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Role of Physiologically Based Kinetic modelling in addressing environmental chemical mixtures – A review

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ABSTRACT

The role of Physiologically Based Kinetic (PBK) modelling in assessing mixture toxicology has been growing for the last three decades. It has been widely used to investigate and address interactions in mixtures. This review describes the current state-of-the-art of PBK models for chemical mixtures and to evaluate the applications of PBK modelling for mixtures with emphasis on their role in chemical risk assessment. A total of 35 mixture PBK models were included after searching web resources (Scopus, PubMed, Web of Science, and Google Scholar), screening for duplicates, and excluding articles based on eligibility criteria. Binary mixtures and volatile organic compounds accounted for two-thirds of the chemical mixtures identified. The most common exposure route and modelled system were found to be inhalation and rats respectively. Twenty two (22) models were for binary mixtures, 5 for ternary mixtures, 3 for quaternary mixtures, and 5 for complex mixtures. Both bottom-up and top-down PBK modelling approaches are described. Whereas bottom-up approaches are based on a series of binary interactions, top-down approaches are based on the lumping of mixture components. Competitive inhibition is the most common type of interaction among the various types of mixtures, and usually becomes a concern at concentrations higher than environmental exposure levels. It leads to reduced biotransformation that either means a decrease in the amount of toxic metabolite formation or an increase in toxic parent chemical accumulation. The consequence is either lower or higher toxicity compared to that estimated for the mixture based on the additivity principle. Therefore, PBK modelling can play a central role in predicting interactions in chemical mixture risk assessment.

1. Introduction

Humans and the environment are exposed to an ever-increasing number of anthropogenic chemicals and to mixtures of chemicals via food, water, air, consumer products etc. However, chemical risk assessment is usually performed for individual substances [1]. The risk assessment of chemical mixtures is particularly challenging due to the (often large) number of chemicals combined in mixtures, limited knowledge on mixture composition, the toxicokinetics and toxicodynamics of mixture components, and the (large) number of potential interactions within a chemical mixture [1,2].

The legal requirements for risk assessment of mixtures depend on the type of mixture and sector. A prospective risk assessment is required for intentional mixtures, e.g., pesticide formulations and multi-component food additives, while it is generally not required for unintentional mixtures [1]. There are growing numbers of methods and novel tools under development that enable understanding of the underlying mechanisms of action and interactions in a mixture. Integrated use of these novel tools (*omics, in silico* approaches, Adverse Outcome Pathways (AOPs), TK modelling) has been shown to hold high potential to support risk assessment of mixtures [1,3].

Two main models are currently used to assess chemical mixtures in a component-based way. These are *Concentration addition* (CA) and *Independent action* (IA). These models are the default approaches in regulatory risk assessment [3,4]. CA is applicable to mixtures composed of chemicals with a similar mode of action, where the overall mixture toxicity equals the sum of the potency-corrected exposure concentrations of individual chemicals. On the other hand, IA (also known as response addition) is applicable to chemicals with dissimilar modes of action. In IA-based approaches, the mixture toxicity will not occur if the individual chemicals are all present at sub-toxic levels, whereas in CA-based approaches all components contribute to the total toxicity depending on their concentration and potency. Both CA and IA are based on the assumption that the components within a mixture have no

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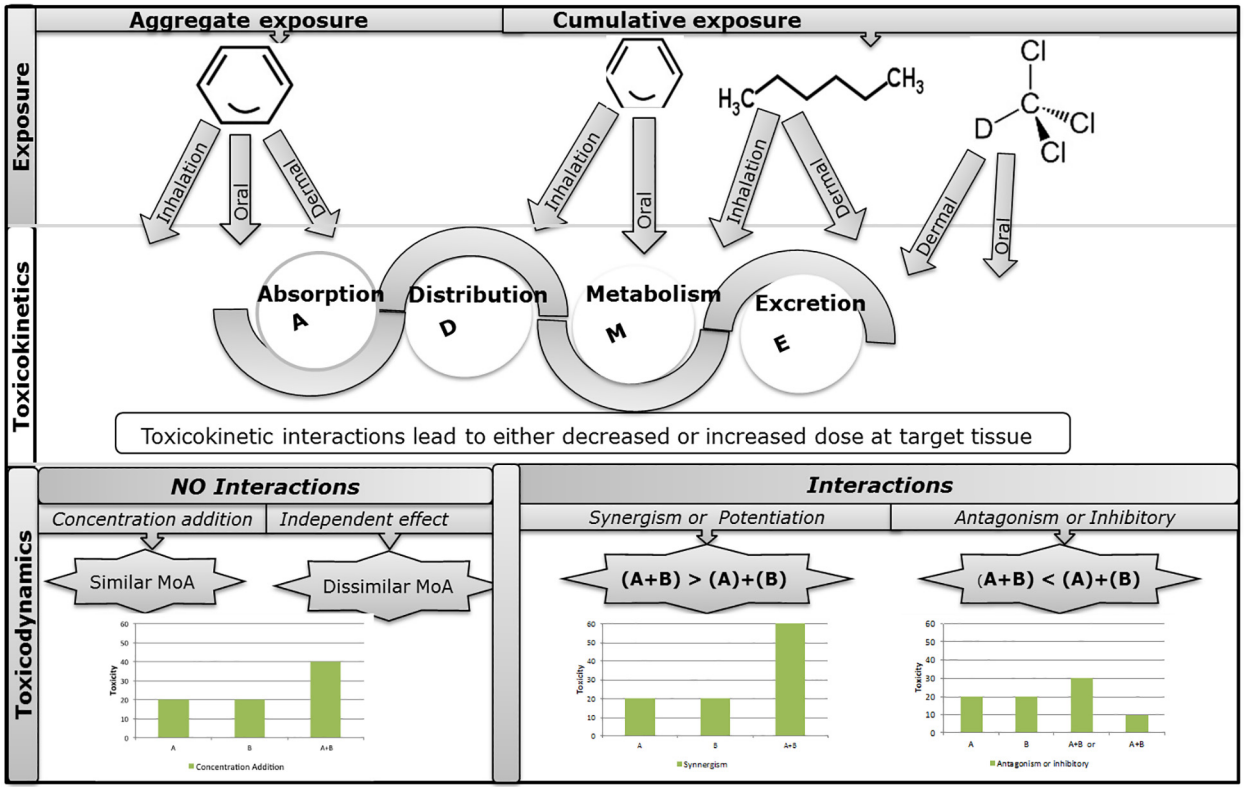

Fig. 1. Schematic representation of exposure to chemical mixtures and consequences of toxicokinetic and toxicodynamic interactions.

interactions with each other [5].

The magnitude of toxicity of some mixtures cannon be explained by CA or IA. In such cases, the components of the mixture influence one another so that the overall toxicity of a mixture is higher or lower than predicted based on additivity. This phenomenon, known as an interaction, can affect both the toxicokinetics (TK) and toxicodynamics (TD) of chemical mixtures in the body. TK interactions are assumed to influence chemicals during the absorption, distribution, metabolism and excretion (ADME) phase within the body, i.e., due to alteration of absorption, induction/inhibition of metabolising enzymes, alteration of physiological barriers, and factors affecting plasma protein binding or excretion. The consequences of TK interactions are usually either an increased or decreased concentration of one or more chemicals at the site of action, which affects the overall toxicity of the mixture (Fig. 1). In general, interactions in a mixture lead to either greater effect (synergism, potentiation) or lower effects (antagonism, inhibition) compared to predictions based on CA or IA (Fig. 1) [3,5,6].

Various approaches have been developed to address the role of interactions in predicting combined effects of mixtures. Adjusted/Weight of evidence Hazard Index (HI) and Physiologically Based Kinetic (PBK) modelling are two of the methodologies that can be used to assess interactions in chemical mixtures [5].

PBK models are represented by set of mass-balance differential equations describing the biokinetic processes of a chemical in the body as a function of physicochemical parameters (e.g., partition coefficient), biochemical parameters (e.g., Michaelis–Menten kinetics: metabolic rate constant, Vmax, and constant, Km), and physiological parameters (e.g., flow, volume). A PBK model has several advantages compared to classical PK modelling approaches, and may be used for various purposes, such as more reliable prediction of the internal dose, supporting biological monitoring, species extrapolation, route-route extrapolation, estimation of response from varying exposure conditions, and estimation of human variability [7–9]. Numerous PBK models have been developed by the scientific community in the last 30 years, as reviewed by Lu et al. [10]. Guidance documents have been developed on best practices on how to build, report, and use these models [7,9].

The role of PBK modelling in assessing mixture toxicity has evolved over the last three decades, by increasingly taking into account the individual responses of mixture constituents and their interactions. The chemicals present in a mixture interact with each other via different mechanisms. In this review, most of the interactions identified take place at the level of toxicokinetics of two or more chemicals. PBK modelling has been widely used to investigate mechanisms of interactions of chemicals in mixtures [5,6]. The purpose of this review is to identify the present state-the-of-the art of PBK models for mixtures and to highlight their role in assessing interactions between environmental chemicals. This review also highlights opportunities and challenges associated with the use of PBK models in the assessment of mixtures.

2. Methodology

The literature search strategy aimed at finding literature published in English on mixture PBK models. For this, a two-step search strategy was carried out to find relevant articles until December 15, 2016. First, a search was conducted on Scopus, PubMed, Web of Sciences, and Google Scholar for titles and abstracts using combination of the following key words, i.e., mixtures, combinations, Physiological Based Pharmacokinetic/Toxicokinetic/Biokinetic modelling, and interactions. Hits obtained in the first step were complemented using bibliographies of relevant literature to add studies missed during the initial phase. The identified articles were further screened for duplicates, and full-text articles were retrieved. Then, exclusion criteria were applied to further screen articles that did not contain PBK model structures or equations, and PBK models that did not apply to two or more chemicals. In the final step, data were extracted following an excel template prepared to collect relevant information, such as class of chemicals, lists of chemicals, number of compartments, types of interaction. The complete list of the relevant articles and extracted information used in this review can be found in the Supplementary material 1 while Fig. 2 summarises the steps followed to implement the search strategy.

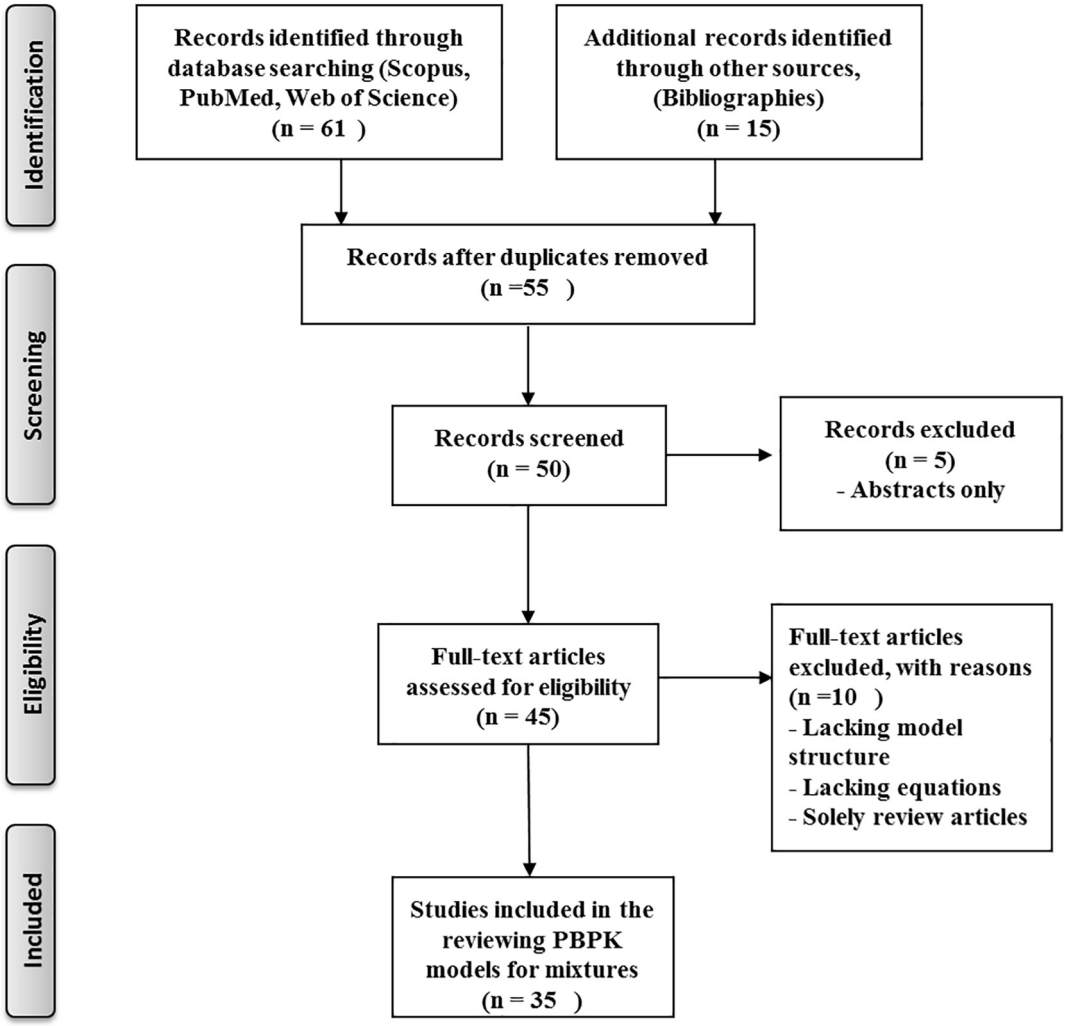

Fig. 2. Flow chart depicting the procedure for selection of studies to include for the review.

3. Results

In this section, the results of the search are presented and a review and a discussion of the state-of-the-art of PBK modelling will follow.

3.1. Main characteristics of identified PBK mixture models

A total of 35 PBK mixture models were included for this review following the search strategy described in the methods section. Binary mixtures and volatile organic compounds accounted for two-thirds of the mixtures reviewed. The most common route of exposure and modelled system in the studies were found to be inhalation and rats, respectively (Fig. 3).

3.2. List of relevant PBK mixture models

A summary of relevant articles for 22 PBK models for binary mixtures is presented in Table 1, whereas Table 2 summarises studies for 5 ternary mixtures, 3 quaternary mixtures, and 5 complex mixtures containing 5 or more defined chemicals. The summary tables describe the type of modelled organism/system, routes of exposure, number of compartments in the PBK model, types of interactions captured in the PBK models, as well as the basis for interaction and development of PBK model for various types and classes of mixtures. Competitive inhibition was found to be the most common type of interaction among the various types of mixtures modelled by the PBK models.

4. Discussion

Various types of mixture PBK models have been developed in the last three decades including models for simultaneous or sequential exposure to two or more defined chemicals involving different species and exposure scenarios. The first PBK model for chemical mixtures was reported to be a type of “one-chemical mixture”. The mixture consisted of the parent chemical, benzene, and its metabolites according to Mumtaz et al. [11] and Yang and Andersen [12]. This was followed by numerous examples of binary mixtures [12,13], ternary [14,15], quaternary [16–18], and complex mixtures [19–22].

The most common type of PBK model applied for mixtures is based on inhalation of binary combinations of volatile organic compounds investigated in rats [23,24]. More than 60% percent of mixture PBK models in this review involve binary mixture interactions at the toxicokinetic level, i.e., metabolic inhibition [25–29].

PBK models for binary or higher order mixtures were constructed by first developing models for each chemical separately, and then connecting the individual models via mass balance equations for metabolism in the liver. The equation for the liver was modified to account for various mechanisms of metabolic interaction. In general, three assumptions were used to account for interactions, i.e., competitive inhibition, uncompetitive inhibition, and non-competitive inhibition. The category of interaction was evaluated by observing how well the PBK simulation curve gives an optimal fit after adjusting the hypothesised interaction terms for each chemical.

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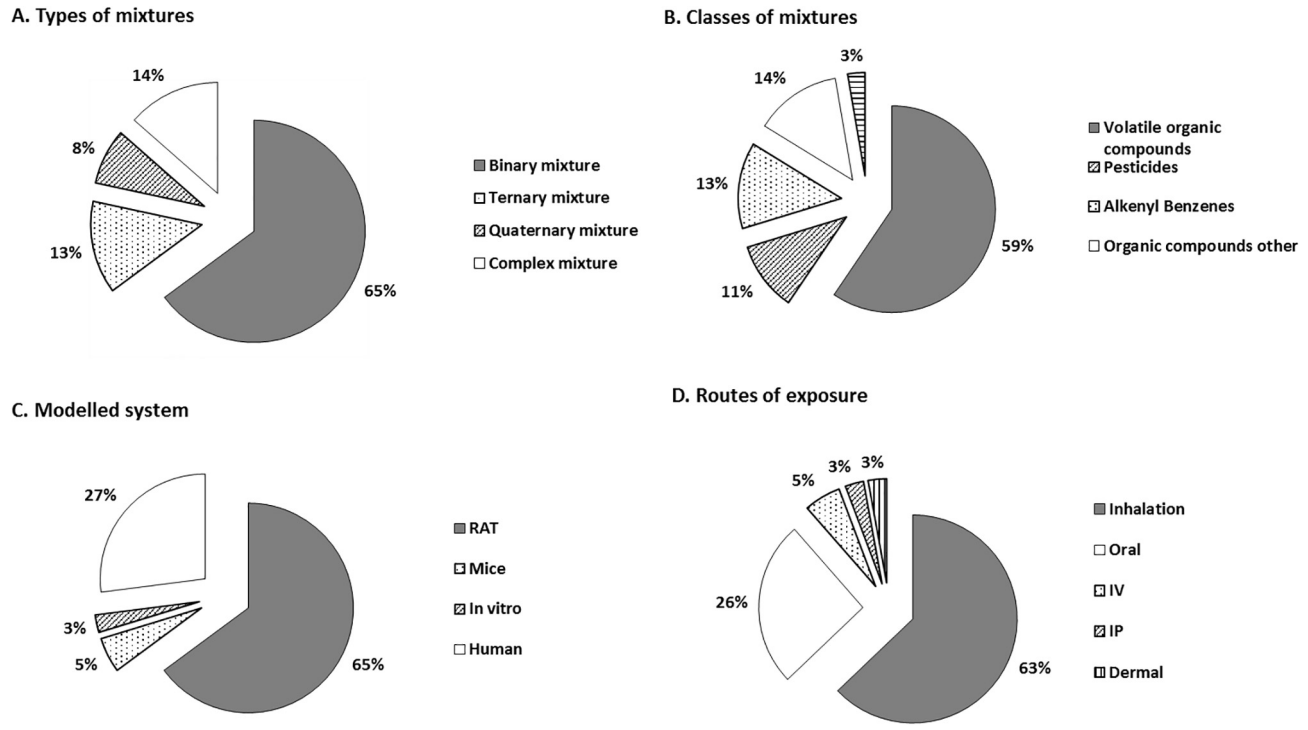

Fig. 3. Summary of the types and classes of mixtures, modeled system, and routes of exposure in the reviewed PBK models.

The interaction in a binary mixture usually occurs via competitive inhibition. The chemical which has the higher concentration in the mixture as a result of either higher dosing or greater blood-air partition coefficient usually acts as an inhibitor compared to the other [30], but inhibition is mostly evident at higher exposure conditions [26,30]. Competitive inhibition was the most common mechanism of interaction during co-exposure of volatile organic compounds probably because most of them are substrates for the same enzyme, CYP 2E1 [27]. Competitive types of interaction usually become a concern at higher concentrations compared to environmental or occupational exposure levels [15].

The common mixture PBK modelling approaches fall into two major categories: bottom-up and top-down.

4.1. Bottom-up PBK modelling of mixtures

The bottom-up PBK modelling methodology for mixtures is based on one or more interactions at a binary level [6,31]. The possible number of interaction increases as the number of chemicals (N) in a mixture increases by $N*(N-1)/2$. This approach has been referred as "bottom-up" mixture modelling methodology as it involves applying binary interactions to predict complex mixtures [6].

There are numerous examples of the "bottom-up" mixture modelling methodology [12,13]. To develop such PBK models, PBK models for each constituent of the mixture first need to be developed and validated. Then linking them together at the binary level (Fig. 4A) based on the mechanism of interaction should follow, and a network of binary metabolic interactions is created (Fig. 4B).

The bottom-up PBK modelling approach is evident in binary, ternary, quaternary and five chemical mixtures reviewed in this paper. In principle, this methodology should be applicable to any mixture as long as information on each interacting pair is available. However, considering the complex mixtures humans are exposed to, characterising every binary interaction in a mixture is difficult since the number of possible interaction increases by $N(N-1)/2$ as the number of chemicals (N) in a mixture increases [13]. Besides, it is impractical or impossible to find data on the increasing number of possible binary combinations in mixtures of increasingly complex compostion. In such cases, a "top-down" or lumping approach is more practical where chemicals with similar characteristics are lumped together and described by a central estimate [6,19].

4.2. Top-down PBK modelling of mixtures

Top-down PBK modelling of mixtures is also referred to as lumping. Lumping simplifies complex mixtures to a level where quantitative study of interactions can be successfully implemented using PBK models. This approach is applicable to more complex, multi-component chemical mixtures where characterisation of every possible binary interaction is impractical or unavailable [6,19,32]. Fig. 5 depicts the approach employed in top-down PBK modelling.

PBK model parameters are employed to lump chemicals in mixtures to enable their description by average parameter values. For example, Dennison et al. [19] used this approach to describe the kinetics of a gasoline mixture. The methodology simplifies the problem by isolating target components for which description is required and treating the rest as a single lump chemical. The gasoline mixture was therefore treated as composed of six chemicals with five target chemicals (benzene, toluene, ethylbenzene, *o*-xylenee, n-hexane) and a lumped chemical group representing the whole gasoline mixture (both for the summer and winter blend). Then using a binary interaction methodology for the six chemicals, it was possible to describe the pharmacokinetics of the five target chemicals as well as the lump using a central estimate value.

The individual PBK models for each chemical and the lump were linked by competitive inhibition of hepatic metabolism at a binary level [19,32]. Similarly, Jasper et al. [21] have evaluated the role of lumping within the target organ in PBK modelling to describe the toxicokinetics of a complex gasoline mixture following inhalation exposure in rats. A total of 109 chemicals were identified and quantified after inhalation exposure, and the mixture was then simplified to 10 target chemicals and various numbers of lumps. The PBK model simulated well the blood concentration for 10 target chemicals compared to the experimental data when enzymatic interaction was incorporated to the PBK model

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Table 1
Summary of relevant PBK models for binary mixtures.

Reference	Modeled organism	Class of chemicals	List of chemicals	Route of administration	Number of compartments	Type/Mechanism of interaction	Basis of interaction	Basis of PBK model
[25]	Rat	VOCs	Trichloroethylene, 1,1-dichloroethylene	Inhalation	4	Competitive inhibition	Optimal fit in PBK simulation	[23–24]
[28]	Rat	VOCs	Benzene, Toluene	Inhalation	4	Non-competitive inhibition	Optimal fit in PBK simulation	[23]
[26]	Rat	VOCs	Toluene, m-xylene	Inhalation	4	Competitive inhibition	Best visual fit in PBK simulation	[23]
[31]	Rat	VOCs	Kepone, Carbon tetrachloride	Oral/inhalation	5	Toxicodynamics (potentiation)	[44]	[45]
[29]	Human	VOCs	Tolune, Xylene	Inhalation	4	Competitive inhibition	Best fit	[26]
[29]	Rat	VOCs	Vinyl chloride, Trichloroethylene	Inhalation	4	Competitive inhibition	Best fit	[23,25]
[27]	Rat	VOCs	Tolune, Dichloromethane			Competitive inhibition	[37]	[26]
[46]	Rat	VOCs	Kepone, Carbon tetrachloride	IP, inhalation	6	Potentiation	[44] in vivo experiment	PBK/PD modelling earliest example
[47]	Human	VOCs	Toluene, n-hexane	inhalation	5	Non-competitive inhibition	*In vivo* & *in vitro* Experiments [48]	[49,50]
[51]	Rat	VOCs	Methylchloroform, m-xylene	Inhalation	4	Competitive inhibition		[24]
[52]	Rat	VOCs	Toluene, n-hexane	Inhalation	4	Non-competitive/ uncompetitive inhibition		[24]
	Human	VOCs	Toluene, n-hexane	Inhalation	4	Non-competitive or uncompetitive inhibition		[24]
[38]	Rat	VOCs	Toluene, Trichloroethylene	IV	4	Competitive inhibition	[26,75]	[26]
[53]	Human	VOCs	Ethylbenzene, Xylene	Inhalation	7	Competitive inhibition		[76]
[54]	Rat	VOCs	Chloroform, Trichloroethylene	IV	7	Competitive inhibition	Simulation	[24]
[55]	Mice	VOCs	Carbon tetrachloride, Tetrachloroethylene	Oral	4	Suicide inhibition		[24]
[56]	Mice	PCB	PCB 153, PCB 126	Oral	5	–		[57]
[58]	Rat	Pesticides	Chlorpyrifos, Parathion	Oral	8	Competitive inhibition at high dose, additivity at low dose	Simulation	
[59]	Rat	Pesticides	Chlorpyrifos, Diazinon	Oral and dermal	7	Non-competitive inhibtion	Simulation	Individual previous models
[33]	Rat and Human	Alkenyl benzene	1'-hydroxyestragole, Nevadensin	Oral	6	Non-competitive Inhibition	Experiment and simulation	[60]
[34]	Rat	Alkenyl benzene	Estragole, Nevadensin	Oral	6	Non-competitive inhibition	[33]	[60]
	Human	Alkenyl benzene	Estragole, Nevadensin	Oral	4	Non-competitive inhibition	[33]	
[35]	Rat and Human	Alkenyl benzene	7-hydroxycoumarin, Malabaricone C	Oral	6	Non-competitive inhibition	Experiment and simulation	
	Rat and Human	Alkenyl benzene	1'-hydroxysafrole, Malabaricone C	Oral	6	Non-competitive inhibition	Experiment and simulation	

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Table 2
Summary relevant PBK models for binary, ternary, quaternary, and more complex mixtures.

Reference	Modeled organism	Mixture type	List of chemicals	Route of administration	Number of compartments	Type/Mechanism of interaction	Basis of interaction	Basis of PBK model
[14]	Rat and Humans	Ternary	Tolune, m-xylene,and ethylbenzene	Inhalation	4	Competitive inhibition	[25]	[24]
[61]	Human	Ternary	Tolune, m-xylene,and ethylbenzene	Inhalation	5	Competitive inhibition	Simulation	
[15]	Rat	Ternary	Trichloroethylene, Tetrachloroethylene, Perchloroethylene	Inhalation	4	Competitive inhibition	Simulation	[24]
[62]	Human	Ternary	Tolune, Ethylbenzene, Xylene	Inhalation	4	[a]Competitive inhibition	Simulation	[14,24]
[63]	In vitro [b,c]	Ternary	R-Bufuralol, Bunitrolol, Debrisoquine	–	3[d]	Competitive inhibition	Experiment	
[16]	Rat	Quaternary	Benezene, Tolune, Ethylbenzene, m-xylene	Inhalation	4	Competitive inhibition	Simulation	
[17]	Rat	Quaternary	Chloroform, Bromoform, Bromodichloromethane, Dibromochloromethane	Oral	5	Metabolic inhibition		
[18]	Rat	Quaternary	Benzene, Toluene, Ethylbenzene, m-xylene	Inhalation	4	Competitive inhibition	Simulation	[16]
[19]	Rat	Complex (5+)	n-hexane, Benzene, Tolune, o-xylene, ethylbenzene, Lump	Inhalation	4	Competitive inhibition	Simulation	[23,28]
[20]	Rat	Complex (5+)	n-hexane, benzene, Tolune, Ethylbenzene, O-xylene, Lumped blend for summer/winter	Inhalation	4	Competitive inhibition	Simulation	[23,28]
[32]	Rat	Complex (5+)	n-hexane, Benzene, Tolune, Ethylbenzene, o-xylene, 1/3 cut, 2/3 cut, whole gas	Inhalation	4	Competitive inhibiton		
[22]	Rat	Complex	(n-tetradecane mix, n-octane mix, lumps)	Inhalation	7	Competitive inhibition	Simulation	
[21]	Rat	Complex (10+)	n-pentane, 2-methlpentane, tolune, n-hexane, cyclohexane, benzene, m-xylene, o-xylene	Inhalation	7	Competitive inhibition	Simulation	

[a] At high concentration.
[b] Microsomal co-incubation.
[c] Liver PBK model.
[d] Within the liver.

and by lumping the 99 non target chemicals [21].

Martin et al. [22] also used PBK modelling and a lumping approach to give a detailed description of the kinetics of aerosolized and vaporised jet fuel. Their model simulated aromatic and lower molecular weight alkanes more accurately than higher molecular weight alkanes, and showed metabolic interaction to be significant at higher concentrations [22].

4.3. PBK modelling of substrate and its inhibitor

In addition to the most common types of PBK models for mixtures based on bottom-up or top-down approaches, PBK models have also been developed to investigate the effect of an inhibitor on substrate metabolism. These substrate and inhibitor combination can be considered as a binary mixture. These PBK models were primarily developed to study the inhibition effect of a chemical on the metabolism of different food additives [33].

A binary PBK model was developed by constructing and validating individual PBK models for both the substrate and inhibitor separately. Then, both models were connected to form a binary PBK model taking into account the type of interaction between the substrate and the inhibitor. The interaction between the inhibitor and substrate is often unidirectional, unlike the conventional PBK models for mixtures where two-way interactions occur among components. For example, Alhusainy and colleagues [34] used this approach to investigate the inhibition of estragole by the basil flavonoid nevadensin following oral administration in rats (Fig. 6).

A non-competitive inhibition type of interaction was used to link the PBK model for estragole and nevadensin based on their previous results [33]. Similarly, the kinetics and inhibition of safrole and 7-hydroxycoumarin by Mace extract containing malabaricone C was described using PBK models in humans and rats [35].

These PBK models describe kinetic interactions in various organs (liver, lung and kidney) and involve various enzymes and phases of metabolism [33–35] unlike conventional PBK models that are limited to metabolism in the liver by a single enzyme. However, these models are of limited applicability since their original purpose was to investigate inhibition in alkenylbenzene food additives. However, the concepts and assumptions used in these models could be applied to conventional PBK models to widen their applicability and relevance.

4.4. Interactions in mixtures

4.4.1. Types of interactions

Exposure to mixtures can lead to combined effects, toxicokinetic and toxicodynamic interactions. The majority of interactions in mixtures occur during the toxicokinetic phase [36]. Toxicokinetic interactions affect the concentration of chemicals reaching the target site, thereby modifying the response or toxicity compared to that predicted using dose addition, and making the mixture risk assessment more challenging [6].

The most common type or mechanism of interaction among the mixture PBK models reviewed was competitive inhibition. In most of the cases, PBK modelling simulations investigated interactions by comparing the observed kinetics of the mixtures with the kinetics predicted by the model containing various scenarios for the type of inhibition (see Table 3).

Results from previous experimental studies were also used in some instances to determine the type of interaction [37]. Sometimes, an experimental study and PBK modelling are combined to validate the type of interactions existing in a mixture [38] or to investigate the mechanism of an inhibitor on various substrates [33,35]. The fact that most chemicals in the investigated mixtures were substrates for a single or specific enzyme was also used to support the hypothesis that most of the interactions in mixtures are competitive in nature [27].

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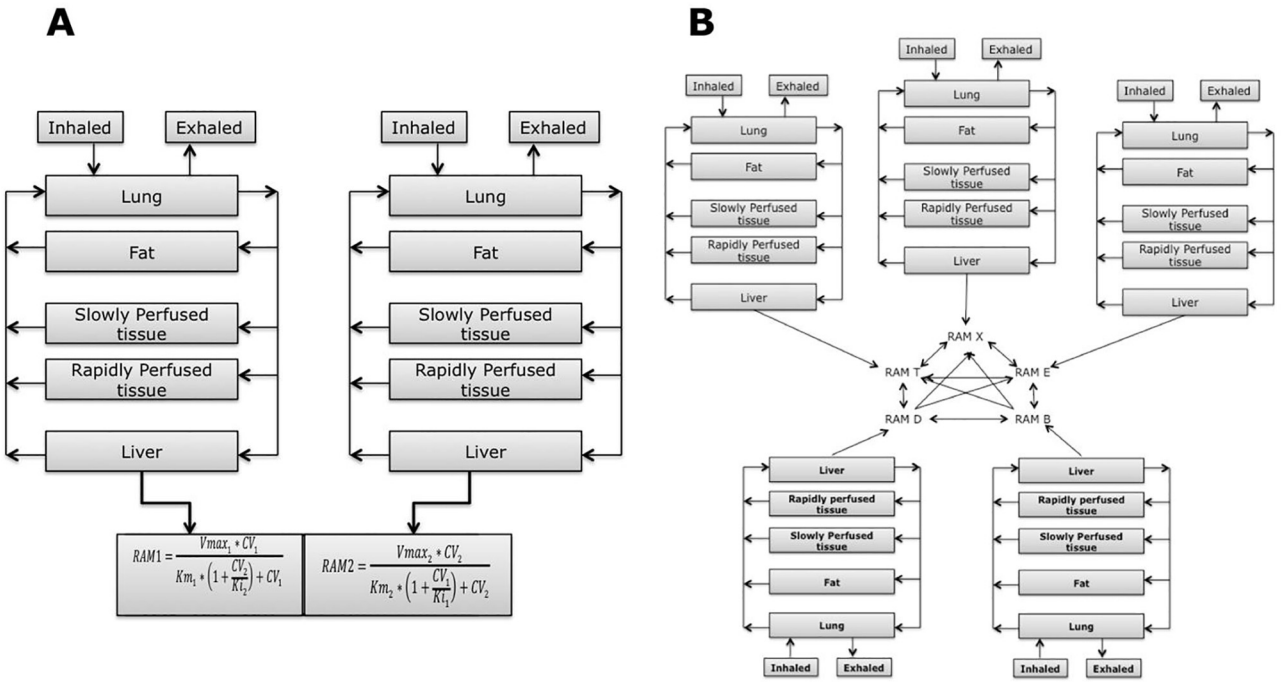

Fig. 4. (A) Conceptual representation of a PBK model for a binary mixture of chemicals 1 and 2 that compete with each other for metabolism; (B) A network of binary pharmacokinetic interactions for a mixture of five volatile organic compounds (Toluene (T), m-Xylene (X), Ethylbenzene (E), Dichloromethane (D), Benzene (B)). Figures are .
adapted from [13,43]

4.4.2. Consequences of interactions

Interactions at the level of toxicokinetics (absorption, distribution, metabolism, excretion) of chemicals affect the concentration reaching the target site, and thereby result in a reduced or increased response/toxicity. Competitive inhibition decreases metabolism of the parent compound, which leads to increased toxicity if the parent compound is more toxic, and reduced toxicity if the toxicity results from the metabolite (Fig. 7).

Some examples of the consequences of toxicokinetic and toxicodynamic interactions are given in Table 4. The inhibitor is usually the one with the higher concentration, e.g., trichloroethylene is a more effective inhibitor in a binary mixture of vinyl chloride and trichloroethylene co-exposure. The relatively higher concentration of trichloroethylene compared to vinyl chloride even in a similar exposure situation is attributed to a larger blood-air partition coefficient for trichloroethylene which leads to an increased concentration in blood

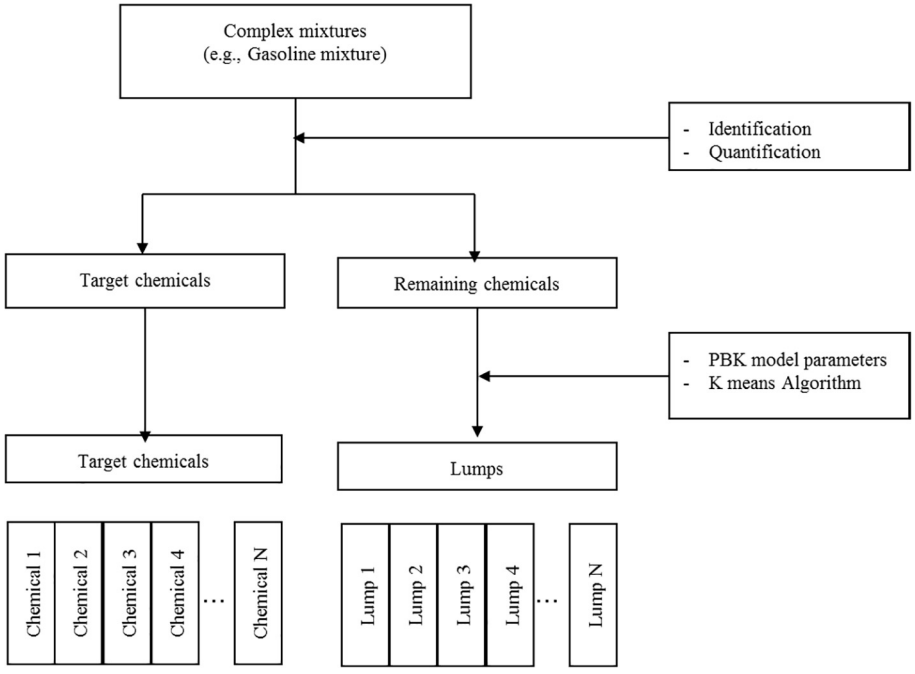

Fig. 5. Flow chart depicting the top-down PBK modelling approach to evaluate interactions in complex gasoline mixtures. The complex gasoline mixture is simplified into 'N' number of target chemicals and sets of chemical lumps using biologically based lumping methodology as described by Jasper and colleagues [21].

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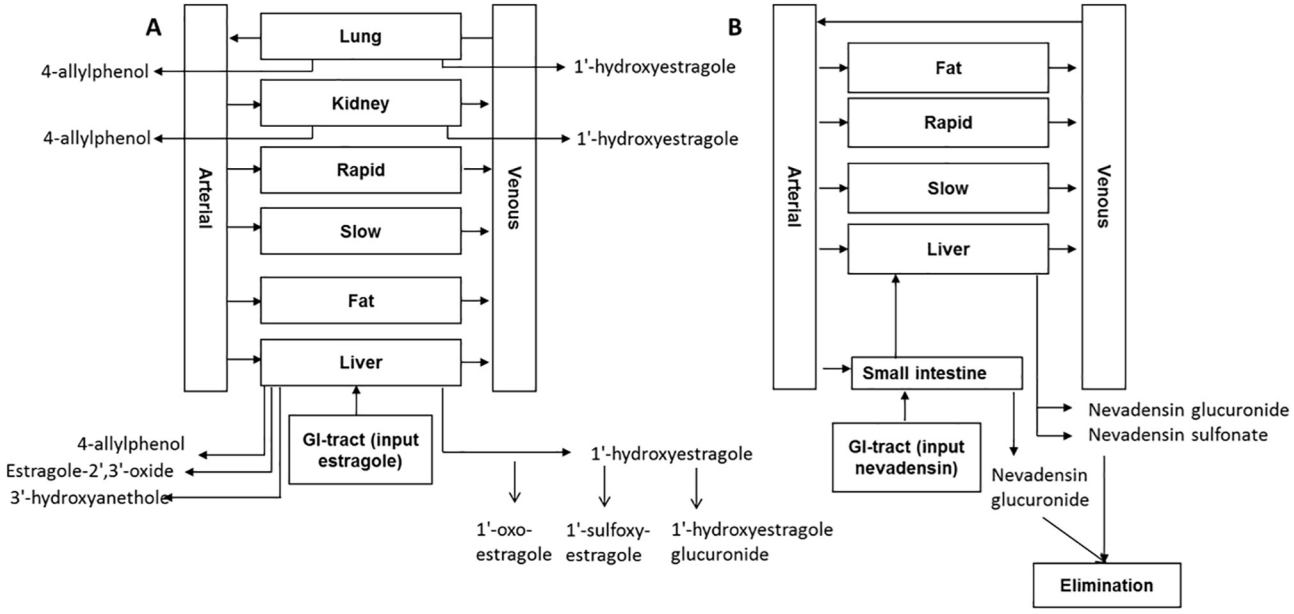

Fig. 6. Schematic diagram of the PBK model for (A) esragole and (B) nevadensin, as reported in Alhusainy et al. [34] and adapted in this figure.

Table 3
Types of inhibition and their corresponding equations used for PBK modelling of a ternary mixture.

Hypothesis tested for interaction in ternary mixture	Chemical 1	Chemical 2	Chemical 3
Equation for rate of metabolism (RAM) for Competitive inhibition	$\frac{Vmax_1 * CV_1}{Km_1 * \left(1 + \frac{CV_2}{Ki_2} + \frac{CV_3}{Ki_3}\right) + CV_1}$	$\frac{Vmax_2 * CV_2}{Km_2 * \left(1 + \frac{CV_1}{Ki_1} + \frac{CV_3}{Ki_3}\right) + CV_2}$	$\frac{Vmax_3 * CV_3}{Km_3 * \left(1 + \frac{CV_2}{Ki_2} + \frac{CV_1}{Ki_1}\right) + CV_3}$
Equation for RAM for Uncompetitive inhibition	$\frac{Vmax_1 * CV_1}{Km_1 + CV_1 * \left(1 + \frac{CV_2}{Ki_2} + \frac{CV_3}{Ki_3}\right)}$	$\frac{Vmax_2 * CV_2}{Km_2 + CV_2 * \left(1 + \frac{CV_1}{Ki_1} + \frac{CV_3}{Ki_3}\right)}$	$\frac{Vmax_3 * CV_3}{Km_3 + CV_3 * \left(1 + \frac{CV_2}{Ki_2} + \frac{CV_1}{Ki_1}\right)}$

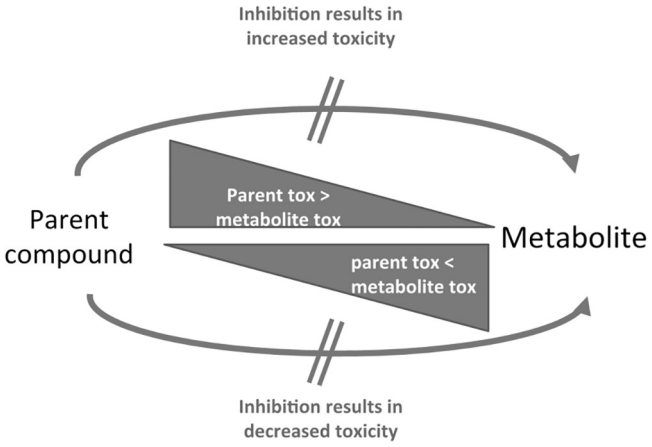

Fig. 7. Consequence of inhibition on toxicity depending on whether the parent compound or metabolite is toxic.

[30]. However, it is also important to note that competitive types of interactions usually become a concern only at higher concentrations than environmental or occupational exposure levels [15].

4.4.3. Relevance of interactions

Evidence in the literature indicates that interactions occur in chemical mixtures, but are observed mainly at higher exposure concentrations. Boobis et al. [39] performed a literature review, identifying 90 studies demonstrating synergisms in mammalian test systems performed at low doses (i.e. close to the point of departure, POD) for individual chemicals. Only 6 of the 90 studies reported useful quantitative information on the magnitude of synergy. In those six studies the difference between observed synergisms and predictions by CA did not deviate by more than a factor of 4. Cedergreen [40] performed a systematic literature review for binary mixtures within three groups of environmentally relevant chemicals (pesticides, metals, antifouling agents). Synergy was defined as a minimum two-fold deviation from CA predictions. Synergy was found in 7%, 3% and 26% of the pesticide, metal and antifoulant mixtures, respectively. The extent of synergy was rarely more than a factor of 10. Based on an in-depth analysis, Cedergreen concluded that true synergistic interactions between chemicals are rare and often occur at high concentrations. Using standard models such as CA is regarded as the most important step in the risk assessment of chemical mixtures.

Our review of PBK models confirmed that interactions mainly occurred at concentrations higher than common environmental or occupational exposure levels. However, there are examples where interactions might be relevant to consider, such as in the context of food safety (reviewed by [41]).

4.5. Challenges and opportunities for PBK modelling of mixtures

One of the major challenges of PBK modelling is the requirement for a large amount of data to build the model. This challenge is even more obvious in mixture PBK modelling since there are more chemicals and interactions to consider in addition to the vast amounts of data for physicochemical and biochemical parameters. A solution to this challenge can be the application of quantitative structure activity relationship (QSAR) modelling. Table 5 shows examples of the chemical kinetic parameters determined using QSAR modelling. Another challenge is the need for trained specialists to develop and validate the models. However, PBK modelling also has several advantages and could

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Table 4
Examples of the consequences of toxicokinetic and toxicodynamic interactions.

Mixtures	Toxicokinetic interaction		Toxicodynamic interaction		References
	Reduced concentration of metabolite	Increased target concentration of substrate	Reduced toxicity from metabolite	Increased toxicity from parent chemicals	
Vinyl chloride and Trichloroethylene		Vinyl chloride		Significant neurobehavioural effect in workers within TLV	[30,64]
Toluene and Trichloroethylene	Hippuric acid	Toluene			[38]
Benzene and Toluene	Phenol and hippuric acid	Benzene	Leukopenia or other toxicities from metabolite of benzene		[28,64–66]
Toluene and m-xylene		Toluene			[26]
Trichloroethylene and Tetrachloroethylene	metabolite of Trichloroethylene				[15]
Trichloroethylene and 1,1-dichloroethylene	Metabolite of 1,1-dichloroethylene		Reduced 1,1-dichloroethylene hepatotoxicity		[25]

Table 5
Some of the QSAR models used to determine toxicokinetics parameters for PBK modelling.

Reference	Toxicokinetics	Parameters predicted	Objective/Target compounds	QSAR modelling Method employed
[68]			Modelling of CYP 450 metabolism of chlorinated organic volatile compounds	
[69]	Metabolism	Vmax/Km	Organophosphorus compounds	
[70]	Metabolism	Km	59 substrates of CYP 3A4	e-state descriptors
[71]	Metabolism	Km (app)	CYP 3A4 inhibitors	Pharmacophore modelling
[72]	Absorption, Elimination	P, Clint, CLh	Volatile compounds	Group combination method
[73]	TD	Toxicity	53 Volatile Organic Compounds	Group combination method
[74]	Metabolism	Km/Vmax	VOCs	Group contribution method

support risk assessment of mixtures since it can account for interactions in a mixture. PBK modelling of mixtures may be relevant in addressing risks from co-exposure of both humans and environmental organisms to multiple chemicals in integrated mixture hazard and risk assessments [42].

Both bottom-up and top-down of PBK modelling approaches are based on binary interactions of parent chemicals whereas in reality interactions could occur between the parent and metabolite, as well as between metabolites. The models assume binary interactions, while in a reality humans are exposed to two or more chemicals at the same time, which is a more complex situation. Published models of mixtures of greater complexity are derived from information on binary interactions to simulate the interactions between all mixture components simultaneously.

Conventional types of PBK modelling can benefit from incorporation of relevant physiological processes. For example, metabolism by a single enzyme is assumed in the conventional bottom-up and top-down PBK modelling approaches for mixtures. However, usually there are different *iso*-enzymes and enzymes metabolising chemicals during phase 1 and phase 2 biotransformation. Moreover, in most of the PBK models reviewed the assumption was that metabolism and interactions occurred mainly in the liver. Even though on one hand such assumptions are important to simplify the model, on the other hand this is also limiting information, since other key organs could play a role in the mode of action of mixtures. Furthermore, relevant interactions in the absorption, distribution, metabolism and clearance phases should at least be added for some relevant chemicals to make the mixture PBK models more relevant and applicable in the future.

Capturing mixture interactions in the development of PBK model platforms (freely available tools, such as, MeGEN, COSMOS-KNIME, IndusChemFate, PLETHEM, R-httk) is another challenge. To our knowledge the above-mentioned platforms do not take into consideration environmental chemical co-exposure and mixture interactions, although some commercial platforms such as SimCyp and Gastroplus address drug to drug interactions.

5. Conclusions

PBK modelling can support the risk assessment of mixtures by including information on kinetics and ADME, thus describing the mechanisms of interaction occurring in mixtures. The risk assessment of mixtures currently relies mostly on concentration addition based approaches, thus neglecting possible interactions. We lay down two approaches that could be implemented in several PBK model software and packages. These approaches, are termed bottom-up and top-down, depending on whether the interactions are described in terms of a series of binary interactions, or by lumping mixture components and using representative parameter values. The choice of approach depends on the complexity of the mixture and availability of binary interaction data. These PBK modelling approaches should be further investigated for their applicability in mixture risk assessment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.comtox.2018.09.001.

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