

Chapter 2

New Developments in Food Safety

Assessment: Innovations in Food Allergy and Toxicological Safety Assessment

Geert Houben, Marty Blom, Jolanda van Bilsen, and Lisette Krul

1 Introduction

Food preferably has to be tasteful, healthy, attractive, and affordable, but should above all be safe. The safety of our daily food nowadays is at a reasonable level, but is far from obvious. With a certain frequency, food safety incidents come up, and the increasing complexity and globalization of our food production networks contribute to an easy outgrowth of such incidents to international crises. There is an increased food demand due to a growing world population, whereas existing food sources are limited. To meet the increasing food demand, industry continues developing new ingredients, sources, and products like novel protein-based products (e.g., insect-derived food proteins); extracts from fruits, vegetables, and herbs; natural fragrances; and flavorings but also introduces new processing methods and innovative food packaging concepts. Furthermore, new food concepts are under development in an attempt to reduce the health burden due to overweight and other (related) western food-related conditions or to specifically treat or prevent food-related diseases such as food allergy. Finally, our current food is still found to pose natural or process-induced hazards, sometimes newly discovered (e.g., acrylamide several years ago), and not seldom its safety is jeopardized due to malpractice such as fraud.

During the past half century, food risk assessment and risk management approaches have developed, particularly with respect to microbiological and chemical safety aspects of food and food production and processing. Yet, several white spots or areas of inefficiency still exist. Until recently, methodologies for assessing and efficiently managing risks posed by allergens in food were lacking. Furthermore, assuring the chemical safety of current and increasingly complex new foods and food concepts and innovations in food as mentioned above requires a

G. Houben (✉) • M. Blom • J. van Bilsen • L. Krul
TNO, 3704 HE Zeist, The Netherlands
e-mail: geert.houben@tno.nl

continuous improvement of our toxicological food safety assessment and management approaches. These should be pragmatic and prevent unnecessary spending of time, money, and animals for safety testing. In this chapter, recent and ongoing innovations in two areas are addressed: innovations in food allergy safety assessment and innovations in toxicological safety assessment of food.

2 Food Allergy Safety Assessment

2.1 Food Allergy

Food allergies are adverse reactions to an otherwise harmless food or food component that involves an abnormal response of the body's immune system to specific protein(s) in foods. True food allergies may involve several types of immunological responses [1]. The most common type of food allergies are mediated by allergen-specific immunoglobulin E (IgE) antibodies which bind to receptors on circulating basophils and mast cells in mucosal tissues. Upon recurrent exposure to the same allergen, cross-linking of cell-bound IgE induces an allergic response by mediator release [2, 3]. The clinical picture of food allergy is pleomorphic and can range from gastrointestinal symptoms to severe anaphylaxis [4]. This adverse hypersensitivity response to food poses a serious public health concern [5–7]. The etiology of food allergy or tolerance, however, continues to be poorly understood. For a more detailed review of the mechanisms of allergy, in particular food allergy, the reader is referred to several excellent review articles [8–10].

2.2 Food Allergens

Virtually all food allergens are proteins, although only a small percentage of proteins are major allergens [11, 12]. Any food that contains protein has the potential to cause allergic reactions in some individuals. However only a few foods or food groups are known to cause allergies on a more frequent base than other foods. The majority (approximately 90 %) of food allergic reactions are caused by eight foods: milk, egg, peanuts, tree nuts, fish, soya, wheat, and shellfish [13]. Although controversy exists as to whether the prevalence of food allergy is increasing, it nonetheless remains an important health issue, affecting approximately 1–2 % of adults and 6–8 % of children [14].

2.3 *Safety Assessment and Risk Management of Known Major Allergenic Foods*

The only option for food allergic individuals to manage their food allergy is the strict avoidance of allergenic food. Medication is available to suppress symptoms, and an increasing number of studies are being published on oral tolerance induction protocols for peanut, milk, egg, and wheat, though these procedures have not yet become standard practice. The majority of the food allergic population therefore relies on rigorous elimination of the allergen in their diet. Legislations in many regions of the world, as for instance for the EU laid down in EU directives 2003/89/EC and 2006/42/EC, prescribe the labeling of food products for several major allergenic foods or products derived from that allergen when added as ingredients to food. In addition, many food producers have incorporated allergen auditing programs and voluntarily warn the allergic consumer as to the potential presence of allergens by using precautionary labeling of food products, e.g., “may contain xxx.” However, despite this, several retrospective studies [15–17] show that many allergic individuals experience an accidental allergic reaction due to hidden allergens or inappropriate labeling. Surveys of commercially available products demonstrate that the presence or the absence of a precautionary warning corresponds poorly with the actual presence of the allergen in the product [18, 19], which can lead to potentially dangerous situations [15, 20]. A recent study in Canada showed that approximately 17 % of allergic individuals experiencing an accidental exposure attributed this to products with unintentional cross-contamination during manufacturing and no precautionary statement on the label [17]. Conversely, many products do not contain the allergen to which the precautionary warning on the label refers. As a consequence, a precautionary warning on products is not always valuable to allergic consumers and they increasingly seem to ignore precautionary labels [21, 22].

To improve this situation, quantitative guidance is needed with advice on maximum levels of unintended allergens in foods (also called action levels) to improve the precautionary labeling. Several initiatives have been set up by both food industry and enforcement bodies with the involvement of various stakeholders to improve allergen management and to introduce more uniform and transparent risk information [23–25]. One of the ultimate goals may be to establish internationally harmonized guidance that includes action levels for labeling unintended allergens. In contrast to many other risk assessment situations, human data on threshold doses to allergenic foods is available and can be used to establish action levels. In very sensitive patients small amounts may elicit severe reactions, but also thresholds for allergic reactions of micrograms or grams of allergenic food have been reported [26–29]. The US FDA Threshold Workgroup [30] therefore concluded that a single threshold level for any of the major food allergens might yield thresholds that are unnecessarily protective and further that additional data were required. A recent study by Taylor et al. [31] combined the threshold dose of peanut allergic individuals from different centers into a peanut threshold distribution curve which can be used to derive the

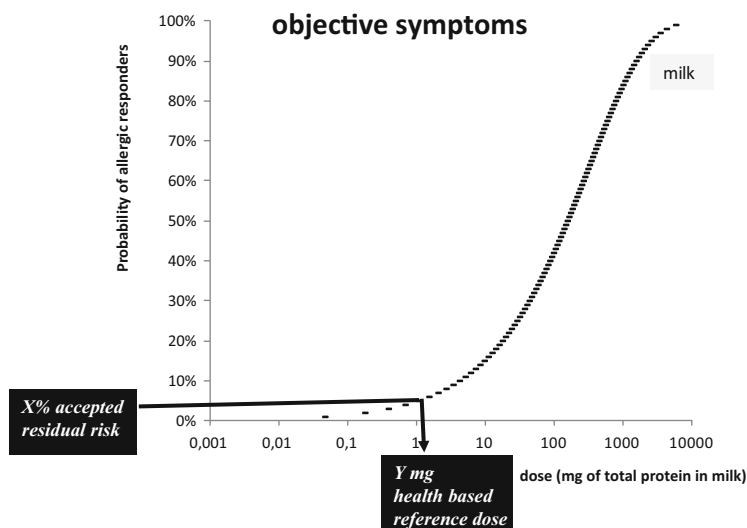


Fig. 2.1 Elaboration of a reference dose protecting the vast majority of allergic individuals (from Blom M.W. et al. Action limits for the potential cross-contamination of a food product with Allergens. Netherlands Journal of Allergy & Asthma (2013) 13: 74–80)

eliciting dose to which a certain portion of the population might respond with objective allergic reactions (ED_p): an approach that was used to establish reference doses for many major allergenic foods by a scientific expert panel that reviewed all published and unpublished threshold data available at the Food Allergy Research and Resources Program (FARRP) of the University of Nebraska and the Netherlands Organization of Applied Scientific Research TNO for the Australian-New Zealand Allergen Bureau [32, 33]. Figure 2.1 illustrates the approach followed by this expert panel for elaboration of reference doses. Starting point for the elaboration of these reference doses was an accepted risk of less than 1 % mild objective reactions, chosen in consultation with stakeholders as an acceptable compromise from the perspectives of food safety objectives, practical feasibility, and scientific feasibility. These reference doses were developed into a guidance for establishing action levels for precautionary labeling by the Allergen Bureau. An incidental reaction, that may occur in view of the accepted remaining risk, will in general be mild and transitory, generally requiring no medical intervention. This quantitative guidance is a big step forward and welcomed by the different stakeholders and increasingly taken up by food-producing companies all over the world. For the allergic consumer this will lead to a greater choice of products and a growing confidence in precautionary labeling.

2.4 *Safety Assessment of Novel Proteins/Protein Sources*

Any food that contains proteins has or will have the potential to cause allergic reactions in some individuals. In order to prevent novel foods from posing a high risk of inducing new allergies, regulatory bodies require the assessment of safety, including the assessment of allergenicity. In Europe the Novel Food Law defines novel foods and novel food ingredients as those that have no history of safe significant use within the EU before 15 May 1997. The absence of a history of safe use can be the result of the food being new to the European Union (e.g., exotic fruits, insects, and algae) or of novel processing techniques. Food existing of or derived from genetically modified (GM) organisms may also have an allergenic hazard and thus also need to be assessed on safety, including allergenic potential.

Even though novel non-GM foods meet less consumer resistance than GM foods [34, 35], reality is that with respect to allergenic potential, it does not matter whether the novel food is from a GM source or a non-GM source; both may have the potential to add to the allergenic burden of the diet of the consumer. However, frameworks for the assessment of the allergenic potential of GM foods have been extensively developed and are more structured than those for other novel proteins/protein sources, for which the assessment will necessarily be more subjective.

2.4.1 **Allergenic Potential of GM Foods**

In the early 1990s it became clear that developments in gene technology might have significant implications for the food supply, particularly in terms of their potential to increase the quantity and quality of available foods. Currently, crops are genetically modified by incorporating new proteins to target deficiencies in nutrients and improve insect or salt resistance so that less pesticide can be used or plants can grow under non-optimal conditions and can often be produced in larger quantities on less land.

Theoretically there are four scenarios in which a novel GM food may be a risk for allergenicity: (1) The transfer of a known allergen or cross-reacting allergen into a food crop: It is generally accepted that the current *in silico* homology searches, combined with serology where appropriate, are sufficient to predict clinically relevant cross-reactivity with known allergens [36]. (2) The potential to increase endogenous allergenicity of the target food by increasing the level of expression of endogenous allergens: This risk to the population is somewhat controversial since it could be argued that at-risk allergic individuals will be avoiding that crop already, although the counterargument is that increasing levels of allergens could increase the number of individuals likely to acquire *de novo* sensitization. (3) There is an unexpected expression of novel proteins/peptides as a result of the introduction of unintended open reading frames (reviewed by [37]). (4) The novel protein may be a *de novo* allergen that has not been previously experienced by the human population. Where there is no previous history of dietary exposure, such as for those

target proteins that are isolated from alternative sources (fungi, bacteria, etc.) or where significant changes have been introduced to the amino acid sequence to confer particular benefits, or cross-linking the proteins results in protein structures with new characteristics, the current battery of tests available will not be sufficient to identify a truly novel allergen. As a consequence, there has been a growing interest in the design and development of appropriate animal models and their potential integration into safety assessment paradigms. In 2010, EFSA's Genetically Modified Organisms (GMO) Panel has adopted a scientific opinion on strategies for assessing the risk of allergenicity of GM plants and microorganisms and derived food and feed which is an update of the 2001 FAO/WHO Decision Tree that was recommended by the joint Food and Agriculture Organization and World Health Organization (FAO/WHO) Expert Consultation on Allergenicity of Foods Derived from Biotechnology [38].

The EFSA panel considers the *weight-of-evidence*, case-by-case approach the most appropriate way of assessing the allergenicity of GM food and feed [39]. In summary, it is recommended that with regard to the search for sequence homology and structural similarities, the local alignment method with a known allergen with a threshold of 35 % sequence identity over a window of at least 80 amino acids is considered a minimal requirement. When IgE binding tests are considered necessary, e.g., when there is sequence homology and/or structure similarity with known allergens, the use of individual sera from allergic individuals rather than pooled sera is recommended. In addition to the pepsin resistance test, it is recommended that the resistance to digestion of the newly expressed proteins is evaluated using other in vitro digestibility tests mimicking physiological conditions of humans; some protein is likely to survive intact into the lower intestine because of the following: (1) Protein does not enter the acid environment of the stomach as a pure test solution, but rather as part of a complex food matrix. Within a bolus of food passing through the stomach, it is unlikely that all protein is exposed to the extremes of acid pH. (2) Upon entering the stomach, proteins continuously leave the acid environment of the stomach, in a non-, partially, and fully digested state (Verhoeckx et al., in prep). In addition, proposals have been made with regard to other additional testing that may improve the assessment, cell-based tests (basophil activation tests (BAT), rat basophil leukemia (RBL) cell line transfected with human IgE receptor activation tests), and sharing of T cell epitopes between transgene-encoded proteins and allergens, e.g., animal models. Even though MHC restrictions in immune responses of animal models currently preclude any conclusions, the use of animal models can be considered as an enhancing step in the weight-of-evidence approach if they are further developed and validated. Animal models can be useful to investigate the capacity to elicit an allergic reaction and/or to act as an adjuvant in different environmental/exposure conditions and analyze the underlying mechanisms. Moreover, animal models can be used as a substitute for allergic human sera for a (pre-)screening of the immunological cross-reactivity of the novel protein with known allergens and may also be appropriate for studying the allergenicity of whole GM foods.

2.4.2 Allergenic Potential of Non-GM Foods

Detailed guidance on how to assess the allergenic potential of novel foods is mainly available for GM foods as described above, but not for “natural” non-GM foods that are newly introduced into the diet, such as alternatively processed proteins or new alternative food protein sources. Since new alternative protein sources (e.g., beet leaves, algae, and insects) are increasingly explored for a sustainable food production, this area will become more important. The assessment of these novel foods will have some similarities to EFSA’s weight-of-evidence approach for novel GM foods, in that the source of the protein needs to be defined. Homology searching is less appropriate for novel proteins/protein sources because there is no specific transgene to sequence. Gubesch et al. [40] designed a methodology to screen novel plant-derived foods for the presence of pan-allergens, IgE binding of food allergens, and clinical relevance of IgE, which illustrates a stepwise approach which could be adopted for the allergenicity assessment of other protein sources as well. Using this approach, cross-reactive allergens can be identified that possibly elicit an allergic reaction in a consumer already sensitized to a known food allergen.

Pan-allergens are ubiquitous proteins responsible for IgE cross-reactivity to a wide variety of related and unrelated allergenic sources. Usually the IgE cross-reactivity is a consequence of structural similarity between homologous proteins, which is translated into conserved sequence regions, three-dimensional folding, and function [41]. However, it has been shown that antibodies also can contribute to cross-reactivity by means of conformational diversity [42] and T cells may also display a cross-reactivity [43].

Although usually considered as minor allergens, sensitization to pan-allergens might be problematic as it bears the risk of developing multiple sensitizations. This may explain the phenomenon that the majority of patients seem to display adverse reactions upon contact to multiple allergen sources. For example, profilin is a pan-allergen that is recognized by IgE from about 20 % of the patients with allergies to birch pollen and plant food [44]. Therefore, as a first screening step, the presence of proteins homologous to known allergens needs to be confirmed by specific animal antibodies or antisera. Thereafter, a specific serum screen to identify potential IgE-binding capacity is appropriate. In this targeted serum screening, it is important to use sera from allergic patients with IgE reactivity to known pan-allergens.

Where the protein in the novel food is unrelated to any major food allergen or comes from an exotic source for which there is little information, e.g., insects or imported fruits, then an investigation into the phylogenetic relationships of the food source with other known foods should first be conducted. This would lead to the design of a targeted serum screen, in which sera from individuals previously sensitized against phylogenetically related foods should be screened for potential cross-reactivity (i.e., to peanuts if screening for a novel legume or to shrimp if screening for mealworms).

Finally, the clinical relevance of *in vitro* IgE binding should be verified by provocation tests (skin-prick tests or a double-blind placebo-controlled food challenge) in a clinical environment. The meaningfulness of such studies with special regard to market authorization of novel proteins/protein sources may be questionable when considering that *in vitro* IgE-binding properties in targeted serum screening and even clinical reactivity in preselected allergic patient groups may be observed with any novel vegetable or fruit [40]. Nonetheless, the continuing performance of comparable studies with novel foods can improve our knowledge about the allergenic potential of novel foods. Having sets of data on different novel foods, those foods with an extraordinary allergenic potential may be easier to identify.

Using the stepwise approach as mentioned above, cross-reactive allergens can be identified. However, this approach will not identify the potency to sensitize a predisposed individual *de novo*. To this end, the assessment should be supplemented with several assays. Since there is no single test available that predicts the *de novo* sensitizing potency of protein (sources), a set of assays should be conducted as described in the previous paragraph. Together with the possible allergen cross-reactivity data, the risk assessment can be performed on a weight-of-evidence base. TNO drafted a generic allergenicity risk assessment flow chart for novel food proteins and protein sources summarizing the key elements to be assessed (Fig. 2.2).

2.4.3 Allergenic Potential Hypoallergenic Food Proteins

In marketing, the term hypoallergenic should only be used when there is little likelihood that a food will cause an allergic reaction. A well-known example is the hypoallergenic infant milk formula. In this chapter, the term hypoallergenic refers to the significant reduction or elimination of individual known allergens from foods which may prove beneficial to human health.

Different approaches may be chosen to reduce the allergenic burden in foods: (1) physical removal of the targeted allergen, (2) genetic modification (RNA silencing or mutational knockout gene expression), and (3) food processing.

In plants, genetic modification may be used, provided that the elimination of allergenic proteins is not deleterious to the plant since such proteins may have a function in the plant or contribute to the nutritional value. To this end, RNA silencing (knockdown gene expression) and mutational knockout gene expression techniques are used. Several examples exist of genetically modified foods with reduced levels of allergenic proteins, such as rice [45], soybean [46], apple [47], peanuts [48], and tomato [49].

However, genetic modification is not the only approach which can be applied to the development of hypoallergenic foods and ingredients. Other novel processing techniques, such as high-pressure processing or extreme heat application, may reduce the allergenicity of problematic foods and ingredients [50]. Needless to say that genetically modified or alternatively processed foods should be assessed for allergenicity with methods and approaches described previously in this chapter.

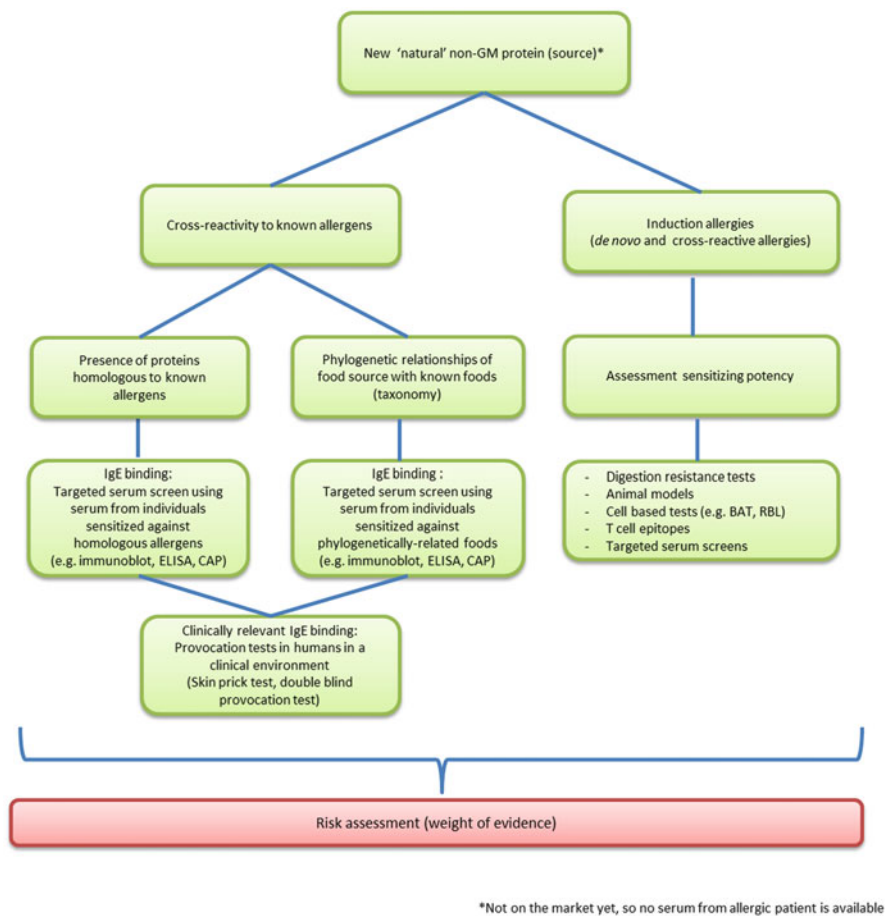


Fig. 2.2 Flow chart of allergenicity risk assessment novel “natural” non-GM protein/sources

2.5 Desirable Improvements in Allergenicity Assessment

Currently, the *weight-of-evidence*, case-by-case approach is considered the most appropriate way of assessing the allergenicity of GM food and feed. This assessment results in a “yes” or “no” verdict to the likelihood of being an allergenic protein (source), which results often in an oversimplification in terms of risk management: for example some novel allergenic protein (sources) may hardly have a significant impact on public health and not require active management but may become unnecessarily banned from the market. For risk management, it is desired to classify novel foods/proteins in a more subtle way. A promising approach is to compare the allergenicity of novel protein (sources) to known allergens with low, intermediate, and high allergenic potency. This relative allergenicity scaling

helps regulatory bodies to decide their priorities and thus improve allergy management by focusing resources to where they are needed.

In order to design a relative allergenicity scale of known allergens, one should first decide what defines allergenicity, i.e., which criteria should be included to identify allergenic foods of public health importance. To this end expert groups under the aegis of the ILSI Europe Food Allergy Task Force [51, 52] have previously proposed and evaluated the following as important criteria: (1) IgE-mediated character of adverse reactions, (2) the required dose of allergenic food to elicit adverse reaction in an already sensitized individual (threshold dose), (3) severity of adverse reactions, and (4) prevalence. It must be acknowledged that the severity of the adverse reactions is a result of the nature, extent, and duration of exposure rather than the inherent allergenic potency of the protein per se. Therefore in order to design a relative allergenicity scale, a risk-scoring system should be developed in which all available data on the IgE-mediated character, threshold dose, and prevalence of a panel of known low/intermediate/high allergens should be collected. Eventually, risk scores should be attributed to the combined available information for each newly introduced food. Such an overall scoring system is currently being developed by TNO as a useful tool for proper risk assessment and management of novel allergens.

3 Toxicological Safety Assessment of Food

3.1 The Toxicological Food Safety Assessment and the Threshold of Toxicological Concern

A toxicological food safety assessment is generally performed by a sequential approach. In this sequential approach traditionally four steps are taken. In the first step the composition of a food product should be completely identified and quantified. Based on this information, the potential hazard of each substance present in the food product should be separately assessed in the second step. For each substance a toxicological threshold has to be derived. Mostly this threshold is derived from animal toxicity data and converted using safety factors to thresholds for human exposure. Subsequently, in the third step an assessment of the past and the current or the expected exposure is performed. Finally, in the fourth step, a risk assessment is performed for each substance by comparing the calculated exposure to the threshold calculated to determine if the product is expected to pose a health concern. It should be noted that it is likely that not each single substance can be identified in a complex food matrix, resulting in data gaps which forces to perform (animal) toxicity testing using the product as a whole.

Foods are chemically complex food matrices (CCFM) and may consist of many substances. Therefore, the abovementioned sequential approach may lead to unnecessary detailed research and as such to a waste of time, animals, and resources. It is therefore essential to make better use of existing toxicological

information. In the past decades, alternative approaches have been developed for the safety assessment of substances. An example of an alternative approach is the Threshold of Toxicological Concern (TTC) concept. Based on a large toxicological dataset, containing chronic toxicity data of a wide variety of substances, the TTC concept provides a predictive safety assessment tool for substances for which no toxicological information is available. Depending on the molecular structure of a substance, a human exposure threshold value can be derived for most substances, below which there is a very low probability of a risk to human health.

The history of development and application of the TTC concept has been extensively reported in several publications [53–59]. Kroes et al. [54] published a decision tree in which different TTC values are proposed for different groups of substances. The decision tree distinguishes between genotoxic and/or high-potency carcinogens, organophosphates, and Cramer class III, II, and I substances. For all these classes of substances a conservative threshold of concern is determined based on a large set of chronic toxicity data. These thresholds are derived for lifetime daily exposure to the substances. Proteins, heavy metals, and polyhalogenated dibenzo-*p*-dioxins and related substances were excluded from the decision tree. Using the decision tree, for many substances a threshold can be derived which can be used in safety assessment (confirmed by the EFSA).

The main difference between the TTC concept and the traditional sequential approach is that the TTC concept is an exposure-driven approach. Since the past decades, exposure-driven safety assessment concepts are applied or developed in several frameworks, e.g., indirect food additives endorsed by the US FDA [60], flavoring components endorsed by JECFA [61], genotoxic impurities in pharmaceuticals endorsed by EMEA [62], contaminants in foods proposed by ILSI [53], and exposure-based waiving under REACH [63]. This is considered as a shift in safety assessment mindset away from the preferred traditional approach to investigate the exact toxicological profile of a substance after which the exposure is considered.

3.2 New Toxicological Safety Assessment Approach for Complex Food Matrices

Many of the compounds present in a complex food matrix might be present at low concentrations resulting in intakes below the TTC thresholds. In order to increase the efficiency of the assessment process of complex food matrices, without making concession to the safety aspects, TNO has developed a complex matrix safety assessment strategy (CoMSAS) which is a stepwise multidisciplinary strategy combining high-end sample preparation, fractionation, and analytical techniques with the TTC concept [64, 65]. The strategy concerns a stepwise analytical approach based on the exclusion of the presence of specific groups of substances using target analytical techniques following the Kroes et al. [54] decision tree and modifications as proposed by Munro et al. [58]. Also the recent conclusions of the EFSA opinion on the TTC concept [59] are taken into consideration. The major

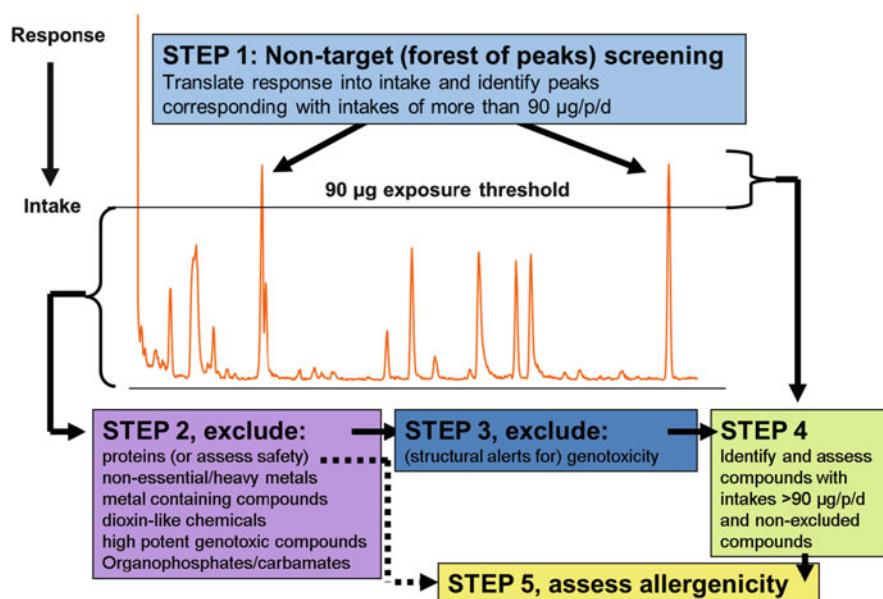


Fig. 2.3 Stepwise approach to assess safety of complex food matrices using CoMSAS

advantage of this strategy is that a full identification of all compounds in the matrix and concurrent compound-specific safety assessment is not needed for substances at low exposure levels. For a detailed description of categories of substances and their thresholds that are considered in the TTC and CoMSAS approach it is referred to Kroes et al. [54], Munro [58], and EFSA [59]. The CoMSAS is schematically presented in Fig. 2.3 and will be further explained below using a stepwise approach.

3.2.1 Step 1

The first step concerns a general analytical screening of the complex food matrix. The goal of this step is to detect as many substances as possible present in the complex food matrix, without identification, and to estimate the concentration at which they are present. This step is performed using a nontarget forest-of-peaks (screening) approach in which a number of analytical techniques is being used to cover a wide variety of physical chemical properties which substances present in the CCFM may possess. Based on the estimated concentration of the detected substances, an exposure estimate for each “peak” can be made based on food consumption information of the total food product concerned. For substances below the exposure threshold of 90 µg/day, no further assessment has to be performed when certain categories of substances can be excluded or are below specific thresholds (see further steps). In principle only the substances with an exposure exceeding 90 µg/day have to be identified after which a substance-specific

safety assessment needs to be performed (see step 4). For identification and quantification of these substances analytical methods like gas chromatography coupled to mass spectrometry, combined with LC and high-resolution mass spectrometry or NMR analysis, may be used. It should be noted that the identity of substances that are present in higher amounts in a complex food matrix is in many cases already known, particularly in case these substances are intentionally present for a specific purpose in the food.

3.2.2 Step 2

As indicated before, the TTC concept cannot be applied to proteins, heavy metals, and polyhalogenated dibenzo-*p*-dioxins and related substances, as these substances have a higher toxicity or are not included in the dataset underlying the TTC concept [54]. Also aflatoxin-like, azoxy- or *N*-nitroso substances do not have a TTC threshold and need a substance-specific safety assessment. Moreover, the threshold of 90 µg/day used in the CoMSAS approach is based on the TTC threshold of Cramer class III substances, which is higher than the threshold for organophosphates and carbamates (18 µg/day). Therefore, for the substances present in the food matrix with an estimated exposure below 90 µg/day, it has to be excluded that they belong to the class of substances as indicated above. Using targeted analytical methods the complex food matrix can be assessed for the presence of proteins, nonessential/heavy metals, metal-containing compounds, dioxin-like substances, highly potent genotoxic substances, and organophosphates/carbamates.

3.2.3 Step 3

For substances with a genotoxic potency a threshold of 0.15 µg/day is set in the TTC decision tree. For applying a general TTC threshold of 90 µg/day, the presence of substances with a genotoxic potency needs to be excluded. The best way to assess this is to test the whole food matrix or extracts thereof for the presence of potential genotoxic substances using a biological assay. Conventional state-of-the-art assays such as the AMES test were not developed to test CCFM. Alternative assays such as the BlueScreen HC assay [66], which is sensitive for gene mutations, clastogenicity, and aneugenicity, can be used as an alternative for this purpose. In case a food or an extract thereof is tested positive in such a genotoxicity assay it is unclear which substance(s) actually causes (cause) the genotoxic response. Fractionation of the food matrix using extraction and separation techniques and testing these fractions for their genotoxic potential may help to finally identify the fraction containing the genotoxic substance(s) and subsequently to find the substance(s) responsible for this effect. This can be followed by identification of the responsible substances present in the positive fraction followed by substance-specific risk assessment. A high-throughput genotoxicity assay such as the BlueScreen HC assay

in combination with fractionation and further analytical research is very helpful for this purpose.

3.2.4 Step 4

The next steps of the CoMSAS approach are related to substances present at levels resulting in intakes above 90 µg/day and assessment of substances that appeared present in step 2 or 3. Step 4 requires assessment of these substances. The safety assessment of the substance can be done based on the TTC threshold of the actual Cramer class, based on substance-specific toxicological data (e.g., retrieved from public literature), or related to legal limit values for the substance (e.g., in case of heavy metals and aflatoxins). In case no substance-specific toxicological information is available, the evaluation can also be performed using available toxicological information from comparable chemical substances (in structure and mode of action). Based on the toxicological evaluation a substance-specific human health limit value can be established which can be compared to the estimated daily intake of the substance. In case it cannot be excluded that a health risk may occur, measures might be taken to reduce or prevent the presence of the substance concerned.

3.2.5 Step 5

In case proteins appear present in the complex food matrix, an allergenicity assessment may be necessary. In case of known food allergens labeling of the product can be performed, and/or an assessment can be made for the probability of an allergic response in an allergic individual. For new proteins/unknown food allergens, new safety assessment approaches will be developed (see later on in this chapter).

3.3 Discussion on CoMSAS

The CoMSAS approach as described above is most efficient in case in a complex food matrix a limited number of substances are present to which the daily exposure exceeds 90 µg/day. To investigate this, TNO has applied the approach to migrants from food contact materials (non-intentionally added substances), natural food supplements, and processing of herbs. The pilot cases demonstrated that the threshold of 90 µg/day would be sufficiently high for a reasonable applicability in the safety assessment of complex food matrices. It should be noted that the TTC concept is not a static concept, but remains under development as more information is expected to be published in near future. The TTC thresholds may therefore also change depending on the information available. The exclusion scheme and strategy

presented in this chapter are based on the most recent literature on TTC at the time of writing. For example, in its evaluation in 2012 the EFSA concluded that the chronic toxicity dataset underlying the threshold for Cramer class II substance (540 µg/day) is not sufficient and that therefore the threshold of Cramer class III substances (90 µg/day) should be applied to Cramer class II substances. Based on this conclusion, the threshold used in the CoMSAS approach was set at 90 µg/day. TNO currently assesses the chronic toxicity dataset underlying the TTC concept to assess whether on a scientifically valid base other thresholds for (sub)classes of the Cramer class III substances can be derived. Details of this study will be submitted for publication upon completion. Based on the outcome of this assessment eventually also the threshold used for the CoMSAS approach may be adapted.

The TTC approach was developed for the evaluation of single substances present in food. The CoMSAS approach is developed for the evaluation of complex food matrices (mixtures). It might occur that in a complex food matrix different substances with similar or interacting toxicological activity are present that will appear as separate “peaks.” The aspect of interaction between (toxicity of) substances at low dosages was evaluated by the Dutch National Institute for Public Health and Environment [67] and Boobis [68]. Both concluded that at low doses interaction between substances such as synergy and antagonism is not likely to occur. However, Pieters and Konemann concluded that dose addition even at low concentrations of substances in mixtures cannot be excluded. In most cases a factor of 10 appeared to be sufficient to cover for dose addition. TNO has assessed this further and concluded that “to some extent cumulative effects at exposure levels for each substance at or below 90 µg/day 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” [69].

The current sequential approach in toxicological safety assessment may lead to unnecessary detailed research, especially considering that more and more complex food matrices have to be assessed. To stimulate food innovation and reduce animal toxicity testing, scientifically sound but pragmatic safety assessment strategies are required in which existing relevant information is used optimally. Besides the TTC principle and read across for single substances, CoMSAS provides a pragmatic approach for the assessment of matrices without the need for full identification. The CoMSAS complies with the current accepted state of the art regarding the TTC concept. In future, the TTC concept might be refined or extended with other (higher) thresholds of concerns for classes of substances due to new knowledge and literature. Moreover, new non-testing concepts might be developed. By making more optimal use of existing information, based on large datasets of toxicological data, these data in combination with for example toxicogenomics information can provide even more pragmatic ways of safety assessment in future.

References

1. Sampson HA, Burks AW (1996) Mechanisms of food allergy. *Annu Rev Nutr* 16:161–177
2. Sampson HA (2004) Update on food allergy. *J Allergy Clin Immunol* 113(5):805–819
3. Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA et al (2003) The biology of IGE and the basis of allergic disease. *Annu Rev Immunol* 21:579–628
4. Eigenmann PA, Beyer K, Wesley BA, Lack G, Liacouras CA, Hourihane JO et al (2008) New visions for food allergy: an iPAC summary and future trends. *Pediatr Allergy Immunol* 19(Suppl 19):26–39
5. Kagan RS, Joseph L, Dufresne C, Gray-Donald K, Turnbull E, Pierre YS et al (2003) Prevalence of peanut allergy in primary-school children in Montreal, Canada. *J Allergy Clin Immunol* 112(6):1223–1228
6. Roehr CC, Edenharter G, Reimann S, Ehlers I, Worm M, Zuberbier T et al (2004) Food allergy and non-allergic food hypersensitivity in children and adolescents. *Clin Exp Allergy* 34(10):1534–1541
7. Prescott S, Allen KJ (2011) Food allergy: riding the second wave of the allergy epidemic. *Pediatr Allergy Immunol* 22(2):155–160
8. Nauta AJ, Engels F, Knippels LM, Garssen J, Nijkamp FP, Redegeld FA (2008) Mechanisms of allergy and asthma. *Eur J Pharmacol* 585(2–3):354–360
9. Berin MC, Mayer L (2009) Immunophysiology of experimental food allergy. *Mucosal Immunol* 2(1):24–32
10. Eigenmann PA (2009) Mechanisms of food allergy. *Pediatr Allergy Immunol* 20(1):5–11
11. Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, Fuchs RL (1996) Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Crit Rev Food Sci Nutr* 36(Suppl):S165–S186
12. Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H (2008) Allergens are distributed into few protein families and possess a restricted number of biochemical functions. *J Allergy Clin Immunol* 121(4):847–852
13. NIAID-Sponsored Expert Panel, Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA et al (2010) Guidelines for the diagnosis and management of food allergy in the United States: Report of the NIAID-sponsored expert panel. *J Allergy Clin Immunol* 126(6 Suppl):S1–S58
14. Selgrade MK, Bowman CC, Ladics GS, Privalle L, Laessig SA (2009) Safety assessment of biotechnology products for potential risk of food allergy: implications of new research. *Toxicol Sci* 110(1):31–39
15. Malmheden Yman I (2004) Detection of inadequate labelling and contamination as causes of allergic reactions to food. *Acta Aliment* 33(4):347–357
16. Boyano-Martínez T, García-Ara C, Pedrosa M, Díaz-Pena JM, Quirce S (2009) Accidental allergic reactions in children allergic to cow's milk proteins. *J Allergy Clin Immunol* 123(4):883–888
17. Sheth SS, Waserman S, Kagan R, Alizadehfar R, Primeau MN, Elliot S et al (2010) Role of food labels in accidental exposures in food-allergic individuals in Canada. *Ann Allergy Asthma Immunol* 104(1):60–65
18. Spanjersberg MQ, Kruizinga AG, Rennen MA, Houben GF (2007) Risk assessment and food allergy: the probabilistic model applied to allergens. *Food Chem Toxicol* 45(1):49–54
19. Pele M, Brohee M, Anklam E, van Hengel AJ (2007) Peanut and hazelnut traces in cookies and chocolates: relationship between analytical results and declaration of food allergens on product labels. *Food Addit Contam* 24(12):1334–1344
20. Spanjersberg MQ, Knulst AC, Kruizinga AG, Van DG, Houben GF (2010) Concentrations of undeclared allergens in food products can reach levels that are relevant for public health. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 27(2):169–174
21. Hefle SL, Furlong TJ, Niemann L, Lemon-Mule H, Sicherer S, Taylor SL (2007) Consumer attitudes and risks associated with packaged foods having advisory labeling regarding the presence of peanuts. *J Allergy Clin Immunol* 120(1):171–176

22. Noimark L, Gardner J, Warner JO (2009) Parents' attitudes when purchasing products for children with nut allergy: a UK perspective. *Pediatr Allergy Immunol* 20(5):500–504
23. Madsen CB, Hattersley S, Buck J, Gendel SM, Houben GF, Hourihane JO et al (2009) Approaches to risk assessment in food allergy: report from a workshop “developing a framework for assessing the risk from allergenic foods”. *Food Chem Toxicol* 47(2):480–489
24. Kerbach S, Alldrick AJ, Crevel R, Domotor L, DunnGalvin A, Mills ENC et al (2009) Managing food allergens in the food supply chain-viewed from different stakeholder perspectives. *Qual Assur Safety Crops Foods* 1:50–60
25. Allergen Bureau (2010) Allergen bureau labelling review survey 2009. *Food Aust* 62(8):344–345
26. Wensing M, Penninks AH, Hefle SL, Koppelman SJ, Bruijnzeel-Koomen CA, Knulst AC (2002) The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. *J Allergy Clin Immunol* 110(6):915–920
27. Wensing M, Penninks AH, Hefle SL, Akkerdaas JH, van Ree R, Koppelman SJ et al (2002) The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. *Clin Exp Allergy* 32(12):1757–1762
28. Bindslev-Jensen C, Briggs D, Osterballe M (2002) Can we determine a threshold level for allergenic foods by statistical analysis of published data in the literature? *Allergy* 57(8):741–746
29. Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C, Bruijnzeel-Koomen C et al (2004) A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? *Clin Exp Allergy* 34(5):689–695
30. Gendel S, Buchanan R, Dennis S, Acheson D, Assimon SA, Beru N et al (2008) Approaches to establish thresholds for major food allergens and for gluten in food. *J Food Protect* 71(5):1043–1088
31. Taylor SL, Crevel RW, Sheffield D, Kabourek J, Baumert J (2009) Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals. *Food Chem Toxicol* 47(6):1198–1204
32. Blom WM, Vlieg-Boerstra BJ, Kruizinga AG, Van Der Heide S, Houben GF, Dubois AEJ (2013) Threshold dose distributions for 5 major allergenic foods in children. *J Allergy Clin Immunol* 131(1):172–179
33. Taylor SL, Baumert JL, Kruizinga AG, Remington BC, Crevel RWR, Brooke-Taylor S, Allen KJ, The Allergy Bureau of Australia & New Zealand, Houben GF (2014) Establishment of reference doses for residues of allergenic foods: report of the VITAL Expert Panel. *Food Chem Toxicol* 63:9–17
34. Frewer L, Lassen J, Kettlitz B, Scholderer J, Beekman V, Berdal KG (2004) Societal aspects of genetically modified foods. *Food Chem Toxicol* 42(7):1181–1193
35. Frewer LJ, Scholderer J, Bredahl L (2003) Communicating about the risks and benefits of genetically modified foods: the mediating role of trust. *Risk Anal* 23(6):1117–1133
36. Aalberse RC, Stadler BM (2006) In silico predictability of allergenicity: from amino acid sequence via 3-D structure to allergenicity. *Mol Nutr Food Res* 50(7):625–627
37. Ladics GS, Cressman RF, Herouet-Guicheney C, Herman RA, Privalle L, Song P et al (2011) Bioinformatics and the allergy assessment of agricultural biotechnology products: industry practices and recommendations. *Regul Toxicol Pharmacol* 60(1):46–53
38. FAO/WHO (2001) Report of joint FAO/WHO expert consultation on foods derived from biotechnology, 22–25 Jan 2001
39. EFSA Panel of Genetically Modified Organisms (2010) Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. *EFSA J* 8(7):1700
40. Gubesch M, Theler B, Dutta M, Baumer B, Mathis A, Holzhauser T et al (2007) Strategy for allergenicity assessment of ‘natural novel foods’: clinical and molecular investigation of exotic vegetables (water spinach, hyacinth bean and Ethiopian eggplant). *Allergy* 62(11):1243–1250
41. Hauser M, Roulias A, Ferreira F, Egger M (2010) Panallergens and their impact on the allergic patient. *Allergy Asthma Clin Immunol* 6(1):1. doi:[10.1186/1710-1492-6-1](https://doi.org/10.1186/1710-1492-6-1)

42. James LC, Roversi P, Tawfik DS (2003) Antibody multispecificity mediated by conformational diversity. *Science* 299(5611):1362–1367
43. Bohle B (2007) The impact of pollen-related food allergens on pollen allergy. *Allergy* 62(1):3–10
44. Scheurer S, Wangorsch A, Nerkamp J, Skov PS, Ballmer-Weber B, Wuthrich B et al (2001) Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (pyr c 4), cherry (pru av 4) and celery (api g 4) with birch pollen profilin bet v 2. *J Chromatogr B Biomed Sci Appl* 756(1–2):315–325
45. Nakamura R, Matsuda T (1996) Rice allergenic protein and molecular-genetic approach for hypoallergenic rice. *Biosci Biotechnol Biochem* 60(8):1215–1221
46. Herman EM, Helm RM, Jung R, Kinney AJ (2003) Genetic modification removes an immunodominant allergen from soybean. *Plant Physiol* 132(1):36–43
47. Gilissen LJ, Bolhaar ST, Matos CI, Rouwendal GJ, Boone MJ, Krens FA et al (2005) Silencing the major apple allergen mal d 1 by using the RNA interference approach. *J Allergy Clin Immunol* 115(2):364–369
48. Dodo H, Konan K, Viquez O (2005) A genetic engineering strategy to eliminate peanut allergy. *Curr Allergy Asthma Rep* 5(1):67–73
49. Lorenz Y, Enrique E, Lequynh L, Fotisch K, Retzek M, Biemelt S et al (2006) Skin prick tests reveal stable and heritable reduction of allergenic potency of gene-silenced tomato fruits. *J Allergy Clin Immunol* 118(3):711–718
50. Davis PJ, Smales CM, James DC (2001) How can thermal processing modify the antigenicity of proteins? *Allergy* 56(Suppl 67):56–60
51. van Bilsen JH, Ronsmans S, Crevel RW, Rona RJ, Przyrembel H, Penninks AH et al (2011) Evaluation of scientific criteria for identifying allergenic foods of public health importance. *Regul Toxicol Pharmacol* 60(3):281–289
52. Björkstén B, Crevel R, Hischenhuber C, Lovik M, Samuels F, Strobel S et al (2008) Criteria for identifying allergenic foods of public health importance. *Regul Toxicol Pharmacol* 51(1):42–52
53. Kroes R, Galli CL, Munro I, Schilter B, Tran L-A, Walker R, Würtzen G (2000) Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. *Food Chem Toxicol* 38:255–312
54. Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Würtzen G (2004) Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chem Toxicol* 42:65–83
55. Kroes R, Kleiner J, Renwick A (2005) The threshold of toxicological concern concept in risk assessment. *Toxicol Sci* 86(2):226–230
56. Renwick A (2004) Toxicology databases and the concept of thresholds of toxicological concern as used by the JECFA for the safety evaluation of flavouring agents. *Toxicol Lett* 149:223–234
57. Renwick A (2005) Structure-based thresholds of toxicological concern—guidance for application to substances present at low levels in the diet. *Toxicol Appl Pharmacol* 207:585–591
58. Munro IC, Renwick AG, Danielewska-Nikiel B (2008) The Threshold of Toxicological Concern (TTC) in risk assessment. *Toxicol Lett* 180:151–156
59. EFSA (2012) Scientific opinion on exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). *EFSA J* 10(7):2750
60. US FDA (1995) US Food and Drug Administration. Food additives: threshold of regulation for substances used in food-contact articles; final rule. *Fed Regist* 60:36582–36596, July 17
61. JECFA (1997) Evaluation of certain food additives and contaminants. Forty-Sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO Technical Report Series (TRS) No. 868

62. Müller L, Mauthe RJ, Riley CM, Andino MM, De Antonis D, Beels C, DeGeorge J, De Knaep AGM, Ellison D, Fagerland JA, Frank R, Fritschel B, Galloway S, Harpur E, Humfrey CDN, Jacks AS, Jagota N, Mackinnon J, Mohan G, Ness DK, O'Donovan MR, Smith MD, Vudathala G, Yotti L (2006) A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Regul Toxicol Pharmacol* 44:198–211
63. Carthew P, Clapp C, Gutsell S (2009) Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. *Food Chem Toxicol* 47:1287–1295
64. Rennen MAJ, Koster S, Krul CAM, Houben GF (2011) Application of the threshold of toxicological concern (TTC) concept to the safety assessment of chemically complex food matrices. *Food Chem Toxicol* 49(4):933–940
65. Koster S, Boobis AR, Cubberley R, Hollnagel HM, Richling E, Wildemann T, Würtzen G, Galli CL (2011) Application of the TTC concept to unknown substances found in analysis of foods. *Food Chem Toxicol* 49:1643–1660
66. Hughes C et al (2012) Development of a high-throughput Gaussia luciferase reporter assay for the activation of the GADD45a gene by mutagens, promutagens, clastogens, and aneugens. *J Biomol Screen* 17(10):1302–1315
67. Pieters MN, Kónneman WH (1997) Toxicology of mixtures: a review of the safety factor of 100 applied in the safety evaluation of chemical mixtures. Dutch National Institute for Public Health and Environment (RIVM), Report number 620110004, Bilthoven, The Netherlands
68. Boobis A (2009) Critical analysis of literature on low dose synergy for use of TTC in screening chemical mixtures for risk assessment. *Toxicol Lett* 189–1:S51
69. Leeman WR, Krul L, Houben GF (2013) Complex mixtures: relevance of combined exposure to substances at low dose levels. *Food Chem Toxicol* 58:141–148

<http://www.springer.com/978-3-319-06150-4>

Pharma-Nutrition

An Overview

Folkerts, G.; Garssen, J. (Eds.)

2014, XV, 484 p. 53 illus., 16 illus. in color., Hardcover

ISBN: 978-3-319-06150-4