance is used uniquely for a specific purpose (e.g., a particular food or cosmetic product) the exposure/intake related to that purpose should be assessed.

The paper by Kroes *et al.* (2004) considered the relationship between the TTC, which is the chronic intake per person per day, and the possible distribution of the chemical in the food supply. Taking a TTC of 90 μg per person per day as an example (e.g., a nongenotoxic, non-organophosphate belonging to structural class III) for a compound that occurs uniformly in the whole diet (1.5 kg of food and 1.5 kg of beverages), this intake is reached by concentrations in the diet of 30 μg/kg of diet. However, in cases where a given compound will not be present in the whole diet but only in a specific product, the human total exposure to this compound is determined by its concentration in the product and the amount of the product that is actually ingested daily by consumers of the product. When the compound is present only in beverages (e.g., 1.5 kg fluids) but does not occur in food, the above-mentioned TTC is equivalent to a concentration of 60 μg/kg of beverage. When the only route of exposure is via ingestion of a single food product, which is consumed in daily amounts of 100 g, the TTC of 90 μg per person per day would be reached by concentrations of 900 μg/kg of the compound in that food.

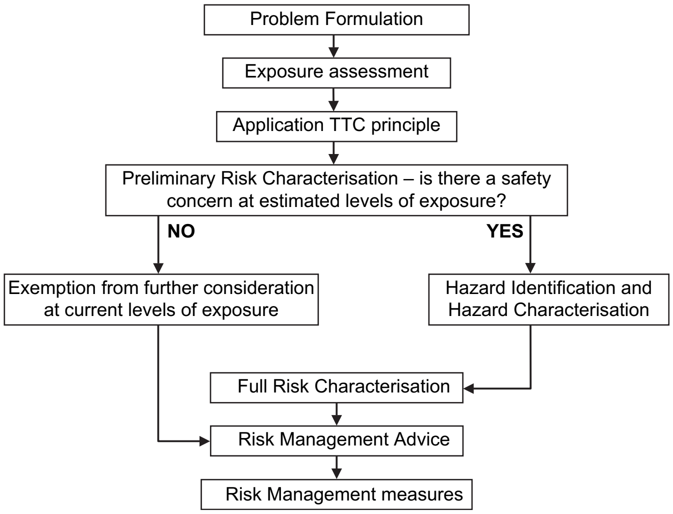

FIG. 3. Application of the TTC principle in the Risk Assessment Paradigm.

If the TTC is applied to an impurity in an approved food chemical, such as an additive with a numerical acceptable daily intake (ADI), the TTC of 90 μg per person per day (or 1.5 μg per kg body weight per day) would give a level of concern for any impurity that was present at more than [(1.5/ADI in μg) × 100]%. For example, if the ADI were 10 mg/kg body weight/day, then there would not be a safety concern for impurities present at 0.015% or less, providing that the impurity did not have structural alerts for genotoxicity and was not an organophosphate compound.

In certain cases it may be necessary to consider combining the exposure to related substances, which are thought to possess a common mechanism of action, such as allyl esters used as flavoring substances. The JECFA (JECFA, 1995, 1999) evaluates structurally related flavoring substances in groups, conducts individual assessments using the TTC approach on each compound, and then considers the safety of the group as a whole under the unlikely condition that all members are consumed simultaneously. Simple addition of the intakes will not allow for differences in potency or interactions, and will assume that the risk from each compound, based on its structural characteristics, is not altered by the presence of the other compounds. In addition, relevant exposures from sources other than food may need to be taken into account.

When dealing with complex mixtures of diverse chemicals, assessment using the TTC approach should focus on the exposure to a ‘‘marker’’ compound or major compound which represents a high proportion of the mixture and is in the highest Cramer class of the known constituents of the mixture.

An appropriate total human exposure estimate is necessary for applying the TTC principle. Because the TTC values are expressed in terms of µg per person per day, special consideration should be given to products designed for specific groups that do not have a body mass of 60 kilograms, such as children, and both the intake estimates and the TTC may need to be related to body weight.

CONCLUSION

The application of the TTC principle is recommended for substances to which humans are exposed at low levels. The approach originated in relation to packaging migrants, was refined for application to flavoring substances, and should now be developed further to allow a wider application to the vast array of low-molecular-weight compounds that are present in humans environment in trace amounts, either naturally or as a result of human activities.

The TTC approach is a preliminary risk characterization step introduced at the beginning of the risk assessment paradigm that will prevent extensive and expensive evaluation and assessment of substances used in or occurring at low levels in the human food and environment, or substances used for specific purposes at low concentrations.

Application of the principle can easily be incorporated into the risk assessment paradigm. Application will stress the importance of an appropriate exposure assessment using the best methodologies in a tiered way. Appropriate route-to-route extrapolation may be necessary for nonfood chemicals, and it is important that methodologies currently used should further be refined.

The application of the TTC approach provides a method that can result in timely advice to risk managers on the possible risks associated with trace chemical constituents humans are exposed to, without the need for chemical-specific data on toxicity and with only simple data on intake.

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