A note of caution: the threshold of toxicological concern (TTC) is unsuited as a decision rule for deciding whether mixture risk assessment is necessary

Thomas Backhaus,

Dep. of Biological and Environmental Sciences

University of Gothenburg

Carl Skottsbergs Gata 22B

Box 461

40530 Göteborg

Sweden

Phone: +46-(0)31-786 2734

Fax: +46-(0)31-786 2560

Email: thomas.backhaus@gu.se

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Abstract

The threshold of toxicological concern has been suggested as a decision rule on whether a mixture risk assessment is required. According to the recent opinion on mixture toxicity, published by the EU scientific committees, mixture effects are not to be expected, if the mixture components do not share the same mode or mechanism of action and are present at or below their individual TTCs. This, however, ignores the statistical error propagation that is encountered when handling multi-component chemical mixtures, which results in a huge probability that one or more mixture components are *de facto* more toxic than estimated by the corresponding TTC. It is hence argued that the TTC is a valuable tool to bridge data gaps in case experimental toxicity data are missing, but it is unsuited without further refinement as a decision criterion on whether a mixture assessment is needed in a given exposure scenario.

Introduction

Chemical Risk Assessment strives to define acceptable levels for chemicals, such as ADIs (acceptable daily intakes), TDIs (tolerable daily intakes) or OEL (occupational health exposure limits), if possible prior to the market introduction of a chemical or its use in a consumer product. Such levels, if set appropriately, allow to implement chemical management and mitigation measures in order to avoid excessive chemical exposures that would impair occupational or public health. However, more than 80 000 chemicals are registered for commercial purposes under the Toxic Substances Control Act in the US alone (http://www.epa.gov/oppt/existingchemicals/pubs/tscainventory/basic.html), and more than 12 000 compounds are registered at the European Chemicals Agency for industrial uses (http://echa.europa.eu/en/information-on-chemicals/registered-substances) even prior to the final REACH deadline in 2018. Additionally, a broad range of ill-characterized by-products and impurities are present even in high-grade commercial chemicals and consumer products. Each of these chemicals might furthermore be metabolized into a whole menagerie of new compounds. The sheer complexity and size of those chemical inventories renders it impossible to generate even the most basic toxicological data for all compounds using standard animal tests. As a consequence, humans are commonly exposed to a broad range of chemicals with unknown toxicity – including for example pesticide metabolites, disinfection byproducts, and chemicals released or migrating from food contact materials [1].

The Thresholds of Toxicological Concern (TTC) concept has been proposed to simplify this complexity by bridging data gaps. Its basic idea is to use the toxicological information from a pool of well tested chemicals to draw conclusions on acceptable levels for untested but chemically similar compounds. The TTC concept has been applied or is under evaluation for a range of different chemicals in different exposure scenarios, for example chemicals in food [2], pesticide metabolites [3], drinking water contaminants [4] and cosmetics [5]. In detail, NOAELs (No Observed Adverse Effect Levels, the highest tested concentration at which no statistically significant adverse effects were observed in an animal experiment) are compiled for a specific group of chemicals, so-called Cramer classes, and a specific toxicological endpoint. For details on the classification of chemicals into groups see e.g. [2,6]. The data are then ranked and the lower 5% percentile of the resulting log-normal distribution is assumed to describe a concentration that is sufficiently protective for all chemicals of that particular class, including toxicologically uncharacterized compounds. The 5% percentile is then divided by the standard assessment factor of 100, which is typically applied to toxicological data in order to account for the uncertainty of extrapolating toxicological data from animals to humans, and for the inter-laboratory variability of data [7]. The resulting final concentration value is the TTC, defined as "*exposure threshold values for chemicals below which there is a very low probability of adverse effects to human health*" [8]. If chemical monitoring data or modeling efforts indicate that exposure to an untested compound exceeds the TTC, the toxicological characterization of the compound in question is warranted, or management measures that reduce exposure need to be taken.

Chemical risk assessment and management is not only challenged by the sheer number of compounds used by society, but also by the fact they co-occur. That is, humans are not exposed to individual chemicals one at a time, but instead to a complex cocktail of toxic chemicals that are simultaneously

ingested via food and water, inhaled and dermally absorbed. Consequently, even limited biomonitoring and modeling approaches indicate or confirm the presence of complex multi-chemical cocktails in human tissues and fluids, e.g. [10,11]. This implies that any strategy for protecting human health needs to factor in the cumulative exposure towards the same chemical or class of chemicals from different sources [9], but also needs to take into consideration the exposure towards mixtures of dissimilar chemicals. Mixture toxicity assessment has therefore been flagged as an area of high priority for policy action by the EU Council of Ministers [12] and the EU Commission [13].

Three of Europe's Scientific Committees, on Health and Environmental Risks (SCHER), Emerging and Newly Identified Health Risks (SCENIHR) and Consumer Safety (SCCS) published a common opinion on the toxicity and assessment of chemical mixtures in 2011, in which the TTC plays a critical role as a criterion to decide whether a mixture risk assessment is warranted in a given exposure scenario [14]. The opinion proposes that a mixture risk assessment is not necessary if exposures from individual compounds or a mode-of-action group do not exceed the individual TTCs.

The following text will evaluate the premises of this conclusion. I will put forward the claim that this proposition is based on untested – and even untestable – assumptions and that it ignores the basic statistical properties of the TTC. Using the TTC as a decision criterion on whether a mixture toxicity assessment is needed leads to a potential underestimation of mixture toxicities and an elevated risk of including misclassified chemicals. This approach hence falls short to safeguard human health against adverse effects of chemical mixtures.

Fundamental concepts for mixture toxicity assessment

There is widespread consensus to use two concepts as a reference framework for analyzing the toxicity of a chemical mixture: Concentration Addition (CA, also termed Dose Addition) and Independent Action (IA, also termed Response Addition) [15,16].

The basic idea of CA is sum up the risk quotient for each mixture component, i.e. the ratio of the individual concentration c_i to the concentration provoking a common effect of x%, ECx_i, providing the mixture risk quotient:

$$\frac{c_{mix}}{ECx_{Mix}} = \sum_{i=1}^{n} \frac{c_i}{ECx_i} \qquad \text{(eq 1)}$$

In principle all *ECx* values have to be recorded for the same toxicological endpoint in the same species of test animals, under identical exposure conditions, and the resulting mixture risk quotient holds true for that particular species and toxicological endpoint only. However, given the fact that such consistent datasets are rarely at hand, the overall risk of a mixture is often estimated in a regulatory context as:

$$\frac{Exp_{mix}}{RfV_{Mix}} = \sum_{i=1}^{n} \frac{Exp_i}{RfVx_i} \qquad \text{(eq 2)}$$

Where Exp_i *and* Exp_{mix} denote the human exposure level to an individual compound respectively the total mixture, and *RfV* is the so-called reference value, a generic term used by the US EPA as an "*estimate of an exposure for a given duration to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime.*" [17]. TTC-, ADI-, TDI- and OEL-values are hence specifications of the generic term *RfV*.

The conceptual counterpart to CA is the concept of Independent Action (IA, also termed Response Addition), which assumes that all compounds in a mixture affect the same toxicological endpoint – but do that via independent, dissimilar modes and mechanisms of action. IA can mathematically be formulated using the law of random, statistically independent events as:

$$E(c_{Mix}) = 1 - \prod_{i=1}^{n} [1 - E(c_i)] \qquad \text{(eq 3)}$$

where $E(c_{Mix})$ denotes the mixture effect at a concentration $c_{Mix} = \sum_{i=1}^{n} c_i$ and $E(c_i)$ describes the effects that the individual components would cause if applied singly at the concentration at which they are present in the mixture.

The contribution of low doses to the toxicity of a mixture

The two approaches lead to fundamentally different conclusions with respect to the contribution of low, individually non-toxic doses. CA assumes that each and every component contributes to the overall mixture toxicity in direct proportion to its individual risk quotient. That is, contributions of individual compounds can be comparatively low, if their toxicological potency and/or concentration in the mixture is low, but it is never zero. The number of components in a mixture is hence a major driver of the overall risk quotient. In contrast, IA, being effect-based, assumes that such low concentrations do not matter for the joint toxicity of a mixture, .i.e. if $E(c_i)=0$ the compound does not contribute to the toxicity of a mixture. Consequently, a mixture that behaves according to IA in the purest sense does not cause any toxicity, even if hundreds or even thousands of toxic chemicals are present at such low concentrations. This argumentation would offer a convenient way to reduce the number of multi-component mixtures that need to be considered for chemical risk assessment.

However, applying IA in order to rule out the contribution of a low dose of a certain compound to an overall joint effect, or to even dismiss the possibility of a mixture effect completely, requires proof of two critical assumptions: First of all, it has to be proven that the exposure level of a given compound does cause absolutely no effect on the toxicological endpoint under consideration. This, however, is simply impossible, given the fact that every animal test and every *in vitro* test has only a limited statistical power to detect effects. The inability of a study to demonstrate an effect is hence, obviously, no proof of its absence. And individually negligible effects cause substantial toxicity, even under the assumption of independence, if multiple chemicals are present. For example, a 50-compound mixture in which each compound would be causing only 1% effect if applied singly – an effect level that is

indistinguishable from a no-effect level in almost any animal study, would still cause 40%effect in total (see eq. 3).

Secondly, it seems highly unlikely that the assumption of a complete independence holds if compounds co-occur in an exposed body, where they might be degraded by similar pathways, transported into similar organs by similar processes, etc.

These conceptual inconsistencies, together with severe practical problems to apply IA in reality [16] has to the unanimous suggestion to start the toxicity and risk assessment of a mixture with CA, independent on whether information on the modes and mechanisms of the involved mixture components is at hand, e.g. [15,16]. Eq. 3 also indicates the possibilities to bridge data gaps using the TTC: In case experimentally validated reference doses are not at hand for any of the mixture components, the TTC can provide surrogate data, in order to enable a screening-level CA-based mixture toxicity and risk estimation [18].

The *a priori* e assumption of a concentration-additive behaviour is not be taken to conclude that all possible or analytically detected chemical mixtures at a workplace or at home are always hazardous. CA does not allow to dismiss potential mixture effects prior to an explicit evaluation, but even a CA-compliant mixture follows an ordinary concentration-response relationship for which standard descriptors such as NOECs or EC50 values, and, consequently, ADIs, TDIs and OELs, can be determined. For example, Houtman and colleagues recently applied CA for assessing the joint human health effects of pharmaceuticals in Dutch drinking water and came to the conclusion that human health effects are negligible [19].

Error propagation renders the TTC unsuitable as a decision criterion for mixture toxicity assessment

Even under the simplistic assumption of a concentration-additive behavior, mixture risk assessments are demanding, in particular because estimates on the simultaneous exposure to all the various mixture components need to be collected. Hence, in an attempt to simplify, it was suggested in the recently published opinion on mixture toxicity assessment [14], to use the TTC as a criterion on whether a mixture risk assessment is warranted in the first place. A mixture effect is not to be expected, so the argument goes, if the mixture components do not share the same mode or mechanism of action and are present at or below their individual TTCs [14]. However, this approach does not only fall short because of the issues outlined in the previous paragraph. Additionally, statistical error propagation renders this approach invalid, if a TTC-based mixture toxicity assessment is supposed to provide the same level of human health protection as a TTC-based single substance assessment.

As mentioned in the introduction, the TTC is calculated as the lower 5% percentile of a pool of assumedly log-normally distributed NOAELs, divided by an assessment factor, usually 100 [2,6]. This implies that a misclassification risk of 5% is accepted. Or in other words, there is a 5% probability that an unknown compound *de facto* has a lower NOAEL than indicated by the lower 5% percentile of the distribution. It is critical to realize that this 5% misclassification risk is a compound specific value. If several compounds are present, the overall misclassification risk increases according to the law of independent random events as follows:

$$Misclassification\ risk = 1-(1-0.05)^n \quad \text{(eq. 4)}$$

where *n* is the number of mixture components, 0.05 is the misclassification risk for a single compound (figure 1). Already a mixture of 14 compounds has a misclassification risk of more than 50%, i.e. there is a more than 50% chance that at least one of the mixture components is misclassified and has an actual NOAEL that is lower than the concentration marked by the 5% percentile of the underlying distribution of NOAEL values.

Equation 1 can be arranged to

$$percentile = 1 - \sqrt[n]{(1 - misclassification\ risk)} \quad \text{(eq 5)}$$

This allows to calculate the lower percentile of the NOAEL distribution that needs to be used in order to ensure that for a given mixture of *n* compounds the misclassification risk does not exceed 5%, in order to achieve the same level of protection as in single substance assessments. The visualization of eq. 5 in Figure 2 shows that for example for a 20 compound mixture the lower 0.26% percentile would need to be used. The percentile that is used for the single substance assessment needs, as a rule of thumb, to be divided by *n*, the number of components in the mixture, an approach that corresponds to the well-known Bonferroni adjustment for multiple comparisons.

However, such low percentiles are unfortunately not practically useful. First of all, their numerical values are not deducible from the available experimental data with a sufficiently level of robustness, as the available data pool does not include more than a couple of hundred chemicals. And secondly, the resulting threshold concentrations get excessively low already for mixtures comprising only a small number of components. This renders the application of a mixture-compliant TTC unsuitable as a filter for simplifying mixture risk assessments.

It warrants pointing out in this context, that the aforementioned assessment factor of 100 does not safeguard against the increased risk of TTC misclassifications, as it accounts for the extrapolation from animal data to humans [7], which is an uncertainty that is encountered for each individual compound and hence needs to be factored in separately.

Conclusions

The TTC can play a valuable role as a surrogate for missing single substance reference values such as ADIs, TDIs or OELs, in order to enable a first CA-based mixture toxicity and risk estimation in the absence of experimental data [18]. However, without further refinements, the TTC is not suitable as a decision rule for deciding whether a mixture toxicity assessment is warranted in the first place. This obviously does not imply that a mixture composed of compounds at their TTCs is always inherently toxic, but it implies that the number of components present is a critical characteristic of the mixture that needs to be factored in, already at the very beginning of the assessment.

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Figure 1: Relationship between the number of chemicals in a mixture and the probability to misclassify at least one mixture component

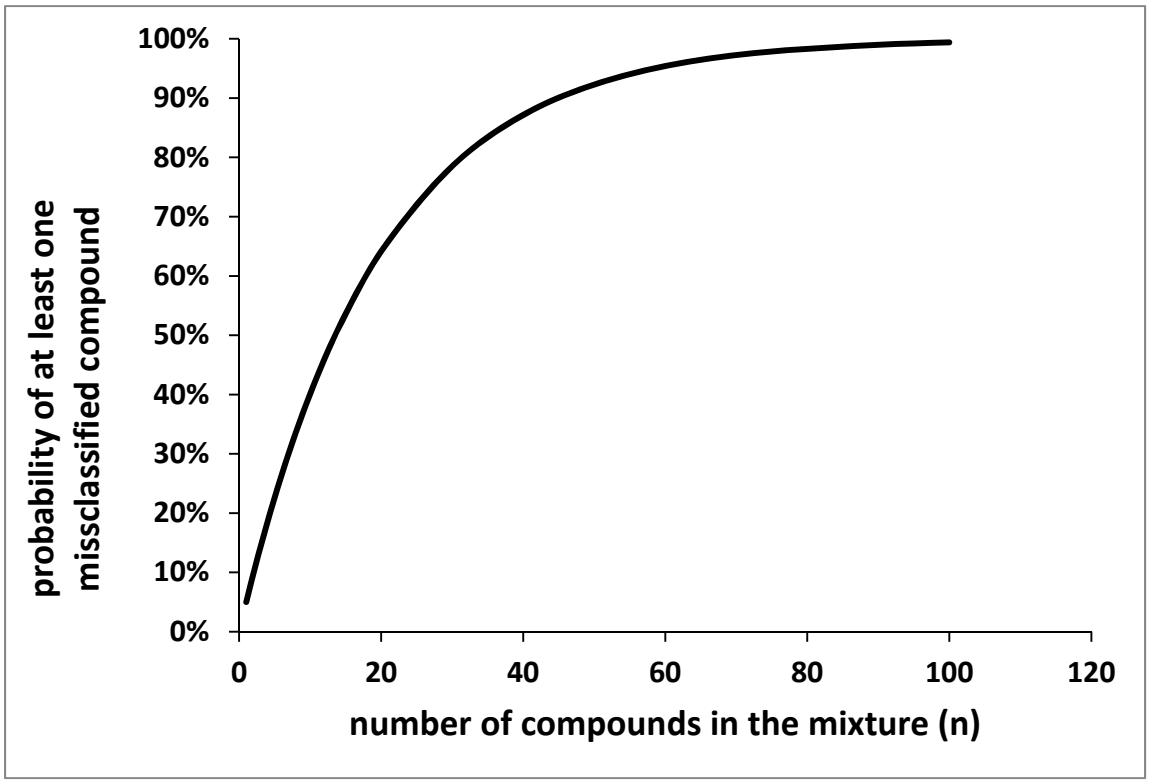

Figure 2: Relationship between the number of chemicals in a mixture and the percentile that is required for the TTC determination in order to achieve the same overall misclassification risk (5%) as in a single substance assessments