Threshold of Toxicological Concern (TTC)

Q&A Document prepared by the ACC LRI

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Introduction

With increasing interest in non-animal alternatives and new approach methodologies (NAMs), a number of researchers in academia, government, and the private sector have increased focus on the potential to use the Threshold of Toxicological Concern (TTC) in tiered risk-based applications for commodity chemicals. Traditionally, the TTC has been used in food safety and consumer product safety evaluations for chemicals with low exposure potentials. However, in cases where the scientific confidence of the TTC approach is appropriate for a given decision context, the TTC can be applied more widely. This Q&A document discusses and illustrates some of these instances. It is intended to provide ACC members a technical summary of the TTC, but is not intended to be a comprehensive scientific review of the TTC. References and hyperlinks are provided for those interested in accessing the primary literature and recent detailed review articles. It is envisioned that this document will be evergreen and will be updated on a periodic basis as new information on the TTC is developed, as new or improved applications of the TTC become available and as new questions on the TTC arise.

The TTC has considerable promise to be an important component of TSCA and product stewardship. Provisions of the amendments to TSCA that were enacted in 2016 specifically require EPA to "reduce and replace, to the extent practicable, scientifically justified, and consistent with the policies of this title, the use of vertebrate animals in the testing of chemical substances or mixtures…" and prior to making a request or adopting a requirement for testing using vertebrate animals, the Agency must take into consideration reasonably available existing information, including traditional animal toxicity testing information, results from new approach methodologies (NAMs) such as computational toxicology, bioinformatics , high-throughput screening, and prediction modeling, grouping chemicals into categories for read-across. It is through this lens — the potential to use the TTC as a NAM within different contexts, such as for risk-based prioritization, or as toxicity value for a safety assessment in lieu of having to conduct additional animal testing to fill a data gap — that this Q&A document was developed.

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Q1. What is the Threshold of Toxicological Concern (TTC)?

Threshold of Toxicological Concern (TTC) can be used in evaluating potential human health risks from exposure to low levels of chemicals when toxicity data are limited or not readily available. The TTC is typically used for systemic toxicity by the oral route of exposure and application of the TTC to other routes of exposure is discussed in Q10. Is there a TTC for ecological toxicity?. The TTC can be used as substitute for substance specific hazard data in specific applications for which sufficient scientific support and justification have been developed.

The TTC is a level of daily exposure (oral) to a chemical over a lifetime that is considered to be of no appreciable risk to human health. In the absence of chemical-specific toxicity data, a TTC value can be used as a surrogate to support risk evaluations and risk-based decision making.

- There is wide agreement among scientists that the underlying principle of the TTC concept is sound.
- TTC values have been deliberately derived to be health protective.
- The benefits of the TTC approach include allowing resources to be efficiently focused on more significant chemical risks and avoiding unnecessary animal testing
- TTC values are similar in many respects to health guidance values such as the EPA Oral Reference Dose, a Tolerable Daily Intake, or the ATSDR Chronic Minimal Risk Level (MRL). These all represent daily exposure levels that can occur over a lifetime, even to sensitive subpopulations (e.g. children), without producing an appreciable degree of health risk
- The TTC approach can be considered to be a New Approach Methodology (NAM) in that it can be used in hazard and risk evaluations for low level substances which lack certain kinds of toxicity testing data in lieu of conducting traditional animal toxicity testing
- The TTC approach is based on the dose-response data for chemicals sharing structural characteristics and uses empirical toxicity datasets from over hundreds of chemicals that covers a wide range of toxicity effects and endpoints including genotoxicity, neurotoxicity, immunotoxicity, reproductive and developmental toxicity, carcinogenic and other chronic/lifetime toxicity.
- A TTC value is assigned to a substance based up an analysis of its chemical structure. The Cramer Classification decision tree approach (discussed in Q5. How were TTC values derived? below) is used. Originally TTC values were calculated as intakes in units of µg/day, but now are expressed as µg/kg-day based on a 60kg body weight. See Exhibit 1. TTC Values.

Exhibit 1. TTC Values

Structural Features	TTC (µg/day)	TTC (µg/kg-day)
Cramer Class III	90 µg/day	1.5 µg/kg-day
Cramer Class II	540 µg/day	9.0 µg/kg-day
Cramer Class I	1800 µg/day	30 µg/kg-day
Acetylcholinesterae Inhibitors (AChEIs)	18 µg/day	0.3 µg/kg-day
Genotoxic alerts	0.15 µg/day	0.0025 µg/kg-day

Adapted from: Kroes et al., 2004

Q2. How can TTC values be used to evaluate the safety of a chemical?

The most common use of the TTC is to compare the calculated or measured exposure of a chemical to that substance's TTC value to determine if the exposure is inconsequential. This is illustrated in Equation 1 in Exhibit 2. Equations for Safety Evaluation below. The TTC approach can be utilized when the exposure to a chemical will be very low. TTC values for the different Cramer classes represent the highest acceptable exposure to a chemical within that class for an intended use scenario. For a chemical of interest, the Cramer Class for the chemical is determined and the exposure is calculated for the use scenario. If the exposure is ≤ TTC value for that Cramer class, there is no hazard associated with that exposure.

In addition, TTC values can also be used in a similar manner as a Reference Dose to determine potential health risks from exposure to a chemical. The calculation of a Margin of Safety is demonstrated in Exhibit 2. Equations for Safety Evaluation below using both the traditional approach and the TTC approach. The greater the Margin of Safety, the lower the risk.

A Reference Dose (RFD) is defined as *"An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments."*

Exhibit 2. Equations for Safety Evaluation

Equation 1. Comparing an Exposure Level to the TTC

Is the Human Exposure Level to a Chemical (in mg/kg/day) greater than, equal to, or less than the TTC?

Equation 2.4 Safety Evaluation Using the Traditional Approach:

$$\frac{\text{Reference Dose for Chemical X}}{\text{Human Exposure Level to Chemical X (in mg/kg/day)}} = \text{Margin of Safety}$$

Equation 3. Safety Evaluation Using the TTC as a NAM:

$$\frac{\text{TTC Value (mg/kg/day)}}{\text{Human Exposure Level to Chemical X (in mg/kg/day)}} = \text{Margin of Safety}$$

Q3. If a TTC were to be used as a NAM in lieu of a traditional in vivo animal toxicity test, what impacts could this have in terms of reducing animal testing and cost and time savings?

To date, the primary use of the TTC has been to evaluate concerns related to trace contaminants or unintended exposure to residuals. While the utility of using the TTC as a NAM in a manner described below has yet to fully explored, given the desire, and in some regions, legislative, regulatory or administrative mandates to reduce, refine or eliminate lab animal toxicity testing, the potential to use the TTC to inform the need, or lack thereof, for additional animal testing of a specific chemical warrants consideration.

Along these lines, as part of the OECD's Integrated Approach to Testing and Assessment (IATA) Case Studies Project, the TTC approach was used as the first decision point, and served as a "possible exit point" for the need for further

testing (see *Chemical Safety Assessment Workflow Based On Exposure Considerations And Non-Animal Methods*). In other words, in this IATA framework, if the predicted exposure would fall below the TTC, additional testing would be waived.

Significant savings in terms of costs, reduction in animals in testing, and time savings can be achieved by using a TTC value to fill a toxicity data gap, as shown in Exhibit 3. Cost and Time Estimates for Different Study Types below. For example, if a chronic toxicity study in 2 species were deemed to be a data need, the use of the TTC as a NAM would save 960 animals, $3.6 million dollars and 3 to 4 years. Time savings is important, as this enables risk-based decision making to occur expeditiously, since the need to wait for conclusion of the in vivo traditional animal test to generate the health passed screening criteria based point of departure for derivation of the reference dose/TDI is eliminated.

Exhibit 3. Cost and Time Estimates for Different Study Types

Type of Study	EPA Test Guideline	Number of animals/study	Cost/study	Approximate time frame for each study (from signed protocol to final report)
Subchronic rat	870.3100	100	$550,000	9 months
Developmental (rat and rabbit)	870.3700	80 per species	$158,900	9 months
DNT rat	870.6300	1,100	$787,000	2 years
Reproductive rat	870.3800	2,600	$440,600	1.5 years
Chronic/Carcinogenicity rat and mouse	870.4300	480 per species	$1,808,800	3 years

Adapted from: Craig et. al, 2019

Q4. Have TTCs been derived specifically for developmental and reproductive toxicity?

Yes. As shown in Exhibit 4. Developmental and Reproductive TTC Values below, the TTC approach can be applied with appropriate level of confidence to these endpoints of interest. In cases where there is a data need for one of these endpoints, the structure of the compound is known and fits within the TTC inclusion criteria, a TTC value can be used in risk assessment. The lowest TTC of 1 µg/kg-day value for developmental toxicity, using an uncertainty factor of 1000, is quite close in value to the 1.5 µg/kg-day systemic toxicity value for chronic exposures.

Bernauer et al. (2008) used a database of 91 substances for which NOAELs/NOAECs for fertility and developmental toxicity were available. They used the lowest oral values for fertility and developmental toxicity, 1.0 mg/kg-day and 1.5 mg/kg-day, respectively, and then divided these values by an uncertainty factor of 1000 to yield TTC values of 1 µg/kg-day and 1.5 µg/kg-day, respectively.

Van Ravenzwaay et al. (2011) evaluated a database consisting of oral developmental toxicity studies in rats conducted using the internally accepted OECD 414 test guideline. The database consisted of 92 and 93 NOAEL values for maternal and developmental toxicity, respectively. This database was combined with the developmental toxicity studies in the Kroes et al. (2004) report. The combined dataset yielded cumulative NOEL distribution that exhibited a 5th percentile NOEL value of 4 mg/kg-day. Van Ravenzway et al. (2011) divided this 5th percentile NOEL value by an uncertainty factor of 500 to "to account for a possible underrepresentation of chemical classes" to yield a TTC value for developmental toxicity of 8 µg/kg-day.

Laufersweiler et al. (2012) compiled a database of 283 chemicals with reliable NOAEL estimates for reproductive or developmental toxicity endpoints in studies using traditional lab animal species (e.g., rats, mice, rabbits). The database

of NOAELs was restricted to oral toxicity studies (substances administered by gavage, dietary, or drinking water). Inclusion / exclusion criteria were applied to ensure only studies of sufficient quality were included in the database. The 5th percentile NOAELs for each Cramer Class were calculated (See Exhibit 4).

Exhibit 4. Developmental and Reproductive TTC Values

End point	Uncertainty Factor applied	Derived Toxicity Threshold (µg/day)	Derived Toxicity Threshold (µg/kg-day)	References
Chronic toxicity (Cramer Class I)	100	90	1.5 µg/kg-day	
Chronic toxicity (Cramer Class I)	100	540	9.0 µg/kg-day	
Chronic toxicity (Cramer Class I)	100	1800	30 µg/kg-day	
Chronic toxicity (AChEIs)	100	18	0.3 µg/kg-day	
Fertility	1000	90*	1.5 µg/kg-day	Bernauer et al. (2008)
Developmental toxicity	1000	60*	1 µg/kg-day	Bernauer et al. (2008)
Developmental toxicity	500	480*	8 µg/kg-day	Van Ravenzwaay et al. (2011)
Reproductive / developmental toxicity (overall)	100	342	6 µg/kg-day**	Laufersweiler et al. (2012)
Reproductive / developmental toxicity (Cramer Class III)	100	186	3 µg/kg-day**	Laufersweiler et al. (2012)
Reproductive /developmental toxicity (Cramer Class II)	100	1122	19 µg/kg-day**	Laufersweiler et al. (2012)
Reproductive / developmental toxicity (Cramer Class I)	100	7860	130 µg/kg-day**	Laufersweiler et al. (2012)

Adapted from: Stanard et al., 2015

* not presented in the publication as µg /day, therefore calculated from the authors' human TTC values assuming 60kg adult woman

** calculated assuming 60kg adult woman and rounded to the nearest whole integer

Q5. How were TTC values derived?

The TTC concept was initially developed by Munro et al. (1996). Munro and colleagues compiled a database of NOELs for 613 substances that had been tested in repeat-dose oral toxicity studies including sub-chronic, chronic, reproductive and developmental toxicity. Where there were multiple NOELs for a given substance, the lowest one was selected (there were a total of 2941 NOELs for the 613 substances).

The substances were then assigned to the appropriate Cramer structural class (see below), and cumulative distributions of the logarithms of NOELs were plotted separately for each of the structural classes. Where necessary, adjustments were also made to extrapolate subchronic NOELs to chronic, and LOELs to NOELs. Then the 5th percentile NOEL was estimated for the distribution of each structural class and this was in turn converted to the TTC limit by applying the conventional default safety/uncertainty factor of 100 (10X to account for extrapolation of animals to humans and 10X for human variability) (Munro et al., 1996).

Three noncancer TTC values were developed based on the three structural classes defined by the Cramer et al. (1978) decision tree. The decision tree comprises 33 questions which utilizes recognized pathways for metabolic deactivation and activation, data on toxicity, and the presence of a substance as a component of traditional foods or as an

endogenous metabolite. The three Cramer structural classes have been defined elsewhere (Munro et al., 2008) as follows:

- Cramer Class 1 are substances of simple chemical structure with known metabolic pathways and are of low potential toxicity.
- Cramer Class II contains substances that are intermediate -- they possess structures that are less innocuous than in the Class 1 but do not have a positive indication of toxicity which are characteristic of Class III.
- Cramer Class III contain structural features that permit no strong initial impression of safety or may even suggest significant toxicity.
- The TTC values established were 1800 µg/person/day for Cramer Class I, 540 µg/person/day for Cramer Class II, and 90 µg/person/day for Cramer Class III substances.

In evaluating the distribution of NOELs, special consideration was given to chemicals shown to be neurotoxicants, immunotoxicants and teratogens. It was concluded that except for neurotoxicants (specifically organophosphate pesticides (OPs) and carbamates), such compounds would still be adequately represented by the TTC approach for systemic toxicity endpoints. The cumulative distribution of NOELs for the three Cramer Classes from the Munro et al. 1996 data are depicted in Exhibit 5. Cumulative Distributions of NOELs.

For organophosphates (OPs) and carbamates, which are neurotoxicants, a human exposure threshold of 18 µg/person/day was derived. This threshold was not intended to replace the normal regulatory assessments and controls for OPs and carbamates used as pesticides but could be used to evaluate their risk should a non-approved or unregulated organophosphate/carbamate be detected as a contaminant. It is noted that the NOELs for the OPs and carbamates were not removed from the original Cramer Class III distribution, and many publications have suggested that this distribution should be re-evaluated without these compounds since an untested OP or carbamate would not be assigned to Cramer Class III.

For a compound with structural alerts for genotoxicity/mutagenicity, Kroes et al. (2004) derived a TTC value of 0.15 µg/d (0.0025 µg/kg-day). This TTC value is based on an evaluation of the distribution of carcinogenic potencies of 730 chemical carcinogens. These were plotted and transformed into a distribution of exposures calculated to represent an estimated lifetime cancer risk of one in a million based on linear low-dose extrapolation. Three groups of carcinogens (aflatoxin-like compounds, azoxy- and N-nitroso-compounds are excluded because many compounds in these classes are characterized as high potency, genotoxic carcinogens.

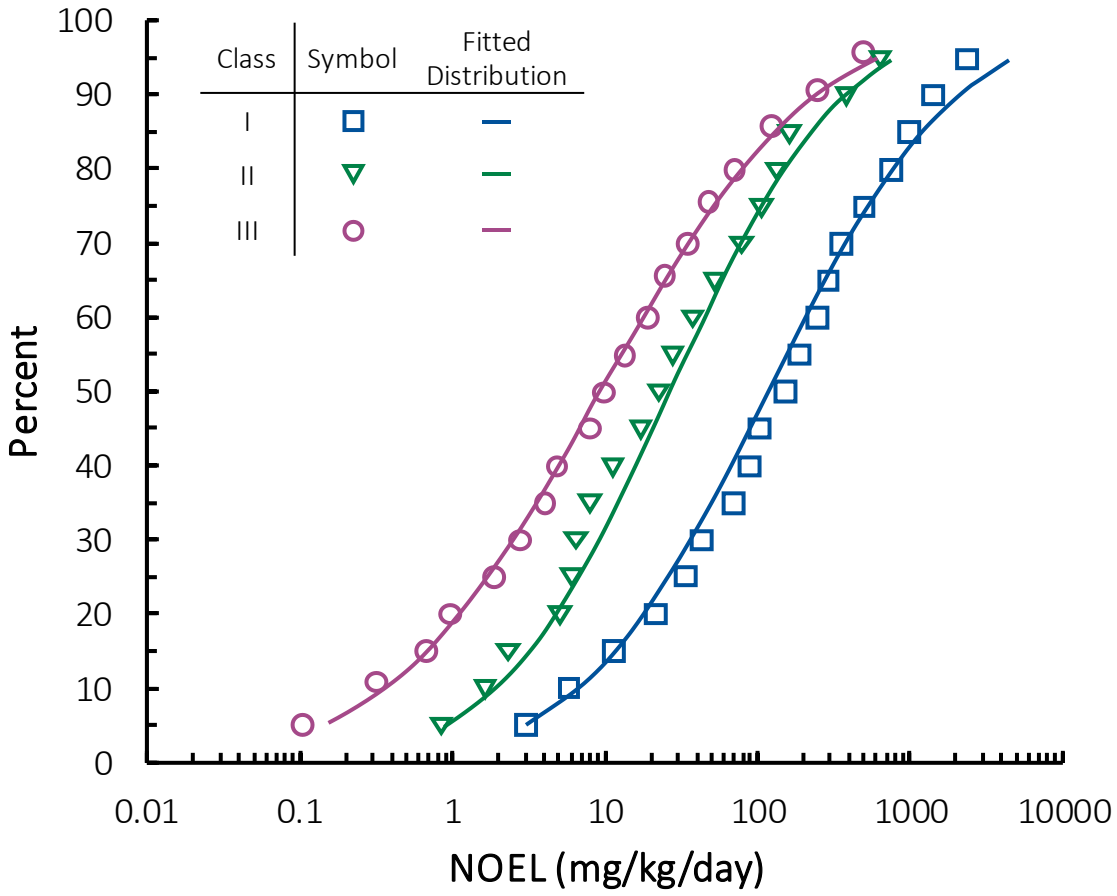
A typical TTC workflow is depicted in Exhibit 6. ILSI Europe TTC Workflow. As illustrated, the following categories of substances are excluded from the TTC approach:

- Inorganic substances, metals and organometallic substances (metals were not in the database used by Munro et al. (1996) to define the TTCs).
- Proteins (proteins were not in the database used by Munro et al. (1996) to define the TTCs).
- Steroids (certain steroids may be non-genotoxic carcinogens which would show a threshold in their dose-response relationships: therefore, the calculation of theoretical upper bound risks using linearized models based on animal carcinogenicity bioassays was considered not to be relevant)
- Nanomaterials (were not in the database used by Munro et al. (1996) to define the TTCs).
- Radioactive substances (were not in the database used by Munro et al. (1996) to define the TTC
- Highly bioaccumulative substances (e.g., compounds with extremely long half-lives that show very large species differences in bioaccumulation, such as TCDD and structural analogues)

- Mixtures of unknown or variable composition. (May use the TTC on a case by case basis – "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" (Kroes et al., 2004).

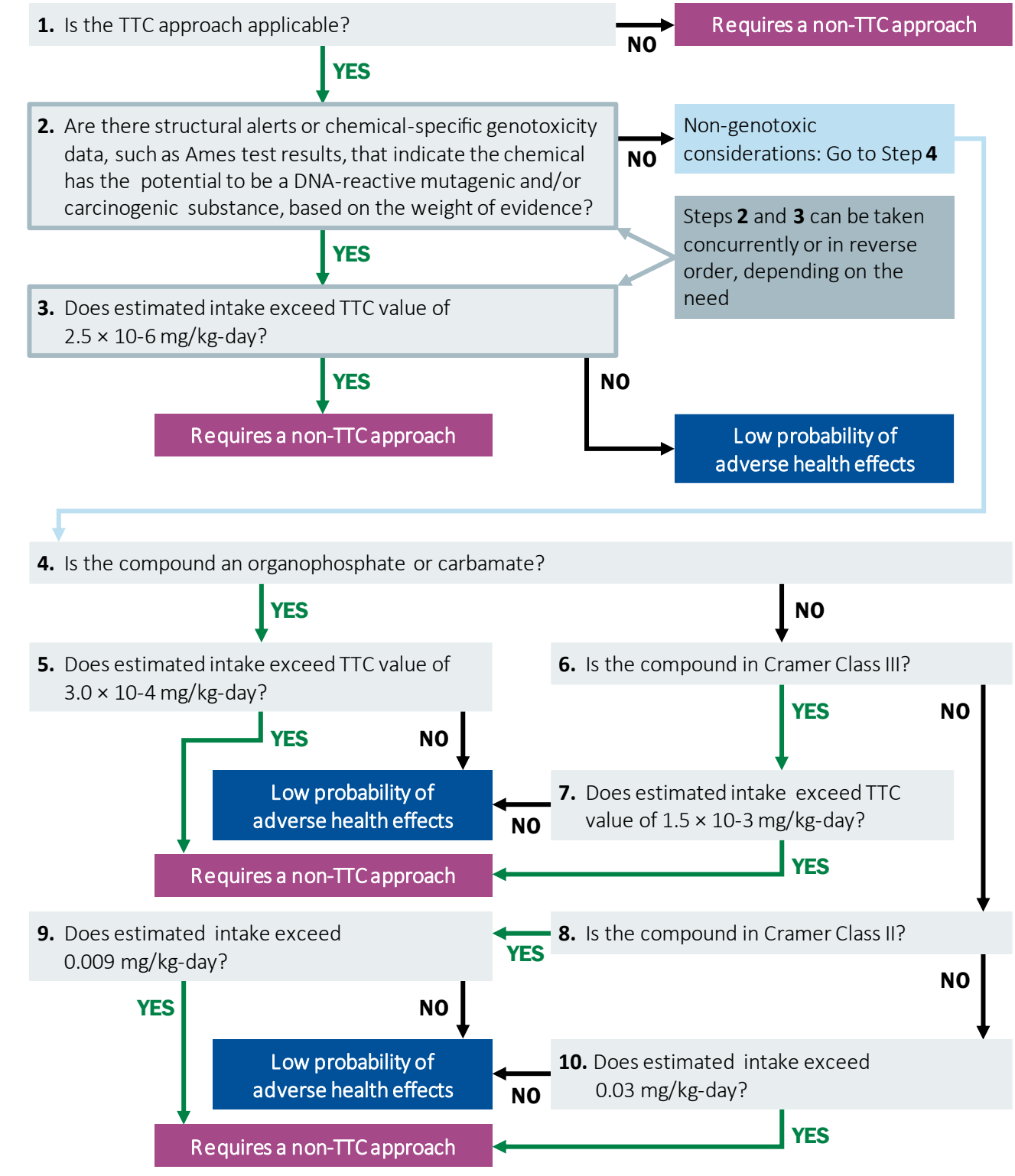
The dataset of substances informing the derivation of TTC values has been extended by Nelms et al, 2019. The TTC values derived from 1304 substances from the ToxValDB were very similar, but not identical to the Munro TTC values; the Cramer I (ToxValDB) was 37.3 µg/kg-day compared to the Munro TTC value of 30, the Cramer II (ToxValDB) was 34.6 ug/kg-day, compared to the Munro TTC 9 ug/kg-day and the Cramer III value was 3.9 ug/kg-day compared to the Munro TTC of 1.5 µg/kg-day. Despite these differences, this research showed that the Munro TTC values are health protective.

Exhibit 5. Cumulative Distributions of NOELs



Adapted from: "Threshold of Toxicological Concern Approach in Regulatory Decision-Making: The Past, Present, and Future" presented by Grace Patlewicz

Exhibit 6. ILSI Europe TTC Workflow



Adapted from: EFSA Scientific Committee et al., 2019

Q6. What regulatory programs are currently using TTCs & how are these being used?

In 1976, Frawley, from the standpoint of food contact materials, put forward the concept that it should be possible to set a threshold for exposure to any individual chemical constituent, regardless of its specific degree of toxicity that would protect against all types of toxicity. The US Food and Drug Agency (FDA) then developed its "Threshold of Regulation" policy for food contact materials (FDA, 1995). Munro el al. (1996) then developed the TTC approach based on an extensive analysis of available chronic toxicity data. Since that time, the TTC concept has been incorporated into a number of regulatory frameworks, including:

- The evaluation of food additives (i.e., flavor chemicals and food contact chemicals)
- Genotoxic impurities in pharmaceuticals (EMA, 2006; FDA, 2015).
- EFSA has published *Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment*
- The Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) issued an *OPINION ON Use of the Threshold of Toxicological Concern (TTC) Approach for Human Safety Assessment of Chemical Substances with focus on Cosmetics and Consumer Products*. These Scientific Committees determined that the TTC approach is "scientifically acceptable for human health risk assessment of systemic toxic effects caused by chemicals present at very low levels, as based on sound exposure information."
- The EC Scientific Committee on Plants has put forward a TTC approach for metabolites of plant protection products in groundwater
- OECD's Integrated Approach to Testing and Assessment (IATA) Case Studies Project *Chemical Safety Assessment Workflow Based On Exposure Considerations And Non-Animal Methods*)

For a comprehensive history of the TTC concept and its application to chemicals in food, the reader is referred to the International Life Sciences Institute (ILSI) monograph (Barlow et al., 2005), the State of the Science review by EFSA/WHO (2016), and other reviews (Hartung et al., 2017).

Health Canada (2016), Australia's National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (NICNAS 2016) and Food Standards Australia New Zealand (FSANZ) (2016) are using or considering TTC as a tool for prioritization and risk-based screening efforts.

Q7. How do I obtain a TTC for a specific chemical?

It can take time, effort, and specific skill sets to derive a TTC value. ScitoVation has developed TTCs for a broad set of chemicals (approximately 45,000 substances described in Mansouri et al., 2016) using Toxtree v2.6.13.

TTC values for tens of thousands of chemicals can now be rapidly accessed using the TTC Applet at this URL https://scitovation.shinyapps.io/TTCApplet/

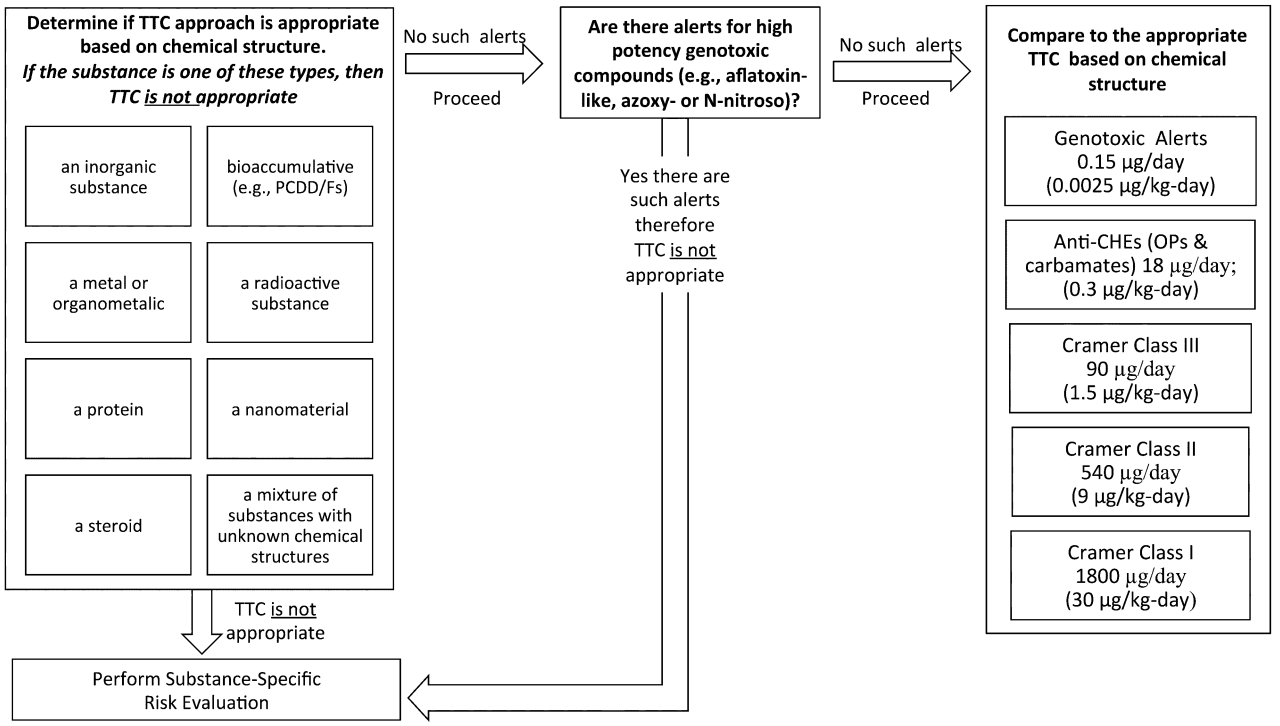
If you need to derive a TTC value for a chemical not contained in the ScitoVation table, you can run Toxtree, an open source application that applies the Cramer rules (Patlewicz et al., 2008) and Kroes TTC decision tree workflows. Specific details on running the Toxtree application can be found at:

- Toxtree (v3.1.0) (IdeaConsult Ltd) https://www.ideaconsult.net/docs/toxtree/license
- Toxtree https://sourceforge.net/projects/toxtree/

Patlewicz et al. (2018) also used custom modules developed for batch processing of thousands of structures to identify carbamates, organophosphates (OPs), and steroids. The conceptual workflow for deriving a TTC is shown in Exhibit 7. Workflow for Deriving a TTC Value.

More recently, Nelms et al. (2019) compared the Cramer TTC values derived from the ToxValDB to the TTC values from the Munro datasets; these were shown to be statistically equivalent. The authors conclude that their study "provides increased confidence in the existing TTC values based on the Munro dataset."

Exhibit 7. Workflow for Deriving a TTC Value



Adapted from: Patlewicz et al., 2018

Toxtree is a free QSAR tool that can be used to determine the Cramer class of chemicals. Toxtree was jointly developed by Ideaconsult Ltd (Sofia, Bulgaria) and the Joint Research Centre (JRC) of the European Commission. For a step by step guide to use Toxtree see this link: https://www.chemsafetypro.com/Topics/CRA/How_to_Use_Toxtree_to_Determine_Cramer_Class_and_Estimate_Toxic_Hazard.html.

The OECD Toolbox also includes a profiler to derive the Cramer class of a chemicals. However, as noted in Bahtia et al. (2015) *Comparison of Cramer classification between Toxtree, the OECD QSAR Toolbox and expert judgment*, different results from the two profiling programs can occur. Therefore, it is important to fully document the procedures used when assigning a Cramer class to a given substance.

Q8. How can TTC be used in TSCA Prioritization?

In accordance with the amendments to TSCA enacted in 2016, following the identification of the active TSCA inventory, EPA is required to eventually conduct risk-based prioritization of all chemicals on the inventory to identify candidates that will be designated as high priority substances for undergoing in-depth risk evaluation. From a human

health perspective, such a risk-based approach for prioritization requires knowledge or data of potential toxicities and information of potential exposure levels for of all such chemicals for conditions of intended use.

In Patlewicz et al. (2018) *Utilizing Threshold of Toxicological Concern (TTC) with high throughput exposure predictions (HTE) as a risk-based prioritization approach for thousands of chemicals* an approach for rapid risk-based prioritization was presented using the TTC combined with heuristic high-throughput exposure (HTE) modelling as an initial proof of concept to perform risk-based ranking of several thousands of chemicals. Chemicals with exposures greater than the TTC were ranked as higher priority for further evaluation whereas substances with exposures less than the TTC were ranked as lower priority. Approximately 6000 substances with modeled median and upper 95% credible interval (UCI) total daily median exposure rates were evaluated. These chemicals were profiled through the TTC workflow published by Kroes et al. (2004) which accounts for known exclusions to the TTC as well as structural alerts. For risk-based prioritization / ranking, the UCI exposures were compared to the appropriate chemical specific TTC values. Overall, as summarized in Exhibit 8. Risk-based Prioritization Results Based on the HTE: TTC Approach, using the TTC with high throughput exposure modeling was shown to be a viable and pragmatic approach for ranking substances as part of a risk-based prioritization approach.

- None of the substances categorized as Cramer Class I or Cramer Class II exceeded their respective TTC values and no more than 2% of substances categorized as Cramer Class III or acetylcholinesterase inhibitors exceeded their respective TTC values.
- The modeled UCI exposures for the majority of the 1853 chemicals with genotoxicity structural alerts exceeded the TTC of 0.0025 µg/kg-day, but only 79 substances exceeded this TTC if median exposure values were used.

Exhibit 8. Risk-based Prioritization Results Based on the HTE: TTC Approach

TTC category	Number of chemicals	TTC (µg/kg-day for 60 kg adult)	Percentage of Substances Exceeding the TTC	
			UCI Exposure Value (number of chemicals)	Median Exposure Value (number of chemicals)
Cramer class III	3214	1.5 µg/kg-day	2% (58)*	0
Cramer class II	332	9.0 µg/kg-day	0	0
Cramer class I	1294	30 µg/kg-day	0	0
AChEIs	102	0.3 µg/kg-day	1% (1)	0
Genotoxic alerts	1853	Kroes 0.0025 µg/kg-day	94% (1740)	4% (79)
		ICH 0.025 µg/kg-day	18% (333)	1% (19)

*Note: if the TTC Cramer Class III value of 3 µg/kg-day were to be used (the re-calculated Cramer Class III TTC developed by Munro et al. following removal of organophosphates from the dataset) then the predicted UCI modeled exposures for less than 1% of the 3214 substances (specifically 28 chemicals) would exceed this TTC.

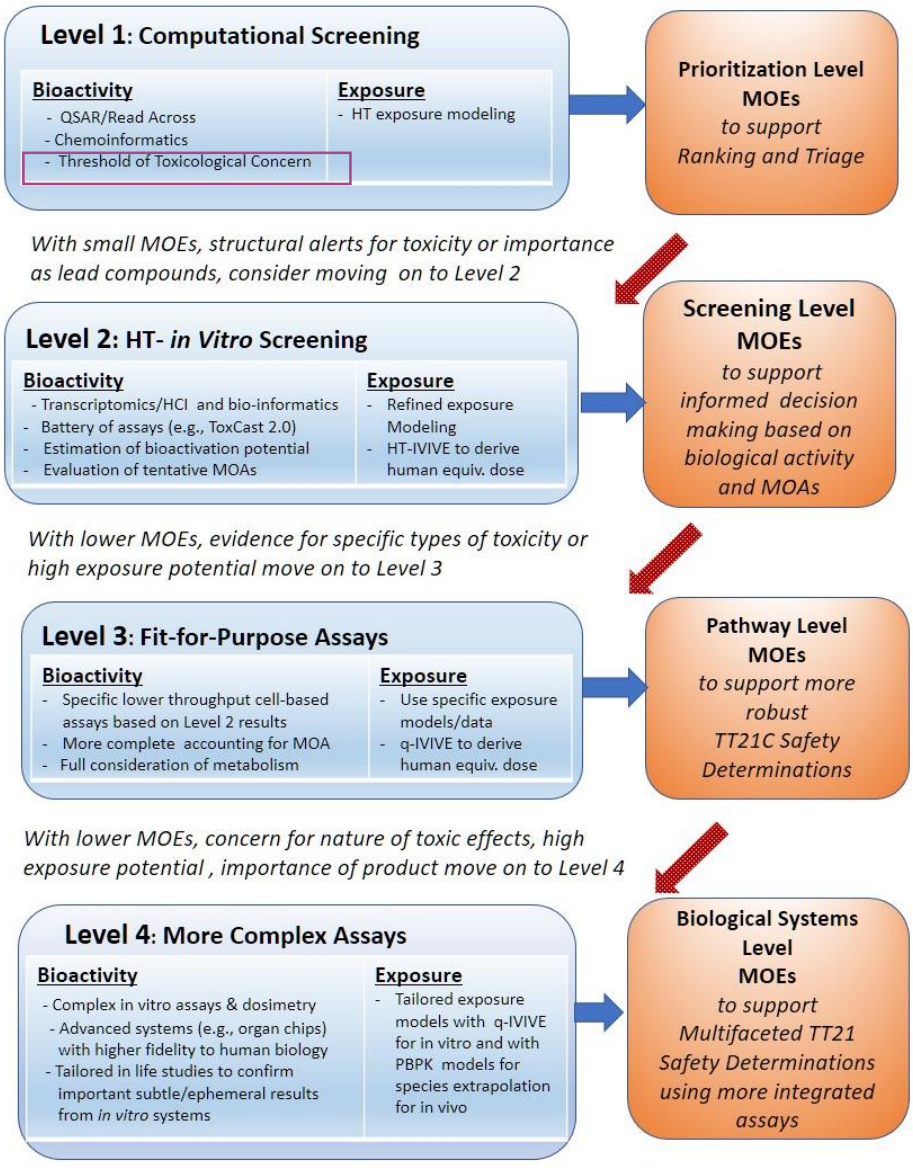
Adapted from: Patlewicz et al., 2018

Q9. How can TTC be used within a tiered integrated approach to testing and assessment?

In OECD's Integrated Approach to Testing and Assessment (IATA) Case Studies Project, the TTC approach was used as the first decision point, and served as a "possible exit point" for the need for further testing (see *Chemical Safety Assessment Workflow Based On Exposure Considerations And Non-Animal Methods*). In other words, in this IATA framework, the TTC was used as a computational NAM.

In Exhibit 9. A multi-level strategy for using new alternative methods and higher-throughput exposure tools for context dependent safety assessments, the tiered testing framework from Andersen et al., (2019) *Developing context appropriate toxicity testing approaches using new alternative methods (NAMs)* is presented. As highlighted, in the initial level of evaluation, similar to the OECD case study cited above, the TTC is compared to the predicted level of human exposure, and if the Margin of Exposure is large, then no further testing would be envisioned as being needed. However, if the MOE is small, then one would proceed to the next level/tier of testing and assessment. (Note: this tiered testing strategy is problem formulation-driven — the level of information available about a chemical would guide the particular testing required for any use conditions, and progression through different levels (orange arrows) would be governed by the decision context. Depending on decision context, a decision-maker might still regard the information at any specific level to be insufficient, regardless of the magnitude of the MOE, leading to consideration of higher-level testing to refine the analysis and provide a greater degree of certainty).

Exhibit 9. A multi-level strategy for using new alternative methods and higher-throughput exposure tools for context dependent safety assessments



Obtained from: Andersen et al., 2019

Q10. Is there a TTC for ecological toxicity?

The EnviroTox Database developed and hosted by HESI, consists of over 91,000 acute or chronic study records for more than 4,000 chemicals, across 1,500 species. It is a web-based platform that includes a calculator to derive predicted-no-effect-concentrations (PNECs) and an ecoTTC tool. Use of the ecoTTC tool is described in the User Guide.

Application of the EnviroTox database is described in Kienzler et al. (2019) *Application of new statistical distribution approaches for environmental mixture risk assessment: A case study*. Two types of "threshold concentration benchmarks" are demonstrated. The first is based on predicted no-effect concentration (PNEC) distributions, i.e., ecotoxicological value of interest for a given chemical to which an assessment factor (AF) is applied. The user can select the AF based on consideration of factors, "such as 1) the coverage of the underlying dataset in terms of acute and chronic data, as well as species and trophic levels; and 2) the geographical region and thus the regulatory framework considered. Once the PNEC distribution is built, the 5th percentile of the distribution gives the threshold concentration, called the ecoTTC value" (Kienzler et al., 2019). Comparing a measured or modeled concentration of a chemical to this ecoTTC value, is akin to deriving a margin of safety.

The second type of threshold concentration that can be used is akin to a margin of exposure, in which no AFs are used. This type of threshold concentration is also derived by calculating the fifth percentile of the distribution of PNEC values. Since no AFs are applied, this benchmark is called the "chemical toxicity distribution-threshold concentration (CTD-TC)."

Additional case studies demonstrating applications of the ecoTTC are in development within the HESI community. These include use of the EnviroTox database tools to set water quality values, evaluate new products, and develop and evaluate alternative test methods. For further information on the EnviroTox Database, contact ecoTTC@hesiglobal.org.

Q11. Is the TTC applicable only to the oral route of exposure? What about inhalation and dermal routes?

Exposure modeling for substances to which the TTC approach is to be applied should, ideally, take into account not only oral exposure, but also any systemic exposure resulting from non-oral routes and sources. The toxicity data used are in deriving the TTC are from oral dosing animal toxicity studies, in which the default assumption is 100% oral bioavailability.

For other routes of exposure, two approaches are possible. One can either develop a route-specific TTC (e.g., a dermal TTC based on empirical toxicity data from dermal application toxicity studies) or adapt an oral TTC using toxicokinetic studies and/or modeling for route to route extrapolation. Williams et al. (2016), in their publication *Assessing the safety of cosmetic chemicals: Consideration of a flux decision tree to predict dermally delivered systemic dose for comparison with oral TTC (Threshold of Toxicological Concern)*, present, and compare, both approaches for deriving a dermal TTC. In comparing the predictive systemic exposure modeling approach to the empirical approach, the investigators concluded that the toxicokinetics modeling approach "… is fit-for-purpose when used as a screening and prioritisation tool."

Escher et al. (2010) and Schuurmann et al. (2016) have explored development of an inhalation TTC. Based on inhalation toxicity studies of 203 industrial chemicals from the database RepDose, Escher and colleagues proposed inhalation TTCs for non-genotoxic compounds of 3.6×10^{-3} ppm (180 µg/person/day) for Cramer class 1 and 2.4×10^{-5} ppm (4 µg/person/day) for Cramer class 3. Schuurmann and colleagues refined the chemical structure classification approach based on structure-activity analysis and reasoning to improve the mechanistic basis for deriving inhalation TTC values.

Partosch and co-workers derived internal TTC (iTTC) by adjusting for oral bioavailability and then applying a reduced uncertainty factor of 25 (instead of 100) to derive iTTC values of 6.9 µg/kg bw/day for Cramer Class I and for Cramer class II/III 0.1 µg/kg bw/day. Most recently, in *Challenges in working towards an internal threshold of toxicological concern (iTTC) for use in the safety assessment of cosmetics: Discussions from the Cosmetics Europe iTTC Working Group workshop* Ellison and colleagues summarize the current state of the science for iTTC and put forward a strategy and plan to develop robust iTTC values, including selection of chemical databases, curation of chemical properties information, toxicokinetic and repeat dose data, and next steps for developing the additional scientific data to underpin the derivation iTTC values for different classes of chemicals.

Q12. What are the limitations of TTCs?

As noted above, there are certain structures for which TTCs are not readily applicable. These include inorganic substances, metals and organometals, bioaccumulative substances proteins, steroids, radionuclides, and complex substances of unknown or variable composition. Therefore, prior to using the TTC approach, it is recommended that one carefully consider the structures of the compounds of interest to determine if the structures are amenable to this approach. Documentation of the rationale for including a chemical with features that some may feel is at or exceeds the boundary of the domain of applicability of the substances underpinning the derivation of TTC values is recommended.

As noted above, route-to-route extrapolation is evolving, as is the iTTC approach. Advances in knowledge will continue to lead to refinement of the method, in particular understanding of biological pathways and advances in quantitative toxicokinetic and toxicodynamic prediction modeling.

To learn more about the ACC LRI, please visit our webpages at https://lri.americanchemistry.com/

If you have specific questions or comments concerning this document please e-mail ACC_LRI@icf.com

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