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Quantitative structure-activity relationships and ecological risk assessment: an overview of predictive aquatic toxicology research

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Abstract

In the field of aquatic toxicology, quantitative structure-activity relationships (QSARs) have developed as scientifically credible tools for predicting the toxicity of chemicals when little or no empirical data are available. A fundamental understanding of toxicological principles has been considered an important component to the acceptance and application of QSAR approaches as biologically relevant in ecological risk assessments. As a consequence, there has been an evolution of QSAR development and application from that of a chemical-class perspective to one that is more consistent with assumptions regarding modes of toxic action. In this review, techniques to assess modes of toxic action from chemical structure are discussed, with consideration that toxicodynamic knowledge bases must be clearly defined with regard to exposure regimes, biological models/endpoints and compounds that adequately span the diversity of chemicals anticipated for future applications. With such knowledge bases, classification systems, including rule-based expert systems, have been established for use in predictive aquatic toxicology applications. The establishment of QSAR techniques that are based on an understanding of toxic mechanisms is needed to provide a link to physiologically based toxicokinetic and toxicodynamic models, which can provide the means to extrapolate adverse effects across species and exposure regimes.

Keywords: Quantitative structure-activity relationships; Mode of toxic action; Aquatic toxicology; Hazard identification

1. Introduction

In the field of environmental toxicology, and especially aquatic toxicology, first generation quantitative structure-activity relationships (QSARs) have developed as scientifically credible

tools for predicting the acute, and in some instances subchronic, toxicity of chemicals when little or no empirical data are available. In addition to using quantitative relationships, analog selection techniques are also employed whereby data associated with 'structurally similar' chemi-

cals are used to estimate risk levels of compounds for which no data are available [1]. In part, the success in establishing these relationships is dependent upon well-defined and quantifiable toxicity endpoints, such as the 4-day LC50 value for the fathead minnow (*Pimephales promelas*). Although the accuracy of toxic potency predictions from QSARs continues to improve, there remains significant uncertainty in the appropriate selection of QSARs for predicting hazards. Thus, the proper application and continued acceptance of these predictive toxicology techniques requires that scientifically credible methods or models be used to systematically assign chemicals to appropriate QSARs or analogues. This fundamental process in the use of predictive techniques represents a major area of uncertainty in prospective ecological risk assessments for chemical stressors [2,3], where in the context of a variety of acute and chronic toxicity endpoints, errors in QSAR selections can result in 10–1000-fold errors in toxic potency estimates.

Traditionally, the selection of structural analogues or QSARs has been based on the implicit assumption that compounds from the same 'chemical class' should behave in a toxicologically similar manner. Although this working hypothesis seems reasonable, the delineation of 'chemical classes' is problematic and research completed over the past several years has challenged the notion that similarity in mode of toxic action is necessarily related to typical chemical classification schemes [4,5]. As a consequence, QSAR development and application have been evolving from a chemical class perspective to one that is more consistent with assumptions regarding modes of toxic action [6]. Thus, the use of mode of action-based QSARs requires a fundamental understanding of both toxic mechanisms and the critical structural characteristics and properties of a chemical that governs its action by a specific mechanism. The need for this level of understanding is critical in other aspects of ecological risk assessments as well. In particular, reducing uncertainties in species extrapolation predictions through the use of biologically based dose response models [7] is fundamentally based on a chemical's mode of toxic action.

This overview provides a summary of our laboratory's effort over the last several years to establish toxicodynamic databases as well as computational chemistry techniques to establish classification systems whereby chemicals are assigned to acute modes of toxic action and their potency subsequently estimated with an appropriate mode of action-based QSAR.

2. Background

Initial hazard identifications for aquatic organisms typically incorporate the use of acute toxicity estimates and in the context of developing QSARs to predict potency, research reported during the early 1980s in both the U.S. and The Netherlands established that the majority of industrial organic chemicals (excluding pesticides and pharmaceutical agents) elicit their acute toxic effects through a narcosis mechanism [8–11]. Narcosis can be defined as a reversible state of arrested activity of protoplasmic structures resulting from exposure to an appropriate xenobiotic. In the context of the intact organism, narcosis and general anesthesia are commonly used interchangeably [12]. Although commonly described in the literature as a non-specific mode of action, the actual mechanism of narcosis and anesthesia remains unknown and is an active area of research, as discussed in several reviews (e.g. see [12,13]). The sense that narcosis is a non-specific mode of toxic action is based, in part, on the diverse group of chemical structures that elicit narcotic effects in aquatic organisms and mammals, including chemically inert gases, aliphatic and aromatic hydrocarbons, chlorinated hydrocarbons, alcohols, ethers, ketones, aldehydes, weak acids and bases, and some aliphatic nitro compounds (e.g. see [12]).

With the development of initial toxicity data sets numerous QSAR investigations were undertaken, and the findings of Veith et al. [8] and Konnemann [10] established that the potency of narcotics was entirely dependent upon a xenobiotic's hydrophobicity. With subsequent experimental studies and modeling efforts it has been generally accepted that the relationships reported by Veith et al. [8] and Konnemann [10] represent the minimum, or baseline, toxicity that a com-

pound can elicit in the absence of a more specific mode of toxic action. With additional study it became clear that there were subclasses of narcotics, more potent than would be predicted from the baseline narcosis QSARs, that could be classified by either acute potency and/or physiological and behavioral characteristics of the narcosis response (e.g. see [12]). Further, it was obvious that some industrial chemicals were significantly more toxic than would be predicted from narcosis QSARs because they were capable of acting as oxidative phosphorylation uncouplers, respiratory chain blockers, or other more specific mechanisms (see review by Broderius [14]).

In an attempt to define the acute toxicity chemical structure space to more completely define the 'universe' of potential predictive models, research was undertaken at our laboratory to establish a database for the fathead minnow 96-h LC50 endpoint. This database now contains LC50 values for approximately 600 chemicals [15–19], which were selected based on an assessment of the U.S. industrial chemical inventory of discrete organic chemicals [20]. A plot of this acute toxicity database, as a function of hydrophobicity (log *n*-octanol:water partition coefficient, or log *P*), is presented in Fig. 1. Overlaid on this plot is a depiction of hypothetical QSARs that represent models that can be developed from this data set. As depicted in this figure the baseline narcosis model (i.e. QSAR 'A', in Fig. 1) provides a minimum potency prediction, while the other relationships predict greater potency for the same log *P* value. Clearly, the process of QSAR selection in ecological and human health hazard identifications represents a major area of uncertainty [2,3], where errors in QSAR selections can result in 10–1000-fold errors in toxic potency estimates. Traditionally, the selection of QSARs has been based on a 'chemical class' perspective; however, the delineation of 'chemical classes' is problematic and research completed over the past several years has challenged the notion that similarity in mode of toxic action is necessarily related to typical chemical classification schemes [5].

3. Mode of action knowledge base and classification scheme: acute toxicity in the fathead minnow

The establishment of toxicologically defensible techniques to assess mode of toxic action from chemical structure requires toxicodynamic knowledge bases that are clearly defined with regard to exposure regimes and biological models/endpoints and based on compounds that adequately span a chemical property space anticipated for future applications. With such knowledge bases, classification schemes ranging from rule-based expert systems to modified neural networks can be established [5]. Failure to adequately specify a knowledge base across the conceptual dimensions of exposure, biological model, and chemical property space can lead to toxicologically meaningless information and statistically inadequate mode of action prediction schemes. As depicted in Fig. 2, a knowledge base of acute lethality in fathead minnows represents a discrete location in the mode of action domain and using information within this plane could potentially lead to a classification system for predicting modes of toxic action for acute toxicity in this and related fish species. Use

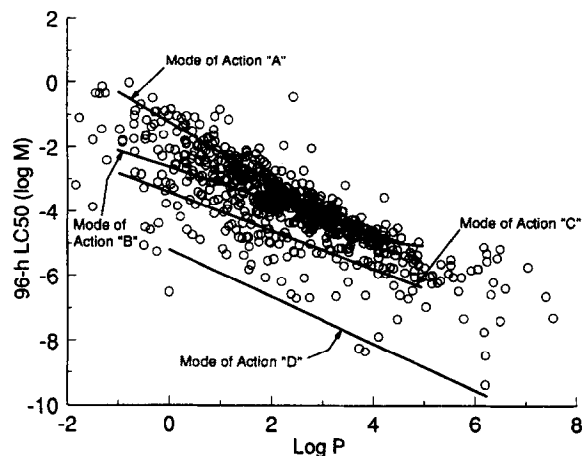


Fig. 1. Plot of the acute toxicity of approximately 600 industrial organic chemicals to the fathead minnow as related to log *P*. Superimposed on this plot are hypothetical QSARs that represent a series of mode of action specific relationships that have been developed for different sets of chemicals within this database. Relationship 'A' can be assumed to represent the baseline, or Narcosis-I, QSAR.

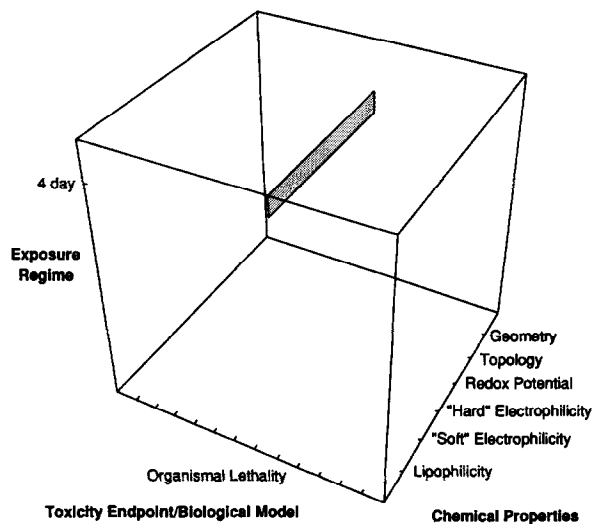


Fig. 2. The domain of information appropriate for assessing acute modes of toxic action in fathead minnows (adapted from [5]).

of this knowledge base to predict mode of toxic action for other biological models could lead to large errors unless the toxicological principles underlying the uncertainties associated with the required extrapolations are understood, i.e. the mechanistic basis of the toxic effect is known and the appropriate dosimetry defined.

3.1. Acute mode of toxic action knowledge base

Using the acute toxicity database described previously [15–19], xenobiotics were empirically assessed for modes of toxic action through the interpretation of joint toxic action studies, physiologically based toxic response syndromes, and single chemical dose-response curves to establish a knowledge base appropriate for the domain represented in Fig. 2. The general approaches and results have been summarized previously [5], and will be briefly highlighted here. Under the assumption that chemicals acting under a common mode of action are concentration additive, the toxicity of chemical mixtures to the fathead minnow was assessed using specific chemicals with known modes of action as reference toxicants [14,21,22]. Subsets from classes of toxicants identified in joint action studies were subsequently evaluated in experiments with rainbow trout (*Onchorhynchus mykiss*) where physiological profi-

les were measured to confirm toxic mode of action classifications [23–27]. Using results from these efforts, in association with behavioral assessments [28,29] and dose-response characteristics of single chemical 4-day LC₅₀ tests, acute modes of toxic action were subsequently assigned to chemicals. Based on the amount of information available for a given chemical, confidence levels of 'A' (high confidence) through 'D' (low confidence) were assigned to each classification.

Of the 617 chemicals in the database, 581 were classified in the following groups: baseline or narcotics-I, narcotics-II, narcotics-III, oxidative phosphorylation uncouplers, respiratory inhibitors, proelectrophiles/electrophiles, acetylcholinesterase (AChE) inhibitors, a diverse group of central nervous system seizure agents and stimulants, and barbiturates. Of the 581 classifications made, 106, 171, 165, and 94 had 'A', 'B', 'C', or 'D' levels of confidence, respectively. There were 45 chemicals assessed that were classified as showing 2 modes of action during acute intoxication; as a consequence, 2 confidence levels were assigned for each classification. For the subset of chemicals that could be classified with an 'A', 'B', or 'C' level of confidence, assessments identified 241 narcotic-I, 13 narcotic-I/narcotic-II, 36 narcotic-II, 26 narcotic-III, 12 oxidative phosphorylation uncouplers, 4 respiratory inhibitors, 97 proelectrophiles/electrophiles, 17 AChE inhibitors, 9 central nervous system convulsants/stimulants, and 6 barbiturates. In addition, 50 chemicals were designated as exhibiting characteristics associated with narcosis as well as side effects that were significantly strong to suggest that an electrophile-based mode of action may have contributed to lethality.

An analysis of the knowledge base illustrated that compounds within a 'chemical class' can be associated with several modes of toxic action groups. For example, aromatic amines and phenolic compounds were associated with narcosis-I, narcosis-II, and oxidative phosphorylation uncoupling. There were also examples of compounds within a class acting as either narcotics or electrophiles (e.g. unsaturated alcohols, aldehydes, alkenes, halogenated aliphatic hydrocarbons, and substituted aromatics). Finally, there was a great

diversity of compounds that were classified as electrophiles.

3.2. Rule-based classification system for predicting acute mode of action

Using the knowledge base, structural fragments were identified for each mode of action. Based on the set of structural fragments an expert system has been developed whereby a chemical structure is screened for specified substructures and, if identified, the compound is assigned to the appropriate mode(s) of toxic action, i.e. *if* a given substructure is present, *then* a given mode of toxic action is assigned to the compound (see [5] for a more detailed summary).

A narcosis-I, or baseline narcosis, mode of toxic action is the initial default assumption made for every chemical. If no structural fragments are subsequently identified, narcosis-I is selected as the mode of action. If a structural fragment(s) is identified in the compound, it is associated with the appropriate mode(s) of action. The mode of action expert system, and series of associated QSARs, have been incorporated within the ASTER (ASsessment Tool for Evaluating Risk) support system. ASTER, which is an integration of the AQUIRE (AQUatic Information RETrieval) toxic effects database [30] and predictive toxicology models, was developed to assist regulators in performing ecological risk assessments [31]. Once a mode of action has been selected, and if a fathead minnow acute toxicity QSAR is available, the estimated LC₅₀ is reported to the user. If more than one mode of action is identified, that mode of action resulting in the greatest toxicity hazard is selected as the default; however, the user is alerted to the possibility of alternative modes of action and associated toxicity predictions, thereby gaining a sense of the uncertainty associated with a QSAR selection.

Recently Verhaar et al. [4] also reported a classification scheme, based primarily on substructural fragment rules, for selecting structure-activity relationships to predict the toxicity of industrial chemicals to aquatic organisms. These investigators primarily relied on an analysis of single chemical and joint action toxicity studies performed in their laboratory, as well as the toxicological literature.

This classification scheme results in the delineation of 4 broad groups of chemicals, including inert chemicals (i.e. narcotic-I compounds), less than inert chemicals (i.e. narcotic-II compounds), reactive chemicals (i.e. oxidative phosphorylation uncouplers, proelectrophile/electrophiles), and specifically acting chemicals (e.g. neuroactive pesticides). Within the latter 2 groups specific rules are provided to delineate subgroupings. Using this scheme, a narcosis-I toxicity value is calculated for a compound and based upon the chemical's assigned class, the toxicity value is reduced by a factor of 1.0, 5–10, or 10–10 000 based on whether the compound has been classified as an inert, less than inert, reactive, or specifically acting chemical, respectively.

Although the prototype expert systems described here provide the basis to identify uncertainties associated with the selection of analogues and QSARs, there still remain significant issues that can lead to prediction errors [5]. Inherent in a rule-based system is the need to specify substructural fragments associated with modes of toxic action, which is based on the subjective bias of the expert(s). Further, a substructurally-based system can become quite difficult to implement in cases where more than one toxicophore is associated with a given structure or where a global property, such as soft-electrophilicity, is combined with a substructure in a rule. In this context, rule-based expert systems do not readily provide the means to relate variation in chemical structure to variation in toxicological properties. More fundamentally, a fragment-based rule system can also be limited because it reduces a chemical structure to a specified substructure and ignores the other topological and potential electronic features of the entire compound which may influence its ability to act under a given mode of toxic action. For example, current expert systems are limited in evaluating reactive toxicants where global and/or local stereoelectronic parameters may be required to assess whether a compound can act as a soft or hard electrophile [32–34] or undergo redox cycling [35]. The potential for using non-rule based artificial intelligence techniques to classify chemicals by mode of toxic action have been discussed elsewhere [5].

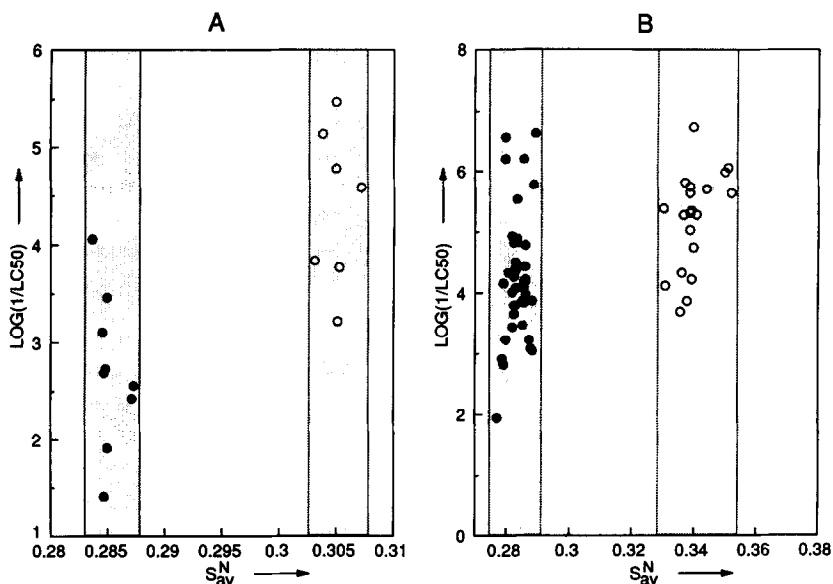


Fig. 3. Isoelectrophilic windows for narcotic-I (○) and reactive (●) unsaturated alcohols, aldehydes and ketones (A) and narcotic-I (○) and reactive (●) substituted benzenes, phenols, and anilines (B; adapted from [37]).

The specific issue of classifying reactive toxicants and subsequently predicting their toxicity has been an area of interest because these compounds are typically among the most potent industrial chemicals and their identification raises concern over potential chronic effects. Recently, a series of studies have described an exploratory approach whereby diverse groups of compounds, found in the fathead minnow acute mode of action database, are being discriminated using global and local measures of reactivity [36–38]. Initial insights were reported in a structure toxicity relationship study for α,β -unsaturated alcohols, aldehydes and ketones and related allene derivatives, which indicated that descriptors of soft electrophilicity, such as average superdelocalizability (S_{av}^N), could differentiate those compounds classified as narcotics from those classified as reactive toxicants [36,37]. As depicted in Fig. 3A, isoelectrophilic windows were established that discriminate alcohols that act as narcotics (average S_{av}^N values of approximately 0.285) from reactive aldehydes and ketones (average S_{av}^N values of approximately 0.305). It was also reported in subsequent investigations [37,38] with a larger set of compounds that was comprised of substituted

benzenes, phenols and anilines (identified as narcotics-I, narcotics-II, oxidative phosphorylation uncouplers, and proelectrophiles/electrophiles in the mode of action knowledge base) that there is a tendency for modes of toxic action to cluster according to soft electrophilicity. In this case, narcotic-I and narcotic-II compounds had average S_{av}^N values of 0.280, and compounds typically classified as uncouplers or proelectrophiles/electrophiles had average S_{av}^N values of 0.345 (Fig. 3B). The clustering of uncouplers and electrophiles within a common range of S_{av}^N suggests that highly reactive compounds with a dissociable proton are capable of disrupting oxidative phosphorylation, while those without dissociable protons produce toxic responses through covalent binding with soft nucleophiles within biomacromolecules [38]. This classification is, of course, not applicable to discerning hard nucleophiles. Within the soft isoelectrophilic windows, QSARs based on log P have been established. These results have led to the suggestion [37,38] that the acute toxicity of chemicals can be described by a nearly orthogonal relationship between molecular descriptors for hydrophobicity and soft electrophilicity (Fig. 4).

The classification of a compound as a reactive toxicant is, of course, complicated by metabolic activation; and the previously described modeling [36–38] is based on an evaluation of parent compounds and assumed metabolic products. In cases where the current acute toxicity database for fat-head minnows does contain compounds that are metabolically activated, e.g. acetylenic and allylic alcohols [36,39], substructural rules indirectly incorporate biotransformation in the classification scheme for the expert system [5] previously summarized. Clearly, there is a need to develop a predictive capability to assess metabolic activation for toxicity predictions.

4. Conclusions

The need to establish models to systematically assign chemicals to appropriate ecotoxicity QSARs has been identified as a major area of uncertainty in risk assessments for chemical stressors in aquatic ecosystems [2,3]. This area of uncertainty is also reflected in the use of predictive toxicology models in the assessment of hu-

man risk [1]. An improved capability for assessing modes of toxic action from chemical structure will address several problems in the field of predictive toxicology, generally. An immediate application of an improved capability lies in the continued development and application of QSAR and analog selection techniques to predict the toxicity of xenobiotics. The establishment of such selection techniques, coupled with a fundamental understanding of toxic mechanisms, is also needed in the development of biologically based dose response models, which can provide the means to better extrapolate adverse effects across species and exposure regimes, when limited empirical data are available [7].

The uncertainties in quantifying chemical similarity of industrial organic compounds for assessing modes of toxic action involves both toxicological and computational chemistry issues. Computational efforts must address similarity methods that are applicable for large chemical data sets (e.g. 500–10 000 structures) that are representative of the heterogeneous industrial organic chemicals in commerce [5]. It must also be recognized that methods designed for assessing similarity within homologous series of structures, although appropriate for drug or pesticide design, will likely be inadequate for large and diverse sets of chemicals [40]. To improve the toxicological basis of these assessments it is essential that mechanistically sound knowledge bases be established so that decisions about chemical similarity incorporate the complexities associated with exposure regimes, biological models and endpoints, and the universe of chemical properties associated with industrial organic chemicals.

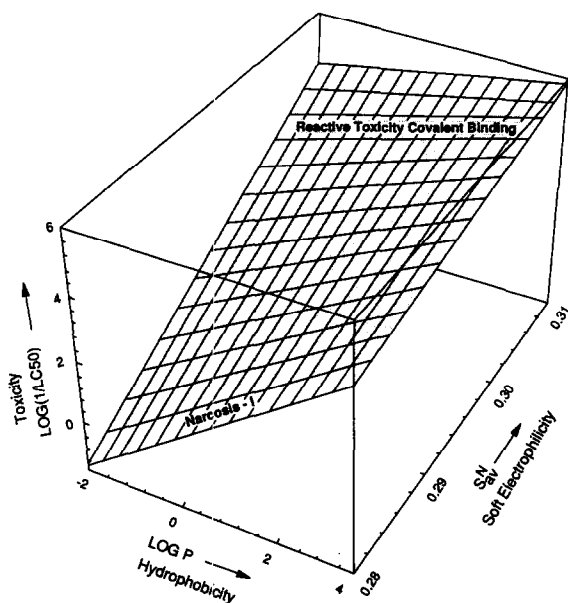


Fig. 4. Response surface showing the relationship between hydrophobicity ($\log P$), soft electrophilicity (S^M), and toxicity (adapted from [37]).

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