

Toxicology of simple and complex mixtures

John P. Groten, Victor J. Feron and Jürgen Sühnel

Humans are exposed to mixtures of chemicals, rather than to individual chemicals. From a public health point of view, it is most relevant to answer the question of whether or not the components in a mixture interact in a way that results in an increase in their overall effect compared with the sum of the effects of the individual components. In this article, options for the hazard identification and risk assessment of simple and complex chemical mixtures will be discussed. In addition, key research needed to continue the development of hazard characterization of chemical mixtures will be described. Clearly, more collaboration among toxicologists, model developers and pharmacologists will be necessary.

Usually, humans are exposed simultaneously or sequentially to large numbers of components (e.g. food, drugs, consumer products and drinking water) at various exposure levels and through multiple exposure routes. However, human health risk is mainly addressed on the basis of *in vivo* toxicity data generated from exposure to individual chemicals at fixed doses through a single exposure route and medium. In addition, application of uncertainty factors to allow for interspecies and interindividual variation generally does not take into account the possibility of combined action or interactions. Thus, new research initiatives in the arena of toxicology are aimed at: (1) identifying whether such potential combined or interactive adverse effects from exposure to mixtures of compounds are

likely or unlikely to occur in humans, and (2) exploring ways in which such joint actions of compounds should be studied. In this article, emerging issues and approaches that are available for the evaluation of risks posed by exposure to chemical mixtures will be discussed and some key research needs will be identified.

Safety evaluation of mixtures

To study the toxicology of chemical mixtures successfully and to assess their potential health risks properly, it is crucial to understand the basic concepts of combined action and interaction of chemicals, and to distinguish between whole-mixture analysis (top-down approach) and component-interaction analysis (bottom-up approach)¹. Moreover, it is equally important to distinguish between simple and complex mixtures, as defined by Feron *et al.*² A simple mixture is a mixture that consists of a relatively small number of chemicals (e.g. ten or less), the composition of which is qualitatively and quantitatively known. A complex mixture is a mixture that comprises tens, hundreds or thousands of chemicals, the composition of which is qualitatively and quantitatively not fully known. Examples of the former are a cocktail of pesticides, a combination of medicines or a group of allyl alcohol esters; examples of the latter are a workplace atmosphere, drinking water, welding fumes or a natural flavoring complex.

A general scheme for the safety evaluation of simple mixtures is proposed in Fig. 1. This scheme starts with a dichotomy between the testing of either entire mixtures or individual components within a mixture. The most pragmatic approach is to test the toxicity of the mixture without identifying the type of interactions between individual chemicals. Whole-mixture effects can be assessed by testing the mixture in its entirety. If adverse effects are found in relevant toxicity studies, these data can be used to characterize the hazard following exposure to the entire mixture; such data can finally be used for risk assessment. However, this approach will not identify the chemicals responsible for interactions, if any, even if sufficient information on the dose–effect relationship is available. In addition, methods that are suitable for testing entire mixtures depend on whether there is information directly available on the mixture of concern, or only on (sufficiently) similar mixtures or groups of similar mixtures.

A more detailed approach in the safety evaluation of mixtures is to assess the combined action of the components in the mixture. In this component-based analysis, several experimental designs can be followed

John P. Groten*
Victor J. Feron
Dept of Explanatory
Toxicology, TNO Nutrition
and Food Research, PO
Box 360, 3700 AJ Zeist,
The Netherlands.
*e-mail:
Groten@voeding.tno.nl
Jürgen Sühnel
Biocomputing Group,
Institut für Molekulare
Biotechnologie,
Beutenbergstr. 11,
D-07745 Jena, Germany.

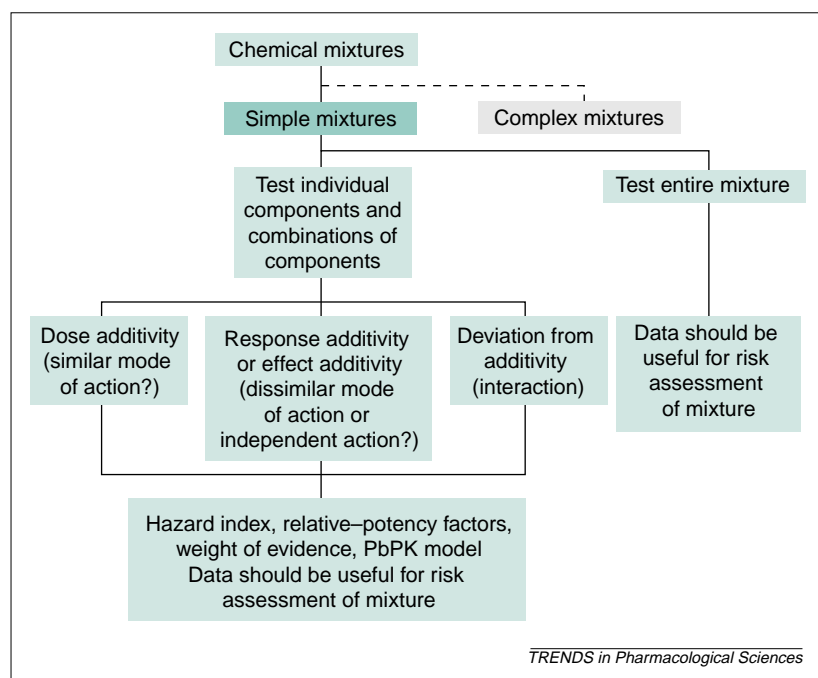


Fig. 1. Safety evaluation of simple mixtures. Either the whole mixture or individual components of the mixture can be analyzed. Abbreviation: PbPK, pharmacokinetic physiologically based modeling.

and the choice of experimental design is merely dependent on the complexity and number of components of the mixture. The major concern in a component-based analysis is whether the data are composed of components that can be thought of as acting via similar toxicological processes, whether the mixture components act by the same mode of action or whether the mode of actions are functionally independent.

For both types of assessment (component based or entire mixture), the outcome shown in Fig. 1 is a quantitative assessment that continues with a complete risk characterization, including uncertainty. The availability and quality of exposure and toxicity data will drive, to a large extent, the type of risk assessment for the entire mixture. One approach on which to base risk assessment is the hazard index (HI), as originally proposed in the US Environmental Protection Agency mixture guidelines³. In this approach, the hazard quotients are calculated for individual compounds and the quotients for each compound in the mixture are then added. A different approach⁴ takes into account both synergistic and antagonistic interactions in the derivation of the HI. In this approach, a weight-of-evidence (WOE) classification is followed to estimate the joint actions (i.e. additivity, antagonism and synergism) for binary mixtures of chemicals, based on information about the individual compounds. Several weighing factors are used in the final classification, such as the mechanistic understanding of the binary interactions, the demonstration of toxicity and additional uncertainty factors (i.e. modifiers of interactions, such as route of exposure and *in vitro* data, among others). To demonstrate its usefulness, the WOE method must first be validated using experimental studies, as illustrated by Mumtaz *et al.*⁵

Another strategy used to assess the hazard of mixed exposure is the toxic equivalency factor (TEF) approach. This method comes from the area of environmental contaminants, where it is widely recognized that structurally related chemicals can exhibit similar toxicity and mode of action, and therefore might show similar joint actions. For example, the group of polychlorinated di-benzo-*p*-dioxins (PCDDs) and dioxin-like PCBs share a common mechanism of toxic action mediated via a specific intracellular binding protein, the Ah receptor. To assess the joint action, each congener has been allocated a TEF relative to the most potent congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The total potency of combined occurrence of PCDDs and dioxin-like PCBs is calculated as the sum of the concentration of each individual congener multiplied by its TEF. The summation of 'concentration \times TEF' for each congener assumes joint action leading to full dose additivity⁶.

The scheme presented in Fig. 1 is deceptively simple, however, because many of the issues that are represented in the diagram require the use of scientific judgement or data that might not be readily available. Furthermore, the true toxicological mechanism is rarely known for a given mixture or even for most of its

components; the judgements that are made of similar action or independence of action, for example, will be uncertain. Thus, one approach that toxicologists can take is to implement several of the methods that are practical to apply and then evaluate the range of health risk estimates that are produced.

How to test for an interaction

An interaction might occur in the toxicokinetic phase (i.e. processes of uptake, distribution, metabolism and excretion) or in the toxicodynamic phase (i.e. effects of chemicals on the receptor, cellular target or organ). The most obvious cases for interactions in the toxicokinetic phase are enzyme induction or inhibition, or both, because metabolism is an important determinant of toxicity (either reactive intermediates are formed – activation – or the toxic agent is removed – detoxification). Compounds that influence the amount of biotransformation enzymes can have large effects on the toxicity of other chemicals. Additionally, uptake and excretion might be active processes. Competition between substrates for the same pumps, in addition to competition between substrates for the biotransformation enzymes themselves, follow the rules for receptors described above.

In principle, to assess interactions, mechanistic or empirical models can be used. 'Empirical' means that only information on doses or concentrations, and effects is available in addition to an often empirically selected quantitative dose–response relationship. However, 'mechanistic' means that additional information on the sequence of reaction steps is available and quantitative parameters are known. For example, to describe the interaction between multiple chemicals and a target receptor or enzyme, the mechanism of joint action can be described by Michaelis–Menten kinetics. In general, this will result in a phenomenon called 'competitive agonism'. The ultimate combined effect will be less than expected on the basis of effect addition because of competition for the same receptor. In fact, this type of interaction can also be considered as a special case of similar joint action (dose additivity). A basic problem with mechanistic models is that in most of the combination studies there is a gap in our information that would allow the application of these models. Therefore, empirical models currently play the dominant role.

Zero interaction

Testing for a possible interaction between two or more agents requires a precise definition of the case of no or zero interaction. A plethora of different empirical methods has been proposed but a generally agreed definition of zero interaction does not yet exist^{7–10}. However, almost all of these methods can be traced back to three basic concepts, termed effect or response additivity, independence (Bliss independence) and dose additivity (Loewe additivity) (Fig. 2). The independence criterion does not seem to be widely used in toxicology, except for special fields such as the toxic effects of combined irradiation.

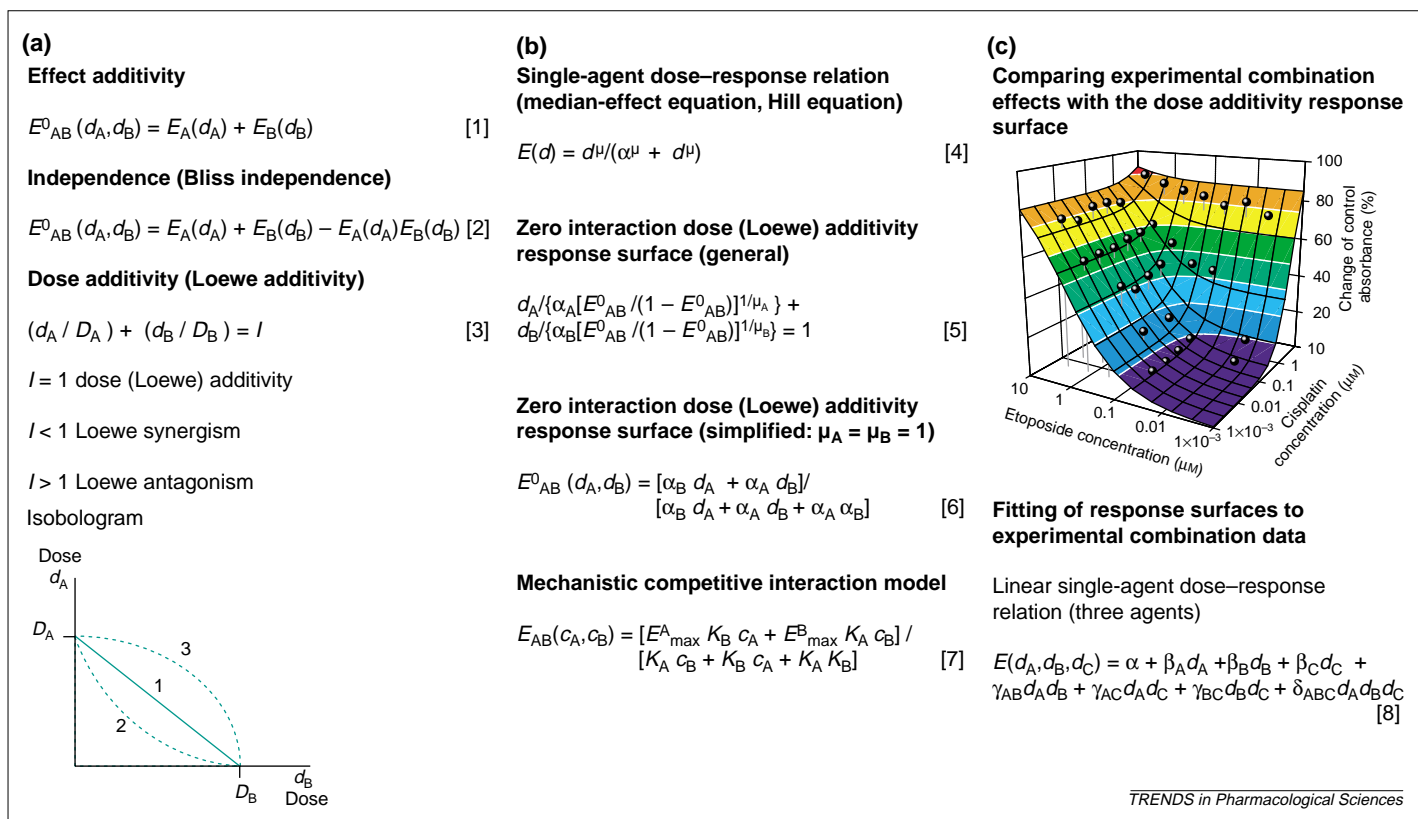


Fig. 2. Testing for interactions between biologically active agents.

(a) Three widely used empirical criteria for defining zero interaction are effect additivity (Eqn 1), independence (Eqn 2) and dose additivity (Eqn 3). A combination effect is said to be non-interactive, according to one of these criteria, if it fulfils the corresponding definition equation. Deviations indicate synergism (effect stronger than expected) or antagonism (effect smaller than expected). So far, there is no consensus on which of these criteria should be considered as the most appropriate. It is also possible that different criteria are appropriate for different mechanisms (e.g. action on one and the same receptor or on different receptors). The calculation of zero interaction combination effects is straightforward for Eqns 1 and 2, whereas the application of Eqn 3 requires the determination of isoboles (lines of equal effects) by a systematic variation of combination doses (isobolographic analysis). The shapes of these isoboles in an isobologram then indicate the type of interaction. For linear dose axes, lines 1–3 represent dose (Loewe) additivity, synergism and antagonism, respectively [d_A , d_B , combination doses; E_A , E_B , E_{AB}^0 , single-agent and zero interaction combination effects; D_A , D_B , doses of agents A and B that produce the same magnitude of effect as a dose combination (d_A, d_B) when used alone; I , interaction index].

(b) Zero interaction response surfaces for dose (Loewe) additivity. To treat the dose additivity criterion on an equal footing with the other criteria, single-agent dose–response relations can be combined with Eqn 3. If this is done for the median effect or the Hill equation (Eqn 4), for example, one arrives at Eqn 5, which describes a response surface that fulfils the dose-additivity criterion throughout the complete dose and effect range. (For a description of the parameters α and μ , see below). Equation 5 is of an implicit nature and has to be solved by iteration. For $\mu_A = \mu_B = 1$, a simplified explicit version is obtained (Eqn 6). For comparison, the equation that describes the mechanistic model of competitive interaction^{11–13} is shown (c_A , c_B ,

concentration of agents A and B; E_{max}^A , E_{max}^B , maximum effects; K_A , K_B , association constants). The dose additivity surface (Eqn 6) is identical to the model of competitive interaction ($E_{max}^A = E_{max}^B = 1$; Eqn 7)¹⁴, which is interesting because the assumptions made in deriving Eqns 6 and 7 are completely independent.

(c) Two types of response surface modeling. In the upper part, a color-coded (Loewe) additivity surface is compared with experimental combination data for the cytotoxic effect of etoposide–cisplatin combinations on NCI-H226 cell lines in ACL-4 medium¹⁵. Adopting an isobolographic approach, it was concluded that there was no synergism at the 50% level. Here, it is shown that with the very same data set, one can determine potential interactions in the complete effect range between 0 and 100%, according to the following approach: determination of the parameters α and μ for the single-agent dose–response relations (Eqn 4); calculation of the dose additivity surface (Eqn 5); and comparison of this surface to experimental data. Whether or not the deviations identified are statistically significant must be determined. The projection of the surface contour lines (which represent equal effects) onto dose plains yields an isobologram for various effect levels. Note that, owing to the logarithmic dose scale, the isoboles are not linear, even though they describe dose additivity. The parameters in Eqn 4 were determined using Origin (<http://www.originlab.com>) and all other calculations were carried out with the program CombiTool (Ref. 16; etoposide: $\alpha = 1.19469 \pm 0.17138$, $\mu = 0.73158 \pm 0.0733$ μM ; cis-diammine-dichloroplatinum(II): $\alpha = 2.72195 \pm 0.2301$; $\mu = 1.33533 \pm 0.13342$ μM ; $\alpha = \text{IC}_{50}$, $\mu = \text{slope}$). Contrary to the approach described above, mathematical expressions for response surfaces can also be fitted directly to experimental data^{17,18}. Equation 8 shows an example with linear single-agent dose–response relations¹⁷. In this case, significant values of the cross term parameters γ and δ directly indicate a deviation from dose additivity. (c) Adapted, with permission, from Ref. 16.

Occasionally, ‘response additivity’ is called ‘absolute response additivity’ and ‘independence’ can be referred to as ‘relative effect additivity’. Therefore, both approaches are subsumed under response–effect additivity in Fig. 1. Note that all of these terms describe the case of zero interaction according to different criteria. For deviations from non-interactive combination effects, there is no uniform terminology. Effects stronger than expected are, for example, often designated as resulting from synergism, potentiation and supra-additivity, and

effects smaller than expected can be designated as resulting from antagonism, sub- or infra-additivity or inhibition. These naming schemes are unnecessarily complex. We therefore prefer a simple and uniform terminology scheme that has been proposed^{17,20}, which uses the terms Loewe synergism–antagonism and Bliss synergism–antagonism to describe deviations from combination effects calculated either according to dose additivity (Loewe additivity) or relative effect additivity (Bliss independence) criteria.

If a particular criterion has been adopted for calculating non-interactive combination effects, combination effects can be tested experimentally for a potential interaction. The effect found must be compared with the theoretically expected non-interactive combination effect. Stronger than expected effects indicate synergism whereas smaller than expected effects indicate antagonism. The calculation of the non-interactive combination effect is very simple for the absolute and relative effect additivity approaches. In the extreme case, nothing more than two single-agent effects and the corresponding combination effect are required. Contrary to these criteria that are defined in terms of effects, the definition equation of dose additivity is given in terms of doses (Fig. 2). This difference has hampered a thorough comparison of the various criteria. An experimental approach that directly applies to the dose additivity definition equation is the widely used isobolographic analysis. An isobologram is a two-dimensional graph with the doses of agents A and B as coordinate axes, in which one or several lines, the isoboles, are shown connecting different dose combinations that all produce the same magnitude of effect (Fig. 2a). From Eqn 3 in Fig. 2, it is clear that dose (Loewe) additivity (interaction index, $I=1$) is characterized by straight lines in an isobologram with linear dose scales touching the dose axes at the single-agent doses D_A and D_B . Hence, significant deviations from this straight line directly indicate antagonism or synergism. It is immediately obvious that the direct use of Eqn 3 (Fig. 2) requires a tedious dose variation testing for the determination of each data point, where a great deal of information is finally lost. However, single-agent dose–response relations can be combined with the dose (Loewe) additivity criterion, which leads to mathematical expressions for the dose additivity response surface (Fig. 2b)¹⁴. After determining this surface from the single-agent dose–response relations, it can be compared with the experimentally observed combination effects. The data that can be analyzed might range from a single dose combination to a dose design that covers the complete surface. Finally, it should be noted that this approach can predict zero interaction response surfaces according to a particular empirical criterion from single-agent dose–response relations. It cannot predict, however, possible interactions between the agents used in the combination experiments.

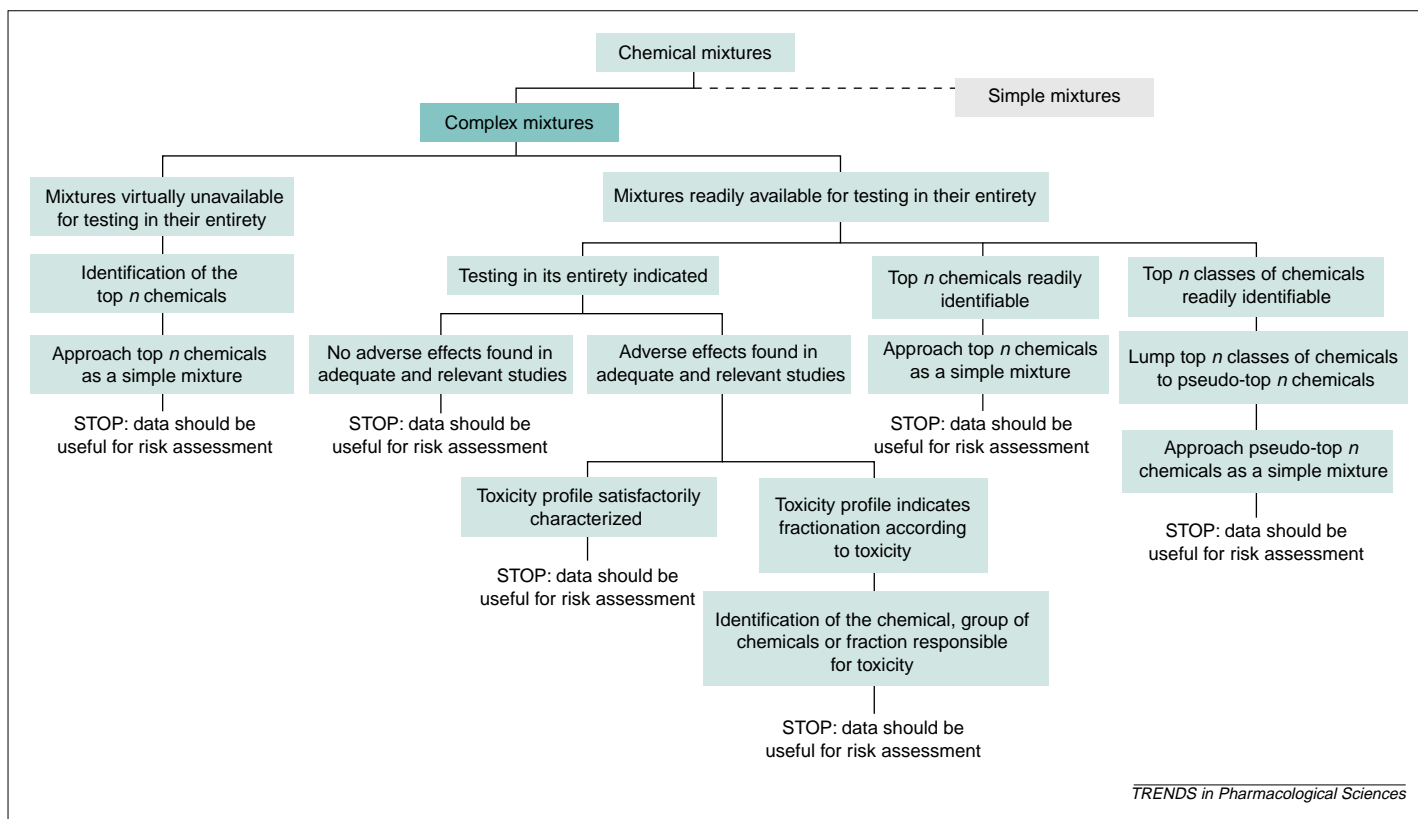
Dose dependency of interactions

In this context, it should be noted that a possible interaction or non-interaction observed in combination experiments might be dose dependent. It is even possible that the interaction is synergistic in one dose range and antagonistic in another dose range. It is one of the basic tenets of pharmacology and toxicology that all biologically active agents have dose–response relationships, even if some are noisy, very steep or flat. Therefore, the response surface methodology is the recommended approach for combined-action

assessment because it provides comprehensive information for the complete dose range under study. In addition, there is a conceptual advantage with response surfaces. Particular dose designs such as the variation of the dose of one agent in the presence of a fixed amount of a second agent or dose variation of two agents but keeping the dose ratio fixed are included as special cases and can thus be analyzed from a general point of view.

Response surface modeling can also be applied to fit mathematical expressions of response surfaces, such as Eqn 8 in Fig. 2c, directly to experimental combination data^{1,17,18}. If one has obtained a reliable response surface it can be used to predict combination effects for any dose combinations within the dose range used for the fitting procedure. One should be very cautious, however, if predictions for doses outside this range are intended. A further problem with this approach is related to the relatively large number of parameters that must be determined by nonlinear regression techniques, which requires a large number of combination experiments and very carefully selected dose designs. These response surfaces can also be analyzed for potential interactions, according to one of the empirical criteria that defines zero interaction. According to the widely adopted dose-additivity criterion for linear dose–response curves, the non-interaction is given by a summation of the single-agent effects. Therefore, when Eqn 8 (Fig. 2) can be used for fitting response surfaces, significant values of the parameters γ or δ directly indicate an interaction. However, this simple reasoning is no longer true for nonlinear dose–response relationships. If mathematical expressions have been determined for both the actual and the zero interaction response surface, a different response surface that provides comprehensive information on the dose dependence of the interaction can easily be derived^{7,14}.

It has already been mentioned that the approaches for combined-action assessment require a thorough statistical analysis. The importance of this analysis cannot be overestimated^{21,22}. The traditional experimental design for these simple mixtures has been to apply full factorial designs²³, which must be practically limited to a small number of chemicals (n) and dose levels (d), but will produce data on interaction effects that can be readily analyzed and interpreted. To implement these designs fully, a large number of dose groups (g) is required ($g=d^n$). Newer approaches for the evaluation of interactive (i.e. non-additive) effects of compounds in mixtures with more than three compounds include statistical designs such as fractional factorial designs, ray designs or dose–effect surface analysis^{22,24–26}. In a fractional factorial design, a carefully selected subset of the potential dose groups from a full-factorial experiment is studied, and the data are analyzed to attribute significant interaction effects to certain groups of chemicals. To make inferences about toxicological interactions for groups of chemicals that were not studied, data on expected single chemical effects are evaluated using expert toxicological judgement, and statistical tests and linear models are



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Fig. 3. Safety evaluation of complex chemical mixtures. Modified, with permission, from Ref. 3, according to Ref. 19.

applied to the experimental data. Another approach, based on dose–effect surface modeling, is to detect departures from additivity²¹. This experimental design is based on the generation of a response surface for the mixture under the assumption that dose addition is operational, using only the dose–response data of the single chemicals. Then, estimates of the response for specific mixture points of interest (using prediction intervals) are constructed and compared with laboratory data on those mixture points. Thus, the experimental design is limited to single chemical dose–response curves and discrete mixture combination points, and the resulting toxicity data can be used directly in a mixtures risk characterization to either estimate risk quantitatively or to qualitatively support a risk estimate made under the assumption of dose addition.

Instead of empirical models using statistical designs, possible interaction between two or more agents can also be given in terms of mechanistic models. As is true for all physiologically based modeling approaches, these type of models have their limitations because they cannot integrate every relevant biological mechanism. For example, despite similar biotransformation pathways, mechanisms of activated toxicants might be very different. In addition, toxicants can influence the biotransformation of one another through induction or inhibition. These might not be included in a toxicokinetic model. Therefore, it is imperative for the modeler to include as much information as possible to limit uncertainties in the model. An example for the analysis of interactive effects by means of mechanistically designed models is pharmacokinetic physiologically based (PbPK) modeling, as used, for example, for binary

mixtures of trichloroethylene and 1,1-dichloroethylene²⁷. Physiologically based modeling is particularly useful for investigating toxicokinetic interactions. So far, mechanistic and empirical analyses of interactions are usually unrelated. It is one of the future challenges to combine information from both approaches in the manner described in Fig. 2 for the dose-additivity and competitive interaction models.

How to deal with complex mixtures

There are several ways to deal with hazard identification and risk assessment of complex chemical mixtures. Different types of complex mixtures require different approaches, and the usefulness of a certain approach depends on the context in which one is confronted with the mixture, and also on the amount, type and quality of the available data on the chemistry and toxicity of the mixture. A scheme for safety evaluation of complex mixtures has been developed^{1,2} and is presented in a modified form in Fig. 3. A conspicuous element of the scheme is the dichotomy between mixtures that are virtually unavailable and mixtures that are readily available for testing in their entirety. Moreover, the inclusion in the scheme of the top *n* and pseudo top *n* approaches (in essence, selection of the *n* most risky components or pseudo-components to be dealt with as a simple mixture) is considered another characteristic aspect of this scheme. The scheme is designed to be used as a flow chart, pursuing in parallel all options until it is decided which option is best. The aim of the scheme is to stimulate safety evaluation of complex mixtures, bringing together and effectively using all

available relevant information, methods, technologies, expertise and experience^{2,19}.

The way in which the safety of natural flavoring complexes (such complexes are derived from higher plants by applying physical separation methods and are used as flavoring substances for food and beverages) is evaluated²⁸ illustrates how the scheme can be used. In fact, natural flavoring complexes are readily available for testing in their entirety, but this is only done when its necessity appears while walking through the step-by-step safety evaluation procedure. The evaluation begins with a review of all available data on the history of dietary use of the natural complex, followed by prioritization of constituents according to their relative intake (from use of the natural complex as a flavoring substance) and their chemical structure¹⁹. The procedure further uses the concept of 'threshold of toxicological concern'²⁹ and assigns constituents to one of three classes of toxic potential³⁰⁻³². The procedure deals with the evaluation of constituents of unknown chemical structure that are recognized as a major issue in the safety evaluation of complex mixtures³³: as a conservative default assumption, the total intake of all unknowns is considered together and placed in the structural class of greatest toxic potential and, thus, is compared with the most conservative exposure threshold. The method also addresses the concept of joint action among structurally related constituents: if a common pathway of intoxication has been identified or can be reasonably predicted on the basis of structure-activity relationships for a group of constituents, the combined intake of those substances will be compared with the appropriate human exposure threshold of concern. Ultimately, the procedure focuses on those constituents or group of constituents that, because of their intake and structure, might pose significant risk from consumption of the natural flavoring complex. This could imply toxicity testing of the complex in its entirety. With the developed strategy, the overall objective of the safety evaluation can be attained: that no reasonably significant risk associated with the intake of natural complexes will go unevaluated²⁸.

Multivariate data analysis approaches³⁴⁻³⁶ can be used for the information-handling problem associated with complex mixtures³⁷. They include, for example, cluster analysis, principal component analysis (PCA) and projection to latent structures (PLS) techniques. Multivariate data analysis approaches fall into two classes: classification and regression. Classification techniques explore the data structure within a particular data set (matrix *x*) and regression approaches establish relationships between different data sets (matrices *x* and *y*). For example, PLS is a regression model that could describe the toxic action as a function of the chemical compounds involved. Pattern recognition techniques represent an important part of multivariate data analysis. They include nonparametric discriminants (neural networks) and similarity-based

classifiers [k-nearest neighbor modeling (KNN) and soft independent modeling by class analogy (SIMCA)].

PCA (Ref. 34) is used to detail the chemical characteristics of a mixture by comparing it with other mixtures, either as 'finger prints' or as detailed information on the identity and quantity of each component. This type of comparison of composition patterns requires a database with compositional information obtained in a standardized way. Pattern recognition can be used here for classification of complex mixtures based on existing toxicity data of related mixtures. This requires the use of multivariate regression techniques, such as PLS (Ref. 34). With PLS, the toxicity of a complex mixture can be described empirically as a function of the physico-chemical data or data on chemical composition: the method can thus be used to identify chemicals that co-vary with toxicity and it might predict the toxicity of unknown, complex mixtures. Recently, Eide *et al.*³⁷ have followed this type of strategy to correlate the GC-MS (gas chromatography-mass spectrometry) analyses of complex mixtures (each containing ~260 components) to the mutagenicity measured in the Ames test. The idea is also to predict mutagenicity from chemical analyses of other types of mixture extracts. An advantage of this approach is that there is not always the need to identify explicitly a complex chemical mixture that is chemically very similar to the mixture of concern. Overall, in the hands of experts, pattern recognition techniques are considered to be powerful tools for the safety evaluation of complex chemical mixtures.

Challenges ahead

There is a marked difference between the complexity of a single compound study and a mixture design study. The experimental plan of a mixture design study will mainly depend on the number of compounds of a mixture and on the question of whether it is desirable to assess possible existing interactions of chemicals in a mixture. Despite their potential and current use, mixture design studies need further extensive testing and cross validation. In these studies, a mixture should, preferably, be tested both at high (effective) concentration and at low (realistic) concentrations.

In vitro tests are becoming an essential part of an integrated toxicology testing strategy. In addition, the scientific progress made in the fields of cellular and molecular biology now permits the use of *in vitro* test systems that can be used to study mechanism of toxicity of mixtures under high-throughput test scenarios. Current developments in the arenas of molecular biology and chip technology, brought forward through efficacy and safety screening in pharmaceutical research, will now be applicable for toxicology to screen effects of mixtures at a molecular level³⁸. In this manner, it is possible to identify proteins altered by the action of a toxic agent and thereby to obtain information on changed biochemical pathways. A compilation of such proteomic signatures

in databases is the first step in elucidating the topological structure and dynamics of metabolic, signaling and regulatory networks. New developments in the area of proteomics and toxicogenomics will significantly increase our information on mechanisms of toxicity and this, in turn, can be expected to have great impact on a better understanding of the approaches for combined-action assessment³³.

Nevertheless, at present, no integrated approach has been undertaken to evaluate the potential predictive value of genomics and proteomics in toxicological assessments. The technologies are complementary because genomics is more sensitive but less predictive for phenotype, whereas proteomics provides information on post-translational modifications and produces better qualitative data without interference from mRNA instability. Once validated, combined genomics and proteomics are expected to map mechanistically the early toxicity-related alterations in cells or animals exposed to

chemicals. Thus, insight into numerous toxicologically relevant cellular processes will simultaneously be obtained. It is likely that (owing to the integrated and holistic nature of genomics and proteomics) changes in these processes can be identified at lower toxicant concentrations than are currently examined. Additionally, because multiple cellular processes are studied within a single experiment, this approach is suitable to evaluate any kind of combinational (e.g. additive and synergistic) effect resulting from combined exposure to more than one toxicant.

Finally, the hazard characterization process of chemical mixtures requires a multidisciplinary approach to make meaningful progress³⁹. This is possible only when experimental toxicologists, mathematicians, model developers and pharmacologists collaborate to ensure parallel research in various areas of this field and to justify the selection of those compounds of particular interest for the hazard characterization of a mixture.

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