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Complex mixtures: Relevance of combined exposure to substances at low dose levels

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ABSTRACT

Upon analysis of chemically complex food matrices a forest of peaks is likely to be found. Identification of these peaks and concurrent determination of the toxicological relevance upon exposure is very time consuming, expensive and often requires animal studies. Recently, a safety assessment framework based on the Threshold of Toxicological Concern (TTC) was published to assess the safety of chemically complex matrices more efficiently. In this safety assessment framework, the toxicological relevance of exposure to unidentified substances in chemically complex food matrices can be related to the Cramer class III TTC threshold, currently set at 90 μg/day. However, possible additive or synergistic effects of combined exposure is not covered.

The current evaluation describes the relevance of combined low dose exposure to unidentified substances in chemically complex food matrices. It is concluded that to some extent cumulative effects at exposure levels for each substance at or below the Cramer class III TTC threshold, being present in a complex mixture including food, might occur. However the health relevance of possible cumulative effects at this dose level is considered to be that low that a need for a correction factor to cover possible cumulative effects is very low to absent.

1. Introduction

When exposed to chemically complex food matrices (CCFMs), exposure to chemicals which might have an additive or synergistic effect, and therefore may induce cumulative effects, should be taken into account in risk assessment. Recently the WHO/IPCS and ECETOC held workshops after which the current state-of-the-art of combined exposure to multiple chemicals in risk assessment was published (Meek et al., 2011; ECETOC, 2011). Furthermore, extensive work was performed on mixture toxicity by Kortenkamp et al. (2009), combined scientific committees of the European Union (EU-SCENIHR, 2012), EFSA (2008), and others. Although several aspects in risk assessment are addressed to account for possible cumulative effects in these evaluations, no conclusions were drawn with respect to the relevance of cumulative effects at low dose exposure in risk assessment. Boobis et al. (2011), evaluated the literature for low-dose synergistic effects but had to expand the survey, also including acute studies, due to very few studies available in literature in which low doses, at or near the respective points of departure (e.g. No Observed Adverse Effect Levels, Benchmark Dose Levels, etc.), were found in chronic studies, which resulted in the inclusion of acute studies with doses well above the chronic points of departure. Furthermore, in most of the evaluated studies in which synergistic effects were seen, the doses of the individual mixture substances exceeded their respective (chronic) points of departure, whereas effects were observed only at the higher doses used in the study and mixtures affecting a common target or mechanism were concerned. Although a significant amount of work is already performed on mixture toxicity, the relevance of combined low dose exposure is still under discussion.

Food is a complex matrix which may contain lots of (trace amounts of) substances, which can be found as a forest of peaks upon analysis. One of the main hurdles in the safety assessment of CCFM, is that it is time consuming and difficult to determine the identity of all substances present at low concentration and concurrently to assess their health risk upon exposure. Koster et al. (2011) and Rennen et al. (2011) published a safety assessment framework for substances in CCFM without the need for full identification of every single substance present at low exposure levels, which may even reduce unnecessary animal testing. This frame-

Abbreviations: AChE, acetyl cholinesterase; ARfD, acute reference dose; ADI, acceptable daily intake; AT, analytical threshold; BMDL, benchmark dose level; CAG, cumulative assessment group; CCFM, chemically complex food matrices; LOAEL, lowest observed adverse effect level; MOA, mode of action; NAEL, no adverse effect level; NOAEL, no observed adverse effect level; TDI, tolerable daily intake; TTC, Threshold of Toxicological Concern.

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work is based on the Threshold of Toxicological Concern (TTC) concept using the Cramer class III threshold, which is currently set at 90 μg/day (Kroes et al., 2004; EFSA, 2012), to deal with the large number of unknown substances present at low exposure levels in CCFM. It should be noted that for this safety assessment framework, target analysis and high-throughput genotoxicity screening is proposed to determine the presence of chemicals of high concern (cohort of concern chemicals, genotoxic substances and organophosphates) present below this threshold or the presence of substances which are not covered by the TTC concept (e.g. endocrine active substances). An uncertainty in the framework is the possibility of cumulative effects upon exposure to unidentified substances present at low exposure levels in the food. The current paper is therefore focused on the relevance of cumulative effects at low exposure levels.

When relating the 90 μg/day TTC threshold to the intended/assumed intake of the CCFM in question, an analytical threshold (AT) related to an intake of 90 μg/person/day for each of the substances in a CCFM was set for comparison of the peaks with the threshold. It should be noted that in the publications of Koster et al. (2011) and Rennen et al. (2011), a Cramer class III threshold was proposed of 540 μg/person/day, being the threshold of the Cramer class II threshold, based on a publication of Munro et al. (2008) which reported a corrected Cramer class III threshold of 600 μg/person/day upon exclusion of organophosphates from the Cramer class III dataset. For the present publication the current threshold for the Cramer class III substances, being 90 μg/person/day, was used to discuss the relevance of cumulative effects at low dose exposure. This threshold is however depending on future re-evaluations of the TTC dataset and may change over time.

Substances exceeding the AT should be considered as potential toxicological relevant substances in a CCFM, assuming that the presence of chemicals of concern in the CCFM can be excluded by targeted analysis and high-throughput genotoxicity screening as described by Koster et al. (2011). These substances should be identified and their risk upon exposure should be evaluated. Substances not exceeding the AT, which would likely be seen as a significant amount of peaks upon analysis, do not have to be taken further into account according to the safety assessment framework. However, possible effects as a result of combination toxicity of these substances cannot be excluded so far. The relevance of combined exposure to unidentified substances at low exposure levels (below the AT) is therefore discussed in this paper.

It is known that combination toxicity of two or more substances may result in either dose addition, independent action or interaction (EU-SCENIHR, 2012). Dose addition may occur when substances have a similar mode of action (MOA). The substances however are likely to have different potencies to derive the effect for which dose addition may be presumed. In general, the effect upon combined exposure can be calculated by the sum of potency corrected exposures. Independent action, also referred to as response addition, simple dissimilar action, simple independent action or independent joint action, occurs where the MOA and possibly, but not necessarily, the nature and sites of toxic effects differ between the chemicals in a mixture and one chemical does not influence the toxicity of another. The effect upon combined exposure is related to the combination of effects induced by each of the substances. Interaction occurs when the combined effect is more (synergistic) or less (antagonistic) than could be expected for each of the individual substances when taken into account a possible dose addition. Comparable to response addition, the substances interacting have a different MOA. However the combined effects interact with each other, e.g. as result of a similar target tissue.

When exposed to a complex mixture, potential effects as a result of both exposure to individual substances as well as combined exposure should be evaluated. For independent action and interaction, cumulative effects may occur when effect levels for two or more substances are exceeded. In this respect one should be aware that e.g. a No Observed Adverse Effect Level (NOAEL) may represent varying risk or response levels, and therefore should be considered as a level of exposure which might induce (slight) effects (EFSA, 2008). This because within a study, effect levels are determined by statistical analysis, which is highly related to the study design among which the size of the test groups and dose-spacing. Therefore a NOAEL might still be an effect level whereas an Acceptable Daily Intake (ADI) or Tolerable Daily Intake (TDI) is usually not, taken into account the additional safety factors applied.

Significant toxic interactions between chemicals are much less likely to occur at doses below the effect levels for the individual substances (EFSA, 2008 and EU-SCENIHR, 2012). Therefore, independent action and interaction are not considered likely to occur in a complex mixture when exposure levels to substances are below their respective effect levels. Co-exposure below the respective ADI, TDI or Benchmark Dose Level (BMDL) of the substances present, is assumed to result in negligible effects if all substances have dissimilar modes of action. The same accounts for the TTC levels in which a safety factor is also included and which is based on the 5th percentile of a parametric lognormal distribution of the concurrent group of chemicals (Munro et al., 1996). For substances in a complex mixture which are present below their effect level, e.g. below the AT which can be considered as threshold for which for the gross majority of chemicals no adverse effects are to be expected, only dose addition might be of relevance. As such, only the relevance of dose addition at low dose exposure via complex matrices, including CCFM, is further evaluated in this paper.

2. Combined exposure in risk assessment

When individual substances share a MOA (e.g. organophosphates or phthalates, etc.) combined exposure may result in dose addition. Even when individual substances are present below their threshold for an effect, combined exposure could result in a measurable effect when taken into account the potency of the individual substances.

For risk assessment purposes, dose addition by combined exposure is evaluated via cumulative assessment groups (CAGs), for which the grouping can be based on general criteria like chemical structure or mechanism of action, or more refined criteria like common toxic effect or toxic MOA. Grouping is based on one or more of the following criteria (EFSA, 2008; Boobis et al., 2008):

- Chemical structure (known or potential toxicophores, based on core molecular structure, specific functional groups, and/or their metabolic precursors).
- Mechanism of action. The mechanism of mammalian toxicity of e.g. a number of pesticides is similar to that responsible for their activity against target pests, e.g. organophosphates.
- General mode/mechanism of mammalian toxicity. This is based on a relatively broad consideration of mode of action, and not a detailed evaluation of key events.
- A specific toxic effect. It is possible that similar toxic effects are caused by structurally unrelated substances via the same MOA. It would be important to consider such substances in the same CAG, as they would exhibit dose addition. However, non-specific effects such as changes in body weight, unless they are due to a specific mechanism, should not be used as a basis for membership of a CAG.

Substances should only be assigned to a CAG upon a definitive identification of those substances that cause the same toxic effect