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A review of quantitative structure-activity relationship modelling approaches to predict the toxicity of mixtures

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ABSTRACT

Exposure to chemicals generally occurs in the form of mixtures. However, the great majority of the toxicity data, upon which chemical safety decisions are based, relates only to single compounds. It is currently unfeasible to test a fully representative proportion of mixtures for potential harmful effects, and as such *in silico* modelling provides a practical solution to inform safety assessment. Traditional methodologies for deriving estimation of mixture effect, exemplified by principles such as concentration addition (CA) and independent action (IA), are limited as regards the scope of chemical combinations to which they can reliably be applied. Development of appropriate quantitative structure-activity relationships (QSARs) has been forwarded as a solution to the shortcomings present within these techniques – allowing for the potential formulation of versatile predictive tools capable of capturing the activities of a full contingent of possible mixtures. This review addresses the current state-of-the-art as regards application of QSAR towards mixture toxicity, discussing the challenges inherent in the task, whilst considering the strengths and limitations of existing approaches. Forty studies are examined within – through reference to several characteristic elements including the nature of the chemicals and endpoints modelled, the form of descriptors adopted, and the principles behind the statistical techniques employed. Recommendations are in turn provided for practices which may assist in further advancing the field, most notably with regards to ensuring confidence in the acquired predictions.

1. Introduction

A significant proportion of toxicological and physicochemical analysis is performed upon single compounds, yet the scenario of one being exposed to a single chemical in isolation is unrealistic [82]. In reality, both humans and environmental species face various, ever-changing mixtures of chemicals throughout daily life [21]. Most, if not all, chemicals are encountered as mixtures, for instance specifically marketed formulated mixtures such as pesticides, food and feed additives and cosmetics (typically referred to as intentional mixtures). In addition, exposure to mixtures of chemicals that may interact is not limited to manufactured products. For example, co-administration of drugs may lead to drug-drug interactions and environmental pollutants may also present themselves unintentionally as mixtures from different sources [36,58]. The prevalence of mixtures occurring either intentionally or unintentionally is evidently large, although only partial regulation of intentional mixture is currently provided [28].

Chemical mixtures can be defined as combinations of two or more chemicals that retain their individual, unaltered chemical identities [21]. In certain circumstances, mixtures may be more problematic when

Abbreviations: AOP, Adverse Outcome Pathway; CA, concentration addition; DHFR, dihydrofolate reductase; E_{LUMO}, energy of the lowest unoccupied molecular orbital; E_{LUMO} + 1, energy of the second lowest unoccupied molecular orbital; ECHA, European Chemicals Agency; EU, European Union; IA, independent action; INFCIM, INtegrated Concentration Addition-Independent action Model; NOEL, no-observed-effect level; QSAR, quantitative structure–activity relationship; QSI, quorum sensing inhibitor; SiRMS, Simplex Representation of Molecular Structure; SMILES, simplified molecular input line entry system; TMP, trimethoprim; UVCB, unknown or variable composition, complex reaction products or biological materials.

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compared to single compounds; a significant concern arises where the individual components are present in mixtures at concentrations where no effect would be anticipated e.g., lower than the no-observed-effect level (NOEL), yet in combination may have the potential to exert unexpected toxicological effects [22,21]. In addition, one of the key actions of the European Union's (EU's) recent "Chemicals Strategy for Sustainability Towards a Toxic-Free Environment" is to take account of the effects of chemical mixtures [23]. However, as the ability to assess the vast number of potential combinations of substances using traditional experimental toxicity testing is unfeasible [21], the value that predictive approaches can provide to mixture toxicity is anticipated to play an increasingly important role in toxicity assessment.

Traditional approaches for hazard assessment of chemical mixtures may either consider the mixture as a whole (top-down), or contributions from the individual components (bottom-up). In general, assessments are typically driven by bottom-up frameworks, where the individual toxicities of all components are known and then modelled mathematically to predict the combined effect of a mixture [29]. In such bottom-up or component-based approaches, it is essential to consider the influence of interactions which may arise between individual components. Where it is presumed that each constituent compound does not impact upon the biological activity of the other, the combined toxicity of a mixture is estimated according to the principle of additivity [21,81]. Should components be understood to operate through similar modes of action, this is typically framed through application of concentration addition (CA) [46]. Alternatively, those with dissimilar modes may be modelled with assumption of independent action (IA) [5]. These have since been termed "first generation" techniques [38]. Whilst the decision on which to adopt is dependent upon the nature of the mixture under examination, the enhanced conservatism inherent within CA has led to its emergence as the generic methodology particularly favoured by risk assessors [3,20,37]. "Second generation" models, further accounting for variation in mode of action and in turn combining elements of both approaches (integrated addition) later emerged – with uptake generally restricted on account of the greater quantities of empirical data required in their training [38].

Deviations from the ideal of additivity may be noted in instances whereby inter-component interactions do occur. The prevalence of such non-additive effects must not be understated, with a recent literature review by Martin et al. [52] observing such behaviours within almost half the experimental mixture studies they review (n = 1220). The term "synergy" describes the phenomenon through which mixture activity is observed as greater than that predicted by simple additivity, and "antagonism" the inverse in which it is less than [2,6,29,63]. Neither CA nor IA is equipped to handle such eventualities, and as such the potential occurrence of either serves to contribute greatly towards uncertainty surrounding estimation of overall mixture toxicity – notably at very low exposure levels [9,29]. Whilst the concept of the "funnel hypothesis" has been forwarded as a means of rationalising the observation that deviation from additivity is less common amongst multi-component (greater-than-binary) mixtures [79], the occurrence of both synergy and antagonism remains challenging to forecast.

In order to assess the toxicity of a greater number and form of mixtures, both additive and non-additive, there is scope for the application of further modelling approaches. One such class of models are quantitative structure-activity relationships (QSARs). QSARs have been used widely in various industrial sectors to predict a range of toxicity endpoints, as well as enabling data gap filling [51]. Predictions are formulated through identifying the correlation between quantifiable properties of the chemical, and the endpoint of concern – thus a model may allow for estimation of missing data by making use of structural information [15]. One of the earliest applications of QSARs towards mixtures was reported by Könemann [41], where it was recognised that the additive toxicity of mixtures could be predicted without use of empirical mechanism of toxic action data. Following this, much effort has been put into further development of related methods – since labelled "third generation". Significant scope exists for utilisation of such approaches, on account both of their practicality and potential predictive power. Ready generation of input parameters through employment of computational techniques may allow for data generation and broadening of applicability domain.

With regard to safety assessment, there is an ever-growing need for the harmonisation of approaches that address the effects of mixtures on human health and the environment. The role of *in silico* methods within the determination of mixture toxicity is deemed essential yet requires careful consideration of the array of challenges and gaps that currently exist [11]. For example, deficiencies in appreciation of realistic co-exposure scenarios, component interactions, mechanistic knowledge and grouping criteria may each impede progress [7]. Ensuring resolution of these issues will undoubtedly require "extensive strategic transdisciplinary initiatives", and as such it is inevitable that *in silico* approaches will be of immense value within mixture safety assessment [16]. However, it is acknowledged that available QSAR workflows for the analysis of mixtures are insufficient (Muratov et al., 2012). To enable a better understanding of the state-of-the-art, this study presents a narrative review of the different QSAR approaches to predict mixture effects within chemical safety assessment (i.e., toxicological studies). Knowledge identified from the review can be utilised to supplement current QSAR uncertainty assessment schemes.

2. Materials and methods

2.1. Collection of literature

Literature relating to the use of QSAR for the assessment of mixture toxicity was identified using the Web of Science database. To ensure that all relevant work was captured, a broad search was conducted for studies from 1970 onwards. Keywords selected within the initial search (performed 25/10/2020) included "QSAR" and "mixture" – this returning 434 publications. The search criteria used resulted in many articles not relevant to this specific topic being identified. These were removed following screening of abstracts. Only articles focusing on QSAR development for mixtures were retained, so reducing the list to 134 taken forward for full text review (for graphical overview of workflow, please refer to Fig. 1).

2.2. Compilation of information

A detailed analysis of the publications identified was undertaken, resulting in a further reduction of the number of articles for reasons including: unavailability of key information, models developed for single chemicals, studies on essential oils/nanoparticles, and mixtures

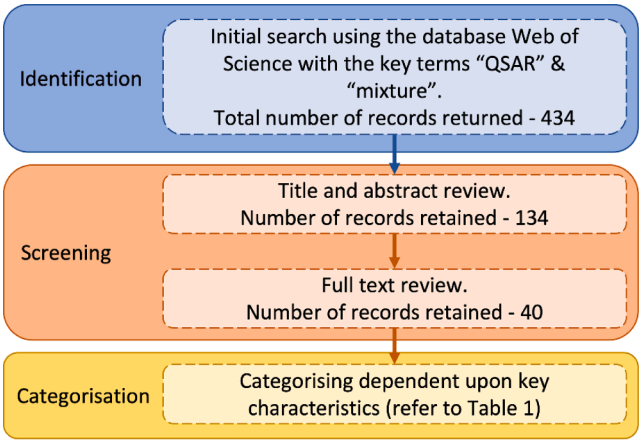

Fig. 1. Overview of workflow adopted in the recovery and screening of literature for inclusion within this study.

predicted solely through either concentration addition or independent action. Although CA and IA are both currently accepted methods used within regulatory approaches [21], the focus of the present study is upon QSAR protocols, and as such the decision was made to remove them. The final list comprised 40 studies, with these being additionally characterised with regards to: mixture composition (number of components, e.g., binary), chemical classification, taxa or testing system, endpoint examined, descriptors adopted (both class of, and conceptual approach applied in generation of mixture descriptors), and finally modelling or statistical technique applied. Table 1 contains overview of the standardised terminology adopted relating to this characterisation.

3. Results and discussion

Evaluation of the literature resulted in identification of 40 relevant publications. As summarised in Table 2 and Fig. 2, the majority of studies could be classified into groupings dependent upon methodology, endpoint etc. Further investigation of these characteristics has enabled the focus of current approaches to be outlined.

3.1. Chemical classification

Classes of chemicals considered in these articles could be classified broadly as belonging to one of four families: industrial chemicals (reported in 22 articles), pharmaceuticals (n = 9), biocides (n = 6) and priority pollutants (n = 5). In general, the majority of articles related to environmental studies, including those for pharmaceuticals, with only a limited number of investigations considering human health effects. Future work into mixture assessments, therefore, should focus upon extending studies of the lesser examined groups, with a particular focus given to human health effects. Cell lines could provide a route towards realising this.

3.2. Mixture composition

Different varieties of mixtures were investigated, ranging from binary to complex. Binary mixtures made up the majority (n = 38) of studies recovered, with comparatively few utilising multi-component combinations, i.e., ternary (n = 10), quaternary (n = 7), quinary (n = 4) and the more realistic supra-quinary (n = 3) –the latter term referring to those containing greater than five constituents. In addition to the number of components within the mixture, it is also important to consider the relative proportions of each, i.e., their ratios. Excluding supra-quinary, there are ten articles that investigated multi-component mixtures. Most of these were of fixed ratio design with some exceptions allowing varied ratios [34,59,73,40,48,17,80,32]. Fixed ratio designs have been demonstrated as favourable within mixture studies, allowing for the distribution of the effect concentration range to be maximised, whilst additionally reducing number of experiments required [39].

Table 1
Summary of defined QSAR characteristics and the categories within.

QSAR Characteristics	Categories
Chemical classification	Biocides, industrial, pharmaceuticals, priority pollutants
Mixture composition	Binary, ternary, quaternary, quinary, supra-quinary[1]
Taxa or testing system	Algae, amphibian, bacteria, cell line, embryos, insect
Endpoint	Acute, chronic, developmental, drug efficacy, growth inhibition, inhibition of reproduction
Descriptor formulation (approach)	Distribution coefficient, fragment non-additive, integral additive, integral non-additive, single variable component, structural similarity
Descriptor formulation (class)	Molecular docking, molecular fragment, molecular structure, physicochemical, quantum chemical
Modelling or statistical technique	CA and IA, CORAL, machine learning, partial order ranking, regression analysis, regression analysis (assumed)

[1] Mixtures containing greater than five components.

Equitoxic ratios were most commonly used - this referring to mixtures where each component exists at the concentration that would result in identical effect if examined separately [25]. The likelihood of a mixture occurring naturally as equitoxic is very small, hence non-equitoxic ratios provide a more realistic representation [78]. Additionally, it has been demonstrated, dependent upon the ratios of chemicals within a mixture, that the type of joint action observed can vary [78,33]. As a result, studies involving the investigation into non-equitoxic mixtures can ensure that changes in joint action are captured.

Binary mixtures studies are limited to predictions within this scope, unless validated otherwise. It is acknowledged that they may serve as an imperfect representation of real-world exposure scenarios [37]. As such, the importance of developing models that can predict the effects of not only binary, but more importantly multi-component mixtures, is crucial. Nevertheless, assessments of binary mixtures can provide invaluable insights into methodology for modelling, as well as being utilised to gain information on mode of action [30].

3.3. Taxa or testing system

A variety of species were used in the toxicological studies; however, the majority investigated bacterial-based bioassays (n = 27). Within this group, use of bioluminescent bacterium *Aliivibrio fischeri* (formerly *Photobacterium phosphoreum*) predominated. Such tests are relatively inexpensive and enable large quantities of consistent data to be generated rapidly. Accordingly, they have been routinely employed as a first screening method within test batteries [60,27]. However, for these tests to effectively monitor an ecosystem, they must be used in combination with other biotests as well as chemical analysis [27].

Various species other than bacteria have nevertheless been subject to investigation. Data from algae, cell lines (mammalian and amphibian), embryos, insects, amphibians, and viruses have all been used to develop mixture QSARs. Algal bioassays make up the second most common grouping (n = 4), with testing upon algae providing an important insight into the balance of aquatic ecosystems as a result of them being primary food producers [50]. Cell lines have been used in only a small number of studies, with such examinations potentially providing insight into specific simple mechanisms of interest. Cell line studies are an important testing procedure enabling the key processes towards a desired endpoint to be captured (Pistollato et al., 2020), however, the extrapolation of such information to entire organisms may prove difficult [90]. In general, QSAR developed to investigate the toxicological effects of mixtures has focused upon environmentally-relevant species, with fewer considering human health.

3.4. Endpoint

The majority of toxicological endpoints for which mixture QSARs were developed related to acute effects. In total, 30 studies have investigated acute toxicity, in comparison to only a few chronic. Examination into the acute effects of chemicals can provide useful and fundamental information, with testing being comparatively simple, interpretable and high throughput. Moreover, such tests can enable underlying mechanisms of toxic action to be defined [18]. However, the use of acute toxicity data for QSAR modelling is not without its limitations. Adverse effects can result from an array of physiological, biokinetic, cellular and molecular events that span different levels of biological organisation. Measuring such complex systems in isolation will inevitably result in a loss of information [42]. In comparison, toxicity following chronic exposure can better provide a realistic contribution to risk assessment of chemicals, particularly within environmental settings where organisms are exposed to the long-term effects of pollutants [71]. However, knowledge of the chronic effects towards organisms of mixture exposure is sparse due to the intricacies of processes required for their determination – compounded by their duration and the costs of analyses [89]. Accordingly, within the scope of the

Table 2
Summary and main characteristics of QSARs used in the mixture toxicity studies identified.

Chemical classification	Mixture composition	Taxa or test system	Endpoint	Molecular descriptor formulation		Modelling or statistical technique	Reference
				Conceptual approach	Descriptor class		
Biocides	Binary	Insect	Acute	Fragment non-additive	Molecular fragment	CORAL	[8]
Priority pollutants	Binary	Cell line	Acute	Integral additive	Molecular structure	Regression analysis	[31]
Industrial	Binary	Bacteria	Acute	Integral additive	Molecular structure	Regression analysis	[12]
Industrial	Binary	Bacteria	Acute	Single variable component	Molecular structure	Regression analysis	[87]
Biocides	Binary	Bacteria	Acute	Integral additive	Molecular structure	Regression analysis and machine learning	Wang et al., 2018a
Priority pollutants	Binary and ternary	Embryos	Developmental	Integral additive	Molecular structure	Regression analysis	[34]
Pharmaceuticals and biocides	Binary, ternary and quaternary	Bacteria	Acute	Integral additive	Molecular structure	Regression analysis	[59]
Pharmaceuticals	Binary and ternary	Bacteria	Acute	Integral additive	Molecular docking	Regression analysis	Wang et al. [73]
Pharmaceuticals	Binary	Bacteria	Acute	Integral additive	Molecular docking	Regression analysis	Wang et al., 2018c[72]
Pharmaceuticals	Binary	Bacteria	Acute and chronic	Integral additive	Molecular docking	Regression analysis	[71]
Pharmaceuticals	Binary	Bacteria	Acute	Integral additive	Molecular docking	Regression analysis	[47]
Pharmaceuticals	Binary	Bacteria	Chronic	Integral additive	Molecular docking and physicochemical	Regression analysis	[24]
Priority pollutants	Binary	Cell line	Acute	Integral additive	Molecular structure and physicochemical	Regression analysis	[26]
Industrial	Binary	Bacteria and algae	Acute	Integral additive	Quantum chemical	Regression analysis	[10]
Industrial	Binary and ternary	Cell line	Organ-level effects	Unclear	Physicochemical	Regression analysis	[40]
Industrial	Binary	Bacteria	Acute	Single variable component	Quantum chemical	Regression analysis	[33]
Biocides	Supra-quinary	Bacteria	Acute	Structural similarity	Molecular structure	Machine learning and CA and IA	Kim et al., 2013b[39]
Pharmaceuticals	Binary	Virus	Drug efficacy	Fragment non-additive	Molecular fragment	Machine learning	[53]
Pharmaceuticals	Binary	Bacteria	Chronic	Integral additive	Molecular docking and physicochemical	Machine learning	[89]
Industrial	Binary	Not Stated	Chronic	Integral additive	Molecular structure and quantum chemical	Regression analysis and machine learning	[49]
Industrial	Binary	Bacteria	Acute	Single variable component	Physicochemical and quantum chemical	Regression analysis	[65]
Industrial	Binary	Bacteria	Acute	Fragment non-additive	Molecular fragment	CORAL	[67]
Priority pollutants and industrial	Binary	Not Stated	Acute	Integral additive	Molecular docking and physicochemical	Assumed regression	[77]
Pharmaceuticals	Binary	Bacteria	Acute and chronic	Integral additive	Molecular docking and quantum chemical	Assumed regression	[88]
Priority pollutants	Binary	Bacteria	Acute	Integral additive and distribution coefficient	Physicochemical	Assumed regression	Wang et al., 2011a[75]
Biocides	Binary, ternary, quaternary and quinary	Embryos	Developmental	Unclear	Physicochemical	Regression analysis	Wang et al., 2011b[74]
Industrial	Binary	Bacteria	Acute	Single variable component	Physicochemical and quantum chemical	Regression analysis	[66]
Industrial	Binary, ternary and quaternary	Bacteria	Acute	Integral additive	Physicochemical and quantum chemical	Regression analysis	[48]
Industrial	Binary	Algae	Growth inhibition	Distribution coefficient	Physicochemical	Regression analysis	[85]
Industrial	Binary, ternary, quaternary and quinary	Bacteria	Acute	Distribution coefficient	Physicochemical	Partial order ranking	[17]
Industrial	Binary	Algae	Growth inhibition	Integral additive	Physicochemical and quantum chemical	Regression analysis	[70]
Industrial	Binary	Bacteria	Acute	Integral non-additive	Quantum chemical	Regression analysis	[86]
Industrial	Binary, ternary, quaternary, quinary and supra-quinary	Bacteria	Acute	Integral additive	Physicochemical	Regression analysis	[69]

(*continued on next page*)

Table 2 (*continued*)

Chemical classification	Mixture composition	Taxa or test system	Endpoint	Molecular descriptor formulation		Modelling or statistical technique	Reference
				Conceptual approach	Descriptor class		
Biocides	Supra-quinary	Algae	Inhibition of reproduction	Structural similarity	Molecular structure	CA and IA	[56]
Industrial	Binary, ternary, quaternary and quinary	Bacteria	Acute	Distribution coefficient	Physicochemical	Assumed regression	[80]
Industrial	Binary, ternary and quaternary	Amphibian	Acute	Integral additive	Physicochemical	Regression analysis	[32]
Industrial	Binary	Bacteria	Acute	Distribution coefficient	Physicochemical	Regression analysis	[44]
Industrial	Binary	Bacteria	Acute	Distribution coefficient	Physicochemical	Assumed regression	[43]
Industrial	Binary	Bacteria	Acute	Single variable component	Quantum chemical	Regression analysis	[84]
Industrial	Binary	Bacteria	Acute	Distribution coefficient	Physicochemical	Regression analysis	[83]

review, few studies utilised QSARs to predict chronic toxicity. However, a small number of successful applications have demonstrated that molecular docking based QSARs may prove a valuable tool for predicting such endpoints [89,24,71]. The current literature available for QSAR chronic mixture toxicity provides a solid foundation to be developed upon, with further research being required in areas of multi-component mixtures, as well as in higher-order species.

3.5. Mixture descriptor formulation

3.5.1. Conceptual approach

A fundamental distinction between the handling of single compounds and chemical mixtures when constructing QSAR lies in the nature of the descriptors which must be employed for each purpose. Whilst generation of molecular descriptors relating to discrete organic substances is generally a trivial process, provision of equivalents suitable for characterising mixtures is an issue of greater complexity. A variety of approaches are attested within literature, based upon differing assumptions regarding the nature and relevance of interactions between member substances (Muratov et al., 2012).

3.5.1.1. Integral additive. The single most popular approach amongst those studies recovered (present within 21 of 40), formation of integral additive descriptors rests upon the intuitive premise that the properties of a mixture may be determined simply through summing those of its individual components – accounting for their relative prevalence and assuming occurrence of no meaningful interaction between each.

$$d_{mix} = \sum x_i d_i \quad (1)$$

Where d_{mix} is a mixture descriptor, d_i the descriptor relating to chemical i, and x_i the fraction of the mixture composed by chemical i.

Application of the methodology in its simplest form is exemplified in the work of Huang et al. [32], whereby toxicity of substituted phenol combinations is inferred solely through reference to a mixture octanol/water partition coefficient log_{kowmix} calculated via fractional addition of the log_{kow} belonging to each component. Versatility of the approach is such that there exist few limitations with respect to the nature of descriptors which may be used alongside it (refer to *Section 3.5.2* and Table 3 for examples). Accordingly, its adoption is noted in investigations employing molecular docking and quantum chemical techniques.

Despite widespread utilisation, shortcomings of this framework remain apparent. Disregarding of the potential impact of inter-component interactions (toxicodynamic, toxicokinetic or physicochemical) when inferring mixture adverse effects is most noteworthy amongst these. Such a limitation almost certainly renders it unapplicable for instances in which non-additivity is present – whilst in principle (despite favourable results) harming its capacity to model even general additive effects.

3.5.1.2. Integral non-additive. By contrast to the above, non-additive approaches envisage the mixture not merely as an agglomeration of mutually-inert components. Instead, they seek to integrate consideration of interactions existing between the molecules within – essentially modelling the mixture as a unit with bulk properties distinct to it (representing a more appropriate approximation of reality). Although appealing as a route towards the addressing issues inherent within additive methodologies, adoption has been limited.

A single study [86] employing an integral, non-additive approach was retrieved. Within, toxicity of a series of binary 1:1 combinations consisting of simple substituted benzenes was modelled through use of quantum chemical descriptors. Properties of a mixture were derived through direct calculation of parameters of the appropriate pooled molecular pair – thus allowing for influence of electronic interactions between members to be accounted for. The rationale behind the lack of widespread uptake of this technique, despite conceptual promise, may lie in the restrictions placed upon its practical application: not only is scope of eligible mixtures constrained to those exhibiting 1:1 component ratio, but requirement to initiate unique calculations relating to each potential combination of substituents is potentially unwieldy.

3.5.1.3. Fragment non-additive. The non-additive principle is extended for application within fragment-based approaches to characterising activity of binary mixtures – forming the basis of three toxicologically-relevant studies. Whilst a thorough overview of core techniques is presented within *Section 3.5.2.4*, it is sufficient when considering generation of mixture descriptors to recognise the parallels which are present between this and "integral non-additive" methodology. In much the same manner, the molecular pair is treated as a unit. Individual fragments may incorporate atoms from either one or both components, and as such may provide descriptors relating both to individual compounds and to the unitary mixture.

3.5.1.4. Distribution coefficient-based. This approach remains suitable for instances in which activity of a mixture is modelled as a function of its partitioning between lipophilic and aqueous phases. Verhaar et al. [68] reported derivation of a formula through which the distribution coefficient representing a mixture may be determined from those of its constituent chemicals.

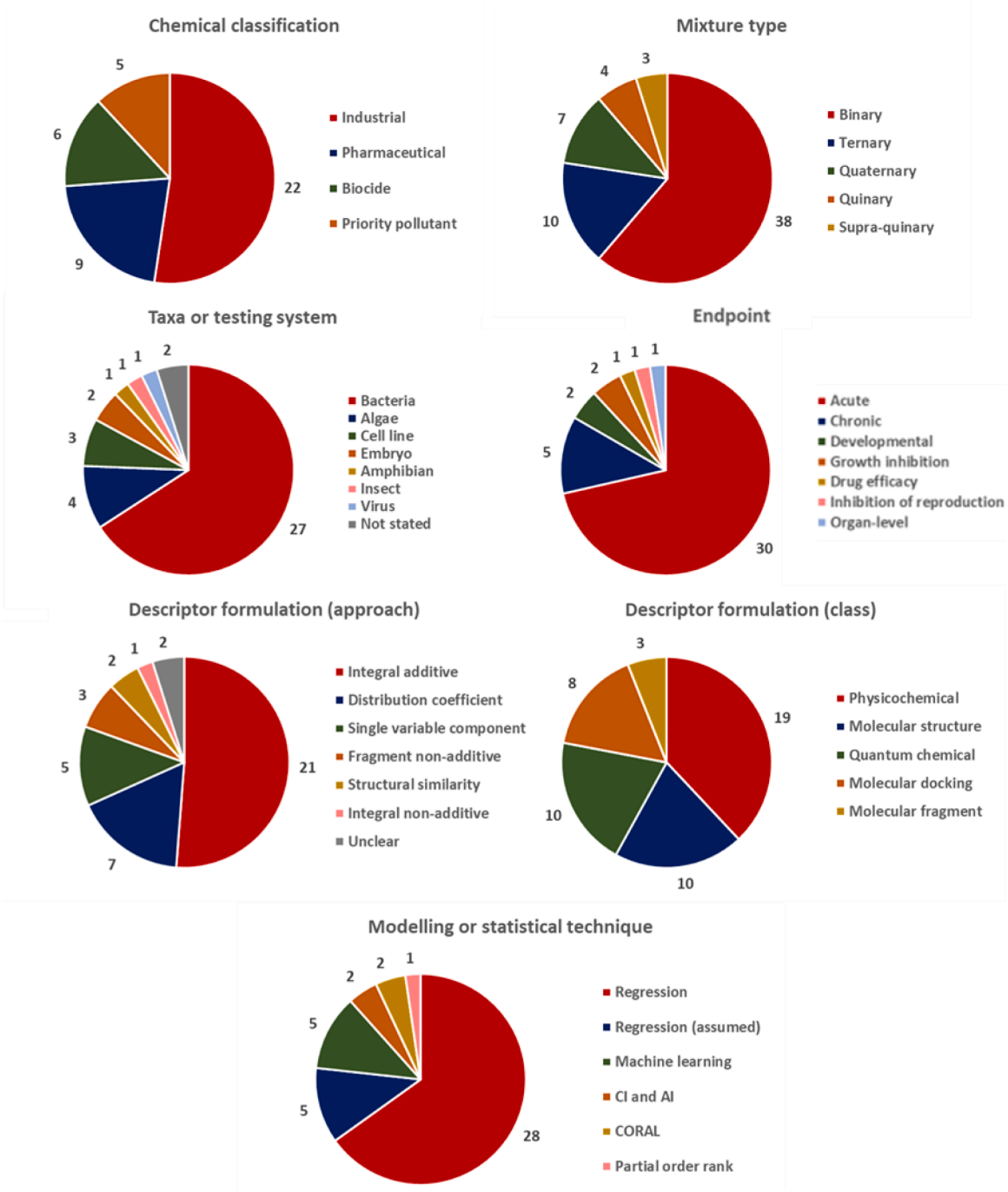

Fig. 2. Quantification of features present amongst those parameters defining key QSAR characteristics.

$$K_{mix} = \frac{W}{V} \times \frac{\sum_{i=1}^{n} \frac{Q^0_{water,i}}{1+(\frac{W}{VK_{SDi}})}}{\sum_{i=1}^{n} Q^0_{water,i} - \sum_{i=1}^{n} \frac{Q^0_{water,i}}{1+(\frac{W}{VK_{SDi}})}}$$

Where K_{mix} is the lipoid/water partition coefficient of the mixture (substances such as *n*-octanol, chloroform and C18-Empore discs having been employed for this function), W the volume of the aqueous phase, V the volume of lipoid, $Q^0_{water,i}$ the initial amount of chemical i in water, $K_{SDi}$the partition coefficient of chemical i, and n the total number of chemical components in the mixture. Seven relevant studies adopting this approach were retrieved, with modifications to the methodology offered on occasion (please refer also to *Section 3.5.2.1*).

3.5.1.5. Single variable component. Each of the aforementioned techniques seeks to characterise toxicity of mixtures through consideration of the contributions of all substances within. However, there exist several studies (five retrieved from literature) in which activity is instead inferred through reference to properties of only a single constituent. In all instances, sequences of binary combinations were examined, whereby-one component was held in common and the other was varied. Typical is the examination by Su et al. [66], within which electronic and physicochemical parameters of a selection of substituted phenols were alone employed in order to model the toxicity of its mixtures alongside elemental lead. Whilst the majority of investigations have focused upon metallic-organic combinations, it should be noted that an early study by Yuan et al. [84] featured solely organic

Table 3
Descriptors calculated using DRAGON software, displayed within their respective blocks.

Descriptor	Title	Block	Publication
piPC06	Molecular multiple path count of order 6	Walk and path counts	[31]
Mor12m	Signal 12 / weighted by mass	3D-MoRSE descriptors	[12]
Mor13s	Signal 13 / weighted by I-state	3D-MoRSE descriptors	
L/Bw	Length-to-breadth ratio by WHIM	Geometrical descriptors	
Eig08_EA (ed)	Eigenvalue n. 8 from edge adjacency mat. weighted by edge degree	Edge adjacency indices	
Eig09_EA (ed)	Eigenvalue n. 9 from edge adjacency mat. weighted by edge degree	Edge adjacency indices	
Eig09_AEA (dm)	Eigenvalue n. 9 from augmented edge adjacency mat. weighted by dipole moment	Edge adjacency indices	
RDF045s	Radial Distribution Function – 045 / weighted by I-state	RDF descriptors	
J_RG	Balaban-like index from reciprocal squared geometrical matrix	3D matrix-based descriptors	
VE2_B(p)	Average coefficient of the last eigenvector from Burden matrix weighted by polarisability	2D matrix-based descriptors	[87]
TIC3	Total Information Content index (neighborhood symmetry of 3-order)	Information indices	
Eig06_AEA (dm)	Eigenvalue n. 6 from augmented edge adjacency mat. weighted by dipole moment	Edge adjacency indices	
PJI2	2D Petitjean shape index	Topological indices	[34]
$^{2}\chi^{v}$	Valence connectivity index of order 2	Connectivity indices	
$^{0}\chi^{v}$	Valence connectivity index of order 0	Connectivity indices	
RDF035m	Radial Distribution Function – 035 / weighted by mass	RDF descriptors	[59]
HATSs	Leverage-weighted total index / weighted by I-state	GETAWAY descriptors	
H-047	H attached to C^{1}(sp3)/C^{0}(sp2)	Atom-centred fragments	
	Independent components[a]	N/A	[56]

components.

3.5.1.6. Similarity. A minority of studies adopt QSAR not as a means of directly inferring the toxic potential of a mixture from the properties of its components, but instead as a means of assessing the similarity of screened compounds against those for which experimental data are present. Both Mwense et al. [56,39] have put forward variations on this theme. Such similarity-based approaches enabled the mixtures components to be separated into clusters, which could then be subjected to CA and IA calculations (see Section 3.6 for further information).

3.5.2. Descriptor class

Many different varieties of molecular descriptors exist, indicating the differing complexity levels of chemical structural representation [13]. In principle, any intrinsic molecular property appropriate for adoption as a descriptor within standard, single-component QSAR is further amenable to application within the domain of the mixture. As such, the range of properties referenced explicitly across the following subsections (on account of appearance within the existing literature) should not be taken as exhaustive.

3.5.2.1. Physicochemical. Considering the modelling of mixture toxicity, physicochemical descriptors have been employed from the very earliest studies. Of particular prominence are those based upon quantitative expression of the distribution of a substance between aqueous and representative lipophilic phases – this in short owing to their applicability in modelling compounds which exhibit a narcotic mode of action. Exemplified by logarithm of the octanol-water partition coefficient, these are acknowledged as being amongst the most effective general parameters to predict toxicity; having seen widespread use in many models for both single chemicals and mixtures [37,43]. It should be noted, however, that utility in handling toxicity mediated through means of chemical reactivity or receptor interaction may be diminished.

Application to mixtures is typically facilitated through adoption of one of two techniques introduced within *Section 3.5.1*: the dedicated method of Verhaar et al. [68], or the more general integral additive approach. Employing the former, models were successfully developed to predict mixture toxicity of non-polar narcotic [83,43] and polar narcotic [44] chemicals. Following on, Wei et al. [80] reported formulation of a simplified model demonstrating strong predictive power for both polar and non-polar mixtures. The aforementioned approaches have been limited to bacterial toxicity with regression-based models. However, additional studies have validated the methodology within algae studies, as well as with Partial Order Ranking methodology [85,17].

Considered by Roberts [62] and by Altenburger et al. [1], the employment of the integral additive approach towards formulation of mixture partition coefficients has since been demonstrated in various environmental studies [32,70,48,69]. One of the few studies to compare both Verhaar and integral additive methodologies directly was completed by Wang et al. [75], in which the mixture toxicity of perfluorinated carboxylic acid was assessed. Results demonstrated that the equivalent Verhaar-adapted approach provided in this instance the stronger at describing the mixtures' hydrophobicity.

3.5.2.2. Molecular docking. Information gathered from molecular docking of chemicals into receptors has been used routinely, particularly as a drug discovery tool enabling the early identification of potentially active candidate molecules. These techniques facilitate the development of mechanism-based models, with interactions between chemicals and receptors being simulated. Specifically, such studies could relate to receptor-mediated molecular initiating events [14]. These simulations enable the interaction energy required for a chemical to bind to its target protein ($E_{binding}$) to be determined [61]. In each of the examples subsequently presented, $E_{binding}$relating to individual components are summed to form mixture descriptors through adoption of the integral additive approach.

Wang et al. [77] were amongst the first to propose the use of binding energy descriptors in modelling mixture toxicity – examining the feasibility of substituting $logK_{owmix}$ with the molecular docking descriptor $E_{binding}$, owing to the linear trend observed between the two. Zou et al. [88] investigated both the acute and chronic toxicities of antibiotics from the sulfonamide family, alongside the sulfonamide potentiator trimethoprim. The study initially identified the receptors responsible for both their acute and chronic effects towards *Aliivibrio fischeri*; determining them to be luciferase, dihydropteroate synthase and dihydrofolate reductase. Models using the binding energies towards each protein, supplemented by pKa, were shown to successfully predict the toxicities of mixtures for both exposures. Further to this study, Zou et al. [89], employed docking in order to curate a library of simulated antibiotic-receptor interactions, spanning several prominent mechanisms of action. Through this, the ready construction of mechanistically-grounded QSAR models relevant to a wide range of potential antibiotic combinations was facilitated.

More recently, Wang et al. [71] also investigated chronic effects of antibiotics. A mechanism-based QSAR model was developed whereby the chronic toxicity of sulfonamides, sulfonamide potentiators tetracyclines could be extrapolated from acute toxicity. Unlike previous extrapolation models, understanding of the differing toxic mechanisms

between acute and chronic exposures was considered. In a variation from Zou et al. [88], in which DHFR served as the sole mediator of TMP toxicity, the targets for the antibiotics reported in this study were represented by surrogate luciferase proteins. Due to a specific target not being considered and instead characterised by surrogates, the model demonstrated promise in predicting the toxicity chemicals for which mechanisms are unknown.

Molecular docking studies have introduced new concepts to the field of QSAR mixture toxicity. [24,47,73] developed mechanistic models derived from binding energies of antibiotics towards target proteins from which they were able to theoretically identify the effective concentration of the mixtures. Wang et al.[73] also proposed equivalent findings but included ternary mixtures. Each study incorporated terms describing the extent to which each specific component contributed towards protein binding, i.e., the effective concentration. Wang et al. [73] further commented upon this, stating that such terms could be interpreted as representing the processes of a component passing through the cell membrane and reaching its target protein. Thus, the component which had a higher probability of interacting with its target protein could be identified depending upon the value of coefficient attached to the term. The authors utilised this knowledge to enable calculation of the actual toxicity ratio – a value which was subsequently used to aid in determining which component had the greater contribution to toxicity.

Wang et al. [72] further employed docking techniques in investigation of mixture effects of the recently popularised antibiotic alternative - quorum sensing inhibitors (QSIs). However, current research remains largely focused upon simple binary mixtures of antibiotics– with only [73] extending examination into multi-component mixtures. It is further noted that existing studies have yet to integrate consideration of mixture toxicokinetics in a manner which would allow conclusions to be drawn regarding likely absolute exposure of targets to components.

3.5.2.3. Molecular structure. Structure-based descriptors (otherwise known as 2D or topological), provide simplistic, interpretable information about molecular structure, as well as being easy and quick to generate [13]. A variety of software is available to calculate these parameters, with DRAGON (previously available at: https://chm.kode-solutions.net/pf/dragon-7–0/) used in several reported mixture studies. DRAGON software calculated over 5000 molecular descriptors, with these being organised into logical blocks. A range of different blocks exists, with these including, but not limited to: constitutional, ring descriptors, topological indices, walk and path counts, and connectivity indices. The DRAGON software was used to obtain descriptors in six studies identified in this analysis, as summarised in Table 3. Due to the range of chemical mixtures and species examined within, it is inevitable that a variety of descriptors were used. For example, Chen et al. [12] and Zhang et al. [87] both utilised edge adjacency indices derived from H-depleted molecular graphs. Both studies utilised toxicity data for bioluminescent bacteria, with Chen et al. [12] investigating aromatic halogenated chemicals and Zhang et al. [87] nitro-substituted benzenes and zinc. These parameters were successful in both instances, additionally proving worth within mixtures of different mixing ratios [87]. Gaskill and Bruce [26] further found that information indices were able to predict mixture toxicity. The authors developed various models to predict impact of polycyclic aromatic hydrocarbon mixtures towards liver cells, with additional topological descriptors being utilised. These topological descriptors, particularly with respect to planar PAHs, proved to be significant in predicting effects, highlighting the role planar characteristics and bond orientation play in causing toxicity.

3.5.2.4. Molecular fragments. Fragment-based descriptors have been described as a promising method for the QSAR modelling of mixtures [13]. However, there are relatively few examples of their use in practice. Muratov et al. [53], predicted combination effects of antivirals against poliovirus-1 through use of Simplex Representation of Molecular Structure (SiRMS) - a framework which enables molecular structures to be represented as a system of simplexes (tetratomic fragments), capable of capturing features at the topological level. Modifications to the approach were undertaken to enable extension for analysis of binary systems, generating descriptors applicable either to single components (bounded simplex), or else drawing elements from across both (unbounded simplex). The latter can be considered as structural descriptors of the mixture as a unit and as such "non-additive". Whilst this approach is highly desirable, that no other recent toxicological report has utilised this methodology suggests that it may only be applicable within certain cases.

Other fragment-based descriptors were utilised by Toropova et al. [67], who demonstrated the ability of the CORAL software (https://www.insilico.eu/coral) to again predict toxicity of binary mixtures. Molecular structures of components were represented by SMILES, using a disconnected approach with a marker (i.e., ".") separating each string. Recently, Carnesecchi et al. [8] further extended this approach, making use of expanded "quasi-SMILES". In this case, the toxic units of each chemical in the binary mixture are incorporated. A classification model predicting potential for non-additivity (either synergism or non-synergism) was simultaneously reported. Results obtained indicated that consideration of toxic units not only enabled greater interpretability of the models, but also improved the statistical performance. In general, models developed by the CORAL software enable frequently occurring molecular features that cause binary mixture toxicity to be identified. However, studies thus far using these procedures (and SiRMS) have only been limited only to binary mixtures.

3.5.2.5. Quantum chemical descriptors. Quantum chemical descriptors are able to describe the electronic and geometric properties, and interactions, of molecules. Although potentially intensive as regards demands upon computational power and running time, they offer greater detail with respect to electronic effects than do traditional empirical methods [35,64]. The most commonly applied quantum chemical descriptors utilised for modelling mixture toxicity were the molecular orbital energies, with energy of the lowest unoccupied molecular orbital (E_{LUMO}), or slight adaptions, being routinely used. This metric accounts for the electrophilicity of a molecule [64], correlated as it is to its electron affinity. Studies extended this parameter to multi-component mixtures [48], and the variation $E_{LUMO}+1$ (energy of the second lowest unoccupied molecular orbital), in combination with total charge weighted partial positively surface area PPSA, have proven superior to previous hydrophobicity-dependent QSARs for non-polar narcotics [49]. Additionally, the difference between the lowest and highest frontier molecular orbitals, i.e., $E_{LUMO}-E_{HOMO}$, or *vice versa*, have been proven effective in mixture calculations. Wang et al. [70] first used this parameter, which is able to determine the stability of the molecule, collectively within a traditional hydrophobicity model to enable better predictions of the joint toxicity of polar narcotics.

In each of the aforementioned instances, orbital mixture descriptors were generated through integral additive means. Quantum chemical descriptors have, however, additionally found employment in a distinct collection of studies introduced within *Section 3.5.1.5*, under the heading "Single variable component". A typical example is provided through Jin et al. [33], whereby models are created considering the energy difference between molecular orbitals- a parameter termed the relative hardness index ($E_{HOMO}-E_{LUMO}$). Multi-pointwise toxicological models (i.e., approaches for mixtures predicting varying effect concentrations) are an under-researched area, although interestingly an additional report studying them, that of Su et al. [65], did employ quantum chemical descriptors. Within, the joint toxicity of nitroaromatics with copper at low, medium, and high concentrations was modelled. The results were similar to those of Jin et al. [33], in that varying the concentrations of the components played a pivotal role on the joint effects

within the mixture.

Currently, the majority of literature describing use of quantum descriptors is focused exclusively on single mixture ratios - typically equitoxic. Realistically-encountered combinations of molecules are expected to deviate from this ideal, thus suggesting that a range of compositions would provide for stronger predictions. These studies, furthermore, concentrate almost exclusively upon industrial compounds – thus serving only a restricted area of chemical space.

3.6. Methods for model development

A variety of statistical approaches were reported across the reviewed literature with regression analysis dominant. Comparatively simple to establish and interpret, regression has been the classical approach in QSAR since its inception. It is, however, not without limitations, with consideration of parameter collinearity required in order to ensure that robust models are developed [45]. As an alternative, machine learning approaches permit nonlinear relationships to be better modelled, which is attractive in mixture toxicity due to the varying nature of underlying combination effects. Two studies developed models using both regression and machine learning, enabling direct comparisons between the performance of both. Results suggested that machine learning approaches, specifically radial basis function neural networks, enable improvements in statistical fit [49,76]. Although, machine learning is a current trend in the area of *in silico* prediction, it is not without its limitations: ensuring that models are well established typically requires a high volume of data. Potential for overfitting must be taken into account, and difficulties in interpretation owing to the black box nature typically hinder derivation of mechanistic knowledge [45].

Whilst studies incorporating exclusively either CA or IA (first generation) are considered beyond the scope of this review, a small quantity of second-generation models are eligible for inclusion on account of their integration of QSAR methodology. Each of the following techniques may be distinguished by the conditional adoption of CA or IA in modelling of inter-component interactions, dependent upon the extent of similarity either in molecular structure or mode/mechanism of action between substances. As such, the combined toxicity of like compounds is determined through the principle of CA, and dissimilar through IA – with ultimate mixture effect being derived from the contributions of both. Mwense et al. [55] introduced an approach termed INtegrated Concentration Addition-Independent action Model (INFCIM), whereby this similarity was determined using computed molecular descriptors. The following equation was employed to calculate overall toxicity:

$$EC_{x,mix} = \omega_A \bullet (CA) + \omega_B \bullet (IA)$$

where coefficients $\omega_A$and ω_B are the weightings for the contributions of CA and IA.

Although this initial model had no theoretical capabilities to provide predictions that would exceed concentration addition, the model was later revised in order to address these limitations [56]. Analogously, [39] developed an approach which incorporated both CA and IA known as a two-stage prediction model. Unlike previous two-stage prediction models which relied on knowledge of modes of toxic action for all components, the authors utilised machine learning clustering techniques to group the constituents – employing CA within-group (stage 1) and IA between-group (stage 2) in determination of absolute mixture effect. Excellent performance against realistic environmental mixtures was reported, highlighting the possibility of success even in absence of mechanistic information. Such models, however, remain at present limited to non-interacting mixtures.

3.7. Uncertainty criteria and assessment for mixture studies

The assessment of chemical mixtures by means of QSAR methodologies is continually generating greater interest. In ensuring that such work is up taken in regulatory settings, it is essential that potential uncertainty associated with models are defined. Cronin et al., [15] recently developed a set of criteria that enabled the full assessment of QSAR models from conception to application, facilitating all aspects of uncertainty to be defined and scored. This was further expanded upon by Belfield et al. [4], where it was demonstrated that the criteria could also be employed to determine fitness-for-purpose. Although these criteria have been developed in order to account for all potential usages of QSAR, completion of the present literature review has elucidated further areas of consideration specifically relevant to construction of QSAR models for prediction of mixture effects. As such, areas have been identified that can be bolstered with lessons learnt to improve the assessment of QSARs for mixture. Specifically, it can be defined that these additional considerations relate to chemical description, descriptor calculation, and statistical performance. These are discussed beneath – with accessory detail provided in Supplementary Table 1.

Firstly, worthy of note is that within the current structure of the QSAR uncertainty criteria, the consideration of chemical mixtures is approached (as clearly defined under criterion 1.1b – "Assessment of significant impurities or mixtures"). However, unambiguous guidance ought to be provided for the assistance of users unfamiliar with mixture handling. To ensure that scorings are assigned correctly, further information on what is to be expected is suggested within the comment section. Not only is it vital that all components within mixtures are fully identifiable, but additionally that the proportion represented by each must be reported. Clearly, measured endpoints will be dependent upon the ratio at which mixtures are investigated, but such information is additionally required to enable accurate calculation of mixture descriptors. Omission of mixture ratios will therefore restrict external reproducibility. Further to this, and in a similar vein (although not discussed further in the present review), guidance to correct reporting techniques of substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCB) as detailed by the European Chemicals Agency (ECHA) are provided [19].

Arguably the most important aspect that changes from modelling single chemicals to mixtures is the handling of descriptors. An entire section of the criteria has been devoted to the consideration of the varieties of descriptors a user may employ (this being 1.3 – "Measurement and/or Estimation of Physico-Chemical Properties and Structural Descriptors"), yet methodologies to convert such features into mixture descriptors are needed. As reviewed in *Section 3.5* many approaches are used to define mixture descriptors. Selection of the correct method in characterising these is not only dependent upon the type of descriptors chosen (such as fragment-based compared to physicochemical), but additionally by the interaction effects within the mixture. Capturing such complex processes and concerns by updating comment guidance to existing criteria would clearly be insufficient; thus, an additional topic must be supplied to fulfil the need. The current structure of the criterion 1.3 enables all plausible descriptors to be considered, relying upon user discretion to evaluate only relevant features that have been employed. As such, supplementing a new point into this section will not alter the validation process, but instead extend applicability of models that may be evaluated. A further criterion 1.3d ("Calculation of mixture descriptors, if utilised") is proposed that will enable the uncertainty level of mixture descriptors to be defined. The main aspect needed to satisfy this recommended criterion is that the selected approach has been derived through thorough consideration of potential interaction effects. Calculating these effects is a topic well studied, with a variety of methods alluded to in the comments for user guidance.

The final section that would benefit from further guidance relates to external validation. Within QSAR modelling, exhaustive validation is required to ensure that predictive performance is correctly evaluated. However, compared to that of traditional QSAR procedures, validation methods for mixtures require further deliberation. Mixtures present further challenges whereby the same components may exist inside different mixtures. Splitting without consideration of this fact will

undoubtedly result in datapoints from the same mixture appearing within both training and testing sets, thus resulting in over-optimistic estimations (Muratov et al., 2012). To combat such occurrences, various strategies have been developed, namely: "points out", "mixtures out", "compounds out", and "everything out" (for detailed discussion of these, please refer to [57,54]. Validating mixture models without consideration of these facts will certainly affect the legitimacy of predictions, as well as the associated uncertainty. As selection of appropriate validation methods is already well defined within criterion 2.2a ("Statement of statistical fit, performance and predictivity"), providing further guidance under the "comment or other information" heading will ensure that mixture strategies can be fully considered.

4. Key findings

The purpose of the current review was not only to identify current trends in QSAR mixture modelling, but also to determine whether existing modelling practices are sufficient to accurately address issues that mixtures present. Regardless of the source of the model or modelling approach, a number of commonalities can be identified. These form a general appraisal, or overview, of the state-of-the-art of QSAR mixture modelling:

4.1. Need for models

- Modelling is a vital approach to assess the toxicity of mixtures. It is inconceivable that all possible combinations of chemicals (and at varied ratios) can be experimentally measured. Therefore, there needs to be a much greater emphasis on modelling approaches for mixture toxicity.

4.2. Need for proper problem formulation

- Much of the current modelling of mixture toxicity has been performed on an *ad hoc* basis. There needs to be greater organisation of these modelling studies to make them realistic of real-life exposures and able to address the problems associated with ensuring environmental and human safety. Utilising the uncertainty criteria proposed by Cronin et al. [15], with guidance previously suggested, would provide a rational foundation for addressing such issues.

4.3. Availability of data for modelling

- This review has demonstrated the paucity of data available for mixtures. Repositories such as PubChem (https://pubchem.ncbi.nlm.nih.gov/), ChEMBL (https://www.ebi.ac.uk/chembl/), DrugBank (https://go.drugbank.com/), IPCheM (https://ipchem.jrc.ec.europa.eu/) and ChemTHEATRE (https://chem-theatre.com/) have been postulated to resolve this issue, yet collating a reliable dataset from such sources is currently unfeasible (Muratov et al., 2012). As such, gathering a larger dataset would likely be reliant upon literature, with the current review highlighting a breadth of publications containing compatible information. It is evident that not only is more data required, but that a more systematic means of storing, distributing and retrieving these data is also essential.

4.4. Understanding data relevance and quality

- There must be greater appreciation of what types of study are useful to assist in environmental risk assessment and will assist in the characterisation of real-life exposure scenarios. Linked to this is the lack of assessment of data quality, with few of the studies being performed to OECD Guidelines or Good Laboratory Practice. If future testing materialises, then there should be a greater emphasis on determining the relevance of experimental studies and ensuring that their quality is suitable for all purposes, including regulatory adoption.

4.5. Identification and incorporation of interaction effects into models

- As yet, there is no consensus on how to approach the inclusion of interaction effects, where they exist, into QSAR models. A better and more complete understanding is required of whether we need to go beyond the typical additive approach. One place where such knowledge could be identified and compiled is via a more extensive review and compilation of drug interaction effects. In addition, there could be a greater understanding and application of our knowledge of mechanisms of toxic action, particularly for acute environmental toxicities. Linked to this, there are obvious opportunities to incorporate knowledge and understanding from Adverse Outcome Pathways (AOPs) into our schemes [14]. Techniques such as read-across could be of particular assistance in this regard.

4.6. Modelling approach (descriptors and statistical methods)

- Models identified with this review used the full range of QSAR descriptors from physicochemical properties to 2D and quantum chemical calculations. There is no ideal descriptor for use in a mixture QSAR study, but those chosen should be pragmatic and give credibility to the model, notably by allowing full mechanistic interpretation. Ideally such descriptors should be simple, unambiguous and easy to calculate. Likewise, there is no consensus on how descriptors can be formalised to account for the mixture contributions and constitution.
- Statistical approaches applied in development of models for mixture toxicity range from simple regression analyses to machine learning. No ideal technique can be recommended at this time. It is appreciated that as the mixtures become more complex, there is likely to be a greater need to adopt machine learning approaches. Whilst rapid and potentially accurate, these typically lack transparency and interpretability, in turn hindering uptake and acceptance.
- A possibility that has yet to be explored fully in terms of mixture toxicity modelling is use of read-across such that effects and even potency may be established from similar or analogous mixtures. Such approaches have seen great acceptance for single chemicals and are increasingly being considered for botanical substances, natural products and UVCBs.

4.7. Towards a unified approach to model meaningful effects for realistic environmental and other mixtures

- Many currently available mixture toxicity QSAR are theoretical and, as such have limited practical application. Despite this, they have provided a wealth of knowledge on which we can build new frameworks and approaches to model such endpoints. Given the possibilities and the appreciated challenges associated with modelling toxicity, there is a great need to develop a unified approach to understanding its application towards mixtures, alongside practical means to developing, evaluating and applying such models to realistic environmental exposures of relevant chemical combinations.

5. Conclusion

The present review has provided a detailed analysis of the differing approaches that have been used throughout QSAR development to predict the effects of mixtures. In general, reoccurring trends presented themselves throughout toxicological-based publications, whereby binary mixtures at a single concentration ratio are examined in an additive manner. Mixture descriptors have commonly been constructed from molecular descriptors through an integral additive approach, and resulting models traditionally developed using regression analysis. The

majority of research on mixtures has been directed towards environmental effects, whilst other fields, for instance human health, have been under-studied. It is expected that to increase the uptake of QSAR predictions, greater respect towards potential interaction effects should be granted, alongside consideration of more realistic exposure scenarios. In general, research up to the current time has provided an excellent foundation, where future work that addresses current limitations may not only improve relevance, quality and therefore uptake of predictions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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